SPECIAL ARTICLE

Variegate porphyria in South Africa, 1688 - 1996 — new developments in an old disease

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Variegate porphyria, an autosomal dominant inherited trait resulting in decreased activity of protoporphyrinogen oxidase, the penultimate haem biosynthetic enzyme, is characterised clinically by photosensitive skin disease and a propensity to acute neurovisceral crises. The disease has an exceptionally high frequency in South Africa, owing to a founder effect. The specific mutation in the protoporphyrinogen oxidase gene sequence which represents this founder gene has been identified. Genetic diagnosis is therefore now possible in families in whom the gene defect is known. However, the exact nature and degree of activity of the porphyria can only be determined by detailed quantitative biochemical analysis of excreted porphyrins. The relative contributions of the acute attack and the skin disease to the total disease burden of patients with variegate porphyria is not static, and in South Africa there have been significant changes over the past 25 years, with fewer patients presenting with acute attacks, leaving a greater proportion to present with skin disease or to remain asymptomatic with the diagnosis being made in the laboratory. The most common precipitating cause of the acute attack of VP is administration of porphyrinogenic drugs. Specific suppression of haem synthesis with intravenous haem arginate is the most useful treatment of a moderate or severe acute attack. Although cutaneous lesions are limited to the sun-exposed areas, management of the skin disease of VP remains inadequate.

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This review, which deals with recent advances in the diagnosis and management of variegate porphyria (VP), was stimulated by the recent description of a gene defect which provides a molecular basis for this condition.[1] This finding provides scientific support for the putative 308-year-old founder effect in the South African form of the disease.

History

Variegate porphyria is possibly the commonest monogenic inherited disease seen in South Africa. The condition was brought to South Africa in 1688 when Ariaantje Adriaanse, an orphan from Rotterdam, was sent to the Cape to marry one of the free burghers, Gerrit Jansz van Deventer.[2] For the next 200 years the relatively benign nature of the disease, coupled with the tendency to produce large families, led to this autosomal dominant trait becoming common among white and coloured South Africans. The last quarter of the 19th century witnessed the discovery of modern pharmaceutical agents and changed the nature of VP. Soon after the introduction of the hypnotic, sulphonal, in the late 19th century, Stokvis[3] in Holland reported the first acute porphyric attack. An elderly woman who had taken sulphonal developed an ultimately fatal crisis characterised by abdominal pain, paralysis and the passage of red urine. This was followed by many other reports and it was soon realised that the hypnotics, including barbiturates (introduced in 1903), could precipitate acute neurovisceral attacks in porphyric subjects.

The first published description of a South African patient with acute porphyria appeared in the University of Cape Town medical students' journal, *I'nyanga*, in October 1939. The authors were medical students Lennox Eales and Jack Chait.[4] In 1951, HD Barnes of the South African Institute for Medical Research was the first to describe a series of cases of 'porphyrinuria' in South Africa.[5] Barnes, together with Geoffrey Dean, a Port Elizabeth physician who had recognised that the disease could manifest with skin lesions, acute attacks or both, coined the terms 'porphyria variegata' and 'South African genetic porphyria'.[6-8] Dean's meticulous genealogical studies subsequently identified the founding couple from whom South African VP patients are descended.[2] Eales' clinical and biochemical studies of 250 cases of porphyria seen in Cape Town documented unambiguously that during the acute attack, patients with VP had increased concentrations of all urinary and faecal porphyrins as well as their precursors, delta-aminolaevulinic acid (ALA) and porphobilinogen (PBG).[9,10] However, in quiescent VP, urinary porphyrins were normal but faecal proto- and coproporphyrin levels remained high. This led Eales to promote the term 'protocoproporphyria' as an alternative to VP. The seminal work of Dean, Barnes and Eales led to the Cape Town group's hosting the first international conference on the porphyrias in 1963.[11] The past decade has seen several important advances in the understanding, diagnosis and management of VP. These are highlighted in this review.

Haem metabolism

Porphyrins are tetrapyrrole macrocycles characterised by their ability to fluoresce a bright red when exposed to ultraviolet light. The iron-porphyrin complex, haem, plays a central role in biological oxidation reactions and is a vital constituent of haemoglobin, myoglobin, all cytochromes including the cytochrome P450 family, and a variety of other proteins central to biological hydroxylation reactions.[12]

The porphyrias are a group of metabolic disorders that result from defects of specific enzymes of the haem synthetic pathway.[13,14] The diagnosis and management of the porphyrias are therefore critically dependent on an understanding of the biochemistry and enzymology of this pathway.[15]

Haem synthesis

All cells are able to produce haem. However, in man most haem is synthesised in the liver and bone marrow. Haem synthesis (Fig. 1) is initiated in the mitochondrion by the condensation of succinyl-CoA and glycine to form ALA. In the cytosol two ALA molecules condense to form the monopyrrole PBG. Four molecules of PBG are assembled and then rearranged to form the first tetrapyrrolic porphyrinogen intermediate, uroporphyrinogen-III, which has 8 carboxylic (COOH) groups. Stepwise decarboxylation of uroporphyrinogen-III through 7-, 6-, and 5-COOH-porphyrinogen intermediates to coproporphyrinogen-III follows. Coproporphyrinogen-III then enters the mitochondrion where it undergoes oxidated decarboxylation to yield protoporphyrinogen-IX which is in turn oxidised to protoporphyrin-IX. This is followed by the insertion of ferrous iron-yielding haem.

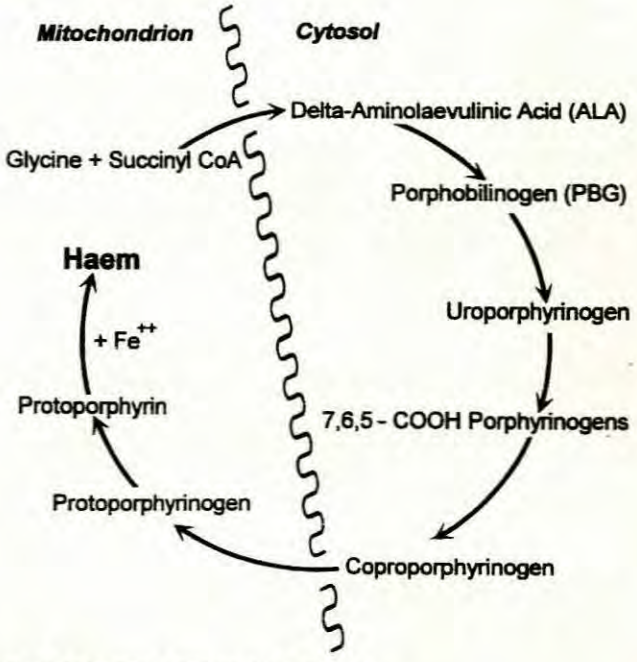

Fig. 1. The haem biosynthetic pathway.

Each of these steps is catalysed by an enzyme (Fig. 2). Deficiency of a particular enzyme (in most instances inherited) may lead to a specific pattern of porphyrin accumulation proximal to the defective enzyme and a characteristic clinical syndrome.[13] The inherited defect in VP involves the enzyme protoporphyrinogen oxidase (PPO).[16-18]

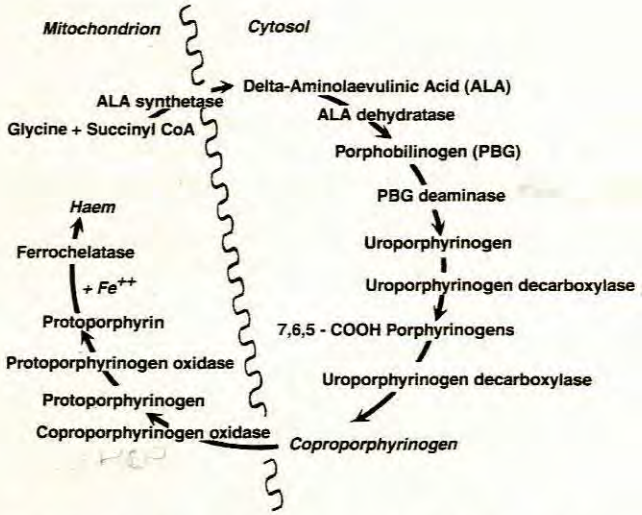

Fig. 2. The eight enzymes which catalyse haem biosynthesis. Deficiency of a particular enzyme (in most instances inherited) may lead to a specific pattern of porphyrin accumulation proximal to the defective enzyme and a characteristic clinical syndrome. The inherited defect in VP involves the penultimate haem synthetic enzyme PPO.

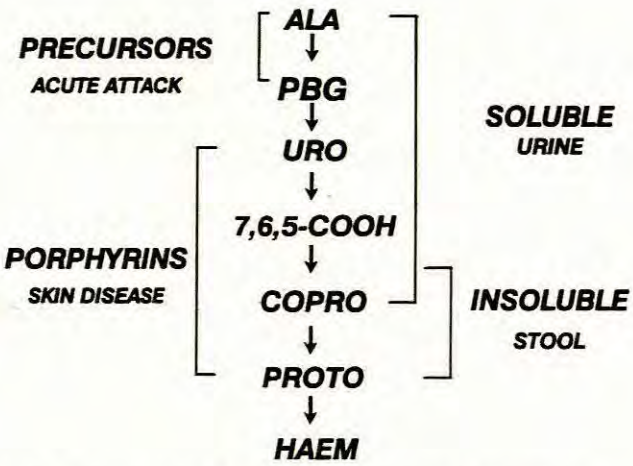

Fig. 3. Accumulation of early haem precursors, ALA and PBG, is associated with acute attacks and late intermediates, the porphyrins, with skin lesions. The early precursors are water-soluble while the late intermediates are hydrophobic.

Haem synthesis is normally an extremely efficient, tightly controlled process. Less than 2.5% of the ALA entering the pathway is lost and the amount of haem produced closely matches the needs of the body.[19] This implies that the pathway is subject to some form of feedback control. Indeed, there is much evidence to suggest that in the liver haem itself modulates its own rate of production, principally at the level of ALA synthase, the rate-determining enzyme of the pathway. In the marrow haem synthesis is also limited by the availability of iron.[15]

The enzyme defects in porphyria are always partial. Indeed, clinically significant haem deficiency is not a feature of these disorders. Any defect in haem synthesis which results in a relative shrinking of the haem pool, will derepress ALA synthase in order to achieve a new steady state of haem synthesis. This increase in ALA synthase, while partially restoring the haem pool, also stresses the synthetic chain to a point where there is an additional accumulation of substrate proximal to the partial block.[20] Many drugs further increase ALA synthase activity, either directly or indirectly, by increasing the demand for haem.[21,22] These drugs may induce an acute attack in VP and in patients with other acute porphyrias.[23]

Biochemistry of the acute attack

Generally, accumulation of early haem precursors, ALA and PBG, are associated with acute attacks and late intermediates, the porphyrins, with skin lesions (Fig. 3).

VP, which is characterised by both photocutaneous lesions and acute neurovisceral attacks,[24] is due to a defect in the penultimate haem synthetic enzyme, PPO.[16-18] This results in accumulation of the distal haem synthetic intermediates protoporphyrinogen IX and coproporphyrinogen III and thus the skin lesions. During acute attacks porphyrinogen concentrations become even higher and, in addition, levels of ALA and PBG, proximal intermediates of the haem synthetic pathway, increase.[25,26] Until recently the mechanism by which ALA and PBG increased in VP was not understood. Acute attacks also occur in two other forms of porphyria, intermittent acute porphyria (IAP) and hereditary coproporphyria (HCP). Although the site of the inherited enzyme defect varies, PBG deaminase in IAP,[27] coproporphyrinogen oxidase in HCP[28] and PPO in VP, neurovisceral attacks in all three acute porphyrias are associated with elevated concentrations of ALA and PBG.[20] The elevated concentrations of ALA and PBG are easily explained in IAP. However, this finding does not tally with the known enzyme abnormality in VP and HCP, where the defective enzymes are situated well downstream of these precursors. That ALA and PBG are not simply increased as a result of sequential damming up of products proximal to a block in the distal portion of the haem synthetic pathway is shown by the observation that increased concentrations of ALA and PBG are not found in porphyria cutanea tarda, where the defective enzyme, uroporphyrinogen decarboxylase, is situated just distal to these precursors (Fig. 2).[29,30] Here, despite the accumulation of large amounts of uroporphyrinogen III, ALA and PBG levels do not increase. Similarly it is unlikely that subjects with VP and HCP have a second inherited defect at the level of PBG deaminase since the haem biosynthetic enzymes are encoded on different chromosomes.[26] We therefore formulated the hypothesis that the obligate haem precursors that accumulate in VP (and in HCP) inhibit PBG deaminase activity and result in rate-limiting effects.[31] The hypothesis was tested by performing a series of kinetic analyses of PBG deaminase using Epstein-Barr virus-transformed lymphocytes from normal and VP subjects. Various porphyrins and porphyrinogens were assessed for their ability to alter the kinetic behaviour of both lymphoblast PBG deaminase and the purified enzyme. Sonicates of lymphoblasts derived from subjects with VP exhibited a significant decrease in PBG deaminase activity and displayed abnormal, sigmoidal, PBG deaminase substrate

velocity curves. These changes could be neutralised by the removal of porphyrinogens from the PBG deaminase-containing fraction of VP lymphoblast sonicates and reproduced by the addition of protoporphyrinogen IX and coproporphyrinogen III (but not uroporphyrinogen III or the porphyrins) to sonicates of lymphoblasts from normal subjects. In addition, kinetic changes similar to those found in VP lymphoblasts were found when protoporphyrinogen was added to purified human PBG deaminase, suggesting that protoporphyrinogen has a direct effect on PBG deaminase activity. The finding that protoporphyrinogen, which accumulates in patients with variegate porphyria, is able to inhibit PBG-deaminase and thus cause elevation of ALA and PBG explains the accumulation of these early precursors during acute attacks of VP.[31]

Clinical features of VP

The MRC/UCT Liver Research Centre has the world's largest experience of variegate porphyria (Fig. 4). Over the past decade we have diagnosed approximately 100 new cases of VP per year.

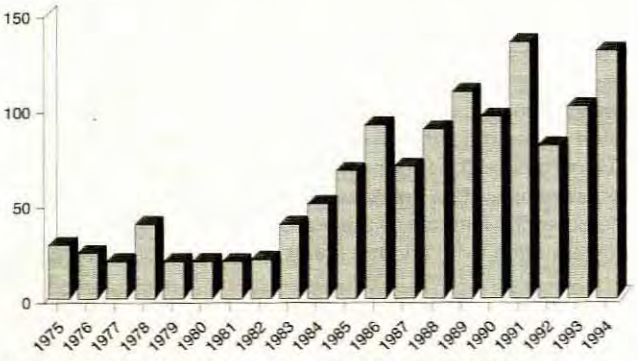

Fig. 4. The number of new cases of variegate porphyria diagnosed annually at the MRC/UCT Liver Research Centre between 1975 and 1994.

VP patients may present at any age. The majority present in the 3rd decade of life but some have presented as late as the 8th decade. Over the past 15 years, the proportions of patients presenting with clinically overt disease, and particularly with an acute attack, have decreased (Fig. 5). This may be attributed to two factors: more intensive family screening, with a higher rate of detection of latent carriers, and better education of doctors and patients leading to avoidance of inducers of the acute attack and a decline in the rate of this serious complication. Currently 40% of patients with variegate porphyria present with skin lesions alone and only 9% with acute attacks. A further 10% initially presented with skin lesions but have subsequently experienced at least one acute attack. Of the total sample, 41% were detected as asymptomatic carriers on the basis of abnormal biochemical findings. In contrast, prior to 1980, 17% presented with acute attacks and 21% with skin lesions and an acute attack.[24] Only 10% were asymptomatic. The proportion of subjects with VP detected at an asymptomatic stage is likely to increase further with the advent of DNA-based testing, which will detect carriers who are both clinically and biochemically quiescent.

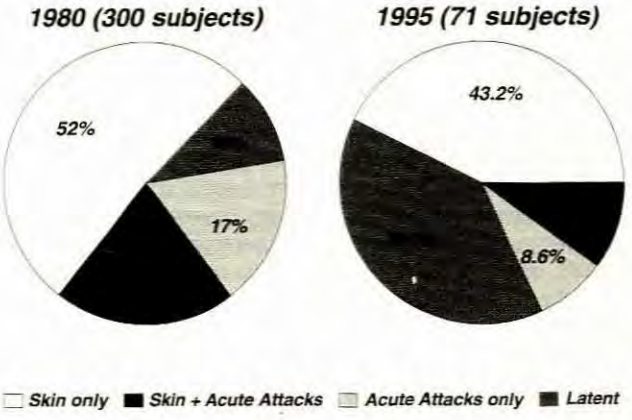

Fig. 5. Contrast between the clinical manifestations of 300 subjects with variegate porphyria diagnosed before 1980 and those of 71 subjects diagnosed in 1995. The increase in the number of asymptomatic subjects and the decrease in persons presenting with an acute attack is thought to be due to aggressive education and counselling programmes.

This change in mode of presentation has been accompanied by a decline in the total number of acute attacks of VP seen at Groote Schuur Hospital, from 56 in the period 1957 - 1966[32] to 24 in the past 10 years (Fig. 6). During the same period the number of acute attacks of IAP has increased considerably, reflecting increasing awareness of this less common form of porphyria in South Africa, with an increased rate of detection and referral.

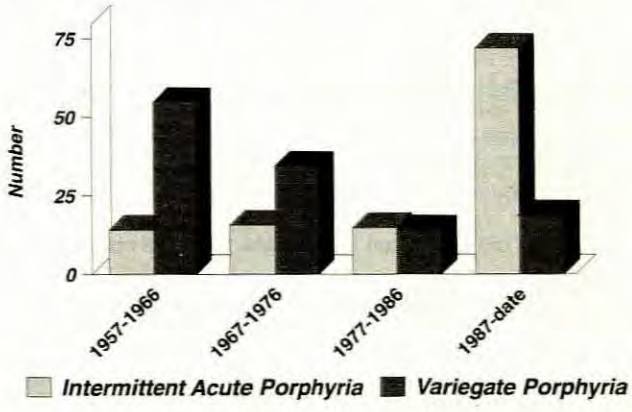

Fig. 6. Decline in the total number of acute attacks of variegate porphyria seen at Groote Schuur Hospital, in the period 1957 - 1996. During the same period the number of acute attacks of intermittent acute porphyria increased, possibly reflecting increasing awareness of this less common form of porphyria in South Africa.

The acute attack

During the 10-year period 1986 - 1996, 20 patients with 107 acute attacks required admission to Groote Schuur Hospital. Of these attacks, 83 were treated in 11 patients with IAP and 24 in 9 patients with VP. The median number of attacks per patient was 1 in VP and 5 in IAP. In addition, a single patient with IAP has more or less continually suffered acute attacks for the past 5 years, with approximately 120 consecutive attacks requiring specific therapy. In view of the extreme

course demonstrated by this patient, she is not included in these figures. The data which follow reflect our recent experience with the 24 consecutive acute attacks in VP.

The median age at the time of the attack was 30 years (range 24 - 36). Five patients were male and 4 female. This is in contrast to IAP, where 80% of the patients experiencing acute attacks were female. Recurrent acute attacks are a rare phenomenon in VP, and it is very unusual for patients to experience more than one or two attacks in a lifetime. Indeed, only 2 of our VP patients were observed to have more than 2 attacks; both were young men who used cannabis frequently. We believe that this may have accounted for their acute attacks. It is noteworthy that repeated attacks are not seen in young women with VP; this is in contrast to IAP, where repeated attacks associated with the menstrual cycle are a feature.

Precipitants

Factors which have been described as potential inducers of the acute attack include drugs, infections, the menstrual cycle and fasting.[33,34] The most common precipitating cause of the acute porphyric attack in VP is the administration of porphyrinogenic agents. No attacks associated with the menstrual cycle were observed in VP, though attacks, in 1 female patient, occurred in early pregnancy. Drugs were thought to be responsible for 16 of the 24 acute attacks. The agents included erythromycin, Rifater, ethanol and cannabis. In no patient with VP could an association with infection be shown. In 6 attacks, no cause was evident.

Clinical features

The most common presenting feature is abdominal pain (Figs 7 and 8). This is severe, felt diffusely through the abdomen and often in the thighs, lower back and buttocks,

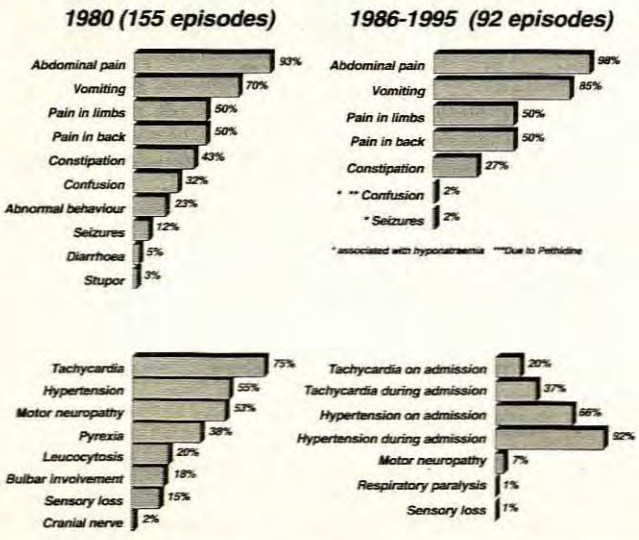

Fig. 7. The acute porphyric attack. The symptoms and signs of 155 acute attacks of porphyria seen at Groote Schuur Hospital prior to 1980 are compared with those of 92 acute attacks seen between 1986 and 1995. The significant decrease in neuropsychiatric features is believed to be due to early diagnosis and treatment with haematin.

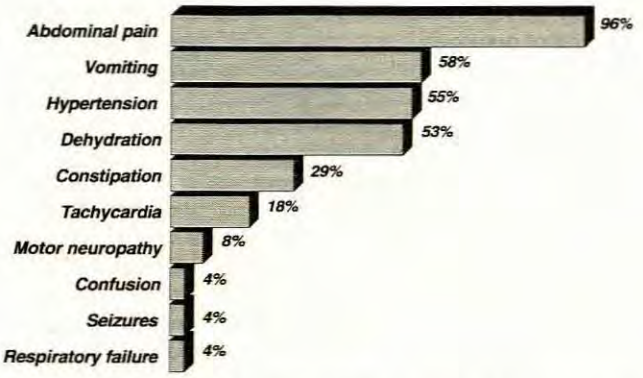

Fig. 8. Manifestations of the acute attack of variegate porphyria.

and is not associated with guarding or rigidity. Only opiates in large doses provide relief, and patients often demand pethidine at frequent intervals, leading to unwarranted suspicions of pethidine dependence.

Other common features are vomiting and dehydration, the serum urea levels being elevated in 53% of subjects. Both hypertension and tachycardia have been reported as common features of the acute attack.[24,32] This was confirmed in our series. The median systolic blood pressure at presentation was 130 mmHg (range 80 - 160 mmHg). However, the increased blood pressure was frequently only noted during the admission. The median pulse rate was 80/min (range 70 - 120/min). Therefore, although these abnormalities, often described in the literature as characteristic features of the acute attack,[34] are common, they are often mild, and are unhelpful as diagnostic features.

Treatment

All patients were admitted and given pethidine, 50 - 100 mg intravenously, at 1 - 2-hourly intervals. The median amount of pethidine per admission was 850 mg, with a maximum of 3 375 mg. Prochlorperazine or metaclopramide was required in 67% of attacks for control of vomiting. Propranolol was used for hypertension in 8%.

Specific suppression of haem synthesis with intravenous haem arginate (Normosang; Leiras Medica) was employed in 46% of episodes.[34] A standard dose of 125 mg dissolved in 100 ml saline, or more latterly, 100 ml human serum albumin, was administered intravenously at daily intervals for a median of 3 days (range 1 - 4).[35,36] This was followed in every case by a prompt decline in ALA and PBG levels and by clinical improvement. Treatment commenced at a median of 1.5 days after admission. In the absence of motor neuropathy we usually allowed a period of observation for spontaneous improvement, in view of the cost of this treatment. The most frequent side-effect of haem administration was phlebitis. This was encountered in 40% of cases. In one instance, administration into a forearm vein was followed by an axillary vein thrombosis. In our experience, haem therapy is highly effective and is the treatment of choice in patients who fail to respond rapidly to conservative therapy, or who show signs of incipient neuropathy.

Carbohydrate loading has traditionally been given because it is reputed to decrease porphyrin synthesis.[37] We have continued to prescribe oral carbohydrate supplements to our patients. However, we use intravenous dextrose-containing fluids with extreme caution as they may aggravate hyponatraemia. We currently do not recommend the use of intravenous fluids that contain dextrose without saline. The suppressive effect of haem dextrose is weak in comparison with that of haem arginate, and it is inadequate therapy for the moderate or severe attack.

Complications and outcome

The median duration of admission was 6 days (range 1 - 56) and outcome was usually good. In contrast to the features described by our unit prior to 1980,[24] we now rarely see motor neuropathy, seizures, confusion or respiratory failure (Fig. 7). In only two instances did patients with VP develop neuropathy; in one, this was limited to a mild distal weakness, whereas the other was profound and required mechanical ventilation for an extended period (Fig. 8). This patient subsequently made a full recovery. The decreased incidence of motor neuropathy may be due in part to better management of the acute attack, particularly the use of haem arginate. However, there is no room for complacency. Though our results at Groote Schuur Hospital are excellent, we are informed from time to time of patients with VP treated elsewhere who have progressed to paralysis and even died. The conclusion must not be that the illness itself is now milder than when reviewed by Eales, but that better management is successful in controlling its severity.

Another significant complication which must always be anticipated is severe hyponatraemia.[34] This is thought to be due to inappropriate antidiuretic hormone release. In our patients the median sodium concentration was frequently decreased, with values below 135 mmol/l being observed in 37% of patients at some stage of the admission. Only 1 patient with VP developed seizures; this was associated with profound hyponatraemia, with a serum sodium concentration of 114 mmol/l, and was followed by a rapid onset of paralysis. Her hyponatraemia did not respond to fluid restriction, and careful administration of hypertonic saline was necessary for its correction. Our experience of IAP is similar; in nearly every instance, seizures were associated with either severe hyponatraemia or massive doses of pethidine, an agent which is epileptogenic in high doses. Again hypertonic saline was necessary for the correction of severe hyponatraemia. Other metabolic disturbances previously described, including hypokalaemia, hypocalcaemia and hypomagnesaemia, have not been a problem in patients with VP.

Skin disease

The cutaneous features of VP are distinctive. However, they are not specific to VP but are also encountered in porphyria cutanea tarda (PCT), HCP and congenital erythropoietic porphyria (CEP). Indeed, a diagnosis of VP cannot be made on the grounds of skin lesions alone. From time to time we encounter patients labelled 'VP' who in fact have PCT. Since the latter is eminently treatable, it is essential that an accurate laboratory diagnosis is made. Typical features of porphyric skin disease are cutaneous fragility with blistering, erosions and scabs.[38] With time, chronic changes are noted. These include hirsutism, thinning and premature ageing, pigmentary changes and milia. Cutaneous lesions are limited to the sun-exposed areas, particularly the backs of the hands, the forearms and, less commonly, the neck and feet.

Unfortunately, management of the skin disease of VP remains inadequate. No systemic photoprotectant has been shown to be effective. Chloroquine and beta-carotene, shown to alleviate the skin disease of PCT and erythopoietic protoporphyria, respectively, have not proved effective in VP.[39,40] Oral activated charcoal, predicted to reduce circulating porphyrin levels by interrupting the enterohepatic cycling of porphyrins, was shown by us to lead to a paradoxical increase in skin disease and porphyrin concentrations, an effect as yet unexplained (unpublished data). The cornerstone of management remains sun avoidance, sensible dress and the use of topical sunfilters with the ability to block the passage of long-wavelength ultraviolet light; sun filters containing zinc or titanium oxide are particularly recommended.[38]

In our extensive experience, the only symptom outside of the acute attack which may be experienced by patients with VP is the skin disease. We have yet to document a single patient with VP in whom chronic abdominal pain, constipation, anxiety, abnormal mood or behaviour could be ascribed to the porphyria. As described above, the acute attack is a well-defined syndrome of severe pain requiring hospitalisation, marked by consistent biochemical findings and an absence of neuropsychiatric abnormalities, except in the setting of profound hyponatraemia, pethidine intoxication or progression to paralysis. The belief prevalent among some doctors and lay people that chronic symptoms can be ascribed to VP is erroneous.

Homozygous VP (HVP)

We have recently reviewed the world experience with this rare disorder — a total of 12 cases.[41] We have studied a single South African child who was presumed to have HVP on the grounds of a compatible clinical presentation, typical VP biochemistry and very low PPO activity. Subsequent DNA studies revealed her to be a compound heterozygote. Indeed, it was our discovery of the two mutations in her PPO gene which led to the identification of the common South African mutation. The fact that, until now, no true homozygote has been identified in South Africa, led us to postulate that true homozygosity for the South African defect may be incompatible with life. Our South African patient, a 10-year-old girl, shows typical features of HVP, including the onset of skin disease within days of birth, subsequent severe photomutilation, deformities of the hands, nystagmus and developmental delay. In addition, she demonstrates an atypical sensory neuropathy. Approximately half of the HVP patients were mentally retarded. Epilepsy has also been described. Our patient has neither of these features.

Diagnosis

Watson-Schwartz reaction for confirmation of the acute attack

The diagnosis during an acute porphyric attack is easily confirmed by a positive Watson-Schwartz test.[42] This simple test, which can be performed in the ward, is described in detail since an early diagnosis may prevent months of paralysis and even save the patient's life. The test is performed by adding 1 ml fresh 'Ehrlich's aldehyde' reagent (*p*-dimethylaminobenzaldehyde in HCl) to 1 ml freshly voided urine. After mixing, a pink-to-red colour, which may require several minutes to develop, indicates the presence of either urobilinogen or PBG. The addition of 2 ml chloroform to the coloured sample, followed by mixing and allowing the mixture to settle, will distinguish between the two possibilities. PBG will remain in the top aqueous layer whereas urobilinogen will be extracted into the bottom solvent phase. However, where the pink colour has not completely extracted into one or other phase, the aqueous phase should be drawn off and mixed with a fresh aliquot of chloroform; this should continue until one or other phase is entirely clear. Provided the reagents are fresh, a negative Watson-Schwartz test effectively excludes the diagnosis of an acute porphyric attack.

Biochemical diagnosis of VP

The exact nature of the porphyria can only be determined by high-performance liquid chromatography (HPLC) or thin-layer chromatography (TLC) of porphyrins extracted from stool, urine, and sometimes blood.[24] The early haem precursors are largely water-soluble and are therefore found in the urine while the distal intermediates are hydrophobic and are found in the stool (Fig. 3).

Typical porphyrin profiles found in the urine and stool of patients with the common hepatic porphyrias can be visualised in Fig. 9, which shows a TLC plate under ultraviolet light. The diagnosis is made on finding a typical pattern of excretion for each individual porphyria. Experience with several thousand porphyrin analyses performed at our Centre has confirmed that TLC or HPLC porphyrin analyses are essential for the accurate diagnosis and typing of the porphyrias. It is of concern that many laboratories investigating the porphyrias only perform qualitative screening tests, very often on urine alone. This practice may miss non-acute VP, and will not differentiate between the three acute porphyrias; neither will it exclude a variety of non-porphyric conditions where raised concentrations of urinary porphyrins, especially coproporphyrin, are common, e.g. some infections, certain anaemias, chemical or heavy-metal intoxications and liver disease, especially alcoholic cirrhosis.[25]

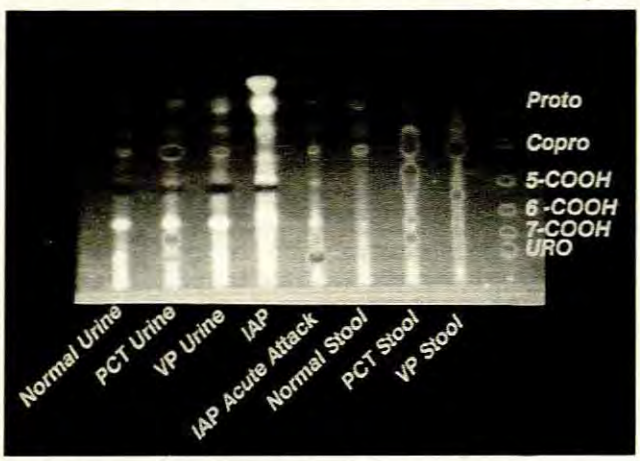

Fig. 9. Thin-layer chromatography of stool, urine and plasma porphyrins exposed to ultraviolet light, illustrating the characteristic porphyrin profiles of VP, IAP and PCT.

The mean concentrations of all stool porphyrins in a series of 221 VP patients who were not in an acute phase and 88 normal subjects are contrasted in Table I. VP subjects had significant elevations of proto-, copro-, pseudo 5-COOH- and 5-COOH-porphyrins. While the classic indicator of VP is considered to be elevated stool protoporphyrin (and, to a lesser extent, coproporphyrin), we found an almost equally important feature to be the presence of either 5-COOH- or pseudo 5-COOH-porphyrin. Since isolated raised stool protoporphyrins are not uncommon in non-porphyric subjects the presence of the 5-COOH forms are crucial for the diagnosis. Pseudo 5-COOH-porphyrin in VP stools has been found to be a modified dicarboxylic porphyrin, presumably derived from the great amounts of protoporphyrin (the primary dicarboxylic porphyrin) present

Table I. Analysis of stool porphyrin data. Porphyrin concentrations are in nmol porphyrin/dry g. *P*-values (Student's *t*-test) are given when the VP group is compared to the normal group.

	Mean	SD (range)	*P*	Proportion (%) > 2 SDs above normal mean
Normal controls (88)				
Uroporphyrin	0.19	0.35 (0 - 1.74)	-	5.9
7-COOH-porphyrin	Trace	- (0 - 0.10)	-	1.1
6-COOH-porphyrin	0	-	-	0
5-COOH-porphyrin	0.13	0.52 (0 - 3.30)	-	4.8
Pseudo 5-COOH	Trace	- (0 - 0.50)	-	2.3
Isocoproporphyrin	0	-	-	0
Coproporphyrin	28.3	25.8 (0.1 - 65.8)	-	0
Protoporphyrin	104.4	64.5 (2.1 - 232)	-	3.4
Quiescent VP (221)				
Uroporphyrin	1.41	4.52 (0 - 47.90)	0.5	21.0
7-COOH-porphyrin	1.14	4.35 (0 - 35.80)	0.048	15.4
6-COOH-porphyrin	0.57	2.86 (0 - 25.6)	0.364	6.6
5-COOH-porphyrin	29.2	16.9 (0 - 261)	$< 10^{-3}$	83.3
Pseudo 5-COOH	27.8	47.1 (0 - 298)	$< 10^{-3}$	46.1
Isocoproporphyrin	2.43	12.73 (0 - 161)	0.131	11.0
Coproporphyrin	286.7	300.0 (22.5 - 1 773)	$< 10^{-3}$	76.3
Protoporphyrin	1 136	992 (207.4 - 5 776)	$< 10^{-3}$	95.6

in stools of these patients. Pseudo 5-COOH- and 5-COOH-porphyrin were more frequently raised than coproporphyrin (83.3% v. 76.3%). During the acute attack of the disease the pattern of porphyrin excretion in the stool is similar to that described for the non-acute phase but is exaggerated.

Urinary porphyrin excretion in non-acute VP patients is highly variable and may be completely normal. The most consistent changes are mild elevations in copro- and uroporphyrin but these are only significantly raised in about 30% of non-acute VP subjects. Urine changes are therefore not useful in non-acute VP. In contrast there are significant elevations in the concentrations of all urinary porphyrins during the acute attack of VP.

Chromatographic analysis of plasma porphyrins is generally troublesome. Certain laboratories have reported that the finding of fluorescence emission spectra between 621 and 627 nm when plasma samles are excited at 405 nm is a highly specific and sensitive marker for VP.[43,44] This finding has yet to be verified in our laboratories using South African VP patients, but it is likely that these findings will hold true.

Enzyme diagnosis

An approximately 50% decreased PPO activity is invariably found in patients with VP. However, the assay is not suitable for routine diagnostic use, because of the extremely low levels of PPO activity in both lymphocytes and fibroblasts, the most available and suitable cell types for assessment of PPO activity.

In our laboratory the most suitable tissue system for measurement of PPO activity has been Epstein-Barr virus-transformed lymphocytes, but it takes some 3 - 7 weeks in culture to prepare sufficient cells for reliable assay.[16,31] This method is therefore not suitable for routine diagnosis of VP.

Genetic diagnosis of VP

Because of its variable clinical and biochemical expression, coupled with the fact that VP seldom manifests before puberty, the diagnosis of VP could be significantly enhanced by the demonstration of an abnormality in the DNA that encodes PPO. A relatively simple test for such a genotype would be highly appropriate, provided that the same defect accounted for the majority of South African VP subjects. Multiple mutations in PBG deaminase have been shown to result in the common phenotype IAP. Here, approximately 70 different mutations have been described. However, the belief that most South African VP subjects were descended from a single founding family[2] suggested that a single PPO genotype might prevail in South Africa.

The search for the South African VP genotype was made possible by the cloning and expression of PPO cDNA from *Bacillus subtilis*[45] by workers at the University of Georgia, USA, in collaboration with members of our Centre. This allowed the cloning, sequencing and expression of PPO cDNAs from *Myxococcus xanthus*,[46] human placenta[47] and mouse liver.[48] Human and mouse PPO cDNAs consist of open reading frames 1 431 nucleotides long, encoding a 477 amino acid protein.[47-49] The human PPO gene contains 13 exons, spanning approximately 4.7 kb.[50]

The availability of the normal gene sequence for human PPO allowed us to study cDNA from South African VP subjects.[1] Initial efforts were directed at identifying significant mutations in the young girl with apparent homozygous VP described above. She was found to have a cytosine (C) to thymidine (T) transition in codon 59 (exon 3), resulting in an arginine to tryptophan substitution (R59W); and a C to T transition in codon 168 (in exon 6) resulting in an arginine to cysteine substitution (R168C). The R59W mutation was present in her mother while the R168C defect was present in her father. These results showed that our patient is, in fact, a double heterozygote, and led us to search for these mutations in the wider South African VP population. In four unrelated patients with VP, who were subsequently examined, the R59W mutation was shown in all, whereas the R168C mutation was absent.

Continuing collaboration with the University of Georgia allowed the production of a genetic construct of the R59W mutation by site-directed mutagenesis. A protein expressed from this construct exhibited substantially reduced enzymatic activity. The observation that activity of the R59W mutant PPO is decreased confirms that this amino acid substitution can account for the decreased PPO activity seen in VP patients. The R59W mutation occurs in the predicted FAD dinucleotide-binding motif of the enzyme, essential for co-factor binding, and it is hence not surprising that PPO activity is affected.

Though direct sequencing of DNA fragments produced by PCR would serve to demonstrate the presence of this mutation in patients, we were able to develop a simpler diagnostic method employing a restriction enzyme digest. Genomic DNA isolated from whole blood is amplified by PCR using appropriate primers and a 252-bp fragment of exon 3 is obtained. The DNA fragment is then digested with the AvaI restriction enzyme. Normal DNA produces a 168-bp and a 84-bp fragment which are clearly visible on gel fractionation and electrophoresis. However, the R59W mutation abolishes the restriction site, and a strong band representing the undigested 252-bp fragment derived from the abnormal allele is seen on the gel (Fig. 10).

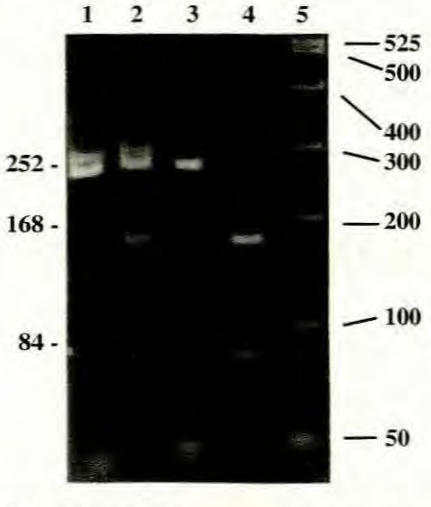

Fig. 10. AvaI restriction enzyme analysis for the protoporphyrinogen oxidase R59W mutation. Lanes 1 and 2 are pre- and post-digested VP DNA, respectively, while lanes 3 and 4 are pre- and post-digested control DNA. Size markers are shown in lane 5. The AvaI site is present in control DNA generating a 168-bp and a 84 bp fragment (lane 4). The AvaI site is absent in VP DNA resulting in an uncut fragment 252-bp long (lane 2). The 168-bp and 84-bp fragments in the VP mutated DNA (lane 2) are due to the heterozygosity of the DNA. (Reprinted from Meissner *et al.*[1] with permission from *Nature Genetics*.)

A larger family study using direct sequencing and AvaI analysis showed that the mutation co-segregated with the disease (Fig. 11). We have shown that the R59W mutation is present in 63 of 66 families investigated (153 of 159 affected individuals). At least one of these families is known to be descended from Ariaantje Adriaanse.

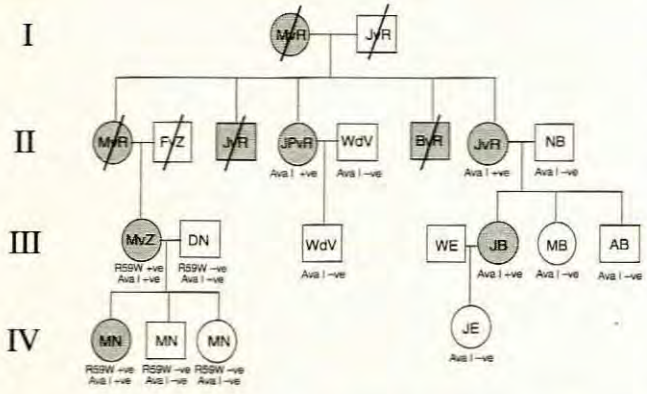

Fig. 11. Pedigree illustrating 4 generations of the VR family. Shading indicates VP. Evidence for VP is anecdotal and obligatory (generation I, circa 1880 - 1910), clinical (generation II prior to 1966) and clinical and/or biochemical (generations II after 1966 and generations III and IV). AvaI +ve/−ve indicates presence or absence of the mutation by restriction analysis while R59W +ve/−ve indicates presence or absence of the mutation by direct sequence analysis. (Reprinted from Meissner *et al.*[1] with permission from *Nature Genetics*.)

DNA testing will now allow a specific diagnosis at any age in those families known to have the R59W mutation. Our preliminary work suggests that this will be the case for most families with VP in South Africa. Indeed, this appears to be the case as all South African laboratories that are offering R59W genetic testing for VP subsequent to our initial report, report an overwhelming predominance of the R59W mutation in subjects investigated for VP (personal communication). Finally, this should allow the elucidation of phenotypic variation seen in VP subjects and provide a mechanism by which the true prevalence and evolution of VP in South Africa may be determined.*

Preventing the acute porphyric attack

The most important steps are early, correct diagnosis, and empowering patients to minimise their exposure to potentially porphyrinogenic medications. The Lennon Porphyria Information Service within the MRC/UCT Liver Research Centre has produced a booklet for patients, their doctors and their pharmacists which is sent to all newly diagnosed porphyrics.[23] This, together with a telephonic information service, advises patients, their families, pharmacists and doctors on all aspects of porphyria.

Over a 5-year period, 2 577 calls, excluding those received directly by the professional members of the staff, were logged on computer. These data indicate that only 32% of enquiries were received from the Western Cape; the rest originated in other provinces, particularly the former Transvaal, confirming that the Centre is performing a national function. The service is well used by both patients and the professions: 46% of queries were from patients, 11% from friends or relatives; 20% from doctors, 8% from pharmacists and 4% from nurses. The remaining 11% were from the general public.

The commonest requests were for general information on porphyria (63%) and about drug safety (31%. Because of this, a computerised drug safety database was established and is continually being updated and improved. Advice on diagnosis was requested by 14% of all callers. It has become apparent that many people believed to have porphyria may not have this condition. Often the diagnosis is made on inappropriate clinical grounds. This is compounded by some laboratories' use of inadequate tests, or may be due to the misinterpretation of data by inexperienced observers. We have become uncomfortably aware of large numbers of people in whom chronic physical and psychological symptoms are erroneously being ascribed to VP. This led to many callers being asked to submit specimens directly to our laboratory for thin-layer chromatography and quantitative fluoroscanning. Written information, in the form of a booklet, printed notes on specific topics and drug lists, was sent out in response to 70% of all requests.

* The R59W and R168C mutations in the protoporhyrinogen oxidase gene mentioned here have more recently been confirmed and a third mutation, termed H20P, shown to exist in at least one South African family.[51] The existence of this mutation in South Africa has been verified by our laboratory.

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