Therapy of Porphyria with Oral Activated Charcoal

R J Hift MBChB (Cape Town) FCP(SA)

MRC/UCT Liver Research Centre and
Dept of Medicine
University of Cape Town

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CONTENTS

CHAPTER 1

INTRODUCTION TO PORPHYRIA

The porphyrias are a group of disorders of the haem biosynthetic pathway. Each is ascribed to a unique deficiency of an enzyme of this pathway. Figure 1.1 shows this pathway and the position of the affected enzyme in each form of porphyria.

PORPHYRINOGENS AND PORPHYRINS

The porphyrinogens are cyclic tetrapyrroles in which the macrocycle exists in the unconjugated state, the four pyyrole rings being connected by single-bonded *methylene bridges*. Four pyrrole rings are connected by single bonds. The corresponding conjugated forms are known as the porphyrins, in which the rings are connected by double-bonded *methene bridges*). The conversion occurs spontaneously in the presence of oxygen, light acid or other oxidising conditions, and is accompanied by a conformational change. Where the porphyrinogens are flexible, non-aromatic compounds, the porphyrins are highly aromatic, rigid, planar macrocycles and are inherently more stable than most porphyrinogens.

With one exception, *ferrochelatase*, which acts upon protoporphyrin, the enzymes of the pathway can use only the porphyrinogens as substrates. Yet most diagnostic and research work in the laboratory is performed on the corresponding porphyrins. This is not only because of the difficulty of maintaining porphyrinogens in the reduced state, but also because the double-bonded structure of the porphyrins confers the property of *fluorescence* on them, which provides a suitable method for their detection and quantitation. This is discussed further in the chapters which follow. The relative proportions in which the porphyrins and porphyrinogens exist in tissues (and indeed in urine, stool and plasma) are largely unknown, though one might expect a considerable proportion to be present as the porphyrin, in view of the rapidity with which porphyrinogens will oxidise.

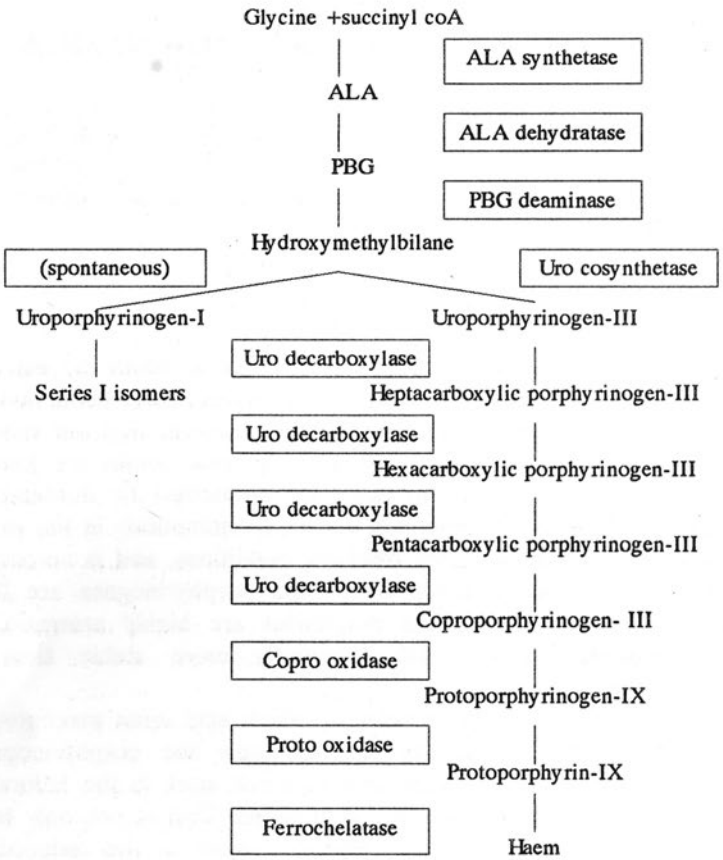

Fig 1.1. The haem synthetic pathway. Enzymes are shown in boxes. (Uro = uroporphyrinogen. Copro =coproporphyrinogen. Proto =protoporphyrinogen.)

GENERAL DESCRIPTION OF THE HAEM SYNTHETIC PATHWAY

A series of chemical reactions and modifications catalysed by a number of enzymes constitute the haem biosynthetic pathway. These enzymes are compartmentalised in the cell; the first and the last three steps occurring in the mitochondrion and the intermediate steps in the cytosol.

The pathway begins with the condensation of the simple amino acid *glycine* with the citric acid cycle product *succinyl coA* to form *delta-aminolaevulinicacid* (ALA). This is catalysed by the mitochondrial enzyme *ALA synthetase*. ALA then leaves the mitochondrion and, in the cytosol, two molecules of ALA condense under the influence of the enzyme *ALA dehydratase* to form *porphobilinogen* (PBG), the pyrrole subunit of the porphyrin ring. Four molecules of porphobilinogen are in turn linked by a head-to-tail condensation to form the linear tetrapyrrole *hydroxymethylbilane* . Working concurrently, the enzyme *uroporphyrinogen cosynthetase* rearranges the pyrrole and closes the ring to form the asymmetric cyclic tetrapyrrole *uroporphyrinogen*-III. In the absence of uroporphyrinogen cosynthetase, hydroxymethylbilane spontaneously cyclises to form the symmetric type I isomer of uroporphyrinogen. However, in mammals, this isomer and the series I isomers derived from it by its successive decarboxylation are functionless and only the type III forms serve as haem intermediates.

Uroporphyrinogen is an octacarboxylic porphyrin, with eight -COOH residues on its side chains. It is now successively decarboxylated by the cytoplasmic enzyme *uroporphyrinogen decarboxylase*. This proceeds in a strict order, giving rise to *heptacarboxylic porphyrinogen*, *hexacarboxylic porphyrinogen*, *pentacarboxylic porphyrinogen* and the tetracarboxylic porphyrinogen, *coproporphyrinogen* (Jackson *et al.*, 1976). The subsequent steps occur in the mitochondrion, where coproporphyrinogen is simultaneously decarboxylated and two of its propionyl side chains oxidised by the enzyme *coproporphyrinogen oxidase* to form the dicarboxylic porphyrinogen, *protoporphyrinogen* (Batlle *et al.*, 1965). The reaction proceeds via the formation of a short-lived tricarboxylic intermediate, *harderoporphyrinogen*. *In vitro*, protoporphyrinogen itself will spontaneously oxidise to *protoporphyrin*. However, in the highly reducing atmosphere of the mitochondrion, this

cannot occur spontaneously and the enzyme *protoporphyrinogen oxidase* is required to effect this reaction. This is a stereo-specific reaction which takes place from one side of the porphyrin ring only, a specificity which would not be encountered under conditions of merely chemical oxidation (Jackson *et al.*, 1974). In a final step catalysed by the enzyme *ferrochelatase*, an atom of iron is inserted into the tetrapyrrole ring (Goldberg *et al.*, 1956). This gives rise to *haem.*

REGULATION OF HAEM SYNTHESIS

The enzyme ALA synthetase is rate-limiting, and is of major importance in the control of haem synthesis. It is believed to be synthesised in the cytoplasm and then to be translocated to the mitochondrion where it is loosely bound to the inner mitochondrial membrane (McKay *et al.*, 1969). It has a short half-life of about 1 hour in mammalian species (Marver *et al.*, 1966). Control over porphyrin synthesis is under most circumstances excercised by the induction of the synthesis of ALA synthetase rather than by a modulation of its activity (Granick, 1958).

Where a relative deficiency of haem exists, synthesis of this enzyme is markedly induced. This may be detected as an increase in activity in an ALA synthetase assay. This is commonly encountered following the administration of certain drugs, particularly those which induce the hepatic cytochrome system. Here the increase in ALA synthetase will give rise to increased production of the haem required for incorporation into the cytochromes. The relative haem deficiency encountered in the porphyrias may result in marked increases in the level of ALA synthetase. This is thought to be responsible in part for the greatly increased porphyrin concentrations encountered in these conditions.

PBG deaminase has the second lowest activity of the enzymes of the haem synthetic pathway, and it has been suggested that this may allow it to function as a secondary control point in the pathway (Brodie *et al.*, 1977a).

SOLUBILITY AND EXCRETION OF PORPHYRINS

The earlier porphyrinogens (uro-, hepta-, hexa- and pentacarboxylic porphyrinogen) are hydrophilic by virtue of their multiple carboxylic residues, and thus water-soluble. The later compounds protoporphyrinogen and protoporphyrin are strongly hydrophobic and insoluble in water by contrast. Coproporphyrinogen is of intermediate solubility. This has considerable importance both *in vivo* and in the laboratory. The water-soluble compounds are excreted mainly in the urine whereas those which are less water-soluble are excreted via the bile and eventually emerge in the stool. Thus both urine and stool are required for an accurate delineation of porphyrin excretion and for the diagnosis and monitoring of porphyria. The varying solubility of the different porphyrins is also believed to be important in determining the type of skin damage associated with the various types of porphyria (Kohn and Kessel, 1979). This is discussed further in the following chapter.

THE PORPHYRIAS

Clinical Features of the Porphyrias

The clinical features of all the porphyrias can be summarised under two headings: the *acute attack* and *cutaneous photosensitivity*.

The Acute Attack

This is characterised by severe abdominal pain, ileus, metabolic disturbance, anxiety, psychosis or central nervous system dysfunction and a predominantly motor ascending peripheral neuropathy which may result in respiratory failure (Moore *et al*, 1987). Its diagnosis and management are comprehensively described elsewhere (Hift *et al*., 1989). These symptoms are all thought to reflect a rapidly developing neuropathy; the abdominal pain, hypertension and tachycardia representing an associated autonomic neuropathy. This manifestation of porphyria may be life-threatening. Only three of the porphyrias have the capacity to cause such attacks: *acute intermittent porphyria* (AIP), *hereditary coproporphyria* (HCP) and *variegate porphyria* (VP). The acute attack is always accompanied by massive elevations in the urinary

concentrations of ALA and PBG, which serve as markers for the condition.

Cutaneous Photosensitivity

All the porphyrias except AIP may be accompanied by cutaneous photosensitivity. This photosensitivity is described in detail in the following chapter.

Other Features of Porphyria

Most clinical effects of porphyria can be ascribed to either the acute attack or to cutaneous photosensitivity. However, *erythropoietic protoporphyria* (EPP), may be accompanied by cholelithiasis and by massive deposits of protoporphyrin as a pigment in the liver, with consequent liver failure (Poh-Fitzpatrick, 1985). *Congenital erythropoietic porphyria* (CEP) may be accompanied by varying degrees of anaemia because of the pronounced disturbance of haem synthesis in the developing erythroblasts. This is may in part be due to haemolysis (Kappas *et al.*, 1983). Anaemia is not encountered in the other porphyrias where there appears to be sufficient flux through the haem synthetic pathway for the maintainance of haem levels within the erythron.

TYPES OF PORPHYRIA

Acute Intermittent Porphyria (AIP)

This condition may give rise to life-threatening acute attacks. AIP differs from all the other porphyrias in that skin lesions are never encountered. In AIP, it is principally ALA and PBG which accumulate rather than the porphyrins, since the defective enzyme, which occurs early in the pathway, is PBG deaminase. Though these precursors have been incriminated in the pathogenesis of the acute attack, they, unlike the porphyrins, are not photoactive, and this is believed to account for the lack of skin involvement in AIP.

AIP is inherited as an autosomal dominant condition. The nature of the gene defect has now been characterised in several families. It has become evident that the condition is heterogeneous and it appears that different mutations have arisen in different families. At least four mutant classes of defective PBG deaminase activity have been identified (Mustajoki and Desnick, 1985). Though associated with Europe and

Scandinavia in particular, several indigenous South African families have now been identified as bearing this condition (Hift and Meissner, 1989).

Congenital Erythropoietic Porphyria (CEP)

Acute attacks are never encountered in CEP, but skin photosensitivity is extremely severe, and the onset of this disabling condition early. The defective enzyme is uroporphyrinogen-III-cosynthetase. In its absence the normal series III isomers of the porphyrins are not produced. However spontaneous cyclisation to the series I isomers continues. Thus large amounts of uroporphyrinogen as well as some heptacarboxylic, hexacarboxylic, pentacarboxylic and coproporphyrinogen are produced, but all of the series I isomer, which cannot be further metabolised (Rimington and With, 1973).

CEP is inherited as an autosomal recessive condition. It, and the extremely rare *plumboporphyria* or ALA dehydratase deficiency, are the only porphyrias which do not show a dominant mode of inheritance.

Porphyria Cutanea Tarda (PCT)

The defective enzyme in this condition is uroporphyrinogen decarboxylase. Large amounts of uroporphyrinogen, heptacarboxylic porphyrinogen, hexacarboxylic porphyrinogen and pentacarboxylic porphyrinogen accumulate, though coproporphyrinogen levels are usually normal. The condition is characterised by photosensitivity, but acute attacks are never encountered.

In most cases PCT is an acquired condition. It arises sporadically in association with liver disease, particularly that associated with alcohol, where it is often associated with states of iron overload. It has also been described following the use of certain drugs, particularly oestrogens (Haberman *et al.*, 1975, Moore *et al.*, 1987). It may also be induced by the administration of polyhalogenated hydrocarbons. A graphic example of this in humans was the outbreak 20 years ago of *Turkish porphyria* amongst young children in Turkey. These children had been fed bread made from wheat whose seeds had been contaminated with hexachlorobenzene (Dogramaci, 1972). This phenomenon is employed in the laboratory to establish test systems for porphyria. Animals are fed these highly substituted hydrocarbons, and a form of porphyria cutanea tarda is readily induced. Though the pathogenesis of acquired PCT is

complex and not well understood, it appears that even here a genetic predisposition may be important.

Occasionally this porphyria is inherited as an autosomal dominant trait. A variant of this condition known as hepato-erythropoietic porphyria (HEP) is described and may represent the homozygous state of the familial form of PCT (Elder *et al.*, 1981). It arises in infancy and is accompanied by severe photosensitivity.

Hereditary Coproporphyria

This form of porphyria is rare, fewer than a hundred cases having been described (Moore *et al.*, 1987). It arises from a deficiency of coproporphyrinogen oxidase. It is one of the acute porphyrias, and may result in fatal acute attacks. Skin photosensitivity, which is usually mild, is also described. In contrast with VP, the skin lesions in HCP appear to arise only during the acute attack (Brodie *et al.*, 1977b). It too is inherited as an autosomal dominant condition.

Variegate Porphyria (VP)

This is the most common form of porphyria in South Africa. It is inherited as an autosomal dominant trait. Its high prevalence is an example of the *founder effect*. In 1688, a Dutch orphan named Ariaantjie Ariens married a *vryburger* at the Cape of Good Hope, Gerrit Jansz van Deventer. It is not known which of them carried the gene for porphyria, but every family with VP in South Africa has been traced back to this couple (Meissner *et al.*, 1987). It has been estimated that 20 000 South Africans now carry the gene. They are found largely amongst Afrikaans-speaking white South Africans, but the gene has now spread more widely and is also encountered among the English-speaking population and among those of mixed descent.

The name *variegate porphyria* arose from the observation that both acute attacks and skin lesions are commonly encountered in this condition (Dean and Barnes, 1959). Fatal acute attacks, particularly in response to the ingestion of certain drugs, were frequent in the past, though much less commonly encountered now (Meissner *et al.*, 1987). Cutaneous involvement is common. About 70% have skin involvement, and in 50% this is the only manifestation (Day, 1986). The skin involvement is clinically indistinguishable from that of the other porphyrias, other than EPP which has a characteristic reaction pattern.

The affected enzyme is protoporphyrinogen oxidase. It has been clearly shown that people with VP have a 50% reduction in the activity of this enzyme (Brenner and Bloomer, 1980; Meissner *et al.*, 1985). This results in the accumulation of large amounts of protoporphyrinogen, and, to a lesser extent, coproporphyrinogen and pentacarboxylic porphyrinogen which may be detected in the stool. During the acute attack, elevations of the earlier, more water-soluble porphyrins are encountered as well as a diagnostic elevation of ALA and PBG, and will be readily demonstrable in the urine.

Erythropoietic Protoporphyria

This form of porphyria does not result in acute attacks. However it may be accompanied by disabling photosensitivity. Three things distinguish this form of porphyria from the others. Its onset is usually during infancy whereas the other porphyrias become manifest only after puberty. Secondly, the photocutaneous sensitivity takes a different form, with an immediate urticarial response to sunlight which is described in more detail in the following chapter. Thirdly, it may result in liver failure, apparently arising from a massive deposition of protoporphyrin in the liver parenchyma.

It is inherited as an autosomal dominant trait. The defective enzyme is ferrochelatase. This results in the accumulation of large amounts of protoporphyrin. As in CEP, it is the haem synthetic pathway of the maturing erythroblasts which is affected; hence most of the accumulated porphyrin observed is derived from the erythrocytes and high levels of porphyrin are seen in these cells.

CHAPTER 2

SKIN DISEASE IN PORPHYRIA

Of the six most common forms of porphyria, skin disease is encountered in five. The skin disease is clearly a manifestation of photosensitivity. Only those parts of the body which are exposed to light develop porphyric skin damage. Under normal circumstances, this includes the dorsal aspect of the hands and forearms, the face and the supraorbital ridges, the ears, nose and lips, and sometimes the feet and ankles. Two distinct patterns of cutaneous disease are encountered (Poh-Fitzpatrick, 1985) and are described below.

THE RAPID PHOTOTOXIC RESPONSE

This pattern is typical of EPP, but may be present to a lesser degree in VP. It has been described as a *solar urticaria*. Following sufficient sun exposure, a burning and stinging sensation arises in the exposed skin. Diffuse oedema develops within hours. This may be accompanied by severe pain. The oedema settles over a few days leaving blotchy petechiae in its wake, which slowly fade. This may be accompanied by photo-onycholysis or light-induced shedding of the nails. This reaction is largely reversible; initially it does not result in as much destruction as the vesicular-erosive form of photosensitivity described below. With time, however, chronic changes may develop. Roughening and coarsening of the skin, particularly over the bridge of the nose, but also on the cheeks and forehead becomes evident. The skin of the dorsal surfaces of the hands becomes leathery, thickened and mottled and the hands take on a weather-beaten appearance. These changes are never as severe or disfiguring as those encountered in the second pattern of skin injury, and the skin fragility so characteristic of the latter is absent. Hence, though the initial sequelae of exposure to light are more severe, the long-term effects on the skin are less.

THE VESICULAR-EROSIVE SCARRING PATTERN

This pattern of photosensitivity is marked by the absence of early symptoms following sun exposure. No immediate discomfort is experienced, and patients may fail to make the connection between exposure to sun and the later development of their skin lesions.

Severe cutaneous fragility is the most frequent symptom. In response to minimal trauma, the skin abrades easily, leaving painful erosions which occupy the full thickness of the epidermis. These may be accompanied by blisters and vesicles. The lesions are painful and unsightly and heal slowly. Chronic scarring, milia, hypopigmentation and hyperpigmentation result (Poh-Fitzpatrick, 1985). Such changes are typical of VP (Day, 1986). In this condition, photocutaneous sensitivity develops in the teens and twenties and may eventually result in an unsightly appearance.

In some cases, the changes may be more severe still and may involve specialised skin structures and associated parts. Thus alopecia, hirsutes, keratoconjunctivitis, osteolysis of the fingers, nail dystrophy, destruction of the nasal and auricular cartilages and scarring of the eyelids and lips may result. Other features include scleroderma-like plaques over the upper chest, back and face, which are not uncommon in PCT, and dystrophic calcification of the scalp, neck and pre-auricular skin (Poh-Fitzpatrick, 1985).

These more severe changes are unusual in the average patient with VP, but are encountered in the more seriously affected cases and where the porphyria has been severe and longstanding. This is particularly so where the onset has been in childhood. This is seen in CEP and HEP (the homozygous form of PCT), and in the few cases of homozygous VP that have been described (Murphy *et al.*, 1986; Mustajoki *et al.*, 1987).

PORPHYRIA AND THE SKIN IN SOUTH AFRICA

The two most common forms of porphyria in South Africa are VP and PCT (Meissner *et al.*, 1987). Occasional patients with severe VP may experience a mild form of acute photosensitivity, and will complain of pain and itch in the skin during sun exposure (Day, 1986; Meissner *et al.*, 1987). However, this is never as severe as the

reaction experienced by patients with EPP, and the skin lesions they develop are of the typical vesicular-erosive type.

The skin lesions of VP and PCT are clinically indistinguishable. CEP also presents with a typical vesicular-erosive pattern, but the symptoms tend to be more severe than those seen in VP, probably because the cumulative damage is greater since blood porphyrin levels are higher and the onset earlier. CEP is rare. A single patient with this condition attends our clinic. He will be described below. EPP, the usual cause of the acute phototoxic response, is encountered in this country, but is uncommon.

PATHOGENESIS OF THE SKIN LESIONS

There is strong evidence that it is the interaction of porphyrins and incidental light, in the skin, that gives rise to the cutaneous disease. The pattern of involvement, with only those areas which are exposed to light being affected, is highly suggestive of such an interaction. A marked photosensitivity indistinguishable from the phototoxic reaction can be induced in man and animals by the administration of exogenous *haematoporphyrin.* This and other porphyrin derivatives have been used for the therapy of tumours (Moore *et al.*, 1987). They undergo a widespread distribution throughout the body following their administration. Ultraviolet light is then directed at the tumour; severe local tissue damage results which may lead to necrosis of the tumour. Such patients have inadvertently been exposed to sunlight and this has resulted in severe, generalised cutaneous photosensitivity (Zalar *et al.*, 1977).

It has been consistently shown that the skin of porphyric patients contains excessive amounts of porphyrin (Poh-Fitzpatrick, 1985). Ultraviolet light microscopy has revealed red fluorescence, typical of porphyrins, in a subepithelial position in skin biopsies from a patient with EPP, a patient with VP and four subjects with PCT (Runge and Watson, 1962). Attempts have also been made to separate and quantify different species of porphyrin in the skin in porphyria. Accumulations of uroporphyrin, coproporphyrin and the intermediate water-soluble porphyrins, as well as a small amount of protoporphyrin, have been measured in skin biopsies in PCT (Malina *et al.*, 1978). Observations by Day from our laboratory suggest that the skin porphyrin

profile in VP is similar (Day 1986). Thus, in contrast to the normal stool excretion pattern of VP where the hydrophobic compound protoporphyrin predominates, it appears that the water-soluble porphyrins have a greater propensity to accumulate in the skin. The significance of this interesting observation will be discussed below.

Histopathology of the Skin in Porphyria

A detailed description of the pathology of the skin in porphyria is beyond the scope of this work. Mention is made here only of those features which aid an understanding of the pathogenesis of the lesions and the rôle that radiation may play in this.

The histology of the skin in the different types of porphyria is surprisingly uniform. The two most common findings are an extensive reduplication of the basal lamina of the small upper dermal blood vessels, and the presence of large clumps of fibrillar material scattered throughout the mid and upper dermis (Epstein *et al.*, 1973). Changes were always most marked in the dermis and lower epidermis. Other important findings are the deposition of immunoglobulin, complement and fibrinogen deposits near the dermal-epidermal basement membrane, though these are inconstant.

Caputo *et al* have studied the sub-microscopic events that give rise to the formation of spontaneous blisters in porphyria cutanea tarda (Caputo *et al.*, 1983). They undertook an ultrastructural examination of skin from unexposed areas and from sun-exposed areas situated far from blisters, near blisters and from the cleavage area of blisters. They found that even unexposed skin is not entirely normal, but shows reduplication of the basal lamina of the blood vessels of the superficial dermis. Occasionally the vessels are embedded in a mass of finely fibrillar material. In exposed areas these changes are more overt; the blood vessels show multiple reduplication of the basal lamina and the vessels are frequently embedded in a mass of finely fibrillar material. Endothelial cells are well preserved. Vacuoles are observed between vessels or beneath the basal lamina. These appear to arise either from cytolysis of dermal cells or from pseudopodia of epidermal cells extending through breaches in the basal lamina into the upper dermis. The relationship of these changes to the presence of porphyrin in this region could not be determined on electron microscopic studies. In

exposed skin near blisters the number and size of these vacuoles are greatly increased and in the cleavage area of blisters the superficial dermis is almost totally occupied by vacuoles whose limiting membranes are fused. Rupture of the limiting membranes of these vacuoles causes the formation of the blister cavity. It thus appears that in the porphyrias, the skin is abnormal even where it is unexposed, but that exposure to light aggravates this greatly, being followed by the development of large numbers of vacuoles which coalesce to form blisters.

Photobiology of Porphyrins

Since porphyrins are present in increased concentrations in the skin in subjects with porphyria and it is principally the light-exposed areas which show evidence of damage, it is reasonable to presume that the interaction of these porphyrin molecules with light underlies the genesis of these skin lesions. Several lines of evidence support this.

Porphyrins and porphyrinogens are indeed active photochemically. Whereas molecules containing single bonds tend to absorb radiation in the far ultraviolet range, those with many conjugated double bonds tend to absorb at longer wavelengths, and may even do so in the visible spectrum. Porphyrinogens, and porphyrins even more so, contain extensive double bond systems and show absorption maxima in both the ultraviolet and the visible spectra. This includes the Soret band at 406 nm, and four lesser peaks of absorption between 500 and 650 nm.

Absorption of light energy *per se* is insufficient to cause tissue damage. An ability to transfer this energy to surrounding structures is necessary for local damage to result. Under normal conditions, most biological compounds exist at the lowest energy level or *ground state* (fig 2.1). Absorption of light energy may result in the promotion of a ground-state electron to an orbital associated with a higher energy level. If the promoted electron retains its original spin, which is opposite to that of its partner in the electron pair, this is know as the *singlet-excited state*. This state is unstable, short-lived, and tends to relax rapidly to the ground state with emission of the surplus energy. This may be in the form of radiation of light or heat, or by a transfer of energy to surrounding chemical structures. Light emission

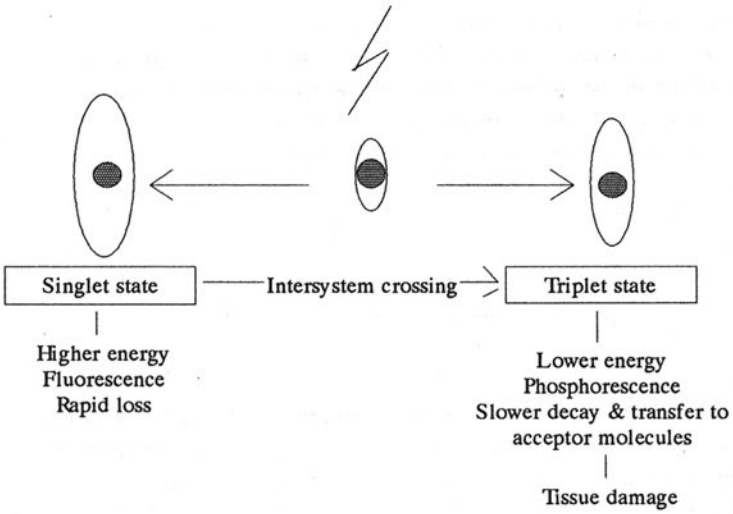

Fig 2.1. Schematic representation of effect of radiation on porphyrins.

from the singlet-excited state is short-lived and is known as *fluorescence.* When the singlet state of porphyrins relaxes to the ground state, the typical red porphyrin fluorescence, with an emitted wave length of about 620 nm, is produced (Moore *et al.*, 1987).

The characteristic red fluorescence of porphyrins under ultraviolet light is widely employed in the laboratory for the detection and quantifying of these compounds. Porphyrinogens, by contrast, do not fluoresce because of the absence of the double-bonded methene bridges. In our own laboratory, biological specimens are screened for the presence of porphyrins by the detection of fluorescence. Following chromatographic separation of the various types of porphyrin from urine, stool or blood, the amounts of individual porphyrin are quantified by measurement of the amount of fluorescence radiated in response to ultraviolet light at the Soret maximum. This is calibrated against the fluorescence of known concentrations of porphyrins in standard solutions. This will be described in more detail below.

If, however, the electron acquires a spin parallel to its partner in the electron pair when promoted to a higher energy level, this is described as the *triplet-excited state*, a so-called *metastable* state which

exists at a lower energy level, is more stable, and has a longer half-life than the singlet-excited state. As it decays back to the ground state, the additional energy may be given out as light which is of a lower intensity and more sustained than the fluorescence of the singlet state and is described as *phosphorescence*. Since the compound is more stable, and the dissipation of energy slower, there is more opportunity for the transfer of the energy to surrounding molecules, resulting in *photochemical reactions*.

Though singlet porphyrin can be involved in energy transfer to other molecules (Land, 1984), the emission of light by fluorescence, although dramatic and extensively employed in the laboratory, is believed less likely to be implicated in the genesis of the skin disease than is the relaxation of the triplet state with its opportunities for transfer of energy to surrounding molecules. However, *intersystem crossing* may occur in which the singlet-excited state transforms to the more stable triplet state.

The nature of this energy transfer from porphyrins within the skin to the surrounding tissues, and the identity of the accepting molecules, are as yet unestablished. There remains a divide between our understanding of the photochemistry of the porphyrins and the morphology of the skin damage seen in porphyria. Several intermediates have been suggested. It seems probable that triplet-excited porphyrin molecules raise ground-state oxygen to the singlet-excited state. Singlet oxygen is known to be a highly reactive agent (Moore et al., 1987) and it may be responsible for oxidising many compounds of biological importance such as lipids, cholesterol and amino acids. This may result in the peroxidation of cell membranes, cross-linking of cell membrane and intra-cellular proteins, inhibition of enzymes associated with membranes, loss of the integrity of membranes, disruption of intracellular organelles and cell death (Poh-Fitzpatrick, 1985). Other mechanisms of energy transfer, perhaps involving the superoxide radical (Athar *et al*, 1988), are possible.

Effect of Radiation Frequency on Skin

It has been clearly shown that the erythema and oedema which develop in the skin of patients with EPP is greatest when the skin is exposed to wavelengths at the Soret maximum of approximately

400 nm (Poh-Fitzpatrick, 1985). This again is strong evidence that it is indeed the photoactive nature of the porphyrins that results in skin damage. However, absorption of light at the lesser peaks in the visible spectrum is also deleterious and this has important therapeutic implications.

Influence of Type of Porphyrin on Biological Damage

A fundamental question which has yet to be convincingly answered is why the photosensitivity of EPP should differ from that of the other porphyrias. Most authorities have attempted to explain this on the basis of the different profiles of porphyrin which accumulate in the various porphyrias, and to relate this to their differing solubilities. Thus it has been suggested that the hydrophobic porphyrin *protoporphyrin* will have the greatest effect on cell membrane structure and function, since it will readily partition into the hydrophobic membrane environment (Sandberg and Romslo, 1981). Indeed, recent work suggests that the insoluble porphyrins *protoporphyrin* and *deuteroporphyrin* in fact bind to the lipid fraction of membranes rather than merely partitioning into it (Rotenberg and Margalit, 1987). This binding is non-specific and is not receptor-mediated. Conversely it has been suggested that *uroporphyrin* may be a more effective damager of water-soluble targets such as the cytosolic enzymes (Sandberg and Romslo, 1981).

Thus it has been suggested that in erythropoietic porphyria protoporphyrin escapes from erythrocytes, circulates in the plasma and is compartmentalised into the lipid environment of cell membranes because of its hydrophobicity. In the skin, protoporphyrin may rapidly be sequestered into dermal capillary endothelial cells. Following exposure to light, tissue damage in this region results in leakage of intravascular contents and oedema. This is followed by the release of other mediators of inflammation resulting in the typical acute phototoxic response of burning pain, erythema and oedema (Poh-Fitzpatrick, 1985). In contrast, hydrophilic porphyrins would be less efficiently localised in membranes and would exert their effects by a different mechanism. This might account for the different pattern of skin reaction seen in PCT and CEP, where the predominant porphyrins are water-soluble. Unfortunately, these other mechanisms are much less well understood. Such a theory also relies on the assumption that skin porphyrins arise from passive diffusion from the circulating plasma pool into the tissues as suggested

by Runge (Runge and Watson, 1962). This is probably simplistic, and the rôle played by porphyrin synthesis in the skin itself is unclear.

A fundamental difficulty with this theory has been the typical vesicular-erosive response of VP which, like EPP, is accompanied by the accumulation of the hydrophobic compound *protoporphyrin*. It may be that the major site of synthesis of protoporphyrin in this condition is hepatic, and that protoporphyrin is efficiently cleared into the bile, with consequently less being available for sequestration in skin, in contrast to EPP where the porphyrin production is predominantly erythropoietic and hence has ready access to the plasma. An alternative explanation holds that a large fraction of the total plasma porphyrin in VP is tightly bound to a carrier molecule, which is thought to be a protein or peptide. It appears that this conjugate consists of a dicarboxylic porphyrin tightly bound to a plasma factor which behaves as a protein on chemical characterisation and has an electrophoretic mobility suggestive of albumin (Longas and Poh-Fitzpatrick, 1982). The resulting complex is conventionally known as *peptide X* or *porphyrin X*. This may prevent protoporphyrin from obtaining access to membranes, allowing only the hydrophilic porphyrins to exert their effects in a manner analogous to that of PCT and CEP. This remains however largely speculative. These theories are based on the assumption that the excess porphyrins noted in the skin are deposited from a plasma pool. However it has been shown that there is no correlation between circulating plasma porphyrin levels and the cutaneous symptoms in either VP or PCT (Day and Pimstone, 1978). This would suggest that local synthesis in the affected tissues may also be of importance. Further work by Day revealed that in PCT and VP, the porphyrin profiles in skin biopsies were remarkably similar and resembled those of the plasma in PCT, with uro- and heptacarboxylic porphyrin predominating (Day, 1986). Though this might be compatible with a deposition of porphyrins from the plasma into the skin in PCT, this would not hold for VP where it is coproporphyrin which predominates in the plasma. An alternative explanation might be that the excess hydrophilic porphyrins seen in the skin of the patients with VP have been synthesised locally in the dermis. Day quotes interesting work by Elder suggesting that uroporphyrin and coproporphyrin are able to focus reactive oxygen generated by light-sensitised porphyrins on to the active site of the enzyme uroporphyrinogen decarboxylase, thereby inactivating it. The accumulation of coproporphyrin might thus result

in a progressive inactivation of uroporphyrinogen decarboxylase and hence a "local" form of PCT. Further work in this line is necessary.

Secondary Mediators of Inflammation

Lysosomal Enzymes

A rôle for lysosomal enzymes in the pathogenesis of the immediate photosensitivity response has been postulated. Exogenous uroporphyrin and protoporphyrin have been shown to accumulate in the lysosomes of human fibroblasts; such cells die after exposure to ultraviolet radiation (Slater and Riley, 1966). Similarly, exogenous uroporphyrin and protoporphyrin are concentrated by lysosomes of mammalian cells in tissue culture and exposure to light radiation at 400 nm is followed by release of lysosomal acid phosphatase into the cytosol (Allison *et al.*, 1966). However, other studies have not supported this. They suggest that the phototoxic response *precedes* the release of lysosomal enzymes (Volden and Thune, 1979; Wakulchik *et al.*, 1980). Thus the inflammatory response evoked by these mediators may follow and aggravate the phototoxic response, but is not essential to it.

Complement

A large body of evidence implicates the complement pathway in the genesis of the skin lesions of porphyria. Deposits of the third component of complement (C3) have frequently been observed at the epidermal junction as well as near the abnormal, thickened dermal capillaries seen in patients with porphyria (Baart De La Faille *et al.*, 1968). Complement components and cleavage products have been found in the blister fluid of patients with EPP following irradiation (Baart De La Faille *et al.*, 1978). Addition of porphyrins to human and animal sera followed by irradiation in both the ultraviolet and visible light spectrum has produced porphyrin-concentration-dependent decreases in the titres of serum complement (Lim and Gigli, 1981a). It has been shown that irradiation of sera *in vitro* containing exogenous uroporphyrin or protoporphyrin with a light source emitting at a wavelength of 400-410 nm results in activation of the complement system and generation of chemotactic activity for human polymorphonuclear leucocytes (Lim *et al.*, 1981b). These investigators subsequently extended these investigations to living subjects. Two patients with EPP, one with PCT

and two normal subjects had one forearm exposed to intense radiation in the 400-410 nm range and blood was immediately drawn from the antecubital vein on that side. The three porphyric subjects all showed a marked decrease in the third and fifth components of complement; the decrease in titres ranging from 23 to 57% for C3 and 19 to 47% for C5. A marked increase in chemotactic activity of blood for polymorphonuclear leucocytes was also shown. These changes were not present in the blood of the control subjects (Lim *et al.*, 1984). Similar results have been obtained by the irradiation of sera from rats rendered porphyric by the administration of hexachlorobenzene (Torinuki *et al.*, 1984). Pigatto and colleagues assessed seven men with active PCT and 10 matched hospital controls (Pigatto *et al.*, 1986). Compared with the controls, plasma levels of C3a were significantly elevated in the porphyric subjects. All were then irradiated, and C3a levels thereafter increased steadily in the porphyric group but remained unchanged in the controls. They were unable to show changes in C5a. An increase in polymorphonuclear chemotactic activity was also shown in the PCT patients following irradiation.

Owing to the absence of immediate symptoms following exposure to light, the vesicular-erosive form of porphyric photosensitivity is less easily investigated. Thus much less is known about the pathogenesis of the skin lesions of VP, PCT and CEP. Presumably acute mediators of inflammation are of less importance than in the phototoxic reaction. The major distinction from EPP appears to be the increased fragility of the skin, perhaps related to Caputo 's demonstration of the coalescence of vacuoles in sun-exposed areas (Caputo *et al*, 1983). The final insult is incidental *trauma,* which acts upon the fragile skin to result in denuded areas and obvious blisters. Other factors may operate as well. For instance, it has been shown that human fibroblasts synthesise new collagen at an increased rate when incubated with uroporphyrin, even in the absence of light (Varigos *et al.*, 1982). This suggests that the presence of porphyrin alone may be sufficient to increase collagen synthesis, and it may be that their photoactivity is not the only property of porphyrins which facilitates skin damage.

SUMMARY

It thus appears that the presence of porphyrin in increased amounts in skin predisposes it to pathological changes which become florid when the skin is exposed to light of a range of wavelengths and is maximal at wavelengths situated around the Soret band. This is almost certainly due to the interaction of light with photoactive porphyrin molecules and involves the promotion of their electrons to the triplet-excited state. These transfer their excess energy to a variety of biological acceptor molecules with consequent skin damage. The details of this are as yet speculative. Various theories have been advanced to account for the different reaction patterns of EPP and VP, but remain unproven.

CHAPTER 3

PREVENTION OF SKIN DAMAGE IN PORPHYRIA

The skin lesions of porphyria appear to arise from the interaction of light with porphyrins or porphyrinogens in the dermis. Thus therapeutic efforts might be directed at several sites. One might attempt to reduce exposure to light or prevent its passage through the epidermis to the deeper regions in which accumulated porphyrins are found, to interrupt the mechanisms responsible for damaging the skin at a molecular level, to suppress the production of porphyrins or to remove excess porphyrins from the skin.

PROTECTION AGAINST LIGHT

People with porphyria are advised to take sensible precautions to reduce their exposure to the sun. Some of these precautions are readily taken; others not. Thus wearing adequately protective clothing, such as a hat, long sleeves and long trousers, avoiding the midday sun and limiting outdoor recreational and work-related pursuits are advised, but personal comfort and current fashion may militate against such advice being accepted. Similarly, while photosensitivity is less of a problem in temperate climates than in the more equatorial regions, it is rarely feasible for the family of an affected individual to move.

In severe cases of photosensitivity extreme measures may be required to limit light exposure. Tinted films pasted on windows will reduce the transmission of light of wavelengths below 550 nm. Fluorescent light strips emit strongly in the 405 nm band, which is very detrimental to the skin in porphyria (Poh-Fitzpatrick, 1985). Replacement of such light strips by incandescent bulbs may be of benefit.

Pharmacological Photoprotectants

Pharmacological photoprotectants have been widely employed to protect the skin in porphyria, but with varying success. They may be divided into two groups, topical sunfilters and systemic photoprotectants.

Topical Sunfilters

Despite widespread use, topical sunscreens are largely ineffective in porphyria. Conventional suntan preparations offer little protection against the longer wavelengths of the ultraviolet and visible spectra, which contain those frequencies most harmful to porphyrics (Poh-Fitzpatrick, 1985). Indeed Gordon has shown that porphyric subjects do not show an undue sensitivity to light in the sunburn range below 320 nm (Gordon, 1963), against which most suntan preparations offer some protection. Thus only those preparations offering significant protection against wavelengths of 400 nm and more are at all likely to diminish the skin reaction in porphyria. A recent analysis of the protective properties of sunscreens available in South Africa shows that almost without exception they offer no protection against wavelengths over 390 nm (Summers and Summers, 1988). They are thus of little use in limiting skin damage in porphyria. Compounds containing *zinc* or *titanium dioxide*, which are opaque and therefore impede the passage of longer wavelengths, are more effective. They tend to be cosmetically unacceptable and have therefore not found wide acceptance amongst porphyrics.

Systemic Photoprotectants

Beta-carotene has been widely employed as a systemic photoprotectant. This yellowish pigment was first noted by Matthews and Sistrom to be effective in protecting a photosensitive bacterial micro-organism from the effects of ultraviolet light (Mathews and Sistrom, 1959). A similar effect was shown in mice sensitised with haematoporphyrin, and subsequently in humans with EPP and other photosensitising disorders (Mathews-Roth *et al.*, 1970; Mathews-Roth *et al.*, 1977).

The mechanism of action of beta-carotene is not well understood. It has been suggested that the yellow colour imparted to the skin by this agent may serve as a physical barrier blocking out certain wavelengths, but this barrier effect is probably insufficient to account

for the degree of relief afforded to people with EPP (Lamola and Blumberg, 1976). Others have suggested that carotenoids may act at the molecular level as scavengers of free radicals and singlet-excited oxygen arising from the photoexcitation of porphyrins in tissues (Foote and Denny, 1968; Foote *et al.*, 1970a).

The results of treatment with beta-carotene have been inconsistent. A review noted that most of the reported studies were uncontrolled and relied on the subject's own perception of his light tolerance, and the only placebo-controlled crossover study (Corbett *et al.*, 1977) showed no benefit, but has been criticised for using an inadequate dose for too short a period (Krook and Haeger-Aronson, 1982). Carotenoids would appear to be more effective in EPP than in the other porphyrias. Though they may have a place in the treatment of PCT when venesection is contraindicated, they are less efficacious in this condition than in EPP. Nor have results been encouraging in VP (Mathews-Roth *et al.*, 1977). If carotenoids indeed behave as free radical scavengers there is a possible theoretical explanation for their greater efficacy in EPP than in the other porphyrias. Carotenoids, like protoporphyrin, are lipophilic compounds and would thus partition among tissues similarly to protoporphyrin. They would thus preferentially seek out those areas where light-induced skin damage is likely to be maximal and would therefore have a greater protective effect (Poh-Fitzpatrick, 1985). It may however merely be that the acute phototoxic reaction is more amenable to interruption by beta-carotene than is the more chronic vesicular-erosive type.

Beta-carotene must be taken in sufficient dose for the development of *carotenodermia,* a yellow discolouration of the skin. This is not cosmetically acceptable to all subjects, and requires about six weeks to develop. An improvement in photosensitivity may take six months to become evident. Beta-carotene has also been used in conjunction with another carotenoid, *canthaxanthin* (Eales, 1978). The pigmentation which follows the use of the combination is of a more natural colour (Haeger-Aronson *et al.*, 1979). However the combination does appear to be accompanied by a risk of deposition of a crystalline pigment on the retina, though this has not yet been shown to be deleterious (Russeau, 1983). Beta-carotene appears otherwise to be safe (Krook and Haeger-Aronson,1982).

INTERRUPTION OF DAMAGE AT A MOLECULAR LEVEL

Carotene, by acting as a free radical scavenger, may exert its effect at this level. Vitamin E (alpha-tocopherol) has also been investigated as an agent for limiting photosensitivity, and efficacy has been claimed for it in several forms of porphyria (Nair *et al.*, 1971). Others have been less convinced by its efficacy, and it appears to have no place in the treatment of VP (Mustajoki, 1972). Alpha-tocopherol is lipid-soluble, hydrophobic and has potent properties as an anti-oxidant and free radical scavenger. It is this latter property which may account for an effect in porphyria. However, unlike beta-carotene, it absorbs light poorly in the 400 nm range and this may explain its less convincing efficacy (Krook and Haeger-Aronson, 1982). Use of vitamin E for prolonged periods is potentially hazardous. Complications reported have included thrombophlebitis, pulmonary embolism, hypertension, gynaecomastia, breast tumours and angina pectoris (Roberts, 1981).

SUPPRESSION OF PORPHYRIN SYNTHESIS

Avoidance of Precipitants

The ability of certain drugs to induce the synthesis of porphyrins is well known, and is best exemplified by the barbiturates. Though the relationship between ingestion of such drugs and photosensitivity is inconstant, it is our impression that skin disease does become worse at a time when VP is shown biochemically to be in an active phase, whether or not symptoms suggestive of an acute attack are present. It therefore seems prudent to avoid those agents known to induce porphyrin synthesis so as not to increase porphyrin concentrations further. Such drugs are however believed to operate by inducing the haem synthetic pathway in the liver. Were it to be shown that most skin porphyrins are indeed synthesised locally and not merely translocated there from the liver via the plasma, then avoiding such precipitants might be expected to play little part in limiting skin disease.

Haematin

Other methods of reducing the synthesis of porphyrins have been suggested. The infusion of *haematin* is widely employed for the suppression of the acute attack. It is believed to work by the negative feedback effect of haem on ALA synthetase, thus diminishing porphyrin synthesis. A study has shown that daily infusion of haem arginate will reduce faecal and plasma levels of porphyrin in VP, but that weekly infusions are unable to maintain lower levels and photosensitivity is not improved (Timonen *et al*, 1990). Such therapy is hence impractical for VP. In addition the long term safety of haematin is unknown and it is not recommended for this purpose (Poh-Fitzpatrick, 1985).

Hypertransfusion

CEP has been treated by *hypertransfusion*, blood transfusions being undertaken sufficiently frequently to reduce the erythropoietic drive, and hence porphyrin production, to negligible levels (Pimstone *et al.*, 1987). This does result in a reduction in porphyrin concentrations, but the risks of repeated transfusion prevent its adoption as a standard measure. It would also not be expected to be efficacious in those porphyrias such as VP and PCT where haem synthesis in the red cells is less effected than is that in other tissues, such as the liver.

Tin-protoporphyrin

More recently attention has focussed on the use of *tin-protoporphyrin* and *tin-mesoporphyrin* as a therapeutic measure. This is an analogue of haem in which the central iron atom is replaced by a tin atom. This functions as a competitive inhibitor of the enzyme haem oxygenase, which is responsible for the destruction of haem. Hence where tin-protoporphyrin is administered, the half life of haem is greatly prolonged. It is thought that this reduces the drive for haem synthesis and thus the production of porphyrins (Berglund *et al.*, 1988; Galbraith *et al.*, 1985). There is considerable interest in this compound. A preliminary report suggests that monthly administration of tin-protoporphyrin may be efficacious in limiting skin disease in VP (Galbraith and Kappas, 1990).

Venesection

Mention has been made of the close relationship between iron overload and PCT. Measures to reduce the total body iron stores have been shown to be effective in moderating the activity of PCT. The simplest form of treatment, which remains the best, is regular *venesection*. This was first applied by Ippen in 1961 (Ippen, 1961). Several other studies subsequently have confirmed its efficacy (Grossman and Poh-Fitzpatrick, 1980). The regimen employed in our clinic is the removal of 500 ml blood at approximately 2-weekly intervals. This is continued until the haemoglobin concentration starts to fall, which normally indicates the onset of mild iron deficiency, or until ferritin levels drop to the lower end of the normal range. This is followed in most cases by a clinical remission. A biochemical remission which may also only develop after several months, is accompanied by a marked decrease in urinary porphyrin excretion. The process of recovery is improved in those who are able to abstain from alcohol or other precipitating causes during therapy.

INCREASING PORPHYRIN CLEARANCE

Chloroquine

Chloroquine is believed to act by complexing with porphyrins within the hepatocytes to form a water-soluble compound which is then released from the liver and rapidly excreted in the urine (Scholnick *et al.*, 1973). It appears to have a rôle in the management of PCT. If standard antimalarial or photoprotective doses are used in patients with PCT, an acute hepatotoxic reaction results marked by fever, malaise, abdominal pain, nausea, vomiting and elevated transaminases (Cripps and Curtis, 1962). Thus a lower dose regimen of 125 mg by mouth twice weekly is usually recommended and is often effective. It has the added advantage of avoiding the irreversible retinal damage which may follow the use of higher doses (Poh-Fitzpatrick, 1985). Remission may take 6 months or more to arise and prolonged compliance is necessary. It has therefore been suggested by Tsega that a short-course high-dose regimen of chloroquine phosphate 500 mg daily for 10 days should be employed (Tsega, 1987). In his study of 46 Ethiopian patients

with PCT, this resulted in a large increase in hepatic transaminase levels, which was presumed to reflect damage to the liver which the author felt was confined to those hepatocytes heavily laden with porphyrins. This statement is however unsupported. Transaminase levels fell below pre-treatment levels after 10 days of therapy, and no patient suffered an adverse outcome. The progression of coexistent liver disease (which was presumably due to alcohol abuse) was thought to have been unaffected by this intercurrent acute hepatitis, and all patients demonstrated a prolonged remission. The major advantage claimed for such a potentially hazardous form of treatment is its brevity, which reduces the problem of non-compliance which may arise with the protracted courses of treatment otherwise employed. Such therapy is not recommended by our clinic.

Plasmapharesis

Plasmapharesis and charcoal haemoperfusion have both been employed to reduce plasma porphyrin levels. Work from this unit suggested that plasmapharesis was useful in treating the skin symptoms of PCT associated with haemodialysis, in which the more conventional therapy of venesection was contra-indicated (Disler *et al.*, 1982). Evidence to support its efficacy has been supplied by others (Grossman *et al.*, 1979) but other reports are less encouraging (Allen *et al.*, 1975).

Sorbent Binding to Porphyrins

A further therapeutic modality which has attracted much interest and forms the basis of the work described here is the oral administration of binding agents such as *cholestyramine* and *activated charcoal*. It is thought that they may interrupt an enterohepatic cycling of porphyrins by binding them in the bowel, thus facilitating their excretion to the exterior and reducing the total body porphyrin load.

An enterohepatic circulation for porphyrins was first postulated in 1949 by Lemberg and Legg (Lemberg and Legg, 1949). It was in 1966 that an attempt was made to interrupt such a cycle with the intention of ameliorating the effects of porphyria. Stathers hypothesised that the administration of a binding agent would result in a complexing of porphyrins in the bowel with consequent interruption of enterohepatic

cycling, a nett loss of porphyrin from the body and therefore clinical improvement (Stathers, 1966). In initial experiments, he observed that cholestyramine forms an unabsorbable complex with bile salts in both human and animal intestine, that it can remove bilirubin from solution, and that it is effective in removing porphyrins from a slightly alkaline solution. He then treated three patients with PCT with cholestyramine, 12 g daily in divided doses. He showed cholestyramine-bound porphyrin fluorescence in the stools and reported a clinical improvement in all three of his subjects. He intubated the duodenum of one patient, demonstrated porphyrin in the duodenal aspirate and was able to show that the porphyrin could be bound by cholestyramine. He suggested that the binding occurs between the propionic side chains of the porphyrin nucleus and the quaternary ammonium groups of cholestyramine. He suggested that clinical improvement in porphyria might follow not only the removal of porphyrin from the body, but also the removal of bile salts. Bile salts would thus compete less strongly with porphyrins for elimination by the liver and the clearance of porphyrins would increase.

The following year Lischner (Lischner, 1966) reported a similar experience in a patient with EPP. He had suspected the presence of a significant enterohepatic cycling of protoporphyrin after observing that the amount of protoporphyrin in a timed collection of duodenal aspirate exceeded its mean faecal elimination rate three-fold. He administered 9-18 grams of cholestyramine per day to his patient and documented an improvement in both clinical and laboratory features during therapy. Yet he warned that the manifestations of the disease in this patient varied so dramatically in response to many known and unknown stimuli that it was impossible to be sure that the improvement was not fortuitous.

These early studies prompted that of Ibrahim and Watson (Ibrahim and Watson, 1968) who set out to gain additional evidence for an enterohepatic circulation of porphyrins. Ibrahim synthesised carbon 14-labelled protoporphyrin and administered it to himself through a duodenal feeding tube. He was able to recover 76% of the administered radio-activity in his stools. He made two observations which led him to conclude that an enterohepatic circulation exists. Firstly, some of the radio-activity recovered in the stools was found to have been incorporated into stercobilin. The authors interpreted this as implying absorption of protoporphyrin, with metabolism in the liver, via haem, to bilirubin

and subsequent re-excretion as stercobilin. The authors discounted the alternative explanation; that the intestinal flora were responsible for this conversion. Secondly, they noted prolonged excretion of radio-activity in the stools, where it was still detectable after eight days. They suggested that in people with a normal bowel habit, all the administered protoporphyrin should have been excreted within four days. This delay in excretion was therefore interpreted as further evidence for an enterohepaticcirculation.

The next attempt to treat porphyria with enteral adsorptive agents was made by Kniffen (Kniffen, 1970), who administered cholestyramine 12 g daily to a 17 year-old girl with EPP accompanied by marked hepatic protoporphyrin deposition. Her photosensitivity abated, and erythrocyte, plasma and faecal protoporphyrin levels decreased. A liver biopsy after 13 months of treatment showed a striking decrease in the degree of accumulation of protoporphyrin in the bile ductules, Kupffer cells and hepatocytes.

Three years later Davidson and colleagues (Davidson *et al.*, 1973) reported their experience with cholestyramine 12 g daily and vitamin E 100 units daily in a 26 year-old female with EPP. Her liver function tests, initially deranged, returned to normal; she tolerated more sun exposure and an improvement in her liver biopsy, with a reduction in its porphyrin content, was noted.

Despite the utility of the hypothesis that porphyrins undergo an enterohepatic circulation and its apparently successful application therapeutically, the existence of an enterohepatic cycling of porphyrins remained unproven. The hypothesis was however lent strong support by Pimstone and colleagues (Pimstone *et al.*, 1982) who studied a patient with CEP. They passed a triple-lumen perfusion study catheter into the duodenum and aspirated samples via the proximal port, below the ampulla of Vater, and via the distal port 15 cm downstream. Polyethylene glycol was infused in isotonic saline as a non-absorbed marker through the infusion port which was positioned just beyond the pylorus. Samples were drawn from the proximal and distal ports and their porphyrin content analysed. This suggested that in excess of 90% of porphyrins were absorbed over a length of 15 cm in the proximal small intestine. This strongly supported the concept of an enterohepatic cycling of porphyrins. However, in view of the large amount of porphyrin contained in bile and the high proportion reabsorbed, they suggested that it seemed unlikely that oral

cholestyramine, with its limited adsorptive capacity for porphyrins, could materially influence this.

Much *in vitro* work has been done by Tishler and colleagues on the potential use of sorbents in the therapy of the porphyrias. They began in 1982 by assessing the efficacy of several sorbents *in vitro* in adsorbing porphyrins, porphyrin precursors and haem proteins from solution (Tishler and Gordon, 1982). They initially used the non-ionic resins amberlite XAD-4 and XAD-7 resins (which are commonly employed in adsorptive columns for haemoperfusion) and coconut-activated charcoal. They showed that these sorbents were able to adsorb large amounts of uroporphyrin, coproporphyrin and protoporphyrin, and significant amounts of myoglobin and haemoglobin. Charcoal also adsorbed ALA and PBG, whereas the XAD resins did not. From this they concluded that charcoal and the amberlite resins are equally effective in adsorbing porphyrins, but that charcoal has a much higher avidity for the precursors ALA and PBG. In performing these experiments, the authors hoped to determine whether charcoal haemo- or plasma perfusion might have any rôle in the treatment of the acute porphyric attack, (during which elevations of ALA and PBG are uniformly encountered). They followed this up with two studies of the efficacy of commercial haemoperfusion cartridges in removing porphyrin precursors from solution (Tishler and Gordon, 1983; Tishler and Winston, 1984). Yet the authors caution that it has never been proven that the accumulation of these precursors is directly causal in the acute attack. Hence, the efficacy of haemoperfusion even in the light of the binding of ALA and PBG by charcoal is by no means assured. However their finding that porphyrins are also bound by charcoal led them to suggest that it may be effective in treatment of the photosensitive porphyrias where porphyrins accumulate. Indeed, charcoal haemoperfusion has not found favour in the treatment of the acute attack, and much more interest has been shown in this latter application.

Subsequently activated charcoal was used in clinical trials in place of cholestyramine. Gandhi and Pimstone showed that an acute challenge with charcoal could reduce plasma porphyrin levels dramatically in CEP, and that its efficacy was superior to that of cholestyramine (Gandhi and Pimstone, 1983). They discovered that all the porphyrins present in this patient's bile would bind to both activated charcoal and cholestyramine *in vitro*. They administered 30 g activated charcoal every 3 hours for 36 hours, and compared this subsequently with a

dose of 4 g cholestyramine given six hourly for 36 hours. Charcoal reduced the plasma porphyrin content by 25% at 3 hours, by 77% at 4 hours, by more than 95% at 6 hours and porphyrins were barely detectable after 13 hours. Following cholestyramine, plasma porphyrin content fluctuated between 25% and 50% of the starting values. This suggested that charcoal was indeed efficacious, and more so than cholestyramine. One notes that the weight of charcoal given far exceeded that of cholestyramine so from this study it is not possible to conclude that charcoal *per se* is superior in its binding capacity to cholestyramine. It certainly does suggest that a much larger dose of charcoal is practical.

They subsequently followed the effects of chronic oral charcoal therapy in this patient in a 12-month follow-up study (Pimstone *et al.*, 1985). The effects of a dose of 60 g three times daily on tissue porphyrin levels, the amount of porphyrin excreted and the clinical activity of the disease were assessed. On chronic therapy, circulating plasma porphyrin levels dropped from approximately 25 g/dl to approximately 1 g/dl (normal range less than 0.5 g/dl). This was accompanied by a 50% drop in urinary porphyrin excretion and a significant diminution in the clinical activity of the disease. The only adverse effects of therapy were the development of deficiencies of vitamin B12, folic acid and vitamin D. These were easily corrected with supplements. They concluded that oral charcoal therapy acts by interrupting the enterohepatic circulation of porphyrins and rapidly diverts skin and circulating plasma porphyrins to the gut lumen from where they are excreted.

These authors also assessed the efficacy of charcoal in a patient with EPP and cirrhosis (Pimstone *et al.*, 1986). A dose of 60 g six-hourly resulted in a significant reduction in skin and liver protoporphyrin levels, as well as a 40% reduction in serum protoporphyrin levels. Tests of liver function improved, and the patient exhibited no photosensitivity. He apparently remained biochemically stable for 2 months, but thereafter liver function deteriorated and he was referred for liver transplantation.

Meanwhile Tishler and colleagues had compared the relative affinity for porphyrins of cholestyramine and eight different formulations of activated charcoal in a solution comparable in some respects with intestinal juice (Tishler and Winston, 1985). The porphyrins were dissolved in 0.5N ammonia, 0.5% sodium desoxycholate and 0.18M

sodium bicarbonate, with a final pH of 8.2. They found that cholestyramine and a particular formulation of charcoal (*Amoco Supersorb PX-21*) had the greatest affinity for uroporphyrin with adsorptive capacities of 26.5 and 17 mg of uroporphyrin per gram of dry sorbent respectively. The other forms of charcoal had adsorptive capacities in the range 1.6 - 5.7 mg of porphyrin per gram of dry sorbent. *Norit A* charcoal, which was used in the trial which forms the basis of this thesis, had a value of 4.1 mg of porphyrin per gram of dry sorbent. However adsorptive capacities of the sorbents for protoporphyrin-IX gave different values with cholestyramine, Amoco Supersorb and five other forms of charcoal all having values ranging from 21-32 mg of protoporphyrin per gram of dry sorbent, whereas Norit A gave a value of 9.9 mg of protoporphyrin per gram of dry sorbent. Thus various formulations of charcoal have differing affinities for the different porphyrins. This may well have implications in the interpretation of clinical studies. They comment that the Amoco Supersorb PX-21 is a highly activated charcoal with an internal surface area some three times that of standard activated charcoals (2800-3500 m^2 per gram versus about 1000 m^2 per gram). Pimstone and colleagues had calculated that approximately 200 mg of porphyrins participate in the daily enterohepatic circulation on their patient with CEP (Pimstone *et al.*, 1982). Using this figure, Tishler estimated that merely a few grams of charcoal should be sufficient to bind all the porphyrin entering the bowel completely. He does however caution that factors such as the gastrointestinal transit time of the sorbent and the rate at which porphyrins appear in the bile make such direct comparisons difficult. Yet he suggests that the extremely high avidity of certain charcoals for porphyrin may allow smaller doses to be used.

In 1987, Pimstone *et al.* summarised their experience with sorbent therapy in their patient with CEP in a comprehensive report (Pimstone *et al.*, 1987). In a review of the short term studies they concluded that oral charcoal was more effective than cholestyramine or hypertransfusion in reducing plasma porphyrin concentration, but that cessation of charcoal was followed by a rise in plasma porphyrin levels to pre-treatment levels within 10 days. In addition, oral charcoal therapy reduced skin porphyrin concentrations by more than 90%. Moreover, a change in the skin porphyrin profile was noted during charcoal therapy. Whereas initially the predominant porphyrins were the hydrophilic porphyrins uroporphyrin and heptacarboxylic porphyrin, the

predominant porphyrins after two days' treatment were pentacarboxylic porphyrin, coproporphyrin and isocoproporphyrin. Fortnightly evaluation for a further nine months confirmed a sustained decrease in plasma porphyrin, which declined to near-normal concentrations, and a fall in skin porphyrin content to 1% of the initial level. Both returned rapidly to pre-treatment levels after cessation of charcoal. The patient had no clinical photocutaneous sensitivity, felt well and experienced no side-effects. The authors advanced three possible mechanisms for the efficacy of charcoal. Firstly, charcoal might work by interfering directly with the enterohepatic circulation of porphyrins; secondly, by preventing the enteral absorption of porphyrin secreted by mucosal cells lining the gut or thirdly, by producing a gradient between plasma and lumenal free porphyrins following the sequestration of porphyrin within the bowel, thus promoting a flux of porphyrin from plasma to the bowel lumen. They suggested that the use of charcoal should be explored in photocutaneous porphyrias other than CEP and EPP.

This study was reviewed by Tishler (Tishler, 1988) who expressed enthusiasm at the outcome of Pimstone's trial. He commented that his own *in vitro* studies had shown considerable variation in the efficacy of different forms of activated charcoal, and that the one used by Pimstone had not been one of the most efficacious. He asked whether another formulation might have had a similar effect at lower doses. He raised certain questions which require answers. Does long-term charcoal therapy confer long-term protection from the cutaneous and other manifestations of these porphyrias? And does charcoal have a role to play in the treatment of other illnesses, such as the hypercholesterolaemias, which are characterised by an enterohepatic circulation of substances in excess? He expressed surprise that the only adverse effects noted were minor deficiencies of three vitamins, and suggested that other deficiencies might develop in time since charcoal is a non-specific sorbent. He cautioned too that charcoal may contain polycyclic aromatic hydrocarbons, the long-term effects of which - and particularly their carcinogenicity - is unknown.

A further development on the theme of sorbent therapy was that of McCullough and colleagues who investigated the combined effect of bile acid and cholestyramine on a patient with EPP (McCullough *et al.*, 1988). They referred to two animal studies which had suggested that oral feeding with a bile acid such as cholic acid could reduce the excessive hepatic accumulation of protoporphyrin and limit its

hepatotoxicity. (Avner and Berenson, 1982; Poh-Fitzpatrick *et al.*, 1983). They therefore administered either cholic acid, cholestyramine or both for periods of approximately one week to a subject with EPP, each treatment being separated from the next by a washout period. No fall in erythrocyte or plasma protoporphyrin concentration, was noted, yet the faecal excretion rate of protoporphyrin increased more than threefold during treatment with cholestyramine or the combination of cholestyramine and bile acid. Bile acid alone had no effect. In a long-term study in this patient they showed a decrease in the total hepatic porphyrin content during cholestyramine therapy, as assessed by a reduction in fluorescence of a liver biopsy sample. Secondly, there was a marked improvement in photocutaneous sensitivity, implying a depletion of the skin protoporphyrin, despite a failure of erythrocyte and plasma porphyrin concentrations to fall. They thus observed that erythrocyte and plasma protoporphyrin levels remained elevated even as tissue porphyrin stores were being depleted. This suggested that an equilibrium between the production of porphyrins, their hepatic excretion and tissue depletion may exist, and plasma protoporphyrin will remain unchanged until the hepatic protoporphyrin deposits are themselves depleted.

In the light of the promise shown by work with charcoal such as that of Pimstone, we undertook a study of its effects in a single patient with CEP, and in eight subjects with VP accompanied by skin disease. This work is described in the chapters which follow.

CHAPTER 4

EXPERIENCE WITH ORAL ACTIVATED CHARCOAL IN CONGENITAL ERYTHROPOIETIC PORPHYRIA

CASE REPORT

The Porphyria Clinic of Groote Schuur Hospital has one patient with CEP under its care. He has been treated with oral activated charcoal for more than two years, and his course is described here.

He was born in the Northern Cape and raised by his grandmother. Following her death, he was admitted to a Place of Safety in Kimberley. He was first seen at Groote Schuur Hospital at the age of 12 for an apparent "arthritis". He was noted to have proximal and distal interphalangeal joint deformities of the hands and was diagnosed as having a burnt-out Still's disease. However he gave a history of skin fragility and frequent blistering of sun-exposed areas since early childhood. Only later was porphyria suspected. It was then realised that his hand deformities were the result of photomutilating skin disease. His face was scarred and showed temporal hirsutism, areas of hypo- and hyperpigmentation (fig 4.1) and large bullae. His teeth fluoresced under ultraviolet light. His porphyrin profile confirmed CEP and is shown in Table 4.1.

	RBC	PLASMA	URINE	STOOL
Uro	81	10.8	3638	1.49
7-COOH	10	1.7	173	0
6-COOH	0	0	82.3	0
5-COOH	0	2.3	432	0
Copro	757	3.6	2845	637
Proto	4795	37.2	7170.3	74.8
Total	6363	55.6		713.29

Table 4.1. Initial porphyrin profile.

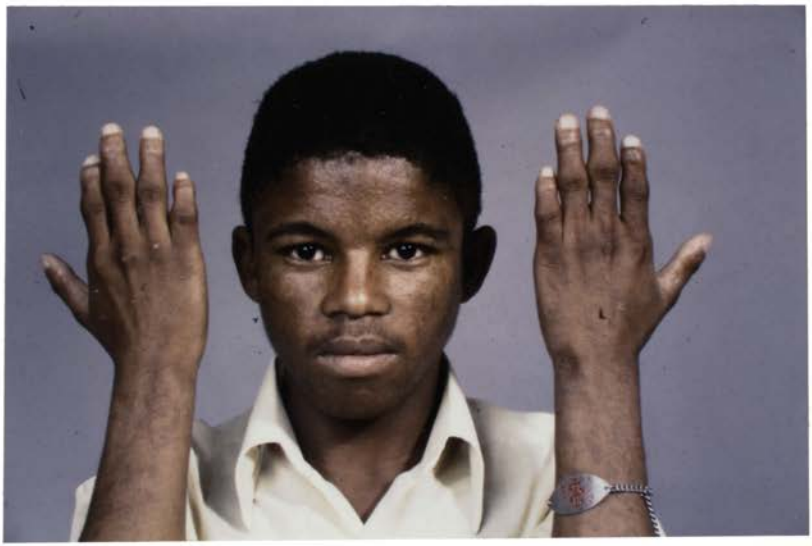

Fig 4.1. Patient with CEP showing photomutilation.

The initial erythrocyte protoporphyrin is higher than is usually encountered in CEP. However, he had features of iron deficiency, with a microcytosis (MCV 74 fl), a reduced serum iron concentration (5 mol/l), transferrin saturation (8%) and serum ferritin concentration (15 mol/l), and a raised total iron binding capacity (65 mol/l). Iron deficiency is sufficient cause for an elevated red cell protoporphyrin.

In the light of the work of Pimstone (Pimstone *et al.*, 1987) therapy with oral activated charcoal was commenced. He was admitted to hospital on 1 April 1987, and discharged to a privately-run children's home six weeks later. Following a ten-day period of observation, he received charcoal in a dose of 60 g every 8 hours. After 12 days, this was reduced to 20 g every 8 hours as he found the charcoal unpalatable. This was further reduced to 10 g twice daily following a month of treatment. He was discharged on this dose and has since been seen frequently. From May 1989 his dose was increased to 30 g bd because porphyrin determinations showed steadily rising levels.

RESULTS

Urine porphyrins

Initially a dramatic fall in urine porphyrin concentration was noted (Figure 4.2a). It remained low for the first 6 months, despite the progressive reduction in dose. However, his course from 5 to 30 months has been marked by a progressive increase in porphyrin concentration to pretreatment levels (Figure 4.2b). The increase in dose to 30 g bd during the last six months appears to have had no impact on this.

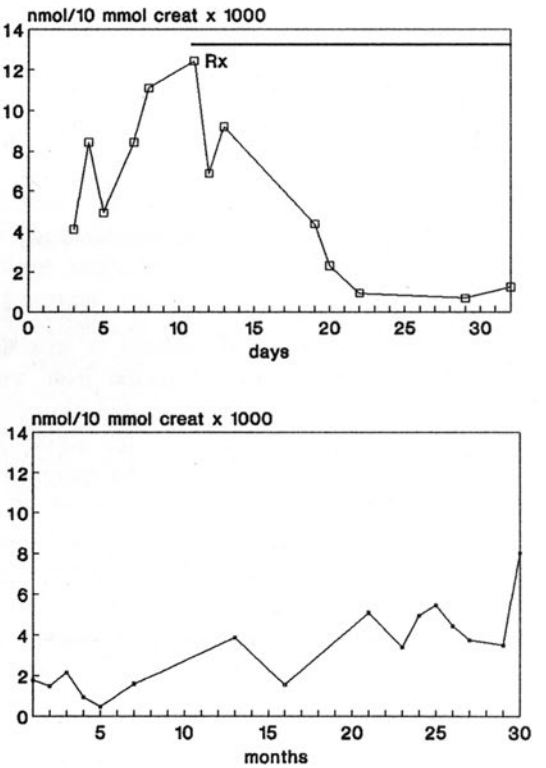

Fig 4.2. Urine porphyrins. (a) at start of therapy, (b) first 30 months.

Erythrocyte porphyrins

Erythrocyte porphyrin concentrations did not show any early change (Figure 4.3a), and remained fairly constant between 4000 and 8000 nmol/l for the first 18 months. There has been a subsequent trend upwards, with a final value of 14000 nmol/l (Figure 4.3b).

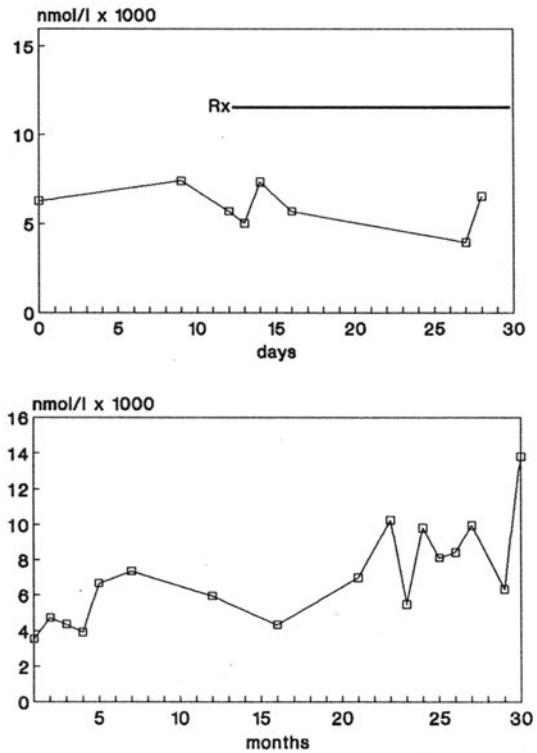

Fig 4.4. Erythrocyte porphyrins. (a) at start of therapy, (b) first 30 months of therapy.

Plasma porphyrins

Plasma porphyrin concentrations, though highly variable, showed a pronounced fall after the start of therapy (Fig 4.4a). They remained low for the first seven months; indeed, porphyrins were undetectable

in the plasma at 3 and 4 months. Following this, porphyrin concentrations have risen dramatically to a peak of 350 nmol/l, a sevenfold rise over the values seen *before* the start of charcoal therapy (Fig 4.4b).

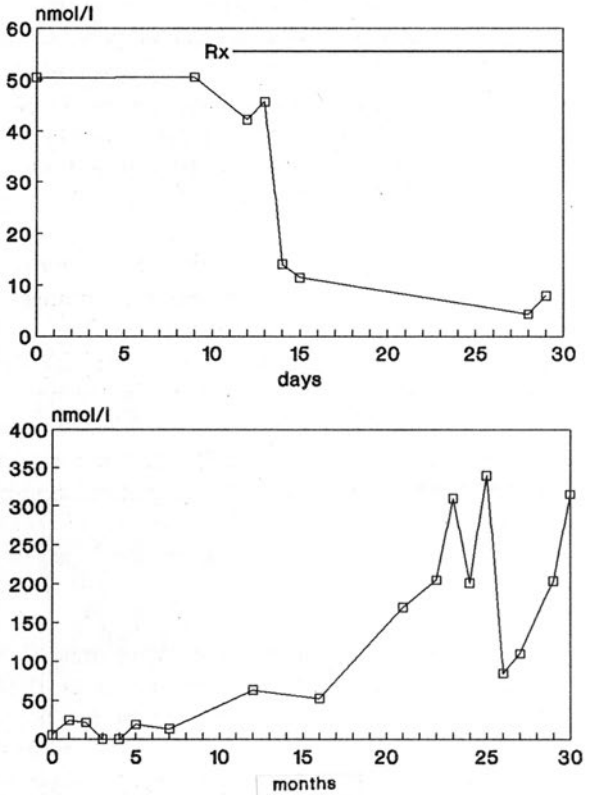

Fig 4.4. Plasma porphyrins. (a) at start of therapy, (b) first 30 months. Note change in scale.

Photosensitivity

The improvement in plasma and urinary porphyrin excretion was attended by a marked improvement in his photosensitivity. Following discharge, his skin remained clear of new erosions and blisters and he was able to enjoy an active school and social life. However, at the

time of writing, he has for the first time since his discharge again developed erosions and blisters in sun-exposed areas.

DISCUSSION

No significant change in erythrocyte porphyrin concentration was seen. This is not unexpected. Since CEP is an erythropoietic porphyria, abnormal porphyrin production is believed to be confined to the erythrocytes. These porphyrins then diffuse into the plasma, and their subsequent binding to charcoal and loss from the body should not decrease erythrocyte porphyrin levels, which appear to reflect the rate of synthesis, rather than removal.

Both plasma and urinary porphyrin levels dropped dramatically at the start of therapy. This suggests that enteral binding has indeed resulted in a flux from plasma into the gut, causing a decrease in plasma concentrations and reducing the amount available for excretion by the kidneys. The early improvement in urinary porphyrin concentrations agrees with the results obtained by Pimstone *et al.* in their patient with CEP (Pimstone *et al.*, 1987). Their patient retained his remission for at least a year. Though our patient appears to have enjoyed a substantial remission, porphyrin levels have steadily risen over the ensuing months, reached pretreatment levels at two years and indeed have exceeded those values subsequently. The reason for this is unclear.

It does not appear to be due to a lack of compliance. He is motivated and willing to co-operate, but has understandably at times found that the demands of a busy school and social life have made strict adherence to his treatment regimen difficult. He has been living in a privately run children's home with dedicated supervision by child-care workers, who accompany him to clinic each month and who supervise the taking of his medication. Periods of particularly close supervision and special encouragement have not resulted in a fall in porphyrin levels. Whether this breakthrough is a feature of long-term therapy remains to be seen, as it cannot be judged from a single case.

Despite the rising porphyrin concentrations, his skin activity remained minimal. Recently his dose was further increased in an attempt to regain control of his porphyrin levels. He has subsequently redeveloped

active skin lesions for the first time in two years. There are two possible explanations for this. Firstly, it may be that his disease has indeed escaped control by charcoal. This in turn requires an explanation. It may be that his disease is becoming more severe independently of his therapy. We have no proof of this, and there is no good precedent for this to be found in the literature. In the light of Tishler's findings on the differing avidities of various brands of charcoal for porphyrins (Tishler and Winston, 1985), the possibility of a change in charcoal formulation to one having a lower affinity for porphyrins has to be considered. Though we have not performed *in vitro* studies on the agent given to him, we do not believe that this is so. The hospital pharmacy has continued to dispense the same brand of charcoal. Furthermore, the porphyrin concentrations have been rising steadily, and have not shown a sudden, unexpected increase as might have been expected had the charcoal suddenly lost its efficacy. It is difficult to find another reason for a progressive failure of therapy. Since charcoal acts by interrupting a physical system, the enterohepatic cycle, anything akin to a "drug resistance" is unlikely.

Secondly, in the light of the work with VP to be presented in the following chapters, the possibility that his recent deterioration might in fact be *due* to his increase in dose should be considered. Against this is the observation that no sudden rise in porphyrins was seen at the start of his treatment, when he was first exposed to charcoal, in contrast to our experience with VP, as will be described below.

Thus far he has remained free of complications of his therapy, except that he now has again developed biochemical evidence of iron deficiency. This may account in part for the recent increase in erythrocyte porphyrin, since an elevated protoporphyrin is characteristic of iron deficiency. To distinguish the contribution of iron deficiency to the increasing levels of porphyrins seen latterly, we have analysed the distribution of the various types of porphyrin in both the red cells and the urine. Figure 4.6 plots erythrocyte uroporphyrin and protoporphyrin on the same axis. It will be seen that they appear to increaseproportionately.

Were iron deficiency alone to account for the increase, one would expect the ratio of protoporphyrin to uroporphyrin to increase, seeing it is the former which is most sensitive to alterations in iron status. This is not seen, suggesting that the increase in porphyrins is a real phenomenon, and not merely secondary to iron deficiency. Similar

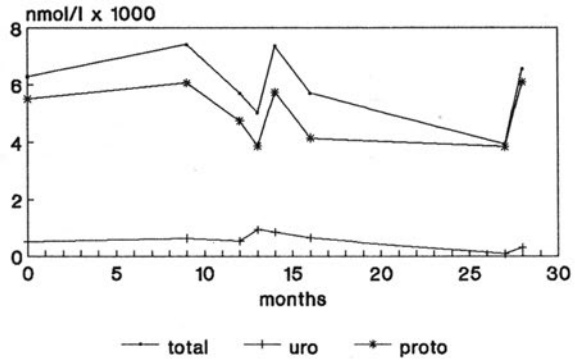

Fig 4.6. Erythocyte uro- and protoporphyrin.

results are suggested by Figure 4.7 which plots the urinary uroporphyrin versus coproporphyrin. They have maintained a more or less constant ratio, again suggesting an increase in porphyrin synthesis.

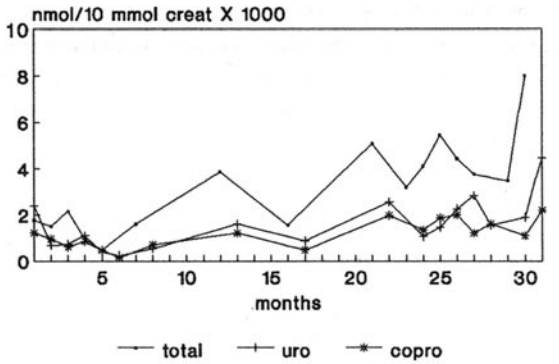

Fig 4.7. Urine uro- and coproporphyrin.

Magnesium and zinc levels have been assessed and are normal. There is no evidence of folate deficiency. He is currently being treated with intensive vitamin and mineral administration on "off days" when he takes no charcoal at all. The effectiveness of this remains to be seen.

Other possibilities remain to be considered. Does the prolonged administration of charcoal perhaps affect the bowel wall and its porphyrin synthesising and absorbing properties, or does it alter the intestinal flora with alterations in microbial porphyrin metabolism? Could this youngster's critical age, peripubertal, have influenced the production of porphyrins independently of his therapy? There is at present insufficient experience with longterm activated charcoal to refute such possibilities.

CONCLUSION

It would therefore appear that charcoal therapy in CEP need not necessarily be as simple as a reading of the case report of Pimstone *et al.* would suggest. We have confirmed that charcoal will reduce the urinary excretion of porphyrins dramatically over 12 months, but have clearly documented biochemical deterioration thereafter. Similarly, it appears that skin photosensitivity has been effectively improved by sorbent therapy. Here the remission has lasted in excess of two years, but the skin disease would now appear to be reasserting itself. The reason for this failure of control is unclear. Our patient will be followed further.

This patient has been under my care since February 1988. The initial stages of his charcoal therapy were supervised by Prof R Kirsch, Mr P Meissner, Dr N White, Dr S Robson and Dr A Unger, whose contribution is acknowledged.

CHAPTER 5

ACTIVATED CHARCOAL IN VARIEGATE PORPHYRIA

Most patients attending the Porphyria Clinic of Groote Schuur Hospital have either VP or PCT. Both are commonly accompanied by typical vesicular-erosive photosensitivity. Venesection is effective in improving the skin disease of PCT, whereas there is no uniformly reliable therapy for the dermatological problems of VP. Though the skin disease of VP is rarely as mutilating as that of CEP, it causes considerable pain, embarrassment and morbidity in many of our patients, whose lifestyle may be quite severely affected by it (Fig 5.1). We therefore embarked on a study to assess the efficacy of activated charcoal in ameliorating the photosensitivity of VP.

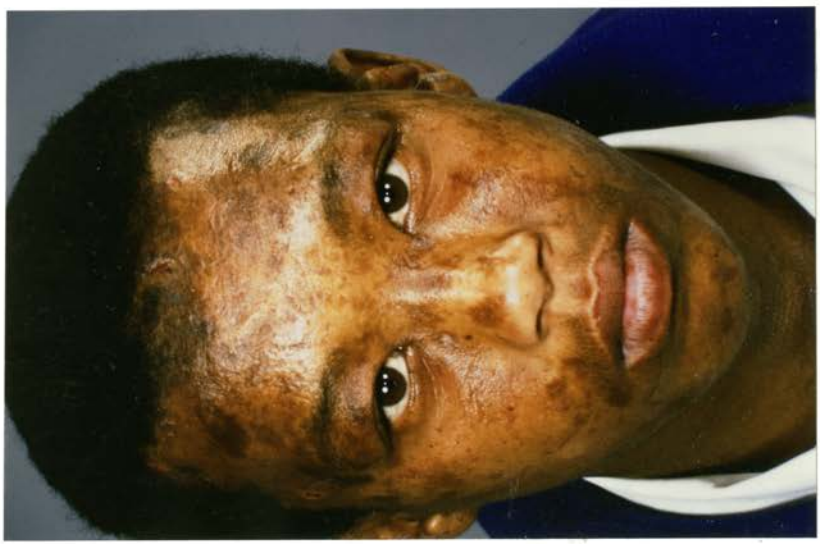

Fig 5.1. Severe skin lesions in a patient with VP.

AIM OF THE STUDY

This study was intended to determine:

- whether the short-term administration of activated charcoal would lead to a fall in urine and plasma porphyrin excretion, as it had in CEP;
- whether charcoal therapy might bring about an improvement in the skin disease of VP;
- whether the administration of charcoal in therapeutic doses is practical and acceptable to patients;
- and thus, in summary, whether the administration of charcoal is an effective form of therapy for the skin disease of VP?

DESIGN OF THE STUDY

The trial was initially planned as a double-blind placebo-controlled crossover study. However the design was subsequently modified to that of an open trial without placebo. The reasons for this, which became evident as planning for the trial progressed, were as follows.

Firstly, there were objections to using the conventional placebos *glucose* or *lactose*. Subjects were to take sixty capsules per day. Large amounts of glucose or lactose would be required to fill such a number of capsules. This might have such deleterious effects as weight-gain and diarrhoea. We did not feel it justified to expose our subjects to this.

Secondly, placebo control appeared impractical. Activated charcoal imparts an unmistakable black colour to the stools. This is impossible to disguise and would hence remove the element of blinding. We considered overcoming this by using *non-activated charcoal* as a placebo. However, this may in itself have some porphyrin-binding capacity, which would prevent its being a true placebo. This might confound the results, and this option was rejected. With the help of the pharmacist, we did experiment with various mixtures of coloured compounds but were unable to reproduce the characteristic colour and texture of charcoal.

Thirdly, a high degree of motivation and compliance is essential when subjects are asked to swallow 60 capsules per day. We felt that subjects would be less likely to comply or indeed to see the

trial out if they suspected they were swallowing an ineffective agent in such quantities.

Since the yardsticks by which the outcome of the trial were to be assessed were predominantly *objective* measures, the possibility of some observer bias would be less critical than it might have been had *subjective* phenomena, such as the participant's sense of his own improvement, been the criterion by which the outcome was assessed. Thus we felt that the lack of placebo control was unlikely to confound the results to a critical degree.

STUDY MEDICATION

Activated charcoal is available commercially as a black powder, as a compressed tablet and as a gelatin capsule. The powder can be taken as a slurry in water. It may also be given in lactulose, which is said to counteract charcoal-induced constipation. Both slurries were offered to a group of people beforehand; the mixture in lactulose was felt to be less acceptable than that in water. In any event the slurry was judged unpleasant. The tablet is more easily swallowed but contains compressed charcoal. We were concerned that this compression might possibly alter the binding characteristics of the charcoal. Accordingly, we avoided charcoal tablets and used charcoal loosely packed into gelatin capsules instead, since this appeared to offer the most acceptable and palatable preparation for the subjects to take. A range of brand names of activated charcoal is available. We used *Norit A* which was imported by Beecham Pharmaceuticals and specially packed for this trial. Size 20 capsules, each holding 300 mg charcoal, were used. Care was taken to avoid overfilling the capsules so as not to compress the charcoal excessively and thus reduce its binding properties.

Dose Employed

One of the intentions of this study was to assess the suitability of charcoal as a practical method of therapy in the average subject with VP. Hence our choice of dose was dictated by the largest dose we felt the subjects could reasonably be expected to swallow. A dose of 15 capsules 4 times daily (or 18 g charcoal daily) was chosen. After the first 6 weeks, Beecham Pharmaceuticals were no longer able to

supply the medication and accordingly Norit A, pre-packed into capsules holding 200 mg of activated charcoal, was imported directly. It was decided to keep the prescription of 60 capsules daily constant; this necessitated a *de facto* reduction in dose to 12 g daily for the final 6 weeks of the study.

Anticipated Side Effects

Charcoal is a relatively innocuous substance. However, little is known about its long-term effects (Tishler 1988). It is non-specific in its binding and we felt that our subjects might be at risk of vitamin and trace element deficiency. We therefore placed them on vitamin supplements. They were instructed not to take these at the same time as their charcoal, but between doses. Administration of charcoal might also be expected to interfere with the absorption of any other medication taken concurrently. We therefore ensured that our subjects were not receiving other medicines, and asked them to alert us should other medication be prescribed for them. A bulk-forming laxative was made available to each subject but in the event constipation was not encountered and nobody made use of it.

CHAPTER 6

SUBJECTS AND METHODS

INCLUSION CRITERIA

Eight subjects fulfilling the following criteria were enrolled. All subjects were unequivocally proven to have VP by the determination of a characteristic pattern of porphyrin excretion in the urine, stool and plasma. This included the demonstration of a raised faecal protoporphyrin, which was accompanied by elevations of coproporphyrin and pentacarboxylic porphyrin, as well as the presence of pseudopentacarboxylic porphyrin and tricarboxylic porphyrin. The latter substances are not encountered in normal people, and their presence is highly characteristic of VP. All subjects were resident in Cape Town, had no intercurrent medical illnesses, and were willing to enter the study, to provide informed written consent and to comply with the prescribed regimen. All had cutaneous involvement; all were free of symptoms suggestive of an acute attack at the time of entry.

SUBJECTS

S. A.: female, aged 33, housewife with a strong family history of VP and marked skin changes.

S. B.: male, aged 30, unemployed. He had consulted our clinic frequently for a variety of minor complaints, none of which had reflected the acute symptoms of porphyria. He showed quiescent skin changes.

C. de R.: female, aged 43. A doctor's receptionist. Despite taking good care of her skin, she found the lesions disfiguring and the restrictions on her lifestyle irksome.

J. G.: male, aged 25, a civil servant. He had recently been to our clinic to seek advice on his skin but was otherwise asymptomatic.

L. G.: a brother of JG, aged 26, a medical graduate performing his national service. His porphyria was asymptomatic apart from the skin lesions which were interfering with his recreational activities.

G. M.: male, aged 18. This young man has had very severe skin lesions since the age of 8, which is unusually early for VP. He has a low standard of education and is unemployed; both these are due in some part to his disfiguring skin lesions.

P. U.: male, aged 32. A bricklayer. VP was diagnosed a few months prior to this trial when he presented to hospital with unexplained abdominal pain. He underwent a laparotomy following induction of anaesthesia with thiopentone and developed a typical acute porphyric attack. He recovered rapidly from this but his skin lesions, which he had ignored previously, became very active thereafter.

F. van Z.: male, aged 35, a bank employee, with a family history of VP. He had never experienced acute symptoms and had mild skin lesions.

METHODS

Timing of Observations

Subjects were assessed at the start of the trial, and at 2, 4, 6 and 12 weeks. At each visit, porphyrin concentrations were determined for specimens of urine, plasma and stool, and the number of lesions on the hands were counted. Photosensitivity testing was performed at the start, and at 2, 4 and 6 weeks.

All subjects were tested concurrently over a three month period. They were seen in two groups, which met on alternate weeks.

Data Handling

All data were entered onto a computer spreadsheet (Lotus 123) for further manipulation. Statistical comparisons of significance were made using the Friedman test (analysis of variance using two ranks). This test takes into account not only the variance within a particular set of observations, but also the variance within the data for each individual. A probability (p) value less than 0.05 was regarded as significant.

Biochemical Estimations

Samples of urine, stool and plasma were analysed for the presence of ALA, PBG and porphyrins at each visit. All reagents were of analytical grade.

Urinary ALA and PBG Determination

ALA and PBG concentrations were assayed according to an established ion-exchange method (Davis and Andelman, 1967) available in kit form (Bio-Rad, Munich, West Germany). The manufacturers instructions were followed. Reaction of the eluted PBG with freshly prepared Ehrlich's aldehyde produced a colour response which could be gauged spectrophotometrically by measuring the absorbance at 553 nm. ALA is measured similarly except that it is first converted to PBG, which then reacts with the Ehrlich's aldehyde.

Plasma, urine and stool porphyrins

A highly sensitive thin-layer chromatography (TLC) assay for porphyrins in blood (plasma and erythrocytes), based on quantitative fluoroscanning, has previously been described, well characterized and standardized (Day *et al.*, 1978a). These methods have been adapted to the assay of porphyrins in stool and urine by simply substituting these for blood as the starting material. The method is as follows.

Sample Preparation

3 ml of urine or plasma, or approximately 0.3 g of stool, were esterified in 30 ml of a 5% (v/v) sulphuric acid/methanol solution overnight, at room temperature in the dark. The wet weight of stool samples was noted. A further aliquot of the same stool sample was weighed, allowed to dry overnight at 60°C, and the dry weight determined, thus allowing determination of a wet weight/dry weight ratio.

Extraction

All procedures were performed in a darkened room. The stool and plasma esterification mixtures were centrifuged at 800 g for 15 minutes. The supernatant of a plasma or stool mixture, and the urine esterification mixture itself, was transferred into a separating funnel and its pH

neutralized with 17% ammonia solution. The porphyrin esters were then extracted into chloroform. Plasma was extracted into a volume of 10 ml (2x5 ml aliquots), urine into a volume of 20 ml (2x10 ml aliquots) and stool into a volume of 45 ml (3x15 ml aliquots). Where initial screening for porphyrins under ultraviolet light suggested a gross excess of porphyrin, proportionately more chloroform was used for the extraction.

Quantitative TLC

The volume of the chloroform extract was noted and precise aliquots of each extract were spotted on to Merck Silica gel-60 TLC plates in partial darkness with a Hamilton microsyringe. Care was taken to adjust the amount of porphyrin esters spotted so that they would fall within the linear portion of the fluorescence-versus-porphyrin concentration curve. The volumes spotted ranged from 30-50ul of stool extract and 100-300ul of urine extract. Plasma was evaporated to dryness, resuspended in 1 ml chloroform and 50-100ul were spotted. Evaporation of the chloroform during the spotting was aided by a constant stream of warm air blown over the working area by a fan. Each plate accommodated 9 spots spaced at 2 cm intervals, two of these lanes being reserved for porphyrin ester standards. Standards containing mixtures of known amounts of the methyl esters of uro-, heptacarboxylic-, hexacarboxylic-, pentacarboxylic-, copro- and mesoporphyrin (which behaves similarly to protoporphyrin on TLC) were spotted in these two lanes. These standards are commercially available from Porphyrin Products, Logan, Utah.

Plates were allowed to run for 45-60 minutes in a solvent system consisting of carbon tetrachloride, dichloromethane, ethyl acetate and ethyl propionate in a 2:2:1:1 ratio by volumes. Each plate was then dipped in an enhancing solvent consisting of chloroform, dodecane, and hexadecane (18:1:1 by volumes), which increases the fluorescence yield. This allows the quantitation of very small amounts of porphyrin ester which would otherwise remain undetected. After drying, the plates were scanned on a fluoroscanning photodensitometer (Vitatron TLD 100) connected to an integrator/recorder unit (Spectra-Physics, Model SP4290). Two interference filters were used, the incident at 399 nm and the fluorescence filter at 620 nm. A trace showing the peaks of porphyrin ester fluorescence was thus produced, and the areas under the peaks were calculated by integration. Individual species

of porphyrin ester could be identified by direct comparison of the retention times of the unknown samples with the standards. An example of TLC plate is shown in Figure 6.1.

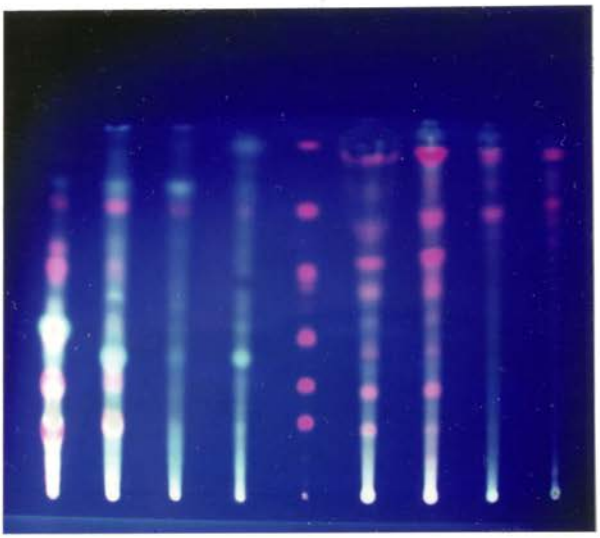

Fig 6.1. TLC plate viewed under ultraviolet light showing separation of porphyrins.

Calculation of Porphyrin Concentration

The areas under the peaks of the various porphyrin esters separated on the TLC plates were compared directly with those of the standards on the same plate. The porphyrin concentrations were calculated according to the following formulae, adapted from Day (Day *et al.*, 1978b).

- *Urine and plasma*

  Concentration(nmol/l) = [(SA/SV)/(PA/PV)][(VF.C)/IV]

- *Stool*

  Concentration (nmol/g dry stool) = [(SA/SV)/(PA/PV)] [(VF.C)(WW/DW)/WS]

where:

SA = peak area of sample porphyrin spot
PA = peak area of relevant standard porphyrin spot
SV = volume of sample applied to TLC plate (ul)

PV = volume of porphyrin standard applied to TLC plate (ul)
VF = final volume of chloroform extract from sample (ml)
IV = Initial sample volume of urine (ml)
IW = Initial sample wet weight of stool (g)
C = Relevant porphyrin ester standard concentration (nmol/l)
WW = wet weight of an aliquot of the stool sample (g)
DW = weight of the same aliquot after drying (g)
SW = wet weight of stool sample esterified

Clinical Observations

Lesion Counts

The hands were inspected and photographed on each occasion. Templates of both hands were drawn. The position of each lesion was marked with a code indicating whether the lesion was a blister, an erosion or a scab. These were defined as follows:

- *Blister* - a fluid-filled vesicle.
- *Erosion* - an open lesion with denuded base.
- *Scab* - an older lesion covered by hardened exudate.

For the purposes of this study, blisters and erosions were presumed to reflect acute or new lesions. Healed lesions and scabs, which may persist for a prolonged period, were not counted as acute lesions and were not used for the analysis.

Photosensitivity Testing

Photosensitivity was assessed by determining the *minimum erythematous dose* (MED) for each subject at a particular visit. This is the length of exposure to light which that person can tolerate before evidence of damage, such as erythema and oedema, develops. Photosensitivity is a phenomenon in which the skin is injured by amounts of light that are harmless to normal people. This may be extended to the concept of skin injury arising from wavelengths of light that are usually harmless. Sunburn is caused by wavelengths of 320 nm and less, and normal people will not develop erythema in response to wavelengths above this. In porphyria by contrast, the offending wavelengths lie at 400 nm and above.

Thus, to distinguish porphyria-induced photosensitivity from the normal reaction of sunburn to short-wavelength ultraviolet light, it is

necessary to prevent the latter from reaching the skin. Two methods are possible. One is the use of a monochromatic light source producing only light of the wavelengths of interest. This can be achieved using arc lamps in tandem with high quality filters. Such equipment is expensive. We attempted to elicit photosensitive reactions by the cheaper expedient of exposing the backs of porphyric patients to a bank of fluorescent tubes (TL 40W/03, Phillips) which emit with maximum intensity at 420 nm, but were unable to induce erythema with this. We therefore made use of a simpler system developed by Gordon (Gordon, 1963). This uses sunlight, the effects of sunburn being controlled for by filtering through glass.

The subjects were lain face down in direct sunlight at midday. Their backs were covered by sheeting except for a 10 cm^2 area on which was placed a template containing six circular openings. Each opening had a surface area of 1 cm^2. Two of these were covered by a microscope slide, the glass excluding short-wavelength ultraviolet light. These openings served to control for the effects of sunburn. The remaining four holes were sequentially uncovered at 15 minute intervals. In an hour's testing, the underlying skin was thus exposed to light for periods of 60, 45, 30 and 15 minutes respectively. The response was assessed by an assessment of immediate pigment darkening, immediate erythema and delayed erythema, for which purpose the subjects were reassessed 6 to 24 hours later. The degree of erythema of each area was noted. The time taken to onset of erythema was labelled as the MED. Figure 6.2 shows the result of such a test.

Subjective Assessment

After 12 weeks, the subjects were asked to answer to the following questions:

- Has charcoal therapy improved your skin?
- How keen would you be to continue with this treatment indefinitely?

The possible answers were *definitely not, probably not, unsure, maybe* and *definitely*. The questions were not answered confidentially, but the subjects were urged to answer truthfully and it was stressed that their answers one way or the other would not have any direct consequences for them or for the investigators.

I am grateful to Mr Peter Meissner for allowing me to adapt the description of the biochemical methodology from the manuscript of his PhD thesis, currently in preparation.

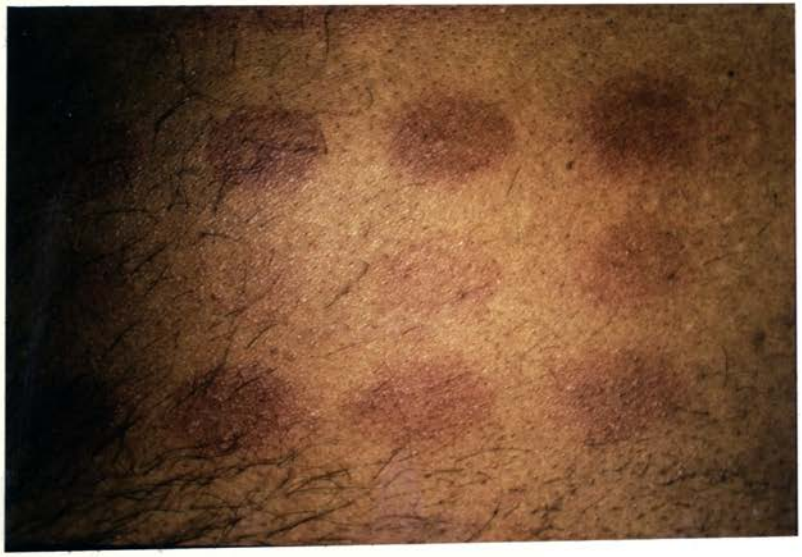

Fig 6.2. Determination of MED.

CHAPTER 7

RESULTS

All eight subjects completed the first 6 weeks of the trial. One (PU) defaulted thereafter. A second subject (SA) confessed to having used the charcoal erratically after six weeks, though she had been compliant till then. Her data at twelve weeks were discarded. Most other data points were available for each subject. Where an observation was unavailable at a particular time, that subject was excluded from the statistical analysis as the Friedman test requires complete pairs. The usual reason for an absent observation was a failure to produce the correct specimen on time, or where processing of a specimen was delayed sufficiently to make the result unreliable, or the inability of a subject to attend the hospital for photosensitivity testing on the relevant day.

ACTIVE SKIN LESIONS

One subject (GM) was excluded from the analysis of the skin lesions, as his lesions were not counted at the start owing to a misunderstanding. It was our impression however that his course was similar to that of the other subjects. Figure 7.1 illustrates the deterioration in the skin. Results are tabulated in Table 7.1, and are shown graphically in Figure 7.2. There is an almost linear increase in the number of active skin lesions from 0-6 weeks. Friedman analysis shows a highly significant difference between 0 and 6 weeks ($p = 0.008$)

Weeks	0	2	4	6	12
SA	1	3	7	4	
SB	3	4	7	5	3
CdeR	0	0	1	0	2
JG	0	6	6	21	7
LG	6	5	5	13	1
PU	13	23	23	34	
FvZ	2	4	10	16	22
MEAN	3.6	6.4	8.4	13.3	7

Table 7.1. Active skin lesions (erosions and blisters)

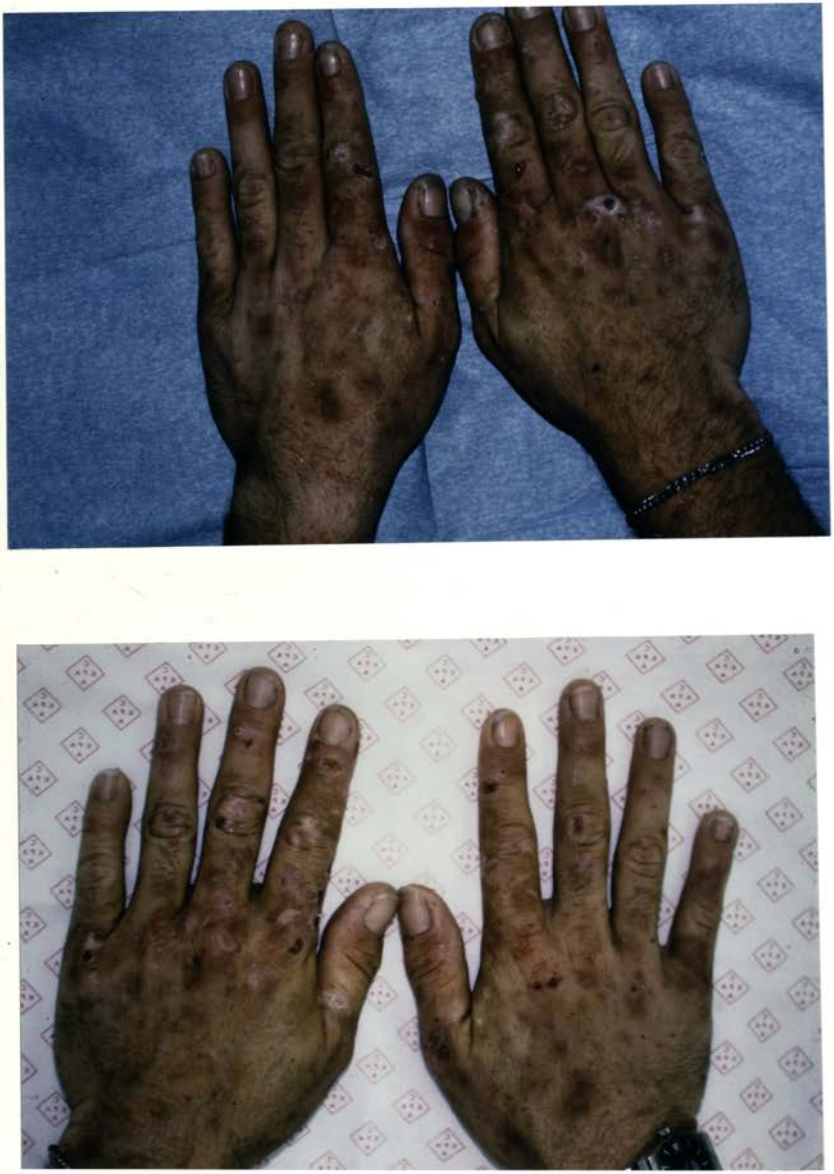

Fig 7.1. Hands of subject PU. (a) Before and (b) after six weeks of charcoal.

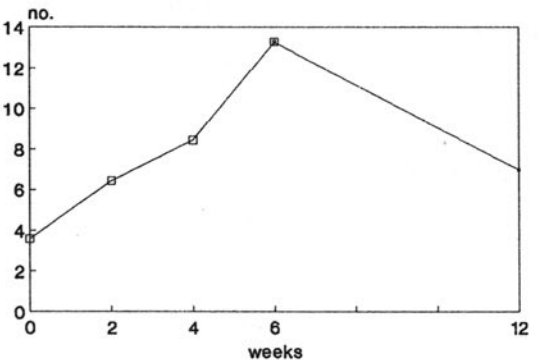

Fig 7.2. Number of active skin lesions (erosions and blisters).

PORPHYRIN CONCENTRATIONS

Plasma Porphyrins

Plasma porphyrin estimations are shown in Table 7.2 and Figure 7.3. Four of the eight subjects had undetectable plasma porphyrins at the start of the trial. However, by the fourth and sixth weeks, all showed porphyrins, usually in highly significant amounts. One subject (SB) was excluded from the analysis because a value for his plasma porphyrins at week 6 was missing. The Friedman test on the remaining 7 subjects gave a highly significant value ($p = 0.0015$). One subject (PU) showed extremely high values. Even if his values are excluded, the change is still significant ($p = 0.002$)The significance of this is discussed below. A decline in porphyrins was noted from 6 to 12 weeks.

Weeks	0	2	4	6	12
SA	0	28.7	37.8	41.7	
SB	5.9	0	14		31.1
CdeR	54.1	0	61	60.7	0
JG	10.1	0	25.5	29.4	30.9
LG	14.7	0	23.5	21.3	19.6
GM	0	22.5	43.8	41.6	34.1
PU	0	72.5	222.7	54.3	
FvZ	0	27.2	49.8	41.7	42.2
MEAN	10.6	18.9	59.8	41.5	26.3

Table 7.2. Plasma porphyrin concentrations (nmol/l).

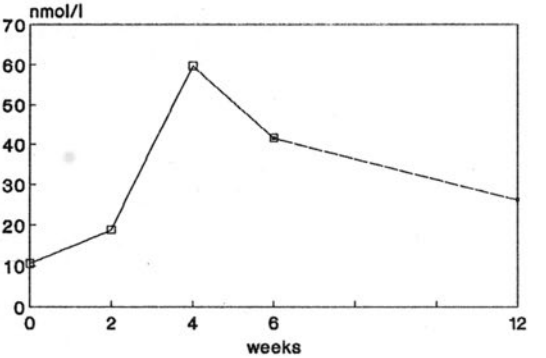

Fig 7.3. Plasma porphyrin concentrations.

Urine Porphyrins

Since urine porphyrins were measured on random urine samples, their concentrations are affected by the degree of urinary concentration. Therefore all have been corrected for this by expressing the result as nanomoles of porphyrin per 10 mmoles of creatinine excreted. Assuming that the creatinine excretion rates are approximately constant, this makes all values comparable. (The figure 10 is chosen as it represents the average daily creatinine excretion; hence porphyrin values per 10 mmol creatinine approximate to the daily porphyrin excretion.)

Urine samples were not available on two subjects at week 6 (SB & CdeR) and these two subjects were excluded from the analysis. The results are shown in Table 7.3 and plotted in Figure 7.4. All subjects other than SB showed a convincing rise in total urine porphyrin

Weeks	0	2	4	6	12
SA	88.4	148.2	412.4	359.9	
SB	235	26	48.4		177.1
CdeR	368.2	394	2163		550.1
JG	63.9	154.5	650.2	302.4	216.4
LG	75.1	33.3	70.5	203.9	311.1
GM	1015.5	1496.8	1744	1984.3	404.9
PU	679	2596.4	2232.8	2599.7	
FvZ	92.9	441.4	326.5	876.9	551.2
MEAN	327.3	661.3	956	1054.5	368.5

Table 7.3. Urine porphyrin concentrations (nmol/l).

excretion. By the Friedman test this result is significant($p = 0.014$). The total urinary porphyrin concentration remained elevated at 12 weeks, but appeared to be declining.

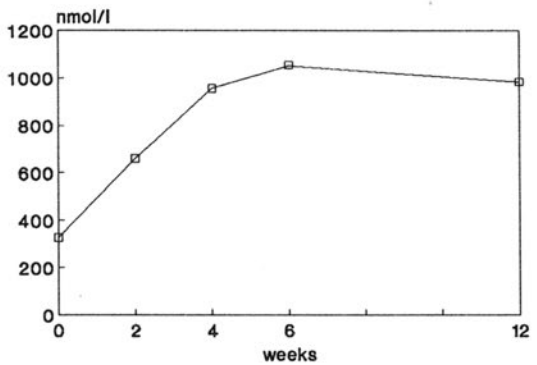

Fig 7.4. Urine porphyrin concentration.

Stool Porphyrins

Results of stool porphyrin testing are shown in Table 7.4 and are portrayed graphically in Figure 7.5. Friedman analysis of variance narrowly misses significance ($p = 0.054$). This was followed by a decline to approximately 70% of the starting amount at 12 weeks. Interpretation of this is difficult since the binding of porphyrin to charcoal is typically irreversible (Tishler 1988). This is discussed further in chapter 9.

Weeks	0	2	4	6	12
SA	1796.1	2045.0	1274.5	3646.9	
SB	773.0	1036.0	1429.2		1080.8
CdR	2505.6	1220.2	1152.7	1445.8	1265.3
JG	2716.0	1463.6	1298.7	1815.0	884.2
LG	1414.4	1721.7	1111.9	342.1	759.8
GM	959.3	1720.9	2624.0	4227.8	878.1
PU	814.9	1114.8	589.1	1477.6	
FvZ	1493.4	2083.7	1056.4	2414.9	1039.0
MEAN	1559.1	1550.7	1317.1	2195.7	984.5

Table 7.4. Stool porphyrin concentrations (nmol/g dry stool).

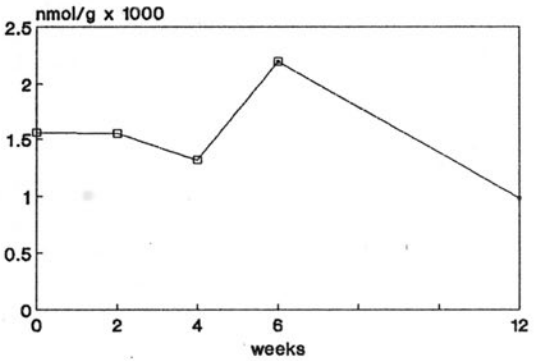

Fig 7.5. Stool porphyrin concentrations.

Urinary ALA

This again is corrected for the creatinine and results are expressed in μmoles per 10 mmol creatinine excreted. ALA concentrations are represented graphically in Figure 7.6a. Again an appreciable rise in ALA levels from week 0 to week 6 is seen, followed by a drop at week 12. On a multi-factorial analysis of variants, this increase to 6 weeks is significant at the 5% confidence interval. However by the Friedman test it does not achieve significance ($p = 0.145$).

Figure 7.6b shows the ALA trends of all the subjects from week 0 to week 6. It is apparent that two subjects, GM and PU, exhibited much higher levels at the start than did the other six subjects. The significance of this is discussed below. If it is assumed that they were subject to some other perturbing influence and their values excluded, the rise in ALA exhibited by the remaining, initially quiescent subjects (Figure 7.6c) is more striking, though again not significant by the Friedman test.

Urinary PBG

This too is expressed in μmoles of PBG per 10 mmol of creatinine excreted. Results are shown igraphically in Figure 7.7a. There is a trend upwards to 6 weeks, but the result does not achieve statistical significance by the Friedman test ($p = 0.16$). As is to be expected, the two subjects who showed elevated ALA levels at the start also have

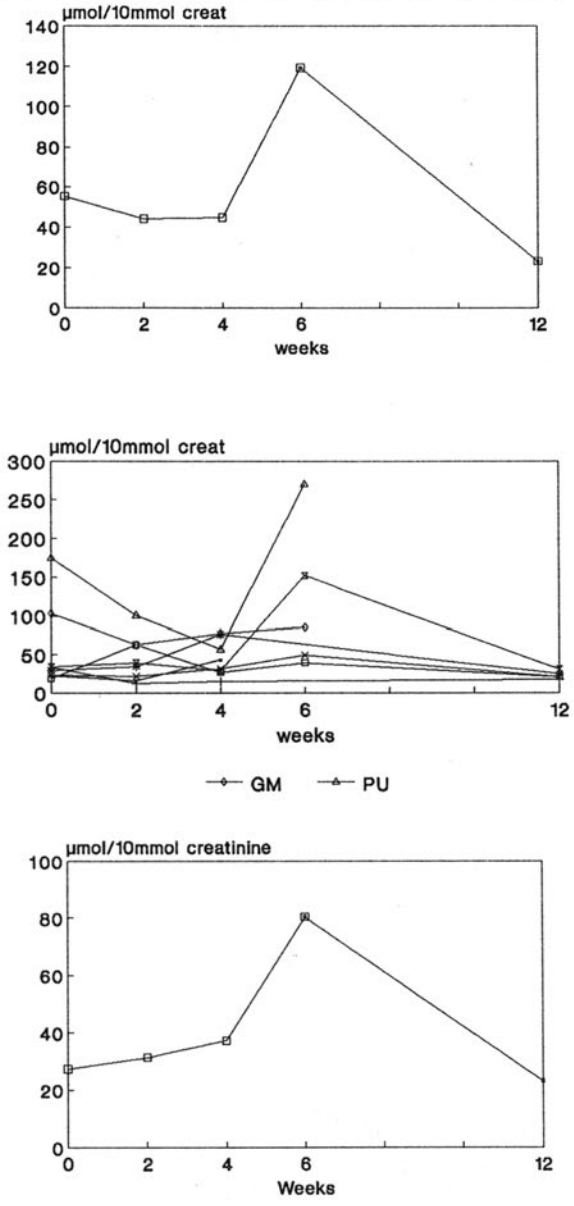

Fig. 7.6. Urinary ALA concentrations. (a) Mean of all subjects. (b) Results of individual determinations, showing the initially high values of subjects GM and PU. (c) Mean of subjects after exclusion of GM and PU.

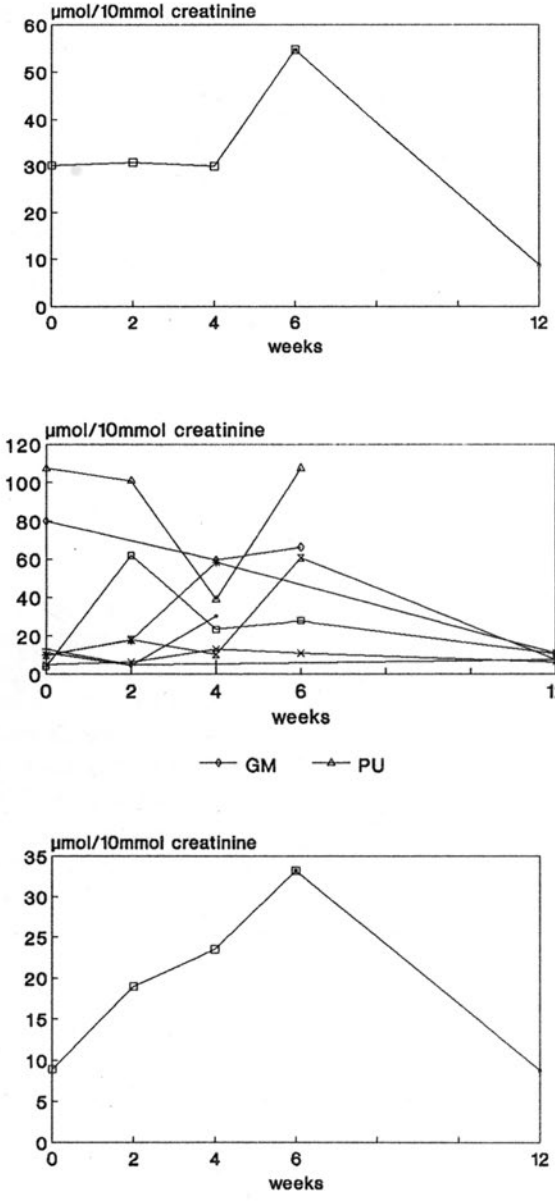

Fig. 7.7. Urinary PBG concentrations. (a) Mean of all subjects. (b) Results of individual determinations, showing the initially high values of subjects GM and PU. (c) Mean of subjects after exclusion of GM and PU.

raised PBG levels. If they are excluded from the analysis (Figure 7.7c) the rise in PBG values of the remaining subjects is more striking.

Plasma Porphyrin Profile

Figure 7.8 represents the proportions of the total porphyrin present in the plasma contributed by each species of porphyrin. There appears to be a change in the porphyrin pattern at two weeks. Whereas the hydrophobic porphyrin *protoporphyrin* constituted 40% of the total at week 0, this had risen to 76% at the end of two weeks, declining thereafter to 62% at the end of 4 weeks and 38% at the end of 6 weeks. The significance of this is uncertain. It so happens that the four subjects who had porphyrins present in the plasma at week 0 had had their plasma cleared of porphyrins at week 2 and vice versa. Statistical comparison is therefore meaningless.

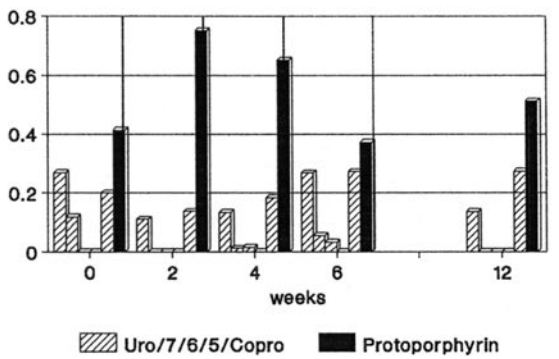

Fig 7.8. Plasma porphyrin profile. The solid bars represent the proportion of the total plasma porphyrin constituted by protoporphyrin; the hatched bars represent the proportions of uro-, hepta-, hexa-,carboxylic porphyrin and coproporphyrin.

PHOTOSENSITIVITY TESTING

Results are shown in Table 7.5 and Figure 7.9. No trend at all is observed ($p = 0.4$). Determinations of MED were not performed at 12 weeks.

Weeks	0	2	4	6
SA	30	30		
SB	45		30	
CdeR	45	15	30	60
JG	30	45	30	
LG	45	45	45	45
GM	0	15	30	30
PU	30	30	15	45
FvZ	30	45	30	30
MEAN	31	32	30	42

Table 7.5. Mean erythematous dose (MED).

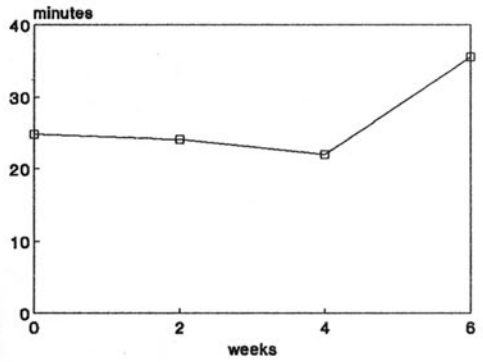

Fig 7.9. Means of MED for all subjects (minutes).

SUBJECTIVE IMPRESSIONS

The results are portrayed graphically in Figure 7.10. It appears that most subjects thought they had benefited from treatment with charcoal and were prepared to continue with it, despite the lack of objective improvement shown above.

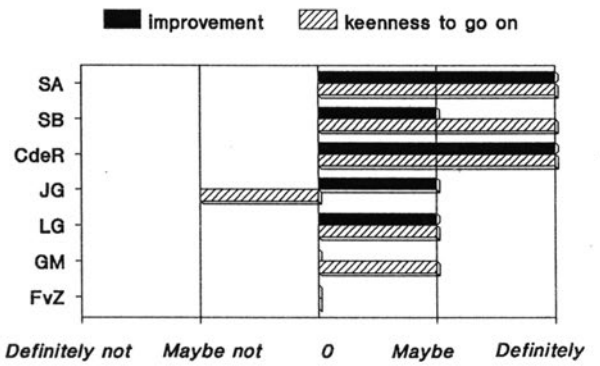

Fig 7.10. Subjects assessment of their own improvement and of their willingness to continue taking charcoal indefinitely.

CHAPTER 8

DISCUSSION

The mechanisms by which activated charcoal and other sorbents are thought to exert a beneficial effect on porphyrin levels in the porphyrias have been described in detail in the preceding chapters. In summary, it is felt that a substantial enterohepatic cycling of porphyrins takes place in the proximal small bowel, and that the administration of sorbents results in an interruption of this cycle. Porphyrins are strongly and irreversibly adsorbed to charcoal in the bowel lumen, and are then excreted, thus depleting the total body porphyrin load.

As yet little clinical experience with this form of therapy for porphyria has been described. Two patients with CEP treated with charcoal have been described in the preceding chapters. Pimstone *et al.* showed an improvement in their case, while the response of our patient is related in chapter 4. We concluded that there was indeed an impressive short-term response to charcoal manifested by a dramatic lowering of urine and, to a lesser extent, plasma porphyrin concentrations, but that this improvement has not been maintained but has been followed by a steady rise in porphyrins to levels approaching those initially seen. Despite this he maintained a good clinical improvement with no evidence of photosensitivity until recently. This is an important result. The reason for the recent breakaway from control is unclear.

The other condition in which there has been some experience with charcoal is EPP. This has been summarised in chapter 4. It would appear that sorbent therapy has been effective in some cases of EPP, and has successfully reduced porphyrin levels, photosensitivity and the protoporphyrin pigment load of the liver.

Thereafter a study to assess the place of activated charcoal in the management of VP was reported. We had felt that charcoal therapy might result in a prompt reduction in porphyrin levels with consequent improvement in skin photosensitivity in VP. The results have not followed our expectations, and are discussed below.

SKIN LESIONS

An unexpected finding has been a marked increase in the number of skin lesions shown by our patients over the first six weeks. The ensuing six weeks were followed by an improvement, but this had not reached starting levels by twelve weeks. Could such a result have occurred for reasons unconnected with the choice of therapy? In particular, does the deterioration of the skin merely reflect increasing exposure to the summer sun? This argument could have been directly refuted by having a placebo-controlled group. This was rejected for the reasons discussed in chapter 6. Similarly, a comparable control group even without placebo followed at the same time would have been instructive. We are at present following some of our subjects over a similar period without charcoal therapy. This may answer this question.

However there are certainly indirect reasons for believing that our observations do represent a direct effect of the therapy. Firstly, the rise in skin lesions paralleled the rise in plasma and urine porphyrin excretion closely. Though it is conceivable that the number of skin lesions may increase as the duration of exposure to the summer sun lengthens, there is no reason at all to believe that porphyrin levels should rise. Secondly, the mean number of skin lesions doubled over four weeks and more than tripled over six weeks. This appears to be far too accelerated a rise to be merely the effect of continuing sun exposure. Similarly, had this been the explanation, the figure at 12 weeks should have been higher still, yet the number of skin lesions was clearly declining.

The time of the trial was carefully chosen to extend for approximately equal periods on either side of the summer solstice, and ran from mid-November to mid-February. The sun exposure was therefore more or less constant throughout the 12 weeks of the trial. This time was also chosen as the weather is clear, with little rain or cloud cover.

In summary we believe that the short term administration of charcoal is indeed followed by a deterioration in the skin disease of VP.

PORPHYRIN LEVELS

Plasma and Urine Porphyrin Concentrations

The porphyrin concentration in both plasma and urine showed a significant early rise following the administration of charcoal. This was six-fold for plasma and three-fold for urine, and was statistically highly significant. No extraneous factors which might result in an increase in porphyrin production was noted in our subjects, and it appears likely that this increase in porphyrins occurs as a direct consequence of the administration of charcoal. There are two principal possibilities to be explored as the mechanism by which this occurs.

Firstly, it is possible that there was no increase in the production of porphyrins, but that there was indeed an increase in traffic of porphyrins from the liver (the major site of porphyrin synthesis in VP) through the plasma, with a consequent increase in filtration through the kidney, elevated urinary porphyrins, increased deposition in the skin and heightened photosensitivity. Such an effect is seen following treatment with chloroquine in PCT, where initiation of therapy has been followed by major increases in plasma porphyrin and urine porphyrin concentrations (Tsega 1987). This was not seen in either Pimstone's patient or our patient with CEP. It may be that this is not the explanation, or that the differing profile of porphyrin accumulation in the two conditions results in PCT and VP behaving differently from CEP. In addition, a mechanism would have to be described to explain why absorption of porphyrins to charcoal in the bowel should be followed by a release in supranormal quantities from the liver into the plasma. The improvement from 6 to 12 weeks might however lend support for such a mechanism. It would suggest an initial redistribution of porphyrins, with a major efflux (presumably from hepatic stores) into the plasma, which declines once the bulk of the stores have been mobilised. This would be in keeping with the findings of McCullough and colleagues in EPP (McCullough *et al.*, 1988). A more mundane explanation would be that the decline merely results from the decrease in dose after six weeks.

The second possibility is that the synthesis of porphyrins was increased *de novo*. This would give rise to serious concern. Any increase in the synthesis of porphyrins in the acute porphyrias places the subject at risk of a potentially fatal acute attack. This fear is so

great that drugs shown in animal or cell culture systems to be inducers of porphyrin synthesis are immediately labelled as potentially dangerous for use by porphyrics. We do not believe that charcoal could increase porphyrin synthesis itself *directly*. It is non-absorbable, and does not induce cytochrome formation in the liver. It would therefore have to induce porphyrin synthesis by an indirect mechanism.

Perhaps, by reducing porphyrin levels, one is disturbing an equilibrium between porphyrin overproduction and the deficient haem synthesis encountered in VP. By removing pathway intermediates, one may be decreasing the substrate available to the deficient enzyme (protoporphyrinogen oxidase), resulting in a slight fall in haem levels with a consequent increase in ALA synthetase activity and an increase in porphyrin synthesis. This could perhaps be directly demonstrated by measuring porphyrin synthesis by the incorporation of radio-labelled glycine into porphyrins. A simpler if less direct method is to examine the levels of the porphyrin precursors ALA and PBG.

Precursor Concentrations

ALA and PBG are the first intermediates of the haem synthetic pathway. During the acute attack, levels of ALA and PBG rise dramatically. Thus there is some correlation between concentrations of ALA and PBG and clinical evidence of the acute attack, though the relationship varies from time to time and subject to subject. Whether these precursors themselves are directly toxic, or merely serve as markers of whatever mechanism operates to cause the acute attack, is unknown. What is clear however is that rising levels of porphyrin precursors should always be viewed with concern in subjects with the acute porphyrias.

Figures 7.6a and 7.7a suggest very strongly that precursor levels rose during initial stages of charcoal therapy. This reached statistical significance by a multivariate analysis, though not by a paired-rank test of variance. The observation should not be discounted since the sample was small and a larger sample may have yielded a more significant result. There are also reasons why porphyrin levels may have risen proportionally more than the precursor levels, following the induction of porphyrin synthesis. Since the principal block in VP lies distally in the pathway, and is marked by an accumulation of the

later porphyrins (particularly protoporphyrin, coproporphyrin and pentacarboxylic porphyrin), there is a rapid passage of intermediates through the earlier steps of the pathway, with proportionately less accumulation of uroporphyrin, ALA and PBG. Work from our laboratory (Meissner, 1990 in press) suggests that only when porphyrin levels rise to a critical level, does the secondary control point at PBG deaminase become rate-limiting. Thus one might expect that precursor levels would rise very quickly once a certain threshold of porphyrin concentration had been reached. Thus, had the pathway in any of our subjects been stressed a little more and the porphyrin levels pushed higher, a more dramatic rise in precursor levels might conceivably have resulted. This is conjectural, but it is of interest that one subject, LG, reported at 4 weeks that he had felt "as though he were developing an acute attack", with some pain and ill-being.

There are hence grounds for believing that we are indeed seeing an increase in precursor production following treatment with charcoal, suggesting that the synthesis of porphyrins has been facilitated.

It will be seen from figures 7.6b and 7.7b that two subjects (GM and PU) showed markedly increased ALA and PBG levels at the start of the trial. Though these levels were three- to four-fold elevated, the subjects had no symptoms of the acute attack, and no precipitating cause for this was evident. Both these individuals have been repeatedly tested for several years, and have frequently shown such elevated levels. Some patients with VP appear to be metabolically more active than others and manifest such high precursor levels without showing the features of an acute attack. Figures 7.6c and 7.7c show that the increase in precursor concentrations for the remaining, more quiescent subjects is shown to be more dramatic when these two subjects with initially high levels are excluded.

However, the possibility that the initial high levels of the precursors in our subjects GM and PU were in themselves predisposing these subjects to an increase in porphyrin levels and skin lesions independently of the charcoal should be considered. Tables 7.1 and 7.2 show that PU indeed had considerably higher skin lesion counts and plasma porphyrin levels than his fellows. GM's plasma porphyrin levels fell in much the same range as the other subjects. Yet it is evident that nearly all the participants showed the same deterioration, and even exclusion of PU from the analysis did not remove statistical significance from the analysis of variance for the skin, or for plasma porphyrins.

We believe that this deterioration is a true phenomenon arising from the ingestion of charcoal by people with VP.

Between 6 and 12 weeks, both the number of skin lesions and the porphyrin levels decreased. There are several possible reasons for this. This may merely reflect the decrease in dose from 18 g to 12 g daily. It might also have reflected the end of a "wash-out" period, a large amount of porphyrin having been mobilised from the liver and tissues into the bowel, with a consequent return to an equilibrium at a lower level. A longer trial using a constant dose of charcoal is required to answer this.

Plasma Porphyrin Profile

In considering the mechanism whereby the administration of charcoal might alter the synthesis or redistribution of porphyrins, we examined the distribution of the different types of porphyrin at the various stages of the trial. The only obvious change was at 2 weeks, where the plasma showed an increased protoporphyrin fraction. Here protoporphyrin comprised 80% of the total porphyrin, as opposed to 40% two weeks earlier (Fig. 7.8). As previously stated, the four subjects who showed any plasma porphyrin at week 0 were not the same four who showed any at week 2. Statistical significance is meaningless, and whether any other significance attaches to the observation is unclear. We did explore the possibility that charcoal was preferentially removing a particular type of porphyrin, and perhaps disturbing porphyrin homeostasis in that fashion. As discussed in chapter 4, Tishler's work has suggested that different formulations of charcoal have differing affinities for the various porphyrins (Tishler and Winston, 1985). Tishler had found that Norit A charcoal, the agent used in this trial, bound protoporphyrin-lX approximately twice as avidly as uroporphyrin-l weight-for-weight. In a similar experiment we have found the same result. Figure 8.1 shows the relative binding of uroporphyrin and mesoporphyrin (which behaves like protoporphyrin) to Norit A charcoal as measured by the extinction of fluorescence in a solution of known porphyrin concentration. Thus, if the shift in plasma porphyrins at 2 weeks from the early to the later intermediates is real, it is not accounted for by a greater affinity of the water soluble porphyrins for charcoal than the hydrophobic later porphyrins.

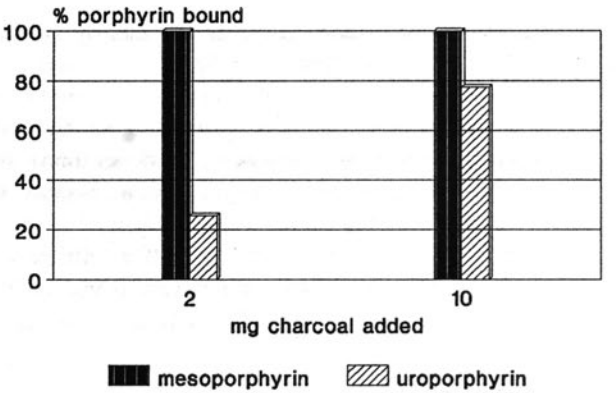

Fig 8.1. Relative affinity of charcoal for uroporphyrin and mesoporphyrin. Identical amounts of each porphyrin were used.

Stool Porphyrins

Since the binding of porphyrin to charcoal is irreversible (Tishler 1988), a unknown amount of stool porphyrin may be unavailable for extraction and measurement because it is complexed with charcoal. Hence the results depicted in Figure 7.5 should be interpreted with caution. It does however appear that the amount of porphyrin contained in the stool increased after charcoal. One may postulate that this reflects increased faecal excretion of porphyrin as a result of adsorption to charcoal within the bowel. The decline at 12 weeks might then represent a decline in porphyrin output should hepatic stores have been depleted to some extent, as hypothesised above. However it may of course merely reflect the lower adsorptive capacity of a smaller dose of charcoal. Alternatively the result may be spurious because of the erratic extraction of bound porphyrins. The four-week lag phase before stool porphyrins rose, for instance, is difficult to reconcile with the rôle postulated for it in immediately increasing the faecal excretion rate, and thus perturbing plasma and urine porphyrin concentrations, which were already altered by two weeks.

PHOTOSENSITIVITY

Despite the dramatic change in the response of the skin with a near-fourfold increase in the number of skin lesions, no trend in the

mean erythematous dose was seen. Gordon has clearly shown that the MED, assessed after exposure to filtered sunlight, is abnormal in subjects with VP. Yet it does not appear in our subjects to have served as an indicator of the severity of the skin disease. This may be because the acute and sub-acute responses (erythema and oedema) assessed by such testing do not correlate well with the vesicular-erosive response to photodamage, or it may relate to the technique of performing the test. Perhaps fifteen minute intervals do not offer sufficient scope to detect small changes, or it may be that slight variations in the intensity of sunlight from one test to the next are sufficient to confound the results.

SELF-ASSESSMENT

By objective criteria the activity of the porphyria in our subjects increased, resulting in higher porphyrin levels and more marked skin disease. Yet five of seven subjects felt that they had benefited from the treatment and five were at least somewhat keen to continue. Only one subject (JG) felt that he would rather not; this was because he had found the ingestion of such vast quantities of capsules irksome. Though possibly they were in some way benefited, perhaps by an increase in the length of sun-exposure they could tolerate before lesions developed, no objective indicator of improvement was obtained and this may merely represent the placebo effect.

CONCLUSIONS

Contrary to the experience in both CEP and EPP, activated charcoal in the dose employed in this study appears to cause an increase in the skin disease of VP and an increase in porphyrin production, at least in the short term. This may be accompanied by a rise in precursor production, suggesting an increase in the rate of porphyrin synthesis, which might potentially be harmful to people with VP.

We conclude therefore that the mode of action of charcoal in VP differs from its effects in other forms of porphyria, is complex and is not at this stage well understood. We would recommend that it should not be used on a casual basis, but only in the setting of a controlled trial designed to determine the effects over a longer period.

BIBLIOGRAPHY

Allen BR, Parker S, Thompson GG, Moore MR, Darby FJ, Hunter JAA. 1975. The effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria. *Br J Dermatol* **93** : 37-42.

Allison AC, Magnus IA, Young MR. 1966. Role of lysosomes and of cell membranes in photosensitization. *Nature* **209** : 874-878.

Athar M, Mukhtar H, Elmets CA, Zaim MT, Lloyd JR, Bickers DR. 1988. In situ evidence for the involvement of superoxide anions in cutaneous porphyrin photosensitization. *Biochem Biophys Res Comm* **151** : 1054-1059.

Avner DL, Berenson MM. 1982. Effect of choleretics on canalicular transport in the rat liver. *Am J Physiol* **242** : G347-353.

Baart De La Faille H, Beerens EGJ, Van Weelden H, et al. 1978. Complement components in blood serum and suction blister fluid in erythropoietic protoporphyria. *Br J Dermatol* **99** : 401-404.

Baart De La Faille-Kuyper EH, Cormane RH. 1968. The occurrence of certain serum factors in the dermo-epidermal junction and vessel walls of the skin in lupus erythematosus and other (skin) diseases. *Acta Dermatol Venereol* **48** : 578-588.

Batlle AM del C, Benson A, Rimington C. 1965. Purification and properties of coproporphyrinogenase. *Biochem J* **97** : 731-740.

Berglund L, Angelin A, Blomstrand R, Drummond G, Kappas A. 1988. Sn-protoporphyrin lowers serum bilirubin levels, decreases biliary bilirubin output, enhances biliary heme excretion and potently inhibits hepatic heme oxygenase activity in normal human subjects. *Hepatol* **8**: 625-631.

Brenner DA, Bloomer JR. 1980. The enzymatic defect in variegate porphyria. *N Engl J Med* **302** : 765-769.

Brodie MJ, Moore MR, Goldberg A. 1977. Enzyme abnormalities in the porphyrias. *Lancet* **2**: 699-701.

Brodie MJ, Thompson GG, Moore MR, Beattie AD, Goldberg A. 1977. Hereditary coproporphyria. *Q J Med* **46**: 229-241.

Caputo R, Berti E, Gasparini G, Monti M. 1983. The morphological events of blister formations in porphyria cutanea tarda. *Int J Dermatol* **22**: 467-472.

Corbett MF, Herxheimer A, Magnus IA, Ramsay CA, Kobza-Black A. 1977. The long term treatment with beta-carotene in erythropoietic protoporphyria: a controlled trial. *Br J Dermatol* **97**:655-662.

Cripps DJ, Curtis AC. 1962. Toxic effect of chloroquine on porphyria hepatica. *Arch Dermatol* **86**: 575-581.

Davidson DL, Bloomer JR, Klatskin G. 1973. Therapy of hepatic disease in erythropoietic protoporphyria. *Gastroenterol* **65**: 535.

Davis JR and Andelman SI. 1967. Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning. I. A modified method for the rapid determination of urinary delta-aminolevulinic acid using disposable ion-exchange chromatography columns. *Arch Environ Health* **15**: 53-59.

Day RS, Pimstone NR, Eales L. 1978a. The diagnostic value of blood plasma porphyrin methyl ester profiles produced by quantitative TLC. *Int J Biochem* **9**: 897-904.

Day RS, Salamanca RE de and Eales L. 1978b. Quantitation of red cell porphyrins by fluorescence scanning after thin layer chromatography. *Clin Chim Acta* **89**: 25-33.

Day RS. 1986. Variegate porphyria. *Seminars in Dermatology*. **5**: 138-154.

Dean GD, Barnes MD. 1959. Porphyria in Sweden and South Africa. *S Afr Med J* **33**: 246-253.

Disler P, Day R, Burman N, Blekkenhorst G, Eales L. 1982. Treatment of hemodialysis related porphyria cutanea tarda with plasma exchange. *Am J Med* **72**: 989-993.

Dogramaci I. 1962. Porphyria turcica (cutaneous porphyria) in Southeastern Turkey. General considerations. *Turk J Paed* **4**: 129-131.

Eales L. 1978. The effects of canthaxanthin - A beta-carotene analogue: On the photocutaneous manifestations of EHP, VP and SP. *S Afr Med J* **54**: 1050.

Elder GH, Smith SG, Herrero C, Mascaro JM, Lecha M, Muniesa AM, Czarnecki DB, Brenan J, Poulos V, Salmanca RE de. 1981. Hepato- erythropoietic porphyria: A new uroporphyrinogen decarboxylase defect or homozygous porphyria cutanea tarda? *Lancet* **1**: 916-919.

Epstein JH, Tuffanelli DL, Epstein WL. 1973. Cutaneous changes in the porphyrias. A microscopic study. *Arch Dermatol* **107** : 689-698.

Foote CS, Chang YC, Denny RW. 1970a. Chemistry of singlet-excited oxygen: IX. Carotenoid quenching parallels biological protection. *J Am Chem Soc* **92**: 5216-5218.

Foote CS, Chang YC, Denny RW. 1970b. Chemistry of singlet oxygen: XI. Cis-trans isomerization of carotenoids by singlet-excited oxygen and a probable quenching mechanism. *J Am Chem Soc* **92**: 5218-5219.

Foote CS, Denny RW. 1968. Chemistry of singlet oxygen: VII. Quenching by beta-carotene. *J Am Chem Soc* **90**: 6233-6235.

Galbraith RA, Drummond GS, Kappas A. 1985. Sn-protoporphyrin suppresses chemically induced experimental hepatic porphyria. *J Clin Invest* **76**: 2436-2439.

Galbraith RA, Kappas A. 1990. Tin-porphyrins decrease urinary excretion of haem pathway intermediates in patients with acute intermittent porphyria. In: Moore M (Ed.) . A century of porphyria. *Molec Aspects Med* **11**: 61.

Gandhi SN, Pimstone NR. 1983. Charcoal far superior to cholestyramine in blocking enterohepatic circulation of porphyrins: A major therapeutic tool in porphyria. *Gastroenterol* **84**: 1372.

Goldberg A, Ashenbrucker M, Cartwright GE, Wintrobe MM. 1956. Studies on the biosynthesis of heme *in vitro* by avian erythrocytes. *Blood* **11**: 821-833.

Gordon W. 1963. The detection of photosensitivity in porphyria using filtered sunlight. *S Afr J Lab Clin Med* **9**: 245-247.

Granick S. 1958. Porphyrin synthesis in erythrocytes. I. Formation of 5-aminolaevulinic acid in erythrocytes. *J Biol Chem* **232** : 1101-1117.

Grossman ME, Bickers DR, Poh-Fitzpatrick MB, De Leo VA and Harber LC. 1979. Porphyria cutanea tarda. Clinical features and laboratory findings in forty patients. *Am J Med* **67** : 277-286

Grossman ME, Poh-Fitzpatrick MB. 1980. Porphyria cutanea tarda. Diagnosis and management. In: Calan J (Ed.). Medical Clinics of North America. Philadelphia: WB Saunders. **64** : 807-827.

Haberman HF, Rosenberg F, Menon IA. 1975. Porphyria cutanea tarda: A comparison of cases precipitated by alcohol and oestrogens. *Can Med Assoc J* **113** : 653-655.

Haeger-Aronson B, Krook G, Abdulla M. 1979. Oral carotenoids for photohypersensitivity in patients with erythrohepatic protoporphyria, polymorphous light eruptions and lupus erythematosus discoides. *Int J Dermatol* **18** : 73-82.

Hift RJ, Meissner PN. 1989. Acute intermittent porphyria - an underdiagnosed cause of abdominal pain. *S Afr Med J* **76** : 44-45.

Hift RJ, Meissner PN and Meissner DM. Porphyria. A guide for people with porphyria and their doctors. 1989. Cape Town: Medical Research Council and University of Cape Town Liver Research Centre.

Ibrahim GW, Watson CJ. 1968. Enterohepatic circulation and conversion of protoporphyrin to bile pigment in man. *Proc Soc Exp Biol Med* **127** : 890-895.

Ippen, H. 1961. Allgemeinsymptome der späten Haut-porphyrie (porphyria cutanea tarda) als Hinweise für den Behandlung. *Deutsche Medizinische Wochenschrift* **86** : 127.

Jackson AH, Games DE, Couch P, Jackson JR, Belcher RB, Smith SG. 1974. Conversion of coproporphyrinogen III to protoporphyrin IX. *Enzyme*. **17** : 81-87.

Jackson AH, Sancovich HA, Ferramola AM, Evans N, Games DE, Matlin SH, Elder GH, Smith SG. 1976. Macrocyclic intermediates in the

biosynthesis of porphyrins. *Phil Trans Roy Soc London (Biology)* **B273** : 191-206.

Kappas A, Sassa S, Anderson KE. 1983. The porphyrias. In: Stanbury JB, Wyngaarden JB, Frederickson DS, et al (Eds). The metabolic bases of inherited disease. 5th Ed. New York: McGraw-Hill. 1301-1384.

Kniffen JC. 1970. Protoporphyrin removal in intrahepatic porphyrastasis. *Gastroenterol* **58** : 1027.

Kohn K, Kessel D. 1979. On the mode of cytotoxic action of photo-activated porphyrins. *Biochem Biopharmacol* **28** : 2465-2470.

Krook G, Haeger-Aronson B. 1982. Beta-carotene in the treatment of erythropoietic protoporphyria. *Acta Dermatol Venereol* (Stockholm) Suppl. **100** : 125-129.

Lamola AA, Blumberg WE. 1976. The effectiveness of beta-carotene and phytoene as systemic sunscreens. Programme of 4th Annual Meeting of American Society for Photobiology. 109.

Land EJ. 1984. Current topics - Porphyrin phototherapy of human cancer. *Int J Rad Biol* **46** : 219-223.

Lemberg R, Legge JW. 1949. Haematin compounds and bile pigments. New York: Interscience.

Lim HW, Gigli I. 1981a. Role of complement in porphyrin-induced photosensitivity. *J Invest Dermatol* **76** : 4-9.

Lim HW, Perez HD, Goldstein IM, Gigli I. 1981b. Complement-derived chemotactic activity is generated in human serum containing uroporphyrin after irradiation with 405 nm light. *J Clin Invest* **67** : 1072-1077.

Lim HW, Poh-Fitzpatrick MB, Gigli I. 1984. Activation of the complement system in patients with porphyrias after irradiation in vivo. *J Clin Invest* **74** : 1961-1965.

Lischner HW. 1966. Cholestyramine and porphyrin-binding. *Lancet* **2** : 1079-1080.

Longas MO, Poh-Fitzpatrick MB. 1982. A tightly bound protein-porphyrin complex isolated from the plasma of a patient with variegate porphyria. *Clin Chim Acta* **118** : 219-228.

Malina L, Miller V, Magnus IA. 1978. Skin porphyrin assay in porphyria. *Clin Chem Acta* **83** : 55-59.

Marver HS, Tschudy DP, Perlroth MG, Collins A. 1966. Delta-aminolaevulinic acid synthetase, 1. Studies in liver homogenates. *J Biol Chem* **241** : 2803-2809.

Mathews MM, Sistrom WR. 1959. Function of carotenoid pigments in non-photosynthetic bacteria. *Nature* **184** : 1892-1893.

Mathews MM. 1964. Effect of beta-carotene against lethal photosensitization by hematoporphyrin. *Nature* **203** : 1092.

Mathews-Roth MM, Pathak MA, Fitzpatrick TB, Harber LC, Kass EH. 1970. Beta-carotene as a photoprotective agent in erythropoietic protoporphyria. *N Engl J Med* **282** : 1231-1235.

Mathews-Roth MM, Pathak MA, Fitzpatrick TB, Harber LC, Kass EH. 1977. Beta-carotene therapy for erythropoietic protoporphyria and other photosensitivity diseases. *Arch Dermatol* **113** : 1229-1232.

McCullough AJ, Barron D, Mullen KE, Petrelli M, Park MC, Mukhtar H, Bickers DR. 1988. Fecal protoporphyrin excretion in erythropoietic protoporphyria: Effect of cholestyramine and bile acid feeding. *Gastroenterol* **94** : 177-181.

McKay R, Druyan R, Getz GS, Rabinowitz M. 1969. Intramitochondrial localization of 5-aminolaevulinate synthase and ferrochelatase in the rat liver. *Biochem J* **114** : 455-461.

Meissner PN, Meissner DM, Sturrock ED, Davidson B, Kirsch RE. 1987. Porphyria - The UCT experience. *S Afr Med J* 72: 755-761.

Meissner PN, Sturrock ED, Moore MR, Disler PB, Maeder DL. 1985. Protoporphyrinogen oxidase, porphobilinogen deaminase and uroporphyrinogen decarboxylase in variegate porphyria. *Biochem Soc Trans* **13** : 203-204.

Moore MR, McColl KEL, Rimington C, Goldberg A. Disorders of porphyrin metabolism. 1987. New York: Plenum Publishing Corporation.

Mukerji SK, Pimstone NR, Gandhi SN, Tan, KT. 1985. Biochemical diagnosis and monitoring therapeutic modulation of disease activity in an unusual case of congenital erythropoietic porphyria. *Clin Chem* **31**: 1946-1951.

Murphy GM, Magnus IA, Barrett DF, Elder GH, Hawk JLM and Smith SG. 1986. *J Roy Soc Med* **79**: 361-363.

Mustajoki P, Desnick RJ. 1985. Genetic heterogeneity in acute intermittant porphyria: Characterisation and frequency of porphobilinogen deaminase mutations in Finland. *Br Med J* **291** : 505-509.

Mustajoki P, Tenhunen R, Niemi KM, Nordmann Y, Kääriäinen and Norio R. 1987. Homozygous variegate porphyria. A severe skin disease of infancy. *Clin Gen* **32**: 300-305.

Mustajoki P. 1972. Vitamin E in porphyria. *J Am Med Assoc* **221** : 714-715.

Nair PP, Mezey E, Murty HS, Quartner J and Mendeloff AI. 1971. Vitamin E and porphyrin metabolism in man. *Arch Intern Med* **128** : 411-415.

Pigatto PD, Polenghi MM, Altomare GF, Giacchetti A, Cirillo R, Finzi AF. 1986. Complement cleavage products in the phototoxic reaction of porphyria cutanea tarda. *Br J Dermatol* **114** : 567-573.

Pimstone NR, Gandhi N, Mukerji S. 1987. Therapeutic efficacy of oral charcoal in congenital erythropoietic porphyria. *N Engl J Med* **316** : 390-393.

Pimstone NR, Gandhi S, Mukerji S. 1985. Therapeutic efficacy of chronic charcoal therapy in photomutilating porphyria. *Gastroenterol* **88**: 1684.

Pimstone NR, Gandhi SN, Mukerji SK, Blekkenhorst G, Day R. 1982. Entero-hepatic circulation of porphyrins in man: does this exist? *Hepatol* **2**: 718

Pimstone NR, Mukerji S, Chow H, Goldstein L. 1986. Oral charcoal therapy for protoporphyric cirrhosis. *Hepatol* **6:** 1176.

Poh-Fitzpatrick MB, Sklar JA, Goldsman C, Lefkowitch JH. 1983. Protoporphyrin hepatopathy effects of cholic acid ingestion in murine griseofulvin-induced protoporphyria. *J Clin Invest* **72** : 1449-1458.

Poh-Fitzpatrick MB. 1985. Porphyrin sensitised cutaneous photosensitivity - Pathogenesis and treatment. In: Disler PB, Moore MR (Eds). Porphyria. *Clinics in dermatology*. Philadelphia: Lippincott, 41-82.

Rimington C, With TK. 1973. Porphyrin studies in congenital erythropoietic porphyria. Studies on a sample of faeces and a sample of urine from a patient with CEP and comparison with a bovine case. *Dan Med Bull* **20** : 5-12.

Roberts, HJ. 1981. Perspective on vitamin E as therapy. *JAMA* **246** : 129-131.

Rotenberg M, Margalit R. 1987. Porphyrin-membrane interactions: binding or partition? *Biochim Biophys Acta*. **905** : 173-180.

Runge W, Watson CJ. 1962. Experimental production of skin lesions in human cutaneous porphyria. *Proc Soc Exp Biol Med* **109** : 809-811.

Rousseau A. 1983. Canthaxanthine deposits in the eye. J Am Acad *Dermatol* **8**: 123-124.

Sandberg S, Romslo I. 1980. Porphyrin-sensitized photodynamic damage of isolated rat liver mitochondria. *Biochem Biophys Acta* **593** : 187-195.

Sandberg S, Romslo I. 1981. Porphyrin-induced photodamage at the cellular and the subcellular level as related to the solubility of the porphyrin. *Clin Chim Acta* **109** : 193-201.

Scholnick PL, Epstein JH, Marver HS. 1973. The molecular basis of chloroquine in porphyria cutanea tarda. *J Invest Dermatol* **61** : 226-232.

Slater TF, Riley PA. 1966. Photosensitisation and lysosomal damage. *Nature*. **209** : 151-154.

Stathers GM. 1966. Porphyrin-binding effect of cholestyramine. *Lancet* **2**: 780-783.

Summers B and Summers RS. 1988. Sunscreen preparations and their use in the prevention of sun-induced skin damage. *S Afr Pharm J* **55**: 369-376.

Summers RS, Summers B. 1989. UVA filters in sunscreen preparations. *Lancet* **2**: 1387.

Timonen P, Mustajoki P, Tenhunen R, Tokola O and Lauharanta J. 1990. Prolonged haem arginate administration in variegate porphyria. In: Moore M (Ed.) . A century of porphyria. *Molec Aspects Med* **11**: 61-62.

Tishler PV, Gordon BJ, O'Connor JA. 1982. The adsorption of porphyrins and porphyrin precursors by sorbents: A potential therapy for the porphyrias. *Meth Find Exp Clin Pharm* **4**: 125-131.

Tishler PV, Gordon BJ. 1983. Sorbent therapy of the porphyrias. II. Experimental plasma for hemoperfusion with a commercial charcoal cartridge. *Meth Find Exp Clin Pharm* **5**: 185-191.

Tishler PV, Winston SH. 1984. Sorbent therapy of the porphyrias. III. Comparative efficacy of experimental plasma perfusion with several commercial hemoperfusion cartridges. *Meth Find Exp Clin Pharmacol* **6**: 389-393.

Tishler PV, Winston SH. 1985. Sorbent therapy of the porphyrias. IV. Adsorption of porphyrins by sorbents in vitro. *Meth Find Exp Clin Pharmacol* **7**: 485-491.

Tishler PV. 1988. Oral charcoal therapy of congenital erythropoietic porphyria. *Hepatol* 183-184.

Torinuki W, Miura T, Tagami H. 1984. *Acta Dermatol Venereol* (Stockholm) **64**: 367-372.

Tsega E. 1987. Long-term effect of high-dose short-course chloroquine therapy on porphyria cutanea tarda. *Q J Med* **65**: 953-957.

Varigos G, Schiltz JR, Bickers DR. 1982. Uroporphyrin I stimulation of collagen biosynthesis in human skin fibroblasts. *J Clin Invest* **69**: 129-135.

Volden G, Thune P. 1979. Photosensitivity and cutaneous acid hydrolases. *Ann Clin Res* **11** : 129-132.

Wakulchik SD, Shiltz JR, Bickers DR. 1980. Photolysis of protoporphyrin treated human fibroblasts *in vitro*: Studies on the mechanism. *J Lab Clin Med* **96** : 158-167.

Zalar GL, Poh-Fitzpatrick MB, Crohn DC, et al. 1977. Induction of drug photosensitization in man following parenteral exposure to hematoporphyrin. *Arch Dermatol* **113** : 1392-1397.