CHAPTER 1

An Historical Introduction to Porphyrin and Chlorophyll Synthesis

Michael R. Moore*

Historical Introduction to Porphyrins and Porphyrias

Introduction

Porphyrins are the extroverts of chemistry. Bright purple and fluorescent, they are used biologically in the processes of energy capture and utilization. Porphyrins are the key to life. It has been suggested that abiotic formation of porphyrins, in particular uroporphyrinogen would have provided the first pigments necessary for the eventual synthesis of the chlorophylls. This would have facilitated the emergence of simple photosynthetic organisms in primordial earth through enhanced efficiency of energy capture and utilisation (Fig. 1).

Communication

The development of the science of porphyrins and chlorophyll has progressed at a breakneck pace over the last century. A key to the development of medicine and science in the 20th century has been the improving levels of communication on an international basis. However, by the 1960s it was becoming increasingly easy for scientists to communicate both in person and electronically. At the same time many national groupings established between the middle and end of the 20th Century have grown and consolidated the interrelationships between scientists and physicians in the study of haem and chlorophyll synthesis. As an illustration of this continuing communication, Table 1 shows the sequence of meetings of the Tetrapyrrole Discussion Group, (TPDG) in the United Kingdom from its inception in 1977.

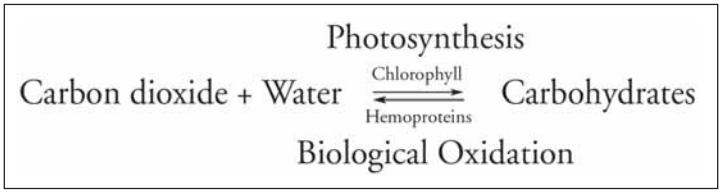

Figure 1. Carbohydrate synthesis and degradation using chlorophyll and hemoproteins.

*Michael R. Moore—National Research Centre for Environmental Toxicology, University of Queensland, 39 Kessels Road, Coopers Plains, Brisbane, Queensland 4108, Australia. Email: m.moore@uq.edu.au

Tetrapyrroles: Birth, Life and Death, edited by Martin J. Warren and Alison G. Smith.

Table 1. The Tetrapyrrole Discussion Group (TPDG) a list of meetings since inception

Year	Date	Site	Organiser
1977	5 July	University College, Cardiff	George Elder/ Stan Brown, Francesco De Matteis
1978	7 April	Middlesex Hospital, London	Francesco De Matteis
1979	29-30 March	University of Leeds	Stan Brown
1980	3-4 January	University of Bristol	Trevor Griffiths, Owen Jones
1980	25-26 September	Western Infirmary, University of Glasgow	Michael Moore
1981	10 April	Queen Mary College, London	Ray Bonnett
1982	23 April	University of Southampton	Peter Jordan
1983	21-22 March	University College, Cardiff	Tony Jackson, George Elder
1984	6 January	Royal Free Hospital, London	Barbara Billing
1984	16-17 July	University of Leeds	Stan Brown
1985	19-20 September	University of Edinburgh	Jenny Houghton
1986	April	The Royal Society	Albert Neuberger
1987	20-23 July	University of Southampton Medical School '10th Anniversary Meeting'	Peter Jordan
1988	11-12 April	University of Leeds	Jenny Houghton
1989	4-7 April	University of Glasgow 'A Century of Porphyria'	Michael Moore
1989	December	The Royal Society	Francesco De Matteis
1990	December	Queen Mary and Westfield College, London	Ray Bonnett
1992	6-7 April	University of Southampton	Peter Jordan
1993	5-6 January	University of Cambridge	Alison Smith
1993	15-16 September	University of Leeds	Jenny Houghton
1994	11-12 July	Queen Mary and Westfield College	Ray Bonnett, MartinWarren
1995	10-12 April	University of Leicester	C-K Lim, Andy Smith
1996	11-12 January	University of Southampton	Peter Shoolingin-Jordan
1996	10-11 September	University of York	Timmins
1997	30 June-1 July	Queen Mary and Westfield College '20th Anniversary Meeting'	Ray Bonnett
1998	23-24 March	University of Cambridge	Alison Smith
1998	7 December	The Royal Society	Martin Warren
1999	1-2 September	University of Essex	Ross Boyle
2001	4-5 January	University of Leicester	Andy Smith
2001	13-14 September	University of Cardiff	Mike Badminton
2002	8-10 April	Heriott-Watt University	Martin Warren
2003	9-10 January	University of Leeds	David Vernon
2003	28-30 August	Lund University, Sweden	Lars Hederstedt, Mats Hansson
2004	16-17 September	University of Cambridge	Alison Smith
2005	1-2 July	University of Hull	Ross Boyle

The porphyrias provide examples of the derangement of the pathway that synthesizes these tetrapyrroles. From the outset, the name "porphyria" described not the diseases but the lustrous purple-red crystalline porphyrins:

πορφνροο
[porphuros or purple].

Again the importance of this topic is reflected in the number of international meetings organized in the last half-century (Table 2). Chapter 5 describes recent advances in this field.

Table 2. A chronology of international porphyrin and porphyria meetings post-1955

Year	Date	Site	Organiser(s)	Background
1955	8-10 Feb	London	Rimington	CIBA Foundation
1959	31 Aug-4 Sept	Canberra	Lemberg, Legge	IUB/AAS Hematin Enzymes
1963	18-24 Sept	Cape Town	Eales	
1968*	8 April	London	Rimington, Neuberger	UCH/ Biochem Soc Symp. 28.
1968*		Crystal Mt	Tschudy	1st Gordon Conference on
to		to	to	Tetrapyrroles GRCs every even
2006		Newport	Ann Smith	year thereafter
1970	2-6 Dec	Cape Town	Eales	
1970				GRC
1972	3-6 April	New York	Adler	NY Acad Sci
1972				GRC
1973*	28-30 June	Marburg	Doss	
1974				GRC
1975*	19-20 Feb	London	Neuberger, Kenner	Royal Society
1975*	1-4 May	Freiburg	Doss	
1975		New York	Adler	NY Acad Sci
1975*	17-19 July	Helsinki	Koskelo & Tenhunen	
1976*	July	Wolfeboro	GRC	
1977	June28-July 1	Marburg	Doss	400th Anniversary Phillips U.
1977*	Sept	Cardiff	Brown, De Matteis	1st Meeting of TPDG Elder/Moore
1978*	July	Wolfeboro	GRC	
1978		Kyoto	Sano	
1979*	29 Oct-1 Nov	Buenos Aires	Batlle	CIPYP
1980*	4-8 Aug	Wolfeboro	Troxler	GRC
1982	23-28 May	Tokyo	Mascaro	Dermatology
1982	1-6 Aug	Wolfeboro	Marks	GRC
1984*	5 Nov	Minneapolis	Pierach	Watson Symposium
1984*	6-10 Aug	New London	Smith (K)	GRC
1985	19-22 June	Paris	Nordmann	
1986*	28 July-1 Aug	Wolfeboro	Sassa	GRC
1986*	27-29 Oct	Rye Brook NY	Silbergeld	NY Acad Sci
1987*	12-13 Nov	London Ont	Haust	
1987*	7-10 Oct	Rome	Topi	
1988*	25-29 July	Wolfeboro	Correia	GRC
1989*	4-7 April	Glasgow	Moore	Century of Porphyria TPDG - Goldberg Festschrift
1990*	23-27 July	Wolfeboro	Beale	GRC
1992*	30 April-4 May	Papendal	Wilson, De Rooij	
1992	Kushner	GRC		
1992*	22-24 Oct	Göttingen	Seubert	Ippen Festschrift
1993*	16-17 March	Oslo	Moan	Rimington 90th Birthday
1993*	22-26 Nov	Melbourne	Blake	
1994	24-29 July	Wolfeboro	McDonagh	GRC
1995*	28 June-2 July	Helsinki	Tenhunen, Mustajoki	
1996*	February	Cape Town	Meissner, Kirsch	International association for the study of liver disease (IASLD)
1996*	14-19 July	Henniker	Bloomer	GRC
1998	12-17 July	Newport	Lagarias, Shoolingin-Jordan	GRC

Table continued on next page

Table 2. Continued

Year	Date	Site	Organiser(s)	Background
1999*	July	Hamburg	Martasek	
2000*	16-21 July	Newport	Ortiz de Montellano	GRC
2000*	10-13 Sept	Paris	Deybach festschrift	Millenium, Nordmann
2000		Dijon	Guillard	ISPP-1
2002	8-10 April	Edinburgh	Heathcote, Warren	Biochem Soc, TPDG
2002	June	Kyoto	Ogoshi	ISPP-2
2002*	14-19 July	Newport	Ferreira	GRC
2003	21-25 Sept	Prague	Martasek	
2004	New Orleans	Smith, Kadish, Sessler		ISPP-3
2004	July	USA	O'Brian	GRC
2005	Feb	Cape Town	Meissner	Kirsch festschrift
2006	July	USA	Smith (A)	GRC
2007	April	Rotterdam	De Rooij	Wilson Festschrift

GRC = Gordon Research Conference

Descriptions in Antiquity

Although the first verifiable cases of porphyria were identified in the middle of the 19th Century, some of the writings of the ancients seem to show clear descriptions of attacks of acute porphyria (Table 3). Hippocrates, 460 to 370BC, gives a splendid description of a woman from Thasus who suffered from many of the features that we now recognise to be associated with the acute attack of porphyria.

The allusions to porphyrins, around 1840, by Lecanu[1] and other workers, preceded the first clinical presentations of porphyria by Schultz[2] and Baumstark[3] by 30 years. The porphyrias belong to that larger group of diseases described by Garrod[4] in 1923 as "inborn errors of metabolism." They demonstrate a unique combination of neurological and dermatological features, which show characteristic variations from one condition to another, the reasons for which may be sought in enzymic change within the haem biosynthetic pathway. The history of various aspects of the porphyrias and porphyrin metabolism has been recorded by Florkin and Stotz[5] and Moore[6] (Table 3).

Structure

The basic porphyrin nucleus is a unique biological structure. It consists of a macrocycle of four pyrrole rings linked by four methene bridges. The normal biological intermediate is not this highly conjugated porphyrin, but the hexa-hydro porphyrin, the porphyrinogen in which each of the methene bridges is reduced.

An important feature of this complex ring structure is its metal-binding capability. The most commonly bound metals are iron and magnesium. In this form the metalloporphyrins reach their true apotheosis. Haem, an iron-containing complex usually bound to various proteins, is central to all biological oxidations. Haemoproteins are also used as oxygen carriers. The chlorophylls are the magnesium-porphyrin compounds, which are central in solar energy utilization in the biosphere.

As well as the systematic formation of porphyrins by biological systems, abiotic synthesis of porphyrins has been described in which a primitive chemical system has produced porphyrin-like compounds through the high entropy of their formation.[7] Such synthesis was important in the ontogenesis of terrestrial life, since it would have facilitated the emergence of life forms on primordial earth by increasing the efficiency of oxido-reductive processes as well as of energy capture.[8] Porphyrins are found in fossil life forms[9] and have even been identified in rocks from the moon.[10]

Table 3. Selected chronology of studies of porphyrins

Year	Researcher	Studies
460-370BC	Hippocrates	Described a case akin to menstrually-related acute porphyria
1831	Laennec	Anaemia of lead poisoning
1837	Lecanu	Acid extraction of hemin from blood
1841	Scherer	Hemin extraction—iron free
1844	Mülder	Purple-red fluid devoid of iron
1864	Hoppe-Seyler	Named hemoglobin
1867	Thudichum	Spectrum and red fluorescence of porphyrins
1871	Hoppe-Seyler	Prepared porphyrins from blood and showed they were pyrroles
1874	Schultz	Congenital porpyria—pemphigus leprosus)
	Baumstark	Urinary porphyrin pigments—an error of biosynthesis
1879	Hoppe-Syeler	Chlorophyll tetrapyrrole, phylloporphyrin, structurally similar to heme
1980	McMunn	Salicylate-induced porphyrinogenesis
1883	Soret	Light absorption around 400nm of hemin
	Krukenberg	Red fluorescence of eggshells
1884	McMunn	Discovery of cytochromes (myohematin)
	Brouwier	Chocolate-colored bones in abbatoir carcasses
1888	Nencki, Sieber	Hematoporphyrin dicarboxylic
1889	Stokvis	Sulfonal-induced acute porphyria
	McMunn	Porphyrins in echinoderms and the black slug
1890	Ranking, Pardington	Described the symptoms of acute porphyria
1891	Geill, Fehr	Induction of acute porphyria in women by sulfonal
	Copeman	Neurosis in female porphyrics
1892	Church	Turacin shown to be a porphyrin
1893	Barnes	Named photosynthesis (carbonic acid and chlorophyll)
1895	Stokvis	Animal porphyria
	Binnendijk	Excess porphyrins in urine of lead-poisoned patient
1896	Saillet	Coproporphyrin (urospectrine)
1903	Zaleski	Mesoporphyrin
	Erbslöh	Axonal degeneration in acute porphyria
1904	Laidlaw	Preparation of protoporphyrin
1906	Willstätter	Chlorophyll characterization commenced
	Dobrschansky	Barbiturate-induced porphyria
1908	Hausmann	Porphyrin photosensitization of mice
1911	Günther	First classification of porphyria
1912	Küster	Correct tetrapyrrolic structure of heme
1913	Meyer-Betz	Hematoporphyrin sensitization of skin
1915	Willstätter	Nobel prize for chlorophyll work
1923	Kämmerer	Porphyrin from putrefying meat—protoporphyrin
	Garrod	Porphyria—inborn error of metabolism
1924	Fischer and Kögl	Ooporphyrin (protoporphyrin) in eggshells
	Policard	Preferential uptake of hematoporphyrin by rat sarcoma
1925	Keilin	Rediscovers cytochromes
	Derrien and Turchini	Oviduct porphyrin deposition on eggshells Intense red fluorescence of harderian gland
1929	Fischer and Zeile	Hemin synthesis confirming Küster's structure Similarities of side chains of heme and chlorophyll

Table continued on next page

Table 3. Continued

Year	Researcher	Studies
1929	Borst and Königsdörffer	Autopsy study of Petry
1930	Fischer	Nobel prize for porphyrin work
1931	Sachs	Chromogen giving red coloration wth Ehrlich's reagent
	Körbler	Porphyrin uptake by tumors
1932	Grotepass	Urinary coproporphyrin III in lead poisoning
	Hijmans van den Bergh	Lead increase in erythrocyte protoporphyrin
1933	Watson	Stercobilins
	Dhéré et al	Studies of microbial porphyrins
1935	Franke, Fikenstscher	Doubling of urinary coproporphyrin excretion after ethanol
1936	Dobriner	First case of what would be called hereditary coproporphyria
	Rimington	Congenital porphyria in cattle
1937	Waldenström	Review, naming of porphyria cutanea tarda and classification of acute porphyria
	Turner	Porphyrins in fox squirrels
	Guildemeister	Albumin binding of porphyrins
1939	Rimington, Hemmings	Coproporphyrinuria following use of sulfanilamide
	Waldenström	Isolation of porphobilinogen
1941	Watson, Schwartz	Simple test for urinary porphobilinogen
1944	Strong	Harderian gland porphyrin and carcinoma in mice
	Clare and Stephens	Congenital porphyria in pigs
1945	Barnes	Variegate porphyria in south Africa
1946	Shemin, Rittenberg	Isotopic study of porphyrin biosynthesis
	Corwin, Erdman	IR investigation of NH tautomerism
1948	Folkers, Smith	Isolation and characterization of vitamin B_{12}
1949	Pauling, Itano	Sickle cell hemoglobin
	Lemberg, Legge	Description of biosynthetic sequence
1950	Neuberger et al, Shemin et al	Uroporphyrin first formed biosynthetically
	Rimington, Sveinsson	Spectral absorption of porphyrins
1951	Watson et al	Erythropoietic and hepatic classification of porphyria
	Shemin, Wittenberg	Requirement for succinyl-CoA
1952	Westall	Isolation of porphobilinogen
1953	Shemin, Russell, Neuberger, Scott	Role of ALA in biosynthesis
	Kosenow, Treibs	Erythropoietic protoporphyria
1955	Goldberg, Rimington	Allylisopropylacetamide-induced experimental porphyria
	Hodgkin et al	X-ray structure of vitamin B12
	Bogorad	Reduced porphyrins—porphyrinogens are biosynthetic intermediates
	Scott	Photosensitizing effect of ALA
	Shemin	The succinate-glycine cycle
	Berger, Goldberg	Named hereditary coproporphyria
	Tio	Porphyria cutanea tarda associated with adenoma
	Schwartz et al, Rassmussen-Taxdal et al	Use of hematoporphyrin derivative on cancer
1956	Goldberg et al	Ferrochelatase—the iron-incorporating enzyme
	Waldenström	Recognition that each porphyria can be localized to a defect in one enzyme

Table continued on next page

Table 3. Continued

Year	Researcher	Studies
1957	Cam	Turkish porphyria—hexachlorobenzene-induced
	Lascelles, Schulman, Richert	ALA synthase—requirement for pyridoxal phosphate
1957	Haeger	ALA in urine in lead poisoning
1958	Granick	ALA synthase the primary control point of heme biosynthesis
1959	Solomon, Figge	3,5-Dicarbethoxy-1,4-dihydrocollidine experimental porphyria
	Becker and Bradley	First application of NMR to porphyrin structure
1961	Calvin	Nobel prize for photosynthesis work
	Magnus et al, and Langhof et al	Named and studied erythropoietic protoporphyria
	Lipson et al	Use of purified hematoporphyrin derivative
	Gajdos et al	Role of ATP in control of porphyrin synthesis
1962	Perutz and Kendrew	Nobel prize for X-ray crystal structure of hemoglobin
1964	Illis	Porphyria and the etiology of werewolves
	Heide et al	Hemopexin, a heme-binding γ-globulin
	Dagg et al	Similarities between acute porphyria and lead poisoning
1965	Woodward	Nobel prize for total synthesis of chlorophyll and vitamin B12
1966	Macalpine and Hunter	"Royal malady"
1969	Pinol-Aguade et al	Hepatoerythropoietic porphyria
	Gajdos et al	Homozygous hereditary coproporphyria
	Romeo and Levin	Uro'gen III cosynthase and congential porphyria
1970	Strand et al	Acute intermittent porphyria related to deficiency of PBG deaminase
	Koskelo et al	Binding of ^{14}C-porphyrins by hemopexin and albumin
1971	Bonkowsky et al	Hematin use in acute porphyria
	Moore et al	Ethanol inhibition of ALA dehydratase
1973	Beale et al	ALA synthesis from glutamate, through DOVA
1974	Jackson et al	Protoporphyrinogen oxidation enzymically controlled
1975	Bonkowsky et al, Bottomley et al,	
	De Goeij et al	Ferrochelatase and erythropoietic protoporphyria
	Beale et al	C_5 Pathway, glutamate → ALA
1976	Elder	Hydroxymethylbilane synthase as secondary control stage
	Wetterberg	International review of drugs in porphyria
	Brodie et al, Elder et al	Coproporphyrinogen oxidase and hereditary coproporphyria
	Kushner et al	Uro'gen decarboxylase and PCT
1977	Brodie et al	Control of acute porphyrias by PBG deaminase
1979	Jordan et al,	Mechanism of formation of uroporphyrinogen III
	Battersby et al	from hydroxymethylbilane (preuroporphyrinogen)
	Bird et al	First description of plumboporphyria (ALA dehydratase deficiency)
	Moore et al	Acute and nonacute classification of porphyria
	Tephly et al	DDC forms *N*-alkylated porphyrins
1980	Meisler et al	Gene for porphobilinogen deaminase on chromosome 11
	Brenner and Bloomer	Protoporphyrinogen oxidase and variegate porphyria
1981	Mustajoki,	Immunological variants of PBG deaminase—
	Anderson et al	CRIM positive and CRIM negative
1982	Day et al	Concurrent (dual) variegate porphyria and PCT
	Nordmann et al	Description of harderoporphyria

Table continued on next page

Table 3. Continued

Year	Researcher	Studies
1983	Eiberg et al	Gene for ALA dehydratase on chromosome 9
	Grandchamp et al	Gene for coproporphyrinogen oxidase on chromosome 3
	Batlle et al	Use of ALA dehydratase replacement therapy
1984	Kordic et al	Homozygous variegate porphyria
	Grandchamp et al	cDNA for PBG deaminase mRNA
	De Verneuil et al	Gene for uroporphyrinogen decarboxylase on chromosome 1
	Youngs	Chester Porphyria
1986	Deybach et al	Homozygous erythropoietic protoporphyria
1987	Jordan and Warren	Dipyrromethane co-factor for PBG Deaminase
	Siepker	FAD cofactor for protoporphyrinogen oxidase
1989	Riddle et al	Two forms of ALA Synthase
1990	Bishop et al	ALA-S2 and X-linked Sideroblastic anaemia—location Xp11.21
1991	Whitcombe et al	Ferrochelatase 18q22
1991	Astrin et al	Uroporphyrinogen co-synthase 10q25.3
1992	Cotteral	X-linked sideroblastic anaemia—due to ALA-S2
1993	Norton	Chester Porphyria on 11q
1994	Dailey et al	Two iron, two sulphur clusters in ferrochelatase
1994	Cacheux et al	Coproporphyrinogen oxidase 3q12
1995	Taketani et al and	Protoporphyrinogen oxidase 1q22-23
	Roberts et al	
1996	Meissner et al	R59W mutation in protoporphyrinogen oxidase—South African variegate porphyria
1998	Röhl, Warren	"Purple Secret" further evaluation of the 'Royal Malady'

Chlorophyll

Prior to 1893, the process by which plants reduce carbon dioxide to organic matter was termed assimilation. However, in that year, Barnes[11] proposed that the biological process for the synthesis of complex carbon compounds in the presence of chlorophyll under the influence of light should be designated as photosynthax or photosynthesis. The history of this process is described by Gest.[12]

The naming of chlorophyll in 1818 was by Pelletier and Caventou[13] from the Greek

χλόροσ θύλλον

[Colour of Leaf]

On our planet chlorophyll synthesis is an extremely conspicuous process. Rüdiger[14] noted that the surge in chlorophyll synthesis in the spring on our planet is the clearest evidence of the presence of life on earth. Elucidation of the structure of chlorophyll by Fischer and Orth[15] occurred in 1940 and a verification of this structure by its total synthesis by Woodward[16] in 1960.

In chlorophyll the four pyrrole rings are linked into a tetrapyrrole with a magnesium atom at the centre of the structure. To form the first of these pyrrole rings there is an alternative route for the synthesis of 5-aminolaevulinate, which starts from glutamate through a three-step pathway, which is called the C5 pathway; in contrast to the synthesis from glycine and succinyl-CoA. This was demonstrated by Beale et al.[17] The overall mechanisms are described by von Wettstein et al.[18]

In the subsequent stages of the pathway, living systems have developed two ways of transforming uroporphyrinogen III into metal complex macrocycles. One is oxidative and leads to the chlorophylls and to haem, which the obligatory acceptor for the enzymic oxidation is oxygen. The second method is nonoxidative and makes use of methylation. This pathway is found in anaerobic organisms and for compounds like cyanocobalamin develops a macrocycle, which is structured to hold the cobalt ion.[19] Recent work on the biosynthetic pathways of haem, chlorophylls and corrins are described in other chapters in this book.

The Early Chemical Era

Early Studies

The history of the porphyrins begins with the work of Lecanu, Berzelius,[20] Scherer,[21] and Mülder.[22] Scherer added concentrated sulfuric acid to dried and powdered blood and washed the precipitate free of iron. He thus had shown that the red coloration of blood was not due to iron. In Mülder's study, he described a "purple-red fluid" without any iron, which he named "Eisenfreises hämatin" (iron-free haematin). This red substance was called "cruentine" by Thudichum[23] in his report to the Privy Council of Great Britain in 1867. He defined its spectrum, and noted that, "it fluoresced with a splendid blood-red color."

Contemporaneously, the first tentative efforts were being made to understand the part played by the tetrapyrroles in living organisms. In 1871, Hoppe-Seyler[24] found that "iron-free haematin" was a mixture of two substances, the main constituent of which he called "hämatoporphryin." Three years later, Schultz[2] published the clinical details of a case of so-called "Pemphigus Leprosus" for his doctoral thesis. The patient was a 33-year old weaver who had suffered from skin photosensitivity from the age of 3 months. His spleen was enlarged and he passed a wine-red urine; this urine was investigated by Baumstark[3] who named two pigments derived from it—"urorubrohaematin" and "urofuscohaematin." The importance of his observations was in his interpretation that the source of the porphyrin pigments was from an error of biosynthesis. The case was a description of congenital porphyria. This was the first association of this class of pigments in urine with a disease in humans. The autopsy records intense red-brown discoloration of the skeleton, a feature of this disease in both animals and humans.

In 1880, MacMunn,[25] who later discovered the cytochromes in 1884, described a dark pigment excreted in the urine of a patient who had been taking sodium salicylate. MacMunn called this pigment "urohaematin," but later, he renamed it "urohaematoporphyrin" because "it bears a very striking resemblance to haematoporphyrin." Hoppe-Seyler[26] studied the porphyrin in chlorophyll and rediscovered the property of red fluorescence first seen by Thudichum. He named it "phylloporphyrin." The term "porphyrin" was used by others such as Church[27] in his description of the porphyrin from Turaco feathers (turacin). Finally, the major spectroscopic feature of porphyrins, the strong absorptions lying around 400 nm was described for haemoglobin in 1883 by Soret.[28] This absorption band for porphyrins is still called the Soret band.

Biochemical Developments

A true biology of the porphyrins could not at that time be formulated because the exact route to their biosynthesis, together with their relationship within intermediary metabolism, were lacking. Many erroneous views were propounded during this period concerning the origins and interrelationships of the porphyrins, views, which obfuscated and retarded progress in the biological sphere. It was thought, for example, that porphyrins arose as degradation products of haemoproteins, such as haemoglobin, by removal of iron and the protein moiety and that protoporphyrin, so formed, was "detoxicated" by progressive carboxylation providing, in uroporphyrin, a more hydrophylic molecule for urinary excretion. These mistaken assumptions inevitably confused any understanding of the porphyrias, although Günther's[29] clinical classification in 1911 helped to resolve some of the misunderstanding. One may also admire Baumstark's foresight in 1874. He believed the urinary pigments of Schultz's 1874 case arose by an error in the biosynthesis of haemoglobin and not by any fault in its degradation, a concept that was not confirmed for another 50 years.

The chemical excreted in the porphyrias remained a matter of debate. The urinary pigment had been thought to be haematoporphyrin.[30-32] Garrod[33] showed that the absorption spectra of the urinary porphyrins were being masked by other chromophores in the urine, but it was not until 1915 that Fischer[34] showed that "urineporphyrin" was quite discrete from "haematoporphyrin." Nencki and his coworker, Sieber[35] contributed to the knowledge of the time by showing that haematoporphyrin was a dicarboxylic porphyrin. Saillet[36] prepared "urospectrine" in 1896 from urine which was subsequently named "coproporphyrin"[37] and also showed the presence of this compound in urine as a colorless chromogen (probably "coproporphyrinogen"). "Protoporphyrin" was also prepared unknowingly at this time by Laidlaw.[38] The correct structure of haem was first proposed by Küster[39] in 1912 but subsequently rejected by other workers, of greater stature, such as Willstätter and Fischer. Following

Figure 2. Richard Willstätter—Nobel laureate in chemistry 1915 (© The Nobel Foundation).

Figure 3. Hans Fischer Nobel laureate in 1930 (© The Nobel Foundation).

the separation studies of Willstätter and coworkers[40,41] for which he was awarded a Nobel Prize (Fig. 2), Fischer began a series of studies, which continued for 30 years until his death in 1945. During this time, he was awarded the Nobel prize for chemistry in 1930 (Fig. 3).

Mathias Petry and Hans Fischer

Mathias Petry was one of Günther's cases of congenital haematoporphyria. Petry became both laboratory aide and source of porphyrins for Fischer. Fischer worked with him until Petry's death in January 1925 when Fischer undertook a chemicopathological autopsy, which he published under the name of "Porphyrinurie".[42] Borst and Königsdörffer[43] published the extensive pathological autopsy. Laidlaw,[38] Fischer,[34] and Schumm[44] differentiated the naturally occurring porphyrin of haem itself from haematoporphyrin and the name "protoporphyrin" was suggested for this substance by Fischer[45]

The Biochemical Descriptive Era

It was during the 1930s that the next generation started their work. During this time, some key names and prominent contributions emerged: the discovery of Ehrlich's positive chromogen in the urine of patients with acute porphyria in attack by Sachs.[46] Waldenstrom studied Sachs' Ehrlich's positive chromogen, which, together with Vahlquist, he named "porphobilinogen"[47] in 1939

Haem Biosynthesis

The next milestone in the development of this subject rested upon the emergence of a systematic biochemical description of the pathway of haem biosynthesis. The names most commonly associated with this are Shemin and Neuberger (Figs. 4, 5). Their work encompassed the early description of how "5N-glycine was incorporated into haem by humans and animals.[48,49] This led first, to the realization that ALA was a precursor of porphyrins[50,51] and at the same time, that the monopyrrole, porphobilinogen, was indeed, the precursor of uro-, copro-, protoporphyrin, and haem.[52]

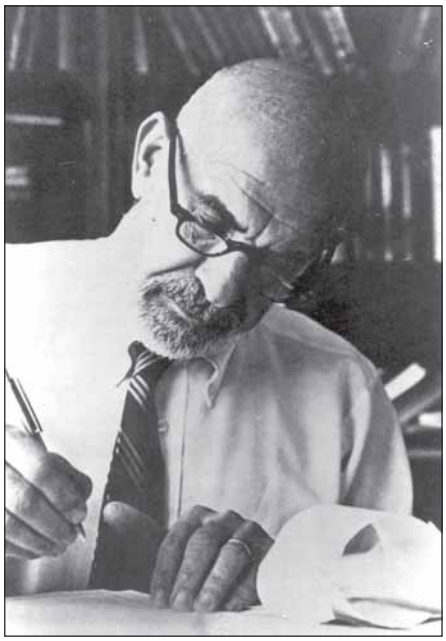

Figure 4. David Shemin.

Figure 5. Albert Neuberger.

A "pyrrolic intermediate" was postulated as a vital step in the biosynthesis of the tetrapyrrole ring. The decisive contribution came in 1952 when the substance called porphobilinogen, excreted by patients suffering from acute intermittent porphyria, was shown to be a monopyrrole.[53,54] It was shown to give rise, enzymatically, to uroporphyrinogen when incubated with a haemolysate of chicken red cells.[52]

Early Description of Porphyria

After sulfonal was introduced as a hypnotic by Kast[55] in 1888, Stokvis[56] reported that an elderly woman who had taken sulfonal excreted dark-red urine and later died. He considered that the pigment in this urine, producing coloration resembling port wine, was similar to, but not identical with, haematoporphyrin. Harley[57] (1890) reported a fatal case of an unusual form of nervous disturbance associated with dark-red urine in a 27 year-old woman who had been given sulfonal and presented many of the neurological features of porphyria. Ranking and Pardington[58] (1890) described two women who excreted "haematoporphyrin" and who exhibited the gastrointestinal and neuropsychiatric manifestations of acute intermittent porphyria. The terms "porphyria" and "porphyrinuria" emerged gradually and slowly replaced "haematoporphyria" and "haematoporphyrinuria." Sometimes sulfonal or the allied drugs, tetronal and trional, had been taken for variable periods prior to the onset of symptoms. Other cases had no obvious relationship to drugs and, presumably, were precipitated by other unknown causes. When barbiturates were introduced into medicine in 1903, it was not long until a report of an acute porphyric attack, precipitated by diethyl barbituric acid, was reported.[59]

Drugs and Chemical Porphyria

The earliest evidence that we have for the drug-related development of porphyria occurred when the drug sulfonal induced the first published example of an attack of acute porphyria by Stokvis.[56] The ability of certain compounds to increase porphyrin synthesis in experimental animals has been used as a means of examining the processes of haem biosynthesis.[60] Experimentation with animals

added much to this area of knowledge but confusingly, some drugs produced different patterns of porphyrin excretion in different species due to differences in response and susceptibility. Some investigators maintained that drugs merely precipitated attacks in patients already suffering from genetic acute porphyria, whilst others disputed this.

Allyl Compounds

Schmid and Schwartz[61] (1952) found that the hypnotic compound Sedormid (allylisopropyl acetylurea) could produce a porphyria in laboratory rodents which was akin to acute intermittent porphyria in humans. Allylisopropylacetamide (AIA), a structural analogue of Sedormid was soon found to be equally effective porphyrinogenic agents[62] (Fig. 6).

DDC

A chance observation by Solomon and Figge[63] (1959) revealed that the substituted dihydropyridine, 3,5-diethoxycarbonyl-1,4 dihydro collidine (DDC) caused an experimental porphyria. Tephly et al (1981),[64] De Matteis and Marks (1983)[65] and others studied the chemical events following experimental administration of the porphyrinogenic drug, DDC. It has been shown that the *N*-alkylated protoporphyrins are the immediate inhibitors of ferrochelatase and that they originate from the haem of the cytochrome P450, DDC being the methyl donor.

Fungicides

Hexachlorobenzene, has also been shown to cause experimental porphyria in humans. An outbreak of cutaneous porphyria in Turkey[66] was related to the ingestion of seed-wheat treated with hexachlorobenzene as a fungicide, which initiated the work, which continues to the present into the effects of polyhalogenated hydrocarbons on haem biosynthesis.[67,68] Another therapeutic fungicide, griseofulvin, was also shown to be porphyrinogenic.[69]

The means by which each of these compounds, AIA, DDC, and hexachlorobenzene, influence the biosynthetic pathway are of interest since they provide a useful pointer to the diversity of influence of chemical compounds upon the processes of control of haem synthesis and provide the rationale for development of the lists of drugs contraindicated for use in acute porphyria.

Classification of Porphyrias

In his papers of 1911 and 1922, Günther[29,70] was the first to classify the diseases of porphyrin metabolism. In the first of these he quoted 14 cases from the literature in which acute symptoms of porphyria arose spontaneously, "haematoporphyria acuta," and 56 cases of "haematoporphyria acuta toxica," in which the symptoms were associated with the ingestion of sulfonal, trional, or veronal. He also defined and named, for the first time, the very rare condition, congenital porphyria, "haematoporphyria congenital" in which the predominating symptoms were due to skin photosensitivity. In Glasgow in 1898, McCall-Anderson[71] had described two brothers, both of whom had solar sensitivity and excreted "haematoporphyrin" in the urine. Meyer-Betz[72] was the first of Fischer's coworkers. He injected 200 mg of haematoporphyrin into his own veins in 1913. Knowing the marked photodynamic effect of this substance on mice, paramecia, and erythrocytes, from the studies of Hausmann,[73,74] he intended to remain indoors and expose himself very cautiously to light. However, he was a practicing physician and soon after the injection he received an urgent message from a sick patient and he felt obligated to make a house call. It was a sunny day and though he strove to avoid direct exposure to the sun, he was unsuccessful. Shortly thereafter, he developed an extreme solar urticaria of the hands and face. This experiment revealed the potent photosensitizing influence of this particular porphyrin.

In Günther's second work in 1922,[70] he elaborated on his first thesis, quoting further cases. He noted the possibility that acute haematoporphyria might be hereditary and suggested that people liable to develop acute or congenital haematoporphyria had a "porphyrism" with certain notable physical and mental characteristics—neurosis, insomnia, dark hair, and pigmented skin. In a survey of the clinical features of acute haematoporphyria he described a triad of symptoms which were commonly present, namely abdominal pain, constipation, and vomiting.

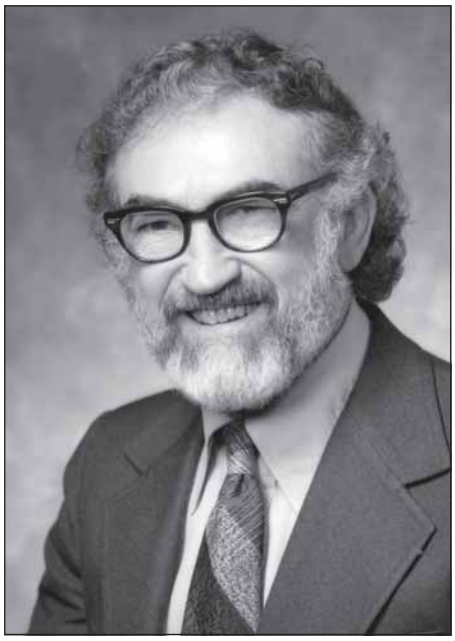

Figure 6. Sam Schwartz (courtesy of Studio Minneapolis).

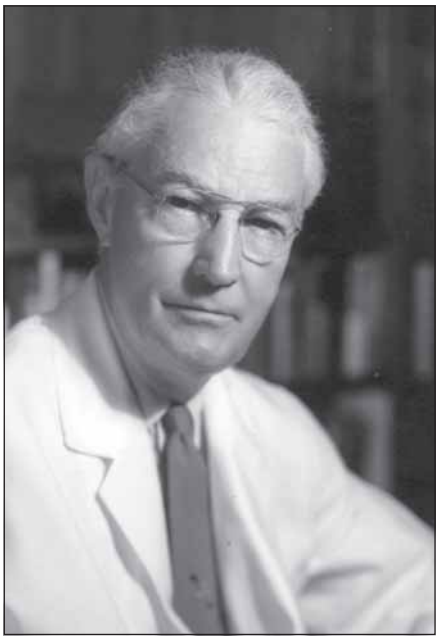

Figure 7. Cecil Watson (courtesy of Dublin Productions).

Hepatic and Erythropoietic Classification

Waldenström (1937)[75] made a clinical survey of 103 cases of acute porphyria found in Sweden. He reviewed some previously published cases of chronic haematoporphyria (Günther's classification) in which light sensitivity occurred some years after birth, at times associated with abdominal pain. For these cases, he substituted the name "porphyria cutanea tarda". This classification was further extended by Watson[76] in 1960, Goldberg and Rimington in 1962[62] and Eales[77] in 1979 (Figs. 7-9).

Classification into Acute and Nonacute Porphyria

The general aim in most classifications was to provide a clinical basis consistent with the known biochemical features. The full elucidation had however, to await the complete description of each of these diseases as a specific enzymic disorder of the haem biosynthetic pathway, a process that evolved over the years between 1955 and 1980.

The clinical manifestations of the porphyrias vary enormously. The traditional classification of the diseases as either hepatic or erythropoietic, dependent upon the primary site (or what was thought to be the primary site) of overproduction of the porphyrins, is inadequate. For that reason the classification into the acute and nonacute types of porphyria based upon the main clinical presentation, offers a more satisfactory means of subdivision of this group of diseases.

The major feature of these diseases is that they may be provoked into an acute attack with a neuropsychiatric or neurovisceral syndrome associated with increases in production and urinary excretion of the porphyrin precursors ALA and PBG. The reasons for these changes have been sought in a number of theories which presently have settled upon an excess of ALA, a deficiency of haem, or a combination of these two.[78] The neuropsychiatric or neurovisceral syndrome is not present in the nonacute porphyrias, which consist of congenital erythropoietic porphyria, erythropoietic protoporphyria, and PCT. The primary presenting feature in the nonacute porphyrias is skin photosensitivity.

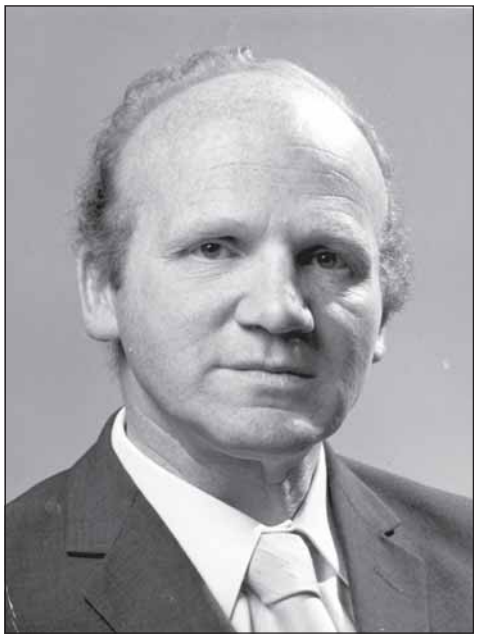

Figure 8. Abe Goldberg.

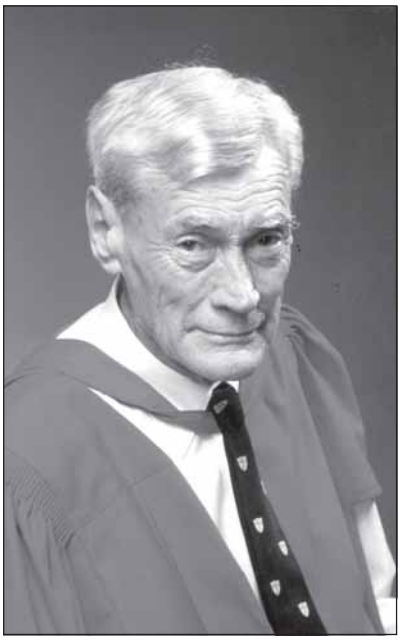

Figure 9. Len Eales.

Enzymes

With the description of the pathway sequence, the time was ripe for the elucidation of the catalytic steps—the enzymic description of the biosynthetic pathway.

Thereafter, each of the stages of the pathway was exhaustively examined. The most important point in this sequence is the first one, the formation of ALA by ALA synthase. Granick provided the evidence from his studies that this was indeed the control point of the biosynthetic pathway[79] (Fig. 10). The last enzyme of the sequence to be described was protoporphyrinogen oxidase.[80] It was clear however, that additional control points would have to be sought in the biosynthetic sequence. One such enzymic control point was demonstrated at porphobilinogen deaminase (PBG-D).[67,81] It was at this site in the pathway that the formation of uroporphyrinogen III was eventually elucidated by Jordan[82] and Battersby et al.[83]

Enzymic Classification

The most important feature of current levels of understanding of the porphyrias is that the metabolic disorder can, in all cases, be localized to one specific enzyme within the haem biosynthetic pathway.[81,84]

Thus, in the acute porphyrias the expression is largely hepatic. In acute intermittent porphyria, the defect has been shown to lie at the level of porphobilinogen deaminase (EC 4.3.1.8). In hereditary coproporphyria the defect lies at the level of coproporphyrinogen oxidase (EC 1.3.3.3), in variegate porphyria at protoporphyrinogen oxidase (EC 1.3.3.4), and in the exceptionally rare "plumboporphyria" at ALA dehydratase (EC 4.2.1.24). The resultant overproduction of coproporphyrinogen and protoporphyrin respectively in these diseases can account for their photocutaneous manifestations.

In the nonacute porphyrias, the expression is both hepatic and erythropoietic. The deficient enzymes are in congenital erythropoietic porphyria, uroporphyrinogen cosynthetase (EC 4.2.1.75), erythropoietic protoporphyria, ferrochelatase (EC 4.99.1.1); and PCT, uroporphyrinogen decarboxylase (EC 4.1.1.37). A classification based upon these enzymic lesions may have merit but

Figure 10. Sam Granick (courtesy of S. Sassa).

Figure 11. Claude Rimington (courtesy of Greta Rimington).

there are logistical problems in the application of such a classification, not the least of which is the difficulty in reproducible measurement of enzyme activities and the nonavailability of biopsy material upon which the estimations might be carried out.

Molecular Genetics

Molecular genetics have allowed a more fundamental recognition of the nature of the genetic defects in these diseases. The early concept that was shown to be true for *Staphylococcus aureus* was that the genes would all lie on one chromosome and be cotransducible; that is unfortunately not so. In humans the early investigations in this sphere have shown clearly that they are not clustered in the human genome, but dispersed among different chromosomes. From the work thus established, cDNA probes have been synthesized for these proteins, which are facilitating further investigation of the molecular defect and of the familial association of these diseases.

Acute Porphyria

Waldenström's studies in Sweden were greatly aided by the presence in his patients' urines of porphobilinogen, which forms a red color with Ehrlich's aldehyde reagent (an acidic solution of paradimethyl-amino-benzaldehyde). As early as 1890, Harley had noted that the urine of a case of sulfonal-induced acute porphyria contained a chromogen which, when oxidized, became a red pigment. Such a substance in the urine of a patient with acute porphyria gave a red coloration insoluble in chloroform, with Ehrlich's aldehyde reagent, and which was therefore not urobilinogen (Sachs, 1931). Waldenström (1937) showed that this was not only excreted in the urine of every one of his patients with acute porphyria, but that some apparently healthy relatives of these patients also excreted it. The idea of a "latent porphyria" was thus conceived. Later, Waldenström and Vahlquist (1939) considered that the Ehrlich-reacting chromogen, which they named porphobilinogen and partly purified, was a dipyrromethane.

The liver and kidney of fatal cases of acute porphyria contained porphobilinogen[85,86] which was isolated from the urine of a patient with acute porphyria in University College Hospital in London,[53] Cookson and Rimington[54] showed it to be a monopyrrole and Haeger[87] found that two-thirds of patients with latent or manifest acute intermittent porphyria excreted excess 5-aminolevulinic acid (ALA) in addition to increased porphobilinogen (Fig. 11).

Concurrent Porphyria

Concurrent porphyria has been defined as two differing types of porphyria occurring in the same individual.[88] A large kindred in Chester have been described, with excretion and enzymic patterns of both acute intermittent porphyria and variegate porphyria (both PBG-deaminase and protoporphyrinogen oxidase with low activity). In another study, Day et al[89] reported 25 patients with a variant of variegate porphyria combined with porphyria cutanea tarda which they called "dual porphyria." Studies performed in six of these dual porphyria patients by Meissner et al[90] showed that erythrocyte uroporphyrinogen decarboxylase activity was reduced as was protoporphyrinogen oxidase activity. Such parallel inheritance need not imply that there is further genetic disadvantage since the effects of multiple enzyme inhibition in the pathway are not necessarily additive, as is shown by the example of lead poisoning.[91] This is not the case for homozygous porphyrias in which the dermatological and other features are usually severe.[92]

Neuropathy

The acute porphyrias have in common the features of acute abdominal pain, limb weakness, and neuropsychiatric presentation. This symptomatology can be explained on a neurogenic basis. Despite advances in genetics and biochemistry, the link between these biochemical abnormalities and the neuropathological and clinical manifestations remains unclear. The earliest case of acute porphyria examined pathologically was that of Campbell[93] in 1898 who failed to find any nervous system abnormality. However, in 1903, Erbslöh[94] described features of axonal demyelination in the femoral nerve from a porphyric patient who had died after treatment with sulfonal. Mason[95] and coworkers (1933) showed that the most characteristic lesions were seen in the nervous system and affected peripheral nerves and sympathetic ganglia.

Psychiatric Aspects

The acute attack may reveal a variety of psychiatric manifestations, including anxiety, depression, and frank psychosis. The literature on the psychiatric features is sparse. The earliest cases were described by Copeman[96] in 1891 and by Campbell in 1898. It is clear that a frequent misdiagnosis of hysteria is made, although Brugsch[97] recognized it as a distinct psychosis in his review of the literature. The available data show that the psychopathology is related to affective neurotic, rather than psychotic, features and a truly schizophreniform presentation has not been observed.[84] Tishler[98] and coworkers in 1985 in 3867 psychiatric inpatients found that 0.2% (8 patients) who experienced episodic psychosis and depression had confirmed acute intermittent porphyria. In general terms, the psychiatric phenomena may be expected in up to 70% of acute attacks.

Monopyrroles in Porphyria and Other Disorders

It is intriguing that disturbances of porphyrin metabolism have been observed in schizophrenics and that excesses of a monopyrrole-hydroxyhaemopyrrole lactam are present in urine of patients with acute porphyria and some psychiatric disorders.[95,96,97,99,100,101] Prior to its discovery in the urine of porphyric patients an association between "mauve factor" excretion and psychiatric illness had been made. Typically the factor was shown to be present in up to 50% of endogenous psychoses and 70% of acute schizophrenia.

Mauve factor was identified using a chromatographic method that suggested it was an alkyl pyrrole. Mass spectrometric analysis of a single component showed a molecular ion that was suggestive of a mono-ethyl dimethyl pyrrole that led to the hypothesis that the "mauve factor" was essentially due to kryptopyrrole. Subsequent studies by Graham et al showed the identification to be incorrect and in fact due to the heterologous pyrrole, haemopyrrole in its lactam form.[101,102]

Nonacute Porphyrias

Congenital Porphyria

Congenital porphyria is one of the rarest of the porphyrias, but was the first recorded case of porphyria in the literature, probably because of the severity and dramatic nature of its symptoms. The case was described by Schultz in 1874. His patient was a 33 year-old weaver who had suffered from photosensitivity of the skin from the age of three months. His urine was wine red and contained pigments which resembled Hoppe-Seyler's acid haematoporphyrin. At autopsy, his bones were found to be dark brown in color. Further case reports were made by other authors.[71,103,104] Günther[29] named the disease haematoporphyria congenital in 1911.

In subsequent autopsies of two cases of congenital porphyria, the porphyrins were found to be concentrated in the bone marrow rather than in the liver. Congenital porphyria was therefore renamed "porphyria erthyropoietica". In subsequent patients examined two different types of normoblast were found, an abnormal type which exhibited marked porphyrin fluorescence, apparently in the nuclei (fluorocytes), and other normal normoblasts without such fluorescence. It was then thought that these abnormal normoblasts which carried the trait representing the inborn error of metabolism.[105]

Porphyria Cutanea Tarda

Porphyria cutanea tarda (PCT) is the most common form of porphyria. Patients present with cutaneous photosensitivity but do not experience attacks of neurovisceral dysfunction. PCT differs from the other porphyrias in that there is no clear pattern of inheritance in the majority of cases although in some, familial transmission can clearly be established. Waldenström[106] introduced the term "porphyria cutanea tarda" (PCT) in 1957.

PCT is usually classified into two main types based on the relative importance of inherited and acquired factors.[107] The majority of patients may be classified as **sporadic**. These patients have no family history of the disorder and its development appears largely related to chronic alcohol ingestion or the use of the contraceptive pill. Erythrocyte URO-D activity is normal in these patients although hepatic URO-D activity is depressed. In the sporadic or toxic disease polyhalogenated hydrocarbons have been shown to be the etiological agent in some cases. The most common precipitant is however, alcohol ingestion, which is known to disturb porphyrin metabolism in normal subjects.[108] Orten et al[109] studied the urinary excretion of porphyrins and precursors in chronic alcoholics and noted increased coproporphyrin excretion but no significant increase in excretion of uroporphyrin, ALA, or PBG.

Toxic Porphyria

If one excludes the porphyria caused by sulfonal, toxic porphyria in humans only became clearly established after 1957 when there was a disastrous outbreak of porphyria among Turkish peasants who had inadvertently ingested the fungicide, hexachlorobenzene, with their wheat and bread.[66,110] In this Turkish population, Günther's postulate of "toxic porphyria" in his early classification was fully substantiated.

Hepatoerythropoietic Porphyria

Hepatoerythropoietic porphyria is a homozygous form of familial PCT first reported by Pinol-Aguade[111] in 1969. This topic is well reviewed by Smith (1986).[112] All have exhibited severe mutilating photosensitivity from birth. In addition, there are hepatic changes and there is usually a mild normochromic normocytic anemia. URO-D activity examined in erythrocytes and fibroblasts is markedly reduced to less than 10% of normal.

All the above forms of PCT present with a similar clinical picture of cutaneous photosensitivity and an identical porphyrin excretion pattern due to reduced hepatic URO-D activity. However the mechanism of the enzyme defect appears to vary, genetic factors being most important in the familial type and acquired factors in the sporadic or toxic type.

Erythropoietic Protoporphyria

Erythropoietic protoporphyria is principally characterized by acute solar photosensitivity. The nature of the disease was first clearly established in 1961 by Magnus et al[113] and Langhof et al[114] although there had been earlier reports.[115] Despite its late description this is a relatively common condition although its prevalence has not been precisely calculated. Onset may occasionally be much later and not necessarily include many of the described features of this disease.[116]

Porphyria in Animals

Hepatic Porphyria

Since acute intermittent porphyria is the most common of the porphyrias affecting humans, one might expect that cases of this condition would have been encountered in animals but, up to the present, no clear example of acute intermittent porphyria in animals has been reported. This might be explained by the difficulties in diagnosis of the disease, should it occur in animals.

Congenital Porphyria in Cattle

Several reports described the finding of chocolate or brown-colored bones in abattoir carcasses. The first such report was by Brouwier[117] in 1884, followed a year later by one from Tappeiner[118] of red-brown bones in swine. Mosselman and Hebrant[119] were convinced that it was formed from haemoglobin, while Ingier[120] thought it to be melanin associated with another pigment derived from chlorophyll. The similarity between this condition in animals and human congenital porphyria was recognized by Schmey,[121] who proposed for it the name "osteohaemochromatosis" instead of the misleading "ochronosis" with which it had previously been known. That the pigment in the bone was a porphyrin seems to have been first clearly realized by Möller-Sorensen[122] in 1920. Credit for the suggestion of its hereditary nature should probably go to Witte.[123]

Studies in South Africa

In 1936, a herd of grade short horn cattle was discovered in South Africa in which no fewer than 13 cases of congenital porphyria were seen.[124] Excessive quantities of uroporphyrin I and coproporphyrin I were found in the organs and body fluids and there was definite evidence of photosensitization. The urine was wine red in color and was rich in uroporphyrin and coproporphyrin; the faeces, erythrocytes, and plasma were also rich in coproporphyrin I. The entire skeleton was deep brown in color and afforded large quantities of uroporphyrin I.[62] The inheritance was relatively easy to trace as autosomal recessive.

Porphyria in Pigs

As with cattle, dark-colored bones had been noticed in pig carcasses long before living cases of congenital porphyria were diagnosed.[118,120,121,125] The first living cases were observed in New Zealand and reported by Clare and Stephens.[126] In Denmark, what appeared to be congenital porphyria in pigs appeared in the Thisted district during 1951 and 1954.[127] Affected animals had discolored teeth displaying pinkish-red fluorescence in ultraviolet light. A closer study of the teeth showed that the porphyrin was not evenly distributed, but was mainly located in the dentine layer just below the enamel.[128]

Cats and Dogs

In 1964, a report appeared of a young kitten whose deciduous teeth were brownish with red fluorescence in ultraviolet light and whose urine had been blood-colored since the cat was two months old; it was otherwise normal. When the permanent teeth erupted they were lighter in color and devoid of fluorescence. A littermate and some kittens from a former litter of the mother also had discolored teeth, suggesting a dominant inheritance.[129] The absence of skin photosensitivity, dominant inheritance, and decrease of porphyrin deposition with age is similar to the porphyria found in pigs.

In studies in Cape Town it has been shown that many dogs have abnormally high excretion of coproporphyrin in urine, which may relate to their carnivorous, rather than omnivorous, eating patterns. Owen[130] et al (1962) described a young dog with permanent teeth showing a transient pink color in ultraviolet light, but both disappeared after several months.

Porphyrin Synthesis in the Animal Kingdom

Free porphyrins in varying amounts are found in widely, although somewhat erratically, distributed in most living organisms. Microbial porphyrins represent a useful source of material for the examination of porphyrin synthesis,[131] for example *Rhodobacter sphaeroides* was used by Lascelles[132] in her studies of the control of porphyrin synthesis.

Among the mammals, a few genera appear to produce much more porphyrin than others; these belong to the family of rodents. The rat produces a relatively large quantity of protoporphyrin, by synthesis in the harderian glands. Squirrels also produce much porphyrin and their bones have a pale-brown color due to deposition of uroporphyrin and fluoresce pale red in ultraviolet light. This is most marked in the American fox squirrel, *Sciurus niger.*[133] Only *Tamias striatus* has this in common with *S. niger*[134] Levin and Flyger[135] found reduced activity of the enzyme uroporphyrinogen III cosynthetase in haemolysates and tissue extracts from fox squirrels as compared with grey squirrels. The animals appear in every way normal without untoward symptoms accompanying their high porphyrin production and must therefore, be regarded as physiological examples of excess porphyrin synthesis. Analogous to this are the molluscs that deposit quantities of uroporphyrin in their shells,[136] or among other higher forms of animal life, the group of birds known as the touracos, or plantain eaters, who utilize the copper complex of uroporphyrin III, called turacin, for the deep-red areas of pigmentation in their flight feathers.[27,137,138] Porphyrins are used in the coloration of eggs,[139] probably by deposition within the oviduct.[140]

The Harderian Gland

Johann Jakob Harder (1656-1711) found and named the retro-orbital gland in the deer, which he named glandula nova lachrymalis in his publication of 1694.[141] Since then, many investigators have found that this gland is present not only in some mammals but also in amphibians, reptiles and birds. The precise purpose of the gland remains an enigma and has probably not been studied in greater depth because of its absence in primates.[142]

The gland has a remarkable capacity to synthesis porphyrins.[140] In particular, the presence of many of the porphyrins of the latter part of the biosynthetic pathway are found in the gland in rodents in such quantities that intermediates such as tri-carboxylic haemoporphyrin can be isolated and characterised in the gland.[143,144,145] Numerous studies have shown that porphyrin production in the gland is profoundly influenced by the presence of steroids.[146,147]

The harderian gland in the golden hamster is an extremely rich source of porphyrins.[148] It has been extensively studied in this rodent. Although the female gland is arguably the richest natural source of porphyrins known, the male gland contains little porphyrin, possibly because the rate-limiting enzyme, 5-aminolevulinic acid (ALA) synthase is more active in the female than the male.[149] All of these, including the role of hormone replacement,[144] emphasize the role that steroids play in porphyrin synthesis and the activities of the enzymes of the biosynthetic pathway.

Phototherapy and Cancer

The early foundation of photochemotherapy may be sought in the work of the 1903 Nobel laureate Neils Finsen. Thereafter, Hausmann in 1908 and 1911[73,74] showed that haematoporphyrin photosensitized both paramecia and mice. Normal human tissue also reacts in a pathological fashion when saturated with porphyrins and exposed to light, as was so clearly demonstrated by Meyer-Betz in 1913.[72] It is a simple step to conclude that this photoreaction might be usefully employed in the destruction of pathological tissue. This was aided by the early description in 1924 by Policard.[150] of the preferential uptake by rat sarcoma of haematoporphyrin. That injected porphyrins accumulated in tumors were observed by Körbler.[151] The work in 1955 of both Schwartz[152] and coworkers and Rassmussen-Taxdal et al[153] is of interest since it pointed the way for future work in humans.

Haematoporphyrin Derivative

The foundation of the present use of haematoporphyrin derivative is based on the work of Lipson et al[154] who showed that some components of haematoporphyrin derivative (HPD) were better localized in malignant tissue than "crude" haematoporphyrin and in the development of lasers. Such chemical treatment of the haematoporphyrin produces a complex mixture of substances, such as

mono- and diacetates of haematoporphyrin, protoporphyrin, deuteroporphyrin, and, in particular, a dihaematoporphyrin ether or ester.[155,156,157]

5 Aminolevulinate

In the second half of the twentieth century it became obvious that 5 Aminolevulinate (ALA) could be converted to porphyrins. In his early study in 1955 Scott[158] showed that, when given to humans, a dose of ALA gave rise to a transient but marked photosensitivity with erythema of the exposed skin resembling mild sunburn reaching a maximum between 9 and 12 hours. The transient nature of the ALA induced photoreaction meant that it too was considered as a potential candidate for use in phototherapy. By bypassing the rate limiting stage in haem biosythesis, through the use of ALA with consequent overproduction of porphyrins 'in situ', would allow such tissues to become photosensitized and subject to photodestruction, given the appropriate dose of photoactivating light. As a consequence of this, ALA based photodynamic therapy has taken its place in the treatment of cancer with considerable successes in the succeeding years.[159,160]

Mechanism of Effect

The fundamental principle behind this therapy is that since tumor tissue will preferentially accumulate porphyrins, these may be used for both identification and therapy of neoplastic tissue. Unfortunately, other metabolically active tissue, such as liver and kidney, also accumulate porphyrins, but being remote from light sources these are relatively safe from the photodestructive effects of therapy. The red fluorescence seen in ultraviolet light of tumors after injection of various porphyrins, has proved of help to the surgeon in the localization of neoplasms during an operation. A serious side effect of therapy is continued light photosensitivity of exposed skin for a considerable time after treatment.

This area of study is one of which work continues to seek better localizers and/or sensitizers, based either on specific synthesis of porphyrin derivatives or through the use of other naturally occurring porphyrins or synthetic porphyrins and in the development of better light sources. At the present time, the clinical interest in this subject has been stimulated by technological developments. These relate principally to the development of better laser light sources such as gold vapor lasers and other forms of tunable lasers and endoseopic probes.

Retrospective Diagnoses

Royal Malady

The history of the porphyrias is naturally only truly reliable from the time at which there was concurrent medical observation and scientific mensuration. Any studies prior to the well-documented works at the end of the last century are, therefore, liable to be steeped in anecdotal inaccuracy. It is however, of interest to consider the hypothesis pro-pounded by MacAlpine and Hunter in 1964 that porphyria, possibly variegate porphyria, was present in the Royal Houses of Stuart and Hanover in the United Kingdom.[161] The ability to carry out such investigations depended not only on the inevitable extensive documentation of royalty, but also on the very precise descriptions conveyed to us over time by their physicians.

Of these, one of the more remarkable is that of Sir Theodore Turquet de Mayerne, physician to James, VI and I. He described one acute episode, following a hunting trip:

> *"On his return he passed blood red urine.... He also told me that he quite frequently passed water, red like Alicante wine but without attendant pain...."*

Mary Queen of Scots had suffered similarly to her son from acute attacks of abdominal colic, described as

> *"...he labored under painful colic from flatus (an affliction from which his mother also suffered)..."*

The history of the "Royal Malady" took a further intriguing turn following the studies of Röhl, Warren and Hunt[162] in 1998. These authors pursued a scientific/medical detective investigation, as a consequence of which, they discovered the bones of Princess Feodora of Reuss in Poland. Following exhumation, bone samples were obtained for DNA analysis. The authors were similarly able to obtain skeletal samples from Theodora's mother, Princess Charlotte of Saxe-Meiningen.

Mitochondrial DNA can be used to establish family relationships provided that there is a continuous maternal line of descent. Czarina Alexandra and Princess Charlotte were first cousins and importantly both were daughters of Queen Victoria's daughters, Princess Alice and Princess Victoria respectively. All should consequentially have inherited the same mitochondrial DNA from Queen Victoria. Such analysis proved that the bones from Princess Feodora's grave did not derive from the Romanov line. However those from Princess Charlotte's grave did derive from that line. As a consequence of the DNA analysis the workers were able to show that there was a novel protoporphyrinogen oxidase gene in Princess Charlotte's bones. Finally the workers found that there was convincing evidence that Prince William of Gloucester had variegate porphyria. Prince William was tragically killed in an aircraft accident in 1972 and in consequence DNA evaluation has never been possible, but the clinical evidence was sufficiently robust to convince Sir Abraham Goldberg and Dr Geoffrey Dean of its merit.

The historical implications of these observations are profound and, if true could imply that the loss by Britain of the American Colonies could be ascribed to a genetic disease[163] and, potentially that Kaiser Wilhelm, whose mother was Princess Victoria could have suffered from the psychiatric features of Variegate Porphyria.[164]

Vincent Van Gogh

Retrospective diagnosis of porphyria continues to be a matter of interest to many workers throughout the world. Wilfred Arnold[165] studied the history of Vincent van Gogh in some detail, his descent into psychiatric illness and the reasons for its causation. Many of the features he exhibited were consistent with acute porphyria precipitated by chemicals such as the solvents and alcohol to which he was exposed. No objective evidence of acute porphyria in his family has yet been discovered. However the subjective evidence is highly suggestive of this condition.

Werewolves

A bizarre suggestion has been that persons with congenital porphyria or hepatoerythropoietic porphyria or other of the homozygous porphyrias were the werewolves or vampires of legend. Lycanthropy (magical transformation of human to wolf) certainly did not take place, but the subjects' skin mutilation, hypertrichosis, and desire to eschew light exposure may have led the superstitious to this conclusion.[166] The medieval descriptions of werewolves included: "pale yellowish excoriated skin"—explicable by haemolytic anemia and pruritis; reddish teeth—erythrodontia; and "habitation in isolated regions, such as Central European valleys"—familial association and inbreeding in such areas. It is not easy to explain either fear of garlic or lust for blood. The effects of garlic might have related to the oxidative metabolism of diallyl disulfide. In this the needs of the patient should be considered. The press hysteria in the United States following these disclosures, established an inaccurate and unjustified perception of porphyria in the public mind and did considerable harm to the porphyric patient.[167]

Ephemera: Porphyrinurias

The heterogeneous group of diseases best described as porphyrinurias are those in which the disturbances of porphyrin metabolism have been brought about by endogenous and exogenous factors other than the genetic ones linked to the porphyrias. The porphyrin normally excreted in excess is coproporphyrin. This category includes lead poisoning, hereditary tyrosinemia,[168] ethanol abuse,[108] myocardial infarction,[169,170] and the effects of drugs like carbamazepine[171] and many other compounds such as polyhalogenated hydrocarbons.[172] Coproporphyrinuria has also been reported in a number of conditions such as liver disease and hepatocellular carcinoma.[37,173] Porphyrins are probably hepatotoxic and may even be associated with the development of neoplasms.[174] When Lithner and Wetterberg[175] carried out a retrospective study of 20 years of the relationship between hepatocellular carcinoma and acute intermittent porphyria, they found this to be significant.

In addition to those porphyrinurias, there are changes in the pattern of porphyrin isomer excretion in the hyperbilirubinemias of the Dubin-Johnson syndrome and in the Rotor syndrome. In these two conditions the quantity of porphyrin synthesized and excreted is not necessarily in excess of normal upper limits, but they are associated with excess production of series I isomer porphyrin.[176-178] A key worker in this area was Peniti Koskelo (Fig. 12).

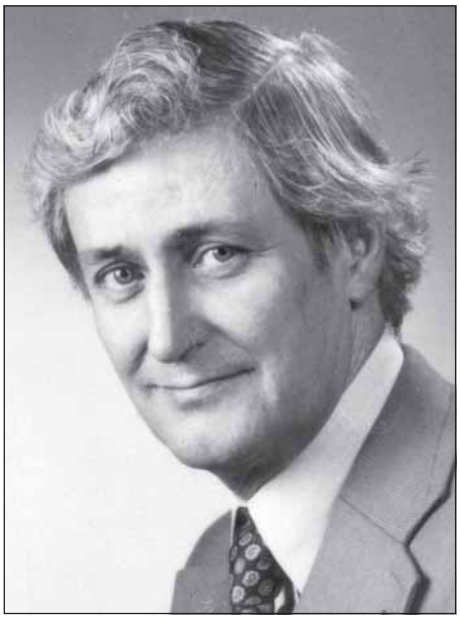

Figure 12. Peniti Koskelo.

Lead

The connection between lead and porphyrin biosynthesis is reputed to have been first made by Binnendjik (cited by Stokvis, 1895), but the connection between this metal and anemia had been made very much earlier by Lannaec[179] in 1831. This was confirmed by Garrod in 1892[33] who observed abnormal porphyrin excretion in the urine of a patient with lead poisoning and then in 1895 by Stokvis[31] who found that lead-poisoned rabbits excreted excess urinary porphyrins. Other effects of lead on the haemopoietic system were reported before the end of the century by Behrend,[180] who observed stippled basophils in the blood of a patient with lead poisoning.

Lead Porphyrinemia and Porphyrinuria

The crude compound that Garrod identified as haematoporphyrin was shown to be coproporphyrin III by Duesberg.[181] Liebig[182] had suggested that it was produced by the action of lead on the bone marrow. By 1932 Grotepass.[183] had demonstrated elevated coproporphyrin in urine in lead poisoning.

The increased concentration of a free porphyrin in blood was identified by Hijmans van den Bergh et al[184] in 1932 as protoporphyrin IX located in the erythrocytes of subjects dosed with lead. In 1958, finally, diminution of ALA dehydratase activity (ALA-D) was identified as a means of lead assessment.[185]

Arsenic

In 1960, studies of hair taken from Napoleon Bonaparte by the University of Glasgow showed that his hair contained considerable concentrations of this metal. Subsequent activation analysis has also found increased arsenic in his hair and would potentially confirm this diagnosis.

Of greatest interest here is arsenic's impact on haem biosynthesis and in particular upon uroporphyrin decarboxylase, especially when one juxtaposes this information against the recent findings by Warren (personal communication) that George III had substantial levels of arsenic in his hair (100 times background). The reasons for such high levels could be sought in the use of arsenic both as a medicine and as a hair powder.

Woods and Fowler[186] showed haem biosynthetic changes associated with arsenic and subsequent studies have confirmed that not only is there increased excretion of porphyrins[187] with decreased activity of uroporphyrinogen decarboxylase which might be used as a biomarker of arsenic exposure.[188,189] Concordant with the hypothesis that As could have contributed to exacerbation of acute porphyria in George III is the finding of precipitation of variegate porphyria in a patient from southern USA who consumed "moonshine" contaminated with lead and arsenic.[190]

Pseudoporphyria

Poh-Fitzpatrick[191] pointed out in 1986 that the term "pseudoporphyria" had been used to describe a bullous dermatosis associated with a number of dermatological conditions that bear some resemblance to porphyria, often induced by many drugs but which were not porphyrias! The term "pseudoporphyria" should not be used to describe them, but only to describe conditions in which alterations of porphyrin metabolism can be found, such as the bullous dermatosis of haemodialysis.[192]

Mycosis Porphyria

In addition to hereditary porphyria in cattle, an acquired form has been described, attributable to a fungal infection.[193] Similar to this was the case reported by Lim et al[194] of a 24 year-old man who had increased faecal porphyrin excretion, resembling that seen in variegate porphyria. The abnormal faecal porphyrins were shown to be the result of excessive consumption of brewer's yeast, which was shown to have a high porphyrin content.

References

1. Lecanu LR. Études chimiques sur le sang humain (cited by Berzelius, 1840), 4º Paris No. 395. 1837.
2. Schultz JH. Ein fall von pemphigus leprosus, complicirt durch lepra visceralis. Griefswald: Inaugural Diss, 1874.
3. Baumstark F. Zwei pathologische Harnfarbstoff. Pflugers Arch ges Physiol 1874; 9:568-584.
4. Garrod AE. Inborn Errors of Metabolism. 2nd ed. London: Hodder and Stoughton, 1923.
5. Biosynthesis of tetrapyrroles and of corrinoids. In: Florkin M, Stotz EH, eds. Comprehensive Biochemistry: A History of Biochemistry. Amsterdam: Elsevier, 1979:193-238.
6. Moore MR, McColl KEL, Goldberg A. The porphyries. Diabet Metab 1979; 5:323-336.
7. Hodgson GW, Baker BL. Porphyrin abiogenesis from pyrrole and formaldehyde under simulated geochemical conditions. Nature 1967; 216:29-32.
8. Mercer-Smith JA, Mauzerall DC. Photochemistry of porphyrins-a model for the origin of photosynthesis. Photochem Photobiol 1984; 39:397-405.
9. Bonnett R, Czechowski F. Metalloporphyrins in coal-gallium porphyrins in bituminous coal. J Lab Chem Sci 1984; 1:125-132.
10. Hodgson GW. Cosmochemical evolution of large organic molecules-illustrative laboratory simulations for porphyrins. Ann NY Acad Sci 1972; 194:86-97.
11. Barnes CR. On the food of green plants. Bot Gaz 1893; 18:403-411.
12. Gest HO. History of the word photosynthesis and evolution of its definition. Photosynthesis Research 2002; 73:7-10.
13. Pelletier F, Caventou JB. Ann Chim Phys 1818; 9(2):194; (J Pharm 1818; 3:486).
14. Rüdiger W. Chlorophyll metabolism: From outer space down to the molecular level. Phytochemistry 1997; 46:1151-1167.
15. Fischer H, Orth H. Die chemie des Pyrrols. Leipzig: Adademische Verlagsgesellschaft, 1940.
16. Woodward RB. The total synthesis of chlorophyll. Pure Appl Chem 1960; 2:383-404.
17. Beale SI, Gough SP, Granick S. Biosynthesis of δ-aminolevulinic acid from the intact carbon skeleton of glutamic acid in greening barley. Proc Natl Acad Sci USA 1975; 72:2719-2723.
18. Von Wettstein D, Gough S, Kannangara CG. Chlorophyll Biosynthesis. The Plant Cell 1995; 7:1039-1057.
19. Battersby AR. Biosynthesis of the pigments of life. Proc R Soc Lond B 1985; 225:1-26.
20. Berzelius JJ. Lehrbuch der Chemie. Arnoldische Buchbandlung. Leipzig: Dresden, 1840:67-69.
21. Scherer J. Chemische-physiologische untersuchungen. Ann Chem Phar 1841; 40:1-64.
22. Mülder GH. Über eisenfreises hämatin. J Prakt Chem 1844; 32:186-197.
23. Thudichum JLW. Report on researches intended to promote an improved chemical identification of disease. 10th Report of The Medical Officer. London: Privy Council, H.M.S.O., 1867:152-233, (App. 7).
24. Hoppe-Seyler F. Das hämatin. Tubinger Med Chem Untersuchungen 1871; 4:523-533.
25. MacMunn CA. Further researches into the colouring-matters of human urine, with an account of their artificial production from bilirubin, and from haematin. Proc Roy Soc Lond Ser B 1880; 31:206-237.
26. Hoppe-Seyler F. Über das chlorophyll der pflanzen. Hoppe-Seyler's Z Physiol Chem 1879; 205:193-197.
27. Church AH. Researches in Turacin an animal pigment containing copper 11. Philos Trans Roy Soc Lond Ser A 1892; 183:511-530.
28. Soret JL. Récherches sur l'absorption des rayons ultraviolets par diverses substances. Arch Sci Phys Nat 1883; 10:430-485.
29. Günther H. Die hämatoporphyrie. Deutsch Arch Klin Med 1911; 105:89-146.
30. Hammarsten O. Tva fall af hamatoporphyrin i urinen. Uppsala Lak For Forh 1891; 26:259-288.
31. Stokvis BJ. Zur pathogenese der hamatoporphyrinurie. Z Klin Med 1895; 28:1-9.
32. Salkowski E. Über vorkommen und nachweis des hämatoporphyrins in härn. Hoppe-Seyler's Z Physiol Chem 1891; 15:286-309.
33. Garrod A. On the occurrence and detection of haematoporphyrin in the urine. J Physiol 1892; 13:598-620.

34. Fischer H. Uber das urinporphyrin. 1. Mitteilung. Hoppe-Seyler's Z Physiol Chem 1915; 95:34-60.
35. Nencki M, Sieber N. Über das hämatoporphyrin. Arch Exp Pathol Pharmacol 1888; 24:430-446.
36. Saillet H. De l'urospectrine (ou urohematoporphyrine urinale normlae). Rev Med 1896; 16:542-552.
37. Fischer H, Zerweck W. Über uroporphyrinogen heptamethylester und eine neue uberfuhrung von uro-koproporphyrin. Hoppe-Seyler's Z Physiol Chem 1924; 137:242-264.
38. Laidlaw PP. Some observations on blood pigments. J Physiol 1904; 31:464-472.
39. Küster W. Beitrage zur Kenntnis des Bilirubins und Hämins. Hoppe Seyler's Z Physiol Chem 1912; 82:463-483.
40. Willstätter R, Mieg W. Untersuchungen über das chlorophyll. Liebigs Ann Chem 1906; 350:1-47.
41. Willstätter R, Stoll A. Untersuchungen über chlorophyll. Methoden und ergebnisse. Berlin: Springer, 1913:424.
42. Fischer H, Hilmer H, Lindner F et al. Chemische befunde bei einem fall von porphyrie (Petry). Hoppe-Seyler's Z Physiol Chem 1925; 150:44-101.
43. Borst M, Königsdörffer H. Untersuchungen uber porphyrie mit besonderer berucksichtigung der Porphyrie Congenita. Leipzig: Hirzel, 1929.
44. Schumm O. Uber die naturlichen porphyrine. Z Physiol Chem 1924; 126:169-202.
45. Fischer H, Orth H. Chemie des Pyrrols. Leipzig: Acad Verlag, 1937.
46. Sachs P. Ein fall von akuter porphyrie mit hochgradiger muskelatrophied. Klin Wschr 1931; 10:1123-1125.
47. Waldenström J, Vahlquist BC. Studien uber die entstehung der roten harnpigmente (uroporphyrin und porphobilin) bein der akuten porphyrie aus ihrer farblosen vorstufe (porphobilinogen). Hoppe-Seyler's Z Physiol Chem 1939; 260:189-209.
48. Shemin D, Russell CS, Abramsky T. The succinate glycine cycle. 1. The mechanism of pyrrole synthesis. J Biol Chem 1955; 215:613.
49. Muir HM, Neuberger A. The biogenesis of porphyrins. 2. The origin of the methyne carbon atoms. Biochem J 1950; 47:97-104.
50. Shemin D, Russell CS. Succinate glycine cycle. J Amer Chem Soc 1953; 75:4873-4875.
51. Neuberger A, Scott JJ. Aminolaevulinic acid and porphyrin synthesis. Nature 1953; 172:1093-1094.
52. Falk JE, Dresel EIB, Rimington C. Porphobilinogen as a porphyrin precursor and interconversion of porphyrins in a tissue system. Nature 1953; 172:292.
53. Westall RG. Isolation of porphobilinogen from the urine of a patient with acute porphyria. Nature 1952; 170:614.
54. Cookson GH, Rimington C. Porphobilinogen. Biochem J 1954; 57:476-484.
55. Kast A. Über die art der darreichung und verordnung des sulfonals. Ther Mh 1888; 11:316-319.
56. Stokvis BJ. Over twee zeldsame kleurstoffen in urine van zicken. Ned Tijds Geneesk 1889; 13:409-417.
57. Harley V. Two fatal cases of an unusual form of nerve disturbance associated with red urine, probably due to defective tissue oxidation. Brit Med J 1890; 11:1169-1170.
58. Ranking JE, Pardington GL. Two cases of haematoporphyrin in the urine. Lancet 1890; ii:607-609.
59. Dobrschansky M. Einiges uber malonal. Wiener Med Presse 1906; 47:2145.
60. De Matteis F. Disturbances of liver metabolism caused by drugs. Pharm Rev 1967; 19:523-550.
61. Schmid R, Schwartz S. Experimental porphyria: Hepatic type produced by sedormid. Proc Soc Exp Biol Med 1952; 81:685-689.
62. Goldberg A, Rimington C. Diseases of Porphyrin Metabolism. Springfield: Thomas, 1962:I:11.
63. Solomon HM, Figge FHJ. Disturbance in porphyrin metabolism caused by feeding diethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate. Proc Soc Exp Biol Med 1959; 100:583-586.
64. Tephly TR, Coffman BL, lngall G et al. Identification of N-methylprotoporphrin IX in livers of untreated mice and mice treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine-source of the methyl group. Arch Biochem Biophys 1981; 212:120-126.
65. De Matteis F, Marks GS. The effect of N-methylprotoporphyrin and succinylacetone on the regulation of haem biosynthesis in checken hepatocytes in culture. FEBS Lett 1983; 159:127-131.
66. Cam C. Report on few cases of congenital porphyria. Nester (Instanbul) 1957; 1:2-6.
67. Elder GH. Acquired disorders of haem synthesis. Essays Med Biochem 1976; 2:75-114.
68. Strik JJTWA, Koeman JH. Chemical Porphyria in Man. Amsterdam: Elsevier North-Holland, 1979.
69. De Matteis F, Rimington C. Disturbance of porphyrin metabolism caused by griseofulvin in mice. Brit J Dermatol 1963; 75:91-104.
70. Günther H. Die bedeutung der hamatoporphyrinurie in der physiologie und pathologie. Ergebnisse der Allgemienen Pathol Anat 1922; 20:608-764.
71. McCall-Anderson T. Hydroa aestivale in two brothers, complicated with the presence of hematoporphyrin in the urine. Brit J Dermatol 1898; 10:1-4.

72. Meyer-Betz F. Untersuchungen über die biologische (photodynamische) wirkung des hämatoporphyrins und anderen derivate des blut und gallenfarbstoffs. Deutsch Arch Klin Med 1913; 112:476-503.
73. Hausmann W. Uber die sensibilisierende wirkung tierischer farbstoffe. Anwendung Biochem Z 1908; 14:275-283.
74. Hausmann W. Die sensibilisierende wirkung des hamatoporphyrins. Biochem Z 1911; 30:276-316
75. Waldenström J. Studien uber porphyries. Acta Med Scand Suppl 1937; 82:120.
76. Watson CJ. The problem of porphyria-some facts and questions. N Engl J Med 1960; 263:1205-1215.
77. Eales L. Clinical chemistry of the porphyries. In: Dolphin D, ed. The Porphyrias. New York: Academic Press, 1979:665-793.
78. Yeung-Laiwah AC, Moore MR, Goldberg A. Pathogenesis of acute porphyria. Quart J Med 1987; 63:377-392.
79. Granick S. Porphyrin synthesis in erythrocytes. 1. Formation of 5-aminolaevulinic acid in erythrocytes. J Biol Chem 1963; 232:1101-1117.
80. Jackson AH, Games DE, Couch P et al. Conversion of coproporphyrinogen III to protoporphyrin IX. Enzyme 1974; 17:81-87.
81. Brodie MJ, Moore MR, Goldberg A. Enzyme abnormalities in the porphyrias. Lancet 1977; ii:699-701.
82. Jordan PM, Burton G, Nordlov H et al. Preuroporphyrinogen-a substrate for uroporphyrinogen III cosynthetase. J Chem Soc Chem Comm 1979; 204-205.
83. Battersby AR, Fookes CJR, Matcham GWJ et al. Biosynthesis of the pigments of life: Formation of the macrocycle. Nature 1980; 285:17-21.
84. Waldenström J. Studies on the incidence and heredity of acute porphyria in Sweden. Acta Genet 1956; 6:122-131.
85. Prunty FTG. Acute porphyria-some properties of porphobilinogen. Biochem J 1945; 39:446-451.
86. Gray CH. Porphyria. Arch Intern Med 1950; 85:459-470.
87. Haeger G. Urinary 5-aminolaevulinic acid and porphobilinogen in different types of porphyria. Lancet 1958; ii:606-607.
88. Moore MR, McColl KEL, Rimington C et al. Disorders of Porphyrin Metabolism. New York: Plenum, 1987.
89. Day RS, Eales L, Meissner D. Coexistent variegate porphyria and porphyria cutanea tarda. N Engl J Med 1982; 307:36-41.
90. Meissner PN, Sturrock ED, Moore MR et al. Protoporphyrinogen oxidase, porphobilinogen-deaminase and uroporphyrinogen decarboxylase in variegate porphyria. Biochem Soc Trans 1985; 13:203-204.
91. Moore MR, Goldberg A. Health implications of the hemopoietic effects of lead. In: Mahaffey K, ed. Dietary and Environmental Lead: Human Health Effects. Amsterdam: Elsevier, 1985.
92. Hift RJ, Meissner PN, Todd G et al. Homozygous variegate porphyria: An evolving clinical syndrome. Postgrad Med J 1993; 69:781-786.
93. Campbell K. A case of haematoporphyrinuria. J Men Sci 1898; 44:305-313.
94. Erbslöh W. Zur pathologie und pathologischen anatomic der toxischer polyneuritis nach sulfonalgebrauch. Deutsch Z Nervenheik 1903; 23:197-204.
95. Mason VR, Courville C, Ziskind E. The porphyrins in human disease. Medicine 1933; 12:355-439.
96. Copeman SM. Porphyrinuria. Proc Pathol Soc Lond Lancet 1891; i:197.
97. Brugsch J. Porphyrine. J Ambrosius Barth, Leipzig 1959.
98. Tishler PV, Woodward B, O'Connor J et al. High prevalence of intermittent acute porphyria in a psychiatric in-patient population. Amer J Psych 1985; 142:1430-1436.
99. Irvine DG, Wetterberg L. Kryptopyrrole-like substance in acute intermittent porhyria. Lancet 1972; 2:1201.
100. Graham DJM, Brodie MJ, McColl KEL et al. Quantitation of 3-ethyl-5-hydroxy-4,5-dimethyl-Δ_3-pyrrolin-2-one in the urine of patients with acute intermittent porphyria. Eur J Clin Invest 1979; 9:40-53.
101. Graham DJM, Moore MR, Thompson GG et al. The effect of 4-ethyl-5hydroxy-3,5-dimethyl- Δ_3 pyrrolin-2-one on porphyrin synthesis in the rat. Biochem Soc Trans 1976; 4:1089-1091.
102. Moore MR, Graham DJM. Monopyrroles in porphyria psychosis and lead exposure. Internat J Biochem 1980; 12:827-832.
103. Gagey PL. Un cas d'hémoglobinurie en cours d'un xeroderma pigmentosum. Thèse de Paris, 1896.
104. Vollmer E. Über hereditare syphilis und haematoporphyrinurie. Arch Dematol and Syphilogy (Berlin) 1903; 65:221-234.
105. Schmid R, Schwartz S, Watson CJ. Porphyrin content of bone marrow and liver in the various forms of porphyria. Arch Intern Med 1954; 93:167-190.
106. Waldenström J. The porphyries as inborn errors of metabolism. Amer J Med 1957; 22:758-773.
107. Tio TH. Beschouwingen over de porphyria cutanea tarda. Doctoral Thesis. Amsterdam: 1956.

108. Moore MR, McColl KEL, Goldberg A. The effects of alcohol on porphyrin biosynthesis and metabolism. In: Rosalki SB, ed. Clinical Biochemistry of Alcoholism. Oxford: Churchill Livingstone: 1984:161-187.
109. Orten JM, Doehr SA, Bond C et al. Urinary excretion of porphyrins and porphyrin intermediates in human alcoholics. Quart J Studies on Alcohol 1963; 24:598-609.
110. Cetingil AI, Ozen MA. Toxic porphyria. Blood 1960l; 16:1002-1010.
111. Pinol-Aguade J, Castells A, Indocochea A et al. A case of biochemically unclassifiable hepatic porphyria. Brit J Dermatol 1969; 81:270-275.
112. Smith SG. Hepatoerythropoietic porphyria. Sem Dermatol 1986; 5:125-137.
113. Magnus IA, Jarret A, Prankerd TAJ et al. Erythropoietic protoporphyria: A new porphyria syndrome with solar urticaria due to protoporphyrinaemia. Lancet 1961; ii:448-451.
114. Langhof H, Müller H, Rietschel I. Untersuchungen zur familiaren protoporphyrinamischen lichurticaria. Arch Klin Exp Dermatol 1961; 212:506-518.
115. Kosenow W, Treibs A. Lichtuberempfindlichtkeit und porphyrinamie. Z Kinderkeikunde 1953; 73:82-92.
116. Murphy GM, Hawk JLM, Magnus IA. Late-onset erythropoietic protoporphyria with unusual cutaneous features. Arch Dermatol 1985; 121:1309-1312.
117. Brouwier L. Quelques observations recueilles dans le service d'inspection de l'abbatoir de Liege: Alteration des os. Echo Vet (Liege) 1884; 271-273.
118. Tappeiner H. Untersuchung pigmentirter knochen vom schweine. Sitzungserber Ges Morph Physiol München 1885; 1:38-41.
119. Mosselman D, Hebrant A. Coloration abnormale du squelette chez une bête de boucherie. Ann Med Vet 1898; 47:201-206.
120. Ingier A. Ochronose bei tieren. Zieglers Beirage zur Pathol Anat 1911; 51:199-208.
121. Schmey M. Über ochronose bei mensch und tier. Frankfurter Z Pathol 1913; 12:218-328.
122. Mö1ler-Sorenson A. On haemochromatosis ossium (Ochronose) has husdyrene. In: Den KGL, ed. Veterinaer-Og Landbohojskoles Aarsskrift. Copenhagen: 1920:122-139.
123. Witte H. Ein fall von ochronose bei einem bullen und einem von ihre stammenden kalbe. Z Fleisch U Milchhyg 1914; 24:334.
124. Rimington C. Some cases of congenital porphyrinuria in cattle-chemical studies upon living animals and postmortem material. Onderstepoort J Vet Sci Animal Ind 1936; 7:567-609.
125. Poulson V. Om ochronotiske tilstande hos mennesker og. dyr. (on ochronotic states in man and animals). Med. Diss., University of Copenhagen, 1910.
126. Clare T, Stephens EH. Congenital porphyria in pigs. Nature (London) 1944; 153:252-253.
127. Jørgensen SK. Congenital porphyria in pigs. Brit Vet J 1959; 115:160-175.
128. Jørgensen SK, With TK. Congenital porphyria in animals other than man. In: Rook AJ, Walton GS, eds. Comparative Physiology and Pathology of the Skin. Oxford: Blackwell, 1965:317-331.
129. Tobias G. Congenital porphyria in a cat. J Amer Vet Med Assoc 1964; 145:462-463.
130. Owen LN, Stevenson DE, Keilin J. Abnormal pigmentation and fluorescence in canine teeth. Res Vet Sci 1962; 3:139-146.
131. Vannotti A. Porphyrins—Their Biological and Chemical Importance. London: Hilger and Watts, 1954.
132. Lascelles J. Tetrapyrrole Biosynthesis and Its Regulation. New York: Benjamin, 1964.
133. Rimington C. Porphyrins. Endeavour 1955; 14:126-135.
134. Turner WJ. Studies on Porphyria. 1. Observations on the fox squirrel (Sciurus niger). J Biol Chem 1937; 118:519-530.
135. Levin EY, Flyger V. Uroporphyrinogen, III. Cosynthetase activity in the fox squirrel (Sciurus niger). Science 1971; 174:59-60.
136. Kennedy GY. Pigments of marine invertebrates. Adv Mar Biol 1979; 16:309-381.
137. Church AH. Researches in turacin, an animal pigment containing copper. Phil Trans R Soc 1869; 159:627-636.
138. Fischer H, Hilger J. Zur kenntnis der naturlichen porphyrine. 8 mitteilung. Uber das vorkommen von uroporphyrin (als kuppfersalz, Turacin) in den turakusvogeln und den nachweis von koproporphyrin in der hefe. Hoppe-Seyler's Z Physiol Chem 1924; 138:49-67.
139. With TK. Porphyrins in egg shells. Biochem J 1973; 137:597-598.
140. Derrien E, Turchini J. Sur les fluoresences rouges de certains tissus on secreta animaux en lumiere ultraviolette-nouvelles observations de fluorescences rouges chez les animaux. C R Soc BW (Paris) 1925; 92:1028-1032.
141. Harder JJ. Glandula nova lacrymalis una cum ducta excretoria in cervis et damis detecta. Acta Eruditorum Lipsiae 1694; 11:49-52.
142. Brownscheidle CM, Niewenhuis RJ. Ultrastructure of the Harderian gland in male albino rats. Anat Rec 1978; 190:735-754.

143. Kennedy GY, Jackson AH, Kenner GW et al. Isolation, structure and synthesis of a tricarboxylic porphyrin from the Harderian glads of the rat. FEBS Lett 1970; 6:9-12.
144. Spike RC, Johnston HS, McGadey J et al. Quantitative studies on the effects of hormones on structure and porphyrin biosynthesis in the Harderian gland of the female golden hamster. I. The effects of ovariectomy and androgen administration. J Anat 1985; 142:59-72.
145. Spike RC, Johnston HS, McGadey J et al. Quantitative studies on the effects of hormones on structure and porphyrin biosynthesis in the Harderian gland of the female golden hamster. II. The time course of changes after ovariectomy. J Anat 1986; 145:67-77.
147. Payne AP, McGadey J, Moore MR et al. Androgenic control of the Harderian gland in the male golden hamster. J Endocr 1977; 75:73-82.
148. Mindegaard J. Studier verrend porphyrinsynthese og porfyrinholdige biologiske pigmentinkorns oprindelse og ultrastruktur, Afdelingen for biokemi og erneering. Danmarks Tekniske Hojskole 1976.
148. Woolley GW, Worley J. Sexual dimorphism in the Harderian gland of the hamster (Cricetus auratus). Anat Rec 1954; 118:416-417.
149. Thompson GG, Hordovatzi X, Moore MR et al. Sex differences in haem biosynthesis and porphyrin content in the Harderian gland of the golden hamster. Int J Biochem 1984; 16:849-852.
150. Policard A. Étude sur les aspects offerts par des tumeurs experimentales examinées a la lumiere de Wood. C R Seances Soc Biol 1924; 91:1423-1424.
151. Körbler J. Untersuchung von krebsgewebe im fluoreszenzerregenden licht. Strahlentherapie 1931; 41:510-518.
152. Schwartz S, Absolon K, Vermund H. Some relationships of porphyrins, X-rays and tumors. Univ Minnesota Med Bull 1955; 27:7-13.
153. Rassmussen-Taxdal DS,Ward GE, Figge FHJ. Fluorescence of human lymphatic and cancer tissues following high doses of intravenous hematoporphyrin derivative. Cancer 1955; 8:78-81.
154. Lipson RL, Baldes EJ, Olsen AM. The use of a derivative of hematoporphyrin in tumor detection. J Nat Cancer Inst 1961; 26:1-11.
155. Kessel D. Components of hematoporphyrin derivatives and their tumor-localizing capacity. Cancer Res 1982; 42:1703-1706.
156. Dougherty TJ, Potter WR, Weishaupt KR. An overview of the status of photoradiation therapy. In: Doiron TJ, Gomer CJ, eds. Porphyrin Localisation and Treatment of Tumors. New York: Alan R. lass, 1984.
157. Kessel D, Cheng ML. Biological and biophysical properties of the tumor-localizing component of hematoporphyrin derivative. Cander Res 1985; 45:3053-3057.
158. Scott JJ. The Metabolism of δ-aminolevinlate acid in CIBA Foundation Symposium on Porphyrin Biosynthesis and Metabolism. In: Wolstenholme GEW, Millar ECP, eds. London: J. and A. Churchill Ltd., 1955.
159. Peng Q, Warloe T, Berg K et al. 5-Aminolevulinic Acid-Based Photodynamic Therapy: Clinical Research and Future Challenges. Cancer 1997; 12(79):2282-2308.
160. Brown JE, Brown SB, Vernon DI. Photodynamic therapy—New light on cancer treatment. JSDC 1999; 115:249-253.
161. MacAlpine I, Hunter R. George the III and the 'Mad Business.' London: Penguin, 1969.
162. Röhl JCG, Warren M, Hunt D. Purple Secret: Genes. 'Madness' and the Royal Houses of Europe 1998.
163. Ware M. Porphyria-A royal malady. London: British Medical Association, 1968.
164. Cox TM, Jack N, Lofthouse S et al. King George 111 and porphyria: An elecental hypothesis and investigation. Lancet 2005; 366:332-335.
165. Arnold WN. Vincent Van Gogh: Chemicals, Crises, and Creativity. Boston: Birkhäuser, 1992.
166. Illis L. On porphyria and the aetiology of werewolves. Proc Roy Soc Med 1964; 57:23-26.
167. Dresser N. Vampires are in the headlines, but patients pay the price. Cal Folk Soc Modesto Calif 1986.
168. Gentz J, Johansson S, Lindblad B et al. Excretion of delta-aminolaevulinic acid in hereditary tyrosinaemia. Clin Chim Acta 1969; 23:257-263.
169. Koskelo P. Studies of urinary coproporphyrin excretion in acute coronary diseases. Ann Med Intern Fenniae 1956.
170. Koskelo P, Heikkila J. Urinary excretion of porphyrin precursors in myocardial infarction. Acta Med Scand 1965; 178:681.
171. Yeung-Laiwah AC, Rapeport WG et al. Carbamazepine-induced nonhereditary acute porphyria. Lancet 1983; i:790-792.
172. Marks GS. The effects of chemicals on hepatic heme biosynthesis. TIPS 1981; 2:59-61.
173. Udagawa M, Horie Y, Hirayama C. Aberrant porphyrin metabolism in hepatocellular carcinoma. Biochem Med 1984; 31:131-139.

174. Tio TH, Leijnse B, Jarrett A et al. Acquired porphyria from a liver tumour. Clin Sci 1957; 16:517-527.
175. Lithner F, Wetterberg L. Hepatocellular carcinoma in patients with acute intermittent porphyria. Acta Med Scand 1984; 215:272-274.
176. Koskelo P, Toivonen I, Adlercreutz H. Urinary coproporphyrin isomer distribution in the Dubin-Johnson syndrome. Clin Chem 1967; 13:1006-1008.
177. Ben Ezzer J, Rimington C, Shani M et al. Abnormal excretion of the isomers of urinary coproporphyrin by patients with Dubin-Johnson syndrome in Israel. Clin Sci 1971; 40:17-30.
178. Cohen C, Kirsch RE, Moore MR. Porphobilinogen-deaminase and the synthesis of porphyrin isomers in Dubin-Johnson syndrome. S Afr Med J 1986; 70:36-39.
179. Lannaec RTM. Traite sur l'auscultation mediate. 4th ed. Chaude Paris 1831.
180. Behrend B. Uber endoglobulare einschlusse volker blutkorperchen. Deutsch Med Wschr 1899; 25:254.
181. Duesberg R. Über die anämien - 1. Porphyrie und erythropoese. Arch Exp Pathol 1931; 162:249.
182. Liebig NS. Uber die experimentelle Bleihämatoporphyrie. Arch Exp Pathol Pharmacol 1927; 125:16-27.
183. Grotepass W. Zur kenntnis des im harn auftretenden porphyrins bei bleivergiftung. Hoppe-Seyler's Z Physiol Chem 1932; 205:193-197.
184. Van den Bergh HAA, Grotepass W, Revers FE. Beitrag über das porphyrin in Blut und Galle. Klin Wschr 1932; 11:1534-1536.
185. Gibson KD, Neuberger A, Scott JJ. The purification and properties of delta-aminolaevulinic acid dehydratase. Biochem J 1955; 61:618-629.
186. Woods JS, Fowler BA. Effects of chronic arsenic exposure on hematopoietic function in adult mammalian liver. Environ Health Perspect 1977; 19:209-13.
187. Fowler BA, Mahaffey KR. Interactions among lead, cadmium and arsenic in relation to porhyrin excretion patterns. Environ Health Perspect 1978; 25:87-90.
188. Ng JC, Qi L, Moore MR. Porphyrin profiles in blood and urine as a biomarker for exposure to various arsenic species. Cell Mol Biol (Noisy-le-grand) 2002; 48:111-23.
189. Wang JP, Qi L, Zheng B et al. Porphyrins as early biomarkers for arsenic exposure in animals and humans. Cell Mol Biol (Noisy-le-grand) 2002; 48:835-43.
190. Hughes GS, Davis L. Variegate porphyria and heavy metal poisoning from ingestion of 'moonshine'. South Med J 198; 76:1027-9.
191. Poh-Fitzpatrick M.B. Porphyria, pseudoporphyria, pseudopseudoporphyria. Arch Dermatol 1986; 122:403-404.
192. Topi GC, D'Alessandro GL, De Costanza F et al. Porphyria and pseudoporphyria in hemodialized patients. Int J Biochem 1980; 12:963-967.
193. Kaneko J, Cornelius CE. Clinical Biochemistry of Domestic Animals. 2nd ed. Vol. 1. New York: Academic Press, 1970.
194. Lim CK, Rideout JM, Peters TJ. Pseudoporphyria associated with consumption of brewer's yeast. Brit Med J 1984; 298:1640.