STUDIES ON THE ACUTE HEPATIC PORPHYRIAS

by

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"No greater misfortune could happen to anyone than that of developing a dislike for argument."

Socrates

PREFACE

When I started my post as Lecturer in the Department of Medicine in 1978 the porphyrias were merely an esoteric group of small-print conditions that had little relevance to me. I soon learnt the frustrating limitations of our knowledge in dealing with the suffering of those few unfortunate individuals afflicted by these rare disorders. Thanks to the guidance and encouragement of Professor Sir Abraham Goldberg I came to realise the unique opportunities that a study of such inborn errors of metabolism could offer in furthering our understanding of basic physiological principles. I am also indebted to Sir Abraham for inculcating in me the constant need to think of the patient's interests first and foremost before satisfying one's often compelling scientific curiosity.

After having spent seven years of clinical involvement with patients suffering from acute intermittent porphyria referred to our Unit, the experience that I have accumulated is embodied in this thesis. Of course, this would not have been possible without the enormous help and stimulating discussions I have had with so many of my colleagues and referring physicians, too numerous to mention individually. Some of the work presented in this thesis has already been published, and the rest will soon follow.

Except where acknowledged the work presented has been carried out personally by myself.

The writing of this thesis is entirely my own work.

CONTENTS

ACKNOWLEDGEMENTS

During the past seven years I have had the great privilege of working as First Assistant to Sir Abraham Goldberg, Regius Professor of the Practice of Medicine at the University of Glasgow. It is thanks to him that I was introduced to the fascinating field of the porphyrias. I acknowledge with gratitude the guidance and advice which he has so generously given me in the course of my clinical and research work. I am grateful to Dr Michael Moore who is an international authority on haem metabolism for his advice and the numerous discussions that we have had. I am deeply indebited to Mr George Thompson, Chief Technician for the measurement of porphyrins and haem enzymes performed in these studies. Moreover he provided me with invaluable help when I was setting up the protoporphyrinogen oxidase assay for the monocyte cultures.

I wish to thank my following colleagues for their contribution in the various studies:

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2. Dr David Doyle, Consultant Neuropathologist at the Institute of Neurological Sciences, Southern General Hospital, Glasgow, for his generous assistance. In the course of our early discussion he initially suggested the measurement of cytochrome oxidase activity

in the muscle biopsies of our porphyric patients. This led to a co-operative study from 1979 to 1984 for which I acted as clinical co-ordinator. All the histochemical measurements were performed in Dr Doyle's laboratory.

3. Professor David Graham, Professor of Neuropathology at the Institute of Neurological Sciences for performing the autopsy on one of our patients at very short notice.

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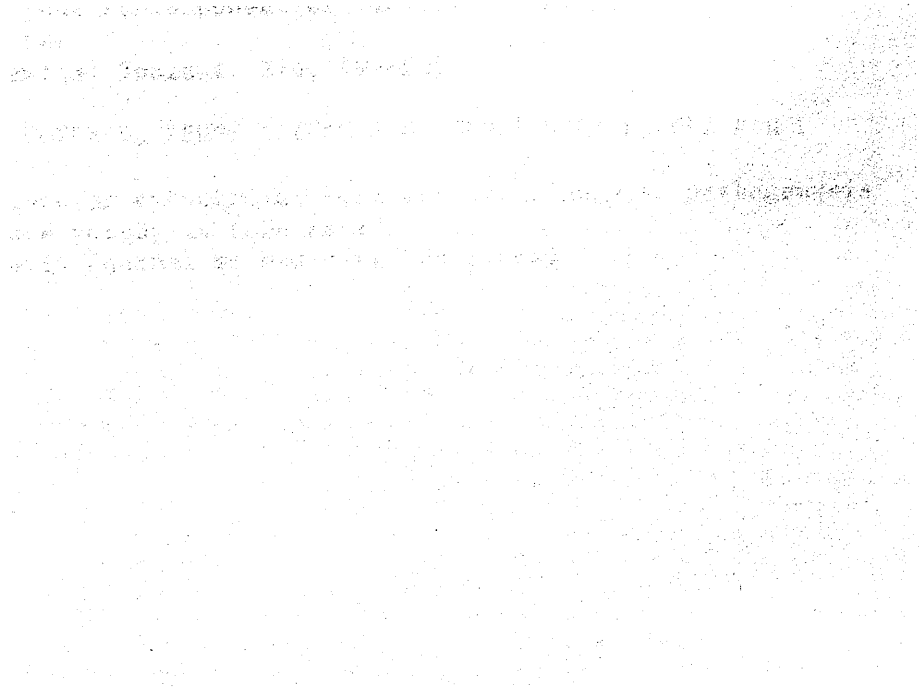
The preparation of the figures and illustrations have been kindly performed by the staff of the Medical Illustration Department at the Western Infirmary, under the direction of Mr Loudon Brown.

Last, but not least, I must thank my wife, Myriam, and my daughter, Roseanne, for their eternal patience and tolerance in the course of my work.



PUBLICATIONS

YEUNG LAIWAH A C, GOLDBERG A, MOORE M R (1983)
Pathogenesis and treatment of acute intermittent porphyria
Journal of the Royal Society of Medicine, 76, 386-392

YEUNG LAIWAH A C, JUNOR B, MACPHEE G J A, THOMPSON G G, McCOLL K E L (1983)
Charcoal haemoperfusion and haemodialysis in acute intermittent porphyria
British Medical Journal, 287, 1746-1747

YEUNG LAIWAH, MACTIER R, McCOLL K E L, MOORE M R, GOLDBERG A (1983)
Early-onset chronic renal failure as a complication of acute intermittent porphyria
Quarterly Journal of Medicine, 205, 92-98

YEUNG LAIWAH A C, RAPEPORT W G, THOMPSON G G, MACPHEE G J A, PHILIP M F, MOORE M R, BRODIE M J, GOLDBERG A (1983)
Carbamazepine-induced non-hereditary acute porphyria
Lancet, 1, 790-792

YEUNG LAIWAH A C, MACPHEE G J A, BOYLE P, MOORE M R, GOLDBERG A (1985)
Autonomic neuropathy in acute intermittent porphyria
Journal of Neurology, Neurosurgery and Psychiatry (in press)

YEUNG LAIWAH A C, JONES L, HAMILTON O, WHALEY K (1985)
Complement-subcomponent-C1-inhibitor synthesis by human monocytes
Biochemical Journal, 226, 199-205

GOLDBERG A, DOYLE D, YEUNG LAIWAH A C, MOORE M R, McCOLL K E L (1985)
Relevance of cytochrome-C-oxidase deficiency to pathogenesis of acute porphyria (abstract)
Quarterly Journal of Medicine (in press)

SUMMARY

This thesis consists of three sections. The first section is a general description of porphyrins and biochemical control of haem biosynthesis followed by an up-to-date review of the acute hepatic porphyrias and their clinical management, with particular emphasis on acute intermittent porphyria (AIP).

The second section addresses itself to the pathogenesis of the neuropathy of AIP. Following a critical review of the histopathological and biochemical work on the subject the results of a study on the cardiovascular autonomic function in AIP are discussed. Objective evidence for parasympathetic and sympathetic dysfunction during an acute attack is provided. Moreover, there is suggestion of parasympathetic involvement both in patients who have fully recovered from the acute episodes and asymptomatic latent cases. These findings support the electrophysiological results of Mustajoki and Seppalainen (1975).

Using two different histochemical techniques, cytochrome oxidase activity was found to be markedly depressed in muscle biopsies taken from AIP patients during an acute attack. Since cytochrome oxidase plays a vital role in the terminal oxidative phosphorylation pathway, the possibility of a myopathic component in the muscle weakness, hitherto attributed to the neuropathy, is considered. But more significantly, depression of cytochrome oxidase activity in muscle tissue suggests that this might be the case in nerve cells. Further support for this concept comes from

other workers who have shown that the activity of the cytochrome-P450-dependent mixed-function oxidase system is also depressed in porphyric patients.

The cytochrome oxidase study is followed by a clinico-pathological discussion on the autopsy findings of a 31-year old AIP patient who died of the disease. Detailed documentation of the patient's clinical course and the purposeful neuropathological autopsy carried out provided us with a rare opportunity to investigate further the relative roles of demyelination and axonal degeneration in the pathogenesis of the disease. It was indeed surprising when independent review of the autopsy material by two experienced neuropathologists failed to reveal any of the characteristic morphological changes previously described.

The newly discovered association between early-onset chronic renal disease and AIP is discussed along three main possibilities: analgesic-induced nephropathy, hypertension-related renal disease and cytotoxic effects of porphyrin precursors on the kidneys. Analgesic-induced nephropathy could be satisfactorily excluded. On balance evidence is in favour of hypertension-related renal disease although a local toxic effect by porphyrin precursors cannot be entirely excluded. A strong case can be made for the porphyria-associated hypertension to the neurogenic in type.

Many AIP patients with severe abdominal pain fail to respond to large dosages of opiate analgesics. A study was performed to determine whether the porphyric pain was truly resistant to opiates. It was found that if the patient failed to respond to

the usual therapeutic dosage of one of three opiates: pethidine, morphine and buprenorphine, increasing the amount of drug administered did not improve the response rate.

In 1981 I observed that the pupils of a porphyric patient in a severe attack failed to undergo miosis after parenteral administration of large doses of morphine; yet they retained a normal response to accommodation and light stimulation. The significance of this finding, only recently appreciated, indicate the possibility of either dysfunction or depletion of the mu-opioid receptors in the optic nervous system.

Attempt to improve the symptoms of an AIP patient by sequential haemodialysis and haemoperfusion was unsuccessful despite a demonstrable fall in the excretion of plasma and urinary porphyrin precursors.

On the basis of these studies I propose a new hypothesis to explain the pathophysiology of acute porphyria:

During an acute attack, as a result of a specific block in the haem biosynthetic pathway, there ensues a deficiency of cytochrome oxidase in the nerve cells. Because neuronal cells have the highest metabolic requirements for oxygen in the body the nervous system becomes particularly susceptible to the effects of cytochrome oxidase deficiency - thus accounting for acute porphyria to become manifest as a neuro-psychiatric syndrome. As the lack of cytochrome oxidase constitutes a "functional" intracellular block it explains why several neuropathological studies have failed to reveal any morphological changes in some porphyric patients dying from the disease. It is only with a more

severe or prolonged attack that degenerative changes develop in the nerve cells. These changes will occur particularly in the metabolically more active anterior horn cells of the spinal cord, neurones with the longest axons and those with larger motor unit innervation.

In the light of recent experimental studies on opioid receptors it is suggested that porphyric neuropathy can lead to a depletion or dysfunction of opioid receptors in the brain stem as well as other parts of the nervous system such as the optic nerves and gut innervation. Lack of functional opioid receptors in the brain stem region would explain the basis of the neurogenic hypertension and tachycardia, inappropriate ADH secretion, and failure of analgesic response to different opioid agonists during the severe attacks. The gut is richly supplied with endogenous opioids and opioid receptors. Depletion of gut opioid receptors could conceivably cause an impairment in the modulating inhibitory effects of endogenous opioids which act as a neurotransmitter. This could provide an explanation for the pain of acute porphyria to localise primarily to the abdomen.

This hypothesis does not explain the nature of the initial peripheral nociceptor stimulus causing the pain. Whether this is due to the porphyric neuropathy, as in diabetes, or to a circulating metabolite is a matter for further speculation.

In the third section of my thesis two new experimental approaches for studying disturbances of the haem pathway in man are explored. The first method follows the discovery of a non-hereditary case of acute porphyria believed to have been

induced by the anticonvulsant carbamazepine. Subsequent patient and volunteer studies confirmed that this drug can produce a pattern of porphyrin excretion similar to that seen in AIP. Carbamazepine has also been shown to exert a significant inhibitory effect on human lymphocyte ALA-dehydratase activity. The second method follows the successful culture of human peripheral blood monocytes in vitro. Monocytes in culture for a period of 10 days were found to produce a mean value of 10.37 pmol protoporphyrin IX/μg DNA/h. This technique would be particularly useful for assessing the potential porphyrinogenicity of drugs and chemicals. Culture of a highly purified population of cells of bone marrow origin could also provide an excellent tool for investigating the molecular basis of the control mechanism for haem biosynthesis. Both in vitro methods described hold much promise in their future development.

ABBREVIATIONS

Abbreviations used in certain parts of the text

ALA	Delta-aminolaevulinic acid
AIA	2-allyl-2-iso-propylacetamide
AIP	Acute intermittent porphyria
DDC	3,5-Diethoxycarbonyl 1-1, 4-dihydrocollidine
HC	Hereditary coproporphyria
HMB	Hydroxymethylbilane
KM	Michaelis-Menten constant
mRNA	Messenger RNA
NEC	Non-enzymatic control for proto.O assay
PBG	Porphobilinogen
Proto IX	Protoporphyrin IX
VP	Variegate porphyria

Haem enzymes

	ALA.D	Erythrocyte Delta-aminolaevulinic Acid Dehydrase <u>or</u> Dehydratase
	ALA.S	Leucocyte Delta-aminolaevulinic Acid Synthase
	Proto.O	Protoporphyrinogen Oxidase
Synonyms [	PBG.D	Erythrocyte Porphobilinogen Deaminase
[	URO.S	Erythrocyte Uroporphyrinogen-1-Synthase

SECTION I

CHAPTER 1

HAEM METABOLISM

1. HAEM METABOLISM

1.1 INTRODUCTION

During the past 30 years much has been learnt about (1) the biochemistry of the porphyrins; (2) the properties of the enzymes involved in the haem biosynthetic pathway; (3) the molecular mechanisms by which these enzymes function and are regulated; and (4) the biological significance of certain disturbances of haem metabolism either inherited, as in the human porphyrias, or induced by drugs and chemicals. The discovery that the haem-containing mixed-function oxidase cytochromes P450 play a major role in the oxidation and detoxification of a large variety of endogenous and exogenous compounds has widened the scope of research in the field of porphyrin and haem metabolism. Consequently the study of the human porphyrias has aroused great interest not only among clinicians but also among biochemists, pharmacologists and other basic scientists: those whose research is concerned with the biotransformation of chemicals in the body and problems of interactions between the gene and the environment. The human porphyrias are particularly relevant for such studies since the clinical expression of these disorders in the genetically susceptible individuals is greatly influenced by distinct hormonal and environmental factors.

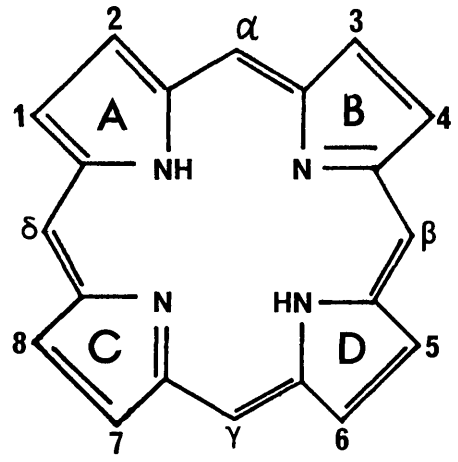
To study the porphyrias a basic understanding of porphyrin chemistry and haem biosynthesis is necessary.

1.2 NOMENCLATURE AND STRUCTURE OF PORPHYRIAS

Porphyrins are cyclic tetrapyrroles. The porphyrin nomenclature hitherto in use was developed by Hans Fisher and his School in Munich (Fisher & Orth, 1937). According to this system the basic structure of porphyrins is represented by the compound which Fisher called "Porphin" - shown in Figure 1a. It consists of four cyclic rings, derived from monopyrroles, designated as A, B, C and D which are joined together by four methene bridges α, β, γ and δ. This macrocycle is a tetrapyrrole. Substitution of the hydrogen ions by alkyl groups at the β positions in the macrocycle (ie positions 2, 4, 6 and 8) permits the formation of a large diversity of porphyrins. Two groups of reference compounds have set the basis for isomeric classification of porphyrins - the aetio- and meso-porphyrins. When each of the four pyrrole rings has one methyl and one ethyl side-chain substitutions the resulting porphyrin is known as an aetioporphyrin. There are four isomeric forms for the aetioporphyrins depending on the specific sequence of methyl and ethyl groups in the eight substituted positions on the ring. Most naturally occurring porphyrins are aetioporphyrins of the I and III isomers (Fisher & Orth, 1937), but only the III isomers have significant functional activity. Mesoporphyrins have a methyl and ethyl substituent on each of two of the adjacent pyrrole rings, and a methyl and propionyl side-chain on the remaining two rings of the macrocycle. Fifteen isomeric forms can exist for the mesoporphyrins.

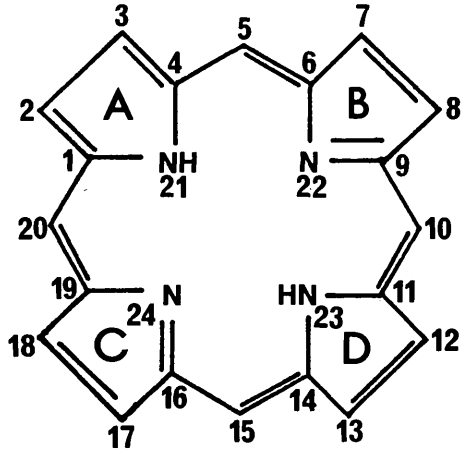
Recently a revised system of nomenclature for tetrapyrroles has been proposed by the International Union of Pure and Applied

a



Porphin (Fischer Numeration)

b



Porphyrin (IUPAC Numeration)

Figure 1: Structure and nomenclature of the porphyrin macrocycle. Porphyrin (Fisher nomenclature) or porphyrin (IUPAC nomenclature) is the basic structure of biologically occurring ring tetrapyrroles.

Chemistry and endorsed by the International Union of Biochemistry (IUPAC-IUB, 1980). Two important features of this nomenclature are that the basic macrocycle is called "a porphyrin" instead of "a porphin" (Fig 1b), and all carbon and nitrogen atoms are numbered from 1 to 24 instead of only numbering the outer carbon atoms of the pyrrole rings. Eleven of the well established trivial names from the Fisher's system are still retained, and these comprise all the terms in use at present for the common porphyrins. This new nomenclature is in effect more systematic and allows for more accurate designation of unusual porphyrin compounds.

1.3 PROPERTIES OF PORPHYRINS

The tetrapyrrole nucleus is essentially planar and hydrophobic. It has a high degree of chemical stability because of the phenomenon of resonance resulting from the conjugated double bond system (Smith, 1975). The water solubility of porphyrins increases with the number of carboxylic side-chains they possess. Thus uroporphyrin (8-carboxylated porphyrin) has a higher water-solubility than 4-carboxylated coproporphyrin which in turn is more water-soluble than 2-carboxylated protoporphyrin. Protoporphyrin is so hydrophobic that it is exclusively excreted in bile whereas uro- and copro-porphyrins can be excreted in the urine (Sano & Rimington, 1963).

Another common property of porphyrins is their distinctive absorption spectrum in the near-ultraviolet and visible regions; this permits their ready identification by spectrophotometry and

spectrofluorimetry. The absorption spectrum of porphyrins consists of a major absorption band around 400 nm known as the Soret band, and four smaller absorption bands between 500 nm and 630 nm. Metal-free porphyrins emit an intense red fluorescence when excited by long wavelength ultraviolet light (400 nm). Porphyrins chelated with diamagnetic metals (eg Mg, Zn, Sn) also fluoresce whereas those chelated with paramagnetic metals (eg Fe, Co, Cr, Ma) do not (Smith, 1975).

Porphyrinogens are the biological precursors of porphyrins. All methine bridges in these compounds are reduced; thus they are colourless and do not fluoresce.

1.4 HAEM STRUCTURE AND FUNCTION

Porphyrins function in living cells only as metal chelates eg the iron porphyrins (haem) and the magnesium porphyrins (Chlorophyll). These compounds provide the prosthetic groups necessary for the catalysis of the basic energy reactions upon which life depends. The evolutional development of haemoproteins has allowed economical utilization of metallic iron for a number of vital biochemical functions. These can be grouped as follows: (1) transport of molecular oxygen (haemoglobin and myoglobin); (2) electron transport for energy transfer (mitochondrial cytochromes); (3) activation of oxygen (cytochrome oxidase, tryptophan pyrrolase and mixed function oxidases including cytochrome P450); (4) activation of hydrogen peroxide (peroxidases); and (5) decomposition of hydrogen peroxide (catalases) (Granick & Gilder, 1947). These specific functions are

determined by the state of oxidation of the chelated iron, the nature of the side-chains on the porphyrin ring and the structure of the protein to which haem is bound. The haem of haemoglobin is the ferrous chelate of protoporphyrin IX (Fig 2).

1.5 HAEM BIOSYNTHETIC PATHWAY

The biosynthesis of 1 haem molecule requires 8 molecules each of glycine and succinyl CoA as starting material. Eight enzymes are involved in the haem biosynthetic pathway, four being localised within the mitochondrion and four within the cytoplasm (Fig 3). The first part of the pathway consists of a series of condensation reactions initiated by the enzyme delta-aminolaevulinic acid synthase to lead towards the formation of an unstable linear tetrapyrrole, hydroxymethylbilane. The first stable intermediate in the pathway is delta-aminolaevulinic acid (ALA), a 5-carbon aminoketone formed by the condensation of 1 molecule of glycine, and 1 molecule of succinyl CoA. Pyridoxal phosphate is an essential co-factor for that reaction. ALA then diffuses out of the mitochondrion into the cytoplasm where 2 ALA molecules are combined under the catalytic action of ALA dehydratase (synonym: porphobilinogen synthase) to form the monopyrrole, porphobilinogen (PBG).

The next step requires the concerted action of two enzymes, PBG deaminase (synonym: uroporphyrinogen-1-synthase) and uroporphyinogen III co-synthetase; this is to convert 4 molecules of PBG into 1 molecule of the tetrapyrrole, uroporphobilinogen III, through the intermediary of hydroxymethylbilane (HMB). HMB

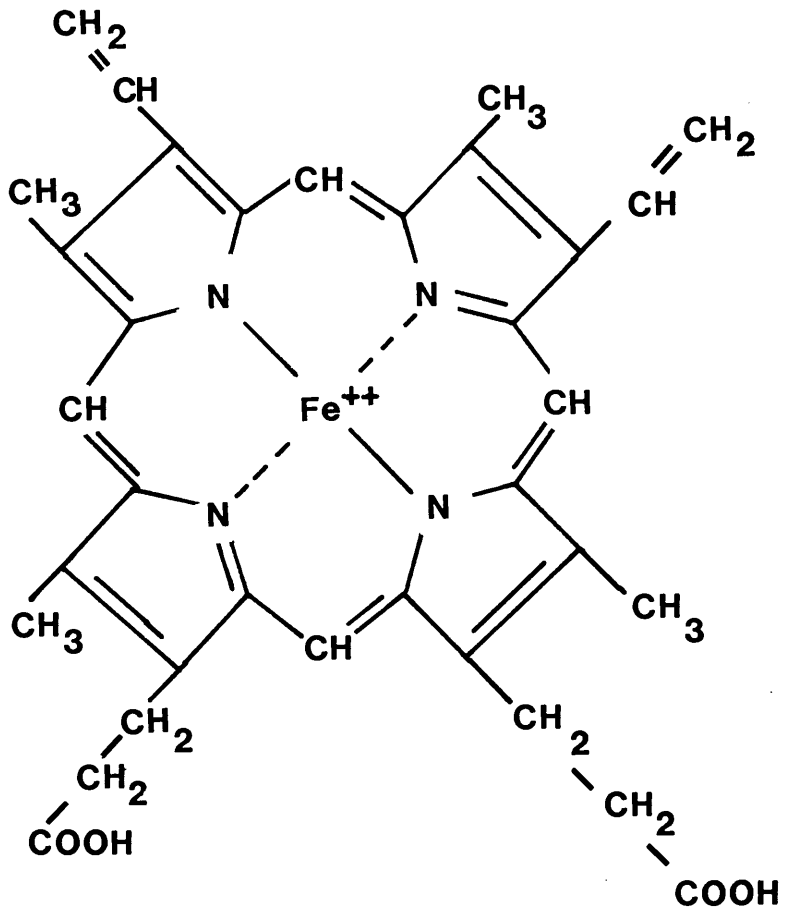
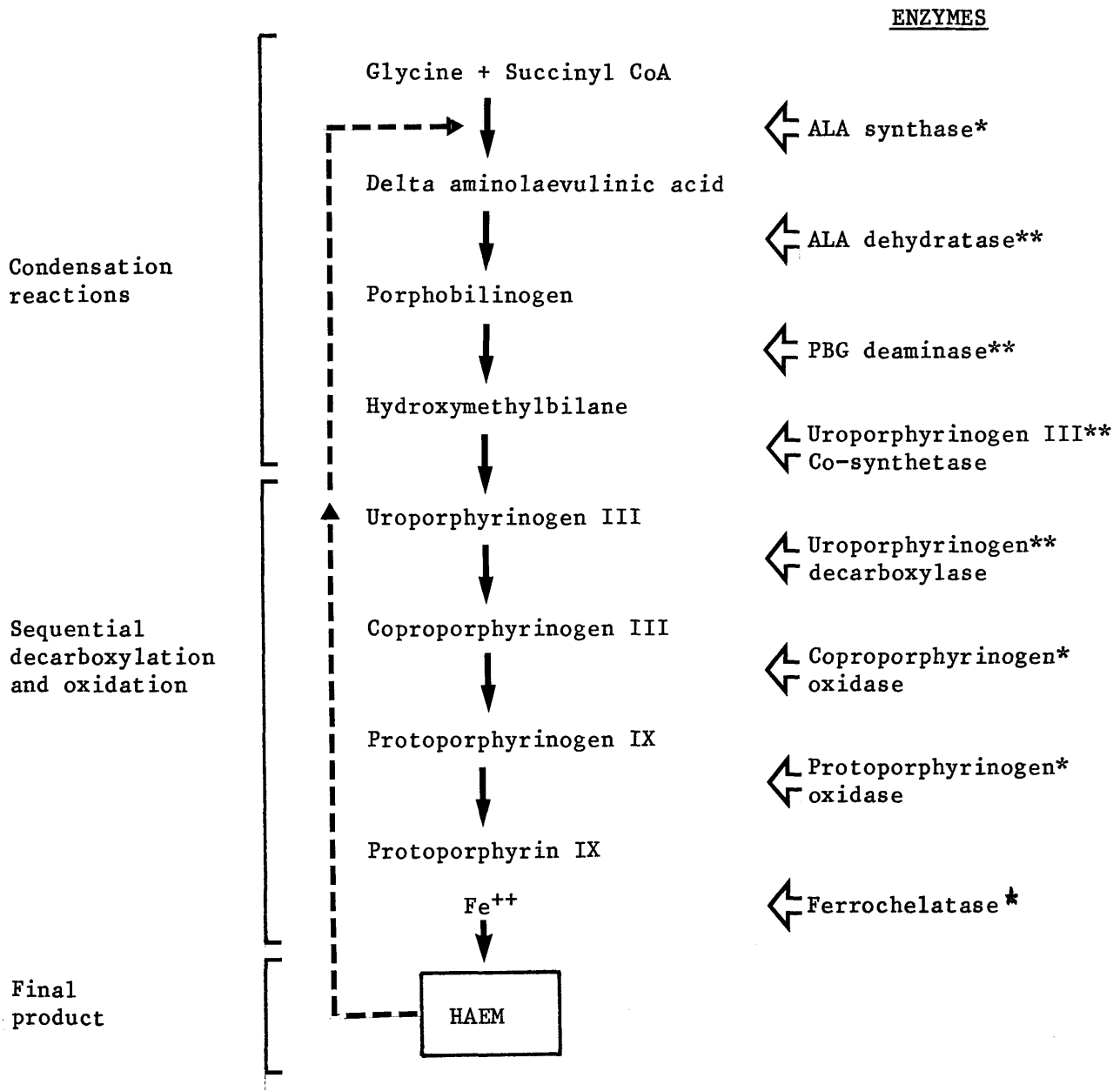


Figure 2: Structure of the haem molecule

HEPATIC HAEM BIOSYNTHETIC PATHWAY



* mitochondrial

** cytosolic

The rate of haem biosynthesis is regulated by the initial enzyme of the pathway ALA.synthase which is under negative feedback control by haem (broken line).

Figure 3

readily undergoes chemical cyclization so that PBG deaminase acting alone will only produce the symmetrical uroporphyrinogen I isomer. But under the catalytic action of uroporphyrinogen III co-synthetase a tetrapyrrole ring is formed with reversal of ring D to form the asymmetrical isomer, uroporphobilinogen III.

Next in the pathway is the sequential decarboxylation of the 8-carboxylate, uroporphyrinogen, to the 4-carboxylate coproporphyrinogen, under the catalytic activity of uroporphyrinogen decarboxylase. Uroporphyrinogen decarboxylase can convert both uroporphyrinogen- I and -III to their corresponding coproporphyrinogen isomers. But only coproporphyrinogen III can enter the mitochondrion where coproporphyrinogen oxidase catalyzes the combined decarboxylation and dehydrogenation of two propionyl residues on rings A and B of the porphyrin nucleus to form protoporphyrinogen IX. As coproporphyrinogen I isomer cannot proceed any further in the pathway it is excreted. Within the mitochondrion protoporphyrinogen is oxidised to protoporphyrin by protoporphyrinogen oxidase. Under the catalytic action of ferrochelatase a ferrous ion is finally inserted into each protoporphyrin molecule to form the <u>haem</u>.

The activities of the haem enzymes from human liver are shown in Table 1 and three important points emerge. First the substrate concentrations measured are much lower than the Michaelis constants (km) of the corresponding enzymes. Therefore the rate of reaction of these enzymes can be increased several-fold by increasing the substrate concentrations until the maximum

Enzyme	Activity*	Substrate concentration nmol/gm wet wt	Apparent Km (uM)
ALA synthase	0.26	-	-
ALA dehydratase	12.9	(0.04)***	270
PBG deaminase	0.14 (0.018)**	1.0	6
Uroporphyrinogen III decarboxylase	11.0	0.03	1.5+
Coproporphyrinogen oxidase	13.0	0.07	0.9
Protoporphyrinogen oxidase	-	-	-
Ferrochelatase	16.6	0.38	1.8 17++

* Activities are expressed as nmol ALA or ALA equivalents produced or utilized/hr/mg protein

** nmol uroporphyrinogen/hr/mg protein

*** rat liver

\+ pentacarboxylate porphyrinogen

++ Fe^{++}

Table 1: Haem biosynthetic enzymes in human liver tissue: Activities, Substrate concentrations and Michaelis constants

velocity, determined by the enzyme concentration, is reached. Second, ALA synthase, the main rate-limiting enzyme of the pathway and PBG deaminase have the lowest enzyme activities. Third, the activities of enzymes converting uroporphyrinogen III to haem are relatively high so that any uroporphyrinogen formed is likely to be rapidly converted to haem. Thus the unstable porphyrinogen intermediates acquire a short half-life which prevents their loss from the pathway by oxidation to porphyrins. The reactions involved in the conversion of ALA to haem therefore form an unbranched irreversible pathway (Elder, 1982): the loss of any intermediates primarily arise from transport or leakage out of the cells and intracellular oxidation of porphyrinogen to porphyrins. Normally the pathway operates very efficiently with little loss of intermediates. And as has been shown in the liver the synthesis and degradation of haem and the protein moieties of haemoproteins are so well co-ordinated that no free haem can be measured (Elder, 1980).

1.6 HAEM BIOSYNTHESIS IN THE LIVER

Liver cells normally produce about 15 per cent of the total haem synthesized in the body, the remainder being made mainly by the bone marrow for haemoglobin production (Granick & Sassa, 1971). About 65 per cent of hepatic haem is utilized for haemoproteins of the cytochrome P450 series; (Tschudy & Lamon, 1980) these constitute a major component of the microsomal mixed-function oxidase system which play an important part in the metabolism and detoxification of drugs, chemicals and other compounds (Meyer & Schmid, 1978; Maines, 1984).

Haem production in the liver is largely controlled by the activity of mitochondrial ALA synthase. The activity of this enzyme is under negative feedback control by the intracellular concentration of haem. The rate-limiting nature of ALA synthase is now well established (Granick & Urata, 1963; Nakao et al, 1966; Marver et al, 1966a; Kappas et al, 1984). Mitochondrial ALA synthase appears to have a rapid turnover rate. Its half-life is about 70 minutes in adult rat liver (Marver et al, 1966b; Hayashi et al, 1969; Tschudy et al, 1965a) and 3 hr in mouse liver (Gayathri et al, 1973) and in cultured chick embryo liver cells (Sassa & Granick, 1970). By comparison the average half-life of mitochondrial proteins in general is about five days. The short half-life and relatively low activity (Table 1) of ALA synthase provide an effective mechanism for its regulation by way of end-product inhibition. It is interesting to note that ALA synthase also has an unusually low affinity for its substrate glycine (km 5-19 mM) and the intracellular concentration of glycine is only about one-fifth to one-twentieth of the km for ALA synthase (Sinclair & Granick, 1977). This suggests that the metabolic control of intracellular glycine concentration may have a significant regulatory role on the activity of ALA synthase.

1.7 CONTROL MECHANISM BY HAEM

After ALA synthase formation in the liver was shown to be controlled by haemin administration (Sass & Granick, 1970) it became clear that depletion of haem could lead to derepression of ALA synthase followed by compensatory increase in its substrate

concentration in order to maintain haem synthesis. Haem-mediated control of ALA synthase seems to occur primarily at the level of enzyme synthesis rather than by direct enzyme inhibition. Experiments using cultured avian embryonic liver cells have shown that haemin could possibly interfere with ALA synthase synthesis at the translational level, shorten the half-life of its mRNA (Sassa & Granick, 1970), or repress the synthesis of mRNA for ALA synthesis at the transcriptional level (Whiting, 1976). It is probable that haem acts by a similar mechanism in human liver but this has not yet been directly demonstrated.

Certain drugs and chemical compounds appear to induce ALA synthase by several different mechanisms in all of which the depletion of a regulatory haem pool appears to be the common final event. There are four main types of situations in which intracellular haem balance can be affected. First, compounds that contain terminal olefinic or acetylinic bonds such as 2-allyl-2-isopropylacetamide (AIA) (De Matteis, 1978) and fluorinated anaesthetics (Ziman et al, 1980) can promote autocatalytic destruction of the haem prosthetic group of cytochrome P450 which then has to be replaced from the regulatory pool. Second, compounds like griseofulvin and the dihydrocollidines can convert haem to n-acetyl porphyrin derivatives which probably act by specific inhibition of ferrochelatase, thus leading to decrease in haem formation (De Matteis et al, 1981a). Third, drugs such as phenobarbitone promote the utilisation of haem for the synthesis of cytochrome P450 dependent mono-oxygenases required for their metabolism. This in turn stimulates the synthesis of apocytochrome P450 which

combine with haem and depletes the regulatory pool (De Matteis, 1978). Fourth, the possibility exists that certain chemicals may decrease the affinity and/or the number of haem binding sites.

For a better understanding of the regulatory role of haem in relation to its own biosynthesis and catabolism the existence of intracellular "free" haem pools has been postulated (De Matteis, 1978; Ortiz de Montelano & Mico, 1981); this is outlined schematically in Figure 4. "Free" haem is considered to be newly synthesized haem that is not yet bound to the prosthetic group of any specific haemoproteins, and possibly haem that has just been released from haemoproteins. "Free" haem probably has a high turnover rate and exists in small quantities in the mitochondria, cytosol and endoplasmic reticulum among which compartments it rapidly reaches equilibrium. To date the existence of "free" haem pools remains speculative because of the unavailability of reliable methods to measure free haem concentrations in subcellular compartments. Nevertheless much indirect evidence is already available in support of this hypothesis. (Kappas et al, 1983; Maines 1984).

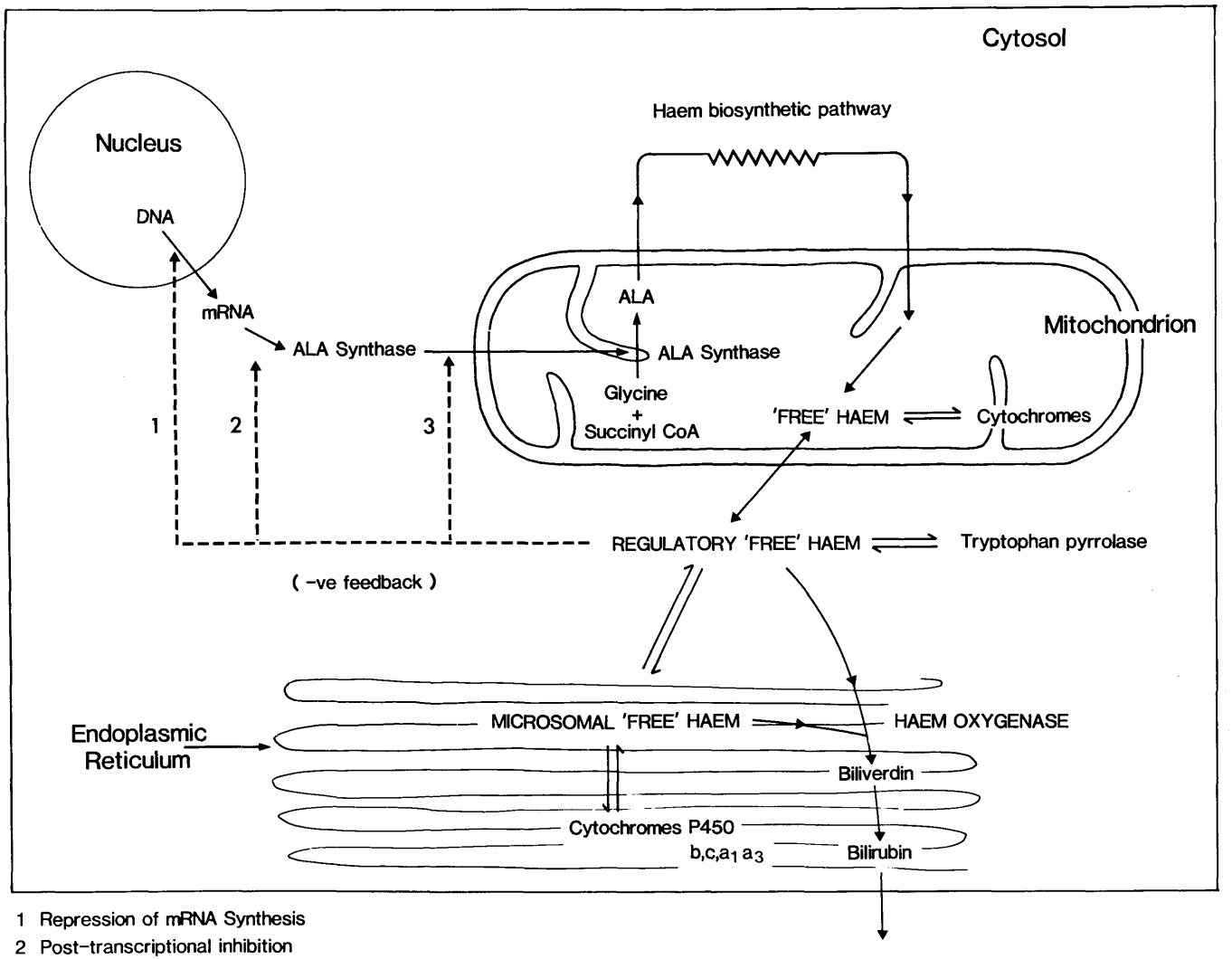
As shown in Figure 4 the "free" haem pools could affect haem biosynthesis and catabolism in several ways:

(1) "free" mitochondrial haem could regulate the synthesis of the mitochondrial cytochrome oxidases;

(2) cytosolic "free" haem could repress the synthesis of ALA synthase, and in addition control the activity of haem oxygenase which degrades haem into bile pigments;

(3) haem oxygenase activity could also be positively controlled by microsomal "free" haem.

SCHEMATIC REPRESENTATION OF THE HAEM BIOSYNTHETIC PATHWAY AND 'FREE HAEM' POOL IN THE HEPATIC CELL



1 Repression of mRNA Synthesis
2 Post-transcriptional inhibition
3 Inhibition of enzyme transfer

Figure 4

Compounds that inhibit haem formation or destroy haem are known to be strong inducers of ALA synthase (De Matteis, 1978; Smith & De Matteis, 1980). Although it is attractive to speculate that induction of ALA synthase is due to derepression of enzyme synthesis following depletion of the "free" haem pool, under certain circumstances induction of ALA synthase seems to occur independently of changes in haem concentration (Kappas et al, 1983).

1.8 HAEM SYNTHESIS IN ERYTHROID CELLS

The mechanism by which haem biosynthesis is regulated in non-hepatic tissues remains unclear and controversial. In analogy to control in the liver it is often assumed that regulation of the haem biosynthetic pathway in non-hepatic tissues also operate by negative feedback of haem on ALA synthase. This view has recently been challenged primarily on the basis of experiments performed in tissue cultures, mainly derived from malignant cell lines or cells transformed by oncogenic viruses. For instance, Sassa and his colleagues performed their studies on cell-culture models using mouse Friend-virus transformed erythro-leukemia cells and human erythroleukemia cell-lines, stimulated with dimethylsulfoxide and a variety of other chemicals. In addition to an increase in ALA synthase, the activities of the other haem enzymes were induced in a sequential fashion (Rutherford et al, 1979). Addition of ALA to the transformed cells in which ALA synthase, ALA dehydratase, and PBG deaminase had already been stimulated by dimethylsulphoxide did not accelerate the rate of haem

biosynthesis (Sassa, 1976). Moreover in the same models treatment with haemin induced ALA synthase activity instead of suppressing it as in hepatic cells (Granick & Sassa, 1978; Hoffman et al, 1980). These workers therefore suggested that ALA synthase might not be the rate-limiting enzyme for haem biosynthesis in erythroid cells; and that ferrochelatase, the final enzyme of the pathway, might instead be more important in controlling the rate of haem formation in erythroid cells (Kappas et al, 1983). Such proposals must obviously be viewed with great caution not only because of the contrived nature of the experimental models and the considerable inter-species differences, but also because of the existence of multiple iso-enzymes with different activities that can occur during foetal and adult life.

1.9 HAEM SYNTHESIS IN OTHER ORGANS

In contrast to the well-characterised pathway of haem metabolism in the liver little is known about haem biosynthesis and degradation in other organs. From what little information there is, the regulatory mechanisms controlling the activity of ALA synthase would appear to differ from that in the liver. For instance, the amount of cytochrome P450-dependent mixed-function oxidase in the kidney of certain strains of rats was found to be only one-tenth of that in the liver, yet the rate of renal and hepatic synthesis and turnover of haem remained comparable (Benedetto et al, 1976). And the renal concentration of porphyrins was found to be several-fold higher than that in the liver (Maines et al, 1978). Renal ALA synthase activity was also

reported to be refractory to the effect of potent inducers of hepatic ALA synthase such as 3, 5, dicarbethoxy-1, 4-dihydrocollidine (DDC). The suggestion that ALA synthase activity in the kidney might not be regulated by haem was supported by the finding that an increase in ALA synthase activity was not necessarily accompanied by a decrease in haem concentration (Maines, 1984).

In steroidogenic organs such as adrenals, testes and ovaries the main function of cytochrome P450 is to catalyse the synthesis of steroids rather than to oxidise and detoxify chemicals. In these organs too, control of ALA synthase activity appears to be governed by factors different from those operating in the liver. For example, following treatment with phenobarbital, AIA or DDC, Condie and his associates (1976a and b) failed to induce ALA synthase activity or decrease cytochrome P450 levels in the adrenals. Furthermore, starvation of the experimental animals caused a four-fold increase in adrenal enzyme activity, but not in the liver (Condie et al, 1976a).

The complexity of ALA synthase regulation in these organs is also reflected in the incomplete refractoriness of ALA synthase induction to metallic ions. Certain metals like copper, cadmium and mercury can increase ALA synthase activity to very high levels (Maines, 1984). These ions do not seem to exert a direct regulatory effect on the enzyme. Instead, the induction could be due to alterations in the hormonal milieu or to other as yet undefined cellular events triggerred only by specific metals (Maines, 1984).

The inability of hepatic ALA synthase inducers to alter ALA synthase activity and cytochrome P450 levels in these organs might be regarded as a biological safeguard against the potentially adverse consequences of unstable swings in steroid metabolism that could be caused by such exogenous agents.

Control of haem biosynthesis in the nervous tissue is discussed later (Chapter 4).

SECTION I

CHAPTER 2

THE PORPHYRIAS

2. THE PORPHYRIAS

2.1 DEFINITION

The porphyrias are inherited and acquired disorders of porphyrin or porphyrin precursor metabolism characterised by specific enzymatic defects in the haem biosynthetic pathway. Consequently, each of the porphyrias has a particular pattern of overproduction, accumulation and excretion of intermediate compounds of haem biosynthesis (Kappas et al, 1983).

2.2 HISTORICAL BACKGROUND

The word "porphyrin" is derived from the Greek "porphoros" meaning purple. The term "haematoporphyrin" was first used by Hoppe-Seyler (1871) to describe the main constituent of the purple-red iron-free haematin. But it was Hans Gunther (1922) who first proposed a classification for the diseases of porphyrin metabolism. Later in 1937, Waldenström introduced the generic term "porphyria" rather than "haematoporphyria" to describe these diseases. Stokvis (1889) described the first recorded case of acute intermittent porphyria in a woman who excreted dark red urine after ingestion of sulphonmethane (Sulfonal^{R}) and died of the disease, but the first observation that it occurred in members of the same family was made by Barker & Eskes in 1912. A familial tendency was similarly noted by Günther (1922) but it was Waldenström (1937) who established its hereditary nature.

2.3 CLASSIFICATION OF THE PORPHYRIAS

The major porphyrias were initially classified as being hepatic or erythropoietic according to the major site of haem synthesis where the metabolic disorder is expressed. In these disorders, with few exceptions such as acquired porphyria cutanea tarda, the enzymatic defect occurs in all the cells of the body. Yet the metabolic consequences of the haem pathway disturbance give rise to diverse clinical syndromes.

Porphyrias that become manifest by acute attacks of neurological symptoms are referred to as the acute porphyrias whereas those characterised by photosensitive skin eruptions, the cutaneous porphyrias. The term "latent porphyria" refers to individuals with a specific enzyme defect who have never had any symptoms of the disease. This term "latent" is somewhat misleading in that the porphyria may still be expressed biochemically causing excessive ALA, PBG and porphyrin production despite a complete lack of symptoms.

2.3.1 Clinical features of inherited porphyrias

The acute porphyrias are acute intermittent porphyria (AIP), hereditary coproporphyria (HC), variegate porphyria (VP) and the recently described homozygous ALA dehydratase deficiency. They are all hepatic in type. The non-acute porphyrias include congenital porphyria, porphyria cutanea tarda and erythropoietic protoporphyria (Table 2). A common feature of the acute hepatic porphyrias is the excessive production and excretion of ALA and typically suffer from abdominal pain often accompanied by various PBG. During an attack patients with the acute hepatic porphyrias

CLINICAL CLASSIFICATION OF THE PORPHYRIAS

The ACUTE HEPATIC PORPHYRIAS

Attacks of abdominal pain and neurodysfunction

- Acute Intermittent Porphyria
- Variegate Porphyria
- Hereditary Coproporphyria

The NON-ACUTE PORPHYRIAS

- Congenital Porphyria
- Porphyria Cutanea Tarda
- Erythropoietic Protoporphyria

Photosensitive skin eruptions

Table 2

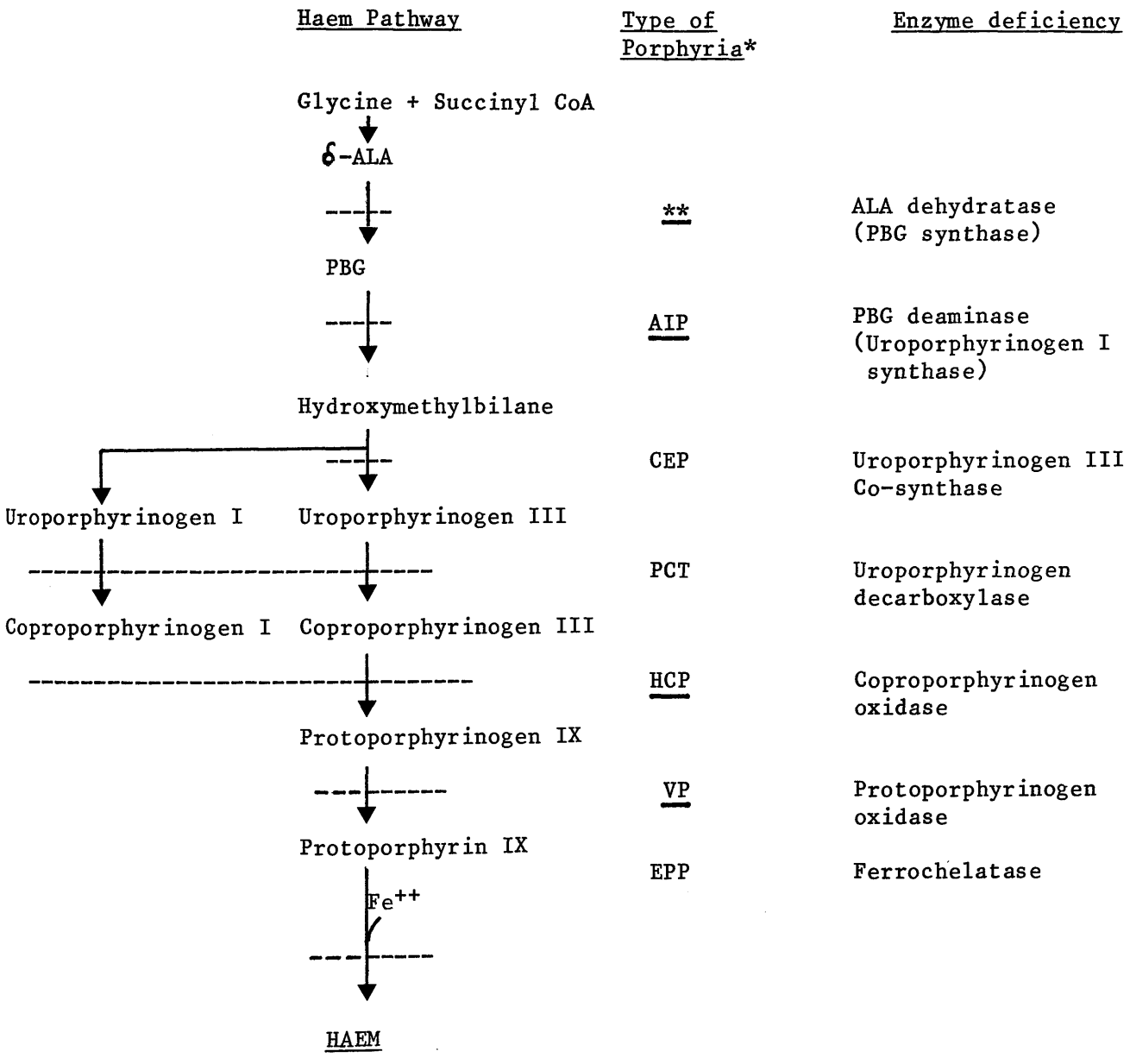
typically suffer from abdominal pain often accompanied by various features of a neuro-psychiatric syndrome. In addition, patients with HC and VP can develop cutaneous lesions due to the deposition of porphyrins in the skin. On the other hand patients with the non-acute porphyrias only display cutaneous manifestations; and they do not excrete increased amounts of ALA and PBG in the urine.

2.3.2 Specific enzyme defects in the porphyrias

Since each type of porphyria is due to a specific haem enzyme defect (Fig 5) they all have a characteristic pattern of porphyrins and porphyrin precursors in blood and excreta, determined by the site of block (Elder, 1982; Kappas et al, 1983; Rimington, 1985).

The enzyme deficiency in AIP is PBG deaminase (PBG.D) (also known as uroporphyrinogen-I-synthase (URO.S)), in HC coproporphyrinogen oxidase, and in VP protoporphyrinogen oxidase. AIP, HC and VP are expressed in the heterozygous state as autosomal dominant conditions. But recently acute poprhyria has been reported in the homozygous state for ALA-dehydratase (Doss et al, 1982c), coproporphyrinogen oxidase (Grandchamp et al, 1977) and protoporphyrinogen oxidase (Korda et al, 1984). In congenital porphyria (CP) the defect is uroporphyrinogen-III cosynthetase, in porphyria cutanea tarda (PCT) uroporphyrinogen decarboxylase and in erythropoietic porphyria (EPP) ferrochelatase. For PCT two types are now recognised: an acquired form and a less common familial form (Kushner et al, 1976; Elder et al, 1978).

ENZYME DEFECTS OF THE HEREDITARY PORPHYRIAS



The horizontal dashed line indicates the site affected by the corresponding enzyme deficiency.

* Abbreviations are explained in the text; those underlined are the acute porphyrias.

** New type recently described - not yet named.

Figure 5

2.4 ACUTE INTERMITTENT PORPHYRIA

2.4.1 Introduction

AIP is the commonest type of acute hepatic porphyria in most countries in the world. Its prevalence has been estimated at 1.9 per 100,000 population in Britain (Beattie, 1973b), 3 per 100,000 (including known latent cases) in Western Australia (Saint & Curnow, 1962) and 7.7 per 100,000 in Sweden (Wetterberg, 1967). The highest known prevalence is in Lapland (100 per 100,000) where all AIP subjects seem to belong to a single kindred (Romeo, 1980).

2.4.2 Establishment of the enzyme defect

In 1963 Granick and Urata demonstrated that synthesis of hepatic ALA-synthase (ALA.S) could be induced in experimental animals by administration of certain chemicals. The first clue to the underlying defect in AIP was provided when Granick (1966) showed that the induction of hepatic ALA.S by allyl-isopropylacetamide (AIA) and dicarbethoxydihydrocollidine (DDC) produced a biochemical abnormality that resembled human hepatic porphyria. Following these observations a similar but genetically-mediated induction of ALA.S was found in AIP patients (Tschudy et al, 1965b) that could explain the excessive production of ALA and PBG (Strand et al, 1970; Granick & Sassa, 1971). Subsequent studies showed that ALA.S activity was increased not only in AIP but also in other types of acute porphyria (Meyer & Schmid, 1973). It thus became apparent that the increase in ALA.S was unlikely to be the primary inherited defect. The primary cause for each type of porphyria could then be explained by a partial block along the haem biosynthetic pathway, which by impairing haem formation

would lead to secondary induction of ALA.S (Meyer, 1973). The resulting overproduction of porphyrin precursors would tend to restore haem synthesis by increasing the intracellular concentration of substrate proximal to the block.

Reduced PBG.D activity in liver cells was the first primary enzyme defect of this type to be demonstrated in patients with AIP (Strand et al, 1970; Miyagi et al, 1971). Subsequently this enzyme defect was shown to occur in all cell types studied; namely, erythrocytes (Meyer et al, 1972; Strand et al, 1972; Sassa et al, 1973), cultured skin fibroblasts (Sassa et al, 1975), transformed lymphocytes (Sassa et al, 1978) and cultured amniotic cells (Sassa et al, 1975). Expression of the mutant gene appears to be relatively constant in so far as the measurable enzymatic activity is about 50 per cent in both latent cases and in patients suffering from the disease (Meyer, 1973; Meyer & Schmid, 1973). Clinical expression of AIP however is highly variable since the great majority of heterozygotes remain clinically latent (McColl et al, 1982a). It is also established that decreased enzyme activity does not alter with disease activity. (Meyer 1973; Sassa et al, 1974).

2.4.3 Molecular basis for the enzyme defect

As a result of studies on the inheritance of rare structural variants and experiments with mouse-human somatic cell hybrids (Wang et al, 1981) it has been possible to localise the structural gene for PBG.D at a single locus on human chromosome 11q 23 - 11q TER. But studies in AIP families have so far failed to link the PBG.D defect with known gene markers on chromosome 1, 2, 6 and 9 (Tongio et al, 1979).

Using anti-human PBG.D antibody recent immunological investigations have shown that AIP can be caused by several types of structural gene mutations. Anderson et al (1981) initially identified an immunoreactive but functionally inactive form of PBG.D in the red cells of 1 of 22 AIP families. This dysfunctional protein was detected in all five gene carriers in that family, and was thought to be the product of a mutant structural gene with different catalytic and substrate-binding properties. The genetic heterogeneity of AIP has been further characterised in Finnish patients in whom 80% of the mutations were of the type without cross-reacting immunological material. The remaining patients had cross-reacting immunological PBG.D material of two types (Mustajoki & Desnick, 1985). One such non-catalytic variant was a protein that was bound to substrate but the resulting complex was more resistant to intra-erythrocytic proteolysis than the normal enzyme (Desnick et al, 1985). Such molecular genetic heterogeneity could well explain in part the variable phenotypic expression of the disease (Goldberg, 1985). The type of mutation(s) that commonly occur in AIP patients of other countries remains conjectural. Other possible gene defects that could arise include defects in structural gene expression, unstable gene products, gene deletions and regulatory gene defects (Stanbury et al, 1983). Further intriguing evidence for genetic heterogeneity in AIP was provided by the recent report of normal erythrocyte PBG.D activity in a large family with affected members having all the other features of the disease (Mustajoki, 1981). The possibility of an enzyme variant with an unusually high Km

value was excluded. The results of PBG.D activity in other tissues pertaining to members of this family is awaited with great interest since failure to express PBG.D deficiency solely in erythrocytes may point to the existence of a gene that controls tissue expression of a particular structural gene.

2.4.4 Clinical features

The clinical manifestations of the acute attack in AIP are very similar to those of other types of acute hepatic porphyrias, with the exception that in hereditary coproporphyria and variegate porphyria photosensitive skin eruptions can also occur. Acute attacks are rare before puberty (Barclay, 1974). The disease tends to become clinically manifest between puberty and 30 years of age, attacks being most frequent in the third decade of life. There is a preponderance of female sufferers (Goldberg & Rimington, 1962; Wetterberg, 1967). Males suffering from the disease tend to do so later than females (on average 10 years) (Stein & Tschudy, 1970). But serious attacks can occur in either sex for the first time at any age. In a third of reported cases no family history can be obtained, the disease presumably having remained latent in the preceding generation or arising from a new mutation (Wetterberg, 1967).

Abdominal pain is the most frequent presenting complaint being the initial symptom in more than 90% of the acute attacks (Waldenstrom, 1957; Goldberg, 1959; Stein & Tschudy, 1970). Location of pain over the abdomen can vary from patient to patient and from attack to attack in the same patient. It frequently fails to conform to any particular neuroanatomical distribution. The

pain is often diffuse but it can be quite localised, especially to the lower part of the abdomen. The intensity of pain can be very severe and commanding. Patients often describe the pain as "nagging" and "unremitting"; it can also acquire a colicky character. Back pain in the lumbar region is a common feature, but there is no renal tenderness. Constipation frequently accompanies, or may even precede, the abdominal pain but in 10% of cases diarrhoea may occur (Goldberg & Rimington, 1962). Nausea and vomiting, at times severe and protracted can cause a problem with oral fluid intake.

Palpation of the abdomen characteristically reveals a mild degree of generalised tenderness which is much less than would be expected from the severity of the pain. Some abdominal distention with reduced bowel sounds may be present. Ileus can be precipitated by administration of narcotic analgesics or drugs with anticholinergic action. Muscle guarding is seldom encountered. The presence of rebound tenderness should alert the clinician to the possibility of peritonitis from other cause. The episodes of abdominal pain usually settle spontaneously after a few days unless there is persistence of some precipitating factor. For a few unfortunate patients, however, the pain can linger on for weeks or months despite treatment.

Motor weakness is the other major feature of the acute attack in some 60% of cases (Goldberg, 1959). The peripheral extensor muscles are often the first to be affected resulting in wrist or foot-drop (Fig 6). At times weakness can initially affect the proximal limb muscles, being more prominent in the arms than in

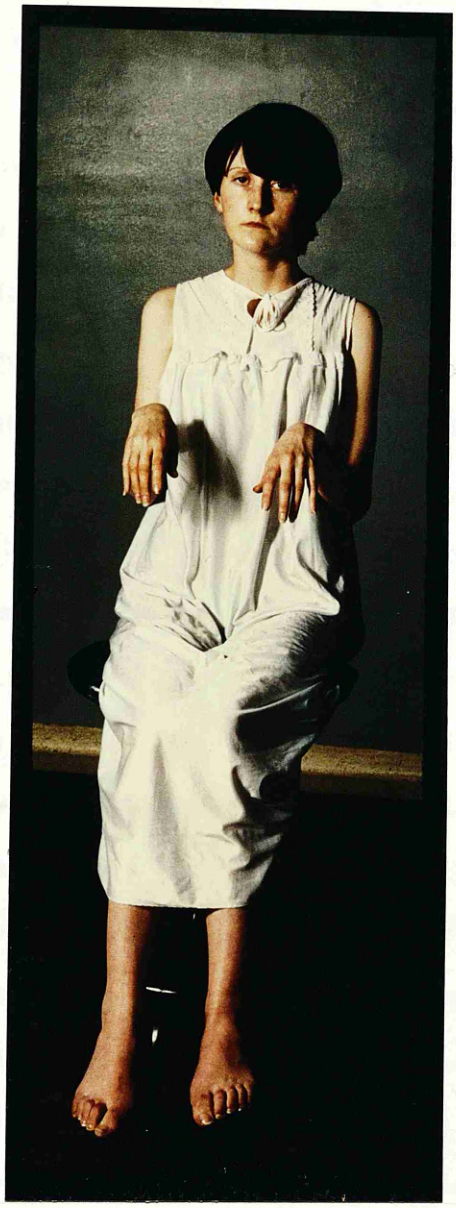


Figure 6 : This 27-year-old woman suffering from AIP developed severe quadriparesis within a week of the onset of abdominal pain

the legs (Ridley, 1969). Its progression is usually gradual but occasionally flaccid paralysis of all extremities can rapidly occur within a matter of days (Brezis et al, 1979; Menewat et al, 1979). During the early stages tendon reflexes can be little affected but when the neuropathy is advanced reflexes are decreased or absent. Unexplained paradoxical preservation of the ankle reflexes has been noted in the presence of quadriparesis (Ridley, 1969). In addition the patient often complains of limb pain accompanied by muscle tenderness. Fasciculation does not occur. Severe wasting can progress rapidly. The serum muscle enzymes however remain normal (Stein & Tschudy, 1970). Abdominal pain and sinus tachycardia nearly always precede the paralytic manifestations, sometimes by several weeks (Ridley et al, 1968). Conversely slowing of the tachycardia signals subsidence of the acute attack. The onset of motor weakness indicates that an attack is likely to be more prolonged and debilitating. Isolated ptosis and lower motor neurone involvement of the cranial nerves, especially of the seventh and tenth, can rarely occur. Progression of muscle weakness can lead to bulbar palsy and eventually respiratory paralysis. These dreaded complications have been responsible for most deaths (Goldberg & Rimington, 1962). Case-reports of blindness due to optic nerve (De Francisco et al, 1979) or occipital lobe involvement (Lai et al, 1977) have been reported in the acute attack. In some cases complete recovery from the muscle weakness can take place within days but in others it may take several months or years (Sorensen & With, 1971). The more severely affected patients often have persistent

wasting of their hand muscles, and eventually develop claw-like contraction deformities of their fingers. (Fig 7)

Sensory symptoms including paraesthesiae and numbness are less common than motor involvement. Although the symptoms may be suggestive of a stocking and glove type of neuropathy, objective signs of sensory deficit are usually sparse and patchy in distribution. Sensory involvement may also have a proximal distribution with a bizarre pattern of sensory loss over the "bathing trunk" or "long johns" area. Alternatively it may be confined to a wide band around the upper arms and thighs when the sensory loss may extend from the hips to the knees "as if a pair of bathing trunks had slipped" (Ridley, 1984). Loss of touch and pain sensation are the most commonly affected modalities. Impairment of vibration sense is less frequent, and joint position sense is mostly preserved.

Cerebral disturbance can trigger tonic-clonic seizures in the absence of other metabolic or structural abnormalities (Ridley, 1969). Rarely abnormal movement disorders have been attributed to basal ganglia involvement but in such cases drug-induced dyskinesia, especially caused by the phenothiazines, must be excluded. No abnormalities have been found in the cerebro-spinal fluid except for an occasional mild increase in its protein content (Stein & Tschudy, 1970).

Features of autonomic dysfunction are frequent during the acute attack (Goldberg, 1959). Sinus tachycardia rising to 160 beats/minute may occur. Hypertension usually accompanies the tachycardia and is characteristically labile. Rarely it can reach

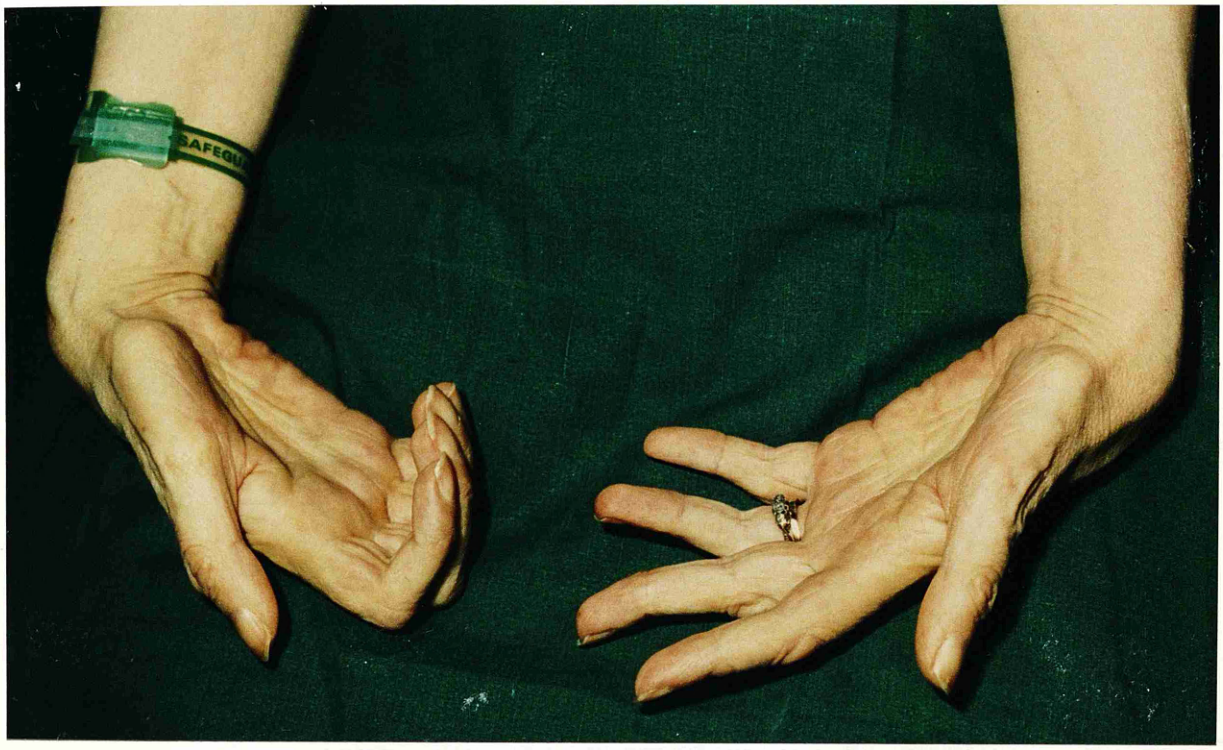


Figure 7: Long-term sequelae of acute porphyria: wasting of the hand muscles especially affecting the extensors leading to claw-like deformity of the fingers. In this photograph patient was attempting to straighten the fingers and wrist.

levels at which retinal exudates appear (Fig 8). The patient may become unduly restless, sweat profusely and display fine tremor of the extremities. Interestingly these sympathomimetic features are often accompanied by increased urinary catecholamine excretion (Schley et al, 1970; Beattie et al, 1973a; Beal et al, 1977). A low-grade fever is not uncommon during the severe attacks. But its presence necessitates the exclusion of any underlying infection, such as a urinary tract infection, which could have precipitated the acute episode in the first instance. A white cell count may be useful since leucocytosis is generally absent in acute porphyria.

Bladder dysfunction can cause urinary retention, or conversely, frequency and urgency of micturition. The urinary output is invariably low unless vigorous measures are taken to ensure adequate fluid replacement.

A wide spectrum of psychological disturbances and psychiatric manifestations has been reported in patients suffering from an acute attack (Wetterberg, 1967). Depression is particularly frequent. An organic brain syndrome can cause excessive irritability, confusion, disorientation, delusions and hallucinations. But when some of these mental manifestations such as chronic anxiety, depression or emotional lability persist after the attack has otherwise settled both biochemically and clinically their relationship to porphyria becomes difficult to evaluate. In such instances other causes like drug dependence have to be considered. Despite previous claims a controlled study could not confirm that psychogenic factors constitute a significant

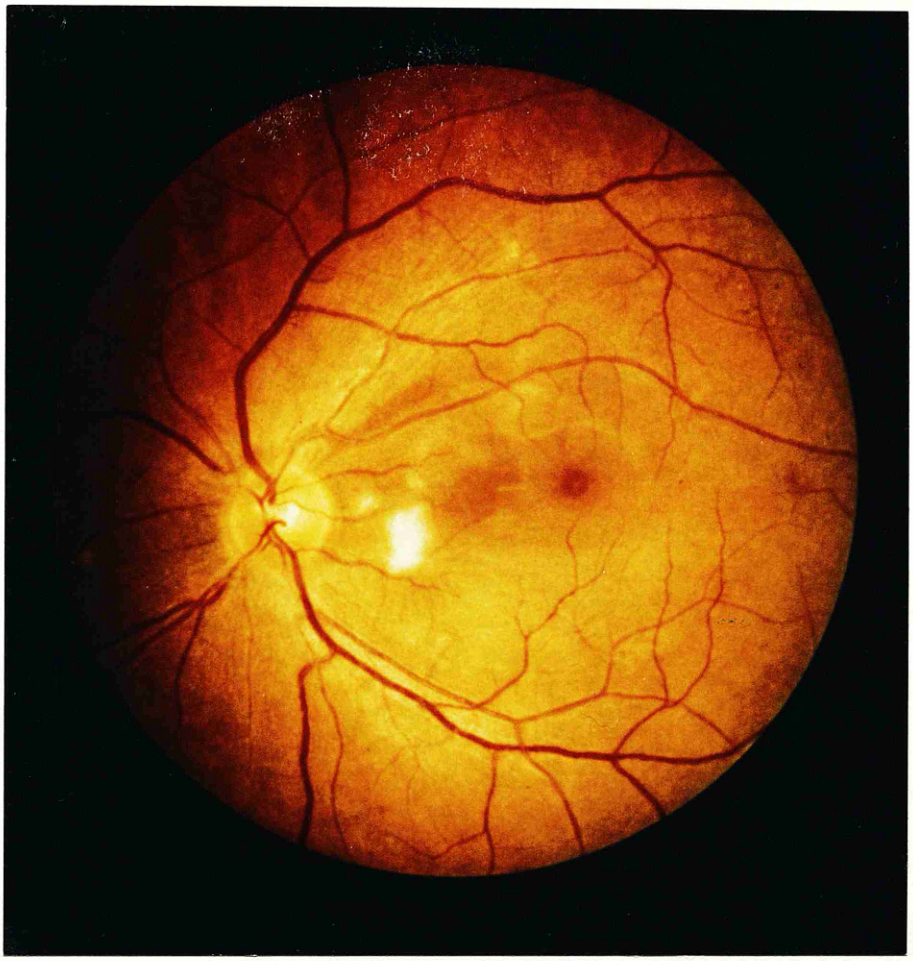


Figure 8: Hypertensive retinal exudates developing during an acute attack in a 31-year-old woman with AIP. Note accompanying retinal artery spasm.

precipitating factor in acute porphyria (Ackner et al, 1962). Among the other metabolic and endocrine disturbances, hyponatraemia is the best-known complication. Although hyponatraemia can be caused by inappropriate ADH secretion (Hellman et al, 1962) it is more commonly the result of protracted vomiting and inadequate or inappropriate fluid and electrolyte balance (Eales et al, 1971). Patients with hyponatraemia should receive intravenous saline if associated with reduced blood volume and not treated with fluid restriction, as is the case for severe hyponatraemia due to inappropriate ADH secretion. Marked hyponatraemia (Na < 125 mM/L) and water intoxication per se can cause seizures and mental disturbances (Andreoli, 1985). Less well defined electrolyte disturbances have been reported; they include hypomagnesaemia (Hellman et al, 1962; Nielsen & Thorn, 1965), and hypercalcaemia following prolonged immobilisation (Barois et al, 1977) and primary hyperaldosteronism (Basiliere & Newcomer, 1971). Mild glucose intolerance seems common but frank diabetes is rare (Waxman et al, 1967). Inappropriate release of growth hormone after a glucose load was initially reported (Perlroth et al, 1967), but this was not confirmed in a later study (Bonkowsky et al, 1976).

During an acute attack total serum thyroxine and tri-iodothyronine are sometimes increased with a concomitant rise in thyroxine-binding globulin (Hollander et al, 1967). Hyperthyroidism has occasionally been reported in AIP patients (Mann & De Nardo, 1965) with the suggestion that it could have been mediated by a "neurogenic" mechanism (Brodie et al, 1978).

AIP has also been reported in association with a variety of uncommon diseases mostly in the form of case-reports many of which were unfortunately poorly documented. These include isolated deficiency of ACTH secretion (Waxman et al, 1969), systemic lupus erythematosus (Harris et al, 1966), relapsing pancreatitis (Kobza et al, 1976), hypermacro-amylasaemia (Hedger & Hardison, 1971) and Gilbert's syndrome (Kobza et al, 1976). It has not yet been possible to establish whether these associations are merely chance-related or whether any pathogenetic links exist between these conditions and AIP. With regard to the endocrine complications of AIP, it is now known that endogenous opioids play a modulatory role in the synthesis and/or release of a number of brain hormones (Snyder, 1977; Copolov & Helme, 1983). Opioid agonists such as morphine facilitates the release of FSH and ACTH. They can also cause an elevation in serum prolactin and growth hormone. But since patients in whom the putative endocrine complications were reported received opiates and other drugs such as chlorpromazine, which can also disturb the endocrine system, many of these associations are now suspect.

Hypercholesterolaemia with an increase in low density B-lipoproteins has been reported in some 40 per cent of AIP patients whereas serum triglycerides were normal (Taddeini et al, 1964). But neither a specific metabolic nor a genetic relationship could be established between hyperlipidaemia and AIP because the possible effect of a high calorie intake on lipid metabolism was not taken into account. It is interesting however that hyperlipidaemia could be induced in animals treated with

chemically-induced porphyria (Taddeini et al, 1964) and in human subjects accidentally exposed to the porphyrinogenic dioxin-related chemical TCDD (Pazderova-Vejlupkova et al 1981).

Other System Involvement

The liver has previously been reported to be histologically normal in AIP (Stein & Tschudy, 1970), but Ostrowski et al (1983) have recently described the presence of diffuse, moderately severe albeit non-specific ultrastructural changes in a high proportion of patients with the acute hepatic porphyrias. These abnormalities were associated with impaired aminopyrine and bile acid clearances. Apart from impaired bromsulphalein (Stein & Tschudy, 1970), antipyrine and bile salt clearances (Anderson et al, 1976; Ostrowski et al, 1983) other measurements of liver function remain unimpaired during the acute attack (Stein & Tschudy, 1976). These biochemical indicators of hepatic involvement do not appear to have any clinical significance.

Anaemia is not a feature of the acute hepatic porphyrias. In one small study, total blood volumes were found to be slightly reduced and red cell survival mildly shortened, but haemoglobin synthesis appeared normal (Bloomer et al, 1971). Patients with AIP also have normal rates of bilirubin production, mostly of erythroid origin (Jones et al, 1971).

2.4.5 Precipitating Factors

In all the acute hepatic porphyrias, the majority of affected individuals remain asymptomatic throughout their entire lifetime since partial deficiency of the haem enzymes does not necessarily cause impairment of haem biosynthesis. Indeed most of the latent

cases have normal urinary excretion of ALA, PBG and porphyrins, indicating that little or no induction of hepatic ALA.S normally takes place (McColl et al, 1982a). Moreover cytochrome P450-mediated drug metabolism is normal in the majority of latent cases (Anderson et al, 1976; Ostrowski et al, 1983). It is now well established that certain precipitating factors can alter a state of latency into one which becomes clinically manifest. Drugs and hormonal influence are the most important precipitating factors although in some patients alcohol, fasting and infection may be contributory to their attack (Goldberg & Rimington, 1962; Eales & Disler, 1962; Kappas et al, 1983; Moore & Disler, 1983). Many of these factors are known to induce ALA.S activity and cytochrome P450 formation in the liver (De Matteis & Gibbs, 1972). The concurrent biochemical changes which result in an accumulation of porphyrin intermediates indicate that acute attacks may develop whenever a demand for additional haem synthesis, such as that imposed by stimulation of cytochrome P450 synthesis, cannot be met. This is because the rate of synthesis that is required exceeds the maximum activity of PBG.D. Consequently this leads to derepression of ALA.S, the rate-limiting enzyme of the pathway, causing a further increase in ALA and PBG formation (Meyer, 1973). As ALA and PBG accumulate behind the enzymatic block, loss of ALA and PBG from the intracellular pool also increases. In turn urinary excretion of these porphyrin precursors becomes correspondingly greater.

Administration of certain drugs can not only precipitate the disease in latent cases but also exacerbate the acute attacks, often with the development of life-threatening neuropathy (Goldberg, 1959; Ridley, 1969). Some patients often appear very sensitive to the porphyrinogenicity of these drugs in that a single dose may be sufficient to induce an attack (Eales & Linder, 1962, Eales, 1971). Paradoxically, even the worst offending drugs, namely the barbiturates, do not consistently lead to an acute attack in some porphyrics (Eales, 1979).

A large number of drugs having very diverse chemical structures has been implicated. The only physical property they appear to share is their high degree of lipid solubility (De Matteis, 1978). Surprisingly perhaps, when these drugs are given to normal individuals or experimental animals even in therapeutically toxic dosage, they fail to induce a porphyric state (Smith & De Matteis, 1980) except in very rare circumstances (Chapter 11). But they can produce measurable changes in the haem biosynthetic pathway in experimental animals and cell cultures (Granick, 1966; Anderson, 1978; De Matteis, 1978, De Verneuil et al, 1983).

Barbiturates have been the most notorious porphyrinogenic drugs (Goldberg, 1959). Since their use has been curtailed acute attacks appear to have become less frequent among populations where porphyria is relatively common (Kramer, 1980). Apart from barbiturates, anticonvulsants and alcohol (Disler & Moore, 1983) are among the drugs which have definitely been shown to induce ALA.S in human liver. Some porphyrinogenic drugs like the sulphonamides seem to have an additional direct inhibitory effect

on hepatic PBG.D which can increase the enzymatic block in AIP (Peters et al, 1980). In some patients, the effect of drugs alone does not appear to be sufficient to precipitate an attack unless other compounding factors such as a change in hormonal milieu favour the induction of ALA.S. This could explain why some porphyric individuals remained asymptomatic despite having taken potentially harmful drugs in the past (Eales, 1979).

The porphyrogenicity of drugs is based on their ability to induce ALA.S. For this purpose either whole animals eg rats or tissue cultures are used to screen drugs and chemicals that may constitute a risk to porphyric patients (De Matteis, 1978; Anderson, 1978). Chick embryo liver in ovo or in culture have been found to provide a particularly sensitive model for studying human hepatic biosynthetic control (Granick, 1966; Kappas & Granick, 1968; De Verneuil et al, 1983). For instance, danazol, a harmful steroidal drug in porphyria was shown to induce ALA.S in chick embryo liver but not in adult rat liver (Lamon et al, 1979). The avian-embryo hepatic-cell culture is also very sensitive to the permissive effects of hormones like insulin, tri-iodothyronine and corticosteroids (Granick & Kappas, 1967). The sensitivity of the culture system to the ALA.S-inducing effect of drugs can be enhanced by primary cells initially with a small dose of haem pathway inhibitors. For example, DDC accentuates porphyrin accumulation in the liver by inhibiting ferrochelatase (Anderson, 1978); and AIA converts liver haem into abnormal products, thereby acting as a potent ALA.S inducer (Smith & De Matteis, 1980). In some instances, the chick embryo model seems

to be so highly sensitive as to be irrelevant to the human situation (Moore & Disler, 1983). Despite the well recognised dangers of extrapolating the results of such experimental models to man, they still provide the best means of determining the potential porphyrinogenicity of drugs. The most valid method, of course, is to demonstrate that the drug in question has caused an acute attack in porphyric patients. For some drugs like barbiturates and sulphonamides the clinical evidence that has accumulated as to their porphyrinogenicity is beyond doubt. But in many instances the case-reports linking the acute attacks to specific drugs are too often anecdotal and lacking in relevant details. In others, the presence of other concomitant diseases and their treatments obfuscate the possibility of a causal relationship.

Several workers have attempted to produce lists of safe and unsafe drugs for use in the acute porphyrias (Rifkind, 1976; Moore & Disler, 1983; Kappas et al, 1983; Moore, 1985). These drugs are often been graded according to their estimated potential porphyrinogenic risks depending on the reliability and completeness of any available clinical details (Moore & Disler, 1983). Unfortunately information available for certain drugs is conflicting and opinions of different authors vary: a good example is in the use of sodium valproate as an anticonvulsant (Doss et al, 1981; Brodie & Goldberg, 1980). These lists of drugs still represent a formidable collection of chemically and often pharmacologically unrelated agents, most of which are of doubtful porphyrinogenicity. But until a better screening method becomes

available, whenever possible, any drug shown to induce ALA.S in the experimental models discussed should be avoided. Used in this way these lists of safe and unsafe drugs offer a useful guide in the management of patients with the acute hepatic porphyrias.

2.4.6 Hormonal influence

Undoubtedly the phenotypic expression of the genetic defects in the acute porphyrias is influenced by hormonal factors. This would explain the following observations:

1. The rarity of acute attacks before puberty (Barclay, 1974).
2. The predominance of female patients suffering from acute porphyria (Waldenström, 1957; Goldberg, 1959).
3. The phasic occurrence of symptoms in the menstrual cycle of a small proportion of women with the disease (perhaps 10-20%). Actually serial measurement of leucocyte ALA.S activity throughout the menstrual cycle in normal women shows a peak prior to menstruation (McColl et al, 1982b).
4. The induction of clinical remission with agents, such as the luteinising-hormone-releasing-factor anologues, which suppress ovulation (Anderson et al, 1984).
5. The paradoxical but well-documented reports that the oral contraceptive hormones can affect porphyrin metabolism and exacerbate the disease in some women (Zimmerman et al, 1966) while suppressing disease activity in others (Perlroth et al, 1965).
6. Reports of activation of the disease during pregnancy (Brodie et al, 1977c).

7. Alterations in steroid hormone metabolism in symptomatic patients with AIP which favour the excessive production of steroid hormone metabolites shown experimentally to be potent hepatic ALA.S inducers (Granick & Kappas, 1967).

When tested experimentally, steroidal hormones with the 5β-H configuration structurally have been found to be potent inducers of ALA.S whereas those with the 5α-H configuration have tended to be inactive in that respect (Bradlow et al, 1973). Steroids with a double-bond in the 4-5 position can be reduced in the liver either to the 5β-H or 5α-H derivatives by two different enzymes: a soluble 5β reductase and an endoplasmic reticulum 5α-reductase. The majority of symptomatic AIP patients, and not the latent cases, have been shown to have about a 50% reduction in the activity of hepatic 5α-reductase (Anderson et al, 1979). This second enzyme defect cannot be explained on the basis of the haem pathway abnormality per se since it is absent in the other porphyrias (Kappas et al, 1972). Family studies have further confirmed that it is an acquired abnormality, but how this comes about remains enigmatic (Kappas et al, 1983). This decreased 5α-reductase activity seems confined to the liver which biotransforms the bulk of steroidal hormones, and not to other organs like the skin. Impairment of 5α-reductase favours the compensatory formation of the more porphyrinogenic 5-β steroidal metabolites. Best known among these are aetiocholanolone and androstenedione (Paxton et al, 1974). Thus it is now clear that both exogenous or endogenous steroid hormones may be porphyrinogenic.

The situation in pregnancy is complex because maternal, placental and foetal tissues all play a role in steroid hormone production and metabolism. Although acute attacks can occur during pregnancy (Brodie et al, 1977c) the outcome for both mother and child is good. Whereas Stein & Tschudy (1970) reported that pregnancy did not significantly affect the disease in 72 of 74 instances, Brodie et al (1977c) found that 54% of 50 AIP women had an acute attack during pregnancy or the puerpurium. In the latter series there was only one recorded maternal death and foetal wastage was not increased. Despite the huge increase in serum concentrations of sex hormones in pregnancy, attacks are perhaps less frequent than expected because these steroids appear to be preferentially reduced to the 5α-compounds instead of their 5β-analogues.

2.4.7 Diagnosis of AIP

During the acute attack, with the exception of homozygous ALA.D deficiency (Doss et al, 1982b), patients suffering from all types of acute hepatic porphyrias excrete excessive amounts of ALA and PBG in their urine. In those latent cases who are already excreting porphyrin precursors excessively, a further increase will occur during the exacerbations (Ackner et al, 1961). Urinary porphyrins will also be increased depending on the site of enzymatic block, but the presence of uroporphyrin may partly be due to its non-enzymatic formation from condensation of PBG. But since urinary porphyrins are predominantly of the III isomer series they are probably derived from the metabolism of porphyrin precursors in non-hepatic tissues such as the kidney (Day et al, 1981). In freshly voided urine PBG is a colourless compound, but

upon exposure to light it turns to reddish-brown. This follows the spontaneous non-enzymatic conversion of PBG to porphyrins and other pigments such as porphobilin, a degradation product. Spontaneous oxidation of the porphyrinogens to porphyrins also contribute to this dark hue.

The presence of PBG can be rapidly tested by the Watson-Schwartz test. This is performed by first mixing equal volumes of urine and Ehrlich's aldehyde reagent (*p*-dimethylamino-benzaldehyde in hydrochloric acid). Sodium acetate is then added to pH4, followed by an extraction step with chloroform or butanol. If PBG is present, it forms a pink or red water-soluble complex. Urobilinogen also forms a red complex, but one which is soluble in the organic solvent following the extraction step (Watson & Schwartz, 1941). A less sensitive variation of the Watson-Schwartz test is the Hoesch test. It is performed by adding urine drop by drop to a large volume of acid Ehrlich's reagent. If PBG chromogen is formed it becomes easily visible by its dark colour whereas urobilinogen fails to produce any significant colour change (Pierach et al, 1977a).

These simple bed-side tests remain very useful for diagnosing the disease during acute attacks.

If a positive Watson-Schwartz or Hoesch test is obtained quantitative measurement of PBG should be carried out.

In addition to urinary PBG the most useful method of confirming the diagnosis of AIP is by erythrocyte PBG.D activity assay (Sassa et al, 1974; Magnussen et al, 1974; Piepkorn et al, 1978). Unfortunately there is much overlap in the range of values for

PBG.D (up to 30%) between normal and AIP individuals (McColl et al, 1982a; Bissell, 1982). Consequently some porphyric patients will fall within the low normal range. Since PBG.D activity is increased several-fold in newly produced reticulocytes and declines with ageing of the red cells (Anderson et al, 1977), interpretation of results must take these factors into account. By combining the measurement of erythrocyte PBG.D and leucocyte ALA.S detection of the latent state in AIP can be increased to 90% (McColl et al, 1982a). Measurement of leucocyte ALA.S, however, is only available in few specialist laboratories.

2.4.8 Prognosis

With greater recognition and avoidance of porphyrinogenic drugs, and better supportive therapy for those in acute attacks the prognosis has greatly improved. In contrast to previously reported high mortality rates eg. 18 to 33% (Waldenstrom, 1957; Ridley, 1969; Sørensen & With, 1971), fatalities from an acute attack are now rare (Kramer, 1980; Ridley, 1984).

2.5 PORPHYRIA VARIEGATA (Variegate Porphyria)

2.5.1 Introduction

Porphyria Variegata (VP) is the name given by Dean & Barnes (1955) to a type of porphyria commonly seen in White South Africans of Afrikaan descent. It was first described by Van der bergh. The disease is inherited as an autosomal dominant trait. In a remarkable epidemiological study Dean (1971) traced back this familial disorder among White South Africans to an early Dutch settler who had married an orphan girl in Cape Town in 1688. VP

has now been reported in many parts of the world. Its prevalence varies from country to country: in Finland it was estimated at 1.3 per 100,000 population whereas in South African Whites it was 300 per 100,000 (Mustajoki, 1978; Dean, 1971).

2.5.2 Underlying Biochemical Abnormality

Ferrochelatase activity was initially reported to be decreased by about 50% of normal in the fibroblasts and normoblasts of patients with the disease (Becker et al, 1977a). But other workers were unable to confirm this finding in leucocyte buffy coat preparations (Brodie et al, 1977a) and skeletal muscle (Pimstone et al, 1973). More recently Brenner and Bloomer (1980a) have measured the activity of ferrochelatase and protoporphyrinogen oxidase in cultured skin fibroblasts. Ferrochelatase activity was found to be normal whereas protoporphyrinogen oxidase was decreased to about 50% of normal, suggesting that protoporphyrinogen oxidase was the primary enzyme defect in VP. This observation has now been confirmed by others (Deyback et al, 1981). Nevertheless there is evidence in the South African cases of VP (and EPP) that a combined deficiency of protoporphyrinogen oxidase and ferrochelatase can exist (Viljoen et al, 1983). In an autopsy report Yamada et al (1984) described a patient with the porphyrin profile of VP and ferrochelatase deficiency, in whom PBG.D was also depressed. A family with homozygous VP in two siblings has recently been recognised by Korda et al (1984). Both children had photodermatosis and severe neurological disturbances from birth.

The most characteristic biochemical finding in VP is a marked increase in faecal protoporphyrin IX accompanied to a lesser extent by an increase in coproporphyrin III (Elder, 1980; Bissell, 1982).

There is also a considerable increase in some hydrophilic porphyrin-peptide conjugates called the X-porphyrin (Rimington et al, 1968). Indeed the excretory findings in VP are more consistent with protoporphyrinogen oxidase deficiency, causing an accumulation of protopophyrinogen which is rapidly oxidised to protoporphyrinogen in the stools. Intracellular oxidation of protoporphyrinogen in the presence of peptides with free-SH groups in the liver can account for the X-porphyrin.

As in the other acute hepatic porphyrias, excessive amounts of ALA and PGB are excreted in the urine during acute attacks.

2.5.3 Clinical features

The clinical features of the acute attack in VP are similar to those described for AIP regarding abdominal pain and neuropsychiatric symptoms. In addition patients with VP can develop skin manifestations. In the absence of skin lesions it is often possible to obtain a family history of increased skin fragility (Mustajoki, 1978; Kramer, 1982).

2.5.4 Dermatological manifestations

Skin lesions can be the initial symptoms of the disease (Eales, 1960). These occur on sun-exposed areas; namely the face, arms and back of hands. Most characteristic is the increased skin fragility which can be easily damaged by relatively minor trauma. Lesions usually start as erythematous patches and progress into

vesicles and bullae. Haemorrhage can occur into these bullae which will eventually heal leaving scars. Development of milia is also typical: milia are small, yellowish-white, spherical subepidermal papules varying from 1 to 5 mm in diameter. Other skin changes include hyperpigmentation, hirsutism, especially over the face and less commonly pseudo-sclerodermatous changes. A history of direct sensitivity to sunlight is somewhat uncommon, even though photosensitivity can be clearly demonstrated by light testing in the majority of these cases (Mustajoki & Koskelo, 1976). Histological examination of the skin typically shows the presence of an amorphous material around the smaller dermal vessels and capillaries. This substance which is strongly PAS positive is rich in 1:2 glycerol groups, as in neutral mucopolysaccharides or mucoproteins (Epstein et al, 1973).

2.6 HEREDITARY COPROPORPHYRIA

2.6.1 Introduction

Hereditary coproporphyria (HC) was described by Berger and Goldberg (1955) when they reported four cases in a Swiss family. HC appears to be the least common type of the acute hepatic porphyrias in Britain. The exact prevalence is not yet known for any country. It is transmitted as an autosomal dominant trait, but recently a rare homozygous form of the disease with a more florid clinical expression has been recognised (Grandchamp et al, 1980).

2.6.2 Biochemical features

The primary enzyme defect in coproporphyrinogen oxidase activity can be demonstrated in the white cell buffy coat preparations (Brodie et al, 1977d), cultured skin fibroblasts (Elder et al, 1976) and lymphocytes (Grandchamp et al, 1980). In the heterozygous form, enzyme activity is reduced to about 50% of normal and in the rare homozygous state to 2-3%. It is surprising that even with gross enzyme deficiency in the homozygous states haem formation seems normal whether the rate rather than the amount of haem formed is impaired remains to be seen. HC is characterised by an excessive urinary and faecal excretion of coproporphyria III. Urinary ALA and PBG excretion is also increased. In the latent phase, however, faecal coproporphyrins are much increased at a time when urinary porphyrin precursor excretion is often normal (Elder, 1980).

2.6.3 Clinical findings

The acute attacks with abdominal pain and neurodysfunction are similar to those described for AIP and VP; but they tend to be of milder severity (Brodie et al, 1977d). The skin manifestations resemble those of VP. Unlike VP, the skin lesions seldom occur without the other biochemical or clinical manifestations of an acute attack.

SECTION I

CHAPTER 3

MANAGEMENT OF THE ACUTE ATTACK OF PORPHYRIA

3. MANAGEMENT OF THE ACUTE ATTACK OF PORPHYRIA

3.1 INTRODUCTION

The first step is to remove or correct any potential porphyrinogenic factor whenever possible. The most common precipitating factors are certain drugs (Moore & Disler, 1983; Goldberg et al, 1983), alcohol (Doss et al, 1982), fasting (Stein & Tschudy, 1970), hormonal influence (Welland et al, 1964; Paxton et al, 1974) and infection (Eales & Linder, 1972). A careful history is important in identifying a precipitating cause because exposure to the porphyrinogenic agent may be quite subtle as was the case in the surreptitious ingestion of a mouthwash preparation containing eucalyptol (Bickers et al, 1975).

There are two aspects to the treatment of the patient:

(I) to provide symptomatic and supportive therapy until the attack subsides spontaneously, as it does in the majority of cases within a few days;

(II) to modify the course of the disease so as to curtail the present attack or to prevent future attacks.

3.2 SYMPTOMATIC AND SUPPORTIVE THERAPY

are directed at controlling pain, ensuring an adequate fluid and nutritional intake, providing appropriate physiotherapy, and last but not least, maintaining the patient's morale.

Pain control

For most patients, abdominal pain is the main problem. It often radiates to the back and is accompanied by pain in the limbs.

Typical abdominal examination reveals few physical signs; the limb muscles may be tender to palpation.

Many patients are able to control their abdominal pain with simple analgesics such as paracetamol, aspirin or dihydrocodeine. But when the pain becomes more severe, opiate analgesics such as pethidine or morphine are needed. Buprenorphine, the long-acting synthetic opiate is also useful given sublingually or intramuscularly, but in our experience often worsens vomiting. In a prolonged attack the patient's response to the severe pain may change to the pattern of behaviour now recognised in other chronic pain situations (Sternbach, 1974). This is where the patient may not look particularly distressed while experiencing and complaining of great discomfort. In such instance, the lack of sympathomimetic features expected of severe acute pain can cause withdrawal of sympathy from inexperienced attending medical and nursing staff. Management of the patient can be very difficult for the clinician when he is confronted with increasing demands for opiate analgesics in a disease where psychological disturbances commonly occur, compounded by the failure to provide adequate pain relief with large doses of opiates. The constant request for analgesia often raises the suspicion of drug addiction. But this danger only exists for those few patients with frequent attacks requiring large doses of narcotic drugs outside hospital. It is therefore important that every attempt should be made to withdraw all opiate drugs during the remission periods. For a few unfortunate patients pain relief cannot be obtained during the waking hours despite administration of large

doses of narcotic agents, even by intravenous infusion (Chapter 9). The patient is therefore encouraged to sleep, undisturbed, but under constant supervision, in a quiet room. This can be achieved by combining standard dosage of opiates with a phenothiazine drug.

Nausea and vomiting usually accompany the abdominal pain. In some cases it can be very severe, especially following the administration of opiate analgesics. These symptoms may be controlled with chlorpromazine, promazine or prochlorperazine. Metoclopramide is best avoided because of possible porphyrinogenicity (Doss et al, 1981). If vomiting is protracted the patient often becomes dehydrated and oliguric.

Constipation, a regular feature of the disease, is made worse by the reduction in food and fluid intake, correction of which often remedies the situation. If there is faecal impaction, "Bisacody" suppositories can be used, failing which a phosphate enema is indicated. Oral neostigmine is considered safe to combat the constipation, but is better avoided because of its other cholinergic side-effects.

Hyponatraemia and hypochloraemia commonly occur. These electrolyte disturbances are more frequently due to inadequate fluid and electrolyte replacement rather than to inappropriate anti-diuretic hormone (ADH) secretion. Differentiation between these two causes of hyponatraemia is important since inappropriate ADH secretion may require fluid restriction whereas hyponatraemia associated with volume-contracted states intravenous saline (Andreoli, 1985). It is desirable for the patient to have a

urinary output of at least 1500 ml daily. Since better methods of nutritional support have become available a state of cachexia can be prevented in porphyric patients. Carbohydrate intake in the form of dextrose (Perlroth et al, 1968; Bonkowsky et al, 1976) or laevulose (Brodie et al, 1977b) has been shown to modify the acute attack both clinically and biochemically. It is recommended that at least 2500 Kcal should be given daily including a minimum of 400 gram of carbohydrate. Should the patient feel nauseated or anorexic and cannot take an adequate carbohydrate intake by mouth, the supplements can be given with minimal discomfort via a fine-bore nasogastric tube. High content carbohydrate solution is conveniently available in proprietary preparations such as "Hycal" (Beecham products) or "Caloreen" (Roussel). For longer term treatment we have found "Clinifeed" (Roussel) which also contains protein and vitamin supplements to be particularly useful. In selected cases the patient has been successfully managed with naso-gastric feeding at home over a period of several weeks. The successful use of naso-gastric feeding has obviated the need for the parenteral administration of nutrients in many of our cases. And since a low-grade pyrexia is a common feature in the more severe attacks of acute porphyria (Waldenström, 1957; Goldberg, 1959; Eales & Linder, 1962) an intravenous access with its risks of infection is to be avoided. Furthermore intravenous infusion of 2 litres of hypertonic solution containing 20% glucose, dextrose as laevulose daily necessitates its administration through a central venous line. Otherwise it has a thrombogenic effect on the peripheral veins. It has been our experience that

the timely use of supplementary feeding aborts the onset of the paralytic phase.

Muscle weakness and limb paralysis require appropriate physiotherapy and splinting to prevent contractures and overstretching of tendons (Figures 9 and 10). Passive exercises should be started as early as possible during the acute phase proceeding to a programme of active movements until full recovery has taken place. Although muscle weakness can rapidly recover after the acute attack, in some cases it may take several months or years (Sorensen & With, 1971).

With the onset of muscle weakness lung function must be monitored to forestall the possibility of respiratory failure. The Wright Peak flow rate meter provides a useful method of assessing the patient's ventilation sequentially. At the earliest signs of respiratory embarrassment, arterial blood gases must be checked, and assisted ventilation considered. Patients who have required intermittent positive pressure ventilation can eventually make a full neurologic recovery (Brezis et al, 1979).

The autonomic manifestations such as hypertension and tachycardia can be controlled with B-adrenergic blockers, and much of the accumulated experience relates to propranolol (Beattie et al, 1973a; Atsmon & Blum, 1970; Menewat et al, 1979). Recently the more selective B_1-adrenergic blocker, atenolol, has been reported to be safe in AIP (Moore, 1985). Propranolol must be started at low dosage ie 10 mg tds because some patients appear to be unduly sensitive to its hypotensive effect (Bonkowsky et al, 1974). The high degree of first-pass hepatic metabolism for propranolol makes

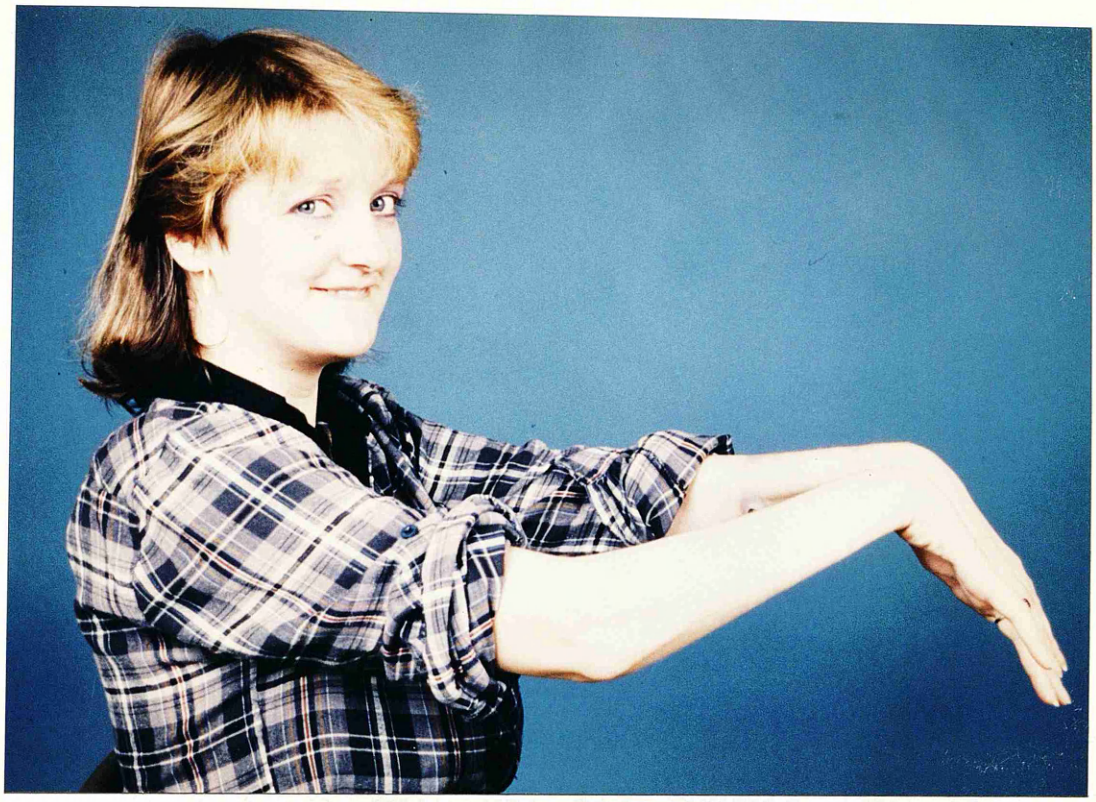


Figure 9: Shows bilateral wrist-drop due to severe weakness of the extensor muscles during an attack of AIP.

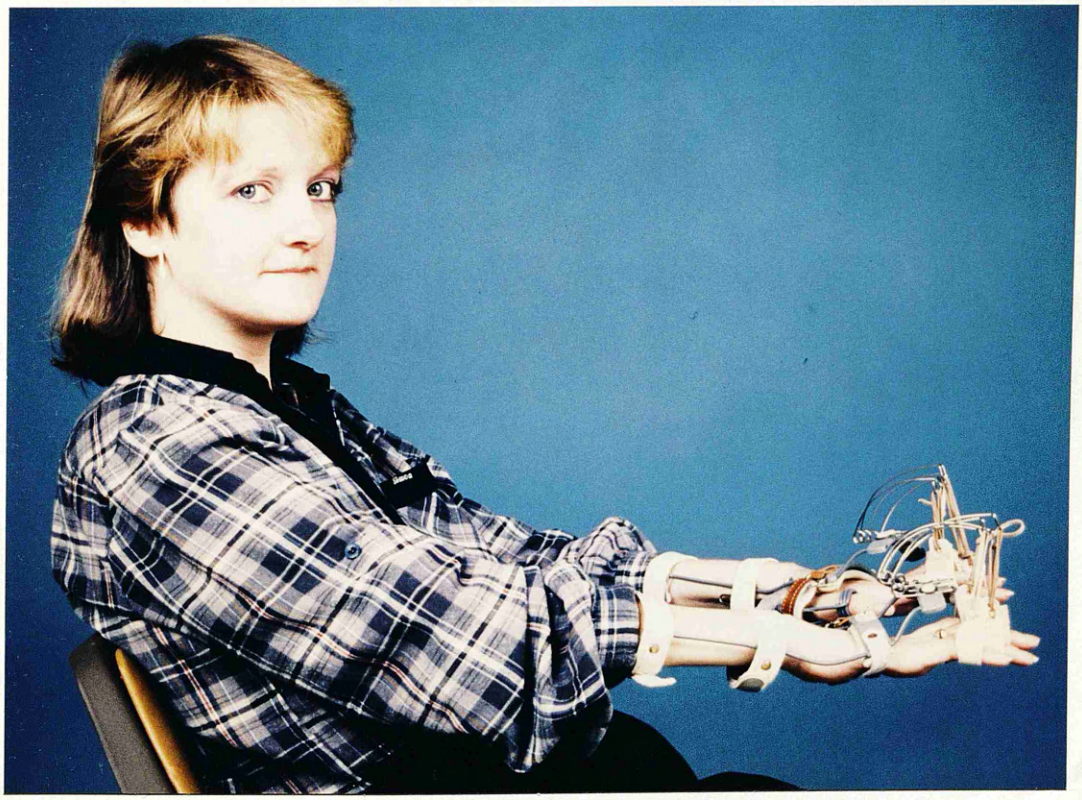


Figure 10: Physiotherapy and splinting are important in preventing contractures. Movable finger splints as illustrated here are particularly useful.

prediction of B-blockade response particularly difficult. But large daily dosages of up to 168 mg intravenously and 960 mg orally have occasionally been employed to curtail an attack (Douer et al, 1978).

Of the psychological disturbances depression, often accompanied by insomnia and emotional lability, frequently plagues the patient. With good nursing care no specific drug treatment is usually required provided pain relief is adequate. Benzodiazepine drugs such as diazepam and chlordiazepoxide must be avoided whenever possible because of alleged porphyrinogenicity (Bonkowsky et al, 1980). Chloral hydrate has been found useful in treating insomnia (Goldberg & Rimington, 1962; Eales & Linder, 1962). Less commonly, frank psychiatric manifestations such as hypomania, hallucinations or an acute organic brain synchrome may necessitate the use of psychotropic agents like chlorpromazine. Phenothiazine drugs are also useful adjuncts to opiate analgesics but postural hypotension can complicate treatment, especially when combined with B-adrenergic blockers.

Seizure control Anticonvulsant therapy is not necessary for treating the occasional seizure that occurs during an attack unless it is prolonged. Other causes such as water and electrolyte disturbances or coincidental meningitis must first be excluded before attributing seizure activity to the acute porphyria. Prolonged seizures can be stopped with intravenous diazepam. The established anticonvulsants like phenobarbitone, primidone, phenytoin and carbamazepine are all unsafe (Magnussen et al, 1975; Moore & Disler, 1983). Indeed before this danger was

appreciated barbiturate use was the commonest precipitating factor that lead to fatal attacks (Hierons, 1957; Goldberg, 1959; Eales & Linder, 1962; Goldberg & Rimington, 1962). Paraldehyde (Goldberg & Rimington, 1962; Reynolds & Miska, 1981), intravenous magnesium sulphate (Taylor, 1981) and oral sodium bromide (Bonkowsky et al, 1980) have all been employed for controlling seizure during the acute attack of AIP but long-term treatment with these agents is impractical and in the case of sodium bromide carries the risk of bromism. Although benzodiazepines and sodium valproate do not appear to induce the hepatic mixed-function mono-oxygenase system they exhibit some porphyrinogenic properties in experimental testing models (Shedlofsky & Bonkowsky, 1984). Thus maintenance anticonvulsant therapy in the porphyric patient who also suffers from epilepsy remains unsatisfactory. Use of any of the currently available drugs entails a calculated risk of porphyrinogenicity. On balance, clonazepam (Larson et al, 1978) and sodium valproate (Biagini et al, 1979; Houston et al, 1977; Brodie & Goldberg, 1980) have been recommended as first-line drugs but these have not gained general support (Shedlofsky & Bonkowsky, 1984; Lai, 1981; Doss et al, 1981).

3.3 DISEASE-MODIFYING THERAPY

Numerous remedies have previously been tried in an attempt to modify the course of the disease, but only two such treatments have been properly evaluated and found to be effective: high carbohydrate and haematin therapy. Hormonal manipulation, at present in the experimental state, promises to be useful in selected cases. Successful claims for the other treatments are

based on anecdotal case reports and small, uncontrolled, often badly-documented studies; these remain unproven at best.

3.3.1 High Carbohydrate therapy

Following the observation that a high carbohydrate intake can reduce urinary porphyrin precursor excretion in experimental AIA-induced porphyria (Rose et al, 1961) it was shown that human AIP (Welland et al, 1964a; Felsher & Redeker, 1967) and VP (Perlroth et al, 1968) responded in a similar way to changes in diet. Refined carbohydrate in the form of glucose, dextrose (Bonkowsky et al, 1976) or laevulose (Brodie et al, 1977b) have been used therapeutically. A high dietary protein content (Welland et al, 1964a) and glycerol supplementation (Bonkowsky et al, 1976) have also been found to be effective but an increased dietary fat content (Bonkowsky et al, 1976) did not confer such benefit. Reduction in the urinary excretion of ALA and PBG by a high carbohydrate intake in acute porphyria is an example of the so-called "glucose effect" where glucose can inhibit the induction of certain hepatic enzymes, in this case ALA-synthase (Tschudy et al, 1964; Marver et al, 1966). The precise mechanism of the "glucose effect" remains speculative (Goldberg, 1974). The biochemical and clinical results of a high carbohydrate regime have ranged from a dramatic improvement to little or no effect on either (Bonkowsky et al, 1976; Dhar et al, 1975; Lamon et al, 1979). The factors governing this inter-individual response have not yet been identified.

3.3.2 Haematin therapy

The interaction between haem and ALA was originally observed in bacteria by Lascelles (1960). Following the initial report that haematin administered intravenously to experimental animals repressed hepatic ALA-synthase (Waxman et al, 1966). Bonkowsky and his associates (1971) showed that the same effect could be obtained in an AIP patient treated for 2 days with haematin by causing a marked diminution of serum and urinary porphyrin precursors.

Haematin is prepared from haemin, a chloride derivative of haemoglobin extracted from out-dated hepatitis B-negative human blood (Pierach, 1982). The final haematin preparation must be sterile, and free from pyrogens and proteins. At present haematin can only be administered intravenously since early studies with oral preparations have been inconclusive (Lamon et al, 1979). Haematin therapy is given as repeated intravenous boluses. It has consistently lowered both plasma and urinary ALA and PBG in the majority of patients receiving it (Watson et al, 1977; Dhar et al, 1975; Lamon et al, 1979; McColl et al, 1981).

A rebound elevation in the serum and urinary porphyrin precursors occurs after haematin therapy, but this does not appear to induce a clinical relapse. In contrast to the consistent biochemical improvement, clinical response has been more variable although generally considered to be favourable. Pierach(1982) reported clinical benefit following 91% of the haematin transfusions administered to 104 porphyric patients whereas McColl et al (1981) noted improvement in only 50% of their patients. The beneficial

response has often been of a partial nature. Therefore in a disease like porphyria, characterised by spontaneous remissions and relapses, it may not be possible to assess the true result of therapy unless a controlled clinical trial is conducted. None has been carried out so far, and consequently enthusiasm for haematin therapy has varied amongst the centres treating these disorders. Undoubtedly, some severely ill patients can make a dramatic recovery following such treatment (Brezis et al, 1979; Menawat et al, 1979) but it does not always prevent a fatal outcome (Lamon et al, 1979). Two main reasons have been given to account for the failure to respond clinically to haematin infusions (Pierach, 1982). First haematin could have been given too late in the course of the attack. Once the neurones become structurally damaged any recovery will invariably be slow, thus masking the potential benefit of such short-term therapy. Second, haematin could have been administered in insufficient amounts or over too short a period of time. Earlier administration of haematin is now being advocated by some workers (Pierach, 1982) since the acute neurological features appear to respond better than the chronic neurological manifestations of the disease (Bosch et al, 1977; Lamon et al, 1979). Haemin has also been reported to promote neurite outgrowth from cultures of embryonic (Bonyhady et al, 1982) and neoplastic (Ishii & Maniatis, 1978) nerve tissue from experimental animals. This neurotrophic property of haemin is unlikely to confer any immediate clinical benefit when used for only a few days, but given over longer term it could possibly fulfil this potential (See Chapter 7). The availability of an

oral preparation would be necessary for this purpose. In this context it is pertinent to note that haem has been found to stimulate formation and stabilisation of mitochondrial membranes in vitro (Gopalan et al, 1984).

Haematin solution has caused troublesome phlebitis in some studies (McColl et al, 1981; Lamon et al, 1979) but not in others (Pierach, 1982). It must therefore be administered with care via a large peripheral vein, or preferably through a central venous line. When given at the recommended dosage, 4 mg/kg B Wt/day at 12 hourly intervals for a 4 to 7 day course, serious side-effects are infrequent (Pierach, 1982). Transitory acute renal failure has been reported in one case given relatively large doses of haematin (Dhar et al, 1978). A transient disturbance of coagulation (Pierach, 1982; Morris et al, 1981) and a temporary fall in platelets (Morris et al, 1981) have been encountered. When examined in vitro haematin has been shown to aggregate platelets, inactivate thrombin and induce the release of serotonin and adenosine triphosphate (Glueck et al, 1983). Experimental studies have indicated that haemin binds to a variety of haemostatic proteins and inhibits their biological activities (Green et al, 1983). Haematin-induced haemolysis has been reported in a 32-year old woman (Petersen & Pierach, 1984) probably due to a direct effect on the erythrocytic membrane (Chou & Fitch, 1980). It can also promote DNA scission in vitro (Aft & Mueller, 1983) but to date no such reaction has been reported with human DNA.

3.3.3 Phenothiazines

In 1956, Melby et al considered chlorpromazine and promazine to be the most effective drugs then available to alleviate the pain and other manifestations of acute porphyria, a view shared by other workers (Goldberg, 1959; Stein & Tschudy, 1970). Their mode of action was attributed to their anti-cholinergic and anti-adrenergic effects since they did not reduce the urinary excretion of porphyrin precursors (Melby et al, 1956). Monaco et al (1957) reported that chlorpromazine did not improve established paralysis although it provided good symptomatic relief. Paradoxically, neostigmine with its cholinergic activity, was also advocated with the belief that porphyrins might interfere with acetylcholine synthesis and function at the neuromuscular junction (Berg, 1945; Gordin, 1948). Ganglion-blocking drugs have also been used in an anecdotal fashion (Wehrmacher, 1952), but the ability of all these agents to modify the course of the disease seems very dubious (Goldberg & Rimington, 1962).

3.3.4 Glucocorticosteroids

Urquhart (1898) originally thought of using suprarenal extract after adrenal disease had been seen in association with acute porphyria. Subsequently, corticotrophin and cortisone therapy have been reported to be of doubtful benefit (Goldberg & Rimington, 1962; Eales & Linder, 1962). At present there is no rational basis for using glucocorticosteroids in porphyria although anecdotal claims of success are still appearing in the literature (Jusic et al, 1976; Sòstarko & Jùsic, 1979).

3.3.5 Vitamin E

Following the observation that the anaemia of vitamin E deficiency was related to a defect in haem biosynthesis (Porter & Fitch, 1966) Murty & Nair (1969) showed that Vitamin E could depress the urinary excretion of porphyrin precursors in AIA-induced experimental porphyria. In an uncontrolled study of four patients, Nair et al (1971) subsequently reported that a similar beneficial effect could be obtained in porphyric patients treated with vitamin E. Pre-treatment blood vitamin E levels in these patients were found to be sub-normal but other possible causes for this finding were not satisfactorily excluded. The neuropathy of vitamin E deficiency, only recently recognised, (Muller et al, 1983) is in fact different from that of acute porphyria being characterised by spinocerebellar degeneration and a sensory central-peripheral axonopathy.

3.3.6 Pyridoxal phosphate (Vitamin B_6)

Pyridoxal phosphate is an essential co-factor for ALA.S and for tryptophan metabolism. Elder & Mengel (1966) studied a single AIP patient in remission whose urinary excretion of ALA and PBG fell significantly after being deprived of pyridoxal phosphate in her diet. Subsequent vitamin B_6 replacement caused an increase in the urinary excretion of these porphyrin precursors. During the entire study she did not have any manifestations of porphyia, but the trial was stopped because she developed optic neuritis due to the deoxypyridoxine prescribed. The results of this experiment have not since been confirmed. (Chapter 4 for further discussion) Price et al (1959) reported that during the acute attack of

porphyria abnormally high levels of copper and zinc are excreted in the urine accompanied by an increase in L-tryptophan metabolites. In the porphyric patients studied the disturbance of tryptophan metabolism could not be corrected by pyridoxine alone, but only when given in combination with chelating agents such as edetic acid. Cavanagh & Ridley (1967) have therefore proposed that in acute porphyria there might be a consumptive depletion of pyridoxal phosphate resulting from the abnormally high activity of ALA.S. This deficiency could then be responsible for the development of the neuropathy when combined with certain, as yet undefined, disturbance of polyvalent cations in the body. Anaology is drawn between the neuropathological similarities in AIP and isoniazid-induced neuropathies in support to this hypothesis, especially since the latter can be prevented by pyridoxine supplementation (Cavanagh, 1967). This hypothesis has not since been properly tested clinically although the sparse published data relating to it have not been particularly encouraging (Price et al, 1959); Hamfelt & Wetterberg, 1968; Kosower & Rock, 1968; Bosch et al, 1977). Several patients with active AIP have recently been treated with large doses of pyridoxal phosphate supplements over a period of several months with no apparent response either to their acute attacks or in preventing further episodes (McColl, Personal communication).

3.3.7 B-adrenergic blockers

Schley et al (1970) found that urinary catecholamines were increased during attacks of porphyria. The possibility of a partial blockade in the re-uptake mechanism of catecholamines by

the adrenergic neurones, as demonstrated in platelets of porphyric patients (Beal et al, 1977), could perhaps explain this observation and provide a rationale for the use of B-adrenergic blockers (Atuk et al, 1975). Several case reports have claimed both clinical and biochemical improvement with propranolol (Atsmon & Blum, 1970; Beattie et al, 1973a; Menawat et al, 1979). Atsmon and his group have produced some evidence in experimentally-induced porphyria which suggest that large doses of propranolol can decrease the activity of ALA-S and urinary excretion of ALA and PBG (Blum et al, 1973; Schoenfeld et al, 1976). Whereas propranolol can undoubtedly control the sympathomimetic features, often in relatively small doses (Menawat et al, 1979), solely by its B-adrenergic blocking action, the clinical benefit of higher dosage (Douer et al, 1978) remains uncertain.

Its putative disease-modifying effects have been attributed to its ability to inhibit aminoacid incorporation into protein (Schoenfeld & Atsmon, 1977) or increase free haem levels (Epstein et al, 1982), thereby reducing ALA.S activity.

3.3.8 Folic acid and specific enzyme replacement

Folic acid and its pteroyl derivatives have been shown to activate PBG.D experimentally (Piper et al, 1976; 1979). Wider et al (1980) have subsequently claimed that folate supplementation can alter the clinical course of the disease but this remains unconfirmed. The same group of investigators have recently attempted to replace specific haem enzyme deficiency, as in the case of ALA-dehydrase deficiency in lead poisoning, by using erythrocyte ghosts as carriers of the missing enzyme (Bustos et al, 1983; Del C Battle et al, 1983).

3.3.9 Hormonal therapy

Some patients suffer recurrent exacerbations of acute porphyria related to their menstrual cycles. Prevention of pre-menstrual attacks by hormonal manipulation is difficult because of the unpredictable and paradoxical effects that these steroids can have. Oral oestrogenic contraceptives, progestogens and androgens have been used in some patients without complications (Perlroth et al, 1965) but in others they have triggered acute attacks (Lamon et al, 1979b; Zimmerman et al, 1966). The reason why cyclically recurring attacks affect only a small percentage of female patients remains unknown. Anderson et al (1984) have recently reported that subcutaneous treatment of an AIP patient with a luteinizing hormone-releasing hormone (LH-RH) agonist successfully prevented her pre-menstrual attacks by abolishing the hormonal swings. The fact that LH-RH suppresses ovarian function by acting at the hypothalamic pituitary level indicates that gonadotrophin secretion or its secondary effects may be responsible for the acute manifestations of the disease. It is interesting to note that the clinical improvement was not accompanied by a concomitant decrease in the excretion of urinary porphyrin precursors. The future availability of an intranasal formulation for LH-RH analogues ("Buserelin") holds much promise not only in the prevention of menstrually-related attacks but also as a mode of contraception for these porphyric patients. Until the safety profile of long-term treatment with LH-RH agonists becomes known, use of these agents must necessarily be circumspect.

Prevention of pre-menstrual attacks has also been achieved by timely intravenous haematin infusions (Lamon et al, 1978) but as previously discussed its place for regular long-term therapy is limited.

3.4 TREATMENT OF PHOTOSENSITIVITY

The photosensitizing lesions of HC and VP are best treated by measures aimed at shielding the patient from sunlight. Most commercial cream barriers are formulated to reduce exposure to light in the range of 290-320 nm, but for optimal screen in porphyria special preparations are required to exclude light within the wavelengths of 390-420 nm. These, unfortunately, contain zinc or titanium oxides which are not cosmetically pleasing because of their heavy consistency. Oral β-carotene, thought to act by quenching singlet oxygen radicals, may be useful. But it causes carotinaemia which sometimes may be converted to a more cosmetically pleasing brown tan by simultaneous oral administration of canthaxanthin (Eales, 1978).

3.5 PREVENTION OF ATTACKS

All patients identified as having acute porphyria should be carefully advised about the nature of the disease and instructed how to avoid known precipitating factors. They should be encouraged to take a regular diet, to avoid fasting and to abstain from alcohol. They should be warned about the dangers of porphyrinogenic drugs. It is helpful for the patient to be provided with a list of drugs which are considered safe or

unsafe. The family doctor must also be informed about the type of the patient's porphyria and given advice about its future management. The patient should be reminded about informing any other medical attendant of their condition. As an additional precaution the patient should be encouraged to wear an identification bracelet or necklace indicating that he or she suffers from porphyria; this is to prevent the administration of dangerous anaesthetics or drugs in case of accident or emergency.

3.6 ANAESTHETICS

Surgery does not carry an increased risk of complications in porphyric patients provided the precautions outlined previously are taken. Safe anaesthetic agents must be carefully selected. Atropine or morphine can be used as premedication. Intravenous ketamine has been found to be a safe inducing agent and under no circumstances must thiopentone or barbiturate derivatives be used. Nitrous oxide is also a safe inhalational agent. Suxamethonium and d-tubocurarine are useful muscle relaxants. If a local anaesthetic is indicated, procaine is the agent of choice (Moore, 1983; De Verneuil et al, 1983). In order to prevent an attack induced by fasting an intravenous infusion of dextrose must be started prior to surgery and continued until a satisfactory oral intake is achieved.

3.7 PREGNANCY

Generally speaking, most porphyric patients have an uneventful pregnancy with no particular danger to the foetus (Stein &

Tschudy, 1970; Brodie et al, 1977c). In some women however the hormonal changes during pregnancy or puerperium can trigger off an attack (Brodie et al, 1977c). Shared care between the obstetrician and a physician with an interest in porphyria is desirable. If vomiting is troublesome during early pregnancy, early administration of intravenous dextrose is indicated. Intravenous dextrose must also be given during labour.

If the patient has had frequent attacks of porphyria, it is our empirical practice to advise such patients against pregnancy until they have been free of symptomatic attacks for at least 18 months. When attacks do occur during pregnancy, they are treated in the usual manner, and particular care is taken to control the blood pressure. Most attacks settle with adequate supportive therapy. As the teratogenic effects of haematin are unknown (Aft & Mueller, 1983), this treatment is better avoided if possible. Therapeutic abortion is rarely indicated unless the attack is uncontrollable and threatens the mother's life.

In cases where the woman decides to delay or avoid pregnancy, she should be warned against using the hormonal contraceptive pills and advised about other forms of contraceptions.

3.8 SCREENING OF RELATIVES

All the blood relatives of any new case of porphyria should be screened. Those with the genetic trait should then be counselled about the nature of their inherited disorder and advised about taking the necessary precautions. Careful counselling is important so as not to engender unnecessary fear or neuroticism

since most of the identified cases will remain completely asymptomatic throughout their lives. Urinary measurement of porphyria precursors and porphyrins is an inadequate method of screening because only about one third of patients will be so identified. The measurement of erythrocytic PBG.D activity is now the standard method of screening AIP patients. But since there is some overlap between the values of PBG.D activity in normal subjects and patients with AIP, measurement of ALA.S in peripheral white cells can be helpful in borderline cases (McColl et al, 1982).

For screening relatives of patients with HC and VP, faecal measurements of porphyhrins must be performed in addition to urinary assays (Mustajoki, 1978; Moore, 1983). But the most sensitive way of identifying the latent cases is by demonstrating reduced activity of the respective haem enzymes in their peripheral leucocytes (Brodie et al, 1977a).

SECTION I

CHAPTER 4

PATHOGENESIS OF THE NEUROPATHY OF ACUTE PORPHYRIA

4. PATHOGENESIS OF THE NEUROPATHY OF ACUTE PORPHYRIA

4.1 INTRODUCTION

Although great strides have been made during the past 40 years in our understanding of the biochemistry and genetics of AIP the pathogenetic mechanism(s) of pain and neuropathy of this condition still remain controversial. Indeed a large amount of published information has already accumulated on this subject, but much of it is derived from animal and in vitro experiments, often under very contrived conditions, and from small uncontrolled clinical studies and anecdotal case reports. This chapter reviews the pathological and investigative data available as a prelude to the studies I have performed in an attempt to clarify the situation further.

4.2 Neuropathological features

The neurological complications of acute porphyria were originally described by Ranking and Parkington and by Harley in 1890. The earliest recorded case examined pathologically was in 1898 when Campbell failed to show any significant abnormality in the central nervous system. In 1903 Erbsloh first described features of axonal degeneration and patchy demyelination in the femoral nerve of a porphyric patient who died following treatment with sulphonal. Several case reports followed and the subject was comprehensively reviewed thirty years later by Mason, Courville and Ziskind (1933) when they sought the relationship between the

clinical syndrome of porphyria and its pathological findings. Detailed histopathological examination of their own four autopsy cases revealed the most characteristic lesions to be in the nervous system particularly affecting the peripheral nerves and sympathetic ganglia. As previously reported by Courcoux et al (1929) and Boström (1920) marked degenerative changes were found in the spinal cord ganglion cells, especially of the anterior horn cells. In addition similar changes were present in the ganglia of the autonomic system. Other abnormalities recorded were extensive degeneration of the axis cylinders and myelin sheaths of the peripheral nerves, chromatolysis of the bulbar motor nuclei and Purkinje cells of the cerebellum. But the cerebrum appeared remarkably unscathed even when the patient displayed psychiatric manifestations during life. It was therefore concluded that the primary neurological damage was axonal degeneration followed by secondary demyelination.

Baker and Watson (1945) reported on the autopsy findings in a 24 year old man who after being treated with a barbiturate developed flaccid quadriplegia and respiratory failure. Although no macroscopic changes were present in the nervous system, marked chromatolysis was noted within the facial and hypoglossal nuclei, and the dorsal nuclei of the vagus nerves. Similar microscopic changes were found in the spinal cord affecting the anterior horn cells but the myelin appeared normal. The spinal roots had remained intact. On the other hand the peripheral nerves, especially those of the lower limbs, were severely damaged with large segments of nerve tissue being totally destroyed. These

pathological changes again supported axonal degeneration as the primary event but an additional demyelinating component could not be excluded. Likewise Denny-Brown & Sciarra (1945) laid stress on the chromatolytic changes in both dorsal and ventral horn cells of the entire spinal cord after finding that the white matter was unaffected. Patchy areas of demyelination were observed along the posterior tibial nerves whereas sections of the sural cutaneous phrenic and vagus nerves had remained intact. Although these authors concluded that the neurological damage could possibly be explained on the basis of a myelin defect due to vasoconstriction-induced ischaemia, the histological features characterising the marked chromatolysis were more supportive of a primary axonal degeneration. Nevertheless the proposal that porphyric neuropathy could be due to segmental demyelination instead of axonal degeneration gained ground (Garçin and Lapresle, 1950; Gibson and Goldberg, 1956).

In their neuro-pathological study of 5 fatal AIP cases Gibson and Goldberg (1956) reported widespread patchy demyelination of the peripheral and autonomic nerves, but axonal damage and Wallerian changes were also noted. In addition they observed marked chromatolysis in the anterior horn cells of the spinal cord and the nerve cells of the medullary nuclei, the dorsal vagal nuclei being particularly affected. Other histological features described were cytoplasmic vacuolar changes and small perivascular haemorrhages. These workers believed that the chromatolytic changes were due to the demyelination of the dependent nerve fibres. And contrary to Denny-Brown and Sciarra (1945) they could

find no evidence for these histopathological changes to be ischaemic in nature. Hierons' (1957) description of the post-mortem findings in five AIP patients was not dissimilar from that of Gibson and Goldberg (1956). Again chromatolysis of nerve cells was a prominent feature of the disease, as was the vacuolated appearance of the affected neuronal cells for which the author could find no satisfactory explanation. Gross demyelination was found in the spinal cord of one of the five cases but meaningful interpretation was hindered by the following atypical features:

(1) the development of bilateral pyrimidal signs;

(2) an elevated cerebrospinal fluid protein level;

(3) focal lymphocytic infiltration of the meninges;

(4) marked post-mortem autolysis with poor histological quality of the axon preparations.

In the single case with good nerve preparations the changes of a primary axonal degeneration was supported by the loss of the large myelinated fibre population with relative preservation of the small myelinated and unmyelinated fibres, indicative of regenerative attempts.

In a review of nine autopsy cases of acute porphyria, Ten Eyck et al (1961) reported neuropathological changes very similar to those described by Mason et al (1933): predominant neuronal degeneration with patchy demyelination. But they stressed on the fact that all these patients had other severe complications unrelated to the porphyria which contributed to their deaths and that could have produced secondary histological changes in the brain.

Analogy has been drawn between the neuropathy of acute porphyria and that of lead intoxication not only because of their striking clinical similarities but also because of their effects on the haem biosynthetic pathway (Dagg et al, 1964). In both conditions there is an excessive accumulation and excretion of the porphyrin precursors, believed by some (Becker & Kramer, 1977b) to be of pathogenetic importance. In chronic lead intoxication both axonal degeneration with nerve loss and demyelination occur in the human peripheral nerves (Buchtal and Behse, 1979) but there is marked species difference in the predominance of these changes in other experimental animals (Dyck et al, 1977). In the rat lead intoxication provide a good model of primary segmental demyelination (Windebank & Dyck, 1984). It is interesting to note that in primary demyelinating disorders such as multiple sclerosis chromatolysis is not a prominent finding despite widespread destruction of the myelin.

Cavanagh and Mellick (1965) provided strong support in favour of axonal degeneration as being the primary lesion in acute porphyria by describing Wallerian-type degeneration and failing to find any demyelination in the peripheral nerves of five fatal cases. Axonal degeneration of the motor nerves was extensive and proceeded as far proximally as the ventral roots, characteristic of the dying-back phenomenon (Cavanagh, 1979). The sensory nerve fibres were less severely affected than their motor counterparts. But an intriguing observation was the sparing of the large-diameter primary sensory fibres from the muscle spindles when the muscles concerned were almost completely depleted of

other types of innervation. Unlike the neuropathy of organo-phosphorus poisoning where the distal muscles with the largest fibres are affected earliest and most severely (Cavanagh, 1954), the dying-back process in porphyric neuropathy occurred irrespective of the distance of the muscle from the spinal cord. Cavanagh & Mellick (1965) also shared the view (Hierons, 1957; Mason et al, 1933) that the neurones of the spinal cord and ganglia seldom underwent necrosis although they became chromatolytic. Cavanagh and Ridley (1967) described axonal degeneration with collateral sprouting in muscles, indicative of previous cycles of nerve degeneration and regeneration, even in those cases where the neuropathy had clinically been of brief duration. They therefore envisaged that porphyric neuropathy was due to a relatively acute and massive "dying-back" degeneration superimposed upon a background of pre-existing subclinical denervation. The dose similarity in the type of denervation between porphyric and isoniazid-induced neuropathies has been highlighted (Cavanagh & Ridley, 1967; Jacobs et al, 1979). And since isoniazid seems to exert its neurotoxic effect by interfering with pyridoxal phosphate formation it was postulated that tissue depletion of this cofactor for ALA synthase might be responsible for the porphyric neuropathy (Cavanagh, 1967).
In a detailed case study, Sweeney et al (1970) again confirmed the presence of axonal degeneration and central chromatolysis with the absence of segmental demyelination. Nerve fibres supplying both proximal and distal muscles were found to be equally affected, supporting the dying-back hypothesis of Cavanagh & Ridley (1967).

A characteristic feature of acute porphyria is proximal muscle weakness. This was explained on the basis that neurones supplying the proximal anti-gravity muscles might be more vulnerable metabolically because of their larger motor units (Ridley, 1969). And the development of weakness in a limb that was subject to unaccustomed exercise supports this possibility (Ridley & Cavanagh, 1972).

It must be emphasized that several workers have also recorded minimal morphological changes in the nervous system of porphyric patients who died during an acute attack, especially if death had occurred within a short time of the onset of paralysis (Mason et al, 1933; Denny-Brown & Sciarra, 1945; Hierons, 1957). This observation is indicative of a functional block resulting from some biochemical disturbance, with the development of structural changes arising from other considerations such as the severity, duration and frequency of the acute attack(s) (Chapter 10). Indeed some patients can make strikingly rapid and complete neurological recovery from virtually complete paralysis (Ridley, 1969; Menawat et al, 1979; Brezis et al, 1979). In such cases the degree of axonal damage must have been comparatively small. Yet in others in whom nerve degeneration has extended far proximally, recovery may not be complete even after several years (Sorensen & With, 1971; Ridley, 1969). For the same reason, the distal limb muscles take longer to recover than the proximal muscles even though the latter may be more severely affected clinically (Ridley, 1969).

Following the work of Cavanagh, Mellick & Ridley on the neuropathological changes in AIP only sporadic case reports of peripheral nerve or muscle biopsies have been published (Masuja, 1969; Nagler, 1971; Thomas, 1971; Wakayama et al, 1975; Anzil and Dozic, 1978; Thorner et al, 1981). They all support the concept of axonal degeneration but some workers (Thomas, 1971; Wakayama et al, 1975; Anzil & Dozac, 1978) have also described the presence of mild segmental demyelination in sural nerve biopsies which could not be attributed to axonal degeneration.

Conclusion

The histopathological data available support the hypothesis that the neuropathy of acute porphyria is caused by a metabolic disturbance which interferes with nerve cell function. Depending on considerations such as severity, duration and frequency of attacks and other as yet unidentified factors the metabolic disturbance can cause a neuronopathy with axonal degeneration of the "dying-back" type. The brunt of the damage is borne by the anterior horn cells of the spinal cord and brain stem ganglia, with relative sparing of the brain cells. Severe axonal degeneration can eventually cause some secondary demyelination. It also appears that the Schwann cells could be directly damaged in an acute attack accounting partly for the demyelination but this is unlikely to be clinically relevant.

4.3 ELECTRODIAGNOSTIC FINDINGS

4.3.1 Motor nerve conduction studies

From the earliest studies it was recognised that even in the presence of severe muscle weakness motor conduction velocity (MCV)

is often normal or only mildly impaired in acute porphyria (Simpson, 1962; Stein & Tschudy, 1970; Maythem & Eales, 1971; Nagler, 1971). This observation has been amply confirmed in larger and more detailed investigations (Flugel & Druschky, 1977; Wochnik-Dyjas et al, 1978; Albers et al, 1978). It favours the concept of primary axonal degeneration since primary demyelination would be expected to cause more marked reduction in conduction velocity. Following an acute attack the impaired MCV can rapidly return to normal in accordance with the speedy removal of a transient functional block. Nagler (1971) suggested that this transient neurological dysfunction could be due to the neurotoxic effects of the porphyrin precursors.

Flügel and Drüschky (1977) reported the results of nerve conduction studies on 16 AIP patients, six of whom had no neurological abnormality, four with neuropathy and the remaining six with tetraplegia. The single patient studied during the acute attack had absent or low-amplitude motor responses except in one nerve with moderate slowing of conduction. Patients studied three to ten years after the acute attack displayed slowing of conduction velocity proportional to amplitude reduction and the presence of fibrillation. The authors concluded that their findings were best explained by an axonal neuropathy. A similar view was reached by Albers et al (1978) after recording normal MCV in eight of eleven AIP patients previously affected by tetraparesis.

Mustajoki and Seppalainen (1975) used the technique of partial antidromal blocking to differentiate between the fast and slow

motor fibres. Patients with AIP and VP were found to have a significant reduction in the conduction velocity of their slow motor fibres and sensory fibres, years after recovery from the acute attacks. Of note similar findings were found in latent cases who have always remained asymptomatic. These results suggest that subclinical neuropathy may be more frequent than clinically suspected - a situation reminiscent of lead neuropathy (Seppalainen & Hernberg, 1972). Since conduction velocity of the slow motor fibres was diminished even in patients with normal urinary porphyrin precursor excretion the neurotoxic role of ALA and PBG becomes questionable.

4.3.2 Sensory nerve conduction studies have been few. In the three patients studied late after recovery from tetraplegia, slowing was noted in one whereas the two others were normal. Albers et al (1978) reported normal measurements in four of six AIP patients with quadriparesis whereas in the remaining two patients sensory-nerve action potentials were absent or of low amplitude with normal latencies. Therefore these findings as for the motor nerve conduction studies are consistent with an axonal neuropathy.

4.3.3 Electromyographic (EMG) Findings

In the early stages of porphyric neuropathy profuse fibrillation potentials have been recorded, and after six to eight weeks polyphasic motor unit potentials characteristic of re-innervation appeared and subsequently increased in amplitude (Nagler, 1971). EMG recordings have conclusively shown that motor nerve fibre degeneration takes place and that the ensuing regenerative

attempts may or may not proceed to completion (Ridley, 1967; Stein & Tschudy, 1970; Maythem & Eales, 1971; Flügel & Druschky, 1977; Wöchnik-Dyjas et al, 1978; Albers et al, 1978). The near-normal MCV accompanied by low amplitude compound muscle action potentials is characteristic of an axonal neuropathy. The low amplitude compound muscle action potential reflect loss of axons and subsequent muscle atrophy; the normal or slightly abnormal conduction velocities indicate that the remaining axons are conducting normally. The early predominance of fibrillation potentials in the proximal muscles suggested to Albers et al (1978) that the major insult occurred at root or cord level, or that the nerve cells with the larger motor units were more vulnerable to damage. The latter possibility supports the hypothesis of Cavanagh & Ridley (1967).

Wöchnik-Dyjas et al (1978) reported that after an acute attack rapid reversibility of EMG changes took place in some patients whereas subclinical disturbances persisted long afterwards in others. They also found that tetanic stimulation tests of neuromuscular function resulted in potentiation rather than fatigue. This was interpreted as suggesting that in porphyric neuropathy there might be a block of neurotransmission at the cholinergic nerve endings reminiscent of botulinum poisoning. No confirmatory report has yet appeared in favour of this intriguing proposal, but in view of the possible pharmacological effects of ALA on neurotransmission it must be further explored.

4.4 PATHOGENESIS OF THE ACUTE ATTACKS

Although the clinical features of acute porphyria can be explained on the basis of a widespread neurological dysfunction (Goldberg, 1959) uncertainty still surrounds its pathogenesis. The relationship between the biochemical abnormalities, predominantly studied in the liver, and the clinical manifestations remains conjectural. Over the past 30 years several hypotheses have been advanced, and as yet there is no conclusive proof for any particular postulate. These postulates are however not mutually exclusive. Unfortunately most of them have been inadequately tested, having been formulated on anecdotal or limited clinical information.

4.4.1 Hypothesis 1

"The neurological manifestations are caused by the depletion of essential substrates or co-factors arising from the haem biosynthetic pathway defect."

Depletion of pyridoxal phosphate (Vitamin B_6)

Pyridoxal phosphate is an essential cofactor for ALA synthase, the rate-limiting enzyme of the haem biosynthetic pathway. It has been suggested that during an acute attack increased ALA synthase activity correspondingly augments the demand and consumption of pyridoxal phosphate with consequent deprivation to the nervous tissues (Price et al, 1959; Cavanagh & Ridley, 1967). Indeed plasma pyridoxal phosphate concentration has been found to be decreased in some AIP patients as compared to normal controls (Hamfelt & Wetterberg, 1968). Unfortunately no details were given about the nutritional status of these porphyric patients who often

take an inadequate diet. Believing that AIP was a disease caused primarily by ALA.S overproduction Elder and Mengel (1966) attempted to reduce ALA.S activity by inducing pyridoxal phosphate deficiency artificially in a patient in remission. This patient seemingly had pre-existing vitamin B_6 deficiency at the outset with abnormal tryptophan metabolism. Following further vitamin deprivation her urinary porphyrin precursors fell significantly. The authors subscribed to the view that a lack of pyridoxal phosphate was responsible for the clinical manifestations of the disease. And from their interpretation the patient ought to have developed porphyric symptoms when her urinary porphyrin precursors fell - and she did not. The belief behind this expected outcome is in fact not supported by current knowledge. Cavanagh (1967) has drawn attention to the close neurohistopathological similarities between porphyric neuropathy and that of vitamin B_6 deficiency; but other differences not seen in porphyria such as demyelination of the posterior column fibres (Follis & Wintrobe, 1945; Swank & Adams, 1948) and swelling of the Betz cells in the motor cortex (Victor & Adams, 1956) have been recorded in experimental animals. The main objection to the pyridoxal phosphate deficiency hypothesis is that the clinical picture of these two conditions is quite different. The neuropathy of vitamin B_6 deficiency is a distal, symmetrical, predominantly sensory polyneuropathy affecting the lower limbs (Vilter et al, 1953; Evans et al, 1960) whereas porphyric neuropathy is primarily motor chiefly affecting the upper limbs (Ridley, 1969). Moreover, Hamfelt & Wetterberg (1968) have pointed out that patients without

porphyria who had the same degree of vitamin B_6 deficiency as those AIP patients with neuropathy showed no evidence of neurological involvement. Pyridoxine supplementation was found not to cause any clinical improvement in the neuropathy despite normalisation of the low vitamin B_6 levels (Hamfelt & Wetterberg, 1968; Price et al, 1959). This, however, could conceivably be due to the advanced stage of nerve damage in so far as any beneficial effect of short-term therapy would not become evident against the background of slow regenerative capacity of the damaged nerve fibres. Cavanagh & Ridley (1967) have advocated the prophylactic use of pyridoxal phosphate to forestall future attacks of porphyria, but doubts have been expressed about this suggestion because pyridoxal phosphate requirement for ALA synthase is probably quite small compared to the other enzymes also dependent on this cofactor (Bonkowsky & Schady, 1982).

Depletion of Zinc

Patients with porphyria have an increased urinary excretion of zinc on account of the chelating properties of porphyrins for divalent ions (Peters et al, 1974). Depletion of zinc is unlikely to explain the porphyric neuropathy for the following reasons. First, the clinical features of zinc deficiency syndromes are totally different from those of acute porphyria. Second, it fails to explain the absence of neurological manifestations in the chronic porphyrias such as PCT and erythropoietic protoporphyria where zinc excretion often exceeds that in AIP (Peters et al, 1974). Third, zinc sulphate supplementation has not shown clinical benefit.

Depletion of glycine

Glycine is a natural substrate for ALA.S which has a high Km. De Matteis & Rimington (1962) have suggested that when ALA.S activity is induced the increased glycine flux into the haem biosynthetic pathway could deplete the supply of this substrate for other important pathways such as that for acetylcholine synthesis - hence causing the neurological manifestations. But in acute porphyria there is no evidence for an acquired deficiency of glycine availability or of acetylcholine synthesis. Sodium benzoate is conjugated with glycine by mitochondrial glycine acyl-transferase to form hippurate which is rapidly excreted in the urine. Piper et al (1973) have reported that diversion of glycine from the haem to the hippurate biosynthetic pathway is theoretically possible. Administration of sodium benzoate to rats with experimentally-induced porphyria led to a decrease in the excretion of ALA and PBG. Contrary to previous suggestion (De Matteis & Rimington, 1962) these results would suggest that limitation in the supply of glycine for ALA.S might blunt the biochemical manifestations of porphyria. But this potential therapeutic use of sodium benzoate in acute porphyria (Becker & Kramer, 1977), has not received confirmation.

4.4.2 Hypothesis 2

The neuropathy is due to the abnormal products derived from the porphyrin precursors.

Irvine and his associates (1961, 1969, 1974) identified a pyrrole compound in the urine of AIP patients, which had previously been found in the urine of patients with certain psychoses, namely

schizophrenia. Initially the compound was thought to be kryptopyrrole (2,4-dimethyl-3-ethyl pyrrole), and it exhibited a mauve colour when reacted with Ehrlich's reagent - hence the term 'mauve factor'. This material was shown to have neurotoxic properties (Irvine et al, 1969). Subsequent studies (Gendler et al, 1978) revealed that the natural compound in porphyric urine was in fact hydroxyhaemopyrrolin-2-one, which under certain conditions was converted to kryptopyrrole and related monopyrroles. Gorchein (1980) failed to find any qualitative or quantitative relationship between the urinary concentrations of hydroxyhaemopyrrolin-2-one and the clinical manifestations of AIP. It is now generally believed that these pyrroles, thought to be mainly derived from PBG, play an insignificant role in the pathogenesis of the disease.

Porphobilin, a dipyrrylmethane derivative of PBG can be found in the urine of porphyric patients after it has been exposed to light. Using a rat phrenic-nerve hemidiaphragm preparation Feldman et al (1971) reported that porphobilin at low concentrations could cause pre-synaptic neuromuscular inhibition. Porphobilin was much more potent than PBG or ALA in this respect. However its role in the pathogenesis of acute porphyria is doubtful since this compound has not been detected in either urine or tissues of porphyric patients. Moreover patients with ALA-dehydratase deficiency would not be expected to make the compound, and yet they exhibit clinical features similar to the other types of acute porphyria (Doss et al, 1980b). Pierach et al (1977b) have also reported that rats given large amounts of

porphobilin displayed no abnormality in blood pressure, pulse or behaviour.

4.4.3 Hypothesis 3

The neuropathy is the result of neurotoxicity from an accumulation of the porphyrin precursors, ALA and/or PBG.

All the types of acute porphyria have in common the excessive production and urinary excretion of ALA. PBG is also increased except in homozygous ALA-dehydrase deficiency. The pathogenetic role of PBG is doubtful since patients with homozygous ALA-dehydratase deficiency can only produce large amounts of ALA without PBG, yet they display the neurological manifestations seen in the other types of acute porphyria (Doss et al, 1979, 1982b). The liver is considered to be the main site of ALA and PBG production. It therefore follows that these precursors must cross the blood brain barrier to exert their neurotoxic effect. Experimental studies in normal rats have shown that the blood-brain barrier is weakly permeable to ALA and PBG. For instance, after intraperitoneal administration the maximal ALA concentration attained in brain ranged from 4% (Shanley et al, 1975) to 12% (McGillion et al, 1975) of maximal blood levels. McGillion et al (1975) have also shown that brain tissue concentrations of ALA remained elevated after the blood concentrations had returned to normal. More significantly, during the acute attacks of porphyria, ALA and PBG can be detected in the cerebro-spinal fluid where they are not normally present. The concentrations of ALA and PBG in the cerebrospinal fluid of patients with AIP (Sweeney et al, 1970, Bonkowsky et al, 1971) and

VP (Percy & Shanley, 1977) (10^{-5} to 10^{-7} M) were reported to be much lower than in the blood (10^{-3} to 10^{-5} M) in keeping with the experimental observations made in the rat. Becker et al (1974) have demonstrated that brain slices can actively concentrate ALA from the surrounding medium with the implication that the neuronal concentration may be much higher than that in the cerebrospinal fluid.

The present hypothesis particularly holds for ALA and is supported by the following:

1. Acute attacks only occur when excretion of the porphyrin precursors is increased. Although some patients can have elevated serum ALA and PBG levels during remission, the onset of symptoms is always accompanied by an even higher excretion of these compounds (Becker & Kramer, 1977b). But the excretion of porphyrin precursors does not correlate well with the clinical severity of the disease (Ackner et al, 1961) (Chapter 9). This is perhaps because different patients display varying degrees of susceptibility to the effects of ALA on account of genetic or other interacting factors. A recent exciting development has been the recognition of molecular heterogeneity in PBG.D accounting for different enzymatic activities (Anderson et al, 1981; Mustajoki & Desnick, 1985); this observation could explain why certain porphyric families seem to suffer from their disease more than others.

2. Acute administration of ALA either intraperitoneally to mice (McGillion et al, 1975) or into the cerebral ventricles of rats (Shanley et al, 1975) produced transient excitatory effects.

When mice were treated with ALA no morphological changes occurred in the nerves but nerve conduction was transiently impaired (Sima et al, 1981). However a number of studies involving oral or parenteral administration of ALA and PBG have produced no evidence of any pharmacological effects except for some skin sensitivity (Berlin et al, 1956; Meyer et al, 1972). These experimental situations merely constitute brief exposures to the effects of the porphyrin precursors, and are clearly not comparable to the more prolonged exposure prevailing during the acute attacks in man.

3. There are two other diseases, one acquired and the other inherited, in which large amounts of ALA are produced and excreted in the urine: lead poisoning and hereditary tyrosinaemia. In both conditions patients can develop clinical features resembling these of the acute attack of porphyria with abdominal pain, motor weakness, tachycardia and hypertension (Dagg et al, 1964; Gentz et al, 1969; Strife et al, 1977). In lead poisoning, the alterations in porphyrin metabolism are mainly due to inhibition of ALA dehydrase and ferrochelatase although other enzymes may also be affected (Tephly et al, 1978). Consequently plasma ALA concentration is increased with brain ALA levels increasing as much as four-fold as compared to controls (Dagg et al, 1964).

Hereditary tyrosinaemia is an inborn error of metabolism, transmitted as autosomal recessive, which is due to a deficiency of 4-fumarylacetate hydrolase (Lindblad et al, 1977). As a result succinylacetone (4,6-dioxoheptanoic acid), an abnormal metabolite, accumulates. Succinylacetone is an extremely potent competitive

inhibitor of ALA dehydrase (Sassa & Kappas, 1983), and in hereditary tyrosinaemia, both plasma concentration and urinary excretion of ALA are increased while PBG level remains unchanged. The occurrence of an AIP-like syndrome in certain patients with hereditary tyrosinaemia (Gentz et al, 1969; Strife et al, 1977) suggests, as in genetically-determined homozygous ALA dehydratase deficiency (Doss et al, 1979, 1982b) that PBG or its derivative porphobilin are unlikely to be the cause of the neurotoxicity.

Whereas clinical and in vivo experimental evidence for porphyrin precursor toxicity has been unimpressive much information is now available to show that ALA and PBG can exert a variety of pharmacological effects in vitro. These in vitro experiments have employed tissues from different animal species including invertebrates and amphibians (Loots et al, 1975; Nicholl, 1976). High concentrations of ALA have also had to be used, often 100-1000 fold higher than those observed in the patient's serum (Sweeney et al, 1970; Miyagi et al, 1971; Percy & Shanley, 1977). Therefore extrapolation of results from such studies to man must be tempered with utmost caution.

Direct effects of ALA

Using isolated rabbit duodenum, Cutler et al (1980) have shown that even after blocking nerve transmission by tetradotoxin ALA (0.2 to 8 x 10^{-3} M) can inhibit muscle tone and both spontaneous and induced contractions. These results indicate that ALA can have a direct effect on rabbit gut smooth muscle. On the other hand, no such effect was observed by Mcgillion (1974) in

guinea-pig ileum, thus highlighting the inter-species variation. Cutler et al (1978) have also previously reported that ALA can inhibit frog gastrocnemius muscle contraction to direct electrical stimulation.

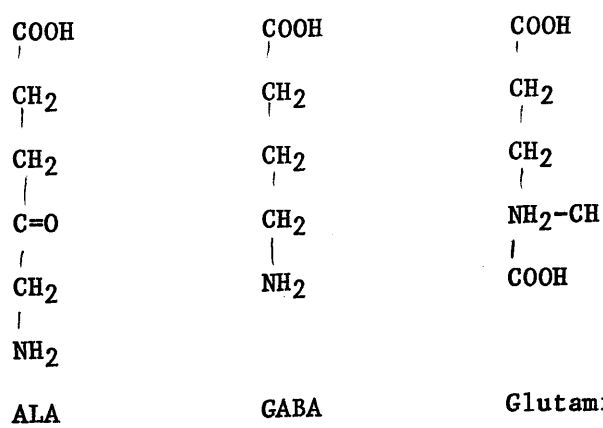
Effects of ALA on neurotransmitter release

Experiments using rat phrenic nerve-hemidiaphragm preparations have shown that ALA inhibits K^+ augmented spontaneous release of acetylcholine (Feldman et al, 1968) and the release of acetylcholine evoked by nerve impulses (Bornstein et al, 1979) at concentrations of 7.4×10^{-3} M and 0.6 to 18×10^{-3} M respectively. ALA (10^{-2} to 10^{-4} M) has also been found to depress electrically-stimulated (Nicholl, 1976) and spontaneous (Loots et al, 1975) dorsal root potentials in isolated hemisected frog spinal cord preparations. Becker et al (1975) have reported that ALA and PBG can depolarise frog muscle fibres. These observations suggest that ALA and/or PBG might have a clinically significant effect at the synaptic level.

ALA and Gamma-aminobutyric acid (GABA).

ALA is structurally similar to GABA and glutamic acid.

Chemical structures of ALA and amino acid neurotransmitters.



GABA is an inhibitory neurotransmitter in both spinal cord and cerebral cortex whereas glutamic acid exerts an excitatory effect (Krnjevic & Schwartz, 1967; Walker, 1983; Fonnum, 1984). GABA seems to act at two receptor sites. At the post-synaptic GABA A receptor GABA increases conductivity through chloride ion channels to cause hyperpolarisation of the post-synaptic neurones (Krnjevic, 1976), thus inhibiting nerve activity. GABA can also bind to GABA B receptors located on the pre-synaptic membrane. Pre-synaptic inhibition mediated by the GABA-B receptor blocks the release of neurotransmitters in both central and autonomic nervous system. ALA selectively competes with GABA for GABA receptor sites on rat brain synaptosomes (Muller & Snyder, 1977; Brennan et al, 1980). Dichter et al (1977) have shown that ALA can mimic GABA A action by increasing chloride ion conductance in the crayfish stretch receptor neurones. At relatively high concentrations (100 uM) ALA can release both GABA and glutamic acid from unstimulated brain cortex synaptosomes, but at lower concentrations (0-5 uM), it can only liberate ALA (Becker et al, 1976). ALA has also been shown to impede Na^+, K^+ - ATPase activity in rabbit brain preparations (Becker et al, 1971). Since inhibition of NA^+, K^+ -ATPase can influence neuromuscular transmission by increasing neurotransmitter release ALA seems to act as a GABA agonist. The situation is more complex because depending on the experimental circumstances ALA can be shown to have opposite effects. Thus at higher concentrations (10^{-4} - 10^{-6} M), ALA can paradoxically inhibit K^+ stimulated release of GABA from preloaded synaptosomes (Brennan & Cantrill, 1979). GABA

activity is terminated through a re-uptake mechanism using a high-affinity transport system in both nerve and glial cells (Mao and Costa, 1978). ALA has been shown to inhibit GABA uptake by brain synaptosomes at high concentrations (1 mm) (Brennan & Cantrill, 1979) but not at lower concentrations (Percy et al, 1981).

Neurotoxicity of ALA

Percy et al (1981) have reported that ALA (10 uM) was neurotoxic to both neuronal and glial components of brain cell cultures from the chick embryo. Such a concentration could possibly occur in porphyric patients since a CSF level of 21 nM has been recorded (Sweeney et al, 1970). Other workers, however, have measured much lower levels: 2.9 uM (Bonkowsky et al, 1971) and 0.3 uM (Percy and Shanley, 1977). ALA neurotoxicity certainly offers an explanation for the histopathological changes seen in acute porphyria; but results obtained with avian cells in vitro does not necessarily reflect the in vivo human situation where cells may be more resistant to toxic agents.

ALA being a small molecule freely crosses the placenta; thus during pregnancy the foetus will be exposed to the high levels of ALA prevailing in the mother's blood. It is remarkable that no porphyria-related teratogenic effects or neurological complications have yet been reported in any of the newborn of women suffering from acute attacks during their pregnancies (Brodie et al, 1977c; Stein & Tschudy, 1970; Kappas et al, 1983). This would seem to indicate that porphyrin precursors are not neurotoxic unless foetal nerve tissue is particularly resistant to

their detrimental effects or unless these complications have passed unnoticed.

Effects of PBG

Earlier studies have indicated that parenterally administered PBG produced no evident toxic effects in rats or rabbits (Goldberg & Rimington, 1954; Jarrett et al, 1956). Feldman et al (1971) found that PBG at relatively low concentrations (2.4 uM) could inhibit neuromuscular transmission in a rat hemi-diaphragm preparation. Paradoxically only weak inhibitory effects were observed on the spontaneous (Nicholl, 1976) and electrically-stimulated (Loots et al, 1975) activity on the frog spinal cord.

4.4.4 Hypothesis 4

The neurological manifestations are due to a deficiency of haem in neural tissues.

Brain tissue contains ALA.S and other enzymes of the haem biosynthetic pathway (Barnes et al, 1971; Paternini et al, 1978). The activities of these enzymes in the rat brain are quantitatively much lower than those in the liver with corresponding ratios ranging from 0.002 to 12.5 (Percy & Shanley, 1979). Sassa et al (1979) have also reported that isolated dorsal root ganglia in culture were able to synthesise a wide spectrum of porphyrins from ALA. Although brain tissue contains low levels of ALA, PBG, total porphyrins, haem and cytochrome P450 it can perform certain haemoprotein-dependent mixed-function oxidase activities (Cohn et al, 1977; Paul et al, 1977).

In considering this hypothesis two key questions need be answered. First, does haem deficiency occur in neural tissues of

patients with acute porphyria? If so, does it fluctuate with the activity of the disease?

Until now direct measurement of the intracellular "haem pool" has not been made possible, its existence being based on much indirect evidence (Muller-Eberhard & Vincent, 1985). As previously discussed (Chapter 1) the "free haem pool" is currently believed to be the regulatory component of total haem in the liver. Deficiency of hepatic haem, however, does not necessarily entail a similar state of depletion in neural tissues unless both organs share a common control mechanism for haem biosynthesis. Earlier studies have reported that brain ALA.S could not be induced in rats after either dietary starvation or treatment with known potent inducers of hepatic ALA.S such as phenobarbital, ethanol, AIA and other allyl compounds (Paternini et al, 1978; Percy & Shanley, 1979). Maines (1984) also found that unlike the liver inhibition of rat brain ALA-synthase by manganese ions was not accompanied by a reciprocal increase in haem oxygenase activity. Both these findings therefore suggested that the brain is perhaps endowed with a different regulatory mechanism for its haem pathway. In contrast, De Matteis et al (1981b, 1982) have provided evidence to indicate that regulation of rat brain ALA.S may after all be affected by haem through a negative feed-back. They have shown that ALA.S could alter its activity during the neonatal period in parallel with the physiological haem requirements of brain tissue and become depressed after intravenous injections of ALA. Inhibition of cerebellar haem biosynthesis by succinylacetone, a specific inhibitor of

ALA-dehydrase, also led to ALA.S induction whereas intra-ventricular injection of haematin caused a depression of its activity. At present there is no satisfactory explanation to account for the discrepancy between these two sets of observations in the control of brain haem synthesis.

Contraction of the haem pool, as may occur in the acute porphyrias, induces ALA.S activity whereas expansion of the haem pool leads to an increase in haem oxygenase (Bissell & Hammaker, 1977) and an accumulation of haem in tryptophan pyrrolase (Al-Badawy & Evans, 1975). Studies in which the fate of ^{14}C-ALA was followed after intra-ventricular injection in the rat brain have supported the existence of a "haem pool" in that organ (Percy & Shanley, 1980; De Matteis & Ray, 1982).

In the human liver at least there is some indirect evidence in favour of the present hypothesis. Most of the haem synthesized in the liver is used up as the prosthetic group for microsomal cytochromes P450. The functional capacity of the cytochrome-P450-dependent mixed-function oxidase system, as assessed by antipyrine clearance, was significantly depressed in patients with AIP (Anderson et al, 1976), HC and VP (Ostrowski et al, 1983). This depression was more marked during the acute exacerbations (Anderson et al, 1976) and in some patients in remission with higher excretion levels of ALA and PBG (Ostrowski et al, 1983). Only certain types of cytochromes-P450 seemed to be affected. Song et al (1974) have reported that AIP patients had decreased rate of metabolism for salicylamide, a salicylic acid derivative that depends on the cytochrome P450 system for its

oxidation. Unfortunately the lack of clinical details on these patients make meaningful interpretation of these results difficult.

As a consequence of intracellular haem deficiency other metabolic disturbances can be expected to arise in those pathways dependent on haem proteins. Labbe (1967) put forward the hypothesis that in the porphyrias the primary defect could be due to inhibition of the mitochondrial terminal electron-transport system, thereby impairing energy production. This proposal was based on studies performed in vitro (Cowger & Labbe, 1967) and in experimental porphyria (De Matteis & Rimington, 1962; Gajdos & Gajdos-Torok, 1969). For instance, several chemical porphyrinogens were shown to impair mitochondrial reduced nicotinamide adenine dinucleotide (NADH) oxidation (Cowger & Labbe, 1967; Gajdos & Gajdos-Torok, 1963) and to deplete hepatic ATP (De Matteis & Rimington, 1962). A wide variety of clinically important porphyrinogenic drugs such as barbiturates and anticonvulsants were also found to increase lactate production in cultured mouse fibroblast cells, presumably by increasing glycolysis (Cowger & Labbe, 1967). Bonkowsky et al (1975) have subsequently reported that mitochondrial NADH oxidation was impaired in fibroblast cultures from AIP patients as compared to controls, in keeping with the in vitro results. But no direct evidence is yet available to show that porphyrinogenic agents either inhibit NADH oxidation or ATP production in human nervous tissue. It is interesting however that some AIP patients have been noted to have an abnormal pyruvate tolerance test (Goldberg, 1959; Stein & Tschudy, 1970; Ridley, 1969; Nagler,

1972). Unfortunately no nutritional documentation of these cases was provided and other factors such as thiamine deficiency which could possibly have given an abnormal pyruvate tolerance test were not excluded (Chapter 6).

The concept that the acute porphyrias constitute a group of "haem deficiency states" (Watson et al, 1977) gained considered support following the successful therapeutic use of parenterally administered haematin in exacerbations of the disease (Bonkowsky et al, 1971; Dhar et al, 1978; Watson et al, 1977; Lamon et al, 1979; McColl et al, 1981). Haematin had been shown to repress hepatic ALA.S activity both in vitro and in experimental animals (Granick, 1966; Waxman et al, 1966; Burnham & Lescelles, 1963). Administration of haematin to porphyric patients consistently reduced the elevated concentrations of ALA and PBG in plasma and urine (Lamon et al, 1979; McColl et al, 1981; Pierach, 1982). Despite these defined effects on the haem pathway the precise mode of action of haematin in relieving the symptomatology of acute porphyria remains unclear.

The transfer of haematin across the blood brain barrier has not been adequately investigated. But of two AIP patients treated with intravenous boluses of haematin, none of it could be measured in the CSF (Lamon et al, 1979). Although this finding has been construed as evidence against the haem deficiency hypothesis in neural tissue (Bonkowsky & Schady, 1982) an alternative explanation is possible. Farrell & Correia (1980) have shown that liver cells can use exogenous haem directly to restitute any haem deficiency and there is no reason to believe that neural tissue

cannot do the same. Since the porphyrin content of brain tissue is normally very low (Percy & Shanley, 1980) only a small amount of exogenous haem may be necessary for restoration of cellular function, without being accompanied by any significant increase in CSF level. The ability of neural cells to utilize haem or porphyrins synthesised extraneurally is supported by the findings that the total porphyrin content of different brain regions does not vary substantially and does not follow the pattern of ALA.S activity (Maines, 1980).

Should the therapeutic effectiveness of haematin not depend on its uptake by the neural tissues for direct use, two further explanations must be considered. First, haematin could simply be repressing ALA.S, thereby stemming hepatic overproduction of the neurotoxic porphyrin precursors. Second, deficiency of haem in the liver could conceivably impair its ability to detoxify some unidentified neurotoxic material or depress the production of some essential factor needed by neural tissues. In that respect it is noteworthy that subtle microscopic abnormalities have recently been described in the liver of porphyric patients whose bile salt and antipyrine clearances were found to be impaired (Ostrowski et al, 1983).

SECTION II

CHAPTER 5

AUTONOMIC NEUROPATHY IN AIP

5. AUTONOMIC NEUROPATHY IN AIP

5.1 INTRODUCTION

The clinical manifestations of AIP have been attributed to a widespread neurological dysfunction caused by the block in haem biosynthesis (Gibson & Goldberg, 1956; Becker & Kramer, 1977b). The abdominal pain has been explained on the basis of splanchnic autonomic dysfunction, providing a mechanism for the intestinal dilatation and stasis occasionally noted radiologically and at laparotomy in patients suffering from an acute attack (Berlin & Cotton, 1950). Indeed many of the accompanying features of an acute attack are suggestive of autonomic neuropathy; namely, the inappropriate sinus tachycardia, labile hypertension, postural hypotension, excessive sweating, severe vomiting, constipation and occasional diarrhoea and sphincteric bladder problems. Ridley et al (1968) have reported that tachycardia invariably preceded the development of peripheral neuropathy and respiratory paralysis, and they postulated that the tachycardia of porphyria might be due to autonomic cardioneuropathy. The transient and labile hypertension which commonly accompanies the acute attack has also been given a neurogenic explanation following damage to the vagal nerves from the underlying disease (Chapter 4).

The development of simple non-invasive tests of cardiovascular reflex function has enabled objective assessment of autonomic neuropathy to be made in a more systematic way than hitherto possible (Ewing, 1978 and 1984). I have therefore studied

autonomic function in patients with AIP by using three tests that reflect cardiac parasympathetic integrity and two tests which give abnormal results with sympathetic nerve dysfunction.

5.2 PATIENTS AND METHODS

Four groups of subjects were studied, the individuals selected being less than 45 years of age. Group I consisted of 28 normal healthy subjects, age- and sex-matched with patients with AIP in groups II, III and IV. Group II comprised eight patients admitted to hospital during an acute attack of AIP prior to receiving any form of drug treatment. Six of these patients were subsequently retested while in remission and were also included in group III. In group III there were 14 asymptomatic patients with AIP in remission, their last attack having occurred at least six months prior to autonomic testing (range 6 months - 10 years). Group IV had ten latent cases of AIP identified after screening relatives of known symptomatic cases. The latent cases denied ever having had any symptoms of acute porphyria, and they belonged to different kindreds. None of the subjects tested were taking any drugs that are known to interfere with autonomic tests. They all had a normal haemoglobin, random blood sugar and blood lead levels. In all symptomatic cases the diagnosis of AIP was made on the basis of excess urinary excretion of ALA, PBG and uroporphyrins, and confirmed by the measurement of the haem enzymes ALA.S and PBG.D in the peripheral leucocytes and erythrocytes respectively. In latent cases in whom urinary excretion of porphyrin precursors and porphyrins were not necessarily elevated the diagnosis

depended on the measurements of ALA.S and PBG.D activity (McColl et al, 1982b). Urinary ALA, PBG and porphyrins; and activities of ALA.S and PBG.D were measured according to the methods described by Moore (1983) and Moore et al (1978) respectively.

The three parasympathetic tests performed consisted of heart rate responses to the Valsalva manoeuvre and to standing from a lying position, and beat-to-beat variation in heart-rate with respiration. The two tests of sympathetic function were blood pressure response to sustained isometric hand-grip and postural drop in blood pressure upon standing (Ewing, 1984).

1. Valsalva manoeuvre (Ewing, 1984): The subjects were asked to blow into a tube connected to an aneroid manometer (Cape Engineering Co Ltd., Warwick, England) with their nostrils closed and to hold their breath at a pressure of 50 mmHg for 15 seconds while a continuous electrocardiogram was recorded. The Valsalva ratio was calculated from the ratio of the longest R-R interval after the manoeuvre (reflecting the rebound bradycardia) to the shortest R-R interval during the manoeuvre (reflecting the tachycardia during strain).

2. Heart-rate response to standing (Ewing et al, 1980a): After lying down in a relaxed state for five minutes, the subject was asked to stand up gently unaided and to remain standing for a further two minutes. A continuous ECG tracing was recorded for the period starting a few beats before until about 60 beats after standing. The R-R intervals were then measured with a ruler. The heart-rate response was expressed as the ratio of the longest R-R interval around the 30th beat to the shortest R-R interval around the 15th beat after standing (the 30 : 15 ratio).

3. Heart rate variation during deep breathing (Hilsted & Jensen, 1979). The patient, resting supine, was instructed to breathe deeply at the rate of six breaths per minute (timed 5 seconds in and 5 seconds out) while an electrocardiogram was recorded. The minimum and maximum R-R intervals during each breathing cycle was measured with a ruler and converted to the equivalent number of beats per minute. The final result was taken as the mean of the difference between the maximal and minimal heart rates for three breathing cycles.

4. Sustained hand-grip test (Ewing, 1984): First the maximum voluntary contraction (MVC) for the dominant hand was determined using a handgrip dynamometer (Tephcotronics Ltd., Edinburgh). The subject was then encouraged to maintain his handgrip steadily at only 30% of that MVC for as long as possible up to a maximum of five minutes. Blood pressure was measured by means of a London School of Tropical Medicine Sphygmomanometer (Rose-Box) on the non-exercising arm with recordings made three times at rest and at one-minute intervals during applied grip. The response to the test was taken as the difference in diastolic blood pressure (phase 5) between the mean of the three resting measurements and the last reading before release of handgrip.

5. Postural drop in blood pressure (Ewing, 1984): The blood pressure of the subject was recorded after ten minutes rest while lying down and again, within 30 to 60 seconds, after standing up. The difference between the systolic blood pressure measured when lying and when standing was taken as the postural fall in blood pressure.

Statistical analysis

The significance of differences between the mean values obtained in groups I, II, III and IV was examined by Analysis of Variance. When this was found to be significant, differences between individual group means were examined, in a standard manner, by follow-up t-tests.

5.3 RESULTS

Table 3 gives the clinical details of patients in group II who were in an acute attack at the time of autonomic testing. They all had severe abdominal pain. Six of the eight patients had detectable motor weakness affecting the limbs, especially the extensor muscles of the wrists. Those with motor involvement had a higher basal heart rate (range 84-119 beats/min) than normal (range 64-78).

All the subjects managed to perform the autonomic tests satisfactorily except for four of the eight patients in group II who had difficulty in sustaining the handgrip (test 4) as described. The test was accordingly modified in that these four patients were encouraged to sustain near maximum handgrip for at least 15 seconds before their blood pressure was measured.

The mean values ($\pm$ SD) for the five tests of autonomic function are presented in Table 4. The three tests of parasympathetic cardiovascular reflex: Valsalva manoeuvre, heart-rate response to standing and heart-rate variation during deep breathing, and one of the two tests of sympathetic function, the blood pressure response to sustained hand-grip were abnormal for group II as compared to normal controls (group I). The differences during the

Patients	Sex	Age (yr)	Time since first acute attack (yr)	Clinical features of acute attack*							
				Resting heart rate beats/	Basal blood pressure (mmHg)	Abdominal pain (0 +++)	Vomiting	Constipation	Excessive sweating	Postural dizziness	Peripheral motor weakness
JC	F	27	6	72	110/65	+++	+	+	-	-	No
JM	F	27	7	84	120/80	+++	-	+	-	-	Yes
CM	F	20	3	119	140/100	+++	-	+	+	-	Yes
HW	F	26	3	92	130/90	++	+	+	-	+	Yes
EL+	F	32	5	102	160/105	+++	-	+	+	-	Yes
JB++	F	28	4	94	155/95	++	-	+	-	-	Yes
RR	M	36	4	86	145/85	+++	-	+	+	-	Yes
AW	M	27	6	78	130/80	++	-	+	-	-	No

* At the time of autonomic testing

\+ Persistent attack lasting more than 2 years

++ Not retested during remission

Table 3: Clinical details of individual porphyric patients in group II

	No of subjects	Age (yr)	Basal heart rate (beats/min)	Parasympathetic tests			Sympathetic tests	
				Valsalva ratio	Heart rate Response to standing (30:15 ratio)	Beat-to-beat variation in heart rate (R-R interval)	Sustained handgrip (diast. BP)	Postural BP drop (mmHg)
Normal control (Gp I)	28	24.7±5.8	70±4	1.72±0.45	1.27±0.13	28.8±7.4	37±12	5±8
AIP in acute attacks (Gp II)	8	27.8±4.6	91±15**	1.38±0.23*	1.04±0.20**	16.5±4.1	16±8**	8±6
AIP in remission (Gp III)	14	27.1±6.4	72±5	1.69±0.39	1.23±0.17	21.6±5.8*	31±12	3±8
Latent AIP (Gp IV)	10	21.2±2.4	73±4	1.36±0.18*	1.22±0.09	27.0±7.2	34±14	4±7

Values = mean ± S.D.

* $p < 0.05$
** $p < 0.001$

Table 4: Results of autonomic function tests in patients with acute intermittent porphyria (AIP)

acute attacks were most marked in the heart-rate response to standing (30 : 15 ratio) and the blood pressure response to sustained handgrip (Figs 11 and 12). It is interesting to note that all these abnormal tests improved on subsequent re-testing during remission.

The Valsalva manoeuvre and heart-rate variation during deep breathing were also significantly reduced for the latent porphyrics (group IV) and those in remission (group III) respectively.

5.4 DISCUSSION

The battery of cardiovascular reflex tests used in our study has already proven to be useful in the overall assessment of the autonomic system in diabetic patients towards a better understanding of the natural history and prognosis of that disease (Ewing et al, 1980b, 1980c). These tests have now provided objective evidence for the common occurrence of both parasympathetic and sympathetic dysfunction during the severe acute attacks of AIP as manifested clinically.

In contrast to diabetic neuropathy in which neurological damage tends to persist once detectable (Ewing et al, 1980b; Watkins & Edmonds, 1984) the autonomic disturbance in the acute attack of porphyria appears to be mostly reversible. Interestingly one of the patients in group II experienced a prolonged attack lasting several months and her autonomic tests were repeatedly found to be grossly abnormal. Undoubtedly persistent peripheral nerve damage can occur in porphyric neuropathy, even after a single severe

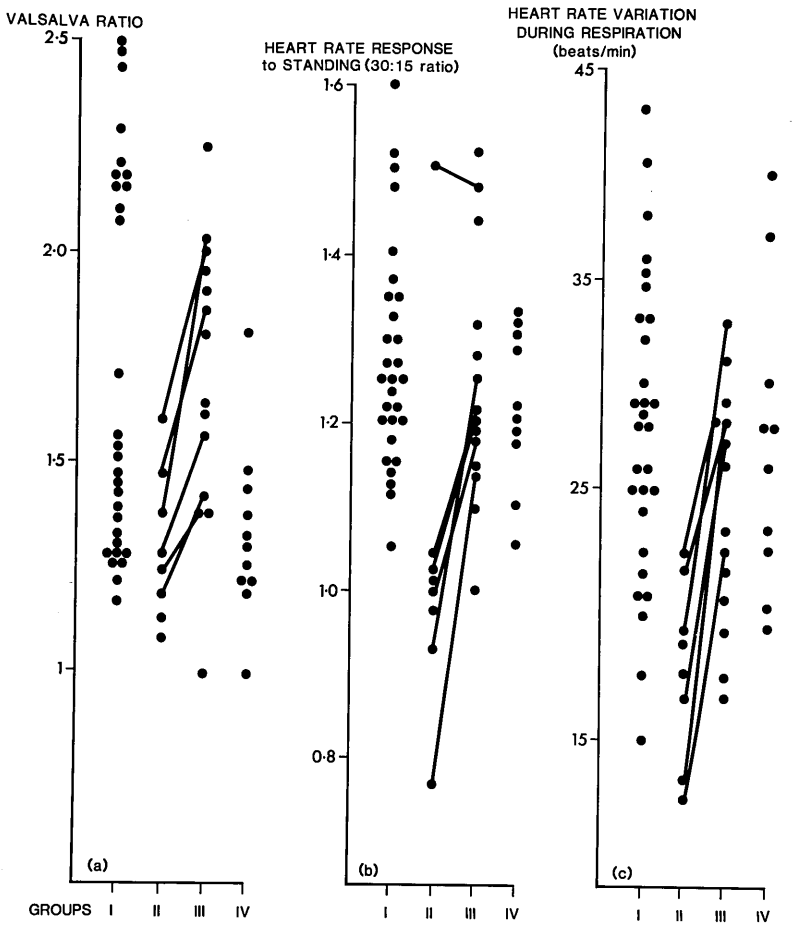


Figure 11: Results of (a) Valsalva manoeuvre; (b) 30 : 15 ratio and (c) heart rate variation during respiration in different groups:
(I) young normal subjects; (II) AIP in acute attack; (III) AIP in remission; and (IV) latent AIP. Each line represents individual patients tested during an acute attack and during remission.

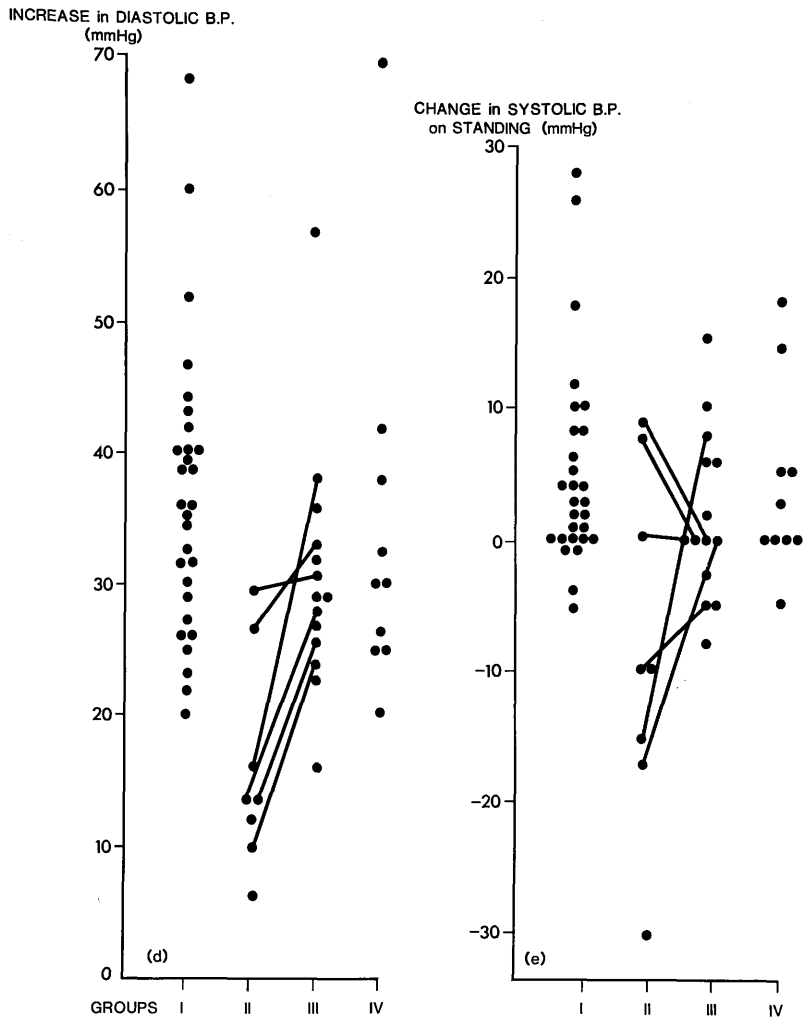


Figure 12: Results of blood pressure response to (d) sustained muscular exercise and (e) posture in groups I to IV.

acute attack (Ridley, 1969; Sorensen & With, 1972). But the reversible nature of the abnormal tests supports the possibility of a transient functional autonomic blockade either resulting from impaired haem biosynthesis within the nerve cells or an accumulation of porphyrin precursors or their derivatives in the body. The actual operative mechanism however remains speculative (Chapter 4).

The Valsalva manoeuvre has previously been reported to be a good indicator of parasympathetic dysfunction in diabetic patients, and in this present study it was found to be abnormal in latent AIP. This observation is in keeping with the subclinical sensorimotor neuropathy often present in these latent AIP cases as detected by nerve conduction studies (Mustajoki & Seppalainen, 1975). Yet the Valsalva manoeuvre remained normal in the AIP patients in remission whereas heart-rate variation during deep breathing was significantly reduced. The discrepancy between these two tests of parasympathetic function could perhaps be accounted for by the relatively small number of patients studied since heart-rate variation during deep breathing is reputedly another sensitive index of parasympathetic nerve damage in diabetic patients, often preceding the manifestation of autonomic symptoms for years (Mackay et al, 1980). Recently Gupta et al (1983) reported that the 30 : 15 ratio was also abnormal in asymptomatic Indian subjects with AIP, but they did not measure other parameters of autonomic function. Nevertheless with cardiac autonomic involvement these studies have shown that in AIP, as in diabetes, the parasympathetic tests become abnormal earlier and more

frequently so than the sympathetic tests (Bennett et al, 1978; Ewing et al, 1980c).

Although the results of the autonomic tests in the groups of porphyric subjects were statistically different from the control group, few individual values were in the unequivocally abnormal range of values published by Ewing et al (Ewing, 1978 & 1984) on the basis of their work in diabetic patients. The reason for this is not clear. A higher basal heart rate, as noted in AIP patients in an acute attack, is presumably not the predominant causative factor since the beat-to-beat variation in heart rate with respiration has at least been shown to be independent of basal heart rate (Ewing et al, 1981). Care was taken in excluding older subjects from the study since increasing age affects sinus arrhythmia (Hellman & Stacy, 1976) postural blood pressure (Caird et al, 1973) and heart-rate response to the Valsalva manoeuvre (Kalbfleisch et al, 1978). The complete absence of symptoms in the latent cases with early parasympathetic damage, and the lack of other autonomic clinical manifestations in a number of AIP patients with severe abdominal pain suggest that the abdominal pain is not entirely due to autonomic dysfunction. Until splanchnic autonomic function can be measured by more direct and sensitive methods in porphyric patients its relationship to abdominal pain will remain conjectural.

It is conceivable that the pain might be causally related to local vasoconstrictor (Denny-Brown & Sciarra, 1945) or spasmodic effects (Cutler et al,1980) of porphyrin precursors (or derivatives) on the gut. The experience of pain by some patients with autonomic

SECTION II

CHAPTER 6

A STUDY OF CYTOCHROME OXIDASE ACTIVITY IN MUSCLE BIOPSIES FROM PATIENTS WITH AIP AND OTHER DISEASES

6. A STUDY OF CYTOCHROME OXIDASE ACTIVITY IN MUSCLE BIOPSIES FROM PATIENTS WITH AIP AND OTHER DISEASES

6.1 INTRODUCTION

The hypothesis that the clinical manifestations of AIP are due to intraneuronal haem deficiency has been difficult to substantiate because of the present limitations in measuring the "free haem" pool. During an acute attack of porphyria, in addition to abdominal pain, the patient often complains of limb pain, hitherto attributed to the neuropathy. Accompanying muscle tenderness can be quite troublesome (Goldberg, 1959; Ridley, 1969) but there is no elevation of serum muscle enzymes (Stein & Tschudy, 1970, Personal observations). The possibility of a myopathic component contributing to the muscle weakness in acute porphyria is supported by the observation that peripheral reflexes are often preserved in the presence of severe limb paralysis (Ridley, 1969). Labbe's group (Labbe, 1967; Cowger & Labbe, 1967) have reported that a wide variety of porphyrinogenic drugs could inhibit mitochondrial terminal oxidation leading to enhanced glycolysis in vitro. The increased glycolysis, as reflected in lactate concentration, was attributed to the Pasteur effect - accelerated glycolysis resulting from impairment of terminal oxidation. Bonkowsky et al (1973) subsequently showed that fibroblast cultures from AIP patients had a defect in mitochondrial oxidation. Indeed energy production in muscles greatly depends on

phosphorylative oxidation which uses many haem-containing cytochromes. This present study was undertaken to determine if cytochrome oxidase activity was impaired in AIP.

6.2 PATIENTS

The following patients were studied:

1. Fourteen AIP patients in an acute attack requiring hospital admission and one latent case of AIP. Their clinical and biochemical findings are summarised in Tables 5, 6 and 7.
2. Two patients with peripheral neuropathy caused by proven chronic lead poisoning.
3. Seven patients with denervation states due to lateral popliteal nerve damage (1), chronic alcohol abuse (2) and diabetes.
4. Two with autoimmune polymyositis.
5. Twelve with post-viral fatigue syndrome, all being sero-positive for Coxsackie B antibody (Behan & Behan, 1980).
6. Ten "normal" controls undergoing hip surgery for degenerative joint disease and rheumatoid arthritis.

6.3 METHODS

Five of the AIP patients underwent an open muscle biopsy under local anaesthesia, and tissue was obtained from the "normal" controls at the time they had their operation. The remaining patients had a needle biopsy performed under local anaesthesia with lignocaine (Edwards et al, 1980). The muscle tissue was immediately snap-frozen in liquid nitrogen before being processed

AIP Patients	Sex	Age (yr)	Age of onset of first attack	No attacks during previous year	Known precipitating factors
JB	F	29	23	3	Menses, ? alcohol
JMcM	F	30	22	> 4	Menses
RR	M	36	30	> 4	Infections
MP	F	50	35	1	Sulphonamide drug
AW	M	26	22	2	? Alcohol
ZP	F	23	22	1 (prolonged)	--
MMc	F	31	30	3	Menses
BB	F	30	28	3	Menses
MF	F	32	29	3	Menses
JC	F	29	20	2	Menses/alcohol
CM	F	27	21	1	Pregnancy
LE	F	28	27	1	--
SW	F	26	23	1	--
EL	F	32	24	1 (prolonged)	--
JMcC*	M	56	--	-	--

* Denotes the latent case, father of EL

Table 5: Clinical features of AIP patients studied

AIP Patients	Abdominal pain	NEUROPATHY					Psychiatric	Others
		Motor		Sensory		Autonomic		
		Clinical	Electrophys.* abnormality	Clinical	Electrophys. abnormality	Clinical		
JB	+++	+++	+	+	+	+++	Depression	Seizures
JMcM	+++	+	++	+	-	-	Hallucinations in depression	Seizures
RR	+++	++	+	+++	+	+	Depression	-
MP	++	+++	ND	-	ND	++	-	-
AW	++	+	-	-	-	-	-	-
ZP	+++	+++	+	-	-	++	-	-
MMc	+++	+	-	-	-	-	-	-
BB	+++	-	ND	-	ND	-	-	Inappropriate ADH
MF	++	-	ND	-	ND	+	-	Seizure
JC	+++	+	ND	+	ND	+	Depression hallucinations	Unilateral ptosis
CM	+++	++	-	-	-	++	Depression	-
LE	++	-	ND	-	ND	-	-	-
SW	+++	-	ND	-	ND	-	-	-
EL	+++	+++	+	+	-	+++	-	-

* Electrophysiological findings compatible with axonal neuropathy

ND = not done

Table 6: Features of acute attacks for the AIP patients studied

	Enzyme activities		24 h-urinary excretion			
AIP Patients	Leucocyte ALA-synthase*	Erythrocyte PBG-deaminase**	Total porphyrins ug	ALA (umol)	PBG (umol)	Uroporphyrins (nmol)
JB	3630	13.5		170	342	697
JMcM	784	16.9		299	213	419
RR	466	16.1		517	759	461
MP	378	18.2		230	428	
AW	1160	14.2		303	746	471
ZP	809	17.9	1086	278	612	
MMc	1322	13.1	1446	516	1446	
BB	636	14.2	606	180	321	
MF	884	15.4		122	430	
JC	368	18.3		51	188	225
CM	448	17.7		268	288	
LE	208	19.4		78	96	
SW	410	16.6		132	112	
EL	3259	21		372	376	308
JMcC (latent)	43	19.4		10	12	
Normal range	20-279	24-54		0-40	0-16	0-49

* nmol ALA/g protein/h

** nmol URO/LRBC/h

Table 7: Haem enzyme activities and urinary excretion of porphyrins and porphyrin precursors during acute attacks

at the Regional Neuropathology Centre, Institute of Neurological Sciences, Glasgow. Tissue sections were stained histochemically for cytochrome oxidase, succinate dehydrogenase, muscle phosphorylase, myosin ATPase and monoadenylate deaminase. Cytochrome oxidase activity was assessed by using the modified Burstone and G-NADI methods.

6.3.1 Histochemical methods

Cytochrome oxidase

(1) Modified Burstone method (Burstone, 1960)

The tissue sections were incubated for periods varying from 10 to 40 minutes at 37°C in Tris-buffered solution containing p-aminodiphenylamine (base) and 8 amino- 1, 2, 3, 4 tetraquinoline at pH 7.4. After the incubation period they were transferred into 10% cobaltous acetate in 10% formalin (PH 5.2) for one hour. The slides were then washed under running water and mounted in glycerin jelly.

(2) Graff's NADI method (Pearse, 1972)

The section were incubated for periods varying from 10 to 40 minutes at 37°C in NADI reagent solution (PH 7.5) containing alpha-naphtol and the oxidase reagent, dimethyl-p-phenylene diamine hydrochloride. After incubation the sections were washed with normal saline and mounted in 20% potassium acetate. The coverslips were ringed with paraffin wax and examined within 24 hours since these preparations are not permanent.

Both cytochrome oxidase methods were used with and without cytochrome C in the incubation medium as an enhancing agent.

Succinate dehydrogenase, a mitochondrial enzyme catalysing the conversion of succinate to fumarate in the Kreb's cycle, was stained histochemically by the method of Pearse (1972).

Muscle phosphorylase, a regulatory enzyme for the conversion of glycogen into glucose residues, was assessed according to the method of Meijer (1968).

Monoadenylate deaminase, a cytosolic enzyme whose deficiency is a recognised cause of myopathy, was stained histochemically by the method of Fishbein et al (1980).

Myosin ATPase staining (Padykulla & Herman, 1955) permitted the differentiation of muscle fibre types. At pH 9.4, the type II fibres stained dark and the type I fibres were light-reacting (Swash & Schwartz, 1984).

6.3.2 Methods of histochemical assessment

1. Cytochrome oxidase activity

The sections were examined after the shortest time interval at which the reaction product became detectable histochemically i.e. 10 minutes, and then at 10 minute intervals till 40 minutes. The sets of sections were coded and always examined with control sections previously shown to stain strongly for cytochrome oxidase. They were examined by an experienced neuro-histopathologist (Dr D Doyle) under light microscopy and scored subjectively for the presence or absence of the reaction products (Fig 13). The following scoring system was adopted:

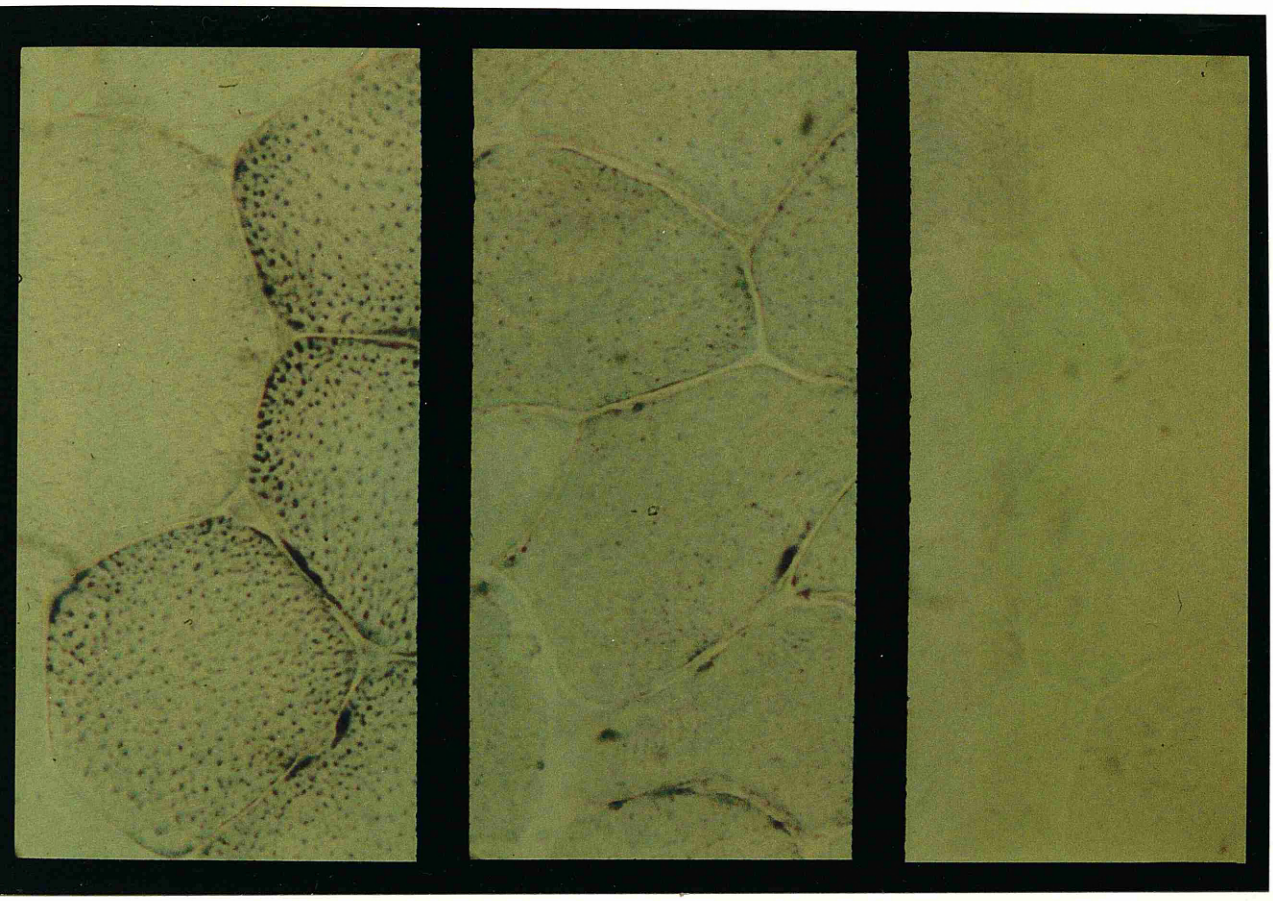


Figure 13: Histochemical staining of muscle sections by the Burstone method. On the left is a strongly positive reaction (graded 5) from a normal control; the middle picture shows a medium reaction graded 3 ; and on the right is a negative reaction (graded (0) from an active AIP patient. (Courtesy of Dr D Doyle)

Numerical Scale	Activity
0	No demonstrable activity evident
1	Only few muscle fibres showed slight activity
2	All fibres showed activity but not sufficient to allow type fibre differentiation
3	Type fibre differentiation was present but weak
4	Type fibre differentiation was moderate
5	Type fibre differentiation was strong.

After the first reading the activities were checked again before final scores were given. This method of assessment was validated after obtaining similar results with the same sections stained and reassessed at a later date.

2. The activities of the other enzymes studied were graded either normal or reduced as compared to the control muscle sections.

Pyruvate Tolerance Test (PTT) was performed in six of the active AIP patients, each matched for age and sex with two normal healthy controls. The patients were given at least 2500 Kcals daily for a minimum period of one week before the test was carried out. After an overnight 12 h fast a baseline venous blood sample was taken and the subject was given 50 gram of glucose dissolved in 300 ml of water. This was followed by a further 50 gram of glucose 30 minutes later. Blood samples were removed at 60 and 90 minutes after the first glucose load. Blood for pyruvate and lactate levels was immediately transferred into ice-cold 10% trichloroacetic acid solution. Pyruvate and lactate were assayed by the methods of Hadjivassiliou & Reider (1968).

6.4 RESULTS

All 14 patients with active AIP showed some depression of cytochrome oxidase activity by both histochemical tests whereas the latent case and the normal controls had good enzymatic activity (Tables 8 and 9). Fewer cases were stained by the Burstone method because of its later development as a routine staining. There was good correlation between the results of the two cytochrome oxidase methods. The 12 AIP patients in whom the activity was more markedly depressed showed virtually no reaction product at the lowest incubation time at 10 minutes. But by increasing the incubation time the activity became perceptible in six and remained undetectable in the others. With the addition of cytochrome C as an enhancing agent, there was a detectable product at 40 minutes in all cases, although the reaction was still weaker. One patient (EL) had two muscle biopsies: the first being an open biopsy and the second a needle biopsy performed 18 months later. On both occasions she had similar staining results. The latent AIP gave a detectable reaction at 10 minutes proceeding to a strong staining of well-defined fibre types at 40 minutes, even without addition of cytochrome C.

The two patients with lead poisoning also showed severe depression of cytochrome oxidase activity. In the non-porphyric groups cytochrome oxidase activity was more variable but generally much stronger than in the AIP patients. Six of the seven patients with peripheral denervation states expressed reduced cytochrome oxidase activity but most developed a reaction short of type differentiation after 40 minutes. Cytochrome oxidase was also

	Total Number	Grading Scale					
		0	1	2	3	4	5
Acute intermittent porphyria	14	6	3	2	1	-	2*
Chronic lead intoxication	2	1	1	-	-	-	-
Peripheral nerve damage	2	-	-	2	-	-	-
Polymyositis	2	-	-	1	-	1	-
Post-viral fatigue syndrome	12	-	-	4	-	4	4
Normal controls	10	-	-	-	2	2	6

* includes the latent case of AIP

Tissue sections were read at 40 minute incubation

Table 8: Results of cytochrome oxidase staining by Burstone method

	Total Number	Grading Scale					
		0	1	2	3	4	5
Acute intermittent porphyria	15	6**	5	2	1	-	1*
Chronic lead intoxication	2	1	1	-	-	-	-
Peeripheral nerve damage	7	-	3	3	-	1	-
Polymyositis	2	-	1	1	-	-	-
Post-viral fatigue syndrome	10	-	2	3	-	4	1
Normal controls	10	-	-	1	3	3	3

* the latent case of AIP

** includes one active AIP biopsied twice

Tissue sections were read at 40 minute incubation

Table 9: Results of cytochrome oxidase staining by NADI method

impaired in the two cases of polymyositis, particularly in the damaged fibre staining at 40 minutes but addition of cytochrome C led to clear distinction of fibre types.

Of the five AIP patients who had an open muscle biopsy, histological changes of denervation were present in three and two of these showed marked features of reinnervation as well. Muscle histology in the remaining two patients was entirely normal although cytochrome oxidase activity was reduced in both. No obvious difference was discernible between the biopsies of those with and those without denervation/reinnervation.

Succinate dehydrogenase and muscle phosphorylase activities were reduced in seven of ten active AIP cases studied, and normal in the latent case. Monoadenylate deaminase staining appeared normal. Myosin ATPase showed a mild predominance of type II fibres in several of the AIP cases, but depression of cytochrome oxidase activity involved both types of muscle fibres.

The pyruvate tolerance test results are shown in Fig 14. Blood lactate levels measured simultaneously during these tests, together with the fasting blood glucose and erythrocyte transketolase activities are given in Table 10. The normal erythrocyte transketolase activities excluded thiamine deficiency as a possible cause of the abnormal pyruvate tolerance test observed in four of the six AIP patients studied. The fasting blood lactate concentrations were normal in the porphyric patients and did not rise significantly after glucose loading.

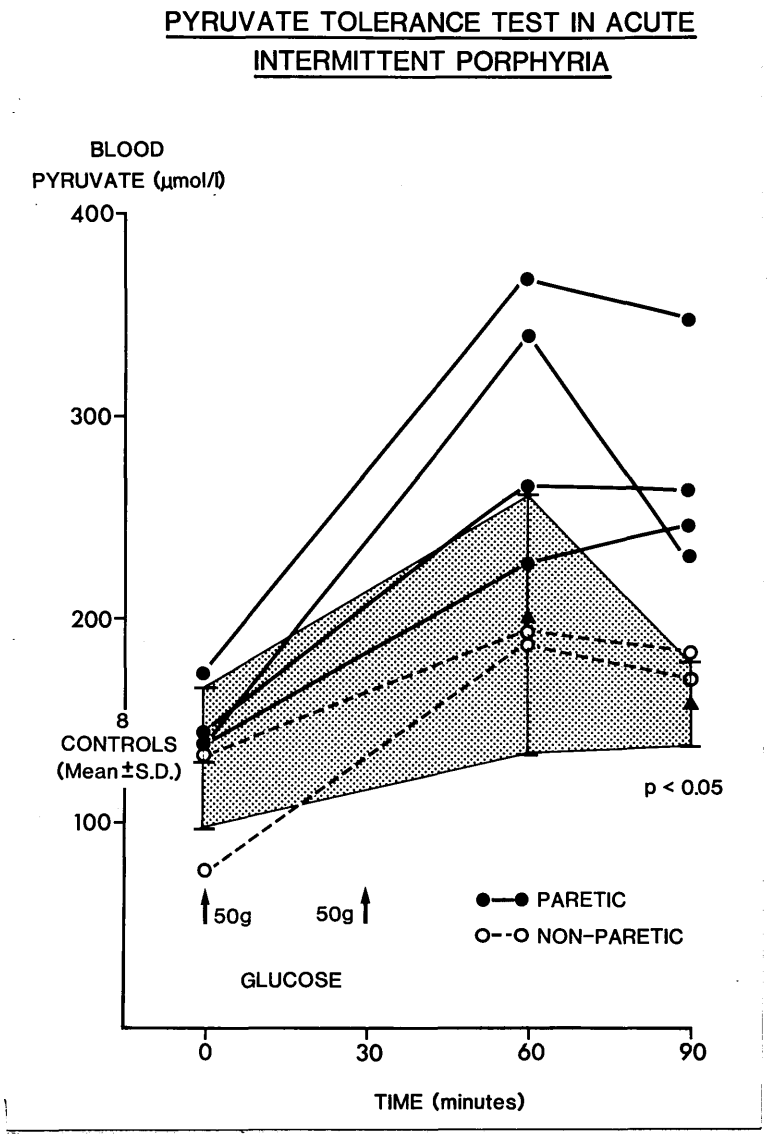


Figure 14: Shaded area represents mean ± S.D. for 12 controls age- and sex-matched with the AIP patients.

AIP	Erythrocyte transketolase activity (% activation)	Fasting glucose (mM/L)	Blood lactate (mM/L)		
			0 min	60 min	90 min
CM	4	4.6	3.9	2.1	2.5
JB	6	5.2	1.4	2.8	2.7
EL	10	6.6	1.8	2.4	2.6
SW	19	5.3	-	-	-
JC	13	4.2	0.5	1.8	1.2
MMc	12	6.0	1.0	2.0	1.9
Mean S.D.	10.6 ± 5.3	5.3 ± 0.9	1.7 ± 1.3	2.2 ± 0.4	2.2 ± 0.6
Normal values*	< 25%	4.7 ± 0.7	1.2 ± 0.5	2.0 ± 1.0	1.6 ± 0.6
Significance value (Wilcoxon's test)		p < 0.5 NS	p < 0.5 NS	p < 0.36 NS	p < 0.09 NS

* refers to n = 10; each AIP patients was age- and sex- matched with two controls

Table 10: Results of erythrocyte transketolase activity, fasting blood glucose and blood lactate levels during pyruvate tolerance tests

6.5 DISCUSSION

Cytochrome oxidase is a complex functional unit which forms part of the inner mitochondrial membrane (Fuller et al, 1979((Fig 15). It consists of two types of haem proteins, cytochromes a and a_3, and it requires copper ions for its activity (Malmstrom, 1979; Wainio, 1983). Its fundamental role is to transfer electrons along the terminal oxidative phosphorylation pathway from cytochrome C_1 to molecular oxygen (Azzi, 1980) (Fig 16). Cytochrome oxidase thus catalyses the final step in a series of redox reactions involved in cellular respiration for ATP synthesis.

Using two different staining techniques cytochrome oxidase activity was found to be markedly depressed in muscle from patients with active AIP and chronic lead poisoning. Interestingly, both tests were normal for the latent case of AIP, who was the father of one of the AIP patients (EL) with severe enzymatic depression. The reduction in cytochrome oxidase activity involved muscle fibres of both types I and II (a and b), although, as is characteristic of neuropathies in general (Swash & Schwartz, 1984), there was a mild predominance of type II fibres in the presence of denervation changes.

Six of the 14 AIP patients studied had evidence of muscle atrophy, partly contributed by disuse and partly by the underlying disease. The remaining eight AIP patients had normal muscle bulk and were fully mobile. Several workers have shown both experimentally (Rifenberick et al, 1973; Nemeth et al, 1980) and in man (Astrom et al, 1976) that although muscle disuse caused

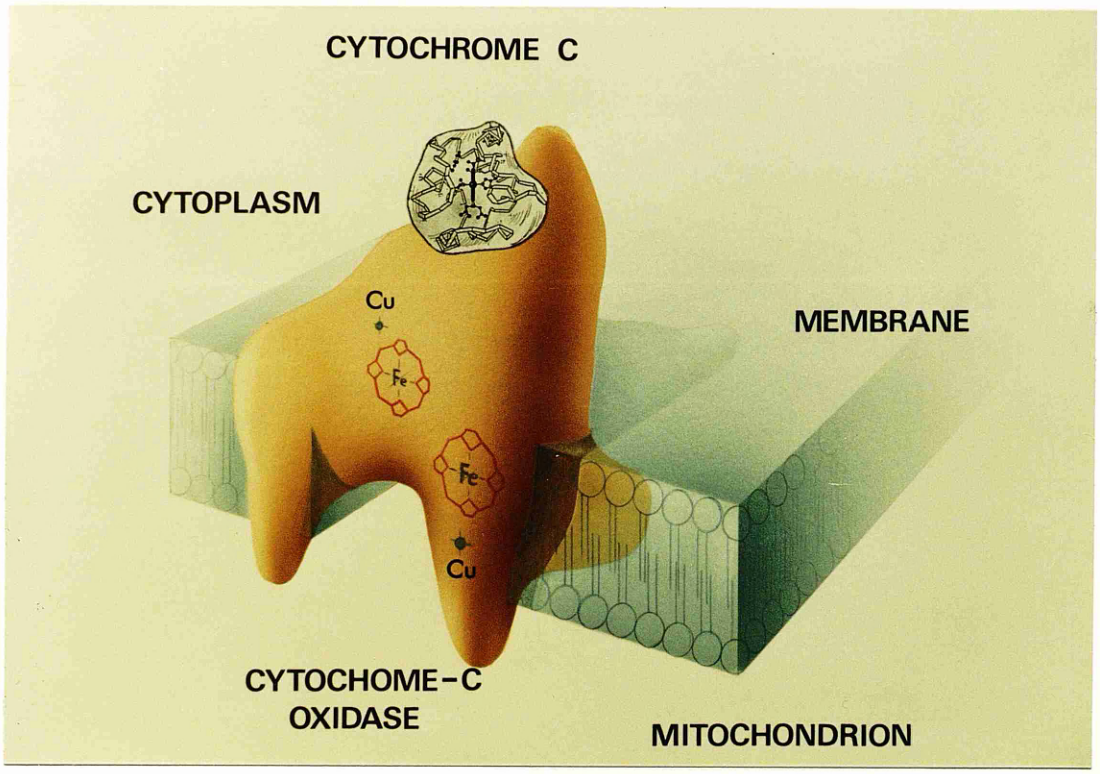


Figure 15: Diagrammatic representation of the cytochrome oxidase molecule within the mitochondrial membrane. Each molecule requires two iron and two copper ions for activity.

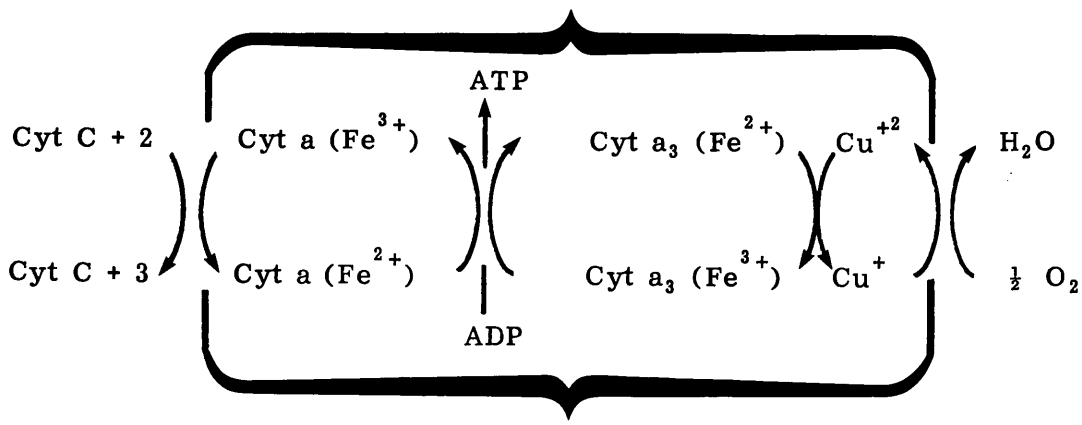


Figure 16: The last stage of the electron transport chain where cytochrome oxidase (i.e. cytochrome aa_3) transfers electrons from cytochrome C to molecular oxygen with the generation of **ATP**

atrophy it did not affect the activities of several mitochondrial enzymes including cytochrome oxidase. On the other hand, muscle denervation in animals led to a marked decline in metabolic rates and enzyme activities, suggesting possible neural regulation of mitochondrial enzymes in type 1 muscle (Nemeth et al, 1980). This explanation might account for the mild depression of cytochrome oxidase activity seen in patients with the other types of denervation states.

It is important to note that cytochrome oxidase activity was depressed in AIP patients even in the absence of clinically evident peripheral neuropathy or denervation changes histologically although it was more markedly so in the presence of these findings. Astrom et al (1976) have reported that muscle mitochondrial enzyme activities also became depressed during viral and mycoplasma infections and slowly rose towards normal in the convalescent period. This might explain the reduced cytochrome oxidase activity observed in patients with the post-viral fatigue syndrome. At present little is known about the genesis of the post-viral fatigue syndrome other than its strong association with Coxsackie B infections and a lack of any consistent physical, biochemical or histological findings (Behan & Behan, 1980). Certain immunological abnormalities have recently been found (Behan, 1983) but, interestingly, Arnold et al (1984) have also discovered a defect in the energy-producing mechanism of such a patient by ^{31}P-nuclear magnetic resonance spectroscopy.

In view of the subjective element inherent in the histochemical methods used the excellent correlation between the results of the

Burstone and G-NADI techniques, performed over a study period of five years, strongly supports our conclusions about the cytochrome oxidase activity. There is indeed some evidence to suggest that cytochrome P450 deficiency exists in the liver of patients with the acute hepatic porphyrias. Anderson et al (1976) have shown that hepatic oxidative drug metabolism, as measured by antipyrine half-lives, was significantly depressed during the active phase of AIP. Ostrowski et al (1983) who have reported that antipyrine clearance was impaired even during the remission phase. Indeed, antipyrine clearance has been found to be a good measure of the activity of the cytochrome-dependent microsomal mixed-function oxygenase system (Hepner & Vessell, 1975).

Gross impairment of cytochrome oxidase would be expected to cause an accumulation of pyruvate and lactate (Fig 17), as has been reported in cases with congenital deficiency of this enzyme (Di Mauro et al, 1980; Minchom et al, 1983; Sengers et al, 1984). The abnormal pyruvate tolerance test found in four of the six AIP patients studied can be explained in the same manner. These four patients had more severe attacks with limb weakness whereas the two patients with normal pyruvate tolerance test only complained of abdominal pain. Blood lactate levels, however, were not significantly raised in any of the five AIP patients, three of whom also had an abnormal pyruvate tolerance test. In McArdle's disease where there is a congenital deficiency of muscle phosphorylase blood lactate fails to increase after exercise (Howell, 1978). Exercising the patients might well have caused an abnormal elevation of blood lactate but it was not possible to exercise these patients because of their clinical condition.

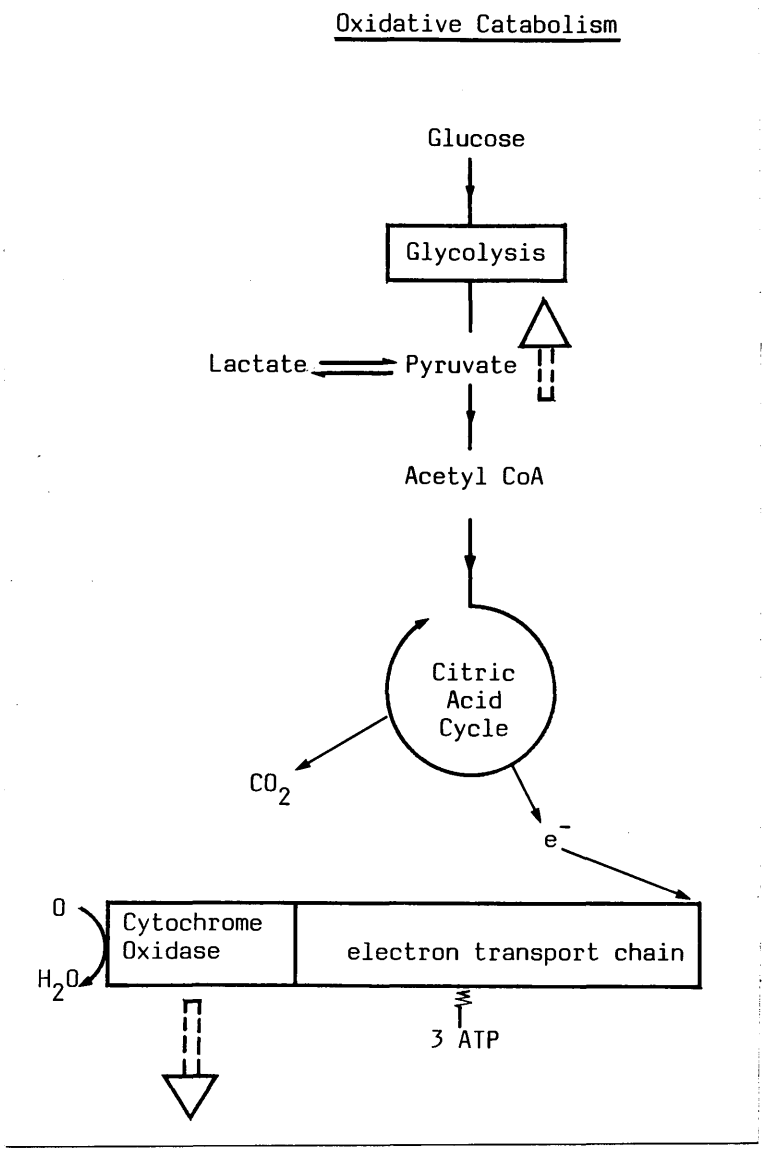


Figure 17: Schematic representation of the effects of cytochrome oxidase deficiency on oxidative catabolism.

The presence of cytochrome oxidase has been demonstrated in both nerve cells and neuroglia of the cerebral cortex (Al Ali & Robinson, 1979). A deficiency of cytochrome oxidase activity in AIP would lend support to the concept of a functional neuronal block operating in the genesis of the clinical manifestations. This would account for the absence of histopathological changes in the fatal case of AIP whose illness, lasting for several years, was complicated by marked quadriparesis and ultimately led to respiratory failure (Chapter 7). Such observation has previously been made by other workers (Mason et al, 1933; Hierons, 1957). It is conceivable that a more severe or prolonged depression of cytochrome oxidase activity could cause the nerve cells to degenerate. Since mitochondria are located within the perikaryon of the nerve cells the distal part of the axons would be the most vulnerable, irrespective of their length. This could explain Cavanagh's observation (1967) that the dying-back type of neuropathy in acute porphyria is "not typical of that seen with organo-phosphorus poisoning" where cells with the longest fibres are affected first, but where fibres of neurones with the largest metabolic requirements are also involved.

Furthermore the brain is metabolically one of the most active organs in the body with relatively enormous rate of oxygen consumption. For a 70 kg man, it is estimated that the brain requires about 40 ml oxygen/min as compared to 250ml oxygen/min for his whole body (Smith & Sokoloff, 1981). The oxygen is used for glucose metabolism upon which the brain almost entirely depends. Not only does it utilize oxygen at a rapid rate but it

is dependent on uninterrupted oxidative metabolism for maintaining its structural integrity and functional activities (Klatzo & Spatz, 1981). This high sensitivity of neuronal cells to oxygen deprivation could account for the nervous system to be the primary organ affected in acute porphyrias.

The potent inhibitory effect of lead on the haem enzymes, especially ALA.D (Moore et al, 1980a) could adequately explain the severe depression of muscle cytochrome oxidase activity in the two patients with chronic lead intoxication.

If cytochrome oxidase deficiency could account for the pathogenesis of the acute types of porphyria the absence of neurological complications in the non-acute types such as porphyria cutanea tarda or erythropoietic porphyria would require further explanation. As previously discussed (Chapter 1) evidence is accumulating for the existence of different control mechanisms operating on the haem pathway in different tissues of the body (Kappas et al, 1983; Maines, 1984). Moreover congenital cytochrome oxidase deficiency is not uniformly present in all tissues. For instance Stansbie et al (1982) described a patient with selective cytochrome oxidase deficiency in skeletal muscle whereas the cases reported by Di Mauro et al (1980) and Van Biervliet et al (1977) also had kidney involvement. In two further cases, the biochemical abnormality was reversible in one (Di Mauro et al, 1983) and was probably a coincidental finding in the other (Rimoldi et al, 1982). Patients with oculo-cranial-somatic syndrome (Versmold et al, 1977; Mitsumoto et al, 1981) are also lacking in cytochrome oxidase but the deficiency in the

muscles is only partial, affecting a proportion of muscle fibres of both types I and II (Johnson et al, 1983). Muscle cytochrome oxidase deficiency has also been reported in association with Leigh's encephalomyelopathy (Willems et al, 1977) and progressive poliodystrophy (Alper's disease) (Prick et al, 1983).

These various phenotypic expressions could conceivably result from mutations involving different cytochrome oxidase isoenzymes in different tissues. The specific block in the haem biosynthetic pathway imposed by the type of porphyria could provide a further mechanism by which the synthesis or functional activity of particular cytochrome oxidase isoenzymes could be regulated. Indeed, Arrese et al (1982) have shown that an abnormal uroporphyrinogen decarboxylase isoenzyme was specifically involved in haem "a" production in a yeast mutant resulting in marked cytochrome oxidase deficiency.

Other specific defects of the respiratory chain affecting cytochromes b and c_1 or NADH.CoQ reductase complex (Morgan-Hughes et al, 1977; Morgan-Hughes, 1983) have been described. All these patients suffered from mild weakness, severe exercise intolerance and a fluctuating acidosis which was either induced or made worse by exercise. None, however, had central nervous manifestations.

Recently it has become possible to observe non-invasively the energy metabolism of human muscle in vivo by using phosphorus nuclear magnetic resonance measurements (Ross et al, 1981; Gadian & Radda, 1981). Application of this technique in acute porphyria to measure intracellular pH and phosphorylated high energy compounds such as ATP would be most helpful in confirming the

existence of a myopathic component due to cytochrome oxidase deficiency. But more precise quantitative measurements of the various types of cytochromes in neural tissue will be required to ascertain their role in porphyric neuropathy.

On the basis of the results presented, it is suggested that a deficiency of cytochrome oxidase, affecting the nerve cells in particular, could link the biochemical, clinical and neuropathological findings in AIP.

SECTION II

CHAPTER 7

CLINICO-PATHOLOGICAL DISCUSSION ON A FATAL CASE OF AIP

7. CLINICO-PATHOLOGICAL DISCUSSION ON A FATAL CASE OF AIP

7.1 INTRODUCTION

As stated in the review (Chapter 4) some controversy still exists as to what part axonal degeneration and segmental demyelination play in the pathogenesis of AIP. The correct assessment and interpretation of the neuropathological changes is indeed an important prerequisite for understanding the mechanism of the neuropathy. Although pharmacological and degenerative processes must be considered separately a unitary explanation must be sought in the final solution. In this chapter, the detailed neuropathological studies undertaken in a well-documented case of AIP who died of the disease are presented.

7.2 CASE REPORT

This 31-year old woman was diagnosed as having AIP in 1971 at the age of 22. Until May 1978 she had already been admitted to hospital on 12 occasions because of severe abdominal pain, constipation and vomiting. Three of these attacks were complicated by mild quadriparesis from which she subsequently recovered. Her attacks were inconstantly related to menstruation and no other precipitating factors could be identified. On most previous admissions she had received parenteral administration of 20% laevulose intravenously in addition to symptomatic treatment with pethidine and chlorpromazine.

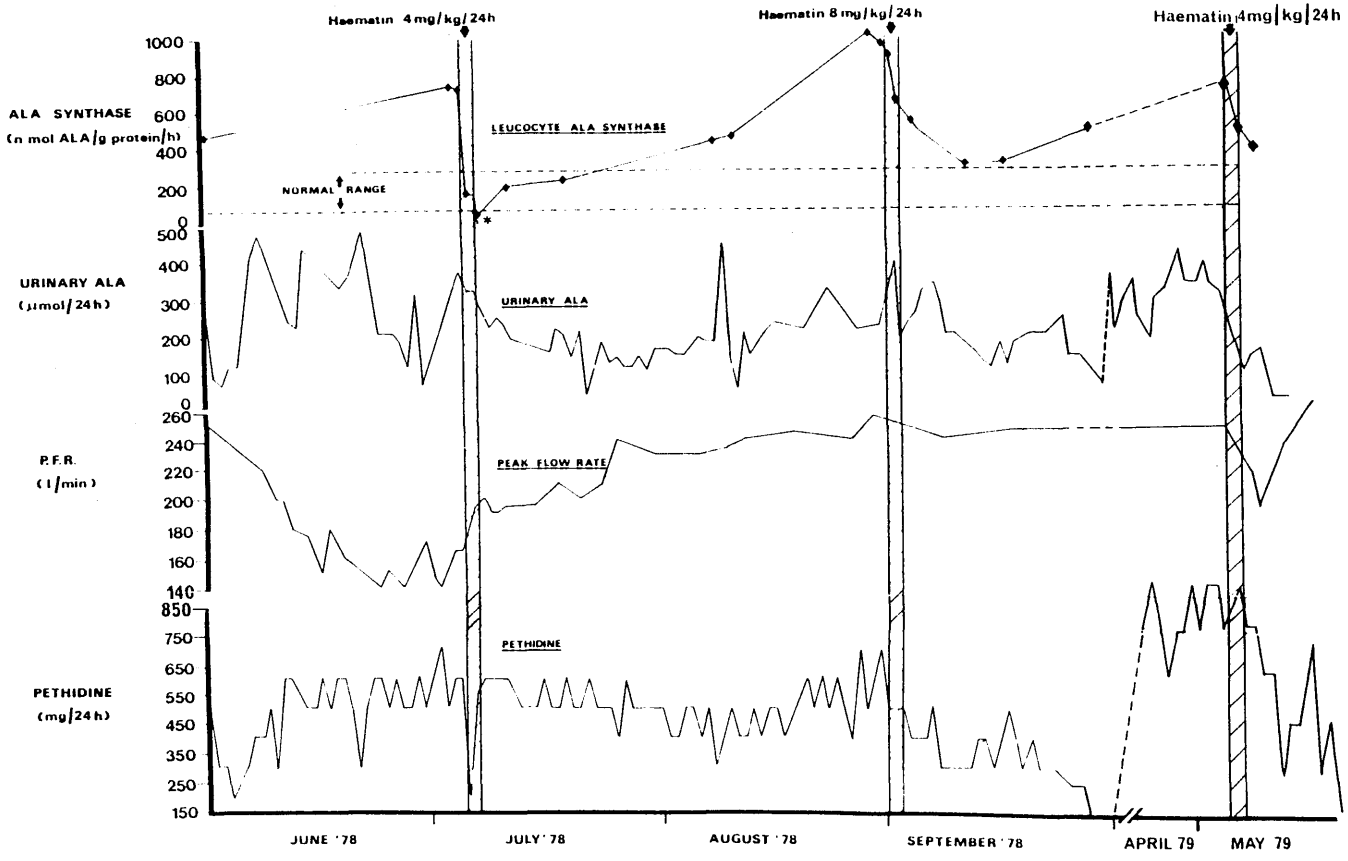


Figure 18: Effect of three courses of haematin on leucocyte ALA synthase activity, urinary ALA excretion, respiratory peak flow rate and analgesic requirements in 31-year-old woman with prolonged attack of acute intermittent porphyria.

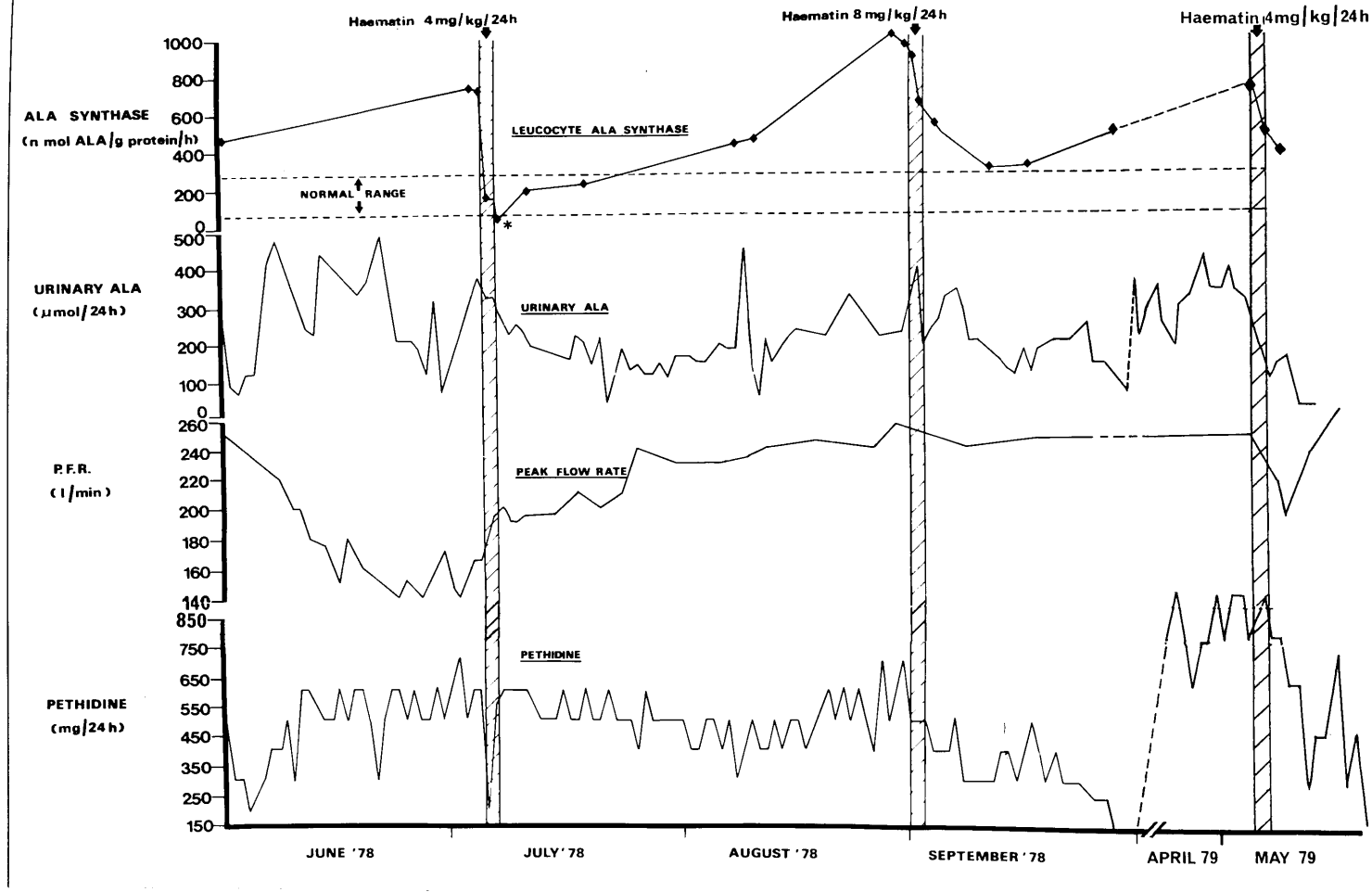


Figure 18: Effect of three courses of haematin on leucocyte ALA synthase activity, urinary ALA excretion, respiratory peak flow rate and analgesic requirements in 31-year-old woman with prolonged attack of acute intermittent porphyria.

On October 9, 1979 she was given a fifth course of haematin for an attack complicated by severe hypertension (210/125 mm Hg) and tachycardia (160/min). On that occasion, she developed a hypertensive exudative retinopathy (Fig 8). The hypertension and accompanying fundal changes settled with propranolol and two weeks later she was discharged home.

Seven months later on May 30, 1980 she was readmitted with severe abdominal pain, hypertension, tachycardia and marked muscle weakness. Her temperature was mildly elevated (38.5°C) in the absence of detectable infection. Nevertheless she was treated with parenteral broad-spectrum antibiotics. She also had three generalised tonic-clonic seizures which did not require specific treatment. Administration of a sixth 2-day course of haematin failed to produce any beneficial effect. On June 5, 1980 the patient was transferred to the intensive care unit. The following day she suddenly went into asystole from which she was successfully resuscitated. Apart from a mild hyponatraemia (Na: 128 mM/L) and hypochloraemia (Cl: 92 mM/L) no other biochemical abnormalities were present at the time. Her arterial blood gases which had been monitored closely were deemed satisfactory. The asystole was considered to be a possible manifestation of the widespread autonomic neuropathy. Although the patient did not appear to have sustained any cerebral damage from her cardiac arrest, she required artificial ventilatory support for the subsequent ten weeks because of severe motor paralysis.

On August 13, 1980 she was weaned off the ventilator. She still experienced constant abdominal pain despite regular administration

of narcotic analgesics. Her hands were deformed showing a claw-like deformity; the muscles of her lower limbs were wasted but no fasciculation was present. Her peripheral reflexes were absent and both plantar reflexes were flexor. Sensory modalities were clinically unimpaired. Her state of nutrition was otherwise remarkably good following the parenteral nutrition she had been receiving. On September 28, 1980 she had another episode of asystole in the ward and died despite resuscitative measures.

Autopsy was performed ten hours after the time of death by Professor David Graham from the Department of Neuropathology, Institute of Neurological Sciences, Glasgow with the specific purpose of correlating the histopathological findings with the clinical features. The histopathological findings were initially reported by Professor Graham and later reviewed by Dr David Doyle, Consultant Neuropathologist.

7.3 NEUROPATHOLOGY

7.3.1 Gross features

The brain weighed 1220 grams and no external abnormality was noted. The meninges were normal with no evidence of tentorial or tonsillar herniation. The hypothalamic structures and oculomotor nerves were normal. The basal vessels were fully patent and free of congenital anomaly.

Coronal sections: No abnormalities were seen in serial sections of cortex, white matter, basal ganglia, thalami and hypothamus including the mamillary bodies. The ventricles were of normal size and there was no shift of the midline structures. Each

medial occipital cortex was also normal. The stems and branches of cerebral arteries were thin-walled and patent. The cerebellum and brain stem were entirely normal externally and at multiple representative cross-sectional levels. The spinal cord was likewise normal externally and at multiple representative levels throughout its length.

7.3.2 Histological findings

Sections were routinely stained by haematoxylin and eosin, Van Giesen, Luxol fast blue counterstained with cresyl violet and silver impregnation after Palmgrin staining.

Multiple representative sections were taken from both right and left cerebral and cerebellar hemispheres. The only abnormality noted was focal loss of nerve cells within the Sommer sector of the hypothalamus. These minor changes were fairly recent in that there was considerable microglial cell reaction to the loss of the nerve cells. Otherwise there were no other abnormalities - in particular no evidence of demyelination.

The sections of hypothalamus and brain stem were completely normal. Particular attention was paid to the vagal nuclei. Similarly sections of the spinal cord taken at multiple levels showed no evidence of demyelination, especially within the ascending fibre systems of the posterior columns. Chromatolytic changes were not seen either in the anterior or posterior horn cells.

Posterior root and autonomic ganglia were completely normal. The posterior roots showed no features of demyelination or axonal loss. Multiple transverse sections of the ulnar, sciatic and sural

nerves showed preservation of normal axonal types with no features of demyelination or Wallerian degeneration. No teased fibre preparations were available for examination.

7.4 DISCUSSION

It is now widely accepted that axonal degeneration, and not demyelination, is the main histopathological lesion that characterises the peripheral neuropathy of acute porphyrin (Mason et al, 1933; Sweeney et al, 1970; Cavanagh & Mellick, 1965). This finding is supported by electrophysiological studies which have often shown normal or only minimal impairment of motor conduction velocities in the presence of severe muscle weakenss (Nagler, 1971; Flugel & Druschky, 1977; Albers et al, 1978; Wocknik-Dyjas et al, 1978). Earlier reports have also noted the absence of any morphological changes in the nervous system of some porphyric patients who died of the disease, but incomplete documentation has made meaningful interpretation difficult (Mason et al, 1933; Denny-Brown & Sciarra, 1945; Hierons, 1957). Following the detailed neuro-histopathological study of five fatal AIP cases by Cavanagh & Mellick (1965), highlighting the neuropathy of acute porphyria as an example of dying-back axonopathy, only sporadic reports have been published, usually of peripheral nerve biopsies (Nagler, 1971; Thomas, 1971; Wakayama et al, 1975; Anzil & Dozic, 1978; Thorner et al, 1981). Apart from few reservations concerning the possible contribution of demyelination in the pathogenesis of the disease (Thomas, 1971; Wakayama et al, 1975; Anzil & Dozor, 1978) axonal degeneration has consistently been

emphasised as the hallmark of porphyric neuropathy. In the autopsy cases whenever axonal changes were found in peripheral nerves chromatolysis was invariably present in the anterior horn cells, vagal and autonomic nuclei (Bostroem, 1920; Mason et al, 1933; Baker & Watson, 1945; Denny-Brown & Sciarra, 1945; Gibson & Goldberg, 1956; Ten Dyck et al, 1961; Sweeney et al, 1970).

The present case is remarkable because of the singular lack of morphological changes, previously described in such cases in both central and peripheral nervous system. This is despite well documented evidence of active disease over a period exceeding eight years during which the patient developed all the severe complications attributed to a widespread neuropathy (Goldberg, 1959). During the later phase of her illness she suffered from constant abdominal pain and had marked tetraparesis. Wasting of the extensor muscles of the upper extremities was particularly evident resulting in claw-like deformity of her fingers. Without examination of teased fibre preparations, more subtle changes of demyelination or Wallerian degeneration in the peripheral nerves could not be excluded. But there were certainly no abnormalities to be found in the multiple cross-sections obtained from the autonomic nerves, and peripheral nerves supplying the severely affected limbs.

Whether haematin therapy could have had a protective effect on the development of morphological changes in the nerve cells remains speculative. In vitro experiments have shown that haematin can promote neurite growth (Ishii & Maniatis, 1978; Bonyhady et al,

1982). The relatively small quantity of haematin administered and the short time course of these treatments could cast some doubt on this possible mode of action. Nevertheless, this matter is undoubtedly of considerable therapeutic importance because earlier and more frequent administration of haematin than hitherto employed may well prevent the chronic sequelae of the acute porphyric attack.

The findings of this case support the concept that porphyric neuropathy is primarily due to a functional block within the nerve cells, possibly arising from a deficiency of haem containing compounds (Meyer, 1973) such as cytochrome oxidase (Chapter 6). If the cellular dysfunction becomes unduly severe or prolonged the recognised morphological changes could ensue and cause permanent damage. The mechanism can also explain why the "dying back" type of porphyric neuropathy is not of the classical type seen in organo-phosphorous poisoning, where the distal portions of the longest nerve fibres are affected first (Cavanagh, 1979) but where neurones with shorter axons (Cavanagh & Ridley, 1967,; Hierons, 1957) and higher metabolic requirements (Ridley, 1969; Ridley & Cavanagh, 1972) are also involved. It is interesting to note that Spencer et al (1979) had proposed a similar hypothesis to explain certain other types of peripheral neuropathies.

SECTION II

CHAPTER 8

EARLY-ONSET CHRONIC RENAL FAILURE AS A COMPLICATION OF AIP

8 EARLY ONSET CHRONIC RENAL FAILURE AS A COMPLICATION OF ACUTE INTERMITTENT PORPHYRIA

8.1 INTRODUCTION

Oliguria is frequently encountered during the acute attacks of AIP. Although it can often be attributed to inadequate fluid intake due to abdominal pain and vomiting, it can persist despite restoration of fluid volume. The transient renal impairment has been noted previously (Day et al, 1981) but permanent renal damage has not been recognised to occur. This chapter describes the close association between early onset chronic renal failure (CRF) that is, renal failure occurring under the age of 65, and AIP. The possible pathogenetic mechanisms involved are also discussed.

8.2 PATIENTS AND METHODS

The records of the six cases presented were retrieved from the files of the Porphyria Research Unit at Glasgow Western Infirmary. These files store information on the majority of symptomatic AIP cases diagnosed in the West of Scotland over the past 20 years. In all six cases, acute intermittent porphyria was diagnosed on the basis of excess urinary excretion of ALA, PBG, and uroporphyrin following clinical presentation. In three cases the diagnosis was further confirmed by measurement of ALA.S and PBG.D in peripheral blood. Urinary ALA, PBG and porphyrins were assayed according to the methods of Moore et al (1978). Renal function was assessed by the measurement of blood urea and serum

creatinine, and whenever possible by 24 hr urinary creatinine clearance and intravenous pyelography (IVP). In three of the four fatal cases, gross and histological examination of the kidneys was possible at autopsy.

A summary of the clinical details, and available laboratory amd autopsy data are given in Tables 11 and 12 respectively.

Since no association has been established hitherto between AIP and CRF the probability of these two conditions occuring together by chance were determined by applying the binomial distribution test.

8.3 RESULTS

In all six patients, none of whom were related, the cause of CRF was attributed to end-stage renal disease accompanied by hypertension (Table 12). No other primary cause could account for the CRF; in particular, there was no underlying infection, diabetes, gout or auto-immune disease. These cases occurred in a population of 65 patients with AIP in remission; that is those patients known to have had an acute attack previously and excluding those latent for the disease. The prevalence of early-onset CRF in subjects under 65 years of age in Scotland is estimated at about 52 per million population (Pendreigh et al, 1972), a figure comparable to that in other parts of the United Kingdom (Dombey et al, 1975). Since the prevalence of symptomatic AIP cases, estimated at about 1.9 per 100 000 (Beattie, 1973b), has not altered in Scotland over the period of observation a highly significant association was found to exist between AIP and early onset CRF ($p < 0.001$). In fact this prevalence is likely to be an underestimate of the exact figure because many cases of AIP

Case no.	Sex	Age (years) when: First attack AIP	Last known normal BP	Sustained hypertension diagnosed*	CRF noted	Analgesic history from: Patient	Relatives	Outcome (age - years)
1	M	37	N.K.	37	37	No	N.K.	Died - 43
2	M	24	26	27	27	No	N.K.	Died - 35
3	F	22**	23	35	35	No	N.K.	Died - 37
4	F	36**	44	52	52	No	No	Died - 55
5	F	14**	28	37	37	No	No	Alive - 45
6	F	22**	25	32	32	No	No	Alive - 34

N.K. = not known

* Hypertension = diastolic > 100 mmHg

** Transient hypertension noted during acute attacks

Table 11: Clinical features

Case No.	Erythrocyte PBG deaminase activity	Urinalysis		Blood urea (mM/1)	Serum creatinine (umol/1)	G.F.R. (ml/min)	IVP findings		Autopsy findings
		Porpho-bilinogen	Protein				Kidney size	Features of analgesic nephropathy	
1	19.0	+	-	13.2*	211*	36*	L = 13 cm*	No*	Bilateral shrunken kidneys: end stage histology with hypertensive changes
2	...	+	-	10.8**	202**	42**	Normal**	No**	As above
3	...	+	tr	11.0***	196***	...	...	...	As above
4	...	+	-	11.6●	201●		Normal	No●	
				57.4●●	620●●	...			
5	11.9	+	-	33.5	446	15	L = 10.5 cm R = 9 cm	No	Still alive - biopsy not possible
6	14.0	+	0.2g/ 24 hr	36	675	7	L = 11 cm R = 12 cm		Still alive - biopsy not possible

Time before death:
* = 6 years; ** = 5 years; *** = 2 years;
● = 3 years; ●● = 2 months

Erythrocyte PBG deaminase activity - normal range = 25-35 nmol/uroform/litre blood

Table 12: Laboratory data, intravenous pyelogram and autopsy findings

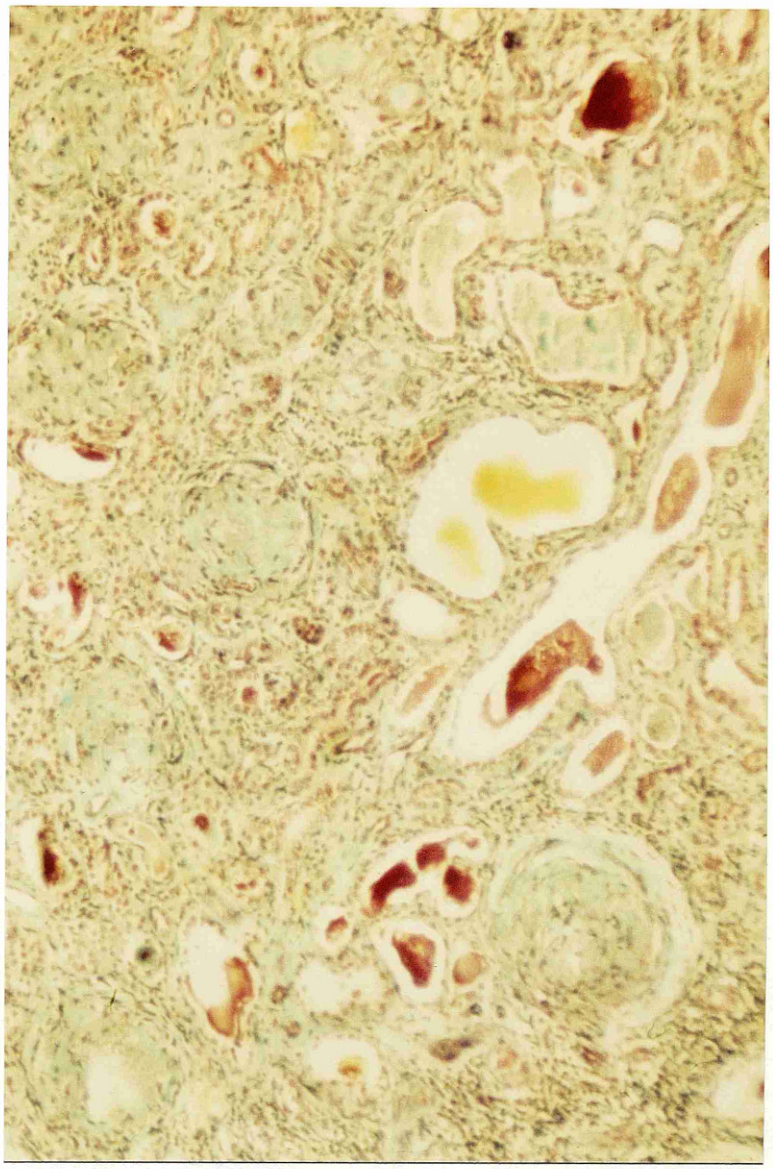


Figure 19: Renal histopathology in AIP patient with early-onset chronic renal failure (Case 1). Section shows prominent tubular loss with atrophy and dilatation of remaining tubules. A number of sclerotic glomeruli are evident and protein casts are easily seen. (Masson stain x 100 magnification)

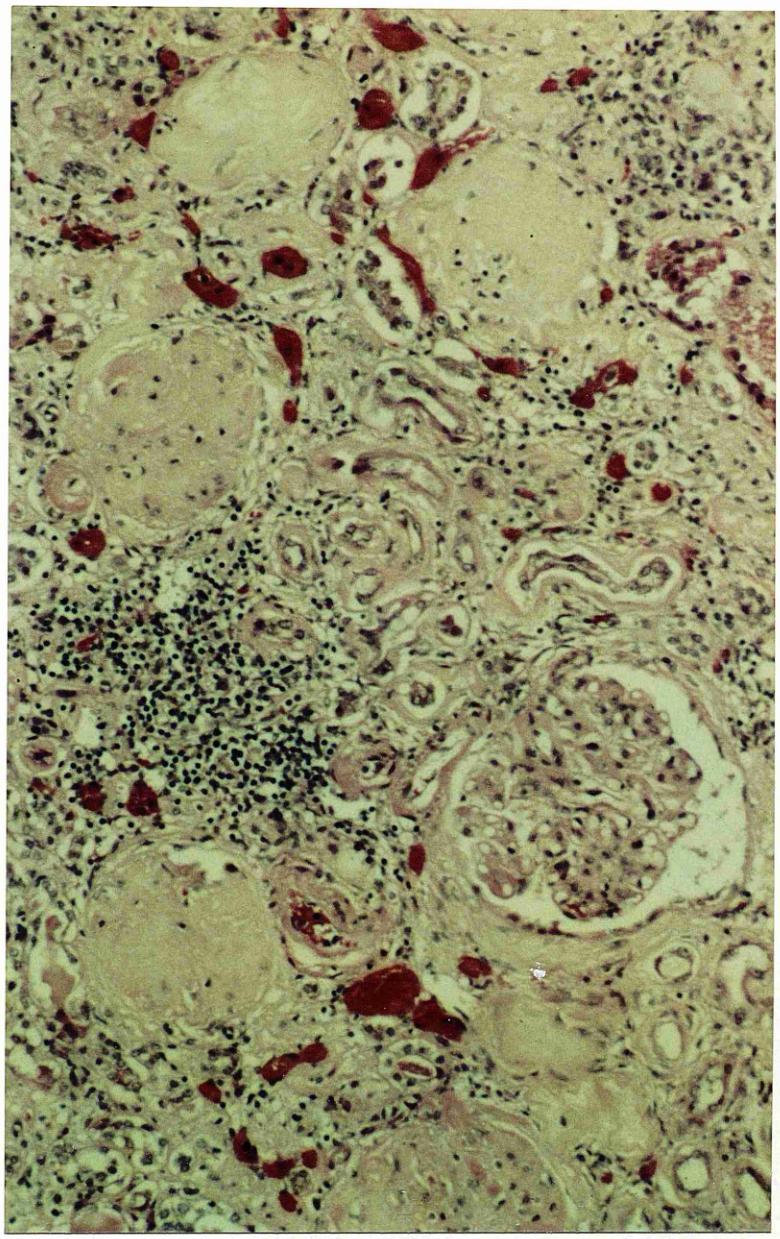


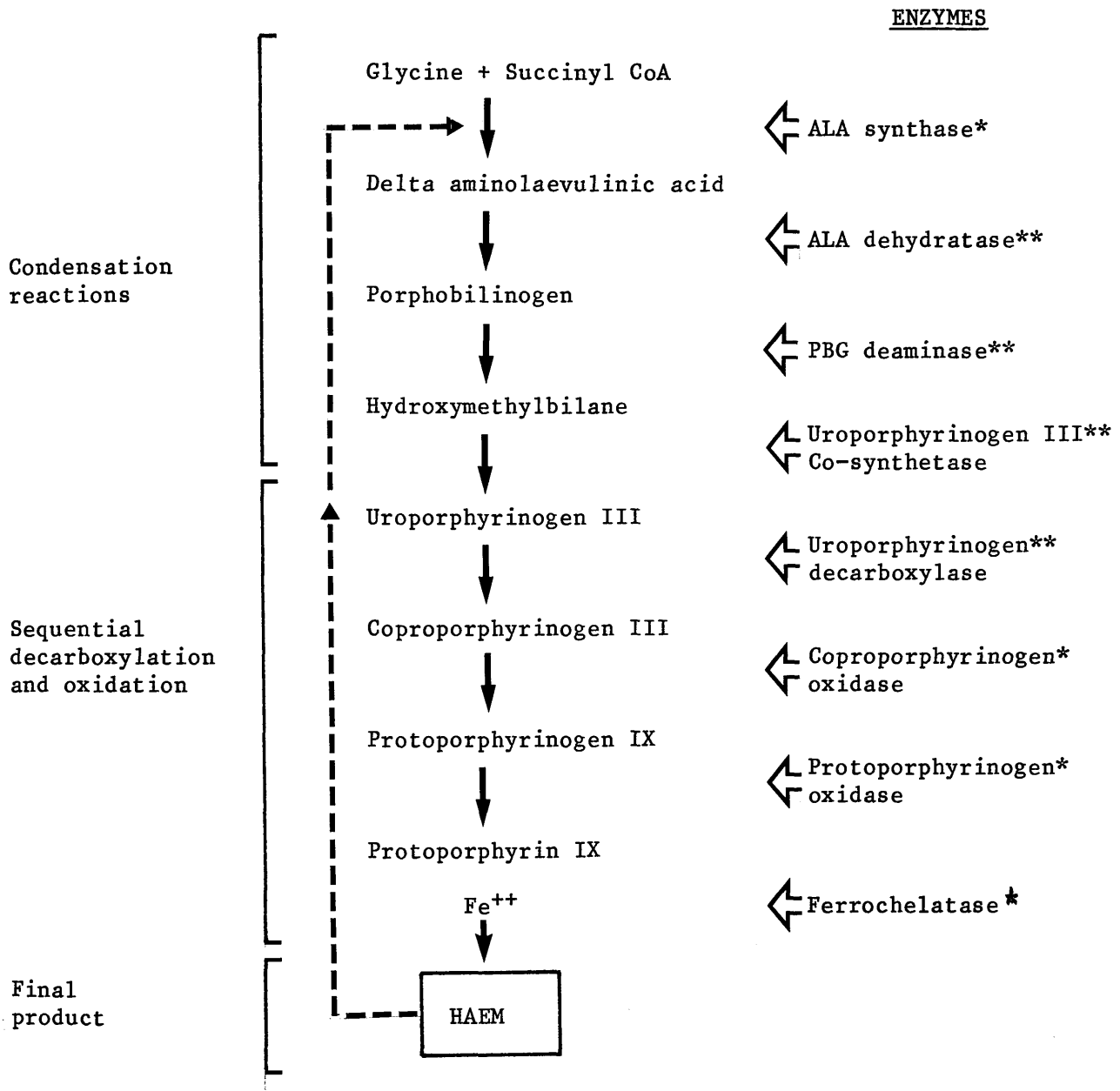
Figure 20: Renal histopathology of Case 3. Ischaemic renal cortex. Majority of glomeruli are totally obsolescent with marked sclerosis and hyalinosis. Changes are evident within the glomerular tufts. Features are compatible with end-stage renal failure. (Masson stain x 100 magnification)

Case No.	Gross		Histology				Overall impression
	External appearance	Internal appearance	Glomeruli	Tubules	Interstitium	Arterioles	
1	Bilateral small and firm kidneys. R = 80 g L = 70 g No scarring	Capsule strips easily. Granular outer surface. Generalized reduction of parenchyma. Renal pelvis unremarkable.	Numerous totally fibrosed. Others show varying ischaemic damage	Severe tubular loss and atrophy. Protein casts++.	Fibrosis + Focal chronic inflammatory cell infiltrate.	Muscular hypertrophy of media++. No fibrinoid changes.	Chronic renal failure with hypertensive changes. No other obvious predisposing conditions
2			*Largely normal although mild changes of glomerular ischaemia	*Tubular atrophy and loss+	*Mild lymphocytic infiltrate	*Thickening of media. No plasmatic vasculosis or arteritis.	*Mild renal ischaemia consistent with early hypertensive changes.
	Bilateral small kidneys.	--	Varying degrees of hyalination some completely fibrosed	Generalized atrophy	Mild chronic inflammatory infiltration	Patchy hyaline thickening of afferent vessels	End-stage renal disease. possibly hypertensive
3	Bilateral small kidneys.	Haemorrhagic areas of necrosis involving cortex	Majority sclerosed. Focal fibrinoid degeneration in remaining	Generalized atrophy and loss	Focal lymphocytic infiltrate	Fibrinoid changes in thickened afferent vessels	End-stage chronic glomerulonephritis with changes of malignant hypertension

*Histology of renal biopsy performed six years before death when IVP = normal and BP = 160/110 before treatment.

Table 13: Pathological renal findings on three patients with AIP who died of chronic renal failure

HEPATIC HAEM BIOSYNTHETIC PATHWAY



* mitochondrial

** cytosolic

The rate of haem biosynthesis is regulated by the initial enzyme of the pathway ALA.synthase which is under negative feedback control by haem (broken line).

Figure 3

Case No.	Gross		Histology				Overall impression
	External appearance	Internal appearance	Glomeruli	Tubules	Interstitium	Arterioles	
1	Bilateral small and firm kidneys. R = 80 g L = 70 g No scarring	Capsule strips easily. Granular outer surface. Generalized reduction of parenchyma. Renal pelvis unremarkable.	Numerous totally fibrosed. Others show varying ischaemic damage	Severe tubular loss and atrophy. Protein casts++.	Fibrosis + Focal chronic inflammatory cell infiltrate.	Muscular hypertrophy of media++. No fibrinoid changes.	Chronic renal failure with hypertensive changes. No other obvious predisposing conditions
2			*Largely normal although mild changes of glomerular ischaemia	*Tubular atrophy and loss+	*Mild lymphocytic infiltrate	*Thickening of media. No plasmatic vasculosis or arteritis.	*Mild renal ischaemia consistent with early hypertensive changes.
	Bilateral small kidneys.	--	Varying degrees of hyalination some completely fibrosed	Generalized atrophy	Mild chronic inflammatory infiltration	Patchy hyaline thickening of afferent vessels	End-stage renal disease. possibly hypertensive
3	Bilateral small kidneys.	Haemorrhagic areas of necrosis involving cortex	Majority sclerosed. Focal fibrinoid degeneration in remaining	Generalized atrophy and loss	Focal lymphocytic infiltrate	Fibrinoid changes in thickened afferent vessels	End-stage chronic glomerulonephritis with changes of malignant hypertension

*Histology of renal biopsy performed six years before death when IVP = normal and BP = 160/110 before treatment.

Table 13: Pathological renal findings on three patients with AIP who died of chronic renal failure

analgesic nephropathy has previously been reported to be relatively high in Glasgow (Murray, 1978). As patients with analgesic nephropathy frequently minimise or even deny their analgesic consumption, interview of their close relatives often provides a more accurate estimate of their analgesic intake (Murray, 1978). Although drug information for three of the deceased patients was limited to what was available from their case-notes, careful interview of the two living patients and the relatives of the decreased patients failed to disclose any history of chronic analgesic abuse. In fact, none of the six cases fulfilled the criteria by which the diagnosis of analgesic nephropathy is made (Nanra et al, 1978; Lindvall, 1978), namely, a history of analgesic abuse and the demonstration of renal papillary necrosis either radiologically or at autopsy. It is relevant to note that a higher prevalence of analgesic nephropathy has been reported in hotter climates (Nanra et al, 1970). Conceivably, therefore conceivable that the state of dehydration that frequently accompanies the recurrent attacks of porphyria might constitute a similar risk even in the absence of excessive analgesic intake. But cases nos 4 and 6 had only one documented acute attack of porphyria.

As the diagnosis of sustained hypertension was made in all six cases at a time when renal damage was already present, the question arises as to whether it preceded or followed the development of CRF. Transient and labile hypertension is a well-recognised feature of the acute attacks of AIP, its frequency ranging from 35-55% in different series (Waldenström, 1957; Goldberg, 1959; Stein & Tschudy, 1970). Even though blood pressure

returns to normal soon after the acute attack, the hypertension can reach levels high enough to cause an exudative retinopathy as was recently seen in a fatal case (Fig 8). Transient hypertension can also arise from a variety of situations causing stress, probably due to adrenergic dysfunction (Frohlich, 1977). But to date no systematic study has been reported on the relationship between the development of acute hypertension and the severity of abdominal pain during the acute attacks.

The central nervous system has an important role in the physiological regulation of the circulation. The brain maintains relatively steady-state conditions within the circulatory system by constantly monitoring the signals received from the cardiovascular mechanoreceptors. Arterial baroreceptor fibres from the aortic depressor regions and carotid sinus are carried by the aortic nerve, a branch of the vagus, and the carotid sinus nerve, a branch of the glossopharyngeal nerve respectively. These then enter the brain-stem before travelling in the tractus solitarius to synapse within the nucleus tractus solitarii (NTS) (Fig 22). The NTS provides one effective locus for regulating autonomic activity since it acts as the primary relay centre of the baroreceptor reflex arc connecting with the hypothalamus and cerebral cortex (Palkovits & Zaborsky, 1977). By sustaining a continuous tonic discharge, dependent on the balanced actions of a "vasopressor" and opposing "vasodepressor" systems in the brain stem, the sympathetic neurones maintain a state of partial vasoconstriction which provides resistance to blood flow and keeps blood pressure in the normal range (Reis & Talman, 1984).

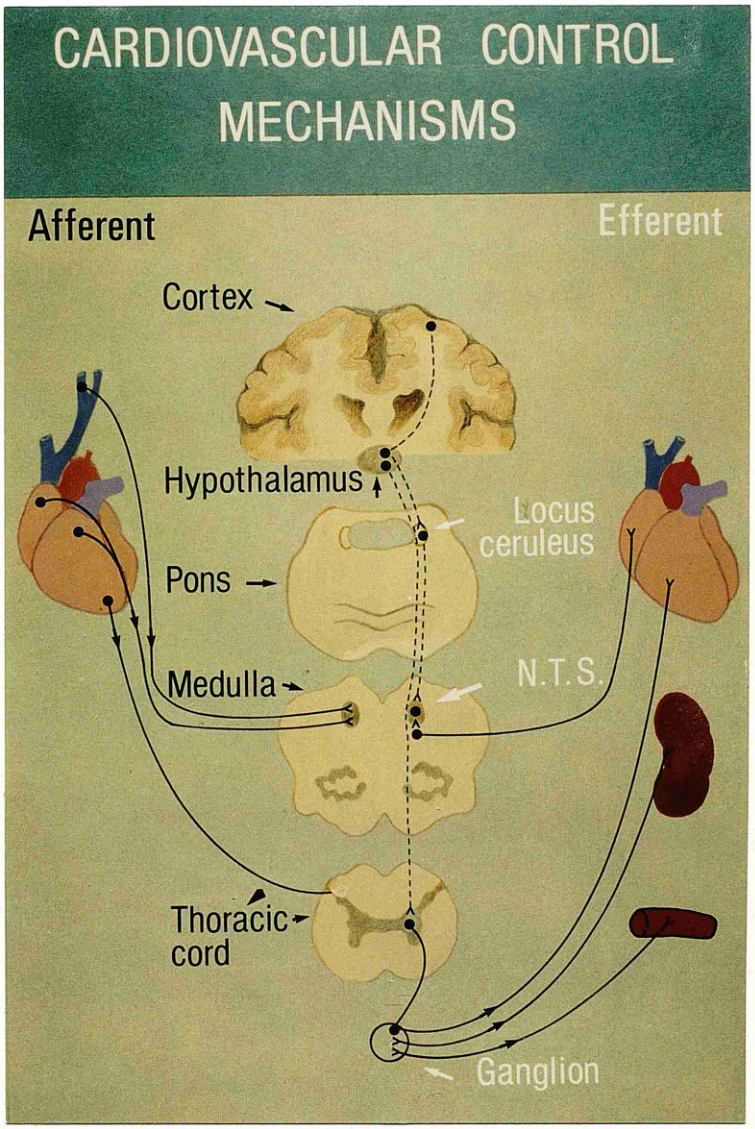


Figure 22: Schematic representation of the anatomical pathways from the arterial baroreceptors via the vagus and glossopharyngeal nerves to the nucleus tractus solitarii in the brain-stem.

Baroreceptor reflexes would therefore appear to stabilise and restrain blood pressure control constantly.

Removal of the baroreceptor input to the CNS produces a sharp increase in arterial pressure, which, if sustained is referred to as "neurogenic" hypertension. Several investigations have shown in different animal species that blood pressure rises when the afferent arm of the baroreceptor reflex arc is interrupted either surgically or pharmacologically (Kumazama et al, 1969; Ferrario et al, 1969; Bustyl et al, 1972; Chalmers & Reid, 1972) ie by sino-aortic de-afferentation. This induced hypertension is characteristically labile (Reis & Talman, 1984) and is accompanied by increased levels of catecholamines (Versteeg et al, 1984).

In fact, Kezdi (1954) has already postulated a similar neurogenic cause for the hypertension of acute porphyria following damage to the vagal and glossopharyngeal nerves from the underlying disease - in the single patient studied neither the blood pressure nor the pulse rate increased following paralysis of the carotid sinus receptors with a local anaesthetic agent. Analogy is drawn to the transient "neurogenic" hypertension (McDowell & Plum, 1954) commonly seen in acute paralytic poliomyelitis and less frequently in Guillain-Barré polyneuritis. It is noteworthy that in a study of four patients with AIP, Schley et al (1970) found an increased 24 hr urinary catecholamine excretion during the acute attacks, the maximal excretion being ten times normal in one patient - somewhat analogous to the excessive production catecholamine production by phaeochromocytoma. Interestingly, brain-stem dysfunction caused by lesions around the floor of the fourth

cerebral ventricle has been reported to cause paroxysmal systemic hypertension and markedly elevated urinary catecholamine excretion in man (Evans et al, 1977).

Vickers (1940) originally reported that 25% of young adults previously affected by paralytic poliomyelitis eventually developed sustained hypertension. This observation suggested that neurogenic hypertension might not be as transient as commonly believed. Indeed structural damage to the hypothalamus and brain-stem nuclei has been described in patients dying of poliomyelitis (Baker et al, 1950; McDowell & Plum, 1954) and AIP (Gibson & Goldberg, 1956). In poliomyelitis the encephalitis can damage the NTS in addition to the other centres for respiratory and cardiovascular control, ultimately leading to respiratory and cardiovascular arrest. Prolonged survival however, has been reported to occur in patients who developed hypertension following damage to the NTS as a result of syringobulbia and ischaemic lesions of the brain-stem (Montgomery, 1961; Magnus et al, 1977). Although bilateral lesions of the NTS can cause a lethal form of hypertension in rats (Doba & Reis, 1973), other animal studies have also shown that discrete experimentally-induced lesions in and around the nucleus of the tractus solitarius can cause specific changes in baroreceptor function capable of producing amore sustained hypertension (Krieger, 1964; Schmitt & Lambie, 1979; Talman et al, 1980; Coleman, 1980). Only partial denervation of the sino-aortic areas was sufficient to produce the hypertension (Schmitt & Lambie, 1979), and interestingly, the chronic type of hypertension caused by NTS lesions was not

particularly labile (Zandberg et al, 1978) Neurogenic hypertension, whether produced by NTS lesions or by sino-aortic deafferentation, arises from the removal of inhibitory baroreceptor influence on sympatho-excitatory centres in the brain. Since NTS neural projection radiate to mid-brain and fore-brain structures as well as to brain-stem vasomotor areas, it is possible that the neural circuit necessary for the full expression of neurogenic hypertension is spread over a wide area (Brody et al, 1984). It is interesting to note that neurogenic hypertension has been found to occur in association with increased levels of plasma vasopressin (Alexander & Morris, 1982), another well-recognised feature of the acute effects of porphyria.

The experimental data support Beattie & Goldberg's original observation (1975) that AIP predisposes to chronic hypertension. After a 20 year follow-up study of 38 AIP patients presenting in an acute attack during early adulthood, about 50% of the surviving patients had a random diastolic blood pressure exceeding 100 mmHg. Six patients had died: one of early onset CRF and malignant hypertension, three of complications of hypertension and two of non-accidental premature deaths at 40 and 42 years, the exact cause of which was unknown. Unfortunately tests of renal function were not carried out in that study and no further up-to-date information is available on this complication.

Apart from these neurogenic mechanisms AIP-associated hypertension could arise from disturbances of the nerve supply to the kidneys since the efferent pathway to the neurogenic control of renin

release is in the sympathetic nervous system. Renin activates the formation of angiotensin I which in turn is converted to angiotensin II. Angiotensin II acts directly as a potent arteriolar constrictor agent. In addition it causes the release of noradrenaline from the adrenergic nerves and acts on specific brain receptors which affect sympathetic nerve outflow (Brody et al, 1984). Indeed high-renin hypertension is a well-established entity both experimentally and in certain clinical states. Elevation of blood pressure in the carotid and aortic baroreceptors can induce a reflex inhibition of renal nerve activity (Ninomoya and Irisawa, 1969), whereas complete baroreceptor denervation can lead to increased renal sympathetic activity (Niijima, 1976), thereby increasing renin release from the renal juxtaglomerular cells. Thus it is possible that activation of the renal neuroendocrine system might be involved in the pathogenesis of the AIP-associated hypertension. In this context it is relevant to mention the case of an 11-year old girl who developed renal failure during an acute attack of porphyria for no apparent reason, the renal impairment then persisting although blood pressure remained normal (Whitelaw, 1974). Furthermore, spasm of retinal (Denny Brown & Sciarra, 1945; De Francisco et al, 1979) and coronary arteries (Eliaser & Kondo, 1942) have also been recorded during an acute attack. Alternatively, as the kidneys are exposed to a relatively high concentration of porphyrins and their precursors, partly synthesised locally (Day et al, 1981) and partly derived from other organs such as the liver, these compounds could possibly

exert a pharmacological effect in reducing renal function either by a cytotoxic or a vasospastic action on renal vessels (Denny-Brown & Sciarra, 1945).

The pathogenesis of early onset CRF in AIP is probably multifactorial. The highly significant association between these two conditions clearly indicates that further studies are needed to define the relative roles of the three factors discussed: porphyria-induced neurogenic hypertension, nephrotoxic effects of porphyrins and precursors, and enhanced susceptibility to analgesic nephropathy. Of these, neurogenic hypertension appears to be the most important. Of note, the renal function of both our living patients has remained stable over the past four years following satisfactory control of their blood pressure with a combination of β-adrenergic blockers and diuretics.

SECTION II

CHAPTER 9

TREATMENT OF PAIN IN THE ACUTE ATTACK OF AIP - A STUDY OF THE RESPONSE TO OPIATE ANALGESICS

9. TREATMENT OF PAIN IN THE ACUTE ATTACK OF AIP - A STUDY OF THE RESPONSE TO OPIATE ANALGESICS

9.1 INTRODUCTION

Abdominal pain is the commonest symptom of acute porphyria occurring in more than 90% of cases (Waldenstrom, 1957; Goldberg & Rimington, 1962; Eales, 1962; Stein & Tschudy, 1970). When the pain is mild simple analgesics seem effective in providing relief, but for more severe attacks narcotic drugs such as pethidine and morphine are required. An acute attack usually lasts a few days but in some cases it can linger for weeks and months.

Most porphyric patients suffering from recurrent attacks learn how to cope with their disease without recourse to hospital treatment. Those who do represent a selected group with more severe attacks, often complicated by protracted vomiting, neuropathy and seizures. It has been our experience that a number of these patients do not appear to respond to opiate analgesics. Administration of narcotic drugs in large amounts without any resulting benefit and the constant demand for still more analgesia inevitably raise the suspicion of drug addiction in some instances. This is particularly so in a disease where psychological and psychiatric disturbances are recognised. Yet these patients display the biochemical manifestations of active porphyria and a stereotype pattern of behaviour which is not that expected of a drug addict. The purpose of this study was to investigate whether opiate resistance occurs in acute porphyria.

9.2 STUDY I

9.2.1 Patient selection

Between June 1981 and March 1984 10 AIP patients admitted to the Porphyria Unit were included in this study after they had satisfied the following criteria:

1. The onset of the attack was less than one week prior to entering hospital.
2. No narcotic analgesics were used during that period of time (but simple analgesics, paracetamol or aspirin-like drugs, were allowed).
3. The patient was well-adjusted to his or her social environment in between attacks.
4. The patient had no other painful conditions requiring treatment.
5. The patient had not been taking any medication that could affect pain response; namely, psychotropic agents.

The clinical details of the patients studied and referred to by their initials are given in Tables 5 and 6. Informed consent was obtained before inclusion in the study.

9.2.2 Pain measurement - Visual Analogue Scale (VAS)

Intensity of abdominal pain was rated according to a visual analogue scale (Scott & Huskisson, 1976).

The scale consisted of a 15 cm line scored into 10 unlabelled segments except at the two ends. One end of the line was marked "0" representing no pain whereas the other end marked "10" indicated the worst pain imaginable: "the sort of pain that would drive one to end it all if it lasted for too long". In the

initial explanation of the pain rating system, the patient was additionally informed that the first third of the line from "0" was equivalent to mild pain; the middle third, moderate pain; and the last third, severe pain.

Pain rating was carried out regularly at 4 hourly intervals unless the patient was asleep. The patient was requested to point with a pencil on the line, and a numerical conversion on the 0 to 10 scale was immediately recorded on a separate chart. When injections were given assessment of pain was carried out before and 5 to 10 minutes after the drug was administered. Correct usage of the VAS by the patient and nursing staff was checked daily by the Principal Investigator.

9.2.3 Diet

On admission, a detailed dietary assessment was carried out by the Hospital Dietitian. If the calorie intake was less than 2500 Kcal/day a fine nasogastric tube was passed to supply at least that amount containing a minimum of 300 gms of carbohydrate. Two of the patients studied could not tolerate a nasogastric tube, and intravenous glucose had to be administered initially. An adequate calorie intake was maintained throughout the study period.

9.2.4 Drugs were obtained from the hospital pharmacy and used in their standard formulation.

Opiate analgesics:

1. Pethidine hydrochloride
2. Morphine sulphage
3. Diamorphine hydrochloride
4. Buprenorphine ("Temgesic" - Reckitt & Colman)

Opiate antagonist:

1. Naloxone ("Narcan" - Du Pont)

Antiemetics

1. Prochlorperazine ("Stemetil" - May & Baker)
2. Cyclizine

Prochlorperazine was always administered with the narcotic analgesics in a dose of 12.5 mg twice to four times daily as an antimetic. In two cases cyclizine 50 mg was used instead.

Hypnotic/sedative 1. Oral diazepam was prescribed to six of the patients.

Antihypertensive 1. Oral propranolol ("Inderal" - ICI) was required to control hypertension and tachycardia in three patients (EL, RR and JMcM) in a dose ranging from 40 to 160 mg daily.

9.2.5 Analgesic regimens

I Because of its proven safety in porphyric patients pethidine was used as the first-line analgesic (Moore & Disler, 1983; De Verneuil et al, 1983). It was initially administered in a dose of 100 mg intramuscularly at regular four hourly intervals. The dose was usually increased to 150 mg if required.

In three patients the dosage was further increased stepwise to a maximum of 1800 mg daily. In two patients failure of pain control necessitated a trial with diamorphine. The starting dose of diamorphine was 5 mg four hourly and increased stepwise to a maximal dose of 20 mg.

II Three patients were given buprenorphine intramuscularly first instead of pethidine. The initial dose of 1.2 mg daily was increased stepwise to 4.8 mg/day. Two of these patients subsequently received pethidine because of failure to control the pain.

III Two patients receiving narcotic analgesics were additionally treated with nitrous oxide inhalation analgesia. A gaseous mixture containing 50% nitrous oxide and 50% oxygen - "Entonox" was self-administered through a rebreathing face mask. The patient was instructed to use the "Entonox" mixture only when the pain was most severe.

The ward staff in our Research Unit nursed and monitored all porphyric patients as for routine "post-operative" management. Blood pressure and pulse were recorded four-hourly except when patient was asleep. The respiratory rate and peak flow expiratory rate were also checked regularly.

9.2.6 Haem enzyme and porphyrin measurements

Erythrocyte URO.S, leucocyte ALA.S and 24 h urinary porphyrin precursor excretion were assayed on the same days.

9.2.7 Results

Pain ratings assessed before and after the intramuscular injections correlated very well with each other.

Analgesic regimen I: Five of the 10 patients studied obtained satisfactory pain relief (ie more than 50% reduction in daily mean pain score) with intramuscular administration of pethidine alone. Pain relief was complete in only a single patient whereas in the others it was only partial. The mean duration of the acute attacks was 8.4 days (range 5 to 12 days). Pain benefit coincided with the expected pharmacological effect of pethidine in three of the five patients. In the other two patients reduction in pain intensity was not evident for at least two days after pethidine had been given at maximal dosages; this is illustrated for patient

RR in Figure 23. This same patient had a recurrent attack during which little benefit was obtained with high-dose pethidine, either as intramuscular injections or intravenous infusion (Fig 24).

The course of three separate attacks for patient JMcM is shown in Figures 25 and 26. In the first attack (Fig 25) she stayed in hospital for six weeks and pethidine given intramuscularly failed to control the pain. At the time of discharge the pain was still rated at 3-4. It persisted for another week before disappearing. During the second attack (Fig 26, A & B) inadequacy of pain relief with pethidine (maximal dose = 1500 mg daily) led to a trial with diamorphine; this was also ineffective in achieving pain control. This lack of response raised the possibility of drug addiction in the patient and the drug was tailed off. When she went home she regularly took oral dihydrocodeine, diazepam and chlorpromazine. The second attack lasted another 17 days before her symptoms settled and all medication was stopped. She suffered a third attack two weeks later just before her menstrual period (Fig 26 C). Pethidine was again found to be ineffective in large doses. This attack lasted for nearly five weeks. Interestingly the patient did not have another attack requiring narcotic analgesics for several months thereafter.

In both patients (RR and JMcM) pethidine did not appear to cause any significant increase in haem enzyme activities or excretion of porphyrin precursors.

Analgesic regimen II Buprenorphine failed to provide adequate pain relief to the first three patients in the study who received that drug (Figs 27, 28 and 29). In one case (Fig 29) the daily

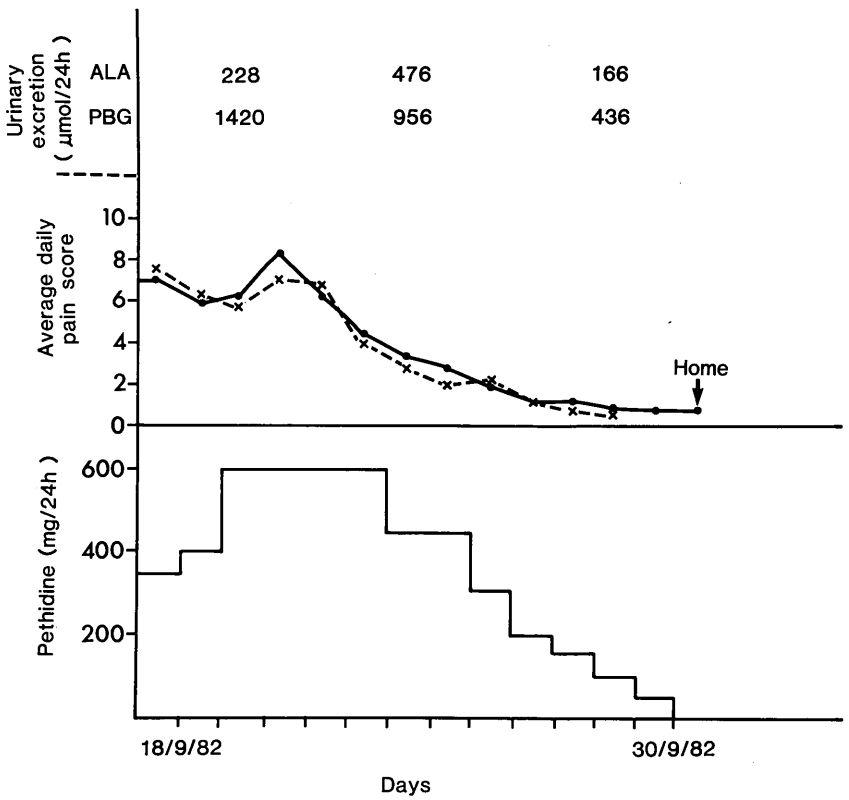


Figure 23: Patient R.R.
Pain ratings during treatment with IM pethidine. Solid line represents ratings recorded before each injection, and dotted line represents re-assessment values obtained 5 to 10 minutes after injection was administered. Urinary porphyrin precursors were measured at intervals.

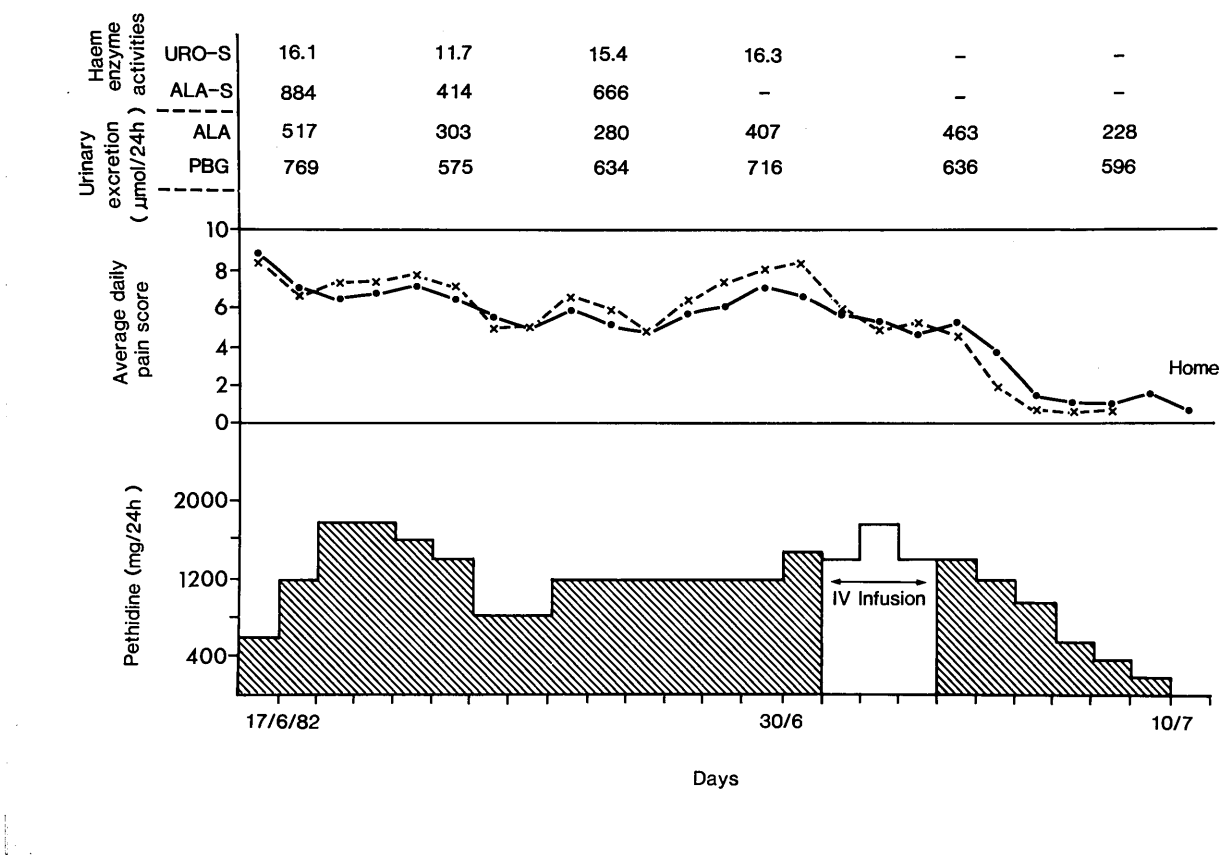


Figure 24: Patient R.R.
Pain ratings following IM and IV administration of pethidine during a recurrent attack. Haem enzyme activities and urinary porphyria precursors were measured at intervals.

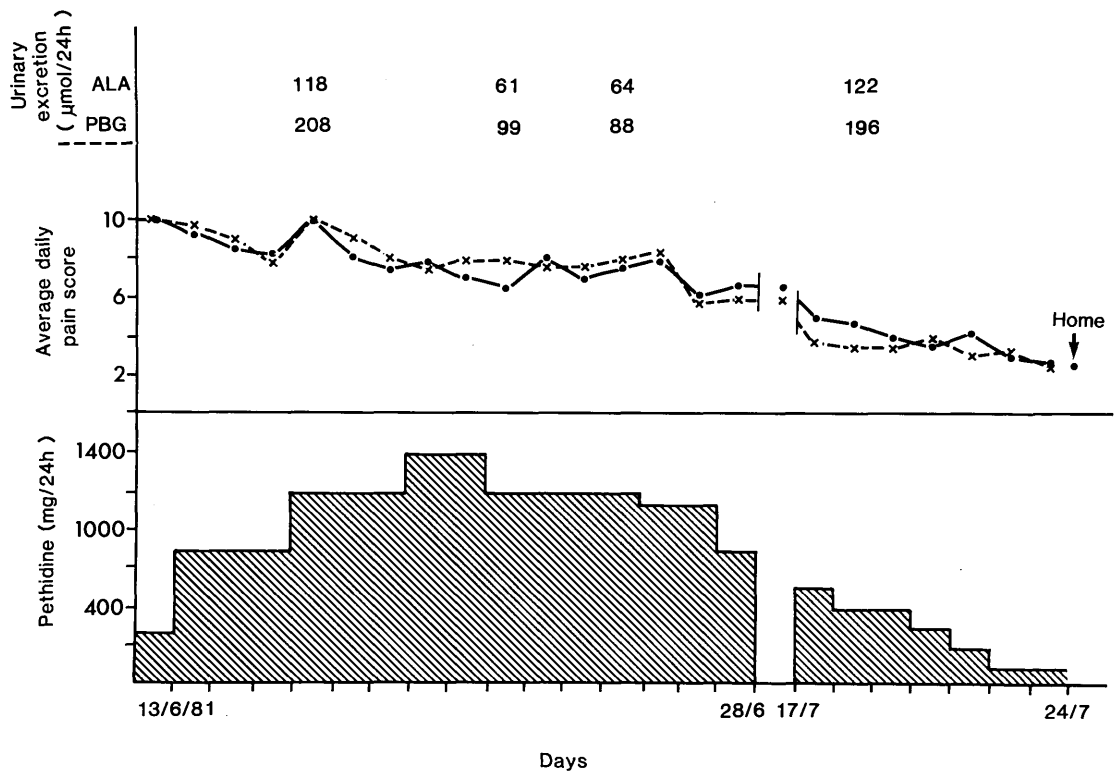


Figure 25: Patient J.McM.
Pain ratings during treatment with IM pethidine in an attack which lasted for more than 6 weeks. Patient still suffered from abdominal pain when she left hospital.

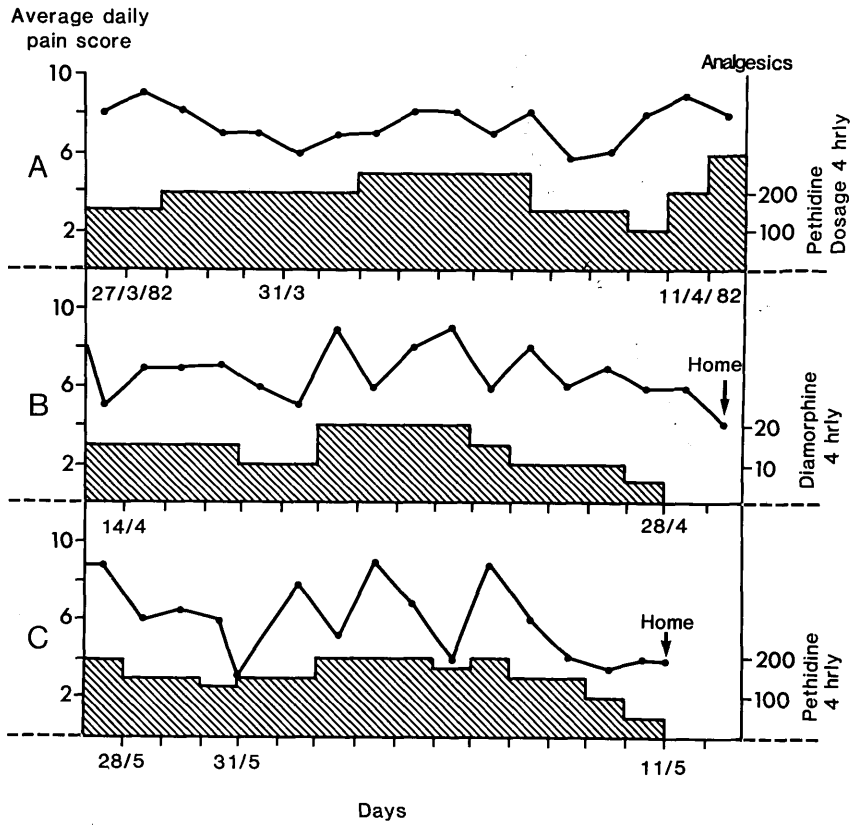


Figure 26: Patient J.McM.
Response to opiate analgesics in two successive attacks. During the first (A & B) neither pethidine nor diamorphine provided any significant relief. In the second attack (C), pethidine was again ineffective. On both occasions attacks settled after 2-3 weeks folowing hospital discharge.

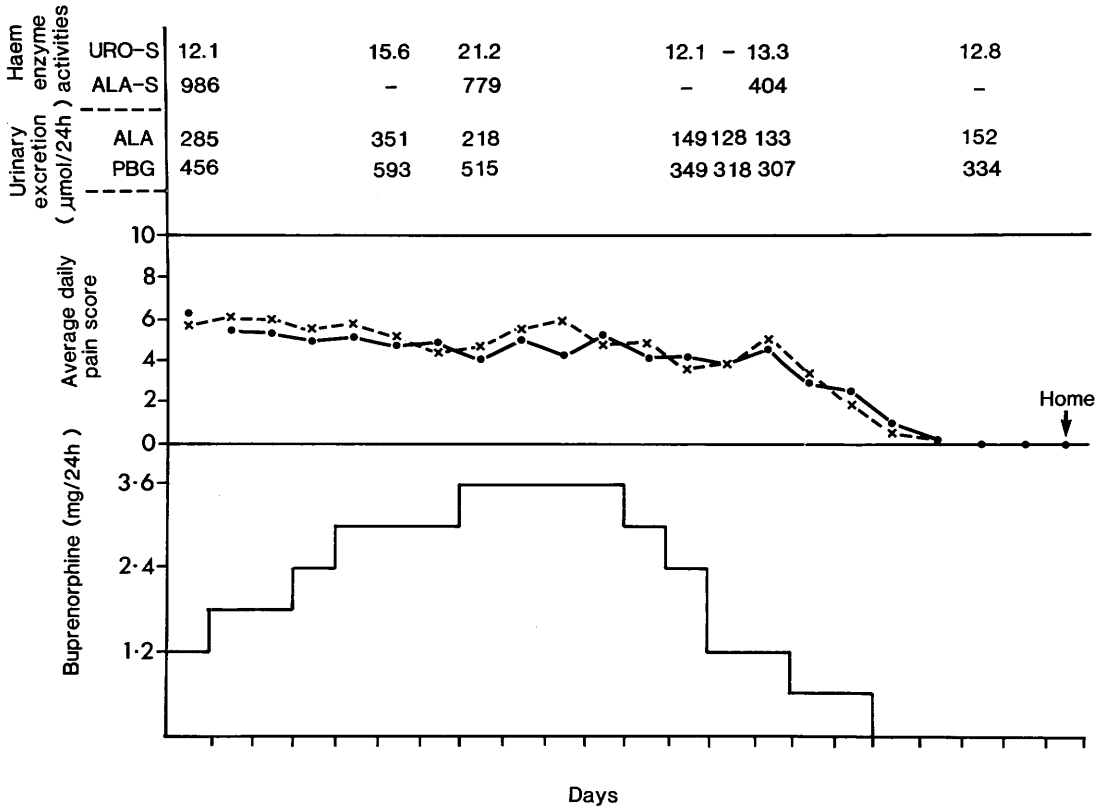


Figure 27: Patient C.M.
Pain ratings while receiving IM buprenorphine. Haem enzyme activities and urinary porphyrin precursors were measured at intervals.

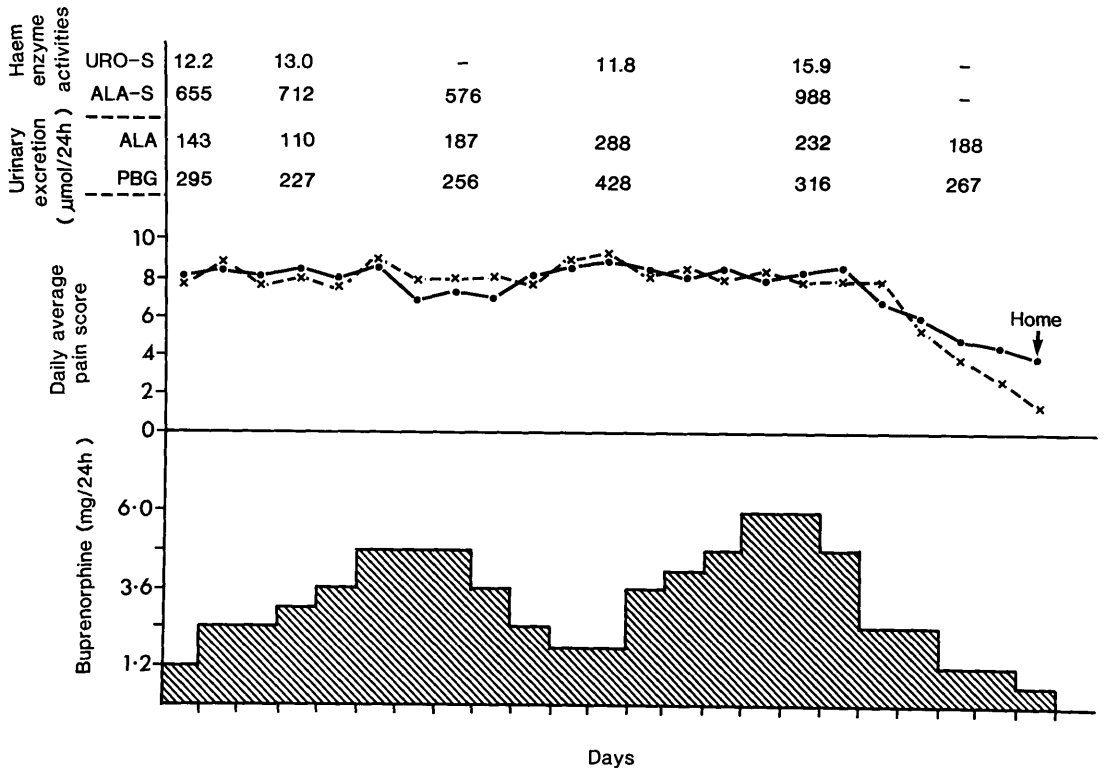


Figure 28: Patient M.S.
Pain ratings with IM buprenorphine during an acute attack of AIP.
Haem enzyme activities and urinary porphyrin precursors were assayed to follow the course of treatment.

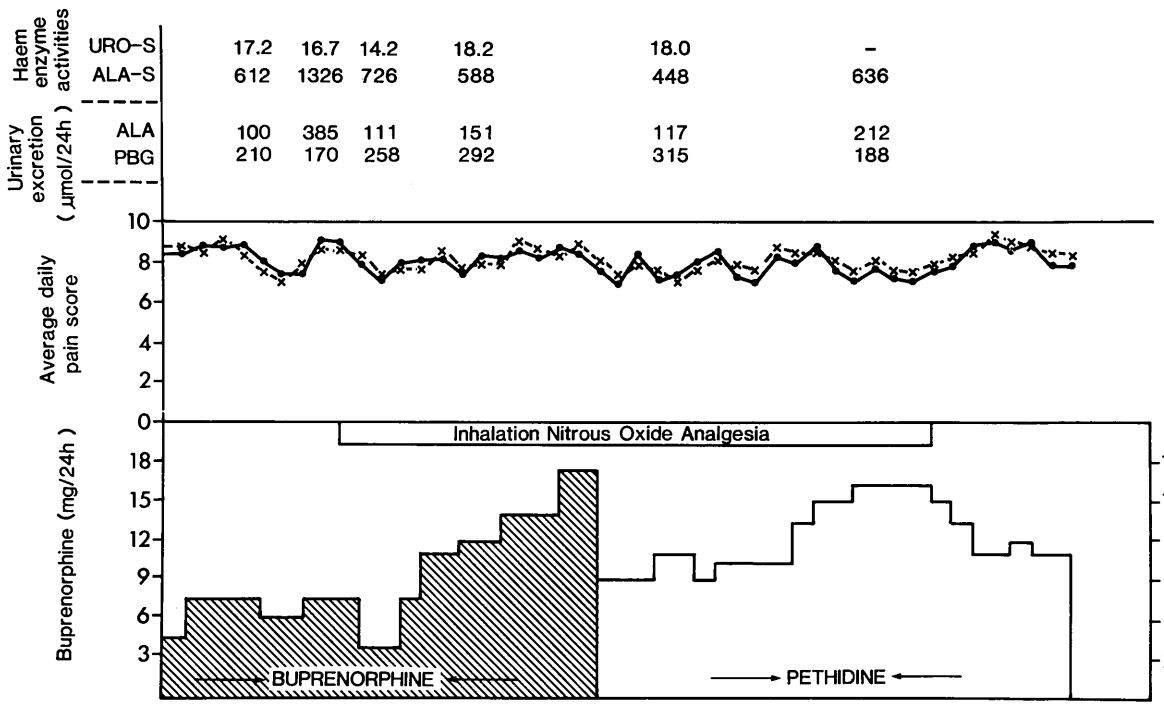


Figure 29: Patient J.C.
Pain ratings during treatment with buprenorphine followed by pethidine administered IM. Supplementary inhalation nitrous oxide analgesia was also provided. Haem enzyme activities and urinary porphyrin precursors were measured at intervals.

dosage was maximally increased to 16.8 mg with no apparent benefit. At this very high dose, careful monitoring of the patient's blood pressure, pulse and respiratory rate showed no significant effect. But she experienced excessive drowsiness and a strong feeling of depersonalisation. These psychotomimetic symptoms disappeared with stoppage of buprenorphine. A change to pethidine made no difference to the pain ratings. Buprenorphine did not cause any significant increase in haem enzyme activities or porphyrin precursor excretions.

Analgesic regimen III: The value of supplementary nitrous oxide inhalation analgesia was assessed in two AIP patients whose pain was resistant to narcotic drugs. In only one case did it seem to provide a mild beneficial effect (Fig 30); no such response was obtained in the other (Fig 29). Both patients overused the nitrous oxide mixture beyond what was recommended. It seemed to induce sleep and a state of "relaxation". Nitrous oxide did not significantly increase haem enzyme activities or urinary excretion of porphyrin precursors.

9.3 STUDY II

9.3.1 Patients

Three patients were studied: two in a severe attack of AIP and a third patient suffering from chronic abdominal pain of unknown cause suspected of being addicted to pethidine.

Case 1: Mrs EL was a 30-year old woman admitted to the Western Infirmary on 29 May 1981 with severe abdominal pain, tachycardia, hypertension and motor weakness. She has had several attacks during the previous six years. On that occasion, her tachycardia

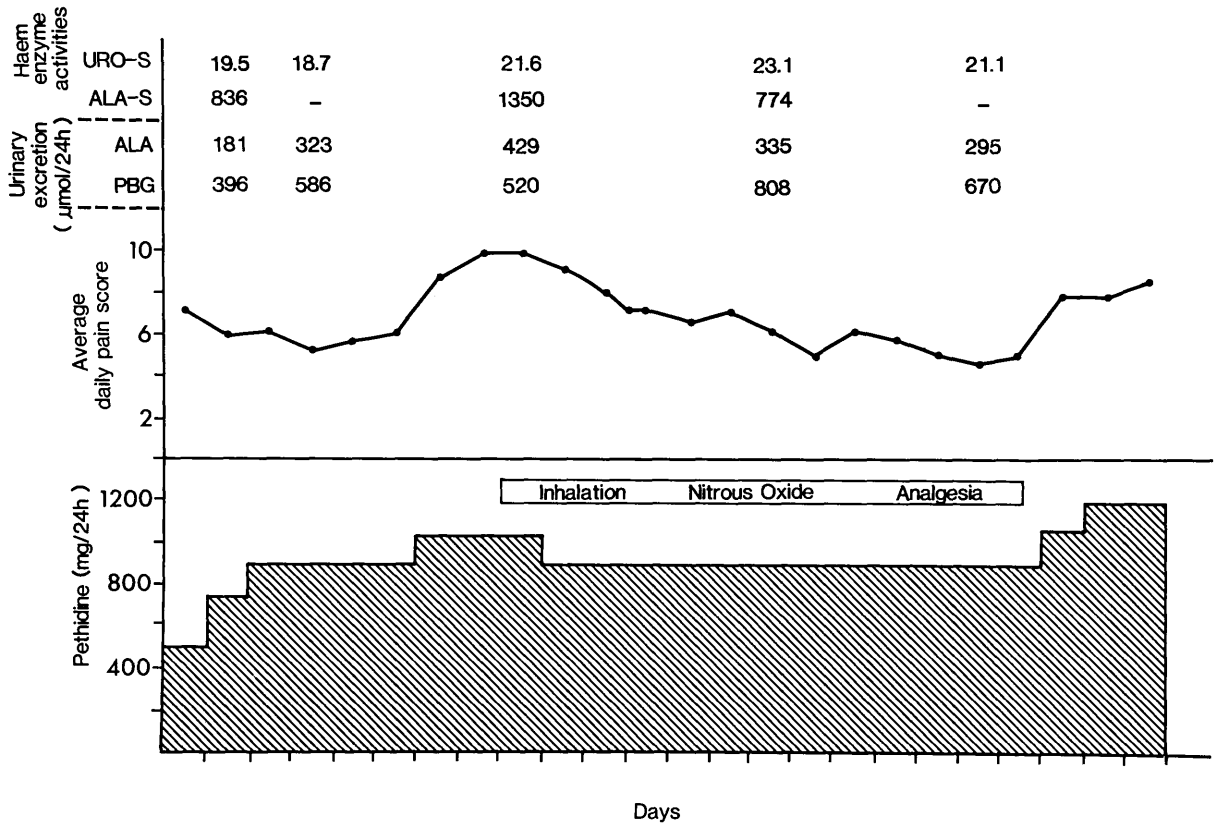


Figure 30: Patient E.L.
Response to treatment with IM pethidine supplemented with inhalation nitrous oxide analgesia.

(108/min) and hypertension (160/112) were controlled with propranolol but large doses of opiates failed to alleviate the pain. On the same evening her unstable condition necessitated her transfer to the intensive care unit (ITU).

On 31 May 1981 the patient was discovered to have received 90 mg of morphine intravenously in less than one hour when that amount was intended for administration over a longer period -she was suspected of having tampered with the sage pump. Naloxone was immediately administered in a dose of 0.4 mg intravenously to prevent respiratory depression. No opiate withdrawal reaction ensued. Surprisingly, by the next morning the abdominal pain had nearly disappeared. Two days later she was discharged home without any analgesics. When she was reviewed as an out-patient two weeks afterwards she was noted to have remained nearly pain-free since her discharge home.

On 8 October 1982 the patient was readmitted with another severe attack. Her pain response to sequential intravenous infusions of pethidine, normal saline as placebo, morphine and naloxone was therefore studied in the hope of repeating the previous favourable outcome.

Case 2: Miss JC was a 26-year old nurse who had experienced her first symptoms of AIP at the age of 20. During the previous year she had suffered from five attacks, precipitated by alcohol on two occasions.

On 25 June 1981 she was admitted to hospital with severe abdominal pain and vomiting. She was initially treated with intramuscular buprenorphine without benefit. After the dosage of buprenorphine had been increased stepwise to 3.6 mg/day over a period of six

days without apparent benefit, she was entered into this present study. Buprenorphine was reduced to 0.6 mg/day for two days, before all subsequent analgesic therapy was administered intravenously.

Case 3: Mrs PJ was a 38-year old woman who had been complaining of constant severe upper abdominal pain for 11 years. She has already had numerous investigations with negative results, including pancreatic function tests, endoscopic retrograde cholangiopancreatography (ERCP), total body CAT scanning and an extensive metabolic screen for rare causes of abdominal pain. She had also been attending the pain clinic in Edinburgh where a coeliac ganglion block was performed without any benefit. She was referred to the Western Infirmary in March 1984 for exclusion of porphyria.

The patient claimed that pethidine, which she could self-administer, was the only drug that could relieve her pain. Any slight suggestion of drug addiction was met with strong protest. But during this admission she consented to see a Consultant Psychiatrist with a special interest in drug abuse. When she was informed that she was suffering from drug dependence on pethidine she refused further psychiatric help. It was discovered that a year previously she was discharged from her post as a high-ranking Nursing Officer after being suspected of securing pethidine fraudulently. Throughout her stay in hospital, her pain ratings were high and her requirements for opiate analgesics showed no abatement. She was keen to undergo further physical tests, and she readily consented to take part in the study.

Case 2

Daily Coding	Agent	Syringe Code No.	Amount of drug	Syringe Code No.	Amount of drug	Syringe Code No.	Amount of drug
A	Buprenorphine	A_1	0.6mg	A_2	1.2mg	A_3	2.4mg
B	Saline (Placebo)	B_1	-	B_2	-	B_3	-
C	Saline (Placebo)	C_1	-	C_2	-	C_3	-
D	Pethidine	D_1	200mg	D_2	400mg	D_3	800mg
E	Saline (Placebo)	E_1	-	E_2	-	E_3	-
F	Saline (Placebo)	F_1	-	F_2	-	F_3	-
G	Naloxone	G_1	0.8mg	G_2	1.6mg	G_3	3.2mg
H	Saline (Placebo)	H_1	-	H_2	-	H_3	-
I	Diamorphine	I_1	10mg	I_2	20mg	I_3	40mg
J	Saline (Placebo)	J_1	-	J_2	-	J_3	-

Case 3

A	Pethidine	A_1	100mg	A_2	150mg	A_3	200mg
B	Saline (Placebo)	B_1	-	B_2	-	B_3	-
C	Diamorphine	C_1	10mg	C_2	15mg	C_3	20mg
D	Placebo	D_1	-	D_2	-	D_3	-
E	Placebo	E_1	-	E_2	-	E_3	-
F	Placebo	F_1	-	F_2	-	F_3	-
G	Placebo	G_1	-	G_2	-	G_3	-
H	Naloxone	H_1	0.8mg	H_2	1.6mg	H_3	3.2mg
I	Placebo	I_1	-	I_2	-	I_3	-
J	Buprenorphine	J_1	0.6mg	J_2	1.2mg	J_3	2.4mg

Table 14: Scheme of drug and placebo administration by intravenous infusions to cases 2 and 3 in Pain Study II

9.3.2 Scheme of drug administration for cases 2 and 3

Following a base-line period during which the patients received relatively low daily dosage of analgesics intramuscularly, the drugs to be tested were administered intravenously by a sage pump. Three externally identical coded syringes containing incremental concentrations of the same agent were prepared for each 24 hour period in the hospital pharmacy. Each syringe contained enough material for an eight hour infusion. The coding scheme, amount and order of drugs administered are shown in Table 14. After use, the empty syringes were returned to the pharmacy and checked for the total amount of drug administered. With the exception of the principal investigator and the pharmacist no other medical, nursing or paramedical staff knew the drug coding. The daily progress of the trial was closely monitored by the principal investigator who was available for immediate consultation in case of emergency; but he did not play an active role in the recording of data.

The patients were informed that they would be receiving various types of potent narcotic analgesics but were not specifically told about the inclusion of placebo.

9.3.3 Results

Case 1: Both pethidine and morphine, increased to a maximal dose of 1600 mg and 100 mg/24 h respectively, failed to provide any significant pain relief. Response to placebo was similar to that obtained with the two opiate agonists (Fig 31). A mild reduction in blood pressure was noted during morphine administration, but concomitant treatment with propranolol and

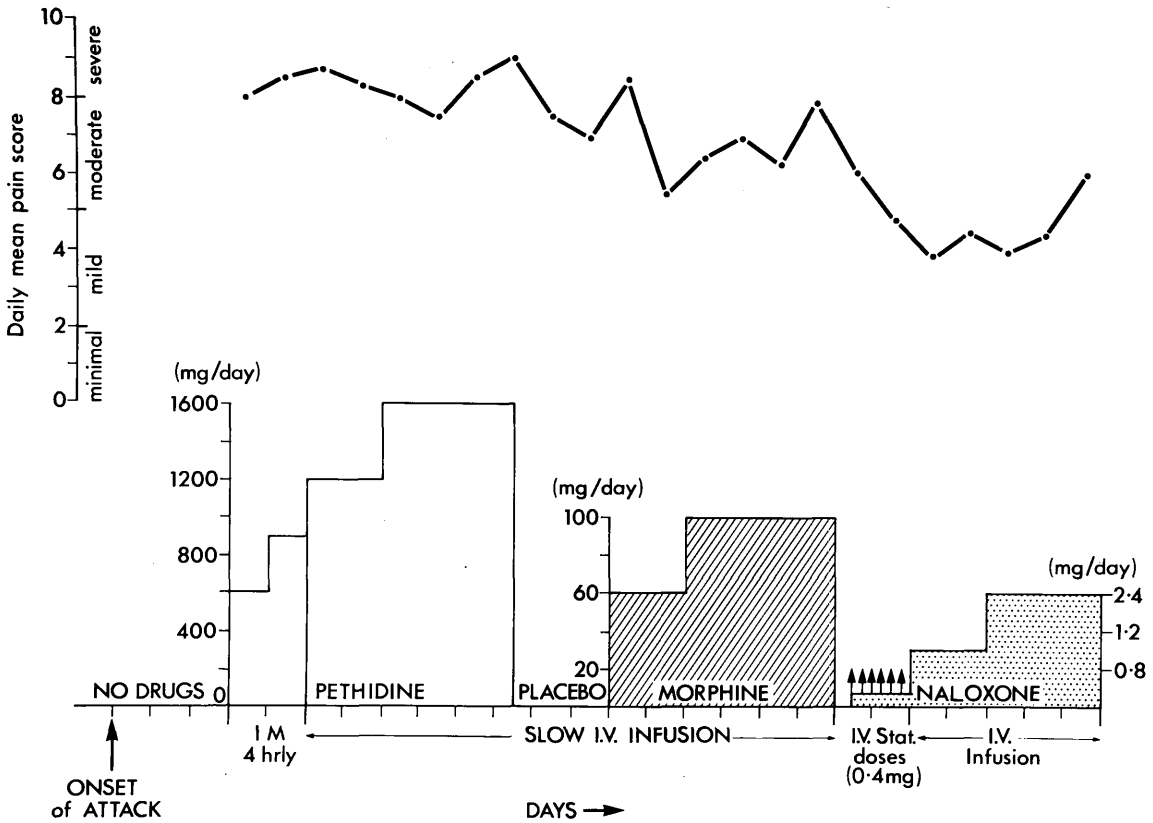


Figure 31: Patient E.L. (Case 1)
Pain ratings during intravenous infusions of pethidine, saline as placebo and morphine followed by naloxone. Note that pain did not increase when the specific opioid antagonist naloxone was administered.

prochlorperazine could have contributed to that effect. Respiratory rate was not depressed, and arterial blood gases remained normal during maximal morphine infusion.

Naxolone was initially administered cautiously by intravenous boluses in the event that she might develop opiate withdrawal syndrome. Following the third naloxone injection, the patient yawned excessively but her pain rating did not increase and other withdrawal symptoms did not develop. Continuation of naloxone infusion was in fact accompanied by favourable pain ratings, yet the dramatic recovery previously experienced did not occur. Following naloxone infusion, administration of buprenorphine did not help the pain.

Case 2: Pain rating apparently improved after initiation of pethidine and diamorphine therapy, but this was not maintained at higher drug dosage (Fig 32). Since a similar transient reduction in pain rating was seen during the second placebo phase it was concluded that these opiate agonists were not particularly effective in relieving pain. Neither was there any significant benefit from buprenorphine or naloxone therapy.

Case 3: In contrast to case 2, the pattern of pain response in this case is different altogether (Fig 33). Whereas pain ratings showed no improvement with low-dose diamorphine or pethidine, an apparent improvement was seen at the higher dosages. But the most remarkable feature was the dramatic swings in pain ratings encountered during the placebo periods.

Administration of a single IV bolus of naloxone (0.4 mg) induced no reaction but infusion of the antagonist at higher dosage caused

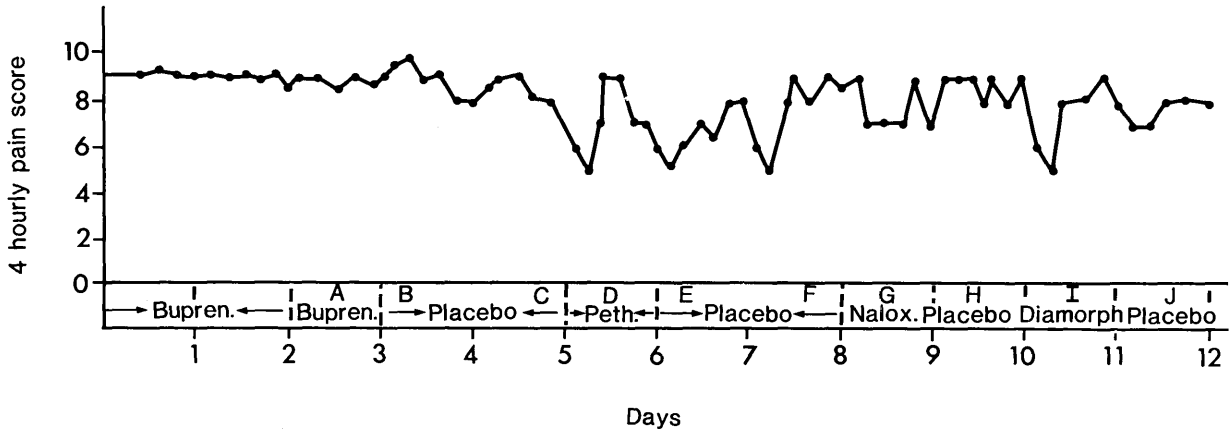


Figure 32: Patient J.C. (Case 2)
Pain ratings during sequential intravenous infusions of buprenorphine (bupren), pethidine (peth), naloxone (nalox) and diamorphine (diamorph) with intervening periods of saline infusions as placebo. All treatments were coded and administered blind.

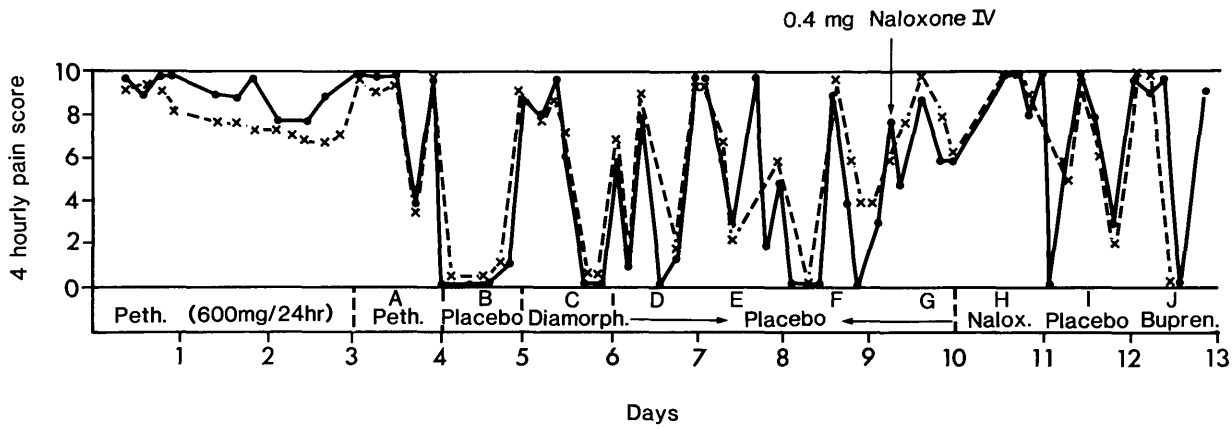


Figure 33: Patient P.J. (Case 3).
Pain rating during sequential IV infusions of pethidine, diamorphine, naloxone and buprenorphine, given blind and coded.
A test-dose of naloxone was given during placego period G without the patient developing any opiate withdrawal reactions.
Solid and dotted lines represent sets of ratings assessed within 10-15 minutes of each other.

a worsening of abdominal pain accompanied by increasing restlessness and aggressiveness. Yet no other manifestations of the opiate withdrawal syndrome emerged.

The erratic pattern of pain ratings to the blind infusion of different opiates and to placebo supports the clinical diagnosis of psychic dependence on narcotic drugs, ie addiction. Failure of withdrawal symptoms to appear following high-dose naloxone is in keeping with the absence of physical dependence.

9.4 DISCUSSION

Pain is an abstract concept which was recently defined by the International Association for the Study of Pain as (Merskey, 1979) "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Such a definition raises as many questions as it seems to answer (Wall, 1985) but it serves a useful purpose in emphasising the two equally important components of the pain experience: the sensory and emotional elements.

The abdominal pain of AIP is typically that of the acute type associated with the generalised activation state referred to as "flight and fight reaction". Although it can retain such characteristics for a few days, it may acquire features of a chronic pain state if it persists for weeks or months. The development of a chronic pain state will cause the disappearance of the sympathomimetic effects of acute pain and the emergence of what Sternback (1981) has called the "vegetative signs"; namely, social withdrawal, psychomotor retardation, agitated depression, abnormal illness behaviour and associated hypochondriasis. Thus

the manifestations of a chronic pain state in AIP may be readily mistaken as part of the psychological and psychiatric disturbances that can also occur in porphyria (Waldenstrom, 1937; Ackner et al, 1961).

The measurement of pain adopted in this study needs some consideration. The patient with pain conveys his or her distress to others through verbal and non-verbal behaviour. Since there are no direct objective measures of pain observation of this behaviour is the only way towards understanding the patient's private experience. Physiological measures have not been found to be reliable indicators of pain. The methods of brain-evoked potentials (Gracely et al, 1978) and signal detection theory (Chen et al, 1979) are potentially more objective indicators of the subjective experience of pain but are quite cumbersome to perform. To date subjective pain responses have been assessed by language analysis (Melzack, 1975; Leavitt et al, 1977), pain category rating (Gracely et al, 1978), pain estimates (Sternback, 1974) and visual analogue scales (Revill et al, 1976; Scott & Huskisson, 1978). Of these specific descriptive methods which have contributed to a more precise measurement of pain intensity, the visual analogue is considered to be the best for measuring pain clinically (Church, 1979; Rutter et al, 1980; Catling et al, 1980; Swanson & Maruta, 1980) - hence its use in this study.

To validate this technique of VAS, pain ratings were recorded twice at each four-hourly time point ie before and 5 to 10 minutes after the intramuscular injections. And indeed the results obtained with these two sets of pain ratings correlated very well with each other for all patients studied.

Pethidine is the recommended standard narcotic analgesic in acute porphyria because of its proven safety record (Moore & Disler, 1983). But in patients requiring large doses of this drug, it often induces muscle hardening at the injection sites, in part accounting for its variable absorption kinetics (Austin et al, 1980). Therefore the high solubility of diamorphine with twice the potency of morphine, provided an advantage in its administration. An alternative to pethidine and morphine was also sought because of the rapid development of tolerance to and dependency on these opiates (Bond, 1984). Moreover repeated pethidine administration can cause central nervous system hyper-irritability due to an accumulation of the active toxic metabolite, norpethidine (Kaiko et al, 1983).

Buprenorphine, a newly developed mixed agonist-antagonist opiate (Appendix IV) (Harcus et al, 1974; Houde, 1979), was used in the study after it was shown to be clinically as effective as morphine and pethidine (Hayes et al, 1979; Harmer et al, 1983) without producing troublesome psychotomimetic effects. In addition it is reputed to be less addictive than the other opiates (Robbie, 1979; Ventafridda et al, 1983). Like other synthetic opiates it can cause some degree of respiratory depression, but a ceiling-effect seems to be reached at about 1.2 mg in normal volunteers. It has been given in a dose of up to 7.0 mg (equivalent to 200 mg morphine) post-operatively without causing any significant respiratory depression (Orwin, 1977). Furthermore at high dosage its effects on the cardiovascular system are relatively minor compared to morphine and pethidine (Malcolm & Coltart, 1977).

In this study the drugs were administered on a regular basis with the pharmacological objective of maintaining plasma drug level above a minimal effective concentration for pain relief. When a switch was made from the intramuscular route to continuous intravenous infusion the starting dose was calculated as the equivalent for a 24-hour period (Foley, 1982).

Three of the ten porphyric patients studied seemed to have had a pharmacological response to the opiate drugs. Although pethidine was used as our first-line narcotic analgesic, diamorphine and buprenorphine were subsequently found to be as effective in some patients. It is interesting to note however that a beneficial placebo response can be expected to occur in about a third of patients treated for other painful conditions irrespective of the methods employed (Beecher, 1959). And this placebo effect, mediated by the release of endogenous opioids, has been reported to be more marked for moderate to severe pain than for mild pain (Levine et al, 1978). While placebo has been advocated as a therapeutic measure for some categories of pain it may cause considerable harm if the patient finds out the stratagem. This can lead to severe loss of confidence in any further treatment and worse in the clinician. That is why it has been difficult to withhold narcotic drugs from porphyric patients in acute attacks when they complained of severe pain.

In some patients who received large amounts of opiates pain relief was not apparent for at least two to three days after the maximal dosage was reached. This beneficial effect is more likely due to the "glucose effect" induced by the high calorie carbohydrate-rich diet than the analgesic drugs.

Previous studies have shown that intravenous administration of pethidine at a median rate of 25 mg/h/Bwt (Chakravarty et al, 1979), buprenorphine at a median rate of 0.048 mg/h/Bwt (Chakravarty et al, 1979) or morphine in the range of 1.5 to 3 mg/h/BWt (Rutter et al, 1980; Catling et al, 1980; Gourlay & Cousins, 1980; Orr et al, 1981) provided effective analgesia to patients undergoing major abdominal and thoracic surgery. The pain of thoracotomy, reputedly one of the most painful operations, was successfully managed with morphine infusion at a rate of less than 1.5 mg/h/BWt. Yet three of the porphyric patients failed to obtain any pain relief following the intravenous administration of pethidine (67 mg/h/BWt), morphine (4.16 mg/h/BWt), diamorphine (2.9 mg/h/BWt) or buprenorphine (0.17 mg/h/BWt). In one instance, buprenorphine was given intramuscularly to a maximal daily dose of 16.8 mg and was completely ineffective. The pain ratings with placebo were similar to those obtained with the different opiates.

The possibility of tachyphylaxis was avoided by selecting patients who have not had narcotic drugs before the study and by rapidly increasing the dosage to a maximum level compatible with safety. Changing from morphine to pethidine, or vice versa, made no difference to the pain ratings. Neither did the pain get worse when buprenorphine followed either morphine or pethidine. These results thus confirm that the abdominal pain of acute porphyria can be truly resistant to opiate analgesics.

Nitrous oxide analgesia appears to act by a non-opioid dependent mechanism since it is not reversed by naloxone. It has been successfully used in terminally ill patients with refractory pain

(Fosberg & Crone, 1979). But only one of the two porphyric patients showed a mild but definite response. Unfortunately the risk of developing megaloblastic anaemia with this agent further limits its usefulness. However nitrous oxide did not exhibit any porphyrinogenic tendency.

Other methods of pain control that might interfere with the peripheral nervous system, spinal circuits and ascending pathways have been considered (Levine, 1984). For instance, peripheral acting analgesics like paracetamol and non-steroidal anti-inflammatory drugs ie aspirin, ibuprofen, flurbiprofen and indomethacin have been used on many occasions, alone or in combination with opiates without any benefit.

Transcutaneous electrical nerve stimulation is a form of counter-irritation that activates both opioid and non-opioid pain-inhibitory mechanisms (Meyer & Watkins, 1984). Attempt at interrupting the transmission of nociceptive impulses by this method has also failed to relieve the abdominal pain. One patient, JC, was trained in self-induced hypnosis in an attempt to modify her pain experience, but after an initial favourable response the patient gave it up.

9.5 ABNORMAL OPIATE RECEPTOR RESPONSE IN AIP

Failure to control the abdominal pain of AIP with large amounts of opiate analgesics implied an abnormal opioid receptor response. The pharmacological basis for this phenomenon was further investigated.

9.5.1 Case history

In October 1981 in the course of a severe attack the pupils of a porphyric patient (EL, Case 1 in the previous study) were noted to be of normal size despite intravenous administration of large doses of morphine (100 mg/24 h). The pupillary responses to accommodation and to light, both direct and consensual, remained normal.

After the acute attack had settled, a complete ophthalmological examination was carried out in the Eye Department on 19 November 1981. No abnormality was detected. The pupillary diameter was 3.5 mm on the right and 4 mm on the left. Fifteen milligrams of morphine was administered intravenously and the pupillary responses reassessed 45 minutes later. The size and responsiveness of the pupils remained unaltered.

Since 1981 the patient has had chronic abdominal pain and persistent quadriparesis with biochemical evidence of active porphyria. She was last admitted to hospital in September 1985 with a severe attack. Diamorphine was given without much benefit and it was stopped on 11 September 1985. On 14 September 1985, the pupillary responses were again examined before and after administration of diamorphine. Fifteen milligrams of diamorphine were initially given intramuscularly with close monitoring of the patient's pulse, blood pressure and respiratory rate. Pain ratings were also recorded. When no significant effect occurred one hour after the injection, a further dose of 30 mg diamorphine was administered and observations were continued for another four hours.

	After IM diamorphine					
	Baseline	15 mg		30 mg		
Time (h)	0	0.5	1	2	3	4
Pupillary diameter (mm)						
Light response - dim light	5.0	5.0	5	4.5	5.0	4.5
- strong light	3.5	3.0	3.0	3.0	3.0	3.5
Accommodation	3.5	3.5	3.0	3.5	3.5	3.5
Pain rating (0 to 10 scale)	5	5	5	4	4	4
Blood pressure lying	124/76	120/78	126/82	120/85	114/80	112/78
standing	-	-	116/80	110/82	102/78	108/64
Pulse rate (min^{-1})	82	82	80	76	78	74
Respiratory rate $(min)^{-1}$	12	11	12	12	11	11

Table 15: Pupillary responses in a patient with acute porphyria following administration of 45 mg diamorphine given in two successive injections at 1 hourly interval. The effects of the opiate agaonist on pain response, blood pressure, pulse and respiratory rate were simultaneously recorded

9.5.2 RESULTS

Before the administration of diamorphine both pupils were equal in size and reacted briskly to accommodation and to light, both directly and consensually. Diamorphine in a dose of 45 mg failed to induce miosis (Table 15) and the pupillary responses remained unaltered (Fig 34). Pain ratings were minimally improved after the second injection. There was a mild reduction in pulse rate and blood pressure but respiratory rate remained unaffected.
Two hours after the 30 mg morphine injection the patient felt nauseated and vomited once. But she remained remarkably alert and denied any feelings of drowsiness. Her only complaint was that her nose felt unduly itchy.

9.5.3 DISCUSSION

Innervation of the iris is exclusively autonomic. Parasympathetic supply to the pupillary constrictor muscles of the iris is carried by the third cranial nerve via the ciliary ganglion and short ciliary nerves. Sympathetic innervation of the pupillary dilator muscles is provided by the long ciliary nerve, a branch of the trigeminal nerve (Fig 35). The light and accommodation reflexes depend on the preservation of both parasympathetic and sympathetic pathways as well as the optic nerve pathways.
Opioid receptors have been described in the inner plexiform and ganglion layers of the retina (Wamsley et al, 1979). They are also found in high concentrations in the pre-optic areas of the mid-brain tectum which receive afferents from the retina. Since section of the optic nerve abolishes morphine-induced miosis while

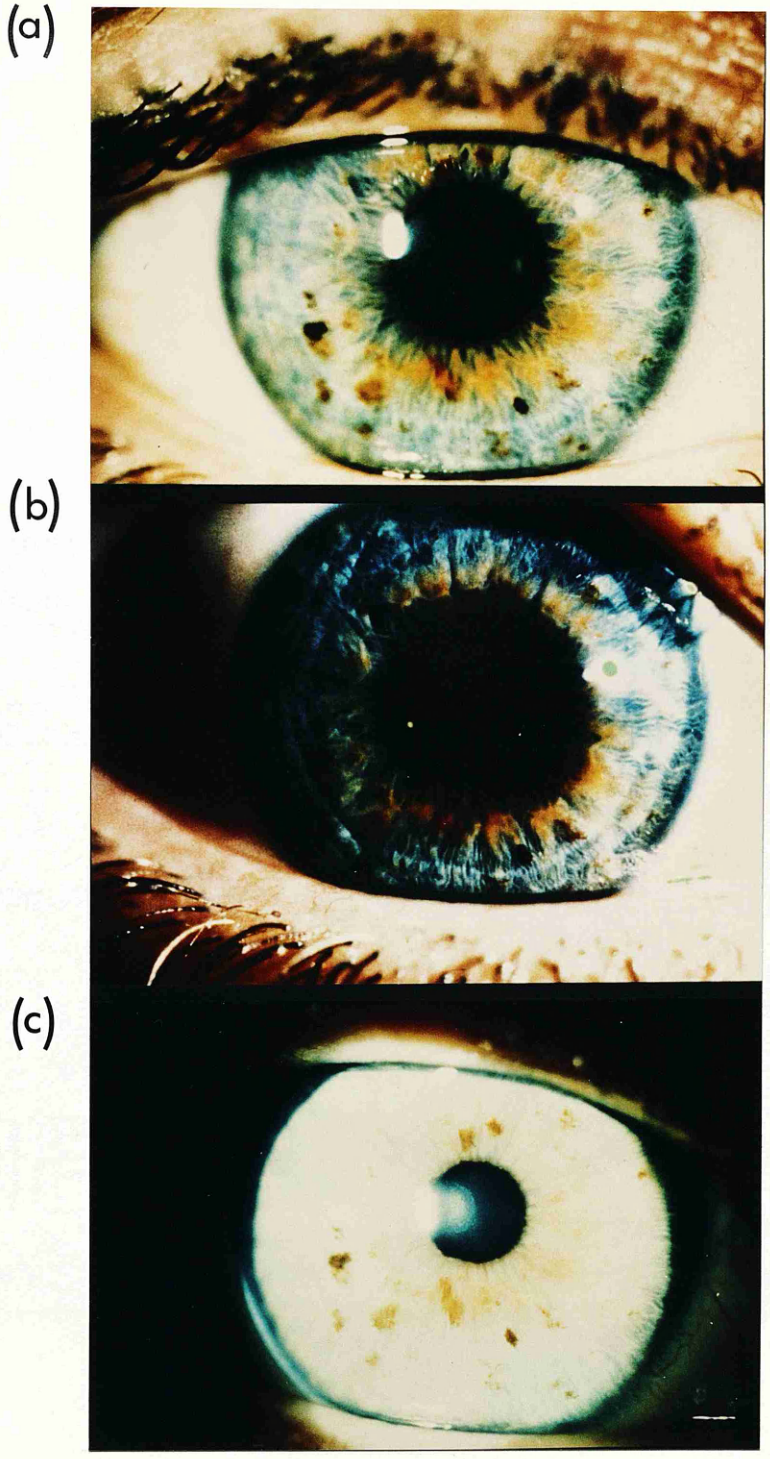


Figure 34: Pupillary responses of AIP patient E.L. after IM administration of 45 mg diamorphine. Patient was comfortable seated at a Haag-Streit slip lamp and photographs of the right pupil were taken successively at the same magnification in one sitting.
(a) in ordinary room-lighting.
(b) after about 10 seconds in complete darkness, showing rapid pupillary dilatation.
(c) responding consensually and briskly to light shone into the left eye.

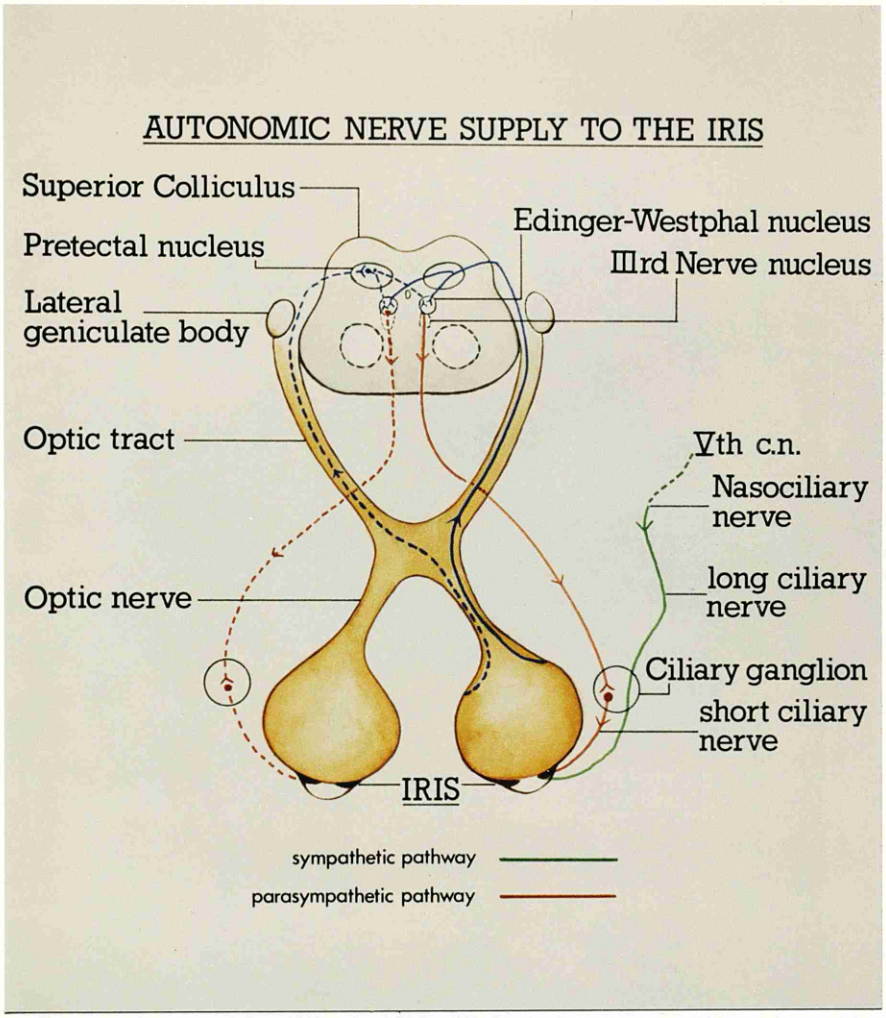


Figure 35: Schematic representation of visual pathways to the mid-brain and autonomic innervation of the iris. Normal pupillary responses to light and accommodation indicate that these pathways are intact.

the consensual light response in the same eye is still preserved (McCrea et al, 1942; Duke-Elder, 1962) local activation of these opioid receptors rather than direct central stimulation would seem to play an important role in its mechanism (Atweh & Kuhar, 1983). Tress and El-Sorby (1979) have shown that although tolerance develops to the miotic effects of diamorphine in drug addicts, miosis can still be induced with a fraction of the daily dose on which they are dependent. Not only was the dose used in the present study much larger than what the patient had been receiving before, but she had no other features to suggest drug dependence. Previously she had been given naloxone without having any withdrawal reactions, and following discharge from hospital she did not require any opiate drugs for maintenance. Failure of diamorphine to induce miosis in the porphyric patient while preserving both light and accommodation reflexes suggests that opioid receptors in the optic system were either non-responsive or depleted.

Morphine mediates its miotic, analgesic and respiratory depressant effects by activation of the mu opioid receptors (Appendix IV). The lack of pain response to such a large dose of diamorphine while maintaining a normal respiratory rate and an alert state of consciousness supports the conclusion derived from the pupillary responses.

A more pronounced depressant cardiovascular effect might have been expected from the dose of diamorphine administered but there was only a slight reduction in the pulse and blood pressure. Endogenous opioids can lower blood pressure - an effect reversible

with naloxone (Lambie et al, 1977; Rubin et al, 1981). But morphine-induced hypotension appears to involve other non-opioid dependent mechanisms interacting with the autonomic system (Mcqueen, 1983). Therefore the explanation for the changes in pulse and blood pressure remains conjectural. It is noteworthy however that in the previous studies in which naloxone was administered after infusion of opioid agonists no rebound hypertension was noted.

Inhibition of pain by endogenous opioids is normally not a tonic phenomenon. It requires a pre-existing noxious stimulus to release the endogenous opioids which then activate the system. This is why naloxone administration to someone not in pain does not cause hyperalgesia. But if opioids, whether endogenous or exogenous, are effectively relieving pain administration of naloxone in sufficient amounts will lead to an exacerbation of pain (Levine et al, 1979; Watkins & Meyer, 1982). Thus failure of naloxone to worsen the pain in the porphyric patients lends further credence to the possibility of underlying abnormal or depleted opioid receptors.

The vagus nerves and their associated brainstem nuclei are important structures in the regulation of autonomic reflexes; for example, cardiovascular control. Damage to these structures can cause neurogenic hypertension - as is the case in acute porphyria (Chapter 8). Opiate receptors have been located along the afferent fibres of the vagus nerves. They are also found in high concentrations in the nucleus tractus solitarius and nucleus commissuralis which receive visceral sensory fibres from the vagus

and glossopharyngeal nerves (Atweh & Kuhar, 1977, 1983; Snyder, 1977). The experimental findings of Atweh and his associates (1978) are therefore particularly relevant in this context. They have reported that lesions of the vagus nerves led to a significant loss of opiate receptors in all parts of the vagal system including the nuclei. Since degeneration of the vagus nerves and their nuclei is a recognised morphological feature in patients who have died from acute porphyria (Mason et al, 1933; Denny Brown & Sciarra, 1945; Gibson & Goldberg, 1956; Hierons, 1957; Ten Eyck, 1961; Jedrzejowska et al, 1974), it is conceivable that porphyric neuropathy might cause a loss of opioid receptors in the brainstem nuclei. This would not only explain the mechanism of neurogenic hypertension in acute porphyria but also the lack of pain sensitivity to opiate analgesics. A similar train of events in the optic nervous system would account for the present observations on pupillary responses.

The feedback regulation of receptor function and eventually of receptor number occurs with a wide variety of neurotransmitters and hormone receptors (Davies et al, 1982; Snavely et al, 1983; Snavely et al, 1985; Lefkowitz et al, 1984). This phenomenon of receptor desensitisation, either by "down-regulation" or by covalent modification e.g. by phosphorylation (Lefkowitz et al, 1984) could offer an alternative explanation for the lack of response to opioid agonists. With intense chronic pain, release of endogenous opioids might cause rapid desensitisation of opioid receptors. By analogy patients with phaeochromocytoma have a normal resting pulse and blood pressure when circulating plasma

catecholamines are greatly increased. In these patients blood pressure response to noradrenaline infusion is muted but not absent (Davies et al, 1982). Thus in desensitisation some response is still expected to occur with the administration of large doses of the agonist, and this should at least show partial reversibility to a specific antagonist with high receptor affinity. The data currently available in the pain studies, although limited, do not support this possibility.

A third possibility to consider is that the porphyrin precurors, especially ALA, or some abnormal metabolite could interfere with opioid receptors. Since urinary excretion of porphyrin precursors does not correlate with the presence or severity of abdominal pain in individual patients having recurrent attacks, as encountered in several of the patients studied, this possibility seems less likely. Indeed, latent cases can excrete large amounts of ALA and PBG and yet remain asymptomatic. Moreover removal of these precursors by haemodialysis and haemoperfusion did not improve the pain (Chapter 10).

Further work is required to define the abnormal opioid receptor response that has been demonstrated in some porphyric patients in acute attacks. Such studies will probably have to take into account the possibility of subtype and tissue-specific "down-regulation" as exists for alpha- and beta-adrenergic receptors (Snavely et al,1983).

Other than the nervous system the gut (Polack et al, 1977; Konturek, 1978; Orwell & Kendall, 1980) also has a rich supply of opioid peptides and opiate receptors. Opioid peptides form an

important group of chemical messengers in a widespread and complex inhibitory system (Duggan, 1983; Watkins & Meyer, 1982) which modulate the transmission of nociceptive impulses from the viscera and peripheral sensory receptors. An abnormality of opioid receptors in the gut could provide a possible explanation as to why pain is primarily abdominal in acute porphyria. Indeed morphological degenerative changes have been described in the coeliac ganglia of these patients (Gibson & Goldberg, 1956; Hierons, 1957). The abnormal opioid receptor hypothesis does not however explain the nature of the initial nociceptive stimulus. Whether this is also due to the porphyric neuropathy, in a manner analogous to diabetic neuropathic pain, or to a circulating metabolic product is a matter of further speculation.

SECTION II

CHAPTER 10

CHARCOAL HAEMOPERFUSION AND HAEMODIALYSIS IN AIP – A CASE REPORT

10. CHARCOAL HAEMOPERFUSION AND HAEMODIALYSIS IN AIP - A CASE REPORT

10.1 INTRODUCTION

The clinical manifestations of AIP have been explained on the basis of neurological dysfunction probably caused by the increased concentrations of circulating ALA. Charcoal haemoperfusion has been advocated as a potential means of removing ALA (Tishler et al, 1982; Tishler & Gordon, 1983). This treatment was tried in the following patient.

Case report

A 34 year old woman presented in 1974 with severe abdominal pain; she was taking the contraceptive pill. Increased urinary concentrations of ALA and PBG confirmed the diagnosis of AIP. After the contraceptive pill was stopped she continued to have recurrent episodes of abdominal pain lasting for up to a week. In 1981 after drinking some wine she developed a severe attack of porphyria with hypertension and peripheral motor neuropathy. Treatment with intravenous haematin produced little clinical benefit, and the attacks persisted for two months. Thereafter the attacks became more frequent and prolonged without any precipitating factor. Her most recent attack before this admission had continued for seven months, being unresponsive to intensive conventional treatment and to high doses of propranolol, vitamin B complex and folic acid. Pain was difficult to control despite administration of large parenteral doses of narcotic analgesics.

After gaining written informed consent haemoperfusion was carried out with a 300 g activated charcoal column (Adsorba 300; Gambro, Sweden) in series with haemodialysis for two hours daily on four consecutive days. Figure 36 shows the effect of this treatment on mean daily pain scores and serum and urinary concentrations of ALA and PBG. Haemoperfusion caused considerable thrombocytopenia, as previously reported with this type of treatment. The platelet count, measured daily, fell from 204 x 10^9/l to 161 x 10^9/l after the first haemoperfusion and then steadily to 75 x 10^9/l after the fourth haemoperfusion, when treatment was stopped; but it had returned to normal by the seventh day after perfusion.

10.2 DISCUSSION

The link between the biochemical disturbance of synthesis of haem and the pathogenesis of pain in acute intermittent porphyria has remained speculative. A strong association exists, however, between raised serum concentrations of ALA and neurodysfunction. All four types of acute porphyria, in which concentrations of ALA are raised, are characterised by neurological dysfunction whereas the non-acute porphyrias, which have normal concentrations of ALA are not. Furthermore, peripheral neuropathy is a feature of lead poisoning (Dagg et al, 1964) and hereditary tyrosinaemia (Gentz et al, 1969) in which blood concentrations of ALA are raised. Tishler et al (1982, 1983) have reported that activated charcoal cartridges effectively removed large amounts of porphyrins and their precursors from solution in vitro, these amounts being considerably greater than estimated daily production during an

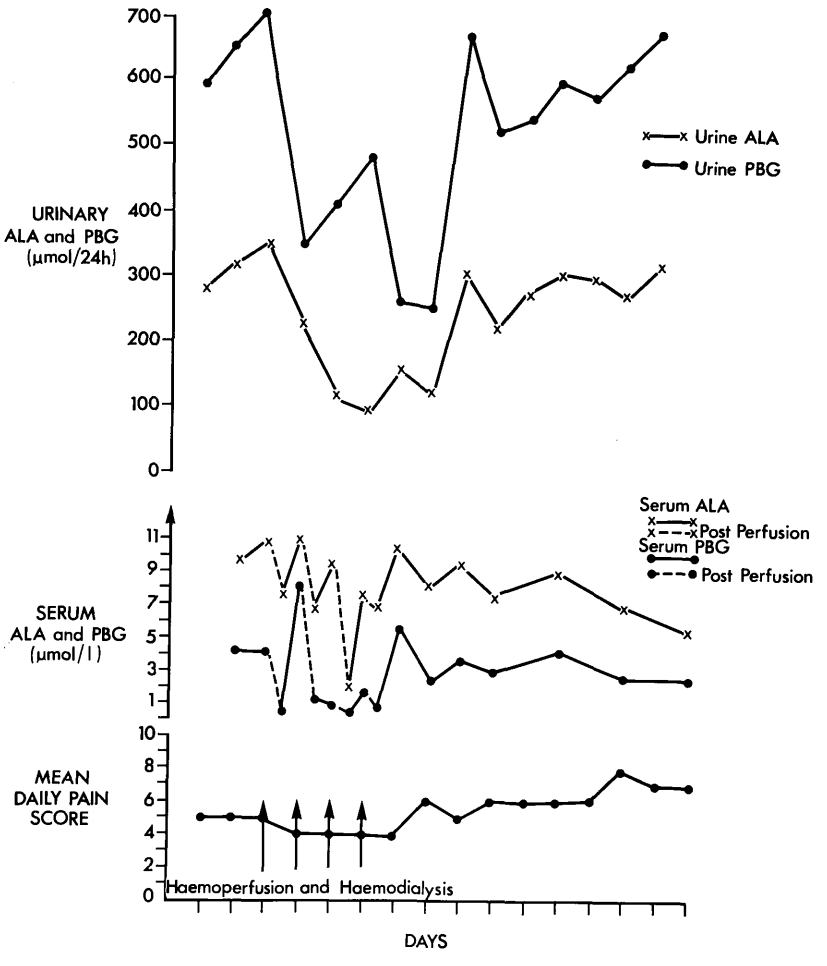


Figure 36: Sequential measurements of urinary and serum concentrations of ALA and PBG in relation to mean daily pain score before and after four days of treatment with haemoperfusion and haemodialysis.
Broken lines indicate concentrations immediately after perfusion.

attack of porphyria. The removal of ALA and PBG, and possibly other neurotoxic compounds, by haemoperfusion through such sorbents might favourably alter the course of acute attacks.

In this study haemoperfusion with activated charcoal columns coupled with haemodialysis considerably reduced serum concentrations of ALA and PBG with a concomitant decrease in urinary porphyrin precursors. But serum concentrations had returned to pretreatment values within 24 hours and abdominal pain was not relieved after four days' treatment. The possibility that a longer course of treatment might have had a more favourable outcome cannot be excluded but this seems unlikely in view of the rapid rebound of serum concentration of ALA after each haemoperfusion. Furthermore, the resulting thrombocytopenia might present a major limiting factor to such treatment.

SECTION III

CHAPTER 11

CARBAMAZEPINE-INDUCED NON-HEREDITARY ACUTE PORPHYRIA

11. CARBAMAZEPINE-INDUCED NON-HEREDITARY ACUTE PORPHYRIA

11.1 INTRODUCTION

In AIP as a consequence of relative haem depletion the activity of the first and rate-limiting enzyme of the pathway, ALA.S, is increased, and excessive amounts of ALA and PBG accumulate in blood and urine. Several types of pyrrolic disturbances have already been produced de novo by drugs and toxins. For instance, many halogenated aromatic hydrocarbons, the best known being hexachlorobenzene, can cause cutaneous hepatic porphyria in all its clinical and biochemical manifestations, in both man and experimental animals by inhibiting uroporphyrinogen decarboxylase activity (Schmid, 1960; Ockner & Schmid, 1961). But there is still no satisfactory animal model for the human acute hepatic porphyrias. Although various chemicals such as AIA and griseofulvin have been shown to induce some of the porphyrin abnormalities seen in the acute porphyrias, none of the models proposed can reproduce any of the neuro-psychiatric features characteristic of these diseases (Smith & De Matteis, 1980). A case of toxic non-hereditary acute porphyhria following carbamazepine treatment of epilepsy is described for the first time, and its relevance as a possible human model of porphyria subsequently discussed.

11.2 PATIENTS AND METHODS

Case 1

A 38-year-old mentally handicapped man was admitted to the Western Infirmary, Glasgow, in January 1982, for investigation of AIP (Fig 37). He had sustained brain damage after contracting measles encephalitis at age 3 years and had soon begun to have frequent generalised tonic-clonic seizures. Throughout his childhood and adolescence epileptic control had been difficult, despite combination therapy with primidone and phenytoin. Carbamazepine was added to his medication in a daily dose of 600 mg when he was 28. Soon afterwards he experienced several episodes of unexplained general malaise and fever. In June 1980, he received a 9-month course of antituberculous chemotherapy for a prolonged bout of fever with radiological changes at the apex of his right lung, although tubercle bacilli were not isolated. At that time phenytoin was replaced by sodium valproate.

In October 1981, the dose of carbamazepine was increased to 1200 mg daily, but seizure activity increased despite theoretically satisfactory drug concentrations. He became unwell and anorexic. He then experienced diffuse abdominal pain accompanied by frequent bouts of vomiting; he also had intermittent dysphagia. Haemoglobin was 13.4 g/dl and serum amylase was normal. Upper gastrointestinal endoscopy, barium enema, and ultrasound scan of the liver and biliary tree were normal. At this time the passage of a dark urine raised the possibility of AIP. There was no family history of porphyria. On physical examination he looked toxic. Blood pressure was 120/55 mm Hg, pulse 118/min (sinus

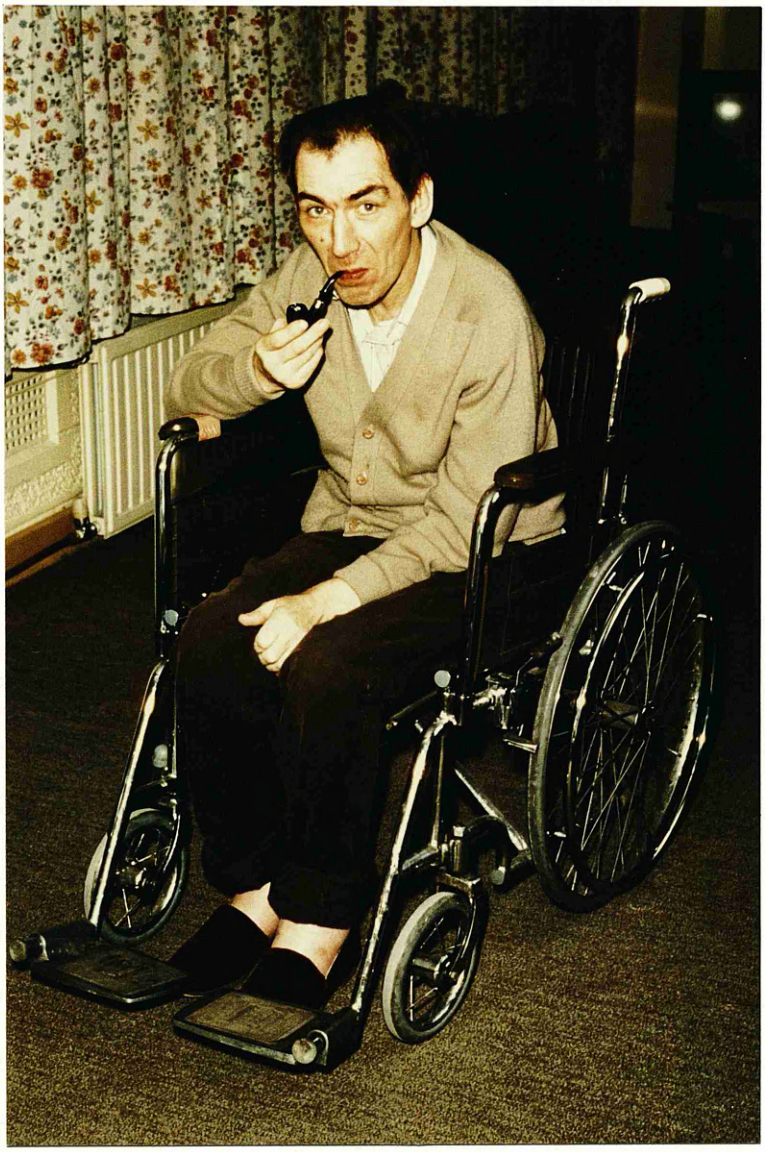


Figure 37: Patient fully recovered from acute attack of porphyria following withdrawal of carbamazepine.

rhythm), respiratory rate 24/min, temperature 37.8oC. He had no lymphadenopathy or skin abnormality. Although he seemed to have abdominal pain, there were few clinical signs in the abdomen. Neurological assessment revealed a mild degree of longstanding left-sided spastic hemiparesis; no nystagmus or signs of peripheral neuropathy were noted. Sensory perception seemed unimpaired, but detailed testing was not possible.

Laboratory data revealed an erythrocyte sedimentation rate of 32 mm in the 1st hour and a mild normochromic normocytic anaemia. Haemoglobin was 10.8 g/dl, white-cell count 8600/ul, platelets 352,000/ul. Reticulocyte count was repeatedly less than 1%. Serum ferritin and vitamin B_{12} levels were normal. Whole-blood folate was initially low at 74 ng/ml but after correction with folic acid supplements the haemoglobin level remained unchanged. Tests for faecal occult blood were negative. Serum sodium was 128 mmol/l. Serum aspartate aminotransferase was 63 U/l and alkaline phosphatase 101 U/l (normal 21-92 U/l). Bacteriological investigation for a low-grade pyrexia of unknown origin, including several blood, urine, and stool cultures, was negative.

Because AIP was supected, the anticonvulsants were gradually withdrawn. This resulted in a temporary worsening of the epilepsy, which necessitated the re-introduction of primidone and sodium valproate. Cessation of carbamazepine therapy resulted in a clear urine and the disappearance of fever, vomiting, and abdominal pain. The mild anaemia also improved.

Case 2

Whereas case 1 displayed both the clinical and biochemical features of AIP after treatment with carbamazepine, the following

patient was symptom-free but had a lowered erythrocyte URO.S activity, as seen in AIP. A 34-year-old woman had severe generalised tonic-clonic epilepsy from age 4 years as a result of birth injury. Epileptic control had previous been very difficult but for 2 years has been satisfactorily maintained with a combination of sodium valproate, phenytoin, and carbamazepine, the last in a daily dose of 1200 mg. She has no abdominal pain or other features suggestive of AIP. No family history of porphyria was obtained. The serum concentrations of all three anticonvulsant drugs were closely monitored and maintained within the recommended therapeutic range.

Epileptic Group Study

Blood samples collected from 53 epileptic patients on a variety of drug regimens and 53 drug-free healthy controls matched for age and sex were assayed for URO.S activity. Nineteen of the epileptic patients were treated with carbamazepine alone, 14 with carbamazepine in combination with other anticonvulsants (phenytoin, phenobarbitone, and sodium valproate), 10 with single-drug anticonvulsant therapy other than carbamazepine, and 10 with combination therapy excluding carbamazepine. The results of the URO.S assays were compared with those of the controls with the Mann-Whitney U test. URO.S activities from a group of 13 patients with symptoms of AIP were included for comparison with the study groups.

Methods

Urinary ALA, PBG and porphyrins; leucocyte ALA.S and erythrocyte URO.S were measured using standard methods.

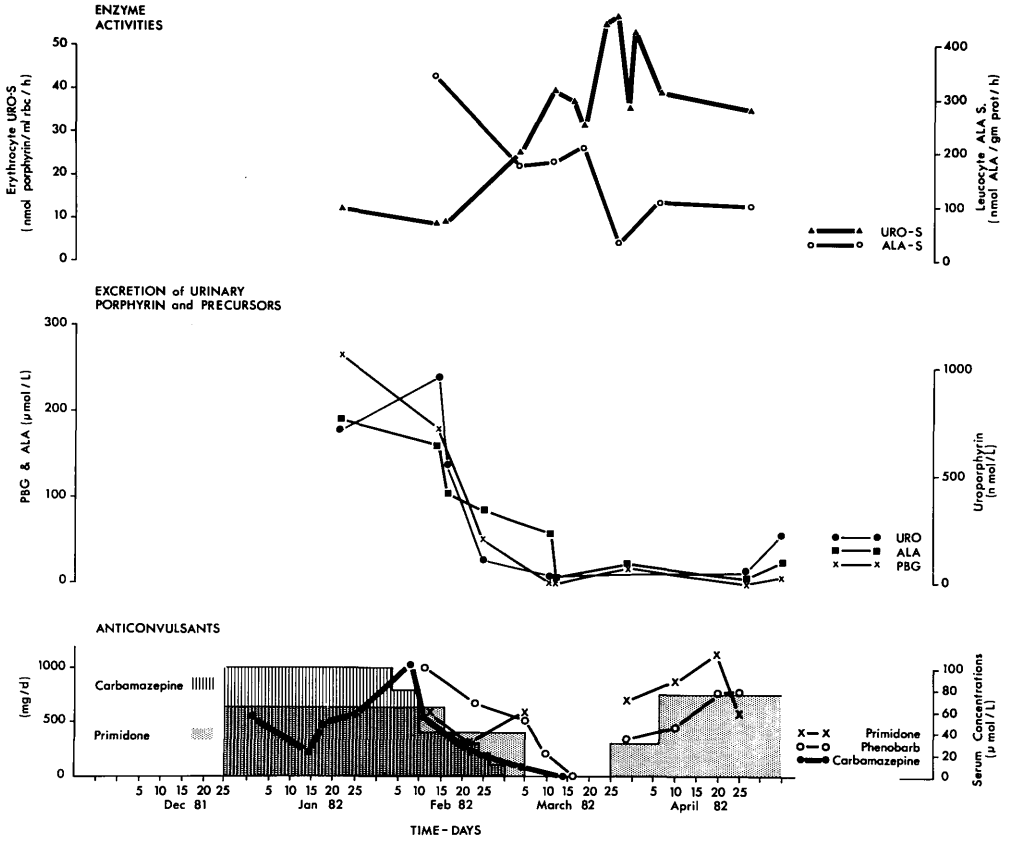


Figure 38: Time course of haem biosynthetic enzyme activities and urinary ALA, PBG, and uroporphyrin excretion following alterations in anticonvulsant therapy.

	Erythrocyte URO.S*	Leucocyte ALA.S**	Urinary ALA (umol/l)	Urinary OBG (umol/l)	Urinary uroporphyrin (nmol/l)
Case 1					
Patient	9	394	171	194	1022
Mother	30	103	0	6	0
Father	29	..	..	..	..
Case 2					
Patient	18	210	10	10	0
(repeat sample)	16	256	5	0	0
Mother	35	181	14	4	0
Father	32	117	0	0	0
Normal ranges	24-54	20-279	0-40	0-16	0-49

* nmol porphyrins formed/ml red cells/h
** nmol ALA/g protein/h

Table 16: Family studies: Enzyme activities and urinary porphyrin precursors and uroporphyrin

uroporphyrin levels were normal, but her HPLC excretion pattern of uroporphyrin, and penta-, hexa- and hepta-carboxylic porphyrins was qualitatively similar to that obtained from AIP patients (Fig 39). Yet, both her parents had normal HPLC porphyrin excretion profiles.

Epileptic Groups Study

The mean erythrocyte URO.S activity was significantly lower in the patients treated with carbamazepine alone (24.8±5.0 ISD nmol porphyrins formed/ml red cells/h) than in the control group (35.8±1.1 nmol porphyrins formed/ml red cells/h) ($p < 0.001$) (Fig 40). URO.S activity was lowest in the group treated with carbamazepine in combination with other anticonvulsants (20.1±10.1 nmol porphyrins formed/ml red cells/h, $p < 0.001$). A small but significant reduction in URO.S activity was also noted in patients treated with anticonvulsants other than carbamazepine, either singly (28.5±5.6 nmol porphyrins formed/ml red cells/h, $p < 0.01$) or in combination (31.0±7.8 nmol porphyrins formed/ml red cells/h, $p < 0.05$).

11.4 DISCUSSION

Of the various neuropsychiatric manifestations of acute porphyria generalised tonic-clonic seizures can occur either alone or as an accompaniment of an organic brain syndrome (Waldenström, 1957; Brodie & Goldberg, 1980). Despite their diverse chemical structures a large number of drugs have been suspected of precipitating an acute attack of AIP (Moore & Disler, 1983). The porphyrinogenicity of such drugs, which seems to depend in part on

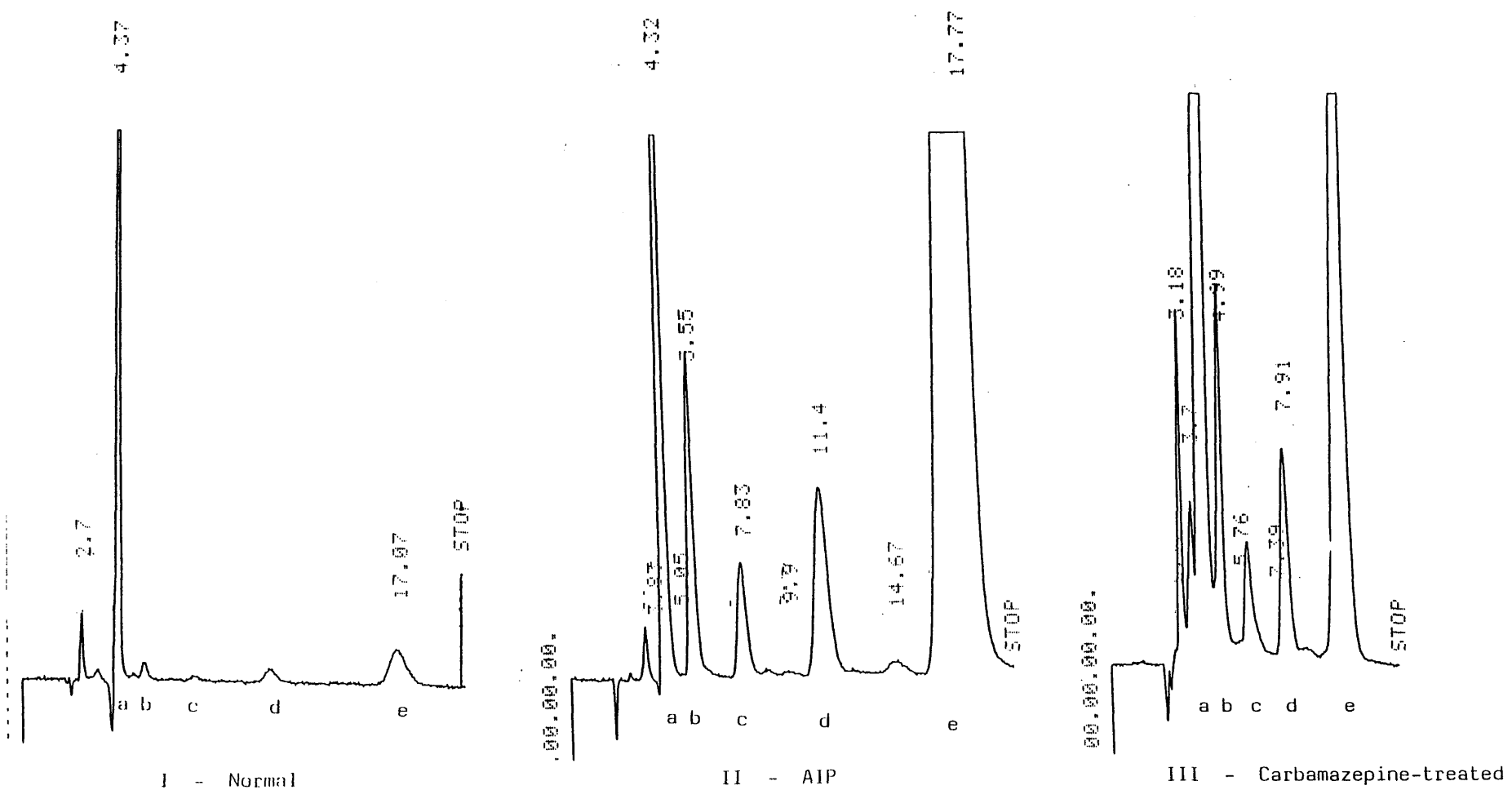


Figure 39: HPLC tracings of concentrated urine (x 50 for I; x 10 for II; x 25 for III) from normal subject, AIP patient and carbamazepine-treated patient demonstrating porphyhrin excretion pattern. Peaks represented by a = coproporphyrins; b = penta-; c = hexa-; d = hepta-; e = uroporphyrins. Excrétion pattern of AIP shows similar characteristics to that of carbamazepine-treated patient.

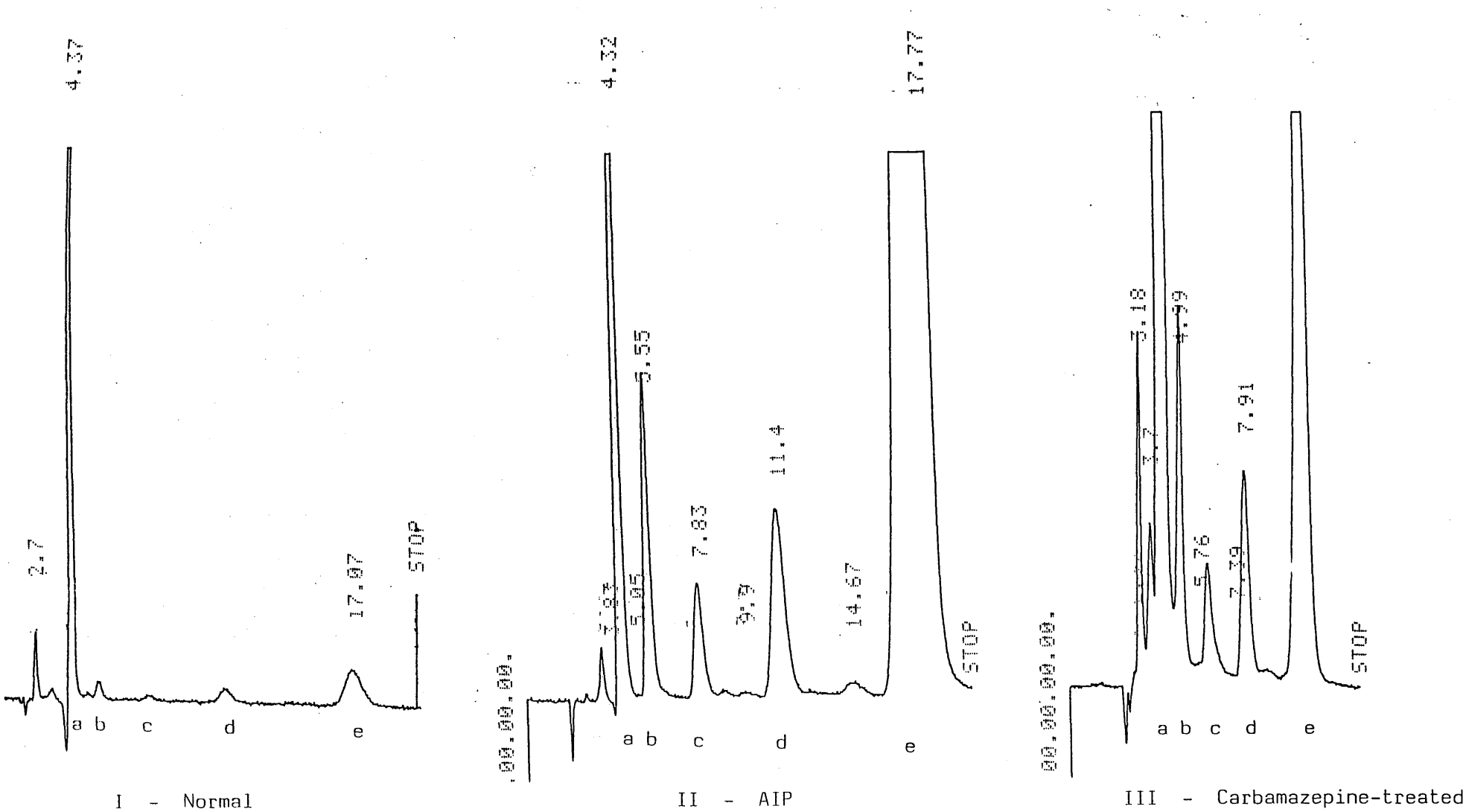
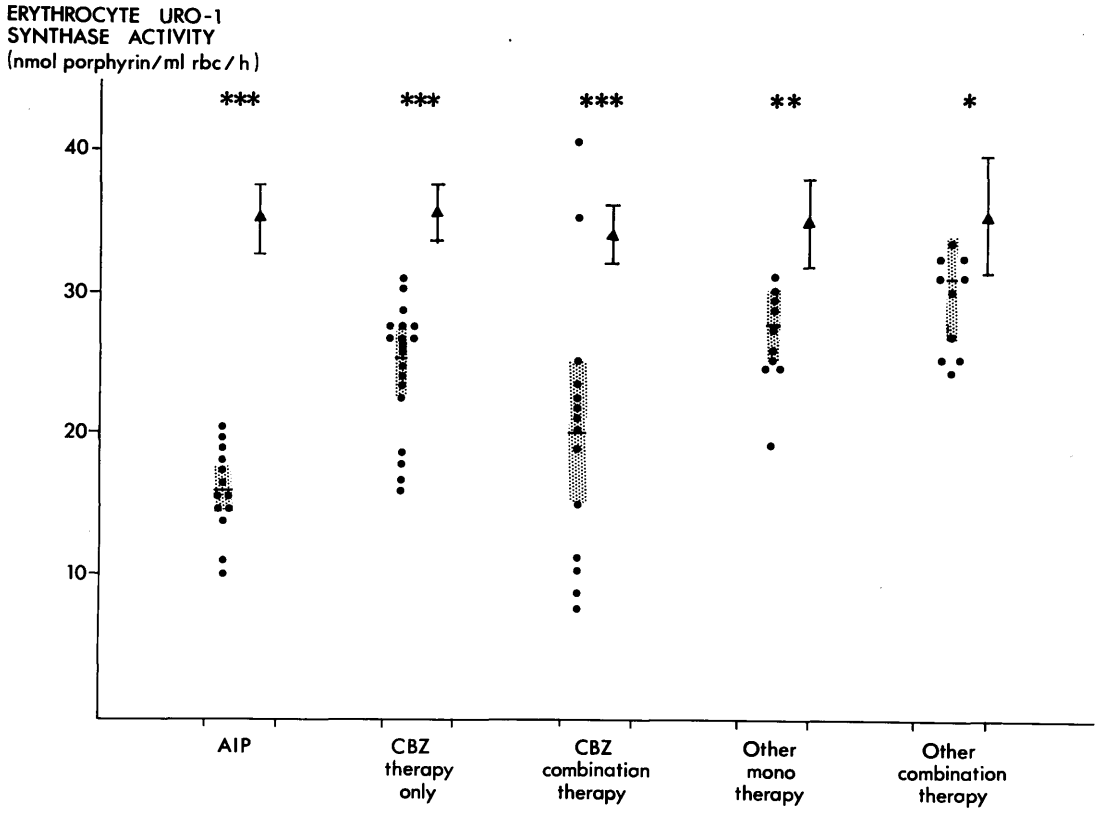


Figure 39: HPLC tracings of concentrated urine (x 50 for I; x 10 for II; x 25 for III) from normal subject, AIP patient and carbamazepine-treated patient demonstrating porphyhrin excretion pattern. Peaks represented by a = coproporphyrins; b = penta-; c = hexa-; d = hepta-; e = uroporphyrins. Excrétion pattern of AIP shows similar characteristics to that of carbamazepine-treated patient.



AIP = Acute intermittent porphyria. CBZ = carbamazepine.
Mean values ± 1 SD.
*$p < 0.005$; **$p \leq 0.01$; ***$p \leq 0.001$.

Figure 40: Erythrocyte URO.S activities in a group of AIP patients and in epileptic patients on different regimens of anticonvulsant therapy (●) and control (I).

their lipophilic properties (Murphy et al, 1975) has been attributed, in some cases, to their ability to induce the cytochrome P450-dependent mono-oxygenase system necessary for their metabolism in the liver (Moore et al, 1970). A partial block in haem biosynthesis can in effect lower intracellular haem concentrations which may then be further depleted by an increase in requirement of haemoprotein for hepatic mono-oxygenase synthesis. As a consequence further de-repression of ALA.S will occur. In case 1 the clinical and biochemical features at presentation were typical of AIP. Despite the past history of brain damage from measles encephalitis the diagnostic problem centred on whether the patient's uncontrolled epilepsy might have been due to an exacerbation of acute porphyria induced by inappropriate anticonvulsant therapy. Barbiturates, carbamazepine and sodium valproate have all been suspected of being porphyrinogenic because they can induce ALA.S activity both _in vivo_ and _in vitro_ (Magnussen et al, 1975; Larson et al, 1978; Bonkowsky et al, 1980). In the hereditary type of AIP URO.S activity would have been expected to remain unchanged after withdrawal of carbamazepine, but in case 1 it rapidly returned to a high normal level. Moreover, after reintroduction of an acceptable therapeutic dose of primidone (which is converted to phenobarbitone in vivo) and sodium valproate, erythrocyte URO.S activity remained normal and leucocyte ALA.S activity failed to increase. This suggests that the increased requirement of cytochrome P450 provoked by the enzyme inducers carbamazepine and primidone is unlikely _by itself_ to account for the pathogenesis of the acute porphyria described.

Although it would have been desirable to carry out a more extensive family study on case 1, this was not possible. But after discussion of the case with the general practitioner looking after the other members of the family and many of their close relations, no suggestion of any porphyric illness was evident amongst them. Being unable to settle beyond doubt the possibility of illegitimacy for case 1, inclusion of the family study on case 2 supports the argument that the porphyrin abnormalties observed are carbamazepine-induced and not due to some rare variants of inherited AIP previously described (Mustajoki, 1981).

The lowered URO.S activity is probably due to a direct depressant effect of carbamazepine on this enzyme system. This possibility is strongly supported by the family studies carried out and by the HPLC excretion pattern of urinary porphyrins from case 1. Whereas the measurement of urinary ALA and PBG is rather insensitive in detecting latent porphyria concurrent assay of erythrocyte URO.S and leucocyte ALA.S activities is much more specific. In this way McColl et al (1982) showed that 49% of 35 symptom free first degree blood relatives of AIP patients had the porphyric enzyme pattern with no sex bias. It is therefore important that results of both urinary porphyrin screening and blood enzymatic assays were normal in the parents. In addition erythrocyte URO.S activity was found to be significantly reduced in epileptic patients receiving carbamazepine. Erythrocyte URO.S activity was further depressed when carbamazepine was given in combination with other anticonvulsants such as phenobarbitone and phenytoin. Surprisingly the latter also appeared to exert a smaller but

significant depressant effect on this enzyme system when given alone. Sulphonamides which are known to provoke attacks of AIP have been shown to inhibit rat hepatic URO.S activity in vitro (Peters et al, 1980). It is still unclear whether the suppressive effect of these drugs on URO.S activity is a consequence of their capability to increase haem requirement by way of induction of the mono-oxygenase enzyme system or an entirely independent property. Although the molecular mechanism by which carbamazepine suppresses URO.S activity remains to be elucidated, the pathogenesis of the acute porphyria in case 1 can still be adequately explained. After the administration of carbamazepine at a high dosage a potent depressant action was exerted on the haem biosynthetic pathway at the level of URO.S. As in hereditary AIP, this partial enzymatic defect produced clinical manifestations only when the system became overloaded with additional porphyrinogenic factors in the form of combination anticonvulsant therapy and reduced carbohydrate intake due to anorexia and vomiting. Carbamazepine itself is also a potent enzyme inducer in man (Rapeport et al, 1983).

Case 1 probably illustrates a genuine case of non-hereditary "Haemato-porphyria acuta toxica" which Günther in 1911 had hoped to differentiate from the familial type of acute porphyria exacerbated by drugs. It might seem surprising that this complication of carbamazepine has not been reported before, considering that a considerable number of patients receiving this drug have erythrocyte URO.S levels within the AIP range. Perhaps it has not been recognised as such before since the problem is

compounded by the knowledge that enzyme-inducing drugs such as carbamazepine can precipitate an acute attack of porphyria. Moreover the majority of latent AIP cases are indeed asymptomatic, and are only brought to light by family screening of overt cases (McColl et al, 1982). In this regard, it is interesting to note that in earlier reports the incidence of acute porphyria in patients prescribed the now obsolete hypnotic drugs sulphonal and trional seemed higher than would have been expected from mere activation of the latent form of AIP (With, 1971). It is therefore probably that drugs other than carbamazepine will produce an acute form of porphyria just as toxic forms of cutaneous hepatic porphyria may be caused by hexachlorobenzene and other polyhalogenated compounds. It is interesting to note that Fuchs et al (1980) described another case of toxic non-hereditary acute porphyria in a 21-year-old man whose illness appeared to have been induced by a combination of phenytoin and phenobarbital.

FURTHER STUDIES OF CARBAMAZEPINE ON HAEM BIOSYNTHESIS

11.5 INTRODUCTION

Further studies were carried out to elucidate the mechanism by which carbamazepine interfered with the haem biosynthetic pathway. It was particularly important to investigate the possibility that carbamazepine might have interfered with the assay of erythrocyte URO.S *in vitro*. And since a depression of URO.S activity in the red blood cells does not necessarily accompany a similar decrease in the liver, as would be required

for the induction of acute porphyria, further evidence for a hepatic URO.S defect was sought. As direct measurement of hepatic URO.S would have required tissue biopsy and was therefore not practical indirect evidence for such a defect was obtained in carbamazepine-treated subjects by measuring their urinary excretion products of porphyrins and precursors.

11.6 PATIENTS AND METHODS

Study 1

Until now carbamazepine has been unavailable in a parenteral form. An *in vitro* cross-incubation experiment was therefore performed using fresh serum obtaining from carbamazepine-treated patients with depressed erythrocyte URO.S activity - case 2 of the previous study. The patient's serum was incubated at different time intervals with erythrocytes from two age- and sex-matched healthy controls of the same blood group. Similarly the patient's erythrocytes were incubated with sera from the controls. The erythrocytes were thoroughly washed three times with normal phosphate buffered saline (PH 7.4) prior to incubation at 37°C. Measurement of URO.S activity was carried out by the standard method.

Study 2 Normal volunteer study

Six healthy male Caucasian subjects were given carbamazepine 400 mg at night for a 22-day period. None of the subjects had evidence of hepatic, renal or haematological disease. Baseline haematology, electrolytes, liver function tests and blood lead concentrations were in the normal reference range. No other drugs

were taken in the month preceding the study or during the study period. Alcohol consumption was not allowed during the study. Only one subject smoked cigarettes. Approval was obtained from the Western Infirmary Ethical Committee and all subjects gave written informed consent.

The subjects were studied one week before treatment with carbamazepine (day 0), and after the fifth (day 4), fifteenth (week 2) and twenty-second (week 3) doses and three weeks after the drug was discontinued (week 6). They were fasted overnight and venous blood was collected for URO.S and ALA.S activities at 0900 h on each occasion. Urine was collected over 24-h periods and measurements of urinary porphyrins and precursors were performed. In addition the pattern of urinary porphyrin excretion was sequentially measured by HPLC method.

11.7 RESULTS

Study 1

As shown in Tables 17a and 17b, the measurement of URO.S activity was unaffected by the presence of carbamazepine contained in serum from Case 2.

Study 2

Significant increases in leucocyte ALA.S activity was noted on the fourth day and were maximal on the seventh day. ALA.S activity slowly returned toward pre-treatment values during the subsequent treatment period, but all values remained elevated above baseline at all time points while carbamazepine treatment continued (Fig 41).

Erythrocyte	In vitro incubation time (hrs)	+ Buffer solution (control)	+ Patient's serum containing carbamazepine (42 umol/L)
Epileptic Patient - Case 2	0	20.4	21.0
	1	18.1	20.7
	17	18.1	17.0
	25	16.0	14.0
	41	15.8	13.0
First Normal Control	0	47.4	53.4
	1	43.9	49.4
	17	45.7	47.8
	25	43.6	42.9
	41	43.4	42.0
Second Normal Control	0	36.0	39.0
	1	36.4	38.6
	17	37.4	37.7
	25	35.2	35.2
	41	38.3	36.3

Table 17a: Erythrocyte URO.S results after incubation at different time intervals

	In vitro incubation time (hr)	Incubated with serum from First normal control	Second normal control	Case 2 (autologous serum)
	0	21.4	20.4	18.6
Washed	1	19.5	19.3	21.2
erythrocytes	17	19.6	18.7	21.5
from	25	20.7	20.6	20.9
Case 2	41	18.3	20.3	18.8

Table 17b: Erythrocyte URO.S results following incubation at different time levels

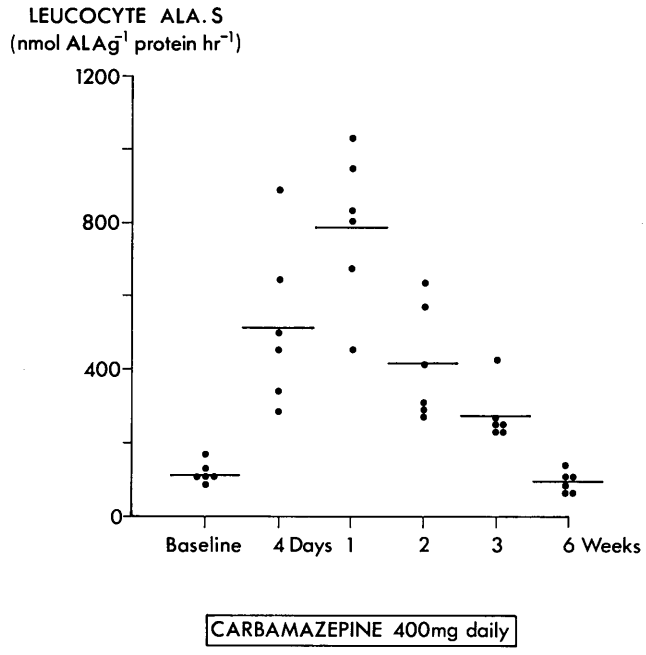


Figure 41: Leucocyte ALA.S activities in six subjects taking carbamazepine for three weeks. Cross bars represent the mean value.

Erythrocyte URO.S activity decreased in all subjects throughout the treatment period. Values were still significantly depressed three weeks following stoppage of carbamazepine (Fig 42). Urinary porphyrins and precursors are shown in Table 18. Significant increases in ALA and total porphyhrin excretion occurred during the first and second week of treatment. PBG excretion showed no consistent change. The HPLC pattern of porphyrin excretion was similar to that seen in AIP during carbamazepine therapy and reverted to normal on cessation of carbamazepine (Fig 43).

11.8 DISCUSSION

The results of the cross-incubation experiment confirmed that carbamazepine and its metabolites in the serum were unlikely to interfere with the assay of URO.S activity in vitro. Neither did they support the possibility of a direct effect on the peripheral red cells to decrease URO.S activity. The URO.S assay employed was an indirect method of measurement in that it used ALA as substrate and depended on a normal erythrocyte ALA.dehydratase, ALA.D activity after activation with zinc and dithiothreitol. Using a different method of URO.S assay with PBG as substrate, Rideout et al (1983) provided some data to suggest that carbamazepine caused a decrease in erythrocyte ALA.D instead of URO.S activity. But Moore et al (1983) used an assay system (Ford et al, 1980) similar to that of Rideout et al; and they confirmed our observations by finding depressed URO.S activity in South African patients treated with carbamazepine. The mean activity ($\pm$ SD) of URO.S for five epileptic patients was 7.23 ($\pm$ 1.41) nmol of

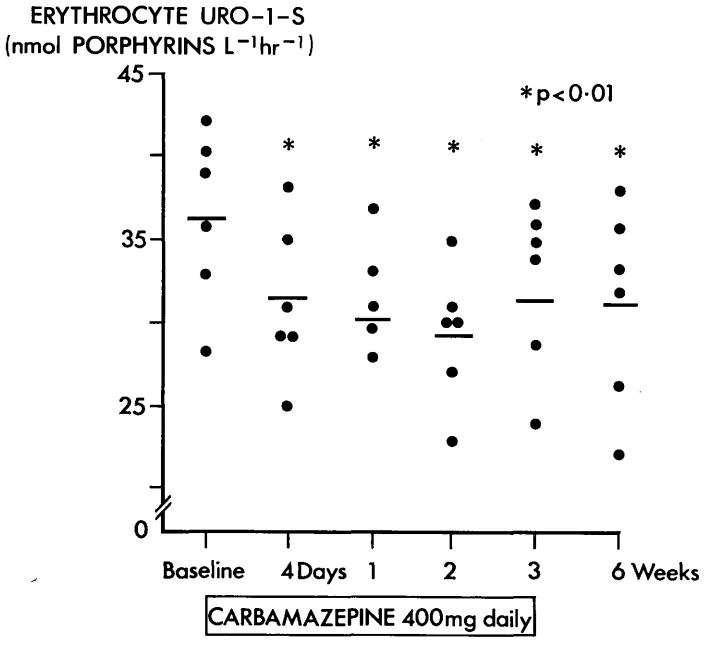


Figure 42: Erythrocyte URO.S activities in six normal subjects givencarbamazepine for three weeks. Cross bars represent the mean values. Statistics by Student's t-test for paired values. Comparisons are with baseline values.

Time of collection	ALA umol $(24h)^{-1}$	PBG umol $(24h)^{-1}$	Porphyrins ug $(24h)^{-1}$
Baseline	17 ± 9.7	Nil	64 ± 9.4
Day 4	28 ± 5.5*	1.5 ± 1.4	85 ± 22.3*
Week 1	23 ± 8.9	2.5 ± 2.9	88 ± 25.1*
Week 2	42 ± 6.0**	Nil	87 ± 47.6
Week 3	26 ± 7.4	7.3 ± 4.0	123 ± 61.1
Week 6	17.5 ± 5.8	8 ± 4.0	61 ± 24.3
Normal range	< 40	< 16	< 306

* $p < 0.05$

** $p < 0.02$

Comparison with baseline by Student's paired t-test

Table 18: Serial daily ALA, PBG and total porphyrin excretion (mean + SD) in six healthy subjects taking 400 mg carbamazepine daily for 22 days

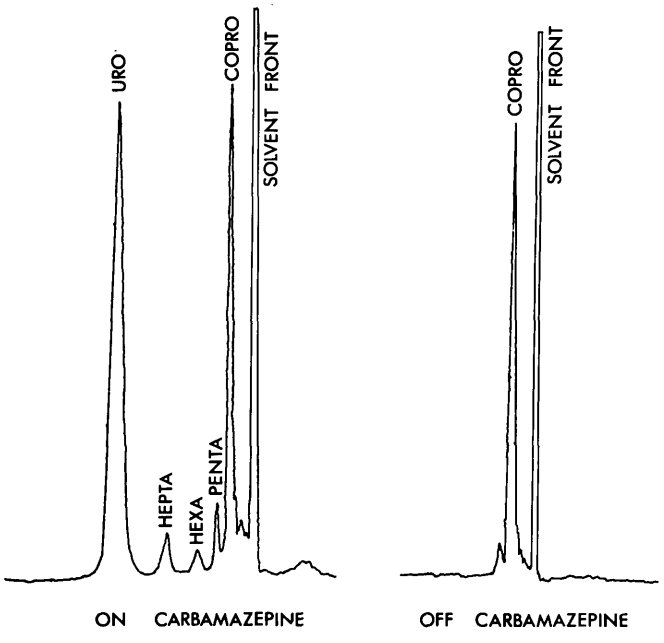


Figure 43: Representative changes in HPLC pattern of urinary porphyrin esters following carbamazepine therapy. These changes were seen in the urine of all subjects after 15 days treatment. The urinary pattern after treatment bears striking abnormalities to that of AIP.

uroporphyrin/L erythrocytes/second as compared to 12.24 (± 2.46) for 31 normal subjects. They did not measure ALA.D activity but they found a significant increase in leucocyte ALA.S activity during the treatment period. Moore et al (1983) therefore concluded that the use of ALA as substrate for the measurement of URO.S activity was quite justifiable.

Previous animal studies have shown that in response to the administration of enzyme-inducing drugs hepatic ALA.S activity increased before a detectable rise in microsomal haemoprotein concentrations (Marver, 1974). In this present study, ALA.S activity increased maximally by the seventh day before returning to baseline levels despite continued carbamazepine administration. This time course was similar to that observed for the increase in 6β-hydroxycortisol excretion following induction with the antibiotic rifampicin (Park, 1981); it may represent the time required for new haemoprotein synthesis of the mono-oxygenase system. A similar pattern of ALA.S elevation has been reported in epileptic patients treated with phenytoin, another potent enzyme-inducer (McColl et al, 1980).

The fall in erythrocyte URO.S activity following administration of carbamazepine to healthy subjects is in keeping with the changes recorded in epileptic patients taking the same drug (Chapter 11.3). Urinary ALA and porphyrins were significantly elevated, although values remained within the normal range for the assays. But this mild elevation was not seen with PBG excretion. In this study depression of hepatic URO.S activity was supported by the changes seen in the HPLC pattern of urinary porphyrin excretion.

The pattern after carbamazepine ingestion closely resembled that of latent AIP.

McGuire et al (1985a) have recently confirmed that administration of carbamazepine can also reduced ALA.D activity (Rideout et al, 1983) both in human erythrocytes and in rat liver. Erythrocyte ALA.D activity was depressed by nearly 50% in epileptic patients receiving long-term carbamazepine therapy. But attempts to reproduce this effect in vitro by direct addition of the drug or its main metabolite, carbamazepine 10, 11-epoxide, to whole blood were unsuccessful. In patients prescribed carbamazepine, depression of erythrocyte ALA.D activity was irreversible and could not be restored by the addition of enzyme activators, as happens with lead intoxication. Further experiments performed in our laboratory (McGuire et al, 1985b) have indicated that both parent carbamazepine and its epoxide derivative could reduce ALA.D activity in mitogen-stimulated human lymphocytes in culture. Contrary to their previous in vitro studies using human erythrocytes and rat liver preparations (McGuire et al, 1985a) exposure of the lymphocytes to carbamazepine for as little as five hours was sufficient to reduce ALA.D activity. These findings suggest that some form of intracellular biotransformation of carbamazepine epoxide must occur before it can reduce ALA.D activity. At present it is not known if a similar mechanism operates for the depressant effect of the drug on URO.S activity. In view of the large difference in the relative activities between erythrocyte ALA.D (2100 umol ALA/L erythrocytes/h) and erythrocyte URO.S (0.27 umol ALA equivalents/L RBC/h); and hepatic ALA.D (15.9

nmol ALA/mg protein/h) and hepatic URO.S (0.15 nmol ALA equivalents/mg protein/h) (Elder, 1982) only a marked diminution in ALA.D activity (> 90%) as seen in lead poisoning would be required to limit substrate availability for URO.S (Moore et al, 1980b). The urinary excretion of large amounts of PBG in the toxic case of acute porphyria described (case 1) suggested that the development of the disease probably arose from a depression of both URO.S and ALA.D activities induced by carbamazepine therapy. Thus the depressant effects of carbamazepine on the haem biosynthetic pathway in man may well provide a possible experimental human model for the acute hepatic porphyrias.

The interpretation of any future studies with this model from the point of view of the human acute hepatic porphyrias would have to take account of the fact that these diseases are all due to single enzyme defects (uroporphyrinogen I synthetase for acute intermittent porphyria, coproporphyrinogen oxidase for hereditary coproporphyria, and protoporphyrinogen oxidase for variegate porphyria) whereas carbamazepine inhibits delta-aminolaevulinic acid dehydrase as well as uroporphyringen I synthetase.

SECTION III

CHAPTER 12

DEVELOPMENT OF AN IN VITRO MODEL USING HUMAN PERIPHERAL BLOOD MONOCYTES FOR STUDYING THE HAEM BIOSYNTHETIC PATHWAY

12. DEVELOPMENT OF AN IN VITRO MODEL USING HUMAN PERIPHERAL BLOOD MONOCYTES FOR STUDYING THE HAEM BIOSYNTHETIC PATHWAY

12.1 INTRODUCTION

Numerous experimental models using either whole animals (With, 1980) or tissue cultures from other species have been described in the study of the haem biosynthetic pathway. None of these however can satisfactorily produce changes that reflect the clinical manifestations of the acute hepatic porphyrias. Efforts to elucidate the pathogenesis of the human disease have had to be approached clinically with the obvious limitations imposed by patient study.

Recent work has established the peripheral blood monocyte as a major secretory cell (Nathan, 1980). Although human monocytes constitute only 2 to 5% of the total white cell count such metabolically active cells are of bone-marrow origin, and if successfully cultured, could provide a useful *in vitro* model for investigating the haem biosynthetic pathway. This would be particularly desirable in view of the easy accessibility and availability of blood monocytes as compared to bone marrow or liver tissue.

In this study I have developed a highly purified human monocyte culture system and shown that proto-O activity can be reliably measured in these cultured cells.

12.2 MATERIALS AND METHODS

Preparation of monocyte cultures

12.2.1 Abbreviations:

1. PBS: phosphate-buffered saline, pH 7.4 containing K_2HPO_4 (0.34 g/L), KH_2PO_4 (1.21 g/L) and NaCl (8 g/L).
2. Human AB serum: human blood group AB serum.
3. RPMI: RPMI-1640 tissue culture medium.
4. RPMI-PBS-EDTA: Equal volumes of RPMI-1640 and PBS containing EDTA (10 mmol L^{-1}).
5. RPMI-FCS: RPMI-1640 containing 20% (V/V) heat-inactivated (2 h at 56°C) foetal calf serum.
6. RPMI-ABS: RPMI-1640 containing 10% (V/V) heat-inactivated (2 h at 56°C) human blood group AB serum.
7. Proto.0 buffer (pH 8.7) consisted of 100 mmol/L Tris HCl 1 mmol/L EDTA and 5 mmol/L glutathione.

12.2.2 Preparation of monocyte monolayers (Appendix II)

Venous blood (60 ml) from normal volunteers was collected in preservative-free heparin. After centrifugation over Ficoll/Hypaque gradients (Boyum, 1968), the mononuclear leucocytes at the interphase were harvested and resuspended in 36 ml of RPMI-FCS. A 12 ml portion of the suspension was added to tissue-culture flasks that had been precoated with the micro-excudate of BHK (baby-hamster kidney) cells (Douglas et al, 1981). After incubation at 37°C for 45 min, the culture medium containing non-adherent cells was decanted, and the monolayer washed three times with warm (37°C) RPMI 1640. Adherent platelets were then removed by a brief exposure (30s) to RPMI-PBS-EDTA. The

adherent monocytes were detached from the flasks by a second incubation with RPMI-PBS-EDTA at 37°C for 15 min. The detached monocytes were washed once in ice-cold RPMI-ABS and then resuspended to 4 x 10^5/ml in RPMI-ABS.

Portions (1 ml) of cell suspensions were added to the wells of Linbro tissue-culture plates, which were then incubated at 37°C for 3 h in a humidified atmosphere consisting of CO_2/air (1:19). The supernatants were removed and the monolayers washed with warm RPMI 1640. The monocytes were finally cultured in 1 ml of RPMI-FCS per well. The incubation was continued for 10 days under the conditions described above to allow the monocytes to mature into macrophages (Strunk et al, 1983).

Characterization of cells in monolayers

All the cells present in monolayers stained positively for non-specific esterase (Horwitz et al, 1977). Over 90% were able to phagocytose latex and serum-treated zymosan particles.

12.2.3 DNA content of monolayers (Appendix III)

The DNA content of the lysates of monolayers was determined by spectrofluorimetry (Cesarone et al, 1979). The volume of the lysate was made up to 5 ml with PBS, and after the addition of 20 ul of H33285 dye the content of DNA was measured by using a Shimadzu fluorimeter with transmission and emission wavelengths of 364 nm and 448 nm respectively. A standard curve of known DNA content was included in all assays. By using this assay 1.1 ug of DNA corresponds to 1 x 10^5 monocytes. Enzyme activity was expressed per ug DNA content of the cultured monocytes.

12.2.4 Monocyte homogenate preparation

After the monocytes had been in culture for 10 days the supernatant was removed and the cells washed once with warm PBS (PH 7.4). The contents of three random wells from the Linbro culture plate were stored for DNA assay. To each of the remaining wells 250 ul of ice-cold proto.O buffer was added. The plate was placed on crushed ice to prevent warming up of the homogenate during disruption of the cells by ultrasonic vibration. The monocytes were subject to two bursts of ultrasonic vibration for a total period of 30 seconds. The cell homogenate from half the number of wells was transferred into a test-tube and heated at 75°C in a water-bath for 20 minutes to destroy proto.O activity (Poulson, 1976). This heated tissue preparation was used in the non-enzymatic control (NEC) cuvettes.

12.2.5 Measurement of protoporphyrinogen oxidase (Proto.O) activity

Proto.O activity was measured by spectrofluorimetry, based on the method of Brenner and Bloomer (1980b) with several modifications. This assay depends on the conversion of protoporphyrinogen IX to protoporphyrin IX (Proto.IX).

Sodium amalgam preparation

The sodium amalgam was used to reduce the proto.IX to protoporphyrinogen. 3 gm metallic sodium, cut into small pieces, were added to a 200 ml volume of toluene which was then boiled on a hot-plate until the sodium formed small globules. 2.5 ml of mercury was slowly added into the toluene until solid amalgam formed at the bottom of the flask. After cooling the amalgam was

washed with petroleum ~~spirit~~, dried and broken into fragments (1.0 cm in diameter). It was stored in a dessicator and discarded if not used within a week.

Preparation of protoporphyrinogen solution

A stock solution of the disodium salt of proto.IX (Sigma Chemicals) - 1.5 mmol/L in 0.01 mol/L KOH containing 20% ethanol (V/V) - was diluted with degassed KOH-ethanol solution to a final concentration of 50 umol/L proto.IX (Sano & Granick, 1961). Immediately before use, the amalgam was finely crushed with a mortar and pestle. It was then transferred to a flask, shielded from light by aluminium foil, containing 3 ml of the proto.IX solution. About 2 gm of amalgam were added for each ml of proto.IX. The chemical reaction was allowed to proceed gently in the dark, under nitrogen, for about 3 minutes while the contents of the flask were gently shaken. The reaction was stopped when the proto.IX fluorescence could no longer be seen under ultraviolet light. The clear solution of protoporphyrinogen was rapidly transferred into a small beaker and its pH adjusted to 8.7 with 40% phosphoric acid (W/V).

Proto.O activity assay

Previous studies have shown that measurement of proto.O activity in mammalian tissue was optimal at pH 8.7 (Poulson, 1976; Brenner & Bloomer, 1980b; Deybach et al, 1981). All reactions were therefore carried out at pH 8.7.

The standard reaction mixture was placed into a disposable fluorimetric cuvette and consisted of:

1. 400 to 1600 ul of either monocyte test homogenate or NEC (200 ul being the volume retrievable from each culture well).

2. 50 ul of freshly prepared protoporphyrinogen solution (1.2 2.5 umol/L).

3. the appropriate volume of proto.O buffer to make up a final volume of 2 ml.

Each assay always included a cell blank and a protoporphyrinogen (proto'ogen) blank in addition to the NEC. Immediately after the protoporphyrinogen solution was added to the cuvette, the reaction mixture was vortexed and allowed to settle for 3 minutes at room temperature in the dark. The fluorescence emission intensity of the whole contents of the cuvette was then measured at 620 nm with an Elmer-Perkin spectrofluorometer (Model 3000) using an excitation wavelength of 400 nm. The cuvettes were then incubated at 37°C in the dark and in air without any shaking. This was done to minimise non-enzymatic formation of protoporphyrin from its precursor. Fluorescence measurements were repeated after 30, 60 and 90 minutes.

12.2.6 Calculation of results

The difference in protoporphyrin concentrations between the untreated cell homogenate and the corresponding non-enzymatic control represented the enzymatically generated protoporphyrin. This was calculated from the fluorescence readings at 90 minutes:

(test sample - Proto'ogen blank)

- (NEC - proto'ogen blank).

The concentration of protoporphyrin was then determined by comparison with the copro I standard.

Proto.O activity was expressed as the amount of protoporphyrin formed per ug DNA of the cultured monocytes per hour (pg proto. ug DNA^{-1} h^{-1}).

12.3 RESULTS

The fluorescence readings of Proto.IX solution was found to become unstable with the passage of time (Figs 44 and 45). In contrast fluorescence measurements of copro I remained constant for the duration of the experiment (Fig 46). The fluorescence-concentration relationship for both copro I and freshly prepared proto.IX solution was linear over the range of 1.2 to 75 nmol/L (Fig 47): in effect they were identical. Therefore copro I solution was employed as the standard in the assay once it was calibrated with a freshly made proto.IX solution.

The results of proto.O activity in the cultured monocytes of the same healthy subject obtained at 5 week intervals are shown in Tables 19 and 20. Proto.O activity was 9.74 and 11.04 pmol proto.IX ug $DNA^{-1}h^{-1}$ on the two successive occasions. When fewer than 1.2×10^6 cultured monocytes were used (i.e. contents of three wells) measurement of proto.O activity in the cell homogenate was not possible, probably because of insufficient enzyme. Using the cell homogenate from three wells in the assay the mean DNA content per well was found to be 3.04 ug, and the mean proto.O activity in the monocytes 10.37 pmol proto.IX ug $DNA^{-1}h^{-1}$ (range 8.86 - 12.12) (Table 21). Since 1.1 ug DNA is equivalent to 10^5 monocytes, the mean proto.O activity per cultured monocyte was 11.41×10^{-5} pmol proto.IX ug $DNA^{-1}h^{-1}$. Increasing the amount of cell homogenate in the assay led to unacceptable nephelometric interference with the fluorescence measurement. Attempts to circumvent this problem by diluting 0.1 ml aliquots of the reaction mixture at the various incubation

EFFECT OF TIME ON STABILITY OF PROTOPORPHYRIN IX IN SOLUTION (PH 8.7)

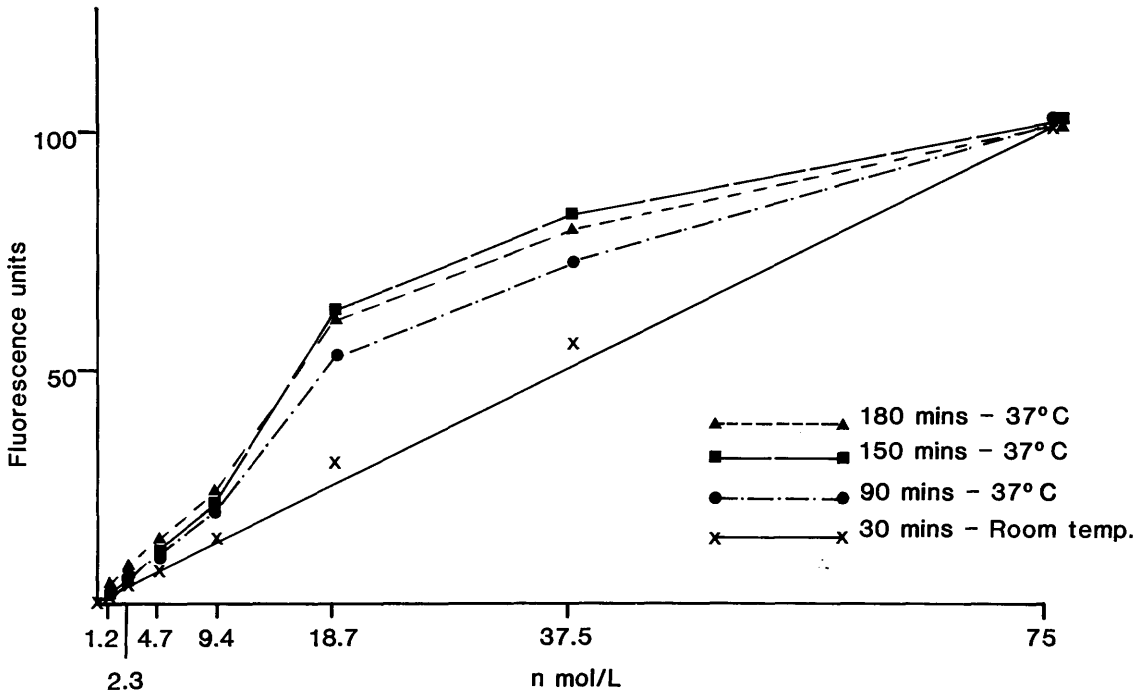


Figure 44

DECAY OF SPECTROFLUORIMETRIC ACTIVITY OF PROTO 1X WITH TIME

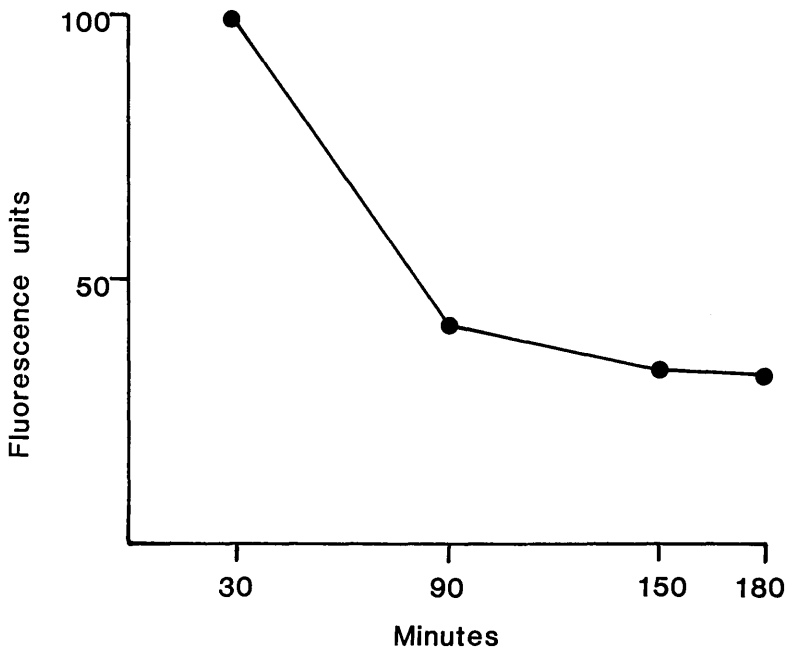


Figure 45

EFFECT OF TIME ON STABILITY OF STANDARD COPROPORPHYRIN SOLUTION

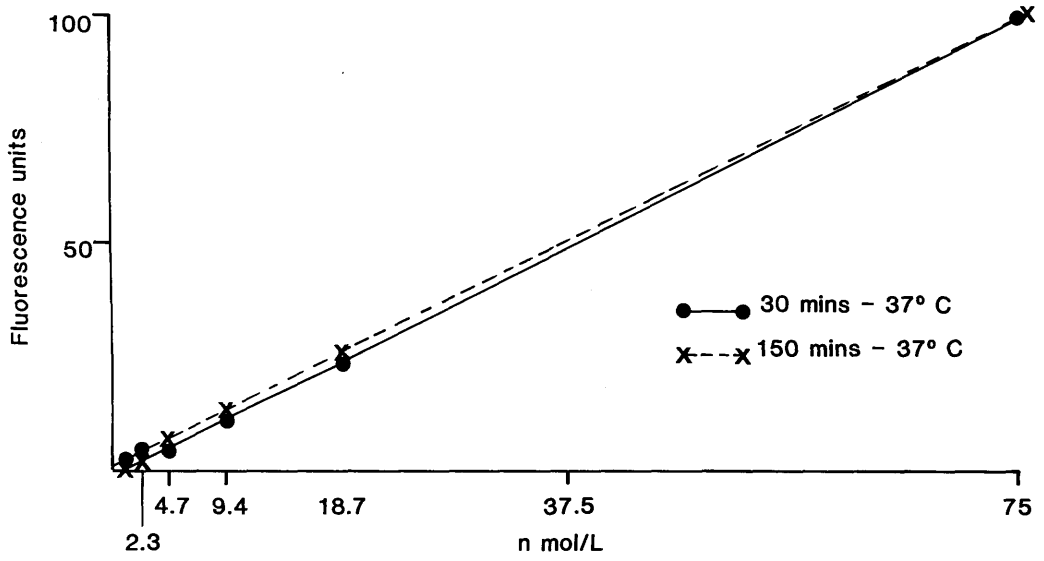


Figure 46

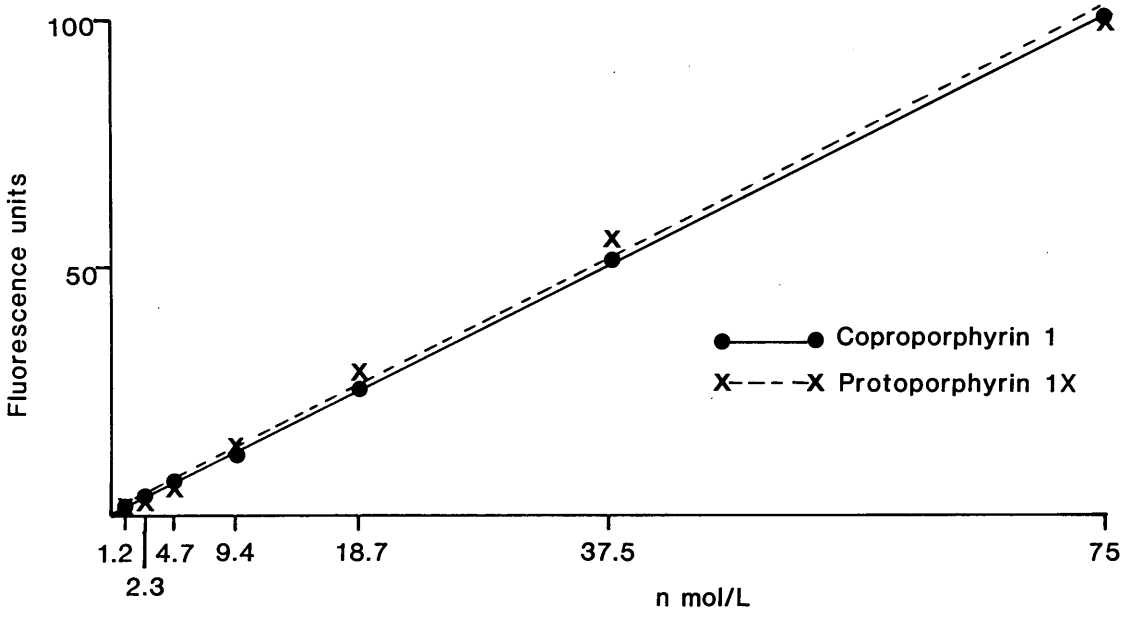


Figure 47: Spectrofluorimetric readings with doubling concentrations of freshly prepared coproporphyrin I and protoporphyrin IX solutions.

Incubation umol(min)	Spectrofluorimetric readings x 10				Maximal change in fluorescence	Proto.O activity pmol proto.IX ug $DNA^{-1}h^{-1}$
	3	30	60	90		
Cell blank	14.5	18.4	22.7	20.5		
Protoporphyrinogen blank	0.7	0.9	1.9	0.5		
Homogenate (XI well)	17.2	22.3	21.7	26.0	3.5	-
*NEC-homogenate (XI well)	19.3	23.2	21.8	22.5		
Homogenate (x 2 wells)	19.8	22.6	28.7	32.6	10.6	3.1
NEC-homogenate (x 2 wells)	16.9	19.8	21.2	21.6		
Homogenate (x 3 wells)	22.7	28.1	36.5	41.9	19.2	11.04
NEC-homogenate (x 3 wells)	19.1	21.3	24.3	22.8		

Mean DNA content/well = 3.2 ug

* NEC = non-enzymatic control

Table 19: Proto.O activity in normal human monocytes after 10 days in culture

Incubation period (min)	Spectrofluorimetric readings (x 10)				Maximal change in fluorescence	Proto.O activity pmol proto.IX ug $DNA^{-1}h^{-1}$
	3	30	60	90		
Cell blank	23.1	27.2	29.8	30.5		
Protoporphyrinogen blank	0.9	1.5	1.8	2.3		
Homogenate (x 3 wells)	28.5	33.4	49.9	59.6	28.2	9.74
*NEC-homogenate (x 3 wells)	29.4	27.7	31.5	31.4		
Homogenate (x 4 wells)	36.7	49.5	68.3	80.3	36.2	11.80
*NEC-homogenate (x 4 wells)	32.4	38.2	40.5	44.1		

Mean DNA content/well = 2.94 ug

*NEC = non-enzymatic control

Table 20: Proto.O activity measured by spectrofluorimetry in human monocytes after 10 days in culture

Normal subjects	Mean DNA content (ug/well)	Proto O activity pmol proto IX ug $DNA^{-1}h^{-1}$
1	3.21	11.04
2	2.98	8.86
3	2.86	9.46
4	3.11	12.12
Mean value	3.04	10.37

Table 21: Results of proto.O activity in cultured monocytes from four healthy male subjects. The cell contents from three culture wells (equivalent to 9.1 ug DNA) were used in these assays.

time-points (Bloomer & Brenner, 1980b) was unsuccessful because of the low fluorescence generated. With the small number of monocytes in use (8×10^6 to 1.6×10^6) the assay was operating at the lowest range of sensitivity of the spectrofluorimeter. Increasing the concentration of protoporphyrinogen in the reaction mixture only caused a rise in the background fluorescence readings since the substrate was already in gross excess as compared to the amount of product formed. Several other changes were made to the operating conditions of the assay without any benefit:

1. Alteration of pH from 8.7 to 9.2 did not improve the sensitivity.
2. Use of protoporphyrinogen derived from porphyrin ester instead of proto.IX (Poulson & Polglase, 1975) conferred no advantage.
3. Addition of Tween 20 to the reaction mixture (Bloomer & Brenner, 1980b; Deybach et al, 1981) caused fluorescence interference and troublesome frothing.
4. Lowering glutathione concentration in the buffer from 5 to 2 mM (Polglase, 1976; Deybach et al, 1981) resulted in similar proto.IX conversion.
5. Reading the fluorescence at emission intensity of 635 nm using an excitation wavelength of 405 nm (Bloomer & Brenner, 1980b) instead of 620 nm and 400 nm respectively made no significant difference.

12.4 DISCUSSION

Peripheral human leucocytes have been shown to possess all the functionally active haem enzymes (Elder et al, 1976; Grandchamp et al, 1977; Brodie et al, 1977d; Sassa et al, 1978; Deybach et al, 1981). Indeed, in our laboratory, the measurements of the mitochondrial haem enzymes can all be performed on the white cell buffy coat. Following the observation that stimulated blood lymphocytes can produce porphyrins (Saillen et al, 1969; Josephson et al, 1972), Sassa and his associates (1978) have reported that URO.S activity is reduced by 50% in similarly transformed lymphocytes obtained from AIP patients. But stimulation of cultured lymphocytes by mitogens like phytohaemaglutinin or by the Epstein-Barr virus inevitably disrupts the normal regulatory mechanism of the cells. Consequently the usefulness of such models is greatly limited.

This study has shown that proto.O activity can be measured in human blood monocytes maintained in culture for a period of 10 days. It is therefore very likely that other haem enzymes can also be measured in these cells. Measurement of ALA.S would be particularly useful for assessing the potential porphyrinogenicity of drugs and chemicals. This human model could solve some of the problems associated with the methods currently employed for testing these drugs eg interspecies differences.

The limitations imposed by the spectrofluometric method necessitated the use of more cells than originally anticipated (ie contents of three instead of single wells). Since the optimal density for monocyte culture in the Linbro plates is between 4-6

10^5 cells, seeding the cells of a higher density will require different culture conditions. Since the yield of monocytes by the present method is on average 1 x 10^7 sufficient cells are available for experimentation. Where a larger supply of monocytes is required I have satisfactorily used whole-blood-pack buffy coats, obtainable from the Regional Blood Transfusion Centre.

The homogeneous cell population provided by the highly purified (more than 99%) monocytes in culture offers an excellent in vitro model for investigating the molecular control of the haem biosynthetic pathway. Indeed the monocyte culture technique developed in the course of this study has already found such an application towards the understanding of the molecular basis of another inherited inborn error of metabolism - hereditary angioneurotic oedema (Yeung Laiwah et al, 1985).

REFERENCES

REFERENCES

ACKNER B, COOPER J E, GRAY C H, KELLY M (1962)
Acute porphyria: a neuropsychiatric and biochemical study.
Journal of Psychosomotor Research, 6, 1-24

ACKNER B, COOPER J E, GRAY C H, KELLY M, NICHOLSON D C (1961)
Excretion of porphobilinogen and delta-aminolaevulinic acid in acute porphyria.
Lancet, 1, 1256-1260

AFT R L, MUELLER G C (1983)
Hemin-mediated DNA strand scission
Journal of Biological Chemistry, 258, 12069-12072

AL-ALI S Y, ROBINSON N (1979)
Ultrastructural demonstration of cytochrome oxidase via cytochrome C in cerebral cortex
Journal of Histochemistry and Cytochemistry, 27, 1261-1266

AL-BADAWY A B, EVANS M (1975)
Regulation of rat liver tryptophan pyrrolase by its cofactor haem. Experiments with haematin and 5 aminolaevulinate and comparison with the substrate and hormonal mechanisms
Biochemical Journal, 150, 511-520

ALBERS J W, ROBERTSON W C, DAUBE J R (1978)
Electrodiagnostic findings in acute porphyric neuropathy
Muscle and Nerve, 1, 292-296

ALEXANDER N, MORRIS M (1982)
Elevated plasma vasopressin in the sinoaortic denervation (SAD) rat
Federation Proceedings, 41, 1094

ANDERSON K E (1978)
Effects of antihypertensive drugs on hepatic heme biosynthesis and evaluation of ferrochelatase inhibitors to simplify testing of drugs for heme pathway induction.
Biochimica et Biophysica Acta, 543, 313-327

ANDERSON K E, ALVARES A P, SASSA S, KAPPAS A (1976)
Studies in porphyria. Drug oxidation rates in hereditary hepatic porphyria.
Clinical Pharmacology & Therapeutics, 19, 47-54

ANDERSON K E, BRADLOW H L, SASSA S, KAPPAS A (1979)
Studies in porphyria. VIII Relationship of the 5 -reductive metabolism of steroid hormones to the clinical expression of the genetic defect in acute intermittent porphyria.
American Journal of Medicine, 66, 644-650

ANDERSON K E, SASSA S, PETERSON C M, KAPPAS A (1977)
Increased erythrocyte uroporphyrinogen-I-synthetase, delta-aminolaevulinic acid dehydratase and protoporphyrin in haemolytic anaemias.
American Journal of Medicine, 63, 359-364

ANDERSON K E, SPITZ I M, SASSA S, BARDIN W, KAPPAS A (1984)
Prevention of cyclical attacks of acute intermittent porphyria with a long-acting agonist of luteinizing hormone-releasing hormone
New England Journal of Medicine, 311, 643-645

ANDERSON P M, REDDY R M, ANDERSON K E, DESNICK R J (1981)
Characterization of the porphobilinogen deaminase deficiency in acute intermittent porphyria.
Journal of Clinical Investigation, 68, 1-12.

ANDREOLI T E (1985)
Disorders of fluid volume, electrolyte and acid-base balance
In "Cecil Textbook of Medicine" pp523-528
eds. Wyngaarden J B, Smith L H Jr, W B Saunders Company, Philadelphia.

ANZIL A P, DOZIC S (1978)
Peripheral nerve changes in porphyric neuropathy: Findings in a sural nerve biopsy.
Acta Neuropathologica (Berlin), 42, 121-126

ARNOLD D L, BORE P J, RADDA G K, STYLES P, TAYLOR D J (1984)
Excessive intracellular acidosis of skeletal muscle on exercise in a patient with a post-viral exhaution/fatigue syndrome.
Lancet, 1, 1367-1369

ARRESE M R, VOJENSKY D MATTOON J (1982)
Characterization of a cytochrome oxidase-deficient yeast mutant which accumulates porphyrias
Biochemical and Biophysical Research Communications, 107, 848-855

ASTRÖM E, FRIMAN G, PILSTROM L (1976)
Effects of viral and mycoplasma infections on ultrastructure and enzyme activities in human skeletal muscle
Acta Pathologica et Microbiologica Scandinavica, 84, 113-122

ATSMON A, BLUM I (1970)
Treatment of acute porphyria variegata with propranolol
Lancet, 1, 196-197.

ATUK N O, OWEN J A, WESTFALL T C (1975)
Acute intermittent porphyria: altered catecholamine metabolism and response to propranolol
Journal of Clinical Pharmacology, 15, 552-553

ATWEH S F, KUHAR M J (1977)
Autoradiographic localization of opiate receptors in rat brain. I Spinal cord and lower medulla.
Brain Research, 124, 53-67

ATWEH S F, KUHAR M J (1983)
Distribution and physiological significance of opiate receptors in the brain.
British Medical Bulletin, 39, 47-52

ATWEH S F, MURRIN L C, KUHAR M J (1978)
Presynaptic localization of opiate receptors in the vagal and accessory optic systems: an autoradiographic study
Neuropharmacology, 17, 65-71

AUSTIN K L, STAPLETON J U, MATHER L E (1980)
Multiple intramuscular injections. A major source of variability in analgesic response to meperidine
Pain, 8, 47-52

AZZI A (1980)
Cytochrome C oxidase. Towards a clarification of its structure, interactions and mechanism
Biochimica et Biophysica Acta, 594, 231-252

BAKER A B, MATZKE H A, BROWN J R (1950)
Poliomyelitis. III. Bulbar poliomyelitis: a study of medullary function
Archives of Neurology and Psychiatry, 63, 257-281

BAKER A B, WATSON C J (1945)
The nervous system in porphyria
Journal of Neuropathology and Experimental Neurology, 4, 68-76

BARCLAY N (1974)
Acute intermittent porphyria in childhood. A neglected diagnosis?
Archives of Diseases in Childhood, 49, 404-406

BARKER L F, ESTES W L (1912)
Family hematoporphyrinuria in association with chronic gastroduodenal dilation, peculiar fits and acute polyneuritis: a preliminary report.
Journal of the American Medical Association, 59, 718-719.

BARNES R, JONES M S, JONES O T G, PORRA R J (1971)
Ferrochelatase and delta-aminolaevulinate synthetase in brain, heart kidney and liver of normal and porphyric rats
Biochemical Journal, 124, 633-637

BAROIS A, GAJDOS P, LIENHART A, GOULON M (1977)
Hypercalcemie au cours de la porphyrie aigue intermittente: A propos de 3 observations.
Seminar Hopital Paris, 53, 1115-1120.

BARTON C, BASBAUM A I, FIELDS H L (1980)
Dissociation of supraspinal and spinal actions of morphine: a quantitative evaluation
Brain Research, 188, 497-498

BASILIERE C J, NEWCOMER A D (1971)
Primary aldosterism associated with acute intermittent porphyria.
New England Journal of Medicine, 285, 595-598.

BEAL M F, ATUK N O, WESTFALL T C, TURNER S M (1977)
Catecholamine uptake, accumulation and release in acute porphyria.
Journal of Clinical Investigation, 60, 1141-1148.

BEATTIE A D (1973b)
Prevalence of the porphyrias
In "Clinical and experimental studies of porphyrin metabolism" pp104-107, MD thesis, University of Glasgow

BEATTIE A D, GOLDBERG A (1975)
Acute intermittent porphyria: natural history and prognosis
In "Porphyrins in Human Diseases", pp245-250
ed. Doss M, Karger, Basel.

BEATTIE A D, MOORE M R, GOLDBERG A, WARD A (1973a)
Acute intermittent porphyria: response of tachycardia and hypertension to propranolol
British Medical Journal, 3, 257-260

BECKER D M, GOLDSTUCK N, KRAMER S (1975)
Effect of delta-aminolaevulinic acid on the resting membrane potential of frog sartorius muscle.
South African Medical Journal, 49, 1790-1792

BECKER D M, KRAMER S (1977b)
The neurological manifestations of porphyria: a review
Medicine (Baltimore), 56, 411-423

BECKER D M, KRAMER S, VILJOEN J D (1974)
Delta-aminolevulinic acid uptake by rabbit brain cerebral cortex
Journal of Neurochemistry, 23, 1019-1023

BECKER D M, VILJOEN J D, KATZ J, KRAMER S (1977a)
Reduced ferrochelatase activity. A defect common to porphyria variegata and protoporphyria.
British Journal of Haematology, 36, 171-179.

BECKER D M, VILJOEN D, KRAMER S (1971)
The inhibition of red cell and brain ATPase by delta-aminolaevulinic acid.
Biochimica et Biophysica Acta, 225, 26-34

BECKER D M, VILJOEN J D, KRAMER A (1976)
Porphyrin precursors and their effects in vitro in some aspects of nerve function
In "Porphyrins in Human Disease. First International Porphyrin Meeting, pp163-172
ed. Doss M, Karger, Basel

BEECHER H K (1959)
Measurement of subjective responses: Quantitative effects of drugs
Oxford University Press, New York

BEHAN P O, BEHAN W M H (1980)
Epidemic myalgic encephalomyelitis.
In "Clinical Neuroepidemiology, pp374-383
ed. Rose F C, Pitman Medical, Tunbridge Wells

BEHAN P O (1983)
Postviral neurological syndrome
British Medical Journal, ii, 853-854

BENNETT T, FARQUHAR I K, HOSKING D J, HAMPTON J R (1978)
Assessment of method for estimating autonomic nervous control of the heart in patients with diabetes mellitus
Diabetes, 27, 1167-1174

BERG M (1945)
Acute porphyria - clinical and pathologic observations
Archives of Internal Medicine, 335-340

BERGER H, GOLDBERG A (1955)
Hereditary coproporphyria.
British Medical Journal, ii, 85-88.

BERLIN L, COTTON R (1950)
Gastrointestinal manifestations of porphyria
American Journal of Digestive Diseases, 17, 110-114

BERLIN N I, NEUBERGER A, SCOTT J S (1956)
The metabolism of delta-aminolaevulinic acid
Biochemical Journal, 64, 90-100

BIAGINI R, TIGNANI R, FIFI A R, NAPPINI L (1979)
Acute intermittent porphyria and epilepsy
Achives of Diseases in Childhood, 54, 644-645

BICKERS D R, MILLER L, KAPPAS A (1975)
Exacerbation of hereditary hepatic porphyria by surreptitious ingestion of an unusual provocative agent - a mouthwash preparation
New England Journal of Medicine, 292, 1115-1116

BISSELL D M (1982)
Laboratory evaluation in porphyria
Seminars in Liver Disease, 2, 100-107

BISSELL D M, HAMMAKER L E (1977)
Effect of endotoxin on tryptophan pyrrolase and delta-aminolaevulinate synthase: evidence for an endogenous regulatory haem fraction in rat liver
Biochemical Journal, 166, 301-304

BLOOMER J R, BERK P D, BONKOWSKY H L, STEIN J A, BERLIN N I, TSCHUDY D P (1971)
Blood volume and bilirubin production in acute intermittent porphyria
New England Journal of Medicine, 284, 17-20

BLUM I, ATSMON A (1976)
Reduction of porphyrin excretion in porphyria variegata by propranolol - a case report
South African Journal of Medicine, 50, 898-899

BLUM I, SCHOENFELD N, ATSMON A (1973)
The effect of DL-propranolol on delta-aminolaevulinic acid synthetase activity and urinary excretion of porphyrins in allylisopropylacetamide-induced experimental porphyria
Biochimica et Biophysica Acta, 320, 242-248

BOND M R (1984)
Pain. Its nature, analysis and treatment. 2nd edition, p.157
Churchill Livingstone, Edinburgh

BONKOWSKY H L, MAGNUSSEN C R, COLLINS A R, DOHERTY J M, HESS R A, TSCHUDY D P (1976)
Comparative effects of glycerol and dextrose on porphyrin precursor excretion in acute intermittent porphyria
Metabolism, 25, 405-414

BONKOWSKY H L, SCHADY W (1982)
Neurologic manifestations of acute porphyria
Seminars in Liver Disease, 2, 108-124

BONKOWSKY H L, SINCLAIR P R, EMERGY S, SINCLAIR J F (1980)
Seizure management in acute porphyria: Risks of valproate and clonazepam
Neurology, 30, 588-592

BONKOWSKY H L, TSCHUDY D P (1974)
Hazard of propranolol in treatment of acute porphyria
British Medical Journal, 3, 47

BONKOWSKY H L, TSCHUDY D P, COLLINS A, DOHERTY J, BOSSENMAIER I, CARDINAL R, WATSON C J (1971)
Repression of the overproduction of porphyrin precursors in acute intermittent porphyria by intravenous infusions of hematin
Proceedings of the National Academy of Sciences of the United States of America, 68, 2725-2729

BONKOWSKY H L, TSCHUDY D P, WEINBACH E C, EBERT P, DOHERTY J M (1975)
Porphyrin synthesis and mitochondrial respiration in acute intermittent porphyria: studies using cultured fibroblasts
Journal of Laboratory and Clinical Medicine, 85, 93-102

BONYHADY R E, HENDRY I A, HILL C E, McLENNAN I S (1982)
Effects of haemin on neurones derived from the neural crest
Developmental Neuroscience, 5, 125-129

BORNSTEIN J C, PICKETT J B, DIAMOND I (1979)
Inhibition of the evoked released of acetylcholine by the porphyrin precursor delta-aminolaevulinic acid
Annals of Neurology, 5, 94-96

BOSCH E P, PIERACH C A, BOSSENMAIER I, CARDINAL R, THORSON M (1977)
Effect of hematin on porphyric neuropathy
Neurology, 27, 1053-1056

BOSTRÖEM A (1920)
Ueber toxisch bedingte aufsteigende Lachmung mit Haematoporphyrie
Zeitschrift fur die gesamte Neurologie und Psychiatrie, 56, 181-196

BOYUM A (1968)
Isolation of leukocytes from human blood: further investigations
Scandinavian Journal of Clinical and Laboratory Investigation, 21 (Suppl), 97, 91-96

BRADBERRY J C, RAEBEL M A (1981)
Continuous infusion of naloxone in the treatment of narcotic overdose
Drug Intelligence and Clinical Pharmacy, 15, 945-950

BRADLOW H L, GILLETTE P N, GALLAGHER T F, KAPPAS A (1973)
Studies in porphyria II. Evidence for a deficiency of steroid 4-5 -reductase activity in acute intermittent porphyria
Journal of Experimental Medicine, 138, 754-763

BRENNAN M J W, CANTRILL R C (1979)
Delta-aminolaevulilnic acid is a potent agonist for GABA autoreceptors
Nature, 280, 514-515

BRENNAN M J W, CANTRILL R C, KRAMER S (1980)
Effect of delta-aminolaevulinic acid on GABA receptor binding in synaptic plasma membranes
International Journal of Biochemistry, 12, 833-835

BRENNER D A, BLOOMER J R (1980a)
The enzymatic effect in variegate porphyria
New England Journal of Medicine, 302, 765-768

BRENNER D A, BLOOMER J R (1980b)
Fluorimetric assay for measurement of protoporphyrinogen oxidase activity in mammalian tissue
Clinica Chimica Acta, 100, 259-264

BREZIS M, GHANEM J, WEILER-RAVELL D, EPSTEIN O, MORRIS D (1979)
Hematin and propranolol in acute intermittent porphyria. Full recovery from quadriplegic coma and respiratory failure
European Neurology, 18, 289-294

BRODIE M J, GOLDBERG A (1980)
Acute hepatic porphyrias
Clinics in Haematology, 9, 253-272

BRODIE M J, GRAHAM D J M, GOLDBERG A, BEASTALL G H, RATCLIFFE W A, RATCLIFFE J G, YEO P B B (1978)
Thyroid function in acute intermittent porphyria: a neurogenic cause of hyperthyroidism
Hormonal and Metabolic Research, 10, 327-331

BRODIE M J, MOORE M R, GOLDBERG A (1977a)
Enzyme abnormalities in the porphyria
Lancet, 2, 699-701

BRODIE M J, MOORE M R, THOMPSON G G, GOLDBERG A (1977b)
The treatment of acute intermittent porphyria with laevulose
Clinical Science and Molecular Medicine, 53, 365-371

BRODIE M J, MOORE M R, THOMPSON G G, GOLDBERG A (1977c)
Pregnancy and the acute porphyrias
British Journal of Obstetrics and Gynaecology, 84, 726-731

BRODIE M J, THOMPSON G G, MOORE M R, BEATTIE A D, GOLDBERG A (1977d)
Hereditary coproporphyria
Quarterly Journal of Medicine, 46, 229-241

BRODY M J, FABER J E, MANGIAPANE M L, PORTER J P (1984)
The central nervous system and prevention of hypertension
In "Handbook of Hypertension", Vol 4: Experimental and Genetic Models of Hypertension", pp474-494
ed. De Jong, Elsevier, Amsterdam

BUCHTAL F, BEHSE F (1979)
Electrophysiology and nerve biopsy in men exposed to lead
British Journal of Industrial Medicine, 36, 135-147

BURNHAM B F, LASCELLES J (1963)
Control of porphyria biosynthesis through a negative feedback mechanism
Biochemical Journal, 87, 462-472

BURSTONE M S (1960)
Modifications of histochemical techniques for the demonstration of cytochrome oxidase
Journal of Histochemistry and Cytochemistry, 8, 59-65

BUSTOS N L, STELLA A M, WIDER E A, DEL C BATLLE A (1983)
Enzyme replacement therapy in porphyrias - III. Potential use of erythrocyte ghosts as carriers of delta-aminolaevulinate dehydratase
International Journal of Biochemistry, 15, 447-452

BUSTYN P G, HORROBIN D F, LLOYD I J (1972)
Chronic hypertension in rabbits induced by bilateral placement of rigid casts around the carotid sinus regions
Cardiovascular Research, 6, 54-56

CAIRD F I, ANDREWS G T, KENNEDY R D (1973)
Effect of posture on blood pressure in the elderly
British Heart Journal, 35, 527-530

CAMPBELL K (1898)
A case of haematoporphyrinuria
Journal of Mental Science, 44, 305-313

CATLING J A, PINTE D M, JORDAN C, JONES J G (1980)
Respiratory effects of analgesia after cholecystectomy: comparison of continuous and intermittent papaveratum
British Medical Journal, 281, 478-480

CAVANAGH J B (1954)
The toxic effect of tri-ortho-cresyl phosphate on the nervous system
Journal of Neurology, Neurosurgery and Psychiatry, 17, 163-172

CAVANAGH J B (1967)
On the pattern of change in peripheral nerves produced by isoniazid intoxication in rats
Journal of Neurology, Neurosurgery and Pscyhiatry, 30, 26-33

CAVANAGH J B (1979)
The "Dying Back" process - a common denominator in many naturally occurring and toxic neuropathies
Archives of Pathology and Laboratory Medicine, 103, 659-664

CAVANAGH J B, MELLICK R S (1965)
On the nature of the peripheral nerve lesions associated with acute intermittent porphyria
Journal of Neurology, Neurosurgery and Psychiatry, 28, 320-327

CAVANAGH J B, RIDLEY A R (1967)
The nature of the neuropathy complicating acute intermittent porphyria
Lancet, 2, 1023-1024

CESARONE C F, BOLOGNESI C, SANTI L (1979)
Improved microfluorometric DNA determination in biological material using 33258 Hoechst
Annals of Biochemistry, 100, 188-197

CHAKRAVARTY K, TUCKER W, ROSEN M, BICKERS M D (1979)
Comparison of buprenorphine and pethidine given intravenously on demand to relieve postoperative pain
British Medical Journal, 2, 895-897

CHALMERS J P, REID J L (1972)
Participation of central adrenergic neurones in arterial baroreceptor reflexes in rabbit: a study with intracisternally administered 6-hydroxydopamine
Circulation Research, 31, 789-804

CHANG K, CUATRECASAS P (1981)
Heterogeneity and properties of opiate receptors
Federation Proceedings, 40, 2729-2733

CHEN A C N, CHAPMAN C R, HARKINS S W (1979)
Brain evoked potentials and functional correlates of induced pain in man
Pain, 6, 365-374

CHOU A C, FITCH C D (1980)
Hemolysis of mouse erythrocytes by ferriprotoporphyrin IX and chloroquine
Journal of Clinical Investigation, 66, 856-858

CHRISTENSEN T, MOAN J, WIBE E, OFTEBRO R (1979)
Photodynamic effect of haemtoporphyrin throughout the cell cycle of the human cell line NHIK 3025 cultivated "in vitro"
British Journal of Cancer, 39, 64-68

CHURCH J J (1979)
Continuous narcotic infusions for relief of postoperative pain
British Medical Journal, 1, 977-979

COHN J, ALVARES A P, KAPPAS A (1977)
On the occurrence of cytochrome P450 and aryl hydrocarbon hydroxylase activity in rat brain
Journal of Experimental Medicine, 145, 1607-1611

COLEMAN T G (1980)
Neurogenic hypertension.
In "Blood Pressure Control" - Vol I., pp80-87
Eden Press Incorporated, Ann Arbor

CONDIE L W, BARON J, TEPHLY T R (1976a)
Studies on adrenal delta-aminolaevulinic acid synthetase
Archives of Biochemistry and Biophysics, 172, 123-129

CONDIE L W, TEPHLY T R, BARON (1976b)
Studies of heme synthesis in the rat adrenals
Annals of Clinical Research, 8, Suppl 17, 83-90

COPOLOV D L, HELME R D (1983)
Encephalins and Endorphins. Clinical, pharmacological and therapeutic implications
Drugs, 26, 503-519

COURCOUX A, LHERMITTE J, BOULANGER-PILLET (1929)
La paralysie extenso-progressive hematoporphyrique
Presse Medicale, 2, 1609-1613

COWGER M L, LABBE R F (1967)
The inhibition of terminal oxidation by porphyrinogenic drugs
Biochemical Pharmacology, 16, 2189-2199

CUTLER M G, DICK J M, MOORE M R (1978)
Effect of delta-aminolaevulinic acid on frog nerve-muscle function.
Life Sciences, 23, 2233-2238

CUTLER M G, MOORE M R, DICK J M (1980)
Effects of -aminolaevulinic acid on contractile activity of rabbit duodenum
European Journal of Pharmacology, 64, 221-230

CUTLER M G, MOORE M R, EWART F G (1979)
Effects of delta-aminolaevulinic acid administration on social behaviour in the laboratory mice
Psychopharmacology, 61, 731-735

DAGG J H, GOLDBERG A, LOCKHEAD A, SMITH J A (1964)
The relationship of lead poisoning to acute intermittent porphyria
Quarterly Journal of Medicine, 34, 163-175

DAVIES I B, MATHIAS C J, SUDERA D, SEVER P S (1982)
Agonist regulation of -adrenergic receptor responses in man
Journal of Cardiovascular Pharmacology, 4, Suppl 1, S139-S144

DAY R S, EALES L, DISLER P B (1981)
Porphyrins and the kidney
Nephron, 28, 261-267

DEAN G (1971)
The Porphyrias. A story of inheritance and environment.
2nd edition
Pitman, London

DEAN G, BARNES H D (1955)
The inheritance of porphyria
British Medical Journal, ii, 89-94

DE FRANCISCO M, SAVINO P J, SCHATZ N J (1979)
Optic atrophy in acute intermittent porphyria
American Journal of Ophthalmology, 87, 221-224

DEL C BATLLE A M, BUSTOS N L, STELLA A M, WIDER E A, CONTI H A, MENDEZ A (1983)
Enzyme replacement therapy in porphyrias - IV. First successful human clinical trial of delta-aminolaevulinate dehydratase-loaded erythrocyte ghosts.
International Journal of Biochemistry, 15, 1261-1265

DE MATTEIS F (1978)
Hepatic porphyrias caused by 2-allyl-2-isopropylacetamide, 3,5-diethoxy-carbonyl-1, 4-dihydrocollidine, griseofulvin and related compounds
In "Heme and Hemoproteins, Handbook of Pharmacology" Vol 44, pp129-155
eds. De Matteis F, Aldridge W N, Springer-Verlag, Berlin

DE MATTEIS F, GIBBS A H (1972)
Stimulation of liver 5-aminolaevulinate synthetase by drugs and its relevance to drug-induced accumulation of cytochrome P450
Biochemical Journal, 126, 1149-1160

DE MATTEIS F, GIBBS A H, SMITH A G (1981a)
Inhibition of protohaem ferrolyase by N-substituted porphyrins
Biochemical Journal, 189, 645-648

DE MATTEIS F, RAY R E (1982)
Studies on cerebellar haem metabolism in the rat in vivo
Journal of Neurochemistry, 39, 551-556

DE MATTEIS F, RIMINGTON C (1962)
The biochemical disturbance in acute intermittent and experimental porphyria
Lancet, 1, 1332-1334

DE MATTEIS F, ZETTERLUND P, WETTERBERG L (1981b)
Brain 5-aminolaevulinate synthase. Developmental aspects and evidence for regulatory role
Biochemical Journal, 196, 811-817

DENNY BROWN D, SCIARRA D (1945)
Changes in the nervous system in acute porphyria
Brain, 68, 1-16

DESNICK R J, OSTASIEWICZ L, TISHLER P, MUSTAJOKI P (1985)
Acute intermittent porphyria: characterization of a novel mutation in the structural gene for porphobilinogen deaminase. Demonstration of non-catalytic enzyme intermediates stabilised by bound protein.
Journal of Clinical Investigation, 76, 865-874

DE VERNEUIL H, DEYBACH J, PHUNG N, DA SILVA V, NORDMANN Y (1983)
Study of anaesthetic agents for their ability to elicit porphyrin biosynthesis in chick embryo liver
Biochemical and Pharmacology, 32, 1011-1028

DEYBACH J C, DE VERNEUIL H, NORDMANN Y (1981)
The inherited enzymatic defect in porphyria variegata
Human genetics, 58, 425-428

DHAR G J, BOSSENMAIER I, CARDINAL R, PETRYKA Z J, WATSON C J (1978)
Transitory renal failure following rapid administration of a relatively large amount of hematin in a patient with acute intermittent porphyria in remission
Acta Medica Scandinavica, 203, 437-443

DHAR G J, BOSSENMAIER I, PETRYKA Z J, CARDINAL R, WATSON C J (1975)
Effects of haematin in acute porphyria
Annals of Internal Medicine, 83, 20-30

DICHTER H N, TADDEINI L, LIN S, AYALA G F (1977)
Delta-aminolaevulinic acid. Effect of a porphyrin precursor on an isolated neuronal preparation
Brain Research, 126, 189-195

DI MAURO S, MENDELL J R, SAHENK A et al (1980)
Fatal infantile mitochondrial myopathy and renal dysfunction due to cytochrome C oxidase deficiency
Neurology (Minneapolis), 30, 795-804

DI MAURO S, NICHOLSON I F, HAYS A P et al (1983)
Benign infantile mitochondrial myopathy due to reversible cytochrome C oxidase deficiency
Annals of Neurology, 14, 226-234

DOBA N, REIS D J (1973)
Acute fulminating neurogenic hypertension produced by brainstem lesions in rat
Circulation Research, 32, 584-593

DOMBEY S L, SAGAR D, KNAPP M S (1975)
Chronic renal failure in Nottingham and requirements for dialysis and transplant facilities
British Medical Journal, 1, 484-485

DOSS M, BAUMANN H, SIXEL F (1982a)
Alcohol in acute porphyria
Lancet, 1, 1307

DOSS M, BECKER U, PETER H-J, KAFFERNIK H (1981)
Drug safety in porphyria: risks of valproate and metoclopramide
Lancet, 2, 91

DOSS M, SCHNEIDER J, TIEPERMAN R, BRANDT A (1982b)
A new type of acute porphyria with porphobilinogen synthase defect in the homozygous state
Clinical Biochemistry, 15, 52-55

DOSS M, VON TIEPERMANN R, SCHNEIDER J, SCHMID H (1979)
New type of hepatic porphyria with porphobilinogen synthase defect and intermittent acute clinical manifestation
Klinische Wochenschrift, 57, 1123-1127

DOUER D, SCHOENFELD N, WEINBERGER A, PINKHAS J, ATSMON A (1978)
Favourable effect of intravenous propranolol in acute intermittent porphyria
Monographs in Human Genetics, 10, 223-226

DOUGLAS S D, ZUCKERMAN S H, ACKERMAN S K (1981)
Obtaining anc culturing human monocytes
In "Methods for studying mononuclear phagocytes", pp33-42 eds. Adams D O, Edelson P J, Joren H S, Academic Press, New York

DUGGAN A W (1983)
Electrophysiology of opioid peptides and sensory systems
British Medical Bulletin, 39, 65-70

DUKE-ELDER S (1962)
System of Ophthalmology. Vol VII, p.606
Henry Kimpton, London

DYCK P J, O'BRIEN P C, OHNISHI A (1977)
Lead neuropathy. 2. Random distribution of segmental demyelination among "old internodes: of myelinated fibres
Journal of Neuropathology and Experimental Neurology, 36, 570-575

EALES L (1960)
Cutaneous porphyria. Observations on 111 cases in three racial groups
South African Journal of Laboratory and Clinical Medicine, 6, 63-86

EALES L (1971)
The acute porphyria attack III. The precipitating and aggravating factors
South African Journal of Laboratory and Clinical Medicine (Special Issue), 17, 120-125

EALES L (1978)
The effects of canthaxanthin - a beta-carotene analogue - on the photocutaneous manifestations of EHP, VP and SP
South African Medical Journal, 54, 1050-1052

EALES L (1979)
Porphyria and the dangerous life-threatening drugs
South African Medical Journal, 56, 914-917

EALES L, DOWDLE E B, SWEENEY G D (1971)
The electrolyte disorder of the acute porphyric attack and the possible role of delta-aminolaevulinic acid
South African Journal of Laboratory and Clinical Medicine, 17, 89-97

EALES L, LINDER G D (1962)
Porphyria - The acute attack. An analysis of 80 cases.
South African Medical Journal, 36, 284-292

EDWARDS R H T (1971)
Percutaneous needle-biopsy of skeletal muscle in diagnosis and research
Lancet, ii, 593-596

EDWARDS R, YOUNG A, WILES M (1980)
Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair
New England Journal of Medicine, 302, 261-271

ELDER G H (1980)
The porphyrias: clinical chemistry, diagnosis and methodology
Clinics in Haematology, 9, 371-398

ELDER G H (1982)
Enzymatic defects in porphyria: an overview
Seminars in Liver Disease, 2, 87-99

ELDER G H, EVANS J O, THOMAS N et al (1976)
The primary enzyme defect in hereditary coproporphyria
Lancet, 2, 1217-1219

ELDER G H, LEE G B, TOVEY J A (1978)
Decreased activity of hepatic uroporphyrinogen decarboxylase in sporadic porphyria cutanea tarda
New England Journal of Medicine, 299, 274-278

ELDER T D, MENGEL C E (1966)
Effect of pyridoxine deficiency on porphyria precursor excretion in acute intermittent porphyria
American Journal of Medicine, 41, 369-374

ELIASER M Jr, KONDO B O (1942)
Electrocardiographic changes associated with acute porphyria
American Heart Journal, 24, 696-702

EPSTEIN J H, TUFFANELLI D L, EPSTEIN W L (1973)
Cutaneous changes in porphyria - a microscopic study
Archives of Dermatology, 107, 689-698

EPSTEIN O, SCHOENFIELD N, GREENBLAT Y, LAHAV M, ATSMON M (1982)
The influence of propranolol in the concentration of heme and on the activity of delta-aminolaevulinate synthase in monolayers of chick embryo liver cells
Biochemical Pharmacology, 31, 485-489

ERBSLÖH W (1903)
Zur Pathologie and pathologischen Anatomie der toxischer Polyneuritis nach Sulfonalgebrauch
Dtsch Z Nervenheik, 23, 197-204

EVANS D A P, MANLEY K A, McKUSICK V A (1960)
Genetic control of isoniazid metabolism in man
British Medical Journal, 2, 485-491

EVANS C H, WESTFALL V, ATUK N O (1972)
Astrocytoma mimicking the features of pheochromocytoma
New England Journal of medicine, 286, 1397-1399

EWING D J (1978)
Cardiovascular reflexes and autonomic neuropathy
Clinical Science and Molecular Medicine, 55, 321-347

EWING D J (1984)
Practical bedside investigation of diabetic autonomic function
In "Autonomic Failure" A textbook of clinical disorders of the autonomic nervous system, pp371-405
ed. Bannister, Sir Roger, Oxford University Press, Oxford

EWING D J, BORSEY D Q, BELLAVERE F, CLARKE B F (1981)
Cardiac autonomic neuropathy in diabetics: comparison of measures of R-R interval variation
Diabetologia, 21, 18-24

EWING D J, CAMPBELL I W, CLARKE B F (1980b)
The natural history of diabetic autonomic neuropathy
Quarterly Journal of Medicine, 49, 95-108

EWING D J, CAMPBELL I W, CLARKE B F (1980c)
Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implication
Annals of Internal Medicine, 92, 308-311

EWING D J, HUME L, CAMPBELL I W, MURRAY A, NEILSON J M M, CLARKE B F (1980a)
Autonomic mechanisms in the initial heart rate response to standing
Journal of Applied Physiology, 49, 809-814

FARRELL G C, CORREIA M A (1980)
Structural and functional reconstitution of hepatic cytochrome P450 in vivo
Journal of Biological Chemistry, 255, 10128-10133

FELDMAN D S, LEVERE R D, LIEBERMAN J S (1968)
Presynaptic neuromuscular inhibition by delta-aminolaevulinic acid - a porphyrin precursor
Transactions of the American Neurological Association, 93, 206-208

FELDMAN D S, LEVERE R D, LIEBERMAN J S (1971)
Presynaptic neuromuscular inhibition by porphobilinogen and porphobilin
Proceedings of the National Academy of Sciences of the United States of America, 68, 383-386

FELSHER B F, REDEKER A G (1967)
Acute intermittent porphyria: effect of diet and griseofulvin
Medicine (Baltimore), 46, 217-223

FERRARIO C M, McCUBBIN T W, PAGE I A (1969)
Hemodynamic characteristics of chronic experimental neurogenic hypertension in unanaesthetized dogs
Circulation Research, 24, 911-922

FISHBEIN W N, GRIFFIN J L, ARMBRUSTMACHER V W (1980)
Stain for skeletal muscle adenylate deaminase: an effective tetrazolium stain for frozen biopsy specimens
Archives of Pathology and Laboratory Medicine, 104, 462-466

FISHER H, ORTH G (1937)
Die Chemïe des pyrrols
Akad Verlagsgesselsh, Liepzig

FLUGEL K A, DRUSCHKY K F (1977)
Electromyogram and nerve conduction in patients with acute intermittent porphyria
Journal of Neurology, 214, 267-279

FOLEY K M (1982)
The practical use of narcotic analgesics
Medical Clinics of North America, 66, 1091-1104

FOLLIS R H Jr, WINTROBE M M (1945)
A comparison of the effects of pyridoxine and pantothenic and deficiencies in the nervous system of swine
Journal of Experimental Medicine, 81, 839-852

FONNUM F (1984)
Glutamate: a neurotransmitter in mammalian brain
Journal of Neurochemistry, 42, 1-11

FORD R E, OU C, ELLEFSON R D (1980)
Assay for uroporphyrinogen I synthase activity with porphobilinogen as substrate
Clinical Chemistry, 26, 1182-1185

FOSBURG M T, CRONE R K (1983)
Nitrous oxide analgesia for refractory pain in the terminally ill
Journal of the American Medical Association, 250, 511-513

FROHLICH E D (1977)
The adrenergic nervous system and hypertension
Mayo Clinic Proceedings, 52, 361-369

FUCHS T, SEUBERT A, SEUBERT S, IPPEN H (1980)
Anticonvulsant-induced unclassified porphyria
International Journal of Biochemistry, 12, 955-957

FULLER S D, CAPALDI R A, HENDERSON R (1979)
Structure of cytochrome C oxidase in deoxycholate-derived two-dimensional crystals
Journal of Molecular Biology, 134, 305-327

GADIAN D G, RADDA G K (1981)
NMR studies of tissue metabolism
Annual Review of Biochemistry, 50, 69-83

GAJDOS A, GAJDOS-TOROK M (1963)
Inhibitory action of adenine and adenine nucleosides and nucleotides on the biosynthesis of porphyrins by Rhodopseudomas spheroides
Nature, 199, 1093-1094

GAJDOS A, GAJDOS-TOROK M (1969)
The quantitative regulation of the biosynthesis of porphyrins by intracellular ATP concentration
Biochemical Medicine, 2, 372-388

GARCIN R, LAPRESLE J (1950)
Manifestations nerveuses des porphyries
Seminar Hopital Paris, 26, 3404-3422

GAYATHRI A K, RAO M R S, PADMANABAN B (1973)
Studies on the induction of delta-aminolaevulinic acid synthase in mouse liver
Archives of Biochemistry and Biophysics, 155, 299-306

GENDLER P L, DUHAM H A, RAPEPORT H (1978)
Haemopyrrole and kyrptopyrrole are absent from the urine of schizophrenics and normal poeple
Clinical Chemistry, 24, 230-233

GENTZ J, JOHANSSON S, LINDBLAD S, LINDSTEDT S, ZETTERSTROM R (1969)
Excretion of delta-aminolevulinic acid in hereditary tyrosinemia
Clinica Chimica Acta, 23, 257-263

GIBSON J B, GOLDBERG A (1956)
The neuropathology of acute porphyria
Journal of Pathology and Bacteriology, 71, 495-509

GILBERT J C, WYLLIE M G, DAVIDSON D V (1975)
Nerve terminal ATPase as a possible trigger for neurotransmitter release
Nature, 255, 237-238

GLUECK R, GREEN D, COHEN I, ISAO C (1983)
Hematin: unique effects on hemostasis
Blood, 61, 243-249

GOLDBERG A (1959)
Acute intermittent porphyria - a study of 50 cases
Quarterly Journal of Medicine, 28, 183-209

GOLDBERG A (1985)
Molecular genetics of acute intermittent porphyria
British Medical Journal, 291, 499-500

GOLDBERG A, MOORE M R, McCOLL K E L, BRODIE M J (1983)
Porphyrin metabolism and the porphyrias
In "Oxford Textbook of Medicine", p.9.81
eds. Weatherall D J, Ledingham J G G, Warrell D A, Oxford University Press, Oxford

GOLDBERG A, RIMINGTON C, (1954)
Fate of porphobilinogen in the rat: relation to acute porphyria in man
Lancet, ii, 172-173

GOLDBERG A, RIMINGTON C (1962)
Diseases of Porphyrin Metabolism, pp64-109
Charles C Thomas, Springfield, Illinois

GOLDBERG M L (1974)
The glucose effect: carbohydrate repression of enzyme induction, RNA synthesis and glucocorticoid activity - a role for cyclic AMP and cyclic GMP
Life Sciences, 17, 1747-1754

GOPALAN G, JAYARAMAN J, RAJAMANICKAM (1984)
Effect of exogenous addition of hemin on the biogenesis of mitochondrial membranes during glucose repression in Saccharomyces cerevisiae
Archives of Biochemistry and Biophysics, 235, 159-166

GORCHEIN A (1980)
Urine concentration of 3-ethyl-5-hydroxy-4,5-dimethyl-^{3}-pyrrolin-2-one (mauve factor) is not causally related to schizophrenia or acute intermittent porphyria
Clinical Science, 58, 469-476

GORDIN R (1948)
Prostigmin vid akut porfyri
Nordisk Medicin, 37, 480-481

GOURLAY G K, COULTHARD K (1983)
The role of naloxone infusions in the treatment of overdoses of long half-life narcotic agonists: application to nor-methadone
British Journal of Clinical Pharmacology, 15, 269-272

GOURLAY G K, COUSINS J M (1984)
Strong analgesics in severe pain
Drugs, 28, 79-91

GRACELY R H, McGRATH P, DUBNER R (1978)
Ratio scales of sensory and affective verbal pain descriptors
Pain, 5, 5-18

GRANDCHAMP B, DEYBACH J C, GRELIER M, DE VERNEUIL H, NORDMANN Y (1980)
Studies of porphyrin synthesis in fibroblasts of patients with congenital erythropoietic protoporphyria and one with homozygous coproporphyria
Biochemica et Biophysica Acta, 629, 577-586

GRANDCHAMP B, NORDMANN Y (1977)
Decreased lymphocyte coproporphyrinogen III oxidase activity in hereditary coproporphyria
Biochemistry and Biophysics Research Communications, 74, 1089-1095

GRANDCHAMP B, PHUNG N, NORDMANN Y (1977)
Homozygous case of hereditary porphyria
Lancet, ii, 1348-1349

GRANICK S (1966)
The induction in vitro of the synthesis of delta-aminolaevulinic acid synthetase in chemical porphyria: a response to certain drugs, sex hormones and foreign chemicals
Journal of Biological Chemistry, 241, 1359-1375

GRANICK S, GILDER H (1947)
Distribution, structure and properties of the tetrapyrroles
Advances in Enzymology and related areas of Molecular Biology, 7, 305-368

GRANICK S, KAPPAS A (1967)
Steroid induction of porphyrin synthesis in liver cell culture
Journal of Biological Chemistry, 242, 4587-4593

GRANICK S, SASSA S (1971)
Delta-aminolevulinic acid synthetase and the control of heme and chlorophyll synthesis
In " Metabolic Regulation" Vol 5, pp77-141
ed. Vogel H J, Academic Press, New York

GRANICK S, SASSA S (1978)
Hemin control of heme biosynthesis in mouse friend-virus transformed erythroleukemia cells in culture
Journal of Biological Chemistry, 253, 5402-5406

GRANICK S, URATA G (1963)
Increase in activity of delta-aminolaevulinic acid synthetase in liver mitochondria induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollildine
Journal of Biological Chemistry, 238, 821-827

GREEN D, REYNOLDS N, KLEIN J, KOHL M, CHUNG-HSIN TSAO (1983)
The inactivation of hemostatic factors by hematin
Journal of Laboratory and Clinical Medicine, 102, 361-369

GÜNTHER H (1911)
Die Haematoporphyrie
Dtsch Arch klin Med, 105, 89-146

GÜNTHER H (1922)
Die Bedeutung der Hematoporphyrie in Physiologie und Pathologie
Ergebnisse der Allgemeinen Pathologie patholigischen
Anatomie des Menschen, 20, 608-704

GUPTA G L, GAKSENA H C, GUPTA B D (1983)
Cardiac dysautonomia in acute intermittent porphyria
Indian Journal of Medical Research, 78, 253-256

HADJIVASSILIOU A G, REIDER S V (1968)
The enzymatic assay of pyruvic and lactic acids - a definitive procedure
Clinica Chimica Acta, 19, 357-361

HAMFELT A, WETTERBERG L (1968)
Neuropathy in porphyria
Lancet, 1, 50

HARCUS A W, WARD A E, SMITH D W (1979)
Methodology of monitored release of a new preparation: buprenorphine
British Medical Journal, 2, 163-165

HARLEY V (1890)
Two fatal cases of an unusual form of nerve disturbance associated with dark red urine probably due to defective tissue oxidation
British Medical Journal, 2, 1169-1170

HARMER M, SLATTERY P J, ROSEN M, VICKERS M D (1983)
Intramuscular on-demand analgesia: double blind controlled trial of pethidine, buprenorphine, morphine and meptazinol
British Medical Journal, 286, 680-682

HARRIS M Y, MILLS G C, LEVIN W C (1966)
Coexistent systemic lupus erythematosis and porphyria
Archives of Internal Medicine, 117, 425

HAYASHI N, YODA B, KIKUCHI G (1969)
Mechanism of allylisopropylacetamide-induced increase of delta-aminolaevulinate synthetase in liver mitochondria. IV. Accumulation of the enzyme in the soluble fraction of rat liver
Archives of Biochemistry and Biophysiology, 131, 83-91

HAYES M J, FRASER A R, HAMPTON J R (1979)
Randomised trial comparing buprenorphine and diamorphine for chest pain in suspected myocardial infarction
British Medical Journal, 2, 300-302

HEDGER R W, HARDISON W G M (1971)
Transient macroamylasemia during an exacerbation of acute intermittent porphyria
Gastroenterology, 60, 903-908

HELLMAN E S, TSCHUDY D P, BARTTER F C (1962)
Abnormal electrolyte and water metabolism in acute intermittent porphyria. The transient inappropriate secretion of antidiuretic hormone
American Journal of Medicine, 32, 734-746

HELLMAN J B, STACY R W (1976)
Variation of respiratory sinus arrhythmia with age
Journal of Applied Physiology, 41, 734-738

HEPNER G W, VESSELL E S (1975)
Quantitative assessment of hepatic function by breath analysis after oral administration of ^{14}C-aminopyrine
Annals of Internal Medicine, 83, 632-638

HIERONS R (1957)
Changes in the nervous system in acute porphyria
Brain, 80, 176-192

HILSTED J, JENSEN S B (1979)
A simple test for autonomic neuropathy in juvenile diabetics
Acta Medica Scandinavica, 205, 385-387

HOLLANDER C S, SCOTT R L, TSCHUDY D P, PERLROTH M, WAXMAN A, STERLING K (1967)
Increased protein-bound iodine and thyroxine-binding globulin in acute intermittent porphyria
New England Journal of Medicine, 277, 995-1000

HOPPE-SEYLER F (1871)
Medizinisch-chemische Untersuchungen, 1-4, p.528
Hirshwald A, Berlin

HORWITZ D A, ALLISON A C, WARD P, KNIGHT N (1977)
Identification of human mononuclear leucocyte populations by esterase staining
Clinical and Experimental Immunology, 30, 289-298

HOUDE R W (1979)
Analgesic effectiveness of the narcotic agonist-antagonists
British Journal of Clinical Pharmacology, 7, 297S-308S

HOUSTON A B, BRODIE M J, MOORE M R, THOMPSON G G, STEPHENSON J B P
Hereditary coproporphyria and epilepsy
Archives of Diseases in Childhood, 52, 646-650

HOWELL R R (1978)
The glycogen storage diseases.
In "The Metabolic Basis of Inherited Disease", 4th edition, pp137-159
eds. Stanbury J B, Wyngaarden J B, Fredrickson D S, McGraw-Hill, New York

IRVINE D G (1961)
Apparently non-indolic Ehrlich-positive substances related to mental illnesses
Journal of Neuropsychiatry, 2,292-305

IRVINE D G (1974)
Kryptopyrrole and other monopyrroles in molecular neurobiology
International Review of Neurobiology, 16, 145-182

IRVINE D G, BAYNE W, MIYASHITA H, MAJER J R (1969)
Identification of kryptopyrrole in human urine and its relation to psychosis
Nature, 224, 811-813

ISHII D N, MANIATIS G M (1978)
Haemin promotes rapid neurite outgrowth in cultured mouse neuroblastoma cells
Nature, 274, 372-373

IUPAC-IUB JOINT COMMISSION ON BIOCHEMICAL NOMENCLATURE (JCBN) (1980)
Nomenclature of tetrapyrroles: Recommendations 1978
European Journal of Biochemistry, 108, 1-30

JACOBS J M, MILLER R H, CAVANAGH J B (1979)
The distribution of degenerative changes in INH neuropathy. Further evidence for focal axonal lesions
Acta Neuropathologica, 48, 1-9

JARRETT A, RIMINGTON C, WILLOUGHBY D A (1956)
Delta-aminolaevulinic acid and porphyria
Lancet, 1, 125-127

JEDRZEJOWSKA H, RAFALOWSKA J, FIDZIANSKA A, SAWICKA E (1974)
Morphological changes in the nervous system and muscles in acute intermittent porphyria
Neuropatologia Polska, 12, 35-50

JOHNSON M A, TURBULL D M, DICK D J, SHERRATT H S A (1983)
A partial deficiency of cytochrome C oxidase in chronic progressive external ophthalmoplegia
Journal of the Neurological Sciences, 60, 31-53

JONES T A, BLOOMER J R, BERLIN N I (1971)
The measurement of the synthetic rate of bilirubin from hepatic hemes in patients with acute intermittent porphyria
Journal of Clinical Investigation, 50, 2259-2265

JOSEPHSON A S, LEVERE R D, LOWENTHAL I, SWERDLOW F, GINSBERG M (1972)
Porphyria synthesis by cultured lymphocytes
Blood, 39, 568-574

JUSIC A, SOSTARKO M, MAJIC D (1976)
Long-term ACTH and corticosteroid therapy in two siblings with polyneuropathy due to acute intermittent porphyria
European Neurology, 14, 294-301

KAIKO R F, FOLEY K M, GRABINSKI P Y et al (1983)
Central nervous excitatory effects of meperidine in cancer patients
Annals of Neurology, 13, 180-185

KALBFLEISCH J H, STOWE D F, SMITH J J (1978)
Evaluation of the heart rate response to the Valsalva manoeuvre
American Heart Journal, 95, 705-715

KAPPAS A, BRADLOW H L, GILLETTE P N, LEVERE R D, GALLAGHER T F (1972)
A defect in steroid hormone metabolism in acute intermittent porphyria
Federation Proceedings, 31, 1293-1297

KAPPAS A, SASSA S, ANDERSON K E (1983)
The Porphyrias
In "The Metabolic Basis of Inherited Diseases", pp1301-1384
eds. Stanbury J B, Wyngaarden J B, Fredrickson D S, Goldstein J L, Brown M S, McGraw-Hill, New York

KAUFMAN L, MARVER H S (1970)
Biochemical defects in two types of human hepatic porphyria
New England Journal of Medicine, 283, 954-958

KEZDI P (1954)
Neurogenic hypertension in man in porphyria
Archives of Internal Medicine, 94, 122-130

KLATZO I, SPATZ M (1981)
Cerebral ischaemia.
In "The Molecular Basis of Neuropathology", pp132-149
eds. Davidson A N, Thompson R H S, Edward Arnold, London

KOBZA K, GYR K, NEUHAUS K, GUDAT F (1976)
Acute intermittent porphyria with relapsing acute pancreatitis and unconjugated hyperbilirubinemia without overt hemolysis
Gastroenterology, 71, 494-496

KONTUREK S J (1978)
Endogenous opiates and the digestive system
Scandinavian Journal of Gastroenterology, 13, 257-261

KORDA V, DEYBACH J C, MARTUSEK P, ZEMAN J, DA SILVA V, NORDMANN Y, HOUSTKOVA H, RUBIN A, HOLUB J (1984)
Homozygous variegate porphyria
Lancet, 1, 851

KOSOWER N S, ROCK R A (1968)
Seizures in experimental porphyria
Nature, 217, 565-567

KRAMER S (1980)
Porphyria variegate
Clinics in Haematology, 9, 303-321

KRIEGER E M (1964)
Neurogenic hypertension in the rat
Circulation Research, 15, 511-521

KRNJEVIC K (1976)
Inhibitory action of GABA and GABA mimetics on vertebrate neurons
In "GABA in nervous sytem function", Kroc Foundation, Series No 5, pp269-282
eds. Roberts E, Chase T N, Tower R B, Raven Press, New York

KRNJEVIC K, SCHWARTZ S (1967)
The action of gamma-aminobutyric on cortical neurons
Experimental Brain Research, 3, 320-336

KUMAZAWA T, BACCELLI G, GUAZZI M (1969)
Hemodynamic patterns during desynchromised sleep in intact cats and cats with sino-aortic deafferentation
Circulation Research, 24, 923-937

KUSHNER J P, BARBUTO A J, LEE G R (1976)
An inherited enzymatic defect in porphyria cutanea tarda: decreased uroporphyrinogen decarboxylase activity
Journal of Clinical Investigation, 58, 1089-1097

LABBE R F (1967)
Metabolic anomalies in porphyria - the result of impaired biological oxidation: a hypothesis
Lancet, 1, 1361-1364

LAI C W (1981)
Carbamazepine in seizure management in acute intermittent porphyria
Neurology, 31, 232

LAI C W, HUNG T, LIN W S J (1977)
Blindness of cerebral origin in acute intermittent porphyria
Archives of Neurology, 34, 310-312

LAMBIE M, SCMITT H, VINCENT, REMOND G (1977)
Central cardiovascular effects on morphinomimetic peptides in dog
European Journal of Pharmacology, 46, 67-71

LAMON J M, FRYKHOLM B C, BENNETT M, TSCHUDY D P (1978)
Prevention of acute porphyric attacks by intravenous haematin
Lancet, 2, 492-494

LAMON J M, FRYKHOLM B C, HERRERA W, TSCHUDY D P (1979B)
Danazol administration to females with menses-associated exacerbation of acute intermittent porphyria
Journal of Clinical Endocrinology and Metabolism, 48, 123-126

LAMON J M, FRYKHOLM B C, HESS R A, TSCHUDY D P (1979a)
Hematin therapy for acute porphyria
Medicine, 58, 252-269

LARSON A W, WASSERSTROM W R, FELSHER B F, SHIH J C (1978)
Post-traumatic epilepsy and acute intermittent porphyria: effects of phenytoin, carbamazepine and clonazepam
Neurology, 28, 824-828

LASCELLES J (1960)
The synthesis of enzymes concerned in bacteria-chlorophyll formation in growing cultures of Rhodopseudomonas spheroides
Journal of General Microbiology, 23, 487-498

LEAVITT F, GARRON D C, WHISLER W W, SHEINKOP M D (1977)
Affective and sensory dimensions of back pain
Pain, 4, 273-281

LEFKOWITZ R J, CARON M G, STILES G L (1984)
Mechanisms of membrane-receptor regulation. Biochemical, physiological and clinical insights derived from studies of the adrenergic receptors
New England Journal of Medicine, 310, 1570-1579

LEVINE J (1984)
Pain and analgesia: the outlook for more rational treatment
Annals of Internal Medicine, 100, 269-276

LEVINE J D, GORDON A C, FIELDS H L (1978)
The mechanism of placebo analgesia
Lancet, 2, 654-657

LINDBLAD B O, LINDSTEDT S, STEENG (1977)
On the enzymatic defects in hereditary tyrosinemia
Proceedings of the National Academy of Sciences of the United States of America, 74, 4641-4645

LINDVALL N (1978)
Radiological changes of renal papillary necrosis
Kidney International, 13, 93-106

LOOTS J M, BECKER D M, MEYER B J, GOLDSTUCK N, KRAMER A (1975)
The effect of porphyrin precursors on monosynaptic reflex activity in isolated hemisected frog spinal cord
Journal of Neural Transmission, 36, 71-81

LUDWIG G D, EPSTEIN I S (1961)
A genetic study of two families having the acute intermittent type of porphyria
Annals of Internal Medicine, 55, 81-93

McCOLL K E L, MOORE M R, THOMPSON G G, GOLDBERG A (1982a)
Screening for latent acute intermittent porphyria: the value of measuring both delta-aminolaevulinic acid synthase and erythrocyte uroporphyrinogen-1-synthase activities
Journal of Medical Genetics, 19, 271-276

McCOLL K E L, MOORE M R, THOMPSON G G, GOLDBERG A (1980)
Induction of delta-aminolaevulinic acid synthase in leucocytes of patients on phenytoin therapy
British Journal of Clinical Pharmacology, 9, 327-331

McCOLL K E L, MOORE M E, THOMPSON G G, GOLDBERG A (1981)
Treatment with haematin in acute hepatic porphyria
Quarterly Journal of Medicine, 50, 161-174

McCOLL K E L, WALLACE A M, MOORE M R, THOMPSON G G, GOLDBERG A (1982b)
Alterations in haem biosynthesis during the human menstrual cycle: studies with normal subjects and patients with latent and active acute intermittent porphyria
Clinical Science, 62, 183-191

McCREA F D, EADIE G S, MORGAN J E (1942)
The mechanism of morphine miosis
Journal of Clinical Pharmacology and Therapeutics, 74, 239-246

McDOWALL F H, PLUM F (1954)
Arterial hypertension associated with acute anterior poliomyelitis
New England Journal of Medicine, 245, 241-245

McGILLION F B (1974)
A possible role for delta-aminolaevulinic acid in acute intermittent porphyria
PhD Thesis. University of Glasgow, p136

McGILLION F B, MOORE M R, GOLDBERG A (1972)
The effect of delta-aminolaevulinic aid on the spontaneous activity of mice
Scottish Medical Journal, 18, 133-134

McGILLION F B, THOMPSON G G, GOLDBERG A (1975)
Tissue uptake of delta-aminolaevulinic acid
Biochemical and Pharmacology, 24, 299-301

McGUIRE G M, BRODIE M J, MOORE M R, GOLDBERG A (1985b)
The effect of carbamazepine on 5-aminolaevulinate dehydratase in cultured human lymphocytes
Biochemical Society Transactions (in press)

McGUIRE G M, MOORE M E, MACPHEE G J, GOLDBERG A, BRODIE M J (1985a)
5-aminolaevulinate dehydratase and carbamazepine in man and rat
Biochemical Society Transactions, 13, 205-206

MACKAY J D, PAGE M M, CAMBRIDGE J, WATKINS P J (1980)
Diabetic autonomic neuropathy. The diagnostic value of the heart rate monitoring
Diabetologia, 18, 471-478

McQUEEN D S (1983)
Opioid peptide interactions with respiratory and circulatory systems
British Medical Bulletin, 39, 77-82

MAGNUS O, KOSTER M, VAN DER DRIF J H A (1977)
Cerebralmechanisms and neurogenic hypertension in man, with special reference to baroreceptor control
Progress in Brain Research, 47, 199-218

MAGNUSSEN C R, DOHERTY J M, HESS R A, TSCHUDY D P (1975)
Grand mal seizures and acute intermittent porphyhria
Neurology, 25, 1121-1125

MAGNUSSEN C R, LEVINE J B, DOCHERTY Y M, CHEESMAN J O, TSCHUDY D P (1974)
A red cell enzyme method for the diagnosis of acute intermittent porphyria
Blood, 44, 857-868

MAINES M D (1980)
Regional distribution of the enzymes of haem biosynthesis and the inhibition of 5-aminolaevulinate synthase by manganese in the rat brain
Biochemical Journal, 190, 315-321

MAINES M D (1984)
New developments in the regulation of heme metabolism and their implications
In "Critical Reviews in Toxicology", 12, pp241-313
ed. Goldberg L, CRC Press Inc., Florida

MAINES M D, TOMIO J M, JANOUSEK V (1978)
Differential patterns of porphyrin biosynthesis in liver and kidney: metal ion blockade of delta-aminolevulinate synthetase induction by porphyrinogenic agents
In "Diagnosis and Therapy of Porphyria and Lead Toxicity", pp8-20, ed. Doss M, Springer-Verlag, New York

MALCOLM A D, COLTART D J (1977)
Cardiocirculatory effects of strong analgesic agents
In "Pain - New Perspectives in Measurement and Management" pp41-45, eds. Harcus A W, Smith R B, Shittle B A, Churchill Livingstone, Edinburgh

MALMSTROM B G (1979)
Cytochrome C oxidase
Biochimica et Biophysica Acta, 549, 281-303

MANN J G, DE NARDO G L (1965)
Acute intermittent porphyria associated with hyperthyroidism
Journal of Clinical Endocrinology and Metabolism, 25, 1151-1155

MAO C C, COSTA E (1978)
Biochemical pharmacology of GABA transmission
In "Psychopharmacology: A Generation of Progress", pp307-318, eds. Lipton M A, Dimascio A, Killam K F, Raven Press, New York

MARVER H S (1974)
The role of heme in the synthesis and repression of microsomal protein
In "Microsomes and Drug Oxidation", pp495-511, eds. Gillette G R, Conney A H, Cosmides A J, Estabrook R W, Fouts J R, Mannering G J, Academic Press, New York

MARVER H S, COLLINS A, TSCHUDY D P, RECHGICL M (1966b)
Delta-aminolaevulinic synthase. II. Induction in rat liver
Journal of Biological Chemistry, 241, 4325-4329

MARVER H S, TSCHUDY D P, PERLROTH M G, COLLINS A (1966a)
Delta-aminolaevulinic acid synthetase. I. Studies in liver homogenates
Journal of Biological Chemistry, 241, 2803-2809

MASON V R, COURVILLE C, ZISKIND E (1933)
The porphyrins in human disease
Medicine (Baltimore), 12, 355-439

MASUJA T (1969)
Pathophysiological observations on porphyrias
Acta Haematologica (Japan), 32, 465-514

MAUZERALL D, GRANICK S (1956)
The occurrence and determination of delta-aminolaevulinic acid and porphobilinogen in urine
Journal of Biological Chemistry, 219, 435-446

MAYER D J, WATKINS L R (1984)
Multiple endogenous opiate and non-opiate analgesia systems
In "Advances in Pain Research and Therapy" Vol 6, pp253-275, eds. Kruger L, Liebeskund J C, Raven Press, New York

MAYTHEM D V, EALES L (1971)
Electrodiagnostic findings in porphyria
South African Journal of Laboratory and Clinical Medicine, 17, 99-100

MEIJER A E F (1968)
Improved histochemical method for the demonstration of the activity of -glucan phosphorylase
Histochemie, 12, 244-252

MELBY T C, STREET T P, WATSON C J (1956)
Chlorpromazine in the treatment of porphyria
Journal of the American Medical Association, 162, 174-178

MELZACK R (1975)
The McGill Pain Questionnaire: major properties and scoring methods
Pain, 1, 277-299

MENEWAT A S, KOCHAR D K, PANWAR R B, JOSHI C K (1979)
Propranolol in acute intermittent porphyria
Postgraduate Medical Journal, 55, 546-547

MERSKEY H (1979)
Pain terms; a list with definitions and notes on usage.
Recommended by the IASP sub-committee on taxonomy
Pain, 6, 249-252

MEYER U A (1973)
Acute intermittent porphyria. Clinical and biochemical studies of disordered heme biosynthesis
Enzyme, 16, 334-342

MEYER U A, SCHMID R (1973)
Hereditary hepatic porphyrias
Federation Proceedings, 32, 1649-1655

MEYER U A, SCHMID R (1978)
The porphyrias.
In "The Metabolic Basis of Inherited Disease" 4th edition, ppl166-1220, eds. Wyngaarden J B, Frederickson D S., McGraw-Hill, New York

MEYER U S, STRAND L J, DOSS M, REES A C, MARVER H S (1972)
Intermittent acute porphyria - demonstration of a genetic defect in porphobilinogen metabolism
New England Journal of Medicine, 286, 1277-1282

MINCHOM P E, DORMER R L, HUGHES I A et al (1983)
Fatal infantile mitochondrial myopathy due to cytochrome oxidase deficiency
Journal of Neurological Sciences, 60, 453-463.

MITSUMOTO H, APRILLE J R, WRAY S H, NEMNI R , BRADLEY W G (1981)
An abnormality of cytochrome aa_3 in progressive external ophthalmoplegia (PEO) with morphologic mitochondrial abnormalities
Neurology (Minneapolis), 31, (Abstract) 58

MIYAGI K, CARDINAL R, BOSSENMAIER I, WATSON C J (1971)
The serum porphobilinogen and hepatic porphobilinogen deaminase in normal and porphyric individuals
Journal of Laboratory and Clinical Medicine, 78, 683-695

MONACO R N, LEEPER R D, ROBBINS J J, CALVY G L (1957)
Intermittent acute porphyria treated with chlorpromazine
New England Journal of Medicine, 256, 309-311

MONTGOMERY B M (1961)
The basilar artery hypertensive syndrome
Archives of Internal Medicine, 108, 115-125

MOORE M R (1980)
International review of drugs in acute porphyria
International Journal of Biochemistry, 12, 1089-1097

MOORE M R (1983)
Laboratory investigation of disturbances of porphyrin metabolism
In "Broadsheet 109, Association of Clinical Pathologists" July, British Medical Association, London

MOORE M R (1985)
Porphyrias - Drug Lists
University of Glasgow publication

MOORE M R, BATTISTINI V, BEATTIE A D, GOLDBERG A (1970)
The effect of certain barbiturates on the hepatic porphyrin metabolism of rats
Biochemical Pharmacology, 19, 751-757

MOORE M R, DISLER P B (1983)
Drug-induction of the acute porphyrias
Adverse Drug Reactions and Acute Poisoning Reviews, 2, 149-189

MOORE M R, McGUIRE G, BRODIE M J, YEUNG LAIWAH A C, GOLDBERG A, MEISSNER P N, KEHOE B (1983)
Carbamazepine and haem biosynthesis
Lancet, 2, 846 (Letter)

MOORE M R, MEREDITH P A, GOLDBERG A (1980)
Lead and haem biosynthesis
In "Lead Toxicity", pp79-118, eds. Singhal R L, Thomas J A, Urban and Schwarzenberg, Baltimore

MOORE M R, THOMPSON G G, GOLDBERG A, IPPEN H, SEUBERT A, SEUBERT S (1978)
The biosynthesis of haem in congenital (erythropoietic) porphyria
International Journal of Biochemistry, 9, 933-938

MORGAN-HUGHES J A (1983)
Disorders of mitochondrial metabolism. Some clinical and biochemical mechanisms
In "Advanced Medicine 19", pp243-260, ed. Saunders K B, Pitman, London

MORGAN-HUGHES J A, DARVENIZA P, KAHN S N et al (1977)
A mitochondrial myopathy characterised by a deficiency of reducible cytochrome b
Brain, 100, 617-620

MORRIS L D, DUDLEY M D, PEARSON R D (1981)
Coagulopathy associated with hematin treatment for acute intermittent porphyria
Annals of Internal Medicine, 95, 700-701

MULLER D P R, LLOYD J K, WOLFF O H (1983)
Vitamin E and neurological function
Lancet, 1, 225-228

MULLER W E, SNYDER S H (1977)
Delta-aminolaevulinic acid: influences on synaptic GABA receptor binding may explain CNS symptoms of porphyria
Annals of Neurology, 2, 340-342

MULLER-EBERHARD U, VINCENT S H (1985)
Concepts of heme distribution within hepatocytes
Biochemical Pharmacology, 34, 719-725

MURPHY F R, KRUPA V, MARKS G S (1975)
Drug induced porphyrin synthesis. XIII. Role of lipophilicity in determining porphyrin-inducing activity of aliphatic amides after blockage of their hydrolysis by bis-[p-nitrophenyl] phosphate
Biochemical Pharmacology, 24, 883-899

MURRAY R M (1978)
Genesis of analgesic nephropathy in the United Kingdom
Kidney International, 13, 50-57

MURTY H S, NAIR P P (1969)
Prevention of allylisopropylacetamide induced porphyrin in the rat by vitamin E
Nature, 223, 200-201

MUSTAJOKI P (1978)
Variegate porphyria
Annals of Internal Medicine, 89, 238-244

MUSTAJOKI P (1981)
Normal erythrocyte uroporphyrinogen I synthase in a kindred with acute intermittent porphyria
Annals of Internal Medicine, 95, 162-166

MUSTAJOKI P, DESNICK R J (1985)
Genetic heterogeneity in acute intermittent porphyria: characterisation and frequency of porphobilinogen deaminase mutations in Finland
British Medical Journal, 291, 505-509

MUSTAJOKI P, KOSKELO P (1976)
Hereditary hepatic porphyrias in Finland
Acta Medica Scandinavica, 200, 171-178

MUSTAJOKI P, SEPPALAINEN A M (1975)
Neuropathy in latent hereditary hepatic porphyria
British Medical Journal, 2, 310-312

NAGLER W (1971)
Peripheral neuropathy in acute intermittent porphyria
Archives of Physical Medicine and Rehabilitation, 52, 426-431

NAGLER W (1972)
Neuropathy in acute intermittent porphyria
Annals of Internal Medicine, 76, 878

NAIR P P, MEZEY E, MURTY H S, QUARTNER J, MENDELOFF A I (1971)
Vitamin E and porphyrin metabolism in man
Archives of Internal Medicine, 128, 411-415

NANRA R S, HICKS J D, McNAMARA J H et al (1970)
Seasonal variation in the post mortem incidence of renal papillary necrosis
Medical Journal of Australia, 1, 293-297

NANRA R S, STUART-TAYLOR J, De LEON A H, WHITE K H (1978)
Analgesic nephropathy: Etiology, clinical syndrome and clinicopathologic correlations in Australia
Kidney International, 13, 79-92

NATHAN C F, MURRAY H W, COHN Z A (1980)
The macrophage as an effector cell
New England Journal of Medicine, 303, 622-626

NEMOTH P M, MEYER D, KARK R A P (1980)
Effects of denervation and simple disuse on rates of oxidation and on activities of four mitochondrial enzymes in type 1 muscle
Journal of Neurochemistry, 35, 1351-1360

NGAI S H, BERKOWITZ B A, YANG J C, HEMPSTEAD J, SPECTOR S (1976)
Pharmacokinetics of naloxone in rats and in man: basis for its potency and short duration of action
Anesthesiology, 44, 398-401

NICOLL R A (1976)
The interaction of porphyria precursors with GABA receptors in the isolated frog spinal cord
Life Sciences, 19, 521-526

NIELSEN B, THORN N A (1965)
Transient excessurinary excretion of antidiuretic material in acute intermittent porphyria with hyponatremia and hypomagnesemia
American Journal of Medicine, 38, 345-348

NIIJIMA A (1976)
Baroreceptor effects on renal and adrenal nerve activity
American Journal of Physiology, 230, 1733-1748

NINOMYA I, IRISAWA H (1969)
Summation of baroreceptor reflex effects on sympathetic nerve activities
American Journal of Physiology, 216, 1330-1336

OCKNER R K, SCHMID R (1961)
Acquired porphyria in man and rat due to hexachlorobenzene intoxication
Nature, 189, 499

ORR I A, KEENAN D J M, DUNDEE J W (1981)
Improved pain relief after thoracotomy: use of cryoprobe and morphine infusion
British Medical Journal, 283, 945-948

ORTIZ de MONTELLANO P R, MICO B A (1981)
Destruction of cytochrome P450 by allylisopropylacetamide is a suicidal process
Archives of Biochemistry and Biophysics, 206, 43-50

ORWELL E S, KENDALL J W (1980)
B-endorphin and adreno-corticotropin in extra pituitary sites: gastrointestinal tract
Endocrinology, 107, 438-442

ORWIN J M (1977)
Buprenorphine - Pharmacological aspects in man
In "Pain - New Perspectives in Measurement and Management" pp141-169, eds. Harcus A W, Smith R, Whittle B, Churchill Livingstone, Edinburgh

OSTROWSKI J, KOSTRZEWSKA E, MICHALAK T, ZAWIRSKA B, MEDRZEJEWSKI W, GREGOR A (1983)
Abnormalities in liver function and morphology and impaired aminopyrine metabolism in hereditary hepatic porphyrias
Gastroenterology, 85, 1131-1137

PADYKULLA HA , HERMAN E (1955)
The specificity of the histochemical method for adenosine triphosphatase
Journal of Histochemical Cytochemistry, 3, 170-183

PALKOVITS M, ZABORSKY L (1977)
Neuroanatomy of central cardiovascular control: nucleus tractus solitarii - afferent and efferent neuronal connections in relation to the baroreceptor reflex arc
Progress in Brain Research, 47, 9-24

PARK B K (1981)
Assessment of urinary 5 B-hydroxycortisol as an in vivo index of mixed function oxygenase activity
British Journal of Clinical Pharmacology, 12, 97-102

PATERNINI J R, SIMONE J J, BEATTIE D S (1978)
Detection and regulation of delta-aminolaevulinic acid synthetase activity in the rat brain
Archives. of Biochemistry and Biophysics, 189, 86-91

PATERSON S J, ROBSON L E, KOSTERLITZ H W (1983)
Classification of opioid receptors
British Medical Bulletin, 39, 31-36

PAUL S M, AXELROD J, DILIBERTO E J (1977)
Catechol estrogen-forming enzyme of brain: demonstration of a cytochrome P450 mono-oxygenase
Endocrinology, 101, 1604-1610

PAXTON J W, MOORE M R, BEATTIE A D, GOLDBERG A (1974)
17-oxosteroid conjugates in plasma and urine of patients with acute intermittent porphyria
Clinical Science and Molecular Medicine, 46, 207-222

PAZDEROVA-VEJLUPKOVA J, NEMCOVA M, PICKOVA J, JIRASEK L, LUKAS E (1981)
The development and prognosis of chronic intoxication by tetra chlorobenzo-p-dioxin in man
Archives of Environmental Health, 36, 5-18

PEARSE A G E (1972)
Histochemistry, theoretical and applied, Vol 2.
Churchill Livingstone, London

PENDREIGH D M, HEASMAN M H, HOWITT L F et al (1972)
Survey of chronic renal failure in Scotland
Lancet, 1, 304-307

PERCY V A, LAMM M C L, TALJAARD (1981)
Delta-aminolaevulinic acid uptake, toxicity, and effect on ^{14}C gamma-aminobutyric acid uptake into neurons and glia in culture
Journal of Neurochemistry, 36, 69-76

PERCY V A, SHANLEY B C (1977)
Porphyrin precursors in blood, urine and cerebrospinal fluid in acute porphyria
South African Medical Journal, 52, 219-222

PERCY V A, SHANLEY B C (1980)
Factors affecting haem degradation in rat brain
Biochemical Pharmacology, 29, 1590-1592

PERLROTH M G, MARVER H S, TSCHUDY D P (1965)
Oral contraceptive agents and the management of acute intermittent porphyria
Journal of the American Medical Association, 194, 1037-1042

PERLROTH M G, TSCHUDY D P, RATNER A (1968)
The effect of diet in variegate (South African genetic) porphyria
Metabolism, 17, 571-581

PERLROTH M G, TSCHUDY D P, WAXMAN A, ODELL W D (1967)
Abnormalities of growth hormone regulation in acute intermittent porphyria
Metabolism, 16, 87-90

PETERS H A, CRIPPS D J, REESE H H (1974)
Porphyria: Theories of etiology and treatment
In "Review of Neurobiology", pp323-335, Academic Press, New York

PETERS P G, SHARMA M L, HARDWICKE D M, PIPER W N (1980)
Sulphonamide inhibition of rat hepatic uroporphyrinogen-I-synthetase activity and the biosynthesis of heme
Archives of Biochemistry and Biophysics, 201, 88-94

PETERSON J M, PIERACH C A (1984)
Hematin-induced hemolysis in acute porphyria
Annals of Internal Medicine, 101, 877

PIEPKORN M W, HAMERNYIK P, LABBE R F (1978)
Modified erythrocyte uroporphyrinogen-I-synthase assay and its clinical interpretation
Clinical Chemistry, 24, 1751-1754

PIERACH C A (1982)
Haematin therapy for the porphyric attack
Seminars in Liver Disease, 2, 125-131

PIERACH C A, CARDINAL R, BOSSENMAIER I, WATSON C J (1977a)
Comparison of the Hoesch and the Watson Schwartz tests for urinary porphobilinogen
Clinical Chemistry, 23, 1666-1668

PIERACH C A, GUIDON L, PETRYKA Z J (1977b)
Effect and fate of intravenously administered porphobilin in rats
Experimentia, 33, 873-874

PIMSTONE N R, BLEKKENHORST G, EALES L (1973)
Enzymatic defects in hepatic porphyria. Preliminary observations in patients with porphyria cutanea tarda and variegate porphyria
Enzyme, 16, 354-366

PIPER W N, CANDIE I W, TEPHLY T R (1973)
The role of substrates for glycine acyltransferase in the reversal of chemically induced porphyria in the rat
Archives of Biochemistry and Biophysics, 159, 671-677

PIPER W N, PETERS P G, SHARMA M L, HARDWICKE D M (1979)
Sulfonamide and folate regulation of uroporphyrinogen I synthase activity
In "Proceedings primer congreso Argentino internacional. Sobre porfiras y porfirinas" p89, Buenos Aires

PIPER W N, VAN LIER R B L, RIOS G, TEPHLY T R (1976)
Isolation of a factor that protects against lead inhibition of hepatic and erythrocytic uroporphyinogen I synthetase activity
Life Sciences, 19, 1225-1234

POLACK J M, SULLIVAN S N, BLOOM S R, FACER P, PEARSE A G E (1977)
Encephalin-like immunoreactivity in the human gastro-intestinal tract
Lancet, 1, 972-974

PORTER R S, FITCH C D (1966)
Vitamin E deficiency in the monkey: X-protoporphyrin and heme synthesis by peripheral blood and bone marrow
Scandinavian Journal of Haematology, 3, 175-185

POULSON R (1976)
The enzymatic conversion of protoporphyrinogen IX to protoporphyrin IX in mammalian mitochondria
Journal of Biological Chemistry, 251, 3730-3733

POULSON R, POLGLASE W J (1975)
The enzymic conversion of protoporphyrinogen IX to protoporphyrin IX
Journal of Biological Chemistry, 250, 1269-1274

PRICE J M, BROWN R R, PETERS HE A (1959)
Tryptophan metabolism in porphyhria, schizophrenia and a variety of neurological and psychiatric diseases
Neurology, 9, 456-468

PRICK M J J, GABREELS F J M, TRIJBELS J M F et al (1983)
Progressive poliodystrophy (Alper's disease) with a defect in cytochrome aa_3 in muscle: a report of two unrelated patients
Clinical Neurology and Neurosurgery, 85, 58-70

RANKING J E, PARDINGTON G L (1890)
Two cases of haemato-porphyrin in the urine
Lancet, 2, 607-609

RAPEPORT W G, McINNES G T, THOMPSON G G, FORREST G, PARK B K, BRODIE M J (1983)
Hepatic enzyme induction and leucocyte delta-aminolaevulinic acid synthase activity: studies with carbamazepine
British Journal of Clinical Pharmacology, 16, 133-137

REIS D J, TALMAN W T (1984)
Brain lesions and hypertension
In "Handbooks of Hypertension" vol 4: Experimental and genetic models of hypertension, pp352-473 ed. De Jong

REVILL S I, ROBINSON J O, ROSEN M, HOGG M I J (1976)
The reliability of a linear analogue for evaluating pain
Anaesthesia, 31, 1191-1198

REYNOLDS N C, MISKA R M (1981)
Safety of anticonvulsants in hepatic porphyrias
Neurology (NY) 31, 480-484

RIDEOUT J M, WRIGHT D J, LIM C K, RINSLER M G, PETERS T J (1983)
Carbamazepine-induced non-hereditary acute porphyria
Lancet, ii, 464 (Letter)

RIDLEY A (1969)
The neuropathy of acute intermittent porphyria
Quarterly Journal of Medicine, 38, 307-333

RIDLEY A (1984)
Porphyric neuropathy
In "Peripheral Neuropathy" vol II, pp1704-1716,
eds. Dyck P J, Thomas P K, Lambert E H, Bunge R, W B Saunders, Philadelphia

RIDLEY A, HIERONS R, CAVANAGH J B (1968)
Tachycardia and the neuropathy of porphyria
Lancet, ii, 708-710

RIDLEY A R, CAVANAGH J B (1972)
Exercise as a factor in the neuropathy of porphyria
Lancet, ii, 87-88

RIFENBERICK D H, GAMBLE J G, MAX S R (1973)
Response of mitochondrial enzymes to decreased muscular activity
American Journal of Physiology, 225, 1295-1299

RIFKIND A B (1976)
Drug-induced exacerbations of porphyria
Primary Care, 3, 665-685

RIMINGTON C (1985)
A review of the enzymatic errors in the various porphyrias
Scandinavian Journal of Clinical and Laboratory Investigation, 45, 291-301

RIMINGTON C, LOCKWOOD W H, BELCHER C V (1968)
The excretion of porphyrin-peptide conjugates in variegate porphyria
Clinical Science, 35, 211-247

RIMOLDI M, BOTTACCHI E, ROSSI L, CORNELIA F, UZIEL G, Di DONATO S (1982)
Cytochrome-C-oxidase deficiency in muscles of a floppy infant without mitochondrial myopathy
Journal of Neurology, 227, 201-207

ROBBIE D S (1979)
A trial of sublingual buprenorphine in cancer pain
British Journal of Clinical Pharmacology, 7, 297S-308S

ROMEO G (1980)
The hepatic porphyrias
In "Progress in Medical Genetics: Genetics of Gastrointestinal Disease" vol 4, p169, eds Steinberg A G, Bearn A G, Motulsky A G, Saunders, Philadelphia

ROSE J A, HELLMAN E S, TSCHUDY D P (1961)
Effect of diet on induction of experimental porphyria
Metabolism, 10, 514-521

ROSS B D, RADDA G K, GADIAN D G, ROCKER G, ESERI M, FALCONER-SMITH J (1981)
Examination of a case of suspected McArdle's syndrome by ^{31}P nuclear magnetic resonance
New England Journal of Medicine, 304, 1338-1342

RUBIN P, BLASCHKE T F, GUILLEMINAULT C (1981)
Effect of naloxone, a specific opioid inhibitor, on blood pressure fall during sleep
Circulation, 63, 117-121

RUTTER P C, MURPHY F, DUDLEY H A F (1980)
Morphine: controlled trial of different methods of administration for post-operative pain relief
British Medical Journal, 1, 12-13

RUTHERFORD T, THOMPSON G G, MOORE M R (1979)
Heme biosynthesis in friend erythroleukemia cells control by ferrochelatase
Cell Biology, 76, 833-836

SAILLEN R, JEQUIER E, VANNOTTI A (1969)
Porphyrin synthesis by the phytohemagglutinin-transformed lymphocytes in vitro
Journal of Reticuloendothelial Society, 6, 175-183

SAINT E G, CURNOW D H (1962)
Porphyria in Western Australia
Lancet, i, 133-136

SANO S, GRANICK S (1961)
Mitochondrial coproporphyrinogen oxidase and protoporphyrin formation
Journal of Biological Chemistry, 236, 1173-1180

SANO S, RIMINGTON C (1963)
Excretion of various porphyrins and their corresponding porphyrinogens by rabbits after intravenous injection
Biochemical Journal, 86, 203-212

SASSA S (1976)
Sequential induction of heme pathway enzymes during erythroid differentiation of mouse Friend leukemia virus-infected cells
Journal of Experimental Medicine, 143, 305-315

SASSA S, GRANICK S (1970)
Induction of delta-aminolaevulinic acid synthetase in chick embryo liver cells in culture
Proceedings of the National Academy of Sciences of the United States of America, 67, 517-520

SASSA S, GRANICK S, BICKERS D R, BRADLOW H L, KAPPAS A (1974)
A microassay for uroporphyrinogen I synthase, one of three enzyme activities in acute intermittent porphyria, and its application to the study of the genetics of this disease
Proceedings of the National Academy of Sciences of the United States of America, 71, 732-736

SASSA S, GRANICK S, BICKERS D R, LEVERE R D, KAPPAS A (1973)
Studies on the inheritance of human erythrocyte delta-aminolaevulinate dehydratase and uroporphobilinogen synthase
Enzyme, 16, 326-333

SASSA S, KAPPAS A (1983)
Hereditary tyrosinemia and the heme biosynthetic pathway - profound inhibition of delta-aminolaevulinic acid dehydratase activity by succinyl acetone
Journal of Clinical Investigation, 71, 625-634

SASSA S, SOLISH G, LEVERE R D, KAPPAS A (1975)
Studies in porphyria IV. Expression of the gene defect of acute intermittent porphyria in cultured human skin fibroblasts and amniotic cells: Prenatal diagnosis of the porphyric trait
Journal of Experimental Medicine, 142, 722-731

SASSA S, WHETSELL W D Jr, KAPPAS A (1979)
Studies on porphyrin-heme biosynthesis in organotypic cultures of chick dorsal root ganglion II. The effect of lead
Environmental Research, 19, 415-426

SASSA S, ZALAR G L, KAPPAS A (1978)
Studies in porphyria. VII. Induction of uroporphyrinogen-I-synthase and expression of the gene defect of acute intermittent porphyria in mitogen-stimulated human lymphocytes
Journal of Clinical Investigation, 61, 499-508

SCHLEY G, BOCK K D, HOCEVAR V, MERGUET P, RAUSCH-STROOMANN J G, SCHRODER E, SCHUEMANN H J (1970)
Hypertension and tachycardia in acute intermittent porphyria
Klinische Wochenschrift, 48, 36-42

SCHMID R (1960)
Cutaneous porphyria in Turkey
New England Journal of Medicine, 263, 397-398

SCHMITT H, LAMBIE M (1979)
Destruction of the nucleus tractus solitarii in dogs: acute effects on blood pressure and hemodynamics, chronic effects on blood pressure - importance of the nucleus for effects of drugs.
In " Nervous System and Hypertension", pp173-179
eds. Meyer P, Schmitt H., Wiley-Flammarion, New York

SCHOENFELD N, ATSMON A (1977)
The effect of agents blocking adrenergic B-receptors on incorporation of amino acids into protein in tissue cultures of chick embryo liver cells
Biochemical Pharmacology, 26, 867-870

SCHOENFELD N, EPSTEIN O, ATSMON A (1976)
The effect of beta-adrenergic blocking agents on experimental porphyria induced by 3,5-diethoxy-carbomyl-1,4-dihydrocolllidine (DDC) in vivo and in vitro
Biochimica et Biophysica Acta, 444, 286-293

SCOTT J, HUSKISSON E C (1976)
Graphic representation of pain
Pain, 2, 175-184

SENGERS R C, TRIJBELS J M F, BAKKEREN J A et al (1984)
Deficiency of cytochromes b and aa_3 in muscle from a floppy infant with cytochrome oxidase deficiency
European Journal of Pediatrics, 141, 178-180

SEPPELAINEN A M, HERNBERG S (1972)
Sensitive technique for detecting subclinical lead neuropathy
British Journal of Industrial Medicine, 29, 443-449

SHANLEY B C, NEETHLING A C, PERCY V A, CARSTENS H (1975)
Neurochemical aspects of porphyria. I. Studies on the possible neurotoxicity of delta-aminolaevulinic acid
South African Medical Journal, 49, 576-680

SHEDLOFSKY S I, BONKOWSKY H L (1984)
Seizure management in the hepatic porphyrias: Results from a cell culture model of porphyria
Neurology, 34, 399

SIMA A A F, KENNEDY J C, BLAKESLEE D, ROBERTSON D M (1981)
Experimental porphyric neuropathy: a preliminary report
Canadian Journal of Neurological Sciences, 8, 105-114

SIMPSON J A (1962)
Conduction velocity of peripheral nerves in human metabolic disorders
In "Progress in electromyography", pp36-42
eds. Pinelli P, Buchthal F, Thiebaut F., Elsevier, Amsterdam

SINCLAIR P, GRANICK S (1977)
Two methods for determining the activity of delta-aminolaevulinate synthetase within intact liver cells in culture
Annals of Biochemistry, 79, 380-393

SMITH A, De MATTEIS F (1980)
Drugs and the hepatic porphyrias
Clinics in Haematology, 9, 399-425

SMITH C B, SOKOLOFF L (1981)
The energy metabolism of the brain
In "The Molecular Basis of Neuropathology" pp104-131
eds. Davidson A N, Thompson R H S., Edward Arnold, London

SMITH K M (1975)
Porphyrins and metalloporphyrins, pp3-58
Elsevier, Amsterdam

SNAVELY M D, MAHAN L C, O'CONNOR D T, INSEL P A (1983)
Selective down-regulation of adrenergic receptor subtypes in tissues from rats with phaeochromocytoma
Endocrinology, 113, 355-361

SNAVELY M D, ZIEGLER M G, INSEL P A (1985)
A new approach to determine rates of receptor appearance and disappearance in vivo. Application to agonist-mediated down-regulation of rat renal cortical B1- and B2-adrenergic receptors
Molecular Pharmacology, 27, 19-25

SNYDER S H (1977)
Opiate receptors in the brain
New England Journal of Medicine, 296, 266-272

SNYDER S H (1980)
Brain peptides as neurotransmitters
Science, 209, 976-983

SONG C S, BONKOWSKY H L, TSCHUDY D P (1974)
Salicylamide metabolism in acute intermittent porphyria
Clinical Pharmacology and Therapeutics, 15, 431-435

SORENSEN A W S, WITH T K (1971)
Persistent paresis after porphyric attacks
South African Journal of Laboratory and Clinical Medicine, 17, 101-103

SOSTARKO M, JUSIC A (1979)
Long-term corticosteroid therapy of polyneuropathy due to acute intermittent porphyria
Neurologija, 27, 1-4

SPENCER P S, SABRI M I S, SCHAUMBERG H H, MOORE C L (1979)
Does a defect of energy metabolism in the nerve fibre underlie axonal degeneration in polyneuropathies?
Annals of Neurology, 5, 501-507

STANBURY J B, WYNGAARDEN J B, FREDRICKSON D S, GOLDSTEIN J L (1983)
Inborn errors of metabolism in the 1980s, ppl-37
McGraw-Hill, New York

STANSBIE D , DORMER R L, HUGHES I A et al (1982)
Mitochondrial myopathy with skeletal muscle cytochrome oxidase deficiency
Journal of Inherited Metabolic Disease, 5, Suppl 1, 27-28

STEIN J A, TSCHUDY D P (1970)
Acute intermittent porphyria - a clinical and biochemical study of 46 patients
Medicine (Baltimore), 49, 1-16

STERNBACH R A (1974)
Pain Patients: Traits and Treatment p81
Academic Press, New York

STERNBACH R A (1981)
Chronic pain as a disease entity
Triangle "Sandoz", 20, 27-33

STOKVIS B J (1889)
Over twee zeldzame kleurstoffen in urine van zicken
Nederlands Tijdschrift voor Geneeskunde, 13, 409-417 (2R2)

STRAND L J, FELSHER B F, REDEKER A G, MARVER H S (1970)
Heme biosynthesis in intermittent acute porphyria: decreased hepatic conversion of porphobilinogen to porphyrins and increased delta aminolevulinic acid synthetase activity
Proceedings of the National Academy of Sciences of the United States of America, 67, 1315-1320

STRAND L J, MEYER U A, FELSHER B F, REDEKER A G, MARVER H S (1972)
Decreased red cell uroporphyrinogen I synthetase activity in intermittent acute porphyria
Journal of Clinical Investigation, 51, 2530-2536

STRIFE C F, ZUROWESTE E L, EMMETT E A, FINELLI V N, PETERING H G, BERRY H K (1977)
Tyrosinemia with acute intermittent porphyria: Aminolaevulinic acid dehydratase deficiency related to elevated urinary aminolaevulinic acid levels
Journal of Pediatrics, 90, 400-404

STRUNK R C, KUNKE K S, GICLAS P C (1983)
Human peripheral blood monocyte-derived macrophages produce haemolytically active C3 in vitro
Immunology, 49, 169-174

SWANK R L, ADAMS R D (1948)
Pyridoxine and pantothenic deficiency in swine
Journal of Neuropathology and Experimental Neurology, 7, 274-286

SWANSON D W, MARUTA T (1980)
Patients complaining of extreme pain
Mayo Clinic Proceedings, 55, 563-566

SWASH M, SCHWARTZ M S (1984)
Biopsy pathology of muscle - Biopsy pathology series
Chapman and Hall, London

SWEENEY V P, PATHAK M A, ASBURY A K (1970)
Acute intermittent porphyria: increased ALA-synthetase activity during an attack
Brain, 93, 369-380

TADDEINI L, NORDSTROM K L, WATSON C J (1964)
Hypercholesterolemia in experimental and human hepatic porphyria
Metabolism, 13, 691-701

TALMAN W T, SNYDER D, REIS D J (1980)
Chronic lability of arterial pressure produced by destruction of A2 catecholaminergic neurons in rat brainstem
Circulation Research, 46, 842-853

TASMAN-JONES C (1980)
Zinc deficiency states
Advances in Internal Medicine, 26, 97-114

TAYLOR R L (1981)
Magnesium sulphate for AIP seizures
Neurology (NY), 31, 1371-1372

TEN EYCK F W, MARTIN W J, KERNOHAN J W (1961)
Acute porphyria: neuropsy studies in nine cases
Proceedings of the Mayo Clinic, 36, 409-422

TEPHLY T R, WAGNER G, SEDMAN R, PIPER W (1978)
Effects of metals on heme biosynthesis and metabolism
Federation Proceedings, 37, 35-39

THOMAS P K (1971)
The morphological basis for alterations in nerve conduction in peripheral neuropathy
Proceedings of the Royal Society of Medicine, 64, 295-298

THOMPSON J W (1983)
Opioid peptides
British Medical Journal, 288, 259-260

THORNER P S, BILBAO J M, SIMA A A F, BRIGGS S (1981)
Porphyric neuropathy: an ultrastructural and quantitative nerve study
Canadian Journal of Neurological Sciences, 8, 281-287

TISHLER P V, GORDON B J (1983)
Sorbent therapy of the porphyrias. II. Experimental plasma or hemoperfusion with a commercial charcoal cartridge.
Methods and Findings in Experimental Clinical Pharmacology, 5, 185-192

TISHLER P V, GORDON B J, O'CONNOR J A (1982)
The absorption of porphyrins and porphyrin precursors by sorbents: a potential therapy for the porphyrias
Methods and Findings in Experimental Clinical Pharmacology, 4, 125-131

TONGIO M M, MAYER S, HAUPTMANN G et al (1979)
Lack of linkage between acute intermittent porphyria and the A and B loci of the HLA system
Tissue Antigens, 13, 273-279

TRESS K H, EL-SOBKY A A (1979)
Pupil responses to intravenous heroin (diamorphine) in dependent and non-dependent users
British Journal of Clinical Pharmacology, 7, 213-217

TSCHUDY D P, LAMON J M (1980)
Porphyrin metabolism and the porphyrias
In "Metabolic Control and Disease", 8th edition, pp939-1008 eds. Bondy P K, Rosenberg L E, W B Saunders, Philadelphia

TSCHUDY D P, MARVER H S, COLLINS A (1965a)
A model for calculating messenger RNA half-life: short-lived messenger RNA in the induction of mammalian delta-aminolaevulinic acid synthetase
Biochemical and Biophysical Research Communications, 21, 480-487

TSCHUDY D P, PELROTH M G, MARVER H S, COLLINS A, HUNTER G Jr, RECHEIGL M Jr (1965b)
Acute intermittent porphyria: the first "overproduction disease" localised to a specific enzyme
Proceedings of the National Academy of Sciences of the United States of America, 53, 841-847

TSCHUDY D P, VALSAMIS M, MAGNUSSEN C R (1975)
Acute intermittent porphyria: clinical selected research aspects
Annals of Internal Medicine, 83, 851-864

TSCHUDY D P, WELLAND F H, COLLINS A et al (1964)
The effect of carbohydrate feeding on the induction of delta-aminolaevulinic acid synthetase
Metabolism, 13, 396-406

TUNG A S, YAKSH T (1982)
In vivo evidence for multiple opiate receptors mediating analgesia on the rat spinal cord
Brain Research, 247, 75-83

VAN BIERVLIET J P, BRUINVIS L, KETTING D et al (1977)
Hereditary mitochondrial myopathy with lactic acidemia, a De Toni-Fanconi-Debré syndrome, and a defective respiratory chain in voluntary striated muscle
Pediatric Research, 11, 1088-1093

VENTAFRIDDA V, De CONNO F, GUARISE G et al (1983)
Chronic analgesic study on buprenorphine action in cancer pain - comparison with pentazocine
Drug Research, 4, 587-590

VERSMOLD H T, BREMER H J, HERZOG V et al (1977)
A metabolic disorder similar to Zellweger syndrome with hepatic acatalasia and absence of peroxisomes, altered content and redox state of cytochromes and infantile cirrhosis with hemosiderosis
European Journal of Paediatrics, 124, 261-275

VERSTEEG D H G, PETTY M A, BOHUS B, De JONG W (1984)
The central system and hypertension: the role of catecholamines and neuropeptides
In "Handbook of Hypertension", vol 4: "Experimental and Genetic Models of Hypertension", pp398-430
ed. de Jong W, Elsevier, Amsterdam

VICKERS H D (1940)
Anterior poliomyelitis: relation to hypertension in young adults
New York State Journal of Medicine, 40, 55-59

VICTOR M, ADAMS R D (1956)
Neuropathology of experimental vitamin B6 deficiency in monkeys
American Journal of Clinical Nutrition, 4, 346-353

VILJOEN D J, CUMMINGS R, ALEXOPOULOS J, KRAMER S (1983)
Protoporphyrinogen oxidase and ferrochelatase in porphyria variegata
European Journal of Clinical Investigation, 13, 283-287

VILTER R W, MUELLER J F, GLAZER H S, JARROLD T, ABRAHAM J, THOMSON C, HAWKINS V R (1953)
The effect of vitamin B6 deficiency induced by desoxypyridoxine in human beings
Journal of Laboratory and Clinical Investigation, 42, 335-357

WAINIO W W (1983)
Cytochrome c oxidase: Two models
Biological Reviews of the Cambridge Philosophical Society, 58, 131-156

WAKAYAMA Y, MUROGA T, HABINO R, MATSUI T, UTSUMI M (1975)
On the study of sural nerve biopsy in a case of acute intermittent porphyria
Clinical Neurology (Tokyo), 15, 333-339

WALDENSTRÖM J (1937)
Studien ueber porphyrie
Acta Medica Scandinavica, 82 (Suppl), 1-254

WALDENSTRÖM J (1957)
The porphyrias as inborn errors of metabolism
American Journal of Medicine, 22, 758-773

WALKER J E (1983)
Glutamate, GABA and CNS disease: a review
Neurochemistry Research, 8, 521-550

WALL P D (1985)
Pain
In "Scientific Basis of Clinical Neurology", ppl63-171
eds. Swash M, Kennard C., Churchill Livingstone, Edinburgh

WAMSLEY J K, ZARBIA M A, YOUNG W S III, KUHAR M J (1982)
Distribution of opiate receptors in the monkey brain: an autoradiographic study
Neuroscience, 7 (3), 595-613

WANG A L, ARREDENDO-VEGA F X, GIAMPIETRO P F, SMITH M, ANDERSON W F, DESNICK R J (1981)
Regional gene assignments for human porphobilinogen deaminase and esterase A4 on chromosome 11
Proceedings of the National Academy of Sciences of the United States of America, 78, 5734-5738

WARE S, MILLWARD-SADLER G H (1980)
Acute liver disease associated with sodium valproate
Lancet, 2, 1110-1113

WATKINS L R, MAYER D I (1982)
Organization of endogenous opiate and nonopiate pain control systems
Science, 216, 1185-1192

WATKINS P J, EDMONDS M E (1984)
Clinical presentation of diabetic autonomic failure
In "Autonomic Failure - A textbook of clinical disorders of the autonomic nervous system", pp337-370
ed. Bannister, Sir Roger, Oxford University Press, Oxford

WATSON C J, PIERACH C A, BOSSENMAIER I, CARDINAL R (1977)

Proceedings of the National Academy of Sciences of the United States of America, 74, 2118-2120

WATSON C J, RUNGE W, BOSSENMAIER I (1962)
Increased urinary porphobilinogen and uroporphyrin after administration of stilboestrol in a case of latent porphyria
Metabolism, 11, 1129-1133

WATSON C J, SCHWARTZ S (1941)
A simple test for urinary porphobilinogen
Proceedings of the Society for Experimental Biology and Medicine, 47, 393-394

WAXMAN A D, BERK P D, SCHALCH D, TSCHUDY D P (1969)
Isolated adrenocorticotropic hormone deficiency in acute intermittent porphyria
Annals of Internal Medicine, 70, 317-323

WAXMAN A D, COLLINS A, TSCHUDY D P (1966)
Oscillations of hepatic delta-aminolaevulinic acid synthetase produced in vivo by heme
Biochemical and Biophysical Research Communications, 24, 675-683

WAXMAN A, SCHALCH D S, ODELL W D, TSCHUDY D P (1967)
Abnormalties of carbohydrate metabolism in acute intermittent porphyria
Journal of Clinical Investigation, 46, 1129-1139

WEHRMACHER W H (1952)
New symptomatic treatment for acute intermittent porphyria
Archives of Internal Medicine, 89, 111-114

WELLAND F H, HELLMAN E S, COLLINS A, HUNTER G W Jr, TSCHUDY D P (1964b)
Factors affecting the excretion of porphyrin precursors by patients with acute intermittent porphyria. II. The effect of ethinyl estradiol
Metabolism, 13, 251-258

WELLAND F H, HELLMAN E S, GADDIS E M, COLLINS A, HUNTER G W Jr, TSCHUDY D P (1964a)
Factors affecting the excretion of porphyrin precursors by patients with acute intermittent porphyria. I. The effect of diet
Metabolism, 13, 232-250

WETTERBERG L (1967)
A neuropsychiatric and genetical investigation of acute intermittent porphyria
Svenska Bokfolaget, Nordedts, Scandinavian University Books

WHITELAW A G L (1974)
Acute intermittent porphyria, hypercholesterolaemia and renal impairment
Archives of Diseases in Childhood, 49, 406-407

WHITING M J (1976)
Synthesis of delta-aminolaevulinate synthase by isolated liver polyribosomes
Biochemical Journal, 158, 391-400

WIDER DE XIFRA E A, DEL C BATLLE A M, STELLA A M, MALAMUD S (1980)
Acute intermittent porphyria - another approach to therapy
International Journal of Biochemistry, 12, 819-822

WILLEMS J F, MONNENS L A H, TRIJBELS J M F et al (1977)
Leigh's encephalomyelopathy in a patient with cytochrome C oxidase deficiency in muscle tissue
Pediatrics, 60, 850-857

WINCHESTER J F, RATCLIFFE J G, CARLYLE E, KENNEDY A C (1978)
Solute, amino acid and hormone changes with coated charcoal hemoperfusion in uremia
Kidney Internatinal, 14, 74-81

WINDEBANK A J, DYCK P J (1984)
Lead intoxication as a model of primary segmental intoxication
In "Peripheral Neuropathy" 2nd edition, pp650-665
eds. Dyck P J, Thomas P K, Lambert E H, Bunge R,
W B Saunders, Philadelphia

WITH T K (1961)
Excretion of porphobilinogen and delta-aminolaevulinic acid in acute porphyria
Lancet, ii, 485-487

WITH T K (1971)
Acute porphyria, toxic and genuine in the light of history
Danish Medical Bulletin, 18, 112-121

WITH T K (1980)
Porphyrias in animals
Clinics in Haematology, 9, 345-370

WOCHNIK-DYJAS D, NIEWIADOMSKA M, KOSTRIZEWSKA E (1978)
Porphyric polyneuropathy and its pathogenesis in the light of electrophysiological investigations
Journal of Neurological Sciences, 35, 243-256

YAMADA M, KONDO M, TANAKA M, OKEDA R, HATAKEYAMA S, FUKUI T, TSUKAGOSHI H (1984)
An autopsy case of acute porphyria with a decrease of both uroporphyrinogen I synthetase and ferrochelatase activities
Acta Neuropathologica (Berlin), 64, 6-11

YEUNG LAIWAH A C, JONES L, HAMILTON A O, WHALEY K (1985)
Complement - subcomponent-C1-inhibitor synthesis by human monocyte
Biochemical Journal, 226, 199-205

ZANDBERG P, PALVOKITS M, de Jong W (1978)
Effect of various lesions in the nucleus tractus solitarii of the rat on blood pressure, heart rate and cardiovascular reflex responses
Clinical and Experimental Hypertension, 1, 355-368

ZHANG A Z, PASTERNAK G W (1980)
Mu and delta-opiate receptors. Correlation with high and low affinity opiate binding sites
European Journal of Pharmacology, 67, 323-324

ZIMAN M R, BRADSHAW J J, IVANETICH M M (1980)
The effect of fluroxene [(2,2,2,-trifluroethoxy) ethane] on heme biosynthesis or degradation
Biochemical Journal, 190, 571-580

ZIMMERMAN T S, McMILLIN J M, WATSON C J (1966)
Onset of manifestations of hepatic porphyhria in relation to the influence of female sex hormones
Archives of Internal Medicine, 118, 229-240

APPENDIX I

EXPLANATION OF NEUROPATHOLOGICAL TERMS

1. Segmental demyelination is the characteristic histopathological abnormality seen in neuropathies due to dysfunction of the Schwann cell. Short segments of myelin break down in a random manner between the nodes of Ranvier; there appears to be no predilection for distal involvement of the nerve cell. Following demyelination the process of remyelination can occur rapidly. And if the axon remains intact recovery can be complete. Segmental demyelination impairs the conduction velocity of the nerve cell considerably. When tested clinically conduction velocity in the affected peripheral nerves is typically reduced by more than 40%.

2. Axonal degeneration of the 'dying-back' type occurs when the most distal end of the nerve cell degenerates with dissolution of the axon and destruction of the accompanying myelin sheath. The myelin breaks up into small lipid globules which are phagocytosed by microglia ie Wallerian degeneration. Despite loss of the axon, the Schwann cells and their basal lamina persist and proliferate to form cords of cells within the endoneural tubes. Soon after the distal part of the axon degenerates the muscle fibres it supplies become atrophied. Axonal degeneration affects the most distal part of the axon first because it is the most vulnerable part to become compromised metabolically, being dependent on the

cell body or perikaryon for somal irrigation. Axonal degeneration may occur in parenchymatous neuropathies, following application of toxic substances, from mechanical compression of the nerve or after death of the cell body.

In the absence of demyelination mild neuropathies of this type may be present without abnormalities of nerve conduction velocities when tested clinically because only a proportion of remaining unaffected nerve cells in the peripheral nerves with their intact myelin sheaths, is required to transmit the electrical impulse of the test unimpeded. Thus diseases which primarily affect the small fibres can have normal nerve conduction velocities. And even those diseases which cause a selective loss of the large, fast-conducting fibres often have a mild slowing of conduction velocities, the velocity being seldom reduced by more than 20 to 30%.

The discussion into demyelinating and axonal neuropathies is somewhat arbitrary as few diseases fall entirely into one group or the other. In severe segmental demyelinating neuropathies some axonal degeneration takes place whereas primary axonal neuropathy can cause some secondary paranodal demyelination.

3. Chromatolysis refers to degenerative and reactive changes in the body of the nerve cell ie perikaryon as a result of axonal injury or destruction. The cell body becomes swollen and pale-staining and its nucleus becomes eccentric in position. The cytoplasmic Nissl granules disintegrate into dust-like basophilic particles. The ultrastructural component of chromatolysis is fragmentation of the granular endoplasmic reticulum.

Chromatolysis is associated with increased nucleic acid and protein turnover. If the axon regenerates the cell body may return to normal. If it does not, or if the axon is damaged close to the cell body, the nerve cell dies.

4. Following axonal damage, attempted regeneration can take place to re-innervate motor end plates. Fine axon sprouts grow from the proximal stumps of the injured cell into the distal region of the nerve where the Schwann cells have formed cords of cells. With repeated axonal degeneration and regeneration, the large myelinated fibres tend to decrease with a relative increase in the small myelinated fibres. Eventually there will be a predominance of non-myelinated fibres as the small myelinated fibres also disappear.

5. Electromyographic findings Normally with a single motor unit stimulation the action potential has a characteristic bi- or tri-phasic form, the amplitude being proportional to the number of muscle fibres, and the duration of the wave form being proportional to the area over which the muscle fibres span. With denervation of the muscle fibres the following changes can be seen on the electromyographic recording:

1. Excessive polyphasic activity (more than 5 phases)
2. Giant potential with amplitude of more than 5 mV (normally less than 4 mV)
3. Spontaneous activity with frequent positive sharp waves ie Fibrillation and Fasciculation
4. Reduction in interference or recruitment pattern on muscle contraction.

APPENDIX II

DEVELOPMENT OF THE HUMAN MONOCYTE CULTURE METHOD

In the original method described by Douglas et al (1981) monocytes were incubated in tissue culture medium (RPMI-1640) containing 20% foetal calf serum (FCS). This method proved to be repeatedly unsuccessful in my hands because by day 7 of culture between 50-90% of the monocytes became non-adherent and were loose in the culture supernatant.

A number of experiments were performed to determine the optimal culture conditions for obtaining a monocyte monolayer. The modifications that ensued have been adopted in the method described.

Peripheral monocytes were obtained from blood of normal healthy volunteers. The cells were separated by centrifugation over Ficoll-Hypaque gradients and passaged through tissue-culture flasks pre-coated with the micro-exudate of baby-hamster kidney cells. The purified monocytes were then cultured at a concentration of 10^5 cells/ml for varying periods of time in RPMI-1640 enriched with sera of different types at various concentrations. At the end of the culture period the supernatant was removed from each well and the non-adherent cells separated by centrifugation. The DNA contents of the adherent and non-adherent cells were then assayed separately.

It was discovered that the type of serum used to enrich the culture medium had a critical effect on the adherence of the monocytes to the culture plate.

The results of representative experiments are presented.

Abbreviations: RPMI = Tissue culture medium RPMI-1640

FCS = Foetal calf serum

Human AB = human blood group AB serum

Expt BHK 3

Culture medium	Duration of culture (days)	% remaining adherent cells	% loose cells	% cells lysed
RPMI-20% FCS	7	29	59	12
RPMI-20% FCS	14	22	55	23

After seven days, 57 ± 14% of the loose cells could be made to readhere if replated in a new well, and they still remained adherent after nine days in culture.

Expt BHK 16 Effect of different types of enrichment sera on the adherence of monocytes in culture for ten days

Type of serum	Adherent cells (ug DNA/well)	Loose cells (ug DNA/well)	Total DNA content	% adherent cells
10% human AB	2.12 ± 0.31	1.04 ± 0.55	3.16 ± 0.42	67 ± 9
10% human autologous	2.02 ± 0.57	1.28 ± 0.41	3.30 ± 0.16	61 ± 14
20% FCS	0.21 ± 0.10	2.72 ± 0.95	2.93 ± 1.05	7 ± 1
*10% human autologous (3h) followed by 20% FCS	0.98 ± 0.27	1.90 ± 0.35	2.88 ± 0.08	34 ± 9

* Monocytes were plated for 3h in 10% human autologous serum. Culture medium was then removed and replaced with 20% FCS.

Inference: 10% human serum achieved best cell adherence in culture.

Expt BHK 18 — Effects of different types of enrichment sera on monocyte adherence after 10 days in culture

Type of serum	Adherent cells (ug DNA/well)	Loose cells (ug DNA/well)	Total DNA content	% adherent cells
20% FCS	0.54 ± 0.01	1.31 ± 0.11	1.85 ± 0.10	30 ± 2
10% human AB (24h) followed by 20% FCS	1.73 ± 0.11	0.54 ± 0.33	2.27 ± 0.34	67 ± 5
10% human AB (72h) followed by 20% FCS	1.58 ± 0.04	0.94 ± 0.08	2.25 ± 0.05	63 ± 2
10% horse serum with 10% FCS	0.56 ± 0.11	1.23 ± 0.23	1.79 ± 0.12	31 ± 6

Inference: Initial incubation of monocytes in human serum seems to be critical for their adherence to the well although they can subsequently be cultured in FCS-containing medium.

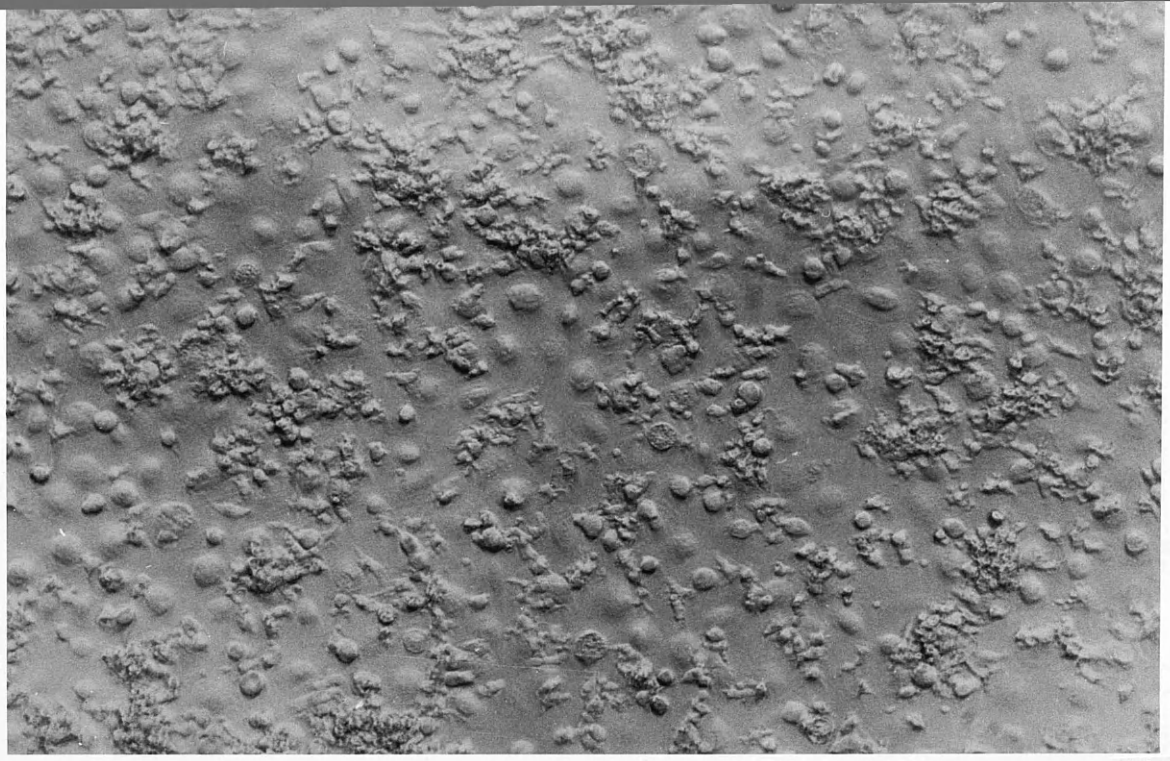
Expt BHK 21 — The monocytes were initially plated in 10% human AB serum
Effects of various types of enrichment sera in the maintenance culture medium on cell adherence after 10 days

Enrichment serum	Adherent cells (ug DNA/well)	Loose cells (ug DNA/cell)	Total DNA content	% adherent cells
10% FCS	1.48 ± 0.01	1.18 ± 0.04	2.66 ± 0.04	55 ± 1
20% FCS	1.70 ± 0.21	1.35 ± 0.08	3.05 ± 0.28	66 ± 2
10% FCS and 10% horse serum	1.79 ± 0.01	1.42 ± 0.03	3.21 ± 0.04	66 ± 3

Conclusion

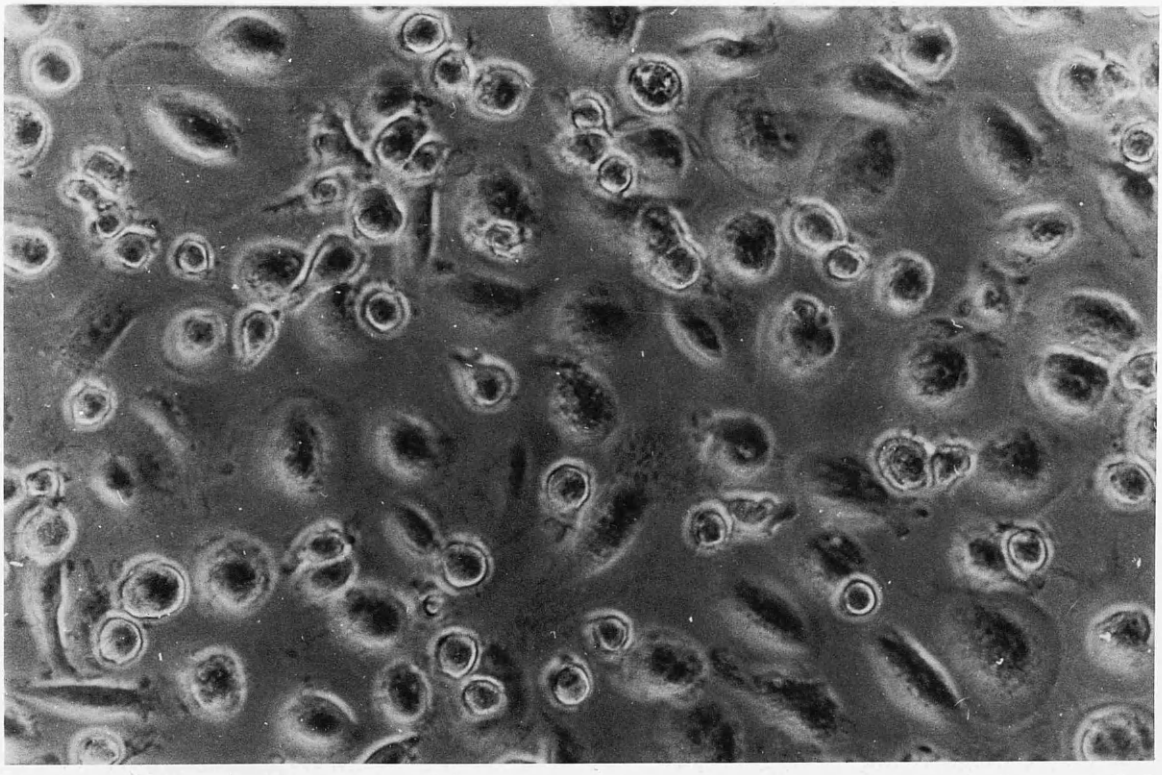
After passage of the monocytes through the BHK-coated flasks the cells were allowed to adhere to the wells of the Linbro culture plates in RPMI-1640 enriched with 10% human AB serum. The medium was then changed to RPMI-1640 containing 20% FCS for the rest of the culture period.

Figure 48 show the microscopic appearances of human monocytes soon after plating and after 10 days in culture respectively.



x 125

(a)



x 250

(b)

Figure 48: Human blood monocytes in culture for 10 days.

(a) well-established cell monolayer.

(b) note considerable spreading and pleomorphism of established monocytes. Identity of these cells cannot be ascertained by morphological appearances alone.

APPENDIX III

HOECHST DNA ASSAY

The main problem that had to be overcome in the development of this assay was to find a detergent that would lyse the monocytes without causing significant interference with the fluorescence measurement.

Method

0.2 ml of the detergent solution was added to each well containing the monocyte monolayer. Thorough mixing was carried out by repeated pipetting. One ml PBS (PH 7.2) was then added to each well and the total contents transferred into a tube containing another 4 ml of PBS. 20 ul of Hoechst 33285 dye (7.5×10^{-5} M) were added to each tube and the contents were vortexed before incubation in the dark for 20 min at room temperature. The reaction mixture was then read spectrofluorimetrically at excitation and emission wavelength of 364 nm and 448 nm respectively.

The effects of different detergents were assessed to the highest concentrations at which fluorescence measurements became significantly altered:

1.	Deoxycholate (Sigma Chemicals)	0.5%:	monocytes were incompletely lysed.
2.	Tween (Sigma Chemicals)	0.5%:	Cell-clumping and incomplete lysis occurred.
3.	Nonidet P40 (BDH)	0.5%:	Similarly caused cell-clumping and incomplete lysis
4.	Triton X100 (BDH)	0.1%:	Clumps of nuclei were observed microscopically

The effects of repeated cycles of freezing (-80°C for 45 minutes) and thawing (37°C) before and after exposure to the different detergents did not significantly improve the problem.

Finally lysis of the monocytes was satisfactorily achieved with 0.05% SDS.

A linear relationship was obtained between the fluorescence readings and the number of monocytes lysed (Fig 49). 10^5 monocytes are equivalent to 1.1 ug DNA using purified calf thymus DNA as standard in the presence of 0.05% SDS.

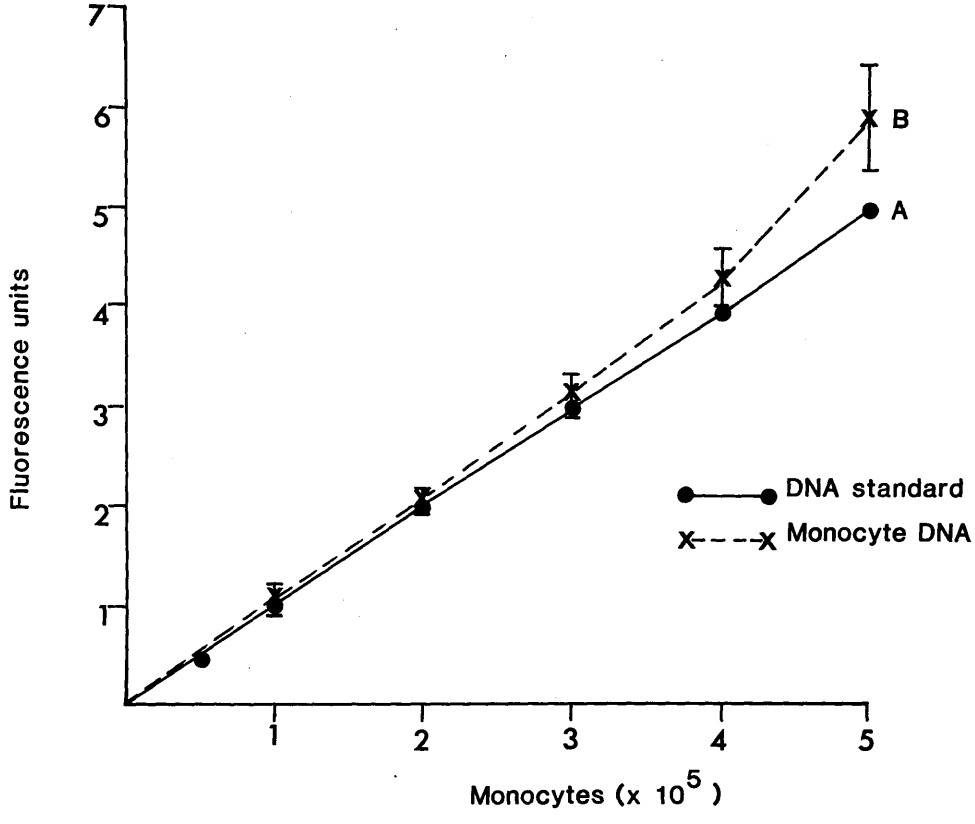


Figure 49: Spectrofluorimetric readings of the DNA content of increasing number of monocytes on day 1 of culture. Purified calf thymus DNA (ug) was used as standard.

APPENDIX IV

OPIOIDS AND OPIATES

"Opiate" refers specifically to the products derived from the juice of the opium poppy (although it has been loosely applied to morphine derivatives). "Opioid" refers to any directly acting compound whose effects are stereospecifically antagonised by naloxone. Peptides with this property are known as "opioid peptides".

Following the discovery of specific brain opiate receptors by Goldstein and his associates in 1971 several types of receptors have been described on the basis of their interaction with morphine and other synthetic non-peptide opiates. It is currently thought that the mu, kappa and delta receptors are the most important physiologically (Paterson et al, 1983; Thompson, 1983). Evidence to date supports the mu receptors as being important in analgesia and the delta receptors in the modulation of emotional behaviour (Snyder, 1980; Zhang & Pasternak, 1980). In addition to mediating analgesia, mu receptors are probably involved in causing respiratory depression and pupillary construction. But more than one class of receptor or ligand seem to function in pain modulation since animals made tolerant to the analgesic actions of morphine can still respond to a relatively specific delta-receptor agonist, D-ALA-D-Leu-encephalin (Tung & Yaksh, 1982).

Opioid receptors are widely distributed in nervous tissue. They are found in highest concentrations in the peri-aquaductal grey matter, rostro-ventral medulla, layers I, IV and VI at the

cerebral cortex, hypothalamus, thalamus and dorsal horn of the spinal cord (Atweh & Kuhar, 1983). Experiments in surgically-induced selective lesions were performed and have indicated that orally or parenterally administered opiates produce analgesia primarily by their actions at the supraspinal sites (Barton et al, 1980).

Morphine and its analogues bind preferentially to mu-receptors which are highly responsive to the blocking action of the specific opioid antagonist naloxone. Naloxone has no agonist activity and is relatively selective for only one receptor type, the mu-receptor (Chang & Cuatrecasas, 1981). It rapidly penetrates the blood-brain barrier and its brain concentration closely follows the changing serum levels (Ngai et al, 1976). Its plasma half-life is about one hour but its duration of action can last four hours after a single injection (Bradberry & Raebel, 1981; Gourlay & Coulthard, 1983).