

## REFLECTIONS

## Fifty years of porphyria at the University of Cape Town

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The porphyrias are a group of disorders resulting from defective haem biosynthesis. One form, variegate porphyria, is common in South Africa as a result of a founder effect. Over the past 50 years, the Faculty of Health Sciences of the University of Cape Town has built and maintained an international reputation for excellence in the field of porphyria. The porphyria group is respected for its

research and for its accumulated experience in the management of these disorders. Equally important has been the comprehensive and holistic care offered to patients with porphyria, and to their families.

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The porphyrias are a group of disorders (Table 1) associated with inherited or acquired defects in the enzymes of the haem biosynthetic pathway (Fig. 1). South Africa has the highest prevalence of porphyria in the world, the consequence of a founder effect which has led to one form of porphyria, variegate porphyria (VP), becoming uniquely and locally common.<sup>1,2</sup>

Geoffrey Dean, a physician working in Port Elizabeth in the 1950s, identified a large number of families of Dutch descent in the Eastern Cape with a disorder, apparently inherited, which manifested as photodermatitis and an alarming tendency to develop a severe and sometimes fatal crisis, characterised by abdominal pain, red urine and paralysis, particularly in response to medication such as barbiturates. Working with Hubert Barnes of the South African Institute for Medical Research in Johannesburg, he identified this illness as a disorder of porphyrin metabolism, and later showed that it represented a previously unrecognised form of porphyria, which he named VP. Dean subsequently traced the origins of VP to the marriage of a Dutch couple in Cape Town in 1688.<sup>3</sup>

The Faculty of Health Sciences of the University of Cape Town (UCT) celebrates its centenary this year and has been active in the study of porphyria for more than half of that century. Indeed, one precocious fifth-year medical student, Lennox Eales, described an early case of porphyria in a paper published in the UCT medical student journal, *I'nyanga*, in 1939.<sup>1</sup>

### The early years

After graduation, Eales joined the Department of Medicine, subsequently rising to occupy the Chair of Clinical Medicine. He developed a passion for the study of porphyria and published extensively on the subject. In 1962 he established a porphyria research group, supported by the Council for Scientific and

Industrial Research, through his Renal Metabolic Research Group, which received international recognition. This research unit was subsequently supported by the South African Medical Research Council (MRC) through the UCT-MRC Porphyria Research Group housed within the Department of Medicine.

Eales, together with a small international group of pioneering scientists and clinicians, laid the foundations of porphyrinology, a composite field which spans the study of both the science of haem biosynthesis and the clinical disorder. This group collaborated during an exciting period in which the link between the porphyrias and disturbances in mammalian haem synthesis was first established and characterised. This link underpins all subsequent work on the biochemical and metabolic basis of these disorders and their clinical consequences.

A subset of the porphyrias, including VP, carries the risk of an acute attack, a metabolic crisis characterised by severe neurovisceral dysfunction that may result in paralysis and death. Many drugs that undergo hepatic metabolism have the capacity to induce haem synthesis and, in the patient with VP, the resulting increase in porphyrins and their precursors may precipitate the acute attack, with potentially fatal consequences. This property is termed *porphyrinogenicity*. Eales and his group were active in investigating porphyrinogenicity, developing laboratory-based experimental systems in which the porphyrinogenicity of specific drugs might be examined, and documenting clinical outcomes in patients exposed to specific drugs. They produced the earliest drug safety recommendations, which found international acceptance, and the drug lists that followed contributed greatly to the improved survival of porphyria patients.<sup>4</sup>

Some of the earliest international conferences on porphyrins and porphyrias, in 1963 and 1971, were hosted at UCT, and 83 publications were produced in the years leading up to Eales's retirement in 1982. A number of clinicians and scientists were trained in Eales's laboratory and subsequently received international recognition for their contributions to the study of porphyria and the clinical care of patients.<sup>5</sup>

### The past 30 years

#### The MRC-UCT Liver Research Centre

Under the leadership of Ralph Kirsch, who succeeded Eales in the Chair of Clinical Medicine, the Porphyria Research Unit was incorporated into the UCT-MRC Liver Research Centre. Eales's legacy

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<sup>5</sup>Drs/Professors Sweeney, Dowdle, Pimstone, Disler, Blekkenhorst and Day in the clinical and scientific arena, and Marietjie Levey and Doreen Meissner in the field of laboratory diagnosis and patient support.

**Table 1. Summary of the haem biosynthetic enzymes and associated porphyrias**

Enzyme	Disorder	Inheritance (predominant mode)	Clinical features
ALA synthase	X-linked protoporphyria (XLPP) (additionally, sideroblastic anaemia)	X-linked	Immediate photosensitivity; liver disease secondary to protoporphyrin accumulation
ALA dehydratase	ALA dehydratase porphyria (ALADP)	Recessive	Acute attacks
HMB synthase	Acute intermittent porphyria (AIP)	Dominant	Acute attacks
Uroporphyrinogen-III synthase	Congenital erythropoietic porphyria (CEP)	Recessive	Severe vesiculo-erosive skin disease
Uroporphyrinogen decarboxylase	Porphyria cutanea tarda (PCT)	Acquired, but may be associated with a dominantly inherited mutation in approximately 30% of cases	Vesiculo-erosive skin disease; usually coexists with iron overload and a number of precipitating factors, particularly alcohol exposure, liver disease and renal failure
Coproporphyrinogen oxidase	Hereditary coproporphyria (HCP)	Dominant	Acute attacks and vesiculo-erosive skin disease
Protoporphyrinogen oxidase	Variete porphyria (VP)	Dominant	Acute attacks and vesiculo-erosive skin disease
Ferrochelatase	Erythropoietic protoporphyria (EPP)	Recessive, but more common than most recessive disorders since the disease-associated mutation is frequently inherited with a low-expression polymorphism which is common in populations of European extraction	Immediate photosensitivity

ALA =  $\delta$ -aminolaevulinic acid; HMB = hydroxymethylbilane.

continued, combining research into the basic and clinical aspects of porphyrinology with excellence in patient care. International conferences were held in Cape Town in 1996 and 2005, and a further 71 porphyria-related articles have emanated from the Centre, bringing the total to 152.

That VP is related causally to deficient activity in the penultimate enzyme of the haem biosynthetic pathway, protoporphyrinogen oxidase (PPOX), was first shown by Brenner and Bloomer in 1980.<sup>5</sup> One of us (PNM) subsequently confirmed that VP is unequivocally associated with a 50% deficiency in PPOX activity and that this deficiency results from a loss of activity of the enzyme through mutated structural encoding in the gene, rather than a defect in its synthesis or regulation. Important follow-on work provided the answer to an otherwise puzzling question: why VP and hereditary coproporphyria (HCP) are associated with acute elevations in  $\delta$ -aminolaevulinic acid (ALA) and porphobilinogen (PBG) and thus the acute attack, whereas porphyria cutanea tarda (PCT) is not, even though the enzymatic defect in PCT occurs more proximally in the haem biosynthetic pathway (Fig. 1). We showed that this probably results from the competitive inhibition of hydroxymethylbilane synthase by coproporphyrinogen and protoporphyrinogen, substrates which accumulate in VP and HCP.<sup>6</sup> Uroporphyrinogen, which accumulates in PCT, appears not to share this ability. Attempts to purify human and porcine PPOX in our laboratory proved unsuccessful; related biochemical work on the kinetics of this enzyme did however provide insights into the functioning of this enzyme.

Much of our work has been performed in collaboration with Professors Harry Dailey of the University of Georgia, USA, George

Elder of the University of Cardiff, UK, and Jean-Charles Deybach of the Centre Français des Porphyries in Paris, France. Collaborative work with Dailey's group resulted in the identification, cloning and publication of a mammalian *PPOX* gene sequence, and subsequently in the identification of the first mutation associated with VP: the R59W mutation.<sup>2</sup> We demonstrated that 95% of South African families with VP carry this mutation, although a small number do not. A critical role for DNA testing in the diagnosis of VP in South Africa was thus established, since inheritance of the R59W mutant gene, easily confirmed by polymerase chain reaction and restriction digest, has a high sensitivity for VP in South Africa. This has greatly increased the speed and accuracy of diagnosis,<sup>1,7</sup> and has subsequently allowed us to determine the sensitivity and specificity of more established biochemical tests for porphyria, the penetrance of VP,<sup>8</sup> and to implement evidence-based, efficient algorithms for the laboratory diagnosis of porphyria.<sup>7</sup> The ability to accurately determine the presence of VP has also allowed us to define the spectrum of clinical presentation of VP more precisely. Contrary to popular belief, VP is quite stereotypic in its presentation, and much of its reputation for presenting in vague and protean guises – and as psychiatric illness in particular – is erroneous, arising from the clinical description of patients who appear not to have had VP<sup>1,8,9</sup> (including, incidentally, the historical figure of King George III<sup>10,11</sup>).

### Structural biochemistry

The ability to isolate and manipulate the *PPOX* gene has allowed us to perform useful work designed to elucidate the structure and function of both normal and mutant PPOX, including work on the

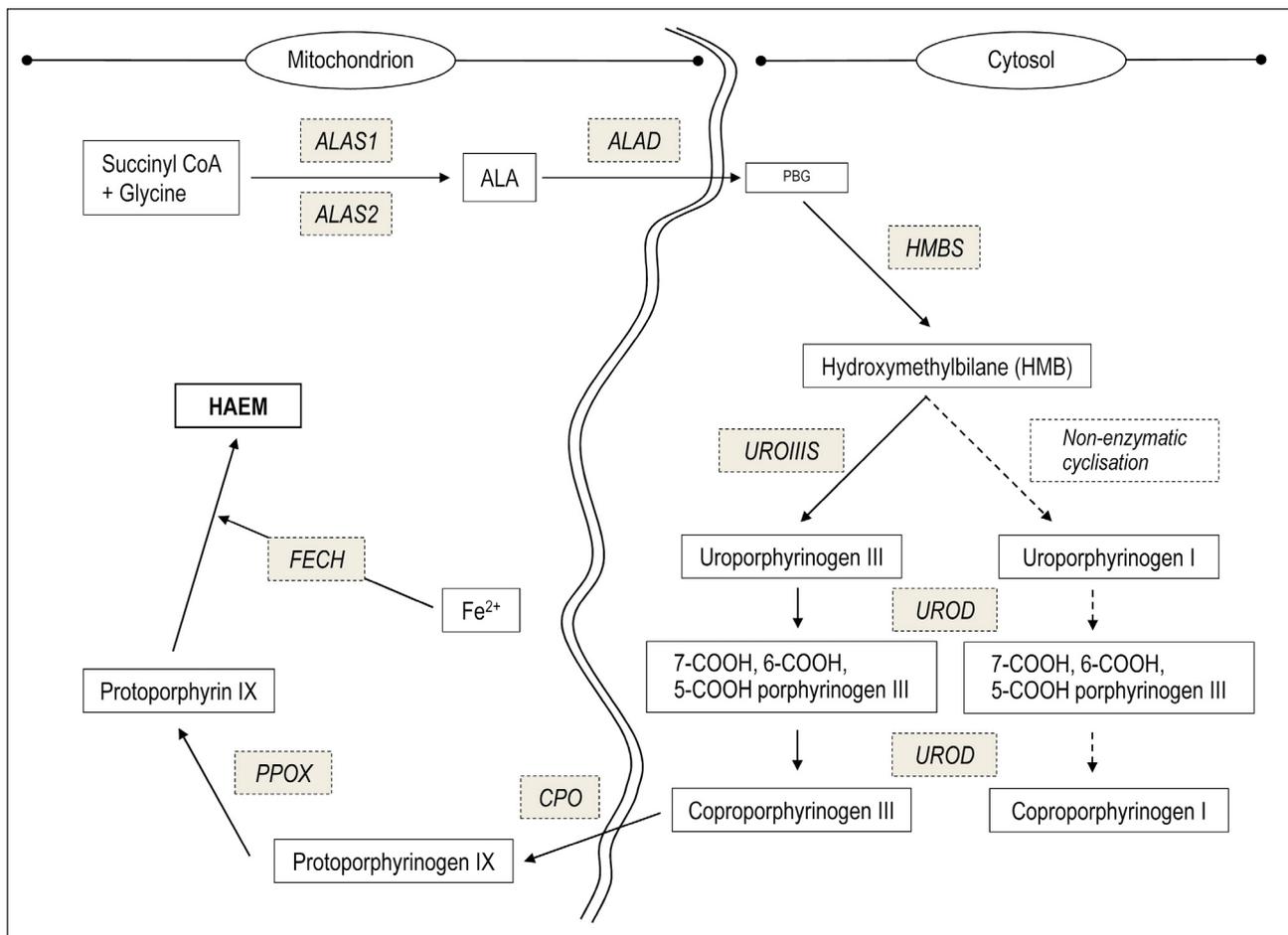


Fig. 1. Enzymes and metabolites of the haem biosynthetic pathway, located within the mitochondrion and the cytosol of a typical mammalian cell. ALAS =  $\delta$ -aminolaevulinic acid synthase; ALAD =  $\delta$ -aminolaevulinic acid dehydratase; HMBS = hydroxymethylbilane synthase; UROIII S = uroporphyrinogen-III synthase; UROD = uroporphyrinogen decarboxylase; COP = coproporphyrinogen oxidase; PPOX = protoporphyrinogen oxidase; FECH = ferrochelatase; ALA =  $\delta$ -aminolaevulinic acid; PBG = porphobilinogen.

crystalline structure of PPOX, its mitochondrial targeting, expression studies of South African VP-associated mutations, expression of the R59W mutation in a knock-in mouse model and the tissue-specific expression of PPOX.

### Drug porphyrogenicity

Avoidance of exposure to porphyrogenic medication is crucial to the control and prevention of the acute attack. Since Eales's early career the group has continued to solicit, collate and collect clinical data on the outcomes of exposure to drugs in patients, more recently as part of an international collaboration centred on the European Porphyria Network (EPNET).<sup>†</sup> Our study of the safety of the injectable anaesthetic propofol in VP patients remains the only controlled trial of the safety of a drug in porphyria;<sup>12</sup> it accompanies other reports on drug safety from our group, which are incorporated into a database established by the Norwegian Porphyria Association (NAPOS) in Bergen, Norway. This is international in scope, fully evidence-based, and premised on the application of predictive algorithms as well as the conventional descriptive (and thus retrospective) approach to

the determination of porphyrogenicity. That it is web-based makes it accessible and of immediate benefit to the porphyria community. One of us (RJH) collaborates with the Scandinavian authors of this database in a state-of-the-art review of the science of drug porphyrogenicity.<sup>13</sup>

### Patient management: diagnosis, treatment and education

Incorporation of our porphyria group within the Liver Research Centre resulted in close links between ourselves as porphyrinologists and the clinicians of the Liver Clinic at Groote Schuur Hospital, one of us (RJH) possessing expertise in both hepatology and porphyriology. This facilitated the development of dedicated patient-centred services for consultation and management, both as in- and outpatients, while collaboration between the clinic and the laboratory promoted excellence in clinical management as well as relevant research. Young clinicians rotating through the clinic as medical registrars have become competent in porphyria management.

The work of the group is multidimensional, effective management of porphyria, demanding diagnosis, treatment and education of patient, family and healthcare professional(s). The diagnostic laboratory serves as the central reference laboratory for southern Africa, has a high throughput and diagnoses more cases of porphyria than any other laboratory in the world. In 2010, 718 individuals were investigated for porphyria, requiring some 2 007 individual analyses, since multiple biochemical and molecular tests on each individual are required to characterise the disorder fully. Diagnosis of 65 cases

<sup>†</sup>EPNET is a network of expert porphyria centres providing specialist testing and clinical advice on all porphyrias. EPNET has expanded from 20 to 32 members in 21 countries (including 4 associate members from outside of Europe, one of which is the UCT group).

of VP, 36 cases of PCT, 6 of acute intermittent porphyria (AIP), 3 of erythropoietic protoporphyria (EPP), 5 of X-linked protoporphyria and 1 of congenital erythropoietic porphyria was confirmed.

An effective modality for the treatment of skin disease in porphyria has proven elusive. We conducted a controlled trial of the use of oral activated charcoal in VP, with the hypothesis that the activated charcoal would interrupt the enterohepatic cycling of porphyrins, thus leading to a decrease in plasma porphyrins and decreased traffic of photoactive porphyrins into the skin. Paradoxically, we found that treatment caused deterioration. Nevertheless, by promoting careful and prudent skin care, most of our patients with VP enjoy a good quality of life.

Many consultations are for patients with PCT, a form of porphyria which typically is acquired, rather than inherited, and frequently precipitated by liver disease, that is associated with hepatic iron overload in particular. Close links between the porphyria group and the liver clinic promote expert and efficient investigation and management of the liver disease (including liver biopsy); this, together with a phlebotomy service established within the porphyria clinic, enables comprehensive patient care.

The most dramatic and dangerous manifestation of porphyria is the acute attack. We have one of the world's largest experiences in management of this, and protocols we have developed are incorporated into international best practice.<sup>9,14</sup>

Introduction of intravenous haem-containing compounds for the management of the acute attack was pivotal in improving outcomes.<sup>15</sup> Augmented hepatocellular haem concentrations repress ALA synthase by a negative feedback mechanism, thus dramatically reducing porphyrin synthesis, halting the acute attack and preventing the onset of serious complications. Haem arginate (Normosang, Orphan Europe), is available to South African patients through our arranging for its importation and registration. Similarly South African patients with EPP have access to beta-carotene and canthaxanthine, agents which, in high doses, may ameliorate the severe photosensitivity associated with the condition.

We have been vigorous in advocacy for the porphyria community and have been active in educating patients, families, health professionals and the public about porphyria and its management. Booklets and information sheets on the subject have been published and incorporated into a range of media resources and a website has been established with full information on the care of porphyria and the safe prescription of drugs. A Porphyria Information Centre, offering a walk-in/phone-in service for patients and health professionals, has been set up.

### Other forms of porphyria: experience and research

Not restricted to VP, the clinic regularly diagnoses, manages and studies patients with less common forms of porphyria (and many of our publications reflect this). PCT is not uncommon in South Africa, and useful research was conducted by Eales, Sweeney, Blekkenhorst and Pimstone,<sup>16</sup> and more recently by the present authors. Sweeney was one of the first to methodically assess the use of chloroquine in the treatment of PCT. Subsequently we have investigated the association of PCT with liver disease and with viral hepatitis and iron overload in particular. More recently we have been studying the association between PCT and HIV.

Although AIP is considerably less prevalent in South Africans than VP, it accounts for the majority of the acute attacks treated.<sup>9</sup> We have participated in molecular studies of AIP in South Africa, delineating the spectrum of mutations in the *hydroxymethylbilane synthase* gene which underlie this disorder.<sup>17</sup> In EPP, we conducted a national study in affected South African families and identified

18 families carrying 4 mutations, 12 of whom carried the same mutation and were shown by haplotype analysis to have a common ancestor.<sup>18</sup> One family proved particularly interesting in that the clinical features were atypical and we were unable to identify any mutation in the *ferrochelatase* gene as would be expected. This South African family, and several others in Europe, were shown to have a novel disease now termed X-linked protoporphyria.<sup>19</sup> This arises pathogenetically from a gain-of-function mutation in the erythroid form of ALA synthase, which results in a greatly increased flux of porphyrins through the haem biosynthetic pathway; ferrochelatase becomes rate-limiting (probably at the level of iron availability) and free and zinc protoporphyrin accumulates, hence the resemblance to classic EPP, where the accumulation of protoporphyrin is caused by an inherited deficiency of ferrochelatase. There appears to be a high incidence of protoporphyria-associated liver disease in these families, a feature noted by Eales in the late 1970s.

A further interest has been the study of those rare patients with unusual – and usually severe – presentations arising from homozygosity or compound heterozygosity for porphyria-associated mutations. We have studied patients with congenital erythropoietic porphyria and hepato-erythropoietic porphyria, arising from double defects in the enzymes uroporphyrinogen cosynthase and uroporphyrinogen decarboxylase respectively, as well as homozygous VP. Given the high prevalence of VP in South Africa, homozygotes would be expected to be commonly encountered. In practice this has not been the case, leading us to hypothesise, along with others, that the homozygous condition is lethal. Our work on the expression of VP-associated PPOX mutations in cell culture, as well as studies in a knock-in mouse model which expresses the R59W mutation,<sup>20</sup> have confirmed this. The R59W mutation results in a defective enzyme with effectively zero kinetic activity, and homozygosity for this condition is lethal since no haem synthesis is possible. Indeed, in the mouse model, homozygous offspring do not survive beyond the ninth day after conception. We have identified 4 young patients with apparent homozygous VP, and shown that, rather than being homozygotes, they are compound heterozygotes, carrying the R59W mutation on one allele, but an unrelated mutation on the other.<sup>2</sup> In expression systems, additional mutations were shown to encode PPOX proteins with some residual enzymatic activity; it is this activity that keeps the patient alive.

### Conclusion

In the past half-century the porphyria group has made significant contributions to the research of porphyrins, porphyrias and haem biosynthesis, matched by holistic and comprehensive care for patients.

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