A C U T E

I N T E R M I T T E N T

P O R P H Y R I A

A clinical and experimental study of the disease

and of related aspects of porphyrin metabolism

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"DO NOT BECOME THE ARCHIVISTS OF FACTS.
TRY TO PENETRATE TO THE SECRET OF
THEIR OCCURRENCE, PERSISTENTLY SEARCH
FOR THE LAWS WHICH GOVERN THEM"

PAVLOV. 1936.

I N D E X

PREFACE

PREFACE

My interest in porphyrin metabolism started in 1951 when, as a medical registrar, I presented a patient with acute porphyria at a clinical meeting in the Western Infirmary, Glasgow. Later, I studied other patients with this disease through the kindness of Dr. J.A.W. McCluskie, Mr. E. Gerstenberg, F.R.C.S.E. and Professor Sir J.W. McNee.

In 1952 I received a Nuffield research fellowship tenable at the department of Chemical Pathology, University College Hospital Medical School, London. From 1952 - 1954 much of the clinical and experimental work in this thesis was done under the guidance of Professor C. Rimington, F.R.S. The experimental work was published as each section reached completion and I enclose reprints of such work. The clinical section and the histories of 50 cases have not been published, with the exception of 2 cases (Nos. 4 and 25) reported by Goldberg, MacDonald and Rimington in 1952.

From September 1954 to January 1956 I held an Eli Lilly travelling fellowship in medicine, awarded by the Medical Research Council. During this period I worked in Professor Wintrobe's department in Salt Lake City, Utah. The major part of my work there was a study of haem synthesis, to which I have briefly referred in Section 2.

The subject I had tackled, at first appearance so circumscribed, quickly revealed attachments to many other branches of medical science. Throughout this thesis I have tried to make it

clear to whom I owe gratitude for such specialised knowledge or aid. I would particularly like to thank Dr. James B. Gibson who was mainly responsible for the joint paper on neuropathology. I would also like to thank Professors Cameron, Dent and Rosenheim and Drs. H.Harris, Harold Heath and Elizabeth Work for their advice.

From the beginning of these studies I have been greatly influenced by the example and benevolence of Professor Rimington and to him I owe much.

A. Goldberg.

1

HISTORY OF ACUTE INTERMITTENT PORPHYRIA

bei welchem anstatt der Kochsalzlösung, das Blut mit Weingeist gemischt und Wasserstoffgas durchgeleitet wurde.

Um die Capacität des Blutserums für Kohlensäure zu prüfen, wurde folgender Versuch angestellt. In eine mit Quecksilber gefüllte graduirte Glasröhre wurden 184 C. C. Kohlensäure geleitet und sodann 60 C. C. Serum von Ochsenblut dazugebracht. Nach 24 Stunden war die Kohlensäure bis auf 60 C. C. verschwunden, folglich 124 C. C. Kohlensäure von dem Blutserum absorbirt worden. Es wurden nun 30 C. C. Essigsäure zu dem Serum gebracht und die Flüssigkeit etwas geschüttelt. Es entwickelten sich darauf wieder 58 C. C. Kohlensäure aus dem Serum.

Bei einem andern Versuche wurden zu 132 C. C. Kohlensäure 56 C. C. Blutserum gebracht. Nach 24 Stunden waren noch 21 C. C. Kohlensäure übrig, also 111 C. C. absorbirt worden. Auf Zusatz von 28 C. C. Essigsäure entwickelten sich wieder 31 C. C. Kohlensäure und 80 C. C. blieben mit dem Serum verbunden.

Es geht daraus hervor, dafs frisches Blutserum von Ochsenblut sein doppeltes Volumen Kohlensäure absorbirt.

Auch über den Farbstoff des Blutes und namentlich über die Frage, ob das Eisen der färbende Theil desselben sey, habe ich, nach der Angabe des Hrn. Prof. Liebig, einige Versuche angestellt. Insbesondere waren es die Versuche von Sanson, welche wiederholt wurden.

Trockener Blutkuchen wurde zu einem feinen Pulver zerrieben und mit concentrirter Schwefelsäure in einem Mörser aufs innigste gemischt. Die Masse wurde sodann in ein Becherglas mit destillirtem Wasser gebracht und an einem kühlen Orte ruhig stehen gelassen. Nach einigen Stunden hatte das Wasser, welches ganz hell und klar blieb, eine bedeutende Menge schwefelsaures Eisenoxyd aufgenommen, was sich durch die gewöhnlichen Reagentien aufs leich-

Plate I. Section I.

The first historical allusion to the porphyrin pigments of blood was by Scherer (1841).

I. HISTORY OF ACUTE INTERMITTENT PORPHYRIA

The first historical allusion to porphyrins was made by Scherer (1841). He added concentrated sulphuric acid to dried and powdered blood and washed the precipitate free of iron. The iron-free residue was then heated with alcohol which took on a blood-red colour. He thus had shown that the red colour of blood was not due to iron (Plate 1). Mulder (1844) carried out a similar study and described a "purple-red fluid" devoid of iron, which he named "iron-free haematin".

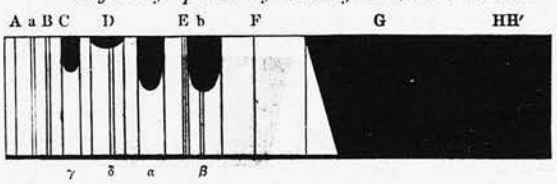
This red substance was called cruentine by Thudicum (1867) in a report to the Privy Council of Great Britain. He defined its spectrum and noted its remarkable fluorescence by a simple method (Plate 2). In 1871 Hoppe-Seyler found that the iron-free haematin, described by Mulder, was a mixture of two substances, the main constituent of which he called haematoporphyrin. (Greek, porphuros - red-purple). Three years later Schultz (1874) published the clinical details of a case of so-called 'Pemphigus Leprosus' for his doctorate thesis. He described the patient as a 33 years old weaver who had suffered from skin photosensitivity since the age of 3 months. His spleen was enlarged and he passed a wine-red urine. The urine of this case was thoroughly investigated by Baumstark (1874), who named two pigments derived from it - urorubrohaematin and urofuscohaematin. Baumstark considered that the spectrum of an acid solution of urorubrohaematin resembled that of Hoppe-Seyler's acid haematoporphyrin, although he did not regard these two substances

Mode of testing Fluids with respect to Fluorescence, by means of a Cone of Sunlight collected by a system of Quartz Lenses.

Bands.	Measurements.		Width.	Intensity.	Rank.
1.	142° 18′ — 142° 3′	=	0° 15′	3	γ
2.	141° 55′ — 141° 30′	=	0° 25′	1	δ
3.	141° 21′ — 141°	=	0° 21′	5	α
4.	140° 45′ — 145° 18′	=	0° 27′	5	β

Blue dark, cut off at 139° 9′.

Diagram of Spectrum of neutral fluorescent Cruentine.



The saturated chloroform solution was one Ctr. thick. The spectrum was measured with the aid of a gaslight only. It fluoresced with a splendid blood-red colour in the sun-cone. This is the first body which is known to fluoresce with homogeneous light, that is to say, the same kind of light or colours which it transmits. The fluorescent cone has an intensity of colour equal to that of a solution about ten times more concentrated. Considering its beauty alone the phenomenon may be placed by the side of those exhibited by quinine, chlorophyll, or cudbear. But by its peculiarity it is perfectly unique.

Alkaline Four-banded Cruentine.—Spectrum 11. plate II., represents

Plate 2. Section I.

Thudicum (1867) first observed the fluorescence of porphyrins ("cruentine")

as identical. It is often written that Baumstark obtained haematoporphyrin from a case of leprosy but it is clear that the case of Schultz and Baumstark was the first description of what we now call congenital porphyria and in fact the first association of this pigment in urine with a disease in man.

In 1880 MacMunn described a dark pigment excreted in the urine of a patient with subacute rheumatism, who had been taking sodium salicylate. He called this pigment urohaematin but later (MacMunn 1885) renamed the pigment urohaematoporphyrin because it "bears a very striking resemblance to haematoporphyrin". Le Nobel (1887) confirmed this finding and also listed a number of diseases, including lead poisoning, in which he had found the pigment. He also found it in normal urine.

Sulphonal was introduced as a hypnotic in 1888. Shortly after this Stokvis (1889) reported that an elderly woman who had taken sulphonal excreted a dark red urine and had later died (Plate 3). He considered that the pigment in this urine was similar to, but not identical with haematoporphyrin. Harley (1890) reported a fatal case of an unusual form of nerve disturbance, associated with dark red urine in a woman of 27 under the care of Dr. John Wyllie, at the Edinburgh Royal Infirmary. This woman had been given sulphonal and presented many of the neurological features of porphyria. Ranking and Pardington (1890) described two women who excreted 'haematoporphyrin' and who exhibited the gastro-intestinal and neuro-psychiatric manifestations of the disease we now call acute intermittent porphyria. These patients were unrelated, though they lived in the same house and

№ 13. 28 September. 1889.

WEEKBLAD

VAN HET

NEDERLANDSCH TIJDSCHRIFT VOOR GENEESKUNDE.

TWEEDE DEEL.

OVER TWEE ZELDZAME KLEURSTOFFEN IN URINE VAN ZIEKEN,

DOOR

Prof. B. J. STOKVIS.

(*Met een Plaat.*)

I. LAKMOES (?) IN URINE NA GEBRUIK VAN RESORCINE.

In Maart l.l. ontving ik door bemiddeling van Dr. C. C. DELPRAT een urine, die zich door haar donkerbruine kleur, haar hoog soortelijk gewicht, haren rijkdom aan ureum en acid. uric., maar bovenal aan indoxyl-zwavelzure zouten onderscheidde. De urine bevatte zoóveel indoxyl-zwavelzuur, dat 5 C.C., met zoutzuur en chloorkalk behandeld, voldoende waren, om 30 —40 C.C. chloroform intensief blauw te kleuren. De urine was afkomstig van een patiëntje, door perityphlitis en peritonitis aangetast, en haar rijkdom aan indoxyl-zwavelzuur was dus zeer verklaarbaar. Maar bij behandeling en schudden met aether, na toevoeging van een weinig phosphorzuur, bleek buitendien uit de urine in den aether een lichtróse kleurstof over te gaan.

Plate 3. Section I.

The first case of "sulphonal porphyria" was reported by Stokvis (1889).

had not taken sulphonal. In the next 20-30 years many other cases were recorded of "haematoporphyrinuria" associated with a definite pattern of symptoms. Günther (1911, 1922) carefully listed these individual cases. Sometimes sulphonal or the allied drugs, tetronal and trional, were taken for long periods prior to the onset of symptoms. Other cases had no obvious relationship to drugs and presumably were precipitated by unknown causes. Barbiturates were introduced into clinical medicine in 1903 and 3 years later Dobrschansky (1906) described a case, which we would now regard as typical of acute porphyria without paralysis, occurring in a patient after prolonged administration of diethyl-barbituric acid.

Hans Günther was the first to classify the diseases of porphyrin metabolism in two comprehensive papers in 1911 and 1922. In the first of these he quoted fourteen cases from the literature in which acute symptoms of porphyria arose spontaneously (haematoporphyria acuta) and fifty-six cases of haematoporphyria acuta toxica, in which the symptoms were associated with the ingestion of sulphonal, trional or veronal. He also defined and named, for the first time, the very rare condition congenital porphyria (haematoporphyria congenita) in which the predominating symptoms are due to skin photosensitivity. There is in this description of congenital porphyria the realization that the disease persists throughout life and Garrod (1923), in the second edition of his classical monograph, credits Günther with the first recognition that this disease was an "inborn error of metabolism." Garrod himself (1892, 1893a, 1893b)and in association with Gowland

Hopkins (1896) had made outstanding contributions to the chemical detection of porphyrins in urine.

In Günther's second work in 1922, he elaborated on his first thesis, quoting further cases. He noted the possibility that acute porphyria might be hereditary and suggested that people liable to develop acute or congenital porphyria had a diathesis (Porphyrism) with certain notable physical and mental characteristics - neurosis, insomnia, dark hair and pigmented skin. In a survey of the clinical features of acute porphyria, he introduced a triad of symptoms which were commonly present - abdominal pain, constipation and vomiting. One of Günther's cases of congenital porphyria was a man called Petry. Hans Fischer commenced a study of the excreta of this patient and between the years 1915 and 1945 made monumental contributions to porphyrin chemistry. By these researches the naturally-occurring porphyrins of excreta - uroporphyrin and coproporphyrin - were found to differ structurally from haematoporphyrin, which was considered to be a chemical artefact. Fischer (1915 a,b), Laidlaw (1904) and Schumm (1924) differentiated the naturally-occurring porphyrin of haem itself from haematoporphyrin and the name protoporphyrin was suggested for this by Fischer. Thus it was that the diseases of porphyrin metabolism came to be termed Porphyrias, by Waldenström (1937), rather than Haematoporphyrias (Günther, 1911).
Waldenström (1937), in his excellent monograph, 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

had occurred some years after birth, at times associated with abdominal pains. For these cases he substituted the name Porphyria Cutanea Tarda. In 1954 Schmid, Schwartz and Watson classified the porphyrias on the basis of the porphyrin content of the livers and bone marrows of 31 cases. In two cases of congenital porphyria they found porphyrins concentrated in the bone marrow, particularly in the normoblastic nucleus, an elaboration of the original observations of Borst and Königsdorffer (1929). This rare group was therefore termed Porphyria Erythropoietica. In the remaining 29 cases porphyrins were mainly found in the liver and they were therefore called Porphyria Hepatica. This included the typical acute intermittent porphyria, a mixed type in which photosensitivity and acute symptoms may occur in the same patient, and Porphyria Cutanea Tarda, a term reserved for a group in which photosensitivity occurs later in life, unassociated with acute symptoms (Table 1).

Waldenström was greatly aided in his studies by the presence in acute porphyria urines of a substance which gives a red colour with Ehrlich's aldehyde reagent (paradimethylamino-benzaldehyde). This substance is now known to be the precursor chromogen of the uroporphyrin found in these urines and with certain very rare exceptions (Watson, Hawkinson and Bossenmaier, 1953) its presence in urine is a specific test for acute porphyria. As early as 1890 Harley had noted that the urine of a case of sulphonal-induced acute porphyria contained a chromogen which, when oxidised, became a red pigment. Paula Sachs (1931) described a substance in the urine of a patient with acute porphyria which gave a red colour,

insoluble in chloroform, with Ehrlich's aldehyde reagent and which was therefore not urobilinogen.

Waldenström (1937) showed that this Ehrlich reacting substance, which he considered to be a chromogen of a porphyrin, was not only excreted in the urine of every case of acute porphyria, but that some apparently healthy relatives of these patients also excreted it. He thus conceived the idea of a latent porphyria and traced examples of acute or latent porphyria to second and even third generations. On the basis of his work, he suggested that acute porphyria was transmitted as a Mendelian dominant characteristic. Later Waldenström and Vahlquist (1939) suggested that the Ehrlich-reacting chromogen, which they partly purified and named porphobilinogen, was a dipyrrylmethane. Prunty (1945) and Gray (1950) found that the liver and kidney of fatal cases of acute porphyria contained porphobilinogen. A most important step occurred when Westall (1952) isolated crystalline porphobilinogen from the urine of a patient with acute porphyria in University College Hospital, London, (Case 36). Cookson and Rimington (1953) elucidated its structure as a monopyrrole. Recently Granick and Schrieck (1955) have suggested that δ-aminolaevulic acid, a precursor of porphobilinogen, is excreted in large amounts in acute porphyria urines.

TABLE I

Classification of Porphyrias

I Günther (1911)

1. Haematoporphyria acuta
2. Haematoporphyria acuta toxica
3. Haematoporphyria chronica
4. Haematoporphyria congenita

II Waldenström (1937)

1. Porphyria congenita
2. Porphyria cutanea tarda
3. Porphyria acuta
   (a) Latent porphyria
   (b) Abdominal form
   (c) Nervous form
   (d) Classical acute porphyria
   (e) Comatose form commencing as (b)

III Schmid, Schwartz and Watson (1954)

1. Porphyria erythropoietica - congenital photosensitive porphyria, usually associated with haemolytic anaemia and splenomegaly.

2. Porphyria hepatica - hepatic disease or functional impairment frequent.

   (a) Intermittent acute type - abdominal and/or nervous manifestations
   (b) 'Cutanea tarda' type - late appearance of photosensitivity without other manifestations.
   (c) 'Mixed' type - photosensitivity with intermittent abdominal and/or nervous manifestations.

PORPHYRINS. CHEMICAL AND BIOCHEMICAL BACKGROUND

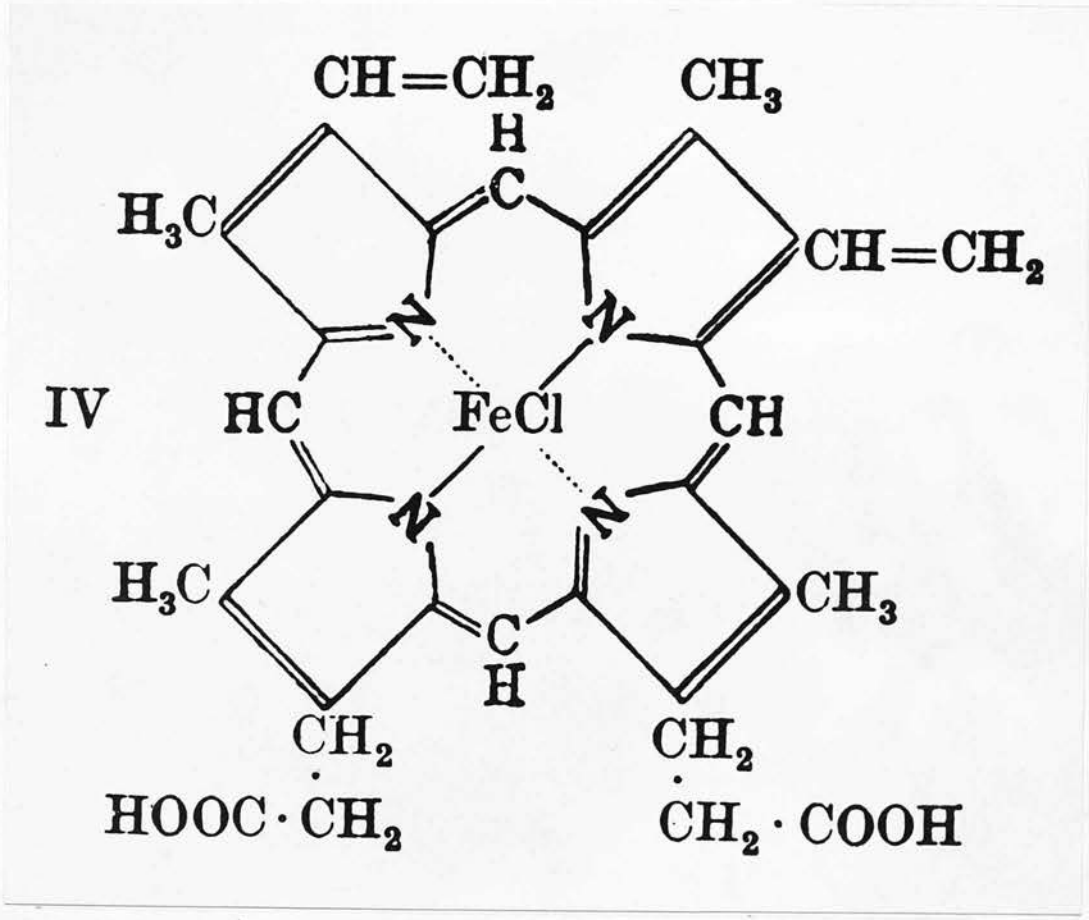
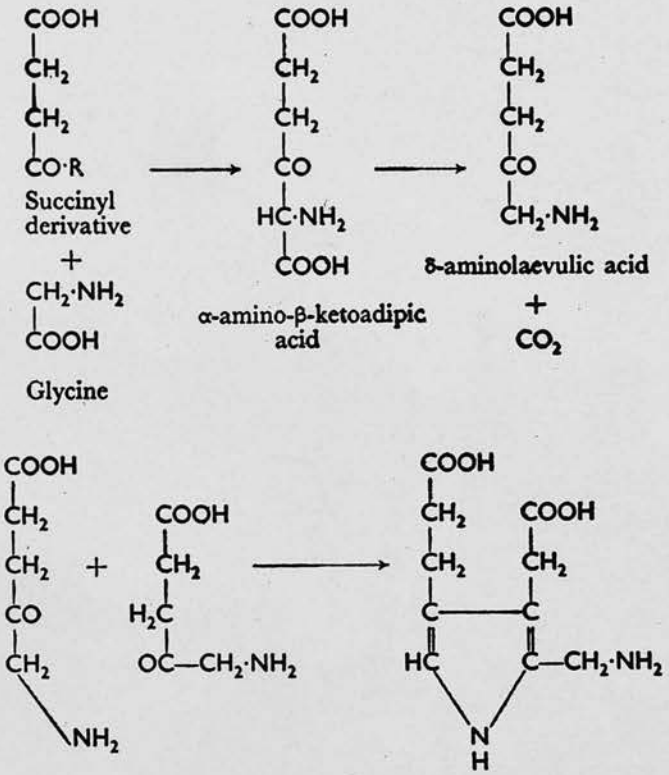


Fig. 1. Section 2.

Haemin chloride.



Scheme summarizing biosynthetic steps from glycine + succinate (from the tricarboxylic acid cycle) to porphobilinogen, the simplest pyrrolic substance known to be a precursor of haem and porphyrins.

Fig. 2. Section 2. (After Rimington, 1955.)

2. PORPHYRINS

Chemical and Biochemical Background

The porphyrins are nitrogen-containing compounds whose chemical structure consists of 4 pyrrole rings united by means of methene bridges. They occur throughout the animal and vegetable kingdomsin the free state or as metal complexes. The iron complex of protoporphyrin IX or haem (Fig. 1) is of especial importance in animals and forms the prosthetic group of haemoglobin, the cytochromes and catalase.

The porphyrins have been known for nearly a hundred years and yet it has only been in the past decade that the main steps in the biosynthesis of haem have been ascertained. This advance has been principally due to the introduction of isotopic labelling techniques. From work carried out primarily in the laboratories of Shemin, Neuberger and Rimington the following pattern emerges (Fig. 2). Active succinate condenses on the α-carbon atom of glycine to give αamino-β-Ketoadipic acid, which is then decarboxylated to give rise to δ-aminolaevulic acid, 2 molecules of which condense to form porphobilinogen. Porphobilinogen is the precursor monopyrrole of all the porphyrins and is also the characteristic excretion product of acute porphyria. The mechanism of conversion of the monopyrrole to the tetrapyrrole structure is not yet known and the biological sequence of porphyrins in the formation of haem from the porphobilinogen stage is not certain.

There was some controversy as to whether protoporphyrin itself was an intermediate in the synthesis of haem (Granick,

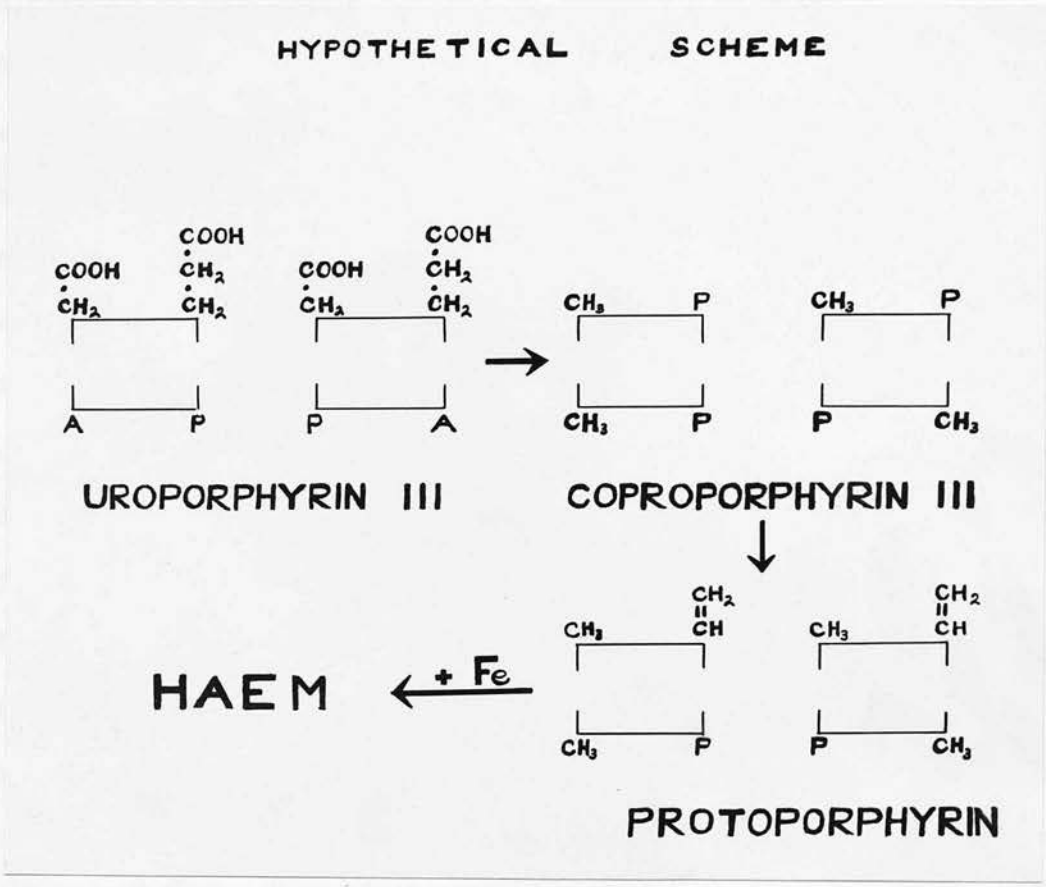


Fig. 3. Section 2.

The probable pathway of haem synthesis. It is likely that the reduced forms of uroporphyrin III and coproporphyrin III are the true intermediates. The porphyrins are illustrated in abbreviated form. They are distinguished by the groups attached to the C.atoms of each pyrrole.

A = acetic. P = Propionic.

1954; Dresel, 1955; Eriksen, 1955; Schwartz and Ikeda, 1955). The present author, while working in Professor Wintrobe's laboratory in Salt Lake City, U.S.A., described a method for the measurement of haem synthesis in a haemolysate of chicken erythrocytes, in which he used radioiron as a tracer for the rate of synthesis. Free protoporphyrin IX, as well as glycine, δ-aminolaevulic acid, and porphobilinogen, significantly stimulated haem synthesis. Furthermore the conversion of protoporphyrin to haem was shown to be enzyme dependent (Goldberg, 1955; Goldberg et al. 1956). A particle-free aqueous extract of chicken erythrocytes contained this enzyme, the activity of which was retained after lyophilisation and storage for at least 4 weeks. This iron-incorporating enzyme requires a co-factor, which is removed by ultrafiltration of the aqueous extract (Goldberg, 1956).

The work of Neve et al. (1955) suggests that reduced uroporphyrin III and coproporphyrin III are also intermediates in haem synthesis. It is thus probable that uroporphyrin III is the first formed tetrapyrrole from porphobilinogen and that by a process of decarboxylation of its reduced form, coproporphyrin III is formed, which is later decarboxylated to protoporphyrin, the precursor of haem itself (Fig. 3).

1 litre of human urine normally contains 10-200 micrograms of a mixture of coproporphyrin I and III, only traces of uroporphyrin and no porphobilinogen. In the diseases of porphyrin metabolism the pattern of excretion is abnormal (Table 1). There are probably abnormalities of porphyrin metabolism in man other than the main group of porphyrias. The use of improved chemical

Disease	Main urinary excretion product
Acute intermittent Porphyria.	Porphobilinogen. δ amino-laevulic acid.
Porphyria Cutanea Tarda.	Uroporphyrins I and III.
Congenital Porphyria.	Uroporphyrin I.
Hereditary Coproporphyria.	Coproporphyrin III. ± trace of uroporphyrin I.

Table 1 Main urinary excretion products in the diseases of porphyrin metabolism.

methods should help to detect them. Hereditary coproporphyria is such a condition, first mentioned by Berger and Goldberg (1955). Four cases of coproporphyria were described in a single family - a boy aged 10 years, his mother and father (first cousins) and his paternal aunt. The symptoms of porphyria were absent. The adults excreted moderately high quantities of coproporphyrin III in the urine and stool, whereas the boy excreted very large quantities of coproporphyrin III in the urine and stool, with a trace of uroporphyrin I in the urine. The boy also suffered from rickets and riboflavine deficiency, both of which were improved by treatment.

ACUTE INTERMITTENT PORPHYRIA.

CLINICAL DISCUSSION OF 50 CASES

AETIOLOGY

Genetics *

The first observation that acute porphyria occurred in members of the same family was made by Barker and Estes (1912). Günther (1922) noted the hereditary implications of the disease but it was Waldenström (1937) who firmly established its hereditary nature. Gates (1946) reviewed Waldenström's data and considered that the disease was inherited as an irregular dominant character. In the present series of 50 cases at least 19 have one or more relatives with the active or latent condition. 9 latent porphyrias were revealed by investigation of relatives. It was only possible to study 11 families in detail (see Appendix). The family trees were constructed on the basis of whether or not porphobilinogen was definitely found in the urine on at least one occasion. There is only one exception to this rule. Case No. 41 died in 1935 and although the urine was noted to be dark in colour it was not tested for porphobilinogen. The clinical and family histories of this patient make the diagnosis of acute porphyria highly probable. The urines were routinely tested by the methods of Watson and Schwartz (1941) and Vahlquist (1939). Where a doubtful positive was obtained about 0.5 to 1 litre of urine was concentrated and any porphobilinogen present was identified by paper chromatography (Westall, 1952; Cookson and Rimington, 1954). Concentrates of normal urine, so treated,

* I am indebted to Dr. H. Harris, London Hospital, for his help and advice in the writing of this portion on genetics.

showed no porphobilinogen. This method proved very helpful in identifying 3 latent porphyrias who excreted small quantities of porphobilinogen - family 2, son of propositus; family 3, father of propositus; family 9, nephew of propositus. It should be noted, however, that out of 18 previously active cases whose urines were tested in a state of remission, 5 had ceased to excrete porphobilinogen (Cases 15, 31, 37, 46 and 47). Case 10 did not excrete porphobilinogen at the age of 8 years but did do so at 11 years. Thus the absence of porphobilinogen in the urine of a relative does not rule out the possession of the trait and this is of particular importance in testing the urine of the child relative.

The findings in this series confirm those of Waldenström (1937, 1956). In 4 families (Nos. 1, 2, 3 and 8) the condition was found in more than 1 generation, apparently transmitted directly from a parent to one or more of the offspring. In another family (No. 7) where it was not possible to test the parents, 2 half-sibs with unrelated fathers were found to be affected. Thus a single gene would seem necessary for the manifestation of the disease. There was no evidence of increased parental consanguinity. This familial distribution is consistent with the hypothesis that the condition is inherited as a Mendelian dominant character, i.e. the individuals are heterozygous for an abnormal gene. In 2 instances, however, (families 1 and 10), where more than one of a group of sibs were affected, it was not possible to detect porphobilinogen in the urines of either of the parents. It must, therefore, be assumed that there is

considerable variation in the degree of the expression of the character and that one can get all grades from the acute case, the latent porphyria, to the apparently normal individual who excretes no porphobilinogen. It is possible that some of the latter group might begin to excrete porphobilinogen, if suitably provoked. In only 2 families (Nos. 1 and 8) is there the association of a parent and a child who have both had the active disease. This is not altogether surprising, because if a gene produces a severe and lethal illness variable in its manifestations, then those with the milder form of the affection are more likely to survive to adult life.

The apparent sporadic occurrence of the disease (families 5 and 11) might perhaps be attributed to mutation, that is the sudden change from the normal gene to its abnormal counterpart in the germ tract of one or other of the parents. Although the occurrence of a mutation in these families cannot be excluded, it is probable that in the majority of instances where an affected child has been derived from normal parents, one or other of the parents is in fact heterozygous for the gene.

From a study of these families, it has been possible to calculate that approximately 25% of the sibs of a case may be expected to have porphobilinogen in the urine. A high proportion of these are liable to have an acute attack. Furthermore, about 1 in 4 of the children of individuals who excrete porphobilinogen may be expected to become similarly affected. If it is true that affected individuals are heterozygous for an abnormal gene, then 2 out of 4 children of parents, only one of whom is affected,

Fig. 1. Section 3

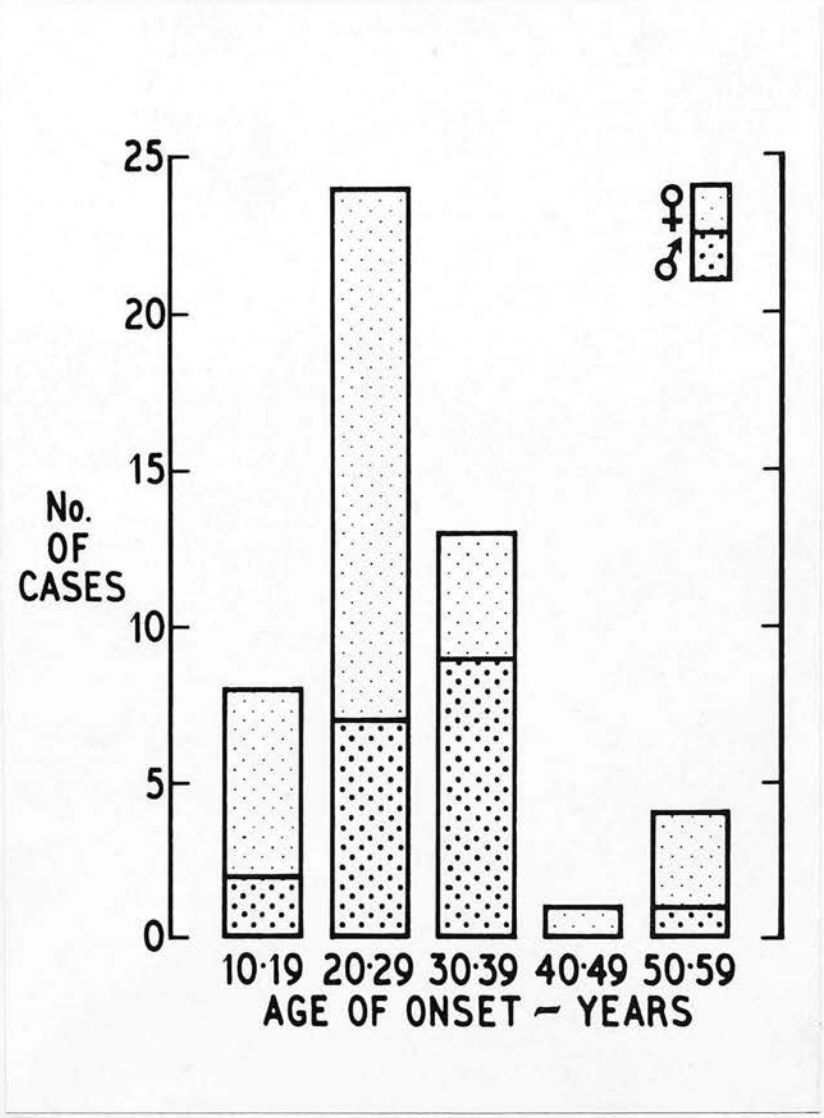


Fig. 1. Section 3.

Age of onset of symptoms
in acute intermittent porphyria

should carry the abnormal gene. Thus half of those carrying the gene may not excrete porphobilinogen.

AGE OF ONSET

The most frequent age of onset (Fig. 1) in this series occurred in the third decade for females and in the fourth decade for males. The high incidence of the third decade in females is confirmatory of Waldenström (1937). The youngest case was a girl of 11 years (No. 10). Rothman (1926) and Bauer (1943) have described acute porphyria in children of 3 years 10 months and 2½ years respectively. 2 of the 5 cases which started after the age of 40 years commenced after prolonged administration of barbiturates. It is possible that a relationship exists in the ages of onset of siblings. Thus Cases No. 17 and 18, brothers, both commenced symptoms at the age of 30 years. Cases No. 21 and 22, brother and sister, started with symptoms at 35 and 36 years, respectively. Cases No. 37 and 38, sisters, started to have symptoms at 23 years of age. Cases No. 40 and 41, brothers, had the commencement of symptoms at 29 and 31 years respectively.

ENDOCRINE FACTORS

(a) Sex incidence Priestley (1894) first drew attention to the preponderance of females with porphyria and Garrod and Hopkins (1896) found that the majority of cases of "Sulphonal Haematoporphyria" were women. Subsequent studies (e.g. Waldenström, 1937) have confirmed this incidence. The present series shows that the 50 cases consist of 31 females and 19 males, i.e. 62% and 38% respectively. It is questionable whether this sex incidence is genetically ordained or whether secondary precipitat-

ing causes, e.g. pregnancy, barbiturate intoxication, predispose to the sex difference. The disease may be no less severe in the male and out of 12 deaths, 33% were male.

(b) Menstruation Waldenström noted the apparent relation of clinical symptoms to menstruation. In the present series the onset of an attack was closely associated with a menstrual period in 7 women (Cases No. 5, 12, 20, 25, 34, 37 and 38). In these, the periods were generally delayed but not suppressed. Case 34 had repeated attacks associated with the onset of her menstrual period. She was put on testosterone and the doctor believed that the testosterone therapy was related to an improvement in her condition. In 6 additional women there was amenorrhoea (Cases No. 6, 9, 26, 30, 32 and 36). Amenorrhoea is not an infrequent accompaniment of a debilitating disease and in Case No. 6 there was also the association of tuberculous endometritis. The incidence of the disease in males, in pre-pubertal girls (Cases 10 and 48) and in post-menopausal women (Cases No. 2 and 15) suggests that, if there is any association between menstruation and clinical symptoms, it is not a primary one.

(c) Pregnancy This is a matter not only of academic interest but of clinical importance since a preponderance of the female cases occurs in the child-bearing period. This point concerned Waldenström (1937) in whose series 2 cases died during pregnancy. He writes, "The question of the importance of pregnancy is more difficult to answer. Among a large number of mature women, some will be pregnant. It is also possible that it is not pregnancy itself which precipitates an attack, but the hypnotic and other

medical drugs given during this time. It would seem important that the doctor realises the possibility of a deterioration in the condition if pregnancy occurs." (He then refers to his Cases No. 65 and 100, both of whom died during pregnancy).

"Another question is of importance in this respect. When the doctor is asked by a woman patient whether she can have children, in spite of her illness, he must also remember the dominant nature of the disease and the danger to the child. He must not underestimate the danger to the mother and child." (Translation).

In the present series several cases show an association with pregnancy. Cases 1, 22 and 39 commenced an attack 5 days, 14 days and 14 days, respectively, after the birth of a child. Cases 3, 30 and 35 started to have symptoms in the very early weeks of pregnancy. Both Cases 3 and 30 died shortly after termination of pregnancy and in the latter case the child also died shortly after birth. Case 20 had a normal pregnancy but had an exacerbation of pain immediately after delivery. Watson (1952) has stressed that an attack might occur after a confinement. On the other hand, there are several examples of normal pregnancies occurring in acute porphyria cases (Cases No. 5 and 26) and some observers have even reported marked improvement in their cases during pregnancy (Rawlings 1950; Freedman et al. 1952).

Thus no dogmatic statement can be made on the relationship between pregnancy and porphyria. In some cases pregnancy may be completed with impunity but in others the impression is gained that pregnancy may gravely and sometimes fatally overtax the

porphyria patient.

ALCOHOL

In Case No. 7 exacerbations of the disease were associated with bouts of alcoholism, although prior to the last attack he had also taken barbiturates as well as alcohol. Martin and Heck (1956) found that alcohol was a precipitating factor in 23 of their 80 acute and photosensitive cases. They did not specify what number of the 23 cases were acute porphyria.

MOTION SICKNESS

In Case No. 45 attacks of severe vomiting were associated with travel. In the last attack, which started during a sea voyage, the urinary porphobilinogen level rose markedly and then fell with improvement in the general condition.

INFECTION

In the present survey there are many examples of the role of infection in precipitating or aggravating an attack of acute porphyria. This is not surprising, since in other metabolic diseases infection may play a similar role. It is well recognised that infection may precipitate a diabetic into diabetic coma, or a patient with Addison's disease into a crisis.

(a) Acute tonsillitis Examples of acute tonsillitis precipitating an attack have been given by Waldenström (1937) and Denny-Brown and Sciarra (1945). This has been confirmed in 5 cases of this series (Nos. 17, 22, 37, 48 and 49). In Case 37 the effect of the throat infection and tonsillectomy on porphobilinogen excretion was recorded (Fig. 2).

(b) Urinary infection Discombe and D'Silva (1945) and Abrahams,

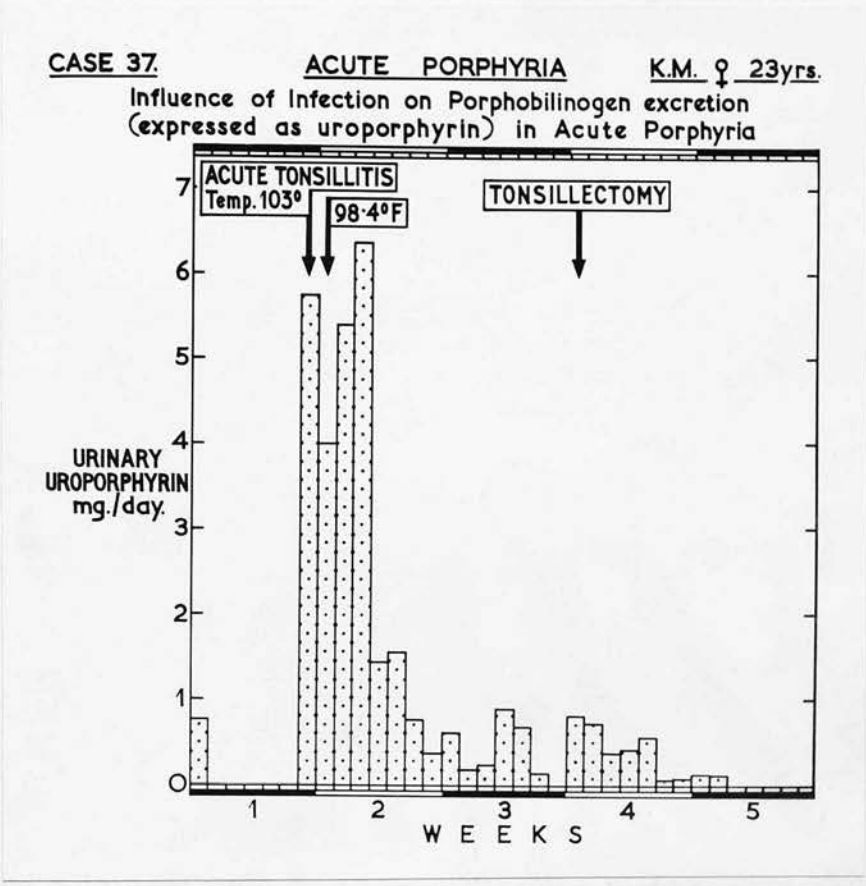


Fig. 2. Section 3.

Gavey and Maclagan (1947) noted the association of a urinary infection in acute porphyria and this has also been recorded in Case 36.

(c) Skin infection This appeared to be a precipitating factor in Cases 4 and 14. In Case 25 erysipelas, which followed injections of A.C.T.H., greatly aggravated an attack already in progress. Skin infection has been noted by Discombe and D'Silva (1945) as such a precipitating factor.

(d) Herpes zoster This occurred in Case 29 just prior to an

attack. Vannotti (1954) has described herpes zoster on the distribution of the ilec-inguinal nerve, occurring with each attack of a case of acute porphyria.

(e) Tuberculous endometritis was associated with an attack in Case 6.

(f) Pulmonary infection This is usually a complication of a severe attack, particularly with immobilisation and respiratory paralysis. Thus an aspiration pneumonia occurred in Case 29 and lobar pneumonia aggravated an attack in Case 4. In Cases 1, 3, 6 and 43 pulmonary infection was a fatal complication in respiratory paralysis.

BARBITURATES

Since the introduction of the barbiturates to clinical medicine in 1903, there have been conflicting reports on their possible relation to human acute porphyria. Drobrschansky (1906) described a typical case of acute porphyria, without paralysis, occurring in a patient after prolonged administration of diethylbarbituric acid. Haxthausen (1927) reported the development of skin photosensitivity and the presence of excessive amounts of porphyrins in the urine of an epileptic who had been taking ethylphenylbarbiturate for some time. Eliaser and Kondo (1942), Denny-Brown and Sciarra (1945), Prunty (1946), Jørgensen and With (1947), Macleod and Grant (1953), and Whittaker and Whitehead (1956) have described deterioration in the clinical state of acute porphyria patients with the development of severe or even fatal paralysis, after the administration of certain barbiturates, among which were ethylphenylbarbituric acid and

ethyl(1-methyl-butyl) barbituric acid. Waldenström (1939, 1940) was convinced that barbiturates may precipitate attacks in cases of latent porphyria and that they seriously affect the prognosis of the disease. On the other hand, Günther (1922), Turner (1938) and Discombe and D'Silva (1945) failed to note any relation between barbiturates and acute porphyria.

Information on the relation of barbiturates to normal human porphyrin metabolism has been obtained from observations on the urinary porphyrin excretion in cases of barbiturate poisoning. Rosendorff (1910), Sowden (1910) and MacLean (1912) found no abnormal porphyrinuria in patients who had ingested single large doses of diethylbarbituric acid, while Rommel (1912) observed dark-red urine in a patient who had taken 25 g. of the same drug.

A study of the cases in the present series suggests 2 types of relationship between barbiturates and acute porphyria.

(1) If barbiturates are given over a prolonged period they precipitate an attack.

(2) If barbiturates are given during the course of an attack they may accelerate the onset of paralysis.

The evidence for the first hypothesis is based on Cases 15, 16 and 50. In these cases barbiturates, mainly phenobarbitone, were given for 1½-2 years. Among the presenting symptoms were drowsiness and memory loss (Case No. 15), incoherence and repetitiousness of speech, convulsions (Case No. 16), hysteria-like behavious and depression (Case No. 50). These symptoms recall the description by Fraser et al. (1954) of chronic barbiturate intoxication or its sudden withdrawal. In each of these

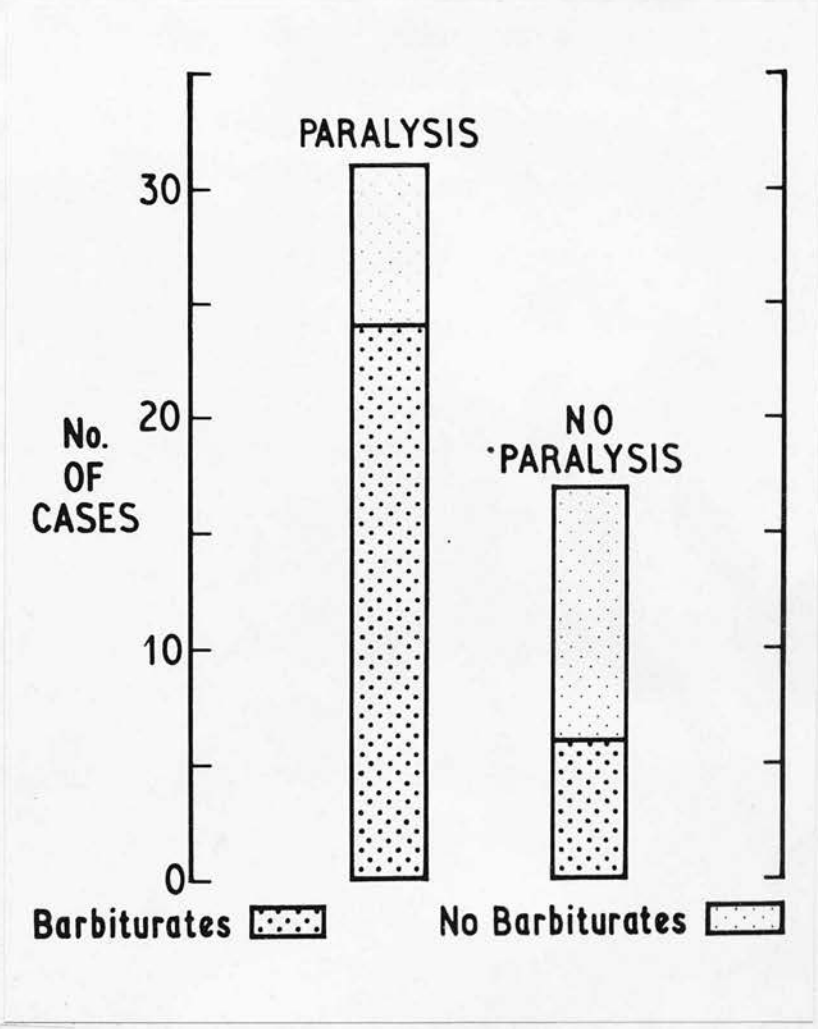


Fig. 3. Section 3.

The relationship between barbiturates and the onset of paralysis in 48 patients with acute porphyria. In 2 cases it was not certain whether barbiturates were given or not, and these were not included in this analysis.

cases the main symptoms and signs departed after the cessation of this drug and there has been no recurrence for several years. On the other hand, there has been evidence that the genetic abnormality exists in this type of case. Thus Case 16 still passed porphobilinogen 3 years after the cessation of barbiturates and the son of Case 15 is a latent case. It is also noteworthy that Cases 15 and 16 were 56 and 59 years of age, respectively, and had suffered no previous symptoms until then. This information suggests that prolonged ingestion of barbiturates may precipitate an attack of acute porphyria where the latent trait exists and in these cases the clinical manifestations bear some resemblance to those of chronic barbiturate intoxication.

The evidence for the second hypothesis - namely that barbiturates may cause neurological manifestations if given during an attack - is summarized in Fig. 3. Thus 77% (24 out of 31) of cases with paralysis or paresis had been given barbiturates, while 35% (6 out of 17) of those without paralysis or paresis had taken barbiturates. This difference is highly significant ($\chi^2 = 7.8$ 1d.f. $p < .01$). Of the 7 cases with paralysis who had not been given barbiturates, 5 (Cases 12, 13, 34, 35, 44) had only mild neurological involvement but Case No. 43 died with general paresis and bronchopneumonia after a prolonged smouldering course and Case No. 47 had quite severe nerve involvement including pyramidal tract signs. Within the group, who had paralysis and barbiturates, are the most severely afflicted patients, e.g. Cases 2, 23, 24, 32 and 33, who had explosive and fatal courses after repeated administration of barbiturates.

In Section 5 the barbiturates are classified according to their effect in disturbing the porphyrin metabolism of rabbits. Those barbiturates with allyl groups, e.g. seconal, were particularly effective. In accordance with this classification an analysis has been made (Table 1) of those barbiturates which were used in patients who had paralysis and those who did not have paralysis. This study reveals one difference in the two groups of cases. In each case in which seconal was given (Cases 2, 7, 21, 24) paralysis of a very severe degree (in two cases fatal) was found. There are an insufficient number of barbiturates used in the group without paralysis to make this difference statistically significant.

Group of Patients	BARBITURATES USED			TOTAL
	II Effective in Rabbit e.g. Seconal	III Slightly Effective in Rabbit e.g. Phenobarbitone, Nembutal, Luminal	IV Ineffective in Rabbit e.g. Soneryl, Amytal	
PARALYSED (a)	4	19	13	36
NOT PARALYSED (b)	0	4	3	7

Table 1. Analysis of barbiturates used in patients (a) who had paralysis and (b) who did not have paralysis, according to the classification of barbiturates in Section 5 with respect to their effectiveness in disturbing the porphyrin metabolism of rabbits. The numbers refer to the number of patients given one of a barbiturate group. Thus 4 patients, who later became paralysed, received seconal. Some patients received more than one barbiturate.

Seconal = sodium allyl(1-methylbutyl)barbiturate

Phenobarbitone = sodium ethylphenylbarbiturate

Nembutal = sodium ethyl(1-methylbutyl)barbiturate

Luminal = sodium diethylbarbiturate

Soneryl = sodium butylethylbarbiturate

Amytal = sodium isoamylethylbarbiturate

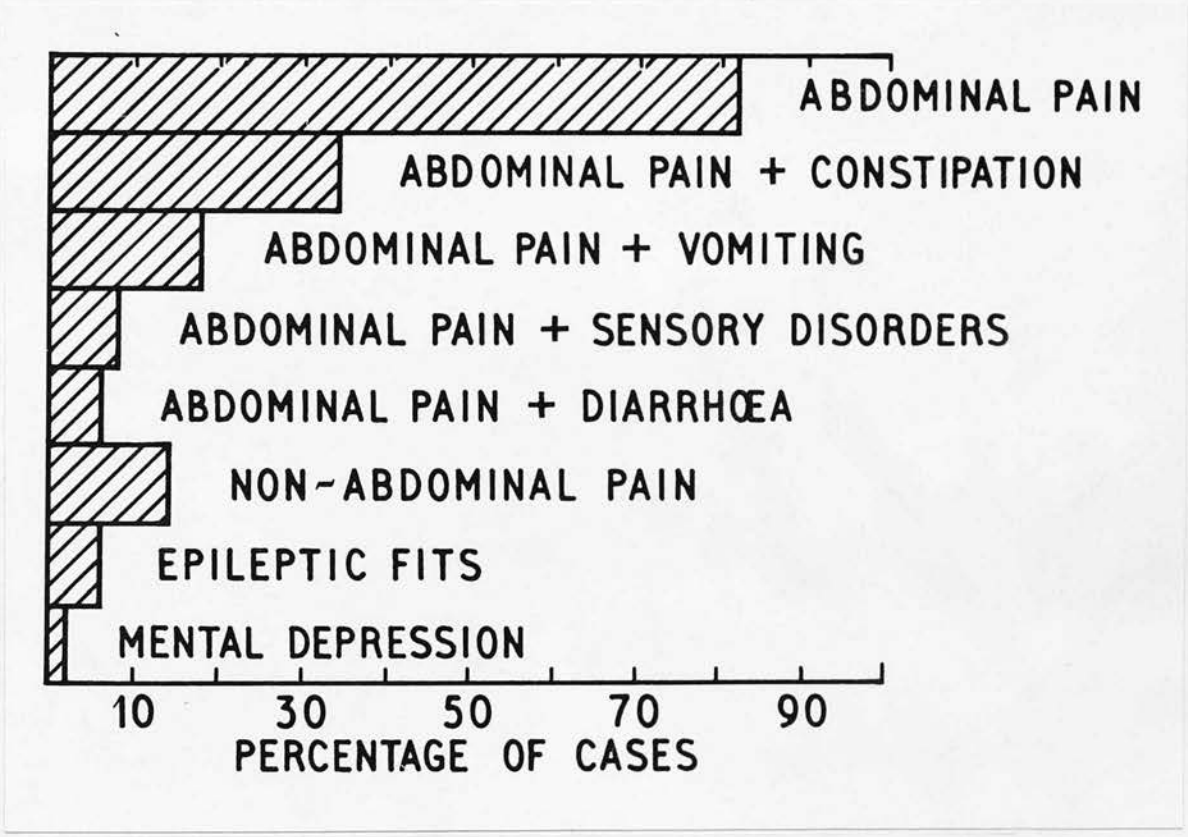


Fig. 4. Section 3.

Main presenting symptoms in
50 cases of acute porphyria

CLINICAL FEATURES

PRESENTING SYMPTOMS

The main presenting symptoms at the onset of the disease are summarised in Fig. 4. These affect 2 systems - gastro-intestinal and neuropsychiatric. Abdominal pain, either alone or associated with constipation, vomiting or more rarely diarrhoea, is the most common complaint. However, in 22% of cases neuro-psychiatric manifestations were predominant and in the majority of these (9 out of 11 cases) there was no complaint of abdominal pain. In a few cases (8%) there was a combination of abdominal pain and disorders of sensation e.g. paraesthesiae or pains of limbs. The 6% of cases which presented with epileptic fits are worthy of note. The danger in the administration of barbiturates to these cases is clear. Many cases had psychological symptoms on admission but only 1 case had such symptoms (mental depression and hysteria in Case 50) as the main presentation.

The incidence of symptoms and signs throughout the course of the disease is shown in Figs. 5 and 6.

GASTRO-INTESTINAL FEATURES

Abdominal pain is predominantly colicky in nature with intermissions when it is constantly present. It may be present in any part of the abdomen but mainly in the epigastrium and (R) iliac fossa or may, in fact, be generalised over the whole abdomen. It is characteristically very severe, boring "deep down" and usually causes great distress to the patient.

All of the patients who complained of abdominal pain and who were examined by the author, did have some abdominal tenderness.

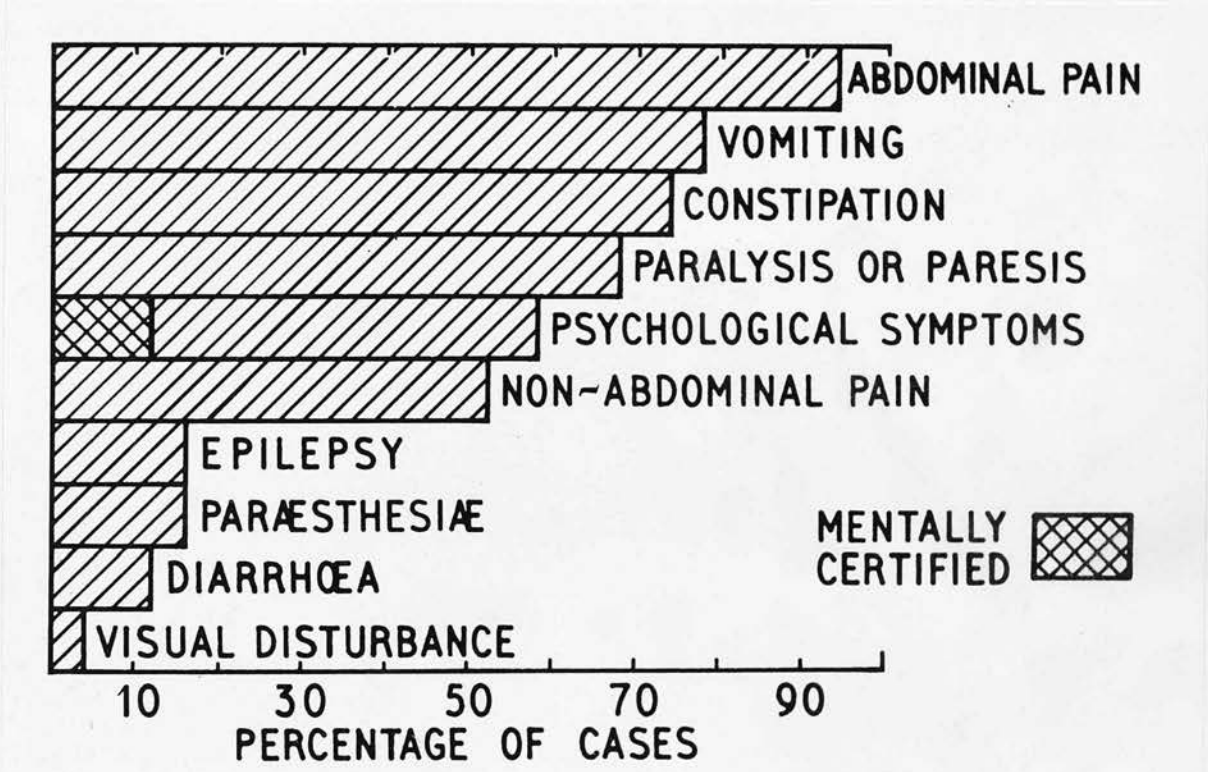


Fig. 5. Section 3.

Incidence of symptoms in
50 cases of acute porphyria

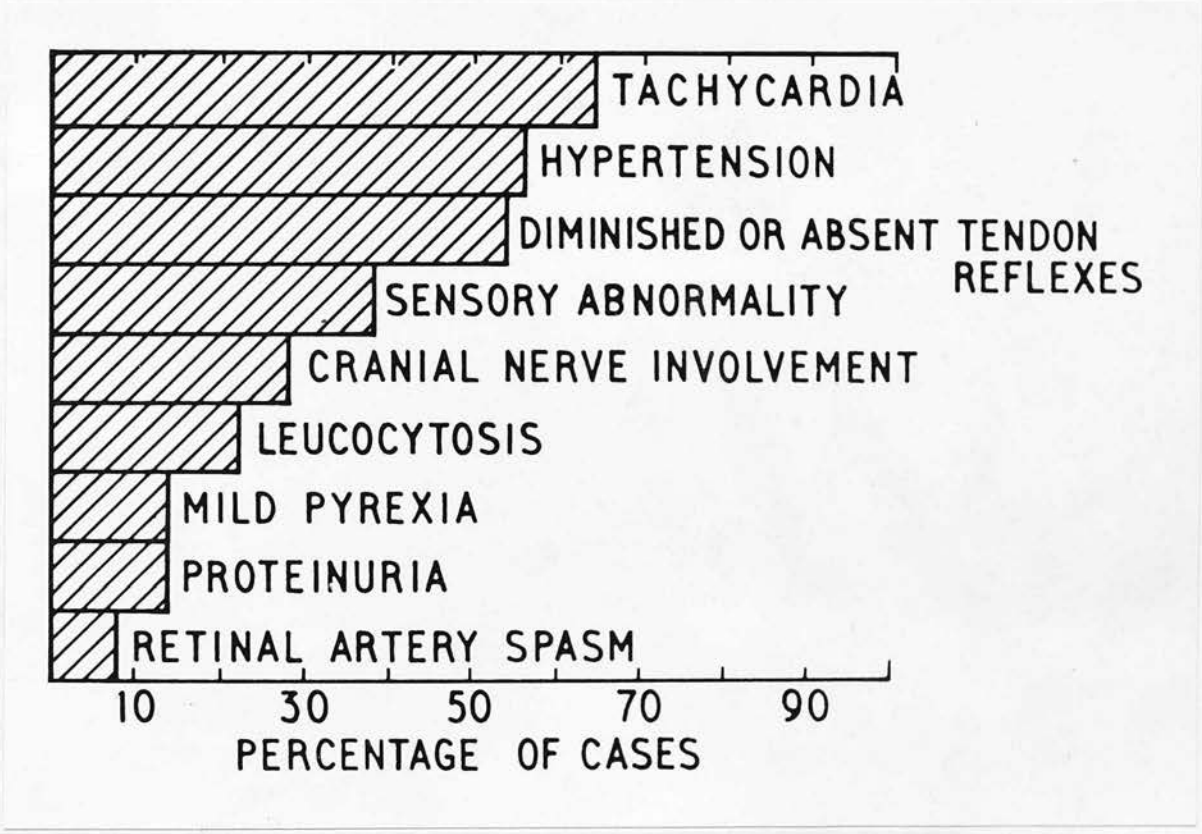


Fig. 6. Section 3.

Incidence of clinical signs in
50 cases of acute porphyria

This was usually much less in degree than the severity of the pain would suggest, but there was no muscular rigidity of the abdomen, with two exceptions. In Case 4 the abdomen became rigid like a case of perforated duodenal ulcer. In Case 21 there was some localised rigidity in the (R) iliac fossa.

Rectal examination was negative in most cases in which it was carried out. In Case 11 there was some rectal tenderness. A large number had vomiting and/or constipation, associated with the abdominal pain. In 1 case (No. 45) vomiting occurred without abdominal pain. Abdominal pain usually preceded the vomiting. 6 cases (Nos. 1, 18, 21, 42, 44 and 50) had diarrhoea and vomiting. It is of diagnostic interest that Case 44 was initially sent to a fever hospital with a diagnosis of gastro-enteritis. In all cases of some severity there was loss of weight during the attack.

NEUROLOGICAL FEATURES

Non-Abdominal pain In 26 cases (52%) pain was felt in the limbs, particularly the legs, in the head and neck, in the large joints, e.g. shoulder and knee, in the lumbar region or in the anterior chest region. In all except 2 cases (Nos. 24 and 27) the pain in these regions was accompanied by abdominal pain. Case 24 had pain predominantly in the large joints. Case 27 had lumbar pain which passed anteriorly and downwards to both groins.

MOTOR DISTURBANCE

Paralysis or Paresis of limb muscles occurred in 34 (68%) of cases. The extent of this varied from weakness of a pair of limbs to complete quadriparalysis. There was paresis of upper limbs only in 1 case (No. 35), paresis of lower limbs only in 5

cases and quadriparalysis or quadriparesis in 28 cases. In the great majority of cases the paralysis was of the lower motor neurone type, although in 5 cases (Nos. 23, 32, 33, 44 and 47) definite extensor plantar responses were obtained and in 1 case (No. 47) there was muscular rigidity of the lower limbs, ankle clonus and increased limb reflexes. In 27 of the 34 cases with paralysis or paresis there were diminished or absent tendon reflexes and in many cases there was marked muscular wasting (Plates 3a and b Appendix). There was a predilection for extensor muscles of wrists and fingers in Cases 5, 14, 25, 41 and 47 (Plate 6b Appendix), but there was no definite pattern of paralysis such as the Landry type which has been suggested in the past. All cases were bilaterally involved but usually unequally so. In very severe cases the muscles of the trunk were also affected. In Case 35 the muscles of the back and upper limbs were particularly weak. Half of the 28 cases with paresis or paralysis of all 4 limbs had cranial nerve involvement (Table 2).

	CASES
Nystagmus	7, 11, 24, 29, 32, 49, 50.
Dysphagia	17, 21, 23, 24, 30, 36.
Aphonia	1, 12, 23, 36.
Diplopia	7, 29.
Facial Palsy	1, 12.
Dysarthria	7.

Table 2. Cranial Nerve Involvement.

The relationship of paralysis or paresis to barbiturates is illustrated in Fig. 3. Incontinence of urine occurred in 6 cases (Nos. 1, 3, 24, 31, 32 and 33) and urinary retention in 1 case (No. 38). In Case 1, incontinence of faeces also occurred. 5 Cases (Nos. 2, 30, 32, 33 and 48) had respiratory paralysis with involvement of the diaphragm and/or intercostal muscles.

SENSORY DISTURBANCE

19 cases (38%) had involvement of sensation (Table 3). Of these all but 2 cases (Nos. 9 and 20) had muscular paralysis or paresis, which came on prior to the sensory loss. 15 cases had muscular paralysis or paresis but no sensory disorder. In 2 cases there was paralysis but the sensory examination was not recorded.

Type of Sensory Disorder	Site	Case No.
Analgesia (Pin-Prick Loss)	Trunk and proximal areas of limbs	13
	Cervical dermatomes 5 & 6	21
	Upper and lower limbs	14
	Lower limbs	11
	Shins	33
	Generalised	2 & 36
Hypo-Algesia	Lower limbs	15
	Bathing trunks region	5 & 24
Hyperaesthesia	Lower limbs	9 & 44
Complete Sensory Loss	Lower limbs	17
	Below mid-thorax	23
Paraesthesiae		
Numbness	Head	7
	Forearms and hands	1 & 7
	Lower limbs	7 & 34
	Waist downwards	50
	Genitalia	23 & 24
Tingling	Feet and calf	9 & 20
Joint and vibration sense loss	Lower limbs to knees	17

Table 3. Summary of Sensory Disorders

VISUAL DISTURBANCE

There was a partial and temporary loss of vision in 3 cases (Nos. 16, 22 and 48) lasting no longer than 2 days.

EPILEPSY

8 cases (Nos. 3, 8, 16, 17, 28, 33, 48 and 50) had epileptic fits. These were generalised in 7 cases. In Case 16 they commenced in the (L) arm, passed to (L) leg and then became generalised. Hypertension was present in 6 of the cases but this was only of a severe degree in 3 cases. In Case 33 there was a B.P. of $\frac{220}{150}$, papilloedema and fits. A prior history of barbiturate ingestion was obtained only in 3 cases (Nos. 16, 28 and 50). Barbiturates were given to control the fits in Cases 17, 28 and 33. In Cases 17 and 33 this was followed by deterioration in the condition and in Case 28 the fits continued until the barbiturates were stopped. Electroencephalograms were performed on 4 of the cases with fits, at the National Hospital, Queen Square, London, at the Maudsley Hospital, London, and at the Central Middlesex Hospital, London. Cases 17 and 50 had normal electroencephalograms. That of Case 33 was abnormal but its pattern was consistent with the stuporose condition of the patient. Case 28 had a mildly abnormal E.E.G. The report is as follows: "A low to medium alpha rhythm is dominant but tends to be unstable in frequency and very mild dysrhythmia is seen at times. There is no significant asymmetry nor focal abnormality, nor disturbance on overbreathing. The record is non-specific and only just outside normal limits, but compatible with the epileptic diathesis".

An electroencephalogram was done at the National Hospital,

Queen Square, London, on Case 29, although she did not have fits. The report of this was as follows: "Slightly abnormal. Confused, choppy type with alpha waves and fast rhythm and enough 5-7 c/s waves to make it abnormal".

MENTAL SYMPTOMS

29 cases (58%) had mental symptoms associated with their illness. These have been arbitrarily classified into 3 grades:

(i) Depressed, nervous, hysterical lacrymose or "peculiar". Case 1, 2, 3, 8, 12, 23, 24, 28, 30, 31, 39, 41, 49, 50. i.e. 14 cases. In Case 50 mental depression was the presenting symptom and she was initially treated as an out-patient in a hospital for mental diseases.

(ii) Confused, hallucinated, disorientated or with personality change. Cases 4, 5, 7, 21, 22, 25, 40, 43, 46. i.e. 9 cases.

(iii) Mentally certified and/or placed in mental institutions. Cases 8, 9, 11, 29, 32, 36. i.e. 6 cases.

The remaining cases were mentally normal. Their reaction to what was, at times, very severe pain, was not excessive.

CEREBRO-SPINAL FLUID

A normal cerebro-spinal fluid, in respect to protein sugar, chloride and cell content, was obtained in 11 cases (Nos. 1, 16, 17, 21, 22, 26, 32, 33, 39, 41 and 48). In Case 31 the chloride content was 670 mg.% (normal 700-750 mg.%). In Case 32 the C.S.F. was tested for porphobilinogen and found to be negative.

CARDIOVASCULAR FINDINGS

Pulse rate 32 cases (64%) had tachycardia (i.e. P.R. over 100/minute) during the acute phase of the disease. In these the P.R. was a good indication of the activity of the condition. In most of these cases the P.R. was 110-120/minute but in a few it rose to 150-160/minute.

Blood pressure 28 cases (56%) had hypertension (i.e. a systolic pressure of 150 mm.Hg. or more and a diastolic pressure of 95 mm. Hg. or more. In some cases (Nos. 3, 4, 7 and 33) there was a marked hypertension. In Case 7 there was a Grade II retinopathy associated with a blood pressure of $\frac{180}{126}$, but the fundus returned to normal when the B.P. became normal. Case 33 had papilloedema and fits associated with the hypertension. Case 2 presented as a case of "Essential Hypertension" and a seconal sedation test was given on 2 occasions, following which the patient's condition rapidly deteriorated and she died. It is of interest that Case 3 developed hypertension in the final attack but not in the previous ones. Case 25 developed hypotension ($\frac{70}{54}$) in 1 phase of her condition.

Electrocardiography Electrocardiograms were reported in 9 cases. Four of these (Nos. 4, 21, 33 and 34) were normal and in Case 43 the E.C.G. showed left axis deviation only; the blood pressure at this time was $\frac{140}{100}$. In the remaining 4 cases I am indebted to Dr. A. Hollman, University College Hospital, London, for help in the interpretation of the E.C.G's.

In these 4 cases the following features were found. P_2 was sharply pointed and there were prominent atrial T waves (Ta),

Plate I. Section 3.

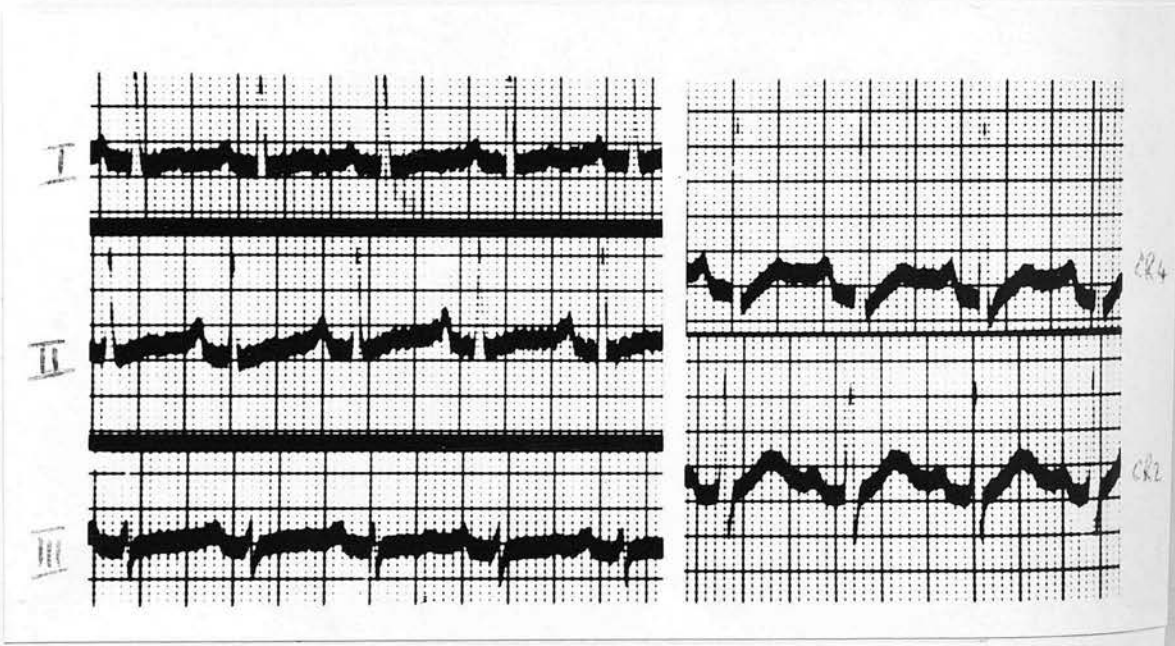


Plate I. Section 3.

Case No. 25. E.C.G. (16/2/52) shows tachycardia, low voltage T waves, pointed P waves in Lead 2, PQ depression in Leads II and CR4.

giving P.Q. and S.T. depression in 2 cases (Nos. 25 and 47). These findings suggest atrial damage or atrial strain and/or hypertrophy, possibly related to respiratory embarrassment. On the other hand, Case 32, in whom (R) atrial dilation and pulmonary collapse was detected post-mortem, did not show these findings.

Low voltage T waves were found in 3 cases (Nos. 25, 32 and 47) which suggests generalised myocardial damage. The serum potassium in these cases was never low enough to account for these changes. There was some cardiac rotation in each of these 4 cases - clockwise in Cases 32 and 36, anti-clockwise in Cases 25 and 47. This was possibly related to (R) heart strain and hypertension respectively.

An example of such an abnormal E.C.G. (Case No. 25) is given in Plate 1.

Ophthalmoscopic Examination Retinal artery spasm was seen in 4 cases (Nos. 30, 36, 37 and 47) (Plate 7 Appendix). As noted above, Cases 7 and 33 had a Grade II retinopathy and papilloedema respectively.

DERMATOLOGICAL FINDINGS

No case had frank photosensitivity of skin, with the exception of Case 47 who had a slight vesicular eruption on her cheeks and bridge of nose at the beginning of her illness. This healed and her facial skin later became pigmented (Plate 6a Appendix). Cases 11, 12, 20, 21, 24, 29, 32 and 36 showed a darkening of the exposed areas of their skin and this returned to a normal colour if the patient recovered.

TEMPERATURE

7 cases had an intermittent low grade pyrexia (99-100.6° F) without obvious infection. These were Cases 11, 19, 20, 25, 31, 43 and 46.

HAEMATOLOGICAL FINDINGS

Haemoglobin determinations were recorded in 27 cases and showed a mean of 14.7 G% (Range 11.8 - 17.3).

The volumes of pack red cells in Cases 32 and 47 were elevated to 55 and 51% respectively. Both these female patients were dehydrated and after adequate hydration the haematocrit of Case 47 became normal. Case 32 died.

The erythrocyte sedimentation rate was recorded in 16 cases by the Westergren or Wintrobe methods. In 11 cases normal values were obtained. In Cases 12, 29, 38, 39 and 49 there were slightly elevated readings by the Westergren method, i.e. 16, 19, 20, 17 and 17 mm./hour respectively.

LEUCOCYTE COUNTS

In 28 cases the leucocyte counts during the active phase were between 4-10,000/cmm. In 11 cases there was an elevated leucocyte count (Table 4), the elevation being due to an increase in polymorphonuclear leucocytes. From Table 4 it can be seen that any substantial leucocytosis was associated with infection or epileptic fits.

Case No.	Leucocyte Count/cmm.	Infection if any or other possible cause
1	24,000	Bronchopneumonia
5	13-45,300	Pulmonary Infection
8	11,600	None
9	12,600	None
16	11,000	None
17	21,000	Post-Epileptic
24	12,000	None
25	29,000	Erysipelas
26	12,000	None
32	12,600	None
39	11,600	None

Table 4. Analysis of leucocyte counts above 10,000/cmm in 11 cases of acute porphyria.

RENAL FINDINGS

Traces of protein were found in the urines of 12 cases, of which 4 also had granular casts. The blood urea was raised in 3 cases (Nos. 2, 11 and 31) but this was probably, in each case, an "extrarenal" type of uraemia due to excessive vomiting. Urinary infections were responsible for aggravations in the condition of Case 36.

PYRUVATE TOLERANCE TEST

Dr. J.N. Cumings, National Hospital, Queen Square, London, carried out pyruvate tolerance tests in Cases 17 and 33 (Table 5). Case 17 showed a slightly abnormal result, while that of Case 33 was frankly abnormal.

TABLE 5

Pyruvate Tolerance Test

Case No.	Results expressed as mg pyruvate/100ml blood Fasting	After 60 mins.	After 90 mins.
*Normal upper limits	1.12	1.26	1.34
Case 17 (30-4-51)	1.02	1.08	1.46
Case 33 (26-8-53)	0.88	-	2.2
(31-8-53)	1.0	1.68	2.08

* Joiner, McArdle, and Thompson (1950).

SERUM ELECTROLYTES

These are recorded in 8 cases (Table 1 Appendix). In 6 of these abnormalities were found. There were diminished sodium and chloride levels in Cases 1 and 47. In Cases 25, 32 and 38 the chlorides were somewhat diminished, while in Case 3 there was a hypochloraemia associated with a hypokalaemia and alkalosis. In each of these cases excessive vomiting had taken place and the results may thus be explained.

EMPIRICAL LIVER FUNCTION TESTS

Some Liver Function tests from 18 cases are recorded in Table 2 (Appendix). It should be emphasised that these were done in different laboratories by different workers. Nevertheless the study of the results bringsforth the following conclusions. In the majority of cases no abnormality was detected. The total protein content of plasma is low in Cases 21 and 34. It is

difficult to know the significance of the abnormality in the Albumen-Globulin ratio in Case 21. The alkaline phosphatase levels in Cases 3 and 33 were raised. It is of interest that in both these cases this test was performed during a very severe phase of a fatal attack. The thymol turbidity was increased in Case 27 and possibly so in Case 30, in which the thymol flocculation was grossly abnormal. The cephalin flocculation was repeatedly abnormal in Case 7, who is an alcoholic. The hippuric acid synthesis test was diminished in Case 4.

Creatinuria Creatine (50 - 700 mg/24 hours) was consistently present in the urine of Case No. 21 in the acute phase of the disease.

CHEMICAL PATHOLOGY OF POST-MORTEM TISSUES

The porphyrin and porphobilinogen analysis of post-mortem tissues in 7 cases of acute porphyria and 2 control patients who did not have porphyria is summarised in Table 3 (Appendix). The liver and kidney contained porphobilinogen in the acute porphyria patients and the liver contained some uroporphyrin where this was tested, in contrast to the control tissues which contained neither porphobilinogen nor uroporphyrin. The livers, bone-marrows and spleens of porphyria patients did not have significantly higher concentrations of coproporphyrin and protoporphyrin than the tissues of control patients. The kidney of 1 case, which was tested, had elevated concentrations of coproporphyrin and protoporphyrin.

The presence of porphobilinogen in the liver and kidney of cases of acute porphyria corroborates the findings of Prunty (1945), Gray (1950) and Schmid et al. (1954).

PATHOLOGICAL FINDINGS

Post-mortems were carried out on Cases 1, 2, 3, 24, 32 and 43 (see case records). Terminal respiratory infection was found in Cases 1, 3 and 43. Pulmonary oedema and (L) ventricular cardiac failure occurred in Case 2. In Case 32 there were small intrapulmonary haemorrhages and areas of collapse, secondary to respiratory paralysis. There was also a small pericardial effusion of about 15 cc. of slightly cloudy fluid, but no pericarditis; the right auricle was dilated; on microscopic examination there was fine patchy atheroma of the coronary arteries and little oedema of the left ventricular myocardium. In Case 24 there was fatty degeneration of the liver and gastric petechial haemorrhages. Gibson and Goldberg (1956) reported on the neuropathology of acute porphyria in 5 Cases, 4 of which belong to the present series (Cases 2, 24, 32 and 43). Concurrently with this, Dr. R.A.B. Drury investigated a biopsy of a dorsal interosseus nerve of the foot of Case 36 (Drury 1956). These neuropathological findings are summarised in Table 4 (Appendix).

COURSE AND PROGNOSIS

The course of acute porphyria in these 50 cases is variable. In a few cases, e.g. 10 and 46, there was merely some intermittent and occasional abdominal pain; in others, e.g. Cases 2, 23, 24, 32 and 33, there was an explosive fatal attack which lasted from 10 days to 10 weeks. The courses in the majority of cases in the present series lie between these 2 extremes. In these, attacks recurred for months or years. A "step ladder"

pattern of increasing severity is seen in Cases 1 and 5. In both of these cases 3 attacks occurred over a period of months, each attack more severe than the last, until the final fatal and paralytic attack took place. In Cases 3 and 30 the patients' condition worsened throughout pregnancy, until death occurred shortly after the birth of the child. The mortality rate in relation to age groups is recorded in Table 6. The overall mortality rate is 24 per cent. These 12 fatal cases are further analysed in Table 7. It can be seen that the majority died from paralyses with or without a terminal respiratory infection. In Case 3 there was also a profound electrolyte disturbance. The most dangerous decade for the patient with acute porphyria is the second in which there was a mortality of 63 per cent.

The duration of the activity of the disease, i.e. the period from the first to the end of the last attack, is some measure of the tendency to relapse. In Table 8 the mean of those periods in the non-fatal cases has been calculated for each 10 year age group. This suggests that after the 4th decade, patients are less liable to relapse, and in fact the 4 patients above the age of 40 who survived their first attack have had no further attacks for several years. The impression is also gained that if a patient in any age group survives a profound and crippling attack, she is unlikely to have a relapse, e.g. Cases 14, 17, 26, 36 and 40.

Age Group (years)	No. of Cases	No. of Fatal Cases	Per cent Mortality
10 - 19	8	5	63%
20 - 29	24	4	17%
30 - 39	13	2	15%
40 - 49	1	0	0
50 - 59	4	1	25%
Total	50	12	24%

Table 6. Relation of mortality rate to age of onset of disease.

Table 7. Analysis of Deaths

Case No.	Age at onset (years)	Duration of illness	Precipitating cause of death	Barbiturates given during attack
1	23	1 year	General Paralysis Bronchopneumonia.	Sodium amytal Phenobarbitone
2	52	4 weeks	General Paralysis Pulmonary oedema.	Seconal
3	29	19 months	End of pregnancy 10 days before death. Pneumonia. Hypochloraemic Alkalosis "Extrarenal Uraemia"	No barbiturates
5	24	6 months	General paresis. Respiratory paralysis.	Sodium amytal, Soneryl.
23	17	10 days	Respiratory paralysis.	Phenobarbitone and soneryl.
24	33	16 days	General Paralysis	Seconal
30	19	6 months	General paresis. Collapse after birth of child.	Nembutal at onset of illness.
32	20	10 weeks	General and respiratory paralysis.	Phenobarbitone
33	13	1 year	Respiratory paralysis. Coma.	Phenobarbitone
41	31	2 years	Respiratory paralysis	Medinal 2 doses
43	18	2 years	Bronchopneumonia General paresis.	No barbiturates
48	15	16 months	Respiratory paralysis	Phenobarbitone

Age Group (years)	No. of non-fatal Cases	Mean duration of activity (years)
10 - 19	3	1.5 (0.5 to 3 yrs).
20 - 29	20	1.9 (10 days - 14 yrs).
30 - 39	11	1.5 (2 weeks - 8 yrs).
40 - 49	1	0.1
50 - 59	3	0.9 (3 weeks - 2 yrs).
Total	38	1.6 (10 days to 14 yrs).

Table 8 Relation of duration of activity of disease in non-fatal cases to age of onset of disease.

DIFFERENTIAL DIAGNOSIS

A definitive diagnosis rests simply on the presence of porphobilinogen in the urine, but the critical point is the recognition that this comparatively rare disease may present with symptoms suggestive of an acute abdominal condition, a psychosis or psychoneurosis, a peripheral neuritis, an epilepsy of unknown causation etc. In this series 6 cases had abdominal operations because of their symptoms - Cases 11, 19, 21, 26, 40 and 47. In Case 19 a gastric ulcer was found on another occasion and a partial gastrectomy was performed. Thus the co-existence of a second condition, unrelated to porphobilinogenuria, may complicate the diagnosis. Case 38 developed abdominal symptoms 4 months after the birth of her child and a diagnosis of post-partum salpingitis was made. Case 26 was diagnosed as a case of poliomyelitis. The articular pain described by Case 24 suggested a diagnosis of acute rheumatism. Case 29 was diagnosed as a "post-herpetic encephalitis". Acute porphyria has been well named "the little simulator" (Waldenström 1939).

TREATMENT

Prophylaxis

In this disease, where definitive treatment awaits a deeper understanding of the biochemical abnormalities, the prevention of an acute attack is important. Whenever an active case is discovered it becomes a duty to test the urines of relatives for the presence of porphobilinogen. The family doctor is thus able to mark the latent porphyria case and if an attack arises,

avoid the dangers of a delayed diagnosis. On the other hand, there is no guarantee that a relative without porphobilinogenuria does not have the genetic defect. It has been observed that individuals heterozygous for the abnormal gene may not excrete porphobilinogen in the urine. Furthermore 5 out of 18 cases did not have porphobilinogen in the urine in states of remission. For these reasons, it is suggested that no member of a family in which there is a known case should be given barbiturates. This is no great therapeutic denial, since there are several excellent non-barbiturate hypnotics.

In dealing with the established case in remission, care should be taken to avoid the several precipitating factors which have been enunciated. Infections should be treated swiftly. Tonsillectomy was a valuable preventive measure in Case 37.

Advice on pregnancy will be asked, but no dogmatic answer can be given. The dangers must be explained and if pregnancy ensues the obstetrician should be alerted. If any surgical procedure becomes necessary, a barbiturate must not be used as an adjuvant to anaesthesia. Careful surveillance throughout the puerperium is necessary since this period, especially the early part of it, is a treacherous one.

Treatment of an acute attack

Usually the patient is a difficult one to treat and nurse. It may be advisable to place her in a side ward rather than in a general ward when gross psychological disturbance and severe abdominal pain are present. If there are signs of paralysis, an artificial respirator should be held in readiness. The relief of

pain can be tried with morphia, pethidine (meperidine), or codeine. Aspirin is not contra-indicated.

The re-establishment and maintenance of salt and water balance is important if excessive vomiting has occurred. The experience should be remembered of Case 47 who was improved by A.C.T.H. administration after enteral and parenteral saline had been given without success. Infection should be sought and treated. Substantial elevations of temperature (more than 101°F), leucocyte count (more than 12,000/c.mm. in the absence of convulsions) or sedimentation rate (more than 20 mm/hour) should give rise to the suspicion that such an infection exists. This aspect of the treatment of the acute porphyria attack resembles the search for a focus of infection in a diabetic coma. The value of this measure is seen in Cases 25, 36 and 37.

Specific Drug Treatment

The efficiency of any treatment is difficult to assess in acute porphyria, in which spontaneous remission and rapid improvement are often encountered. In spite of this, it is of value to attempt an objective judgment of the various kinds of specific drug therapy used in these 50 cases.

A.C.T.H. It is of interest to note that Urquart (1898) suggested that suprarenal extract should be tried in cases of porphyria, since disease of the suprarenals had been recorded in this condition. Campbell (1898) gave "suprarenal tabloids" to a porphyria patient without effect. On the other hand, Prunty (1949) found evidence of adrenal cortical hyperplasia in his case. A.C.T.H. was given in 5 cases in this series. In 3 of these (Nos.

3, 12 and 43) there was no obvious effect, but in cases 25 and 47 there was some improvement of the clinical condition associated with the administration of the drug. In the latter case (No. 47) the hypochloraemia did not respond to enteral or parenteral salt therapy, until A.C.T.H. was given. This may be compared with the severe post-operative hyponatraemia and hypochloraemia, persisting despite adequate intake of sodium, potassium, and chloride, which is quickly corrected by cortisone (Graber et al. 1956). A.C.T.H. is worth trying in those cases in which there is generalised weakness and/or salt and water loss (Goldberg, Macdonald and Rimington, 1952). Watson (1954) has stressed that A.C.T.H. should be given as early as possible in the course of the disease. (Figs. 1 and 3 Appendix).

Cortisone In 3 cases (13, 36, 43) cortisone had no effect, while in Cases 5 and 13, there was some improvement associated with its administration.

Neostigmine (Prostigmine) is a parasympathomimetic drug, which stimulates postganglionic cholinergic nerve endings by means of its anti-cholinesterase activity. Besides this action it exerts a component of direct action on voluntary muscle fibres (Goodman and Gilman, 1955). In many cases of acute porphyria there is an apparent depression of parasympathetic activity (hypertension, tachycardia, constipation, although diarrhoea has less frequently been observed). A myasthenia-gravis-like picture has also been described (Denny-Brown and Sciarra, 1945). It is not surprising, therefore, that neostigmine has been used in the treatment of this condition - in some cases with good effect

(Waldenström, 1944; Berg, 1945; Gordin, 1948; Veflingstad, 1949; Oigard and Roos, 1953; Gillhespy and Smith, 1954) and in other instances without benefit (Ashby and Bulmer, 1950; Fawcett, 1954). Berg (1945) and Berlin and Cotton (1950) observed by kymograph and X-ray examination, respectively, an improvement in the peristaltic activity of the stomach and small intestine of acute porphyria cases, after the injection of prostigmine. In the present series neostigmine had no effect on the paralysis or pain in Cases 5, 6, 9, 11 and 30 but did relieve constipation and retention of urine in Case 30. In Case 36 there was a marked improvement of the mental state associated with administration of neostigmine, but this was probably fortuitous. It is possible that some cases of acute porphyria might be symptomatically helped by this drug, particularly where there is a diminished action of smooth muscle.

Hexamethonium may diminish hypertension (Case 23, 36, 30). It does not alter the course of the disease; it may not relieve pain pari passu with the depression of hypertension (Case 30). In Case 33 this drug was only effective as a hypotensive agent for short periods after its injection. Wehrmacher (1952) has described the variable effects of tetraethyl ammonium chloride and tubocurarine chloride on pain in a case of acute porphyria.

Other Drugs The following drugs were used without apparent benefit - calcium gluconate I.V. (Cases 4 and 25), benadryl (Case 25), priscol (Case 25), probanthine (Case 39), eucortone (Cases 4 and 47). Vitamin B therapy (aneurin and riboflavin) was given enterally or parenterally in several cases (7, 9, 17, 33, 46). With the possible exception of Case 7, an

alcoholic, it had no benefit.

Psychological Attitude In many patients a definite diagnosis is delayed for some time. The patient may begin to accept the statement that the very real pain she is suffering is due to "nervousness" or even "imagination" and in some cases this situation may in itself aggravate the mental state of the patient. Case 29 said she felt greatly relieved when she realized that her doctors had found a cause for her pain, even though her symptoms were not immediately relieved. For this reason it is a kindness to explain that the symptoms have been brought about by a definite disease. In family 10, a brother of Cases 43 and 44, developed an hysterical equivalent of the disease, but had no porphobilinogenuria. The fear of the disease which may be present in healthy relatives should be noted.

Physiotherapy Where paralysis or paresis has occurred the limbs should be correctly maintained in positions of function and active physiotherapy should be started as soon as possible and continued until complete recovery has been attained (see below).

Treatment after a severe attack

The patient may recover quickly and completely. On the other hand, she may be left severely crippled or even insane after the acute attack. It is important to realize that complete restitution of physical and mental function is the rule in these patients, but this may take 4 or 5 years. Thus Cases 36 and 40 were mentally ill for several years, but both eventually returned to a normal state. Cases 14, 17, 21, 26 and 36 had marked wasting and weakness of musculature, but showed remarkable recoveries.

That the pathological changes of the nervous tissue in this disease are so completely reversible should be of great encouragement to the medical attendant and patient.

DISCUSSION OF CLINICAL FEATURES

Günther (1922) suggested that 3 symptoms commonly occurred in acute porphyria - abdominal pain, vomiting and constipation. This is in agreement with the incidence of symptoms in this survey (Fig. 5). Attacks may, however, occur entirely without abdominal pain. The pain may be felt in the limbs, chest, head, neck or the joints. This point has been repeatedly made since Ranking and Pardington (1890) and Harley (1890) referred to non-abdominal pain in the disease. Diarrhoea has also been noted (Garrod and Hopkins, 1896; Günther, 1922; Van den Bergh et al. 1937). Waldenström (1937) described 3 cases with mucous colitis, a condition present in Case No. 42 of the present series. Hypertension in acute porphyria was first described by Melkersson (1926), and Waldenström (1937) noted the significance of the hypertension and tachycardia, but Bostroem (1920) recorded a B.P. of 95/30 in one case. This may be compared to Case 25, who developed hypotension in one of her attacks. Essential hypertension may occur simultaneously as in Case 2 and in Saint et al. (1954).

Some of the electrocardiographic findings are substantiated by those of other workers. Thus Berlin and Cotton (1950) noted P.Q. depression in one case. Eliaser and Kondo (1942) found marked elevation of the S-T segment, which they attributed to coronary arterial spasm. Degenerative changes of the mycocardium

have been described by van den Bergh et al. (1934) and Saint et al. (1954).

The type of neurological involvement - patchy, predominantly motor and this mainly lower motor neurone - is the same as that found by Waldenström (1937). The evidence for pyramidal tract involvement in 5 cases of the present series corroborates similar findings by Melkersson (1926) and Waldenström (1937). Waldenström also found that 60 per cent of his cases had neuropsychiatric manifestations. Five of these were mentally certified. These figures are in agreement with the present series. The association of epileptiform seizures with acute porphyria was noted early in the history of the disease (Campbell, 1898; Brown and Williams, 1909).

Abnormal E.E.G. patterns were obtained in Cases 28, 29 and 33. Berlin and Cotton (1950) and Perrault et al. (1953) also reported definite abnormalities. The former workers noted low voltage waves of 5-7 cycles/sec present throughout the tracing, suggestive of diffuse cortical damage (cf. Case 29 in this series). The latter found signs of paroxysmal activity likely to be basal in origin; this activity disappeared between attacks.

The finding of skin pigmentation in several of the cases in this series corroborates Waldenström (1937) and Vannotti (1954). The latter also described "mild cutaneous lesions of a vesicular nature", which may occur in acute porphyria and this description recalls Case No. 47, in whom such a vesicular eruption occurred on the face at the very onset of the condition. It may be argued that Case 47 should be classified as a porphyria cutanea tarda in view

of this skin manifestation and also because of the raised faecal porphyrin obtained in remission (MacGregor, Nicholas and Rimington, 1952). On the other hand, the skin manifestations were mild and fleeting, while the acute attack was the predominant clinical manifestation.

Proteinuria was present in about 25 per cent of cases. Such a finding has been noted by many observers, e.g. Garrod and Hopkins (1896), Günther (1911, 1922), Grund (1919) and Melkersson (1926). Hymans Van den Bergh et al. (1937) and Harbitz (1924) also described the association of subacute and chronic nephritis respectively, while Stern (1894), Denny-Brown and Sciarra (1945) and Prunty (1949) obtained renal tubular necrosis at the post-mortems of their cases.

Excessive vomiting was suggested as a cause of the serum electrolyte changes. Similar changes have been explained on this basis (Vannotti, 1954), but Prunty (1949) suggested that the pathological lesions he found in the renal tubules might be the cause of the diminished plasma sodium and chloride levels of his patient. The empirical liver function tests show only slight or moderate changes in a few cases. Vannotti (1954) found the galactose tolerance test positive in 30 per cent of his cases. The diminished hippuric acid synthesis in Case 4 may be related to a diminished glycine pool in the liver.

In experimentally induced porphyria in the rabbit, where much porphyrin was formed, there was a diminished urinary glycine excretion, revealed by paper chromatography (Section 6). In cases of human acute porphyria no such change was found, but the

hippuric acid synthesis test may be a more sensitive index of available glycine. The mild diabetic curve in Case 11 and the abnormal pyruvate tolerance test in Case 33 suggest that abnormalities in carbohydrate metabolism, particularly in the brain, may exist in this disease and might repay further study.

The marked creatinuria in Case 36 emphasises the muscle wasting in this condition. Vannotti (1954) recorded creatinuria in a rare form of acute porphyria, which he called "Myoporphyria", and in which muscular atrophy and decolorisation were present.

The presence of a raised blood cholesterol in Case 8 is of interest. Vannotti (1954) recorded that a lowered level was more commonly found.

In Cases 45 and 46 large amounts of coproporphyrin as well as porphobilinogen were found in the urines. The significance of this finding is not known.

4

PHARMACOLOGY OF THE PORPHYRINS AND PORPHOBILINOGEN

4. PHARMACOLOGY OF THE PORPHYRINS AND PORPHOBILINOGEN

The work described in this section attempts to define the relationship between the abnormal substances excreted in acute porphyria and the clinical manifestations of this disorder of porphyrin metabolism. During an attack of acute porphyria, patients usually excrete large quantities of porphobilinogen either alone or with certain porphyrins. The excretion of the porphyrins and porphobilinogen is usually in direct proportion to the severity of the symptoms, suggesting a causal relation, although Waldenström (1939) has reported an authenticated case in which the patient did not pass uroporphyrin or porphobilinogen in the urine or bile during the attack, but did so on other occasions. Several authors have claimed that porphyrins may influence the intestine or uterus (Supniewski, 1927; Günther, 1922; Reitlinger and Klee, 1928; Vannotti, 1937; and Simici, 1938). Critical appraisal of these reports has led to the repetition of this work using porphyrins of the kind known to be excreted in porphyria, which were obtained by improved methods of purification. The isolation of porphobilinogen in crystalline form (Westall, 1952) has for the first time allowed pharmacological testing of the pure substance, although Waldenström and Wendt (1939) and Prunty (1945) had injected partially purified porphobilinogen into rabbits.

METHODS

Animal Experiments Observations were made on the blood pressure (recorded with a cannula in the carotid artery) and on the

respiration of 13 cats and 3 rabbits (anaesthetized with chloralose (80mg./kg) after induction with ether) and of 1 pithed cat, 1 pithed and eviscerate cat, and 1 decerebrate cat. Injections were made into the right femoral vein or the splenic vein.

Stimulation of the distal end of the vagus, separated and cut in the neck, was with supramaximal 0.5 msec. shocks at 10 c./s.

Isolated Organs Experiments were also made on isolated strips of guinea-pig ileum, non-pregnant rabbit uterus, rabbit jejunum and ileum, or cat ileum, set up in Tyrode's solution at 34°C. Contractions were recorded on smoked paper by a frontal writing lever. Experiments with light irradiation were done with an electric bulb of 300 w. at 25 cm. from the tissue in the organ bath.

Drugs The porphyrins, with the exception of haematoporphyrin, had been isolated from biological material as the methyl esters. Before use the esters were hydrolysed with 7 N HCl for 36 hours, at room temperature, the excess of HCl being then removed in a vacuum desiccator over KOH. Haematoporphyrin was prepared and used as the dihydrochloride. Pure crystalline porphobilinogen (Westall, 1952) was used; the porphobilin was obtained by Mr. R. G. Westall as a by-product in the preparation of porphobilinogen.

Porphobilinogen was dissolved in a minimum volume of 0.1 N $NH_4.OH$ and then made up to the required volume with 0.9% saline. For the porphyrins and porphobilin M/7 sodium bicarbonate was used as the solvent.

RESULTS *

Anaesthetized Cats and Rabbits Recordings were made of the direct effect of porphobilinogen - and, in one experiment, of uroporphyrin I - on the blood pressure, respiration and vascular responses of the treated animals to acetylcholine, histamine, nicotine, adrenaline, noradrenaline, and vagal stimulation. The amount of porphobilinogen injected (up to 100 μg/kg.) was limited by the amount available; but from the known rate of excretion in patients (40-160 mg./day), and from the fact that tests for plasma porphobilinogen concentrations at the height of an attack were not greater than 1.5 μg/ml. (see Section 7B), it is likely that the blood levels obtained in our experiments were comparable to or even greater than those obtaining in acute porphyria. The only effect observed was an apparent potentiation by porphobilinogen of the response to adrenaline and noradrenaline in a few of the early experiments (Fig. 1). This apparent potentiation of adrenaline and noradrenaline could not, however, be repeated and its interpretation is complicated by the fact that considerable spontaneous fluctuations in sensitivity to these drugs may occur.

* The work on anaesthetised cats and rabbits was performed by Drs. W.D.M. Paton and J.W. Thompson. The remainder of the work on isolated tissues and unanaesthetised animals was done by the author of this thesis.

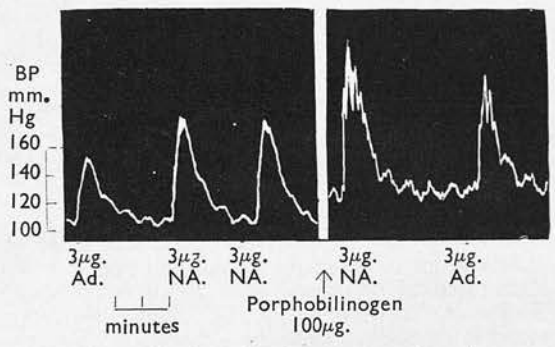


FIG. 1.—Cat.; chloralose; blood pressure recording; intravenous injections. Responses to 3 μg. adrenaline and noradrenaline before and after 100 μg. porphobilinogen.

Isolated Organs After obtaining records of spontaneous activity and tone, and of consistent responses to acetylcholine, histamine, adrenaline, and 5-hydroxytryptamine, the effect of adding porphyrins, porphobilinogen, and porphobilin to the preparation was investigated. With uroporphyrin I the effect of light irradiation was also determined. A summary of these results is given in Table I.

TABLE I
TESTS OF PORPHOBILINOGEN AND PORPHYRINS ON ISOLATED TISSUES

		Porphobilinogen	Uroporphyrin I	Uroporphyrin III	Coproporphyrin I	Coproporphyrin III	Haematoporphyrin	Porphobilin
Rabbit uterus	Drug concn.	1/40,000	1/10,000	1/40,000	1/40,000	1/40,000		
	Effect on spontaneous activity and tone	0	0	0	0	0		
	Effect on response to adrenaline	0	0	0	0	0		
Rabbit ileum or jejunum	Drug concn.	1/40,000	1/10,000				1/10,000	
	Effect on spontaneous activity and tone	0	0				0	
	Effect of combining with light irradiation		0					
	Effect on response to histamine, acetylcholine, adrenaline						0	
Guinea-pig ileum	Drug concn.	1/20,000	1/40–10,000	1/10,000	1/20,000	1/20,000	1/8,000	1/20,000
	Effect on spontaneous activity and tone	0	Slight + twice only	0	+ twice only	0	–	+ (histamine contaminant ?)
	Effect on response to acetylcholine	0					–	
	Effect on response to 5-OH tryptamine	0		0	0	0		
	Effect on response to histamine		+ (50%) once only				–	

0 = No effect: + = increase; – = decrease.

The only significant responses were those to haematoporphyrin (1/8,000) and to porphobilin. The former produced a distinct waning contraction of guinea-pig ileum, followed by inactivity of the intestine and a refractoriness - which became complete - to histamine and acetylcholine. Rabbit intestine was unaffected. Porphobilin produced a histamine-like contraction, sensitive to mepyramine, but less so to atropine (Fig. 2); it was considered likely that the effect was due to contamination (c. 0.4 mg./g) with histamine itself.

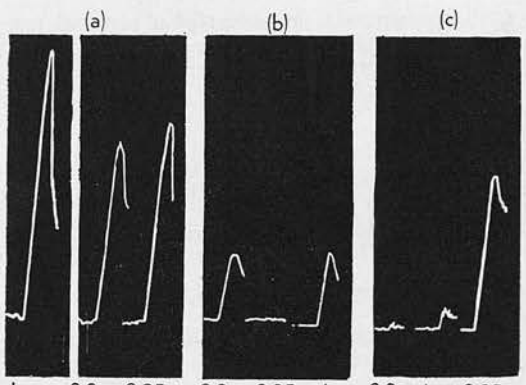


FIG. 2.—Isolated guinea-pig ileum. Response to 1 mg. porphobilin, 0.3 µg. histamine and 0.25 µg. acetylcholine (a) normally, (b) in presence of 1.5×10^{-9} atropine, (c) in presence of 10^{-9} mepyramine.

Test of Porphobilinogen on Unanaesthetized Rabbit

10 mg. porphobilinogen was injected intravenously into a rabbit (2.2 kg.) with an external biliary fistula. The animal showed no abnormal symptoms in the 3 days following the injection. There was a slight rise in the level of bile protoporphyrin during this period and a trace of uroporphyrin was noted in the urine several hours after the injection. No porphobilinogen was found

in the urine.

Test of Whole Urine from Patients with Acute Porphyria

As a final test, to cover the possibility that in porphyria some unidentified pharmacologically active substance is excreted, a sterile specimen of urine from a patient suffering a moderately severe attack of acute porphyria (with hypertension and abdominal pain) was infused into an anaesthetized cat at a rate of approximately 4 ml./min. for 15 min. This by itself produced no effect on blood pressure or respiration; and its effect on the responses to adrenaline, noradrenaline, histamine, acetylcholine, and nicotine was indistinguishable from that of a specimen of normal urine.

DISCUSSION

Despite a great deal of research on acute porphyria the mechanism of the production of symptoms remains obscure. Recent work has tended to minimize the possible direct influence of the porphyrins (Waldenström, 1939) and to emphasize the importance of the pathological features of patchy myelin change observed in the peripheral and autonomic nerves (Denny-Brown and Sciarra, 1945). These authors considered that the changes might be caused by an intermittent ischaemia, probably due to a circulating vaso-constrictor substance. Following this, Wehrmacher (1952) reported clinical improvement in acute porphyria with the use of ganglion-blocking agents. A search for some such vasoconstrictor substance, which might be present only in active cases of acute porphyria, would be a reasonable approach to the problem. The pharmacological testing of the known and already purified

excretion products was clearly necessary. It would be unlikely that uroporphyrin and coproporphyrin could fulfil this role, since these are excreted in increased amounts in both congenital porphyria (as the series I isomers), and in porphyria cutanea tarda (as the series I and III isomers) where skin photosensitivity may be the only symptom. Porphobilinogen, however, is always excreted in the urine in attacks of acute porphyria and in those phases of porphyria cutanea tarda where acute symptoms are superimposed on the cutaneous syndrome. For this reason, porphobilinogen or some closely related substance has been strongly suspected of being the materia peccans of acute porphyria (Lowry et al., 1950).

These experiments have failed to show that either the porphyrins or porphobilinogen have any significant pharmacological action. The initial animal experiments, in which porphobilinogen appeared to potentiate the blood pressure responses of the cat to adrenaline and noradrenaline, could not be repeated. These interesting results cannot be explained, although it is just possible that there may be an individual tissue and animal sensitivity to such drugs. Their inactivity is at variance with the results of some previous investigators. The difficulty of isolating porphyrins from biological materials, such as urine, in a state of purity that will guarantee freedom from possible histamine contamination, is very great. It is suggested that the contradictory results obtained by previous workers may possibly have been due to histamine contamination.

The results of intravenous injection of porphobilinogen

into a rabbit confirm Prunty's (1945) findings, but contradict those of Waldenström and Wendt (1939), who found porphobilinogen in the urine of a rabbit into which they had previously injected the partially purified substance (amount used unknown). Experiments on the excretion of porphobilinogen after enteral and parenteral administration to rats have shown that this substance is removed by glomerular filtration without significant tubular re-absorption (Section 7A). The reason for the present results in the rabbit is probably due to the loss of porphobilinogen through the external bile fistula.

Further work has tended to substantiate the absence of pharmacological activity of the porphyrins and porphobilinogen. An "experimental porphyria" or disturbance of porphyrin metabolism has been produced in rabbits by the non-hypnotic substance allyl isopropyl acetamide (Goldberg, 1953); very large quantities of uroporphyrin and porphobilinogen were excreted - in one animal for as long as three weeks - without obvious pharmacological effect. Apart from constipation, there is no evidence that the state of these rabbits compared with the clinical state of human acute porphyria, although the animals excreted proportionately greater quantities of the substances. Falk, Dresel, and Rimington (1953) have shown that porphobilinogen is a porphyrin precursor in a tissue system. This emphasizes that in porphobilinogen we are probably dealing with a physiological substance. While these experiments therefore do not rule out the possibility that an unidentified vasoconstrictor substance may be produced in acute porphyria, or that the known excretion products may exert

a pressor action by a mechanism at present unknown, they render these suggestions unlikely.

SUMMARY

1. The porphobilinogen and the porphyrins usually excreted in acute porphyria, as well as haematoporphyrin and porphobilin, have been tested pharmacologically.
2. Apart from slight and variable action of uroporphyrin I, coproporphyrin I, and porphobilin, these substances show no pharmacological action.
3. The significance of the results in relation to the symptoms of acute porphyria is discussed.

5

THE EFFECT OF CERTAIN BARBITURATES ON THE PORPHYRIN METABOLISM OF RABBITS

5. THE EFFECT OF CERTAIN BARBITURATES ON THE PORPHYRIN METABOLISM OF RABBITS

Introduction

In Section 3, evidence was put forward that the barbiturates may precipitate an attack of acute porphyria or may be associated with the paralytic phase of the disease. Lehmann and Zinn (1910) first carried out an experiment with diethyl barbituric acid, which had caused a porphyrinuria in one of their patients. They gave this drug in prolonged dosage to one rabbit without the production of similar pigments. Animal experiments were also carried out by Laubender and Monden (1938), who investigated the effects of diethyl, bromallylisopropyl and ethyl-cyclohexenyl-barbituric acids on the urinary porphyrins of rabbits. They failed to find any such effect in all these experiments with the exception of one animal treated with diethylbarbituric acid, which showed a slight rise of urinary porphyrins.

The barbiturates comprise a group of chemically related drugs, while acute porphyria is a rare disease. Moreover, it is usually impossible to tell from published records whether the barbiturates had precipitated the overt disease in a case of latent porphyria or had in fact provoked acute porphyria in a formerly healthy subject. To overcome this difficulty and the uncertainty in assessment of these clinical impressions, the effects of different barbiturates on the porphyrin metabolism of rabbits were studied. Out of nine barbiturates so tested, six

had a significant effect on the urinary porphyrin excretion; those containing one or more allyl groups were particularly effective.

METHODS

Seventeen rabbits (average wt. 2.8 kg.) were housed in individual metabolism cages allowing the separation of urine and faeces. A preliminary base-line period of 4-7 days before drug administration was allowed during which the total daily urinary coproporphyrin and sometimes also faecal porphyrins were determined. If the animal died during an experiment, tissue porphyrins and porphobilinogen were also determined. The average duration of each course of barbiturate was 12 days. The types of barbiturates and their route of administration are recorded in Table 1. If the rabbit survived this course, analyses were continued during a recovery period of 4-7 days. After such an interval, some rabbits were started on a different barbiturate as indicated in Table 1. The maximum sub-lethal dose of each drug was employed.

Quantitative analyses

Urinary porphyrins. These were determined by the method of Rimington and Sveinsson (1950) with the following modifications. Ether-soluble porphyrins were extracted by ether: acetic acid (10:1 by vol.) and after the ether had been washed three times with water, were transferred directly into 1.4N-HCl for spectrophotometric determination (Beckman, model DU). One part of urine containing porphobilinogen, was diluted with 4 parts 2N sodium acetate buffer pH 4.22, and placed in boiling water for

Table 1. *Effect of barbiturates on the urinary excretion of coproporphyrin in rabbits*

The sodium barbiturates were given in equally divided dosage twice daily. A letter after a rabbit number signifies that the rabbit obtained the corresponding barbiturate on a primary course *a*, or on subsequent courses *b*, *c*, etc. Doses were administered by intramuscular injection unless indicated otherwise.

Drug	Rabbit no.	Dosage: Average level (mg./kg./day)	Dosage: Duration (days)	Mean urinary coproporphyrin: Pre-dosage level (μg./day)	Mean urinary coproporphyrin: Increase (μg./day)
5:5-Diallylbarbituric acid	2*a*	113*	15	19·7	+193·7
	1*b*	103*	35	7·5	+169·5
	3	100	17	5·2	+119·2
	4*a*	128*	12	8·7	+88·3
	5	101·6	7	8	+86
	6	125	9	5·3	+84
	7	114	7	2	+51
	8	148	7	3·3	+47
Sodium 5-allyl-5-(1-methylbutyl)-barbiturate	9	172	7	3·7	+42·6
	10	115	12	3·8	+22·9
	11*b*	100	12	9·8	+20·2
	2*d*	82·8	12	19·7	+17·3
5-Allyl-5-*iso*propylbarbituric acid	2*b*	67·8†	12	19·7	+81·6
	12	76†	13	7	+40
	13	74†	12	7	+27
Sodium 5:5-diethylbarbiturate	14*b*	171	13	5	+ 9·7
	13*d*	140	12	7	+ 9·6
	15	169	13	1·8	+ 8·7
	16	144	12	2·4	+ 5·6
Sodium 5-ethyl-5-(1-methylbutyl)-barbiturate	11*a*	98·5	12	9·8	+15·2
	2*c*	110·5	12	19·7	+ 7·3
	4*b*	128	14	8·7	+ 2·3
Sodium 5-ethyl-5-phenylbarbiturate	14*a*	71	9	5	+14·7
	1*a*	67	20	7·5	+13·5
	13*c*	81	12	7	+11·7
	16*b*	82	12	2·4	+ 6·8
Sodium 5-*iso*amyl-5-ethylbarbiturate	13*b*	90	12	7	− 2
	2*e*	83	12	19·7	− 5·2
Sodium 5-butyl-5-ethylbarbiturate	17*a*	109·5	12	11·5	+ 5·5
	16*a*	98	12	2·4	+ 2·3
Sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate	17*b*	218	12	11·5	+ 5·2
	2*f*	164	12	19·7	− 1·7

* Some doses given by gastric intubation.

† Given by gastric intubation.

20 min. Appropriate dilution for spectrophotometric determination of the total uroporphyrin was made with HCl so that the final concentration of acid was 0.5N.

Faecal coproporphyrin and tissue copro- and proto-porphyrin. Faeces and tissues were ground thoroughly in a mortar. Liver tissue was in addition comminuted in a Waring Blendor. The ether-soluble porphyrins from weighed portions of faeces and tissue were extracted by repeated shaking with ether:acetic acid (10:1, v/v) until the supernatant after centrifuging showed no porphyrin fluorescence. The porphyrins were then analysed as in the case of urine.

Porphobilinogen. Porphobilinogen in urine was determined by the method of Vahlquist (1939). Tissue dispersions were treated with about an equal volume of 20% (w/v) trichloroacetic acid, the mixture was centrifuged and the supernatant analysed as in the case of urine.

Blood examination. Haemoglobin was determined by the method of Rimington (1942). Erythrocyte counts and reticulocyte counts were carried out using standard techniques.

Urinary amino acids. These were examined by methods of Datta, Dent and Harris (1950).

Identification of porphyrins

The urines from rabbits treated with sodium ally (1-methylbutyl) barbiturate or allylisopropylbarbituric acid contained increased amounts of porphyrins, all of which were ether soluble. These urines were preserved with toluene and stored at 3°. The porphyrins were extracted with ether:acetic acid and, after the ether had been washed 3 times with water, were transferred into a minimal volume of 2.8N-HCl and then precipitated at the isoelectric point (pH3.1). The precipitate was centrifuged, dried, and esterified by contact with methanolic HCl for 24 hr. The porphyrin esters were transferred into $CHCl_3$ by the addition of saturated sodium acetate, the $CHCl_3$ layer washed once with water, once with dilute ammonia, and twice with water, in that order. The α band (absorption band at longest wavelength) of the porphyrin ester at this stage was noted on the Hartridge Reversion Spectroscope. A portion of the ester was dried, hydrolysed with 7N-HCl for 36 hr. and chromatographed

on paper (Nicholas and Rimington, 1951). The remainder was crystallized from $CHCl_3$: methanol (1:5, v/v.). Melting points, which were not corrected, were determined on an electrically heated micro apparatus (A. Gallenkamp and Co. Ltd.). Isomer analysis was done by the method of Chu, Green and Chu (1951).

Urines from some rabbits treated with diallylbarbituric acid contained uroporphyrin and porphobilinogen as well as an increase in ether-soluble porphyrins. These combined urines were brought to pH3-4 with glacial acetic acid and sufficient talc was added to adsorb all porphyrins. The talc was filtered, dried, and repeatedly eluted with 2N ammonia until no porphyrin fluorescence appeared in the supernatant. The total alkaline eluates were then brought to the isoelectric point and the porphyrins thus precipitated were dried, esterified and taken into $CHCl_3$ as described above. The porphyrin esters were fractionated by chromatography on columns of aluminium oxide Grade IV and magnesium oxide Grade III (Nicholas, 1951). The fractions were defined by α band estimations and, after hydrolysis, by paper chromatography (Nicholas and Rimington, 1951). Isomer analysis of octacarboxylic porphyrin esters was performed by the method of Falk and Benson (1953), while that on tetra and penta-carboxylic porphyrins was done by the method of Chu et al. (1951). The uroporphyrin ester was crystallized from chloroform:methanol.

RESULTS

Those barbiturates which produced the greatest increase of urinary porphyrins also caused the most prolonged hypnosis. Diallybarbituric acid was the most effective drug, while

allylisopropylbarbituric acid and sodium allyl (1-methylbutyl) barbiturate, caused a higher excretion of urinary coproporphyrin and a more prolonged hypnosis than the remaining barbiturates. In each of three rabbits treated with diallylbarbituric acid, the drug was given sometimes by gastric intubation and sometimes by intramuscular injection. No significant quantitative difference of urinary porphyrin excretion was obtained by thus changing the route.

Urinary coproporphyrin Table 1 records the average daily excretion of coproporphyrin in the urine during the pre-dosage period and the average deviation from this figure during administration of the drug. Coefficients of variation are not included since in almost every instance the results were quite obviously significant. Where an effective barbiturate was used, the urine coproporphyrin excretion showed a prompt increase after the 1st or 2nd day, rose to a maximum level on about the 6-7th day, remained at about that level during the subsequent administration of the drug and fell to normal within 2-3 days of its cessation.

Urinary uroporphyrin and porphobilinogen In three rabbits (1b, 2a and 3) treated with diallylbarbituric acid, uroporphyrin and porphobilinogen appeared in the urine on the 7th, 7th and 8th days, respectively, and generally continued to be excreted for as long as the drug was given (Table 2 and Fig. 1).

Faecal coproporphyrin In rabbit 1b the faecal coproporphyrin was determined before and during administration of diallylbarbituric acid. This showed that the faecal coproporphyrin rose at the same rate as the urine coproporphyrin from a previous normal

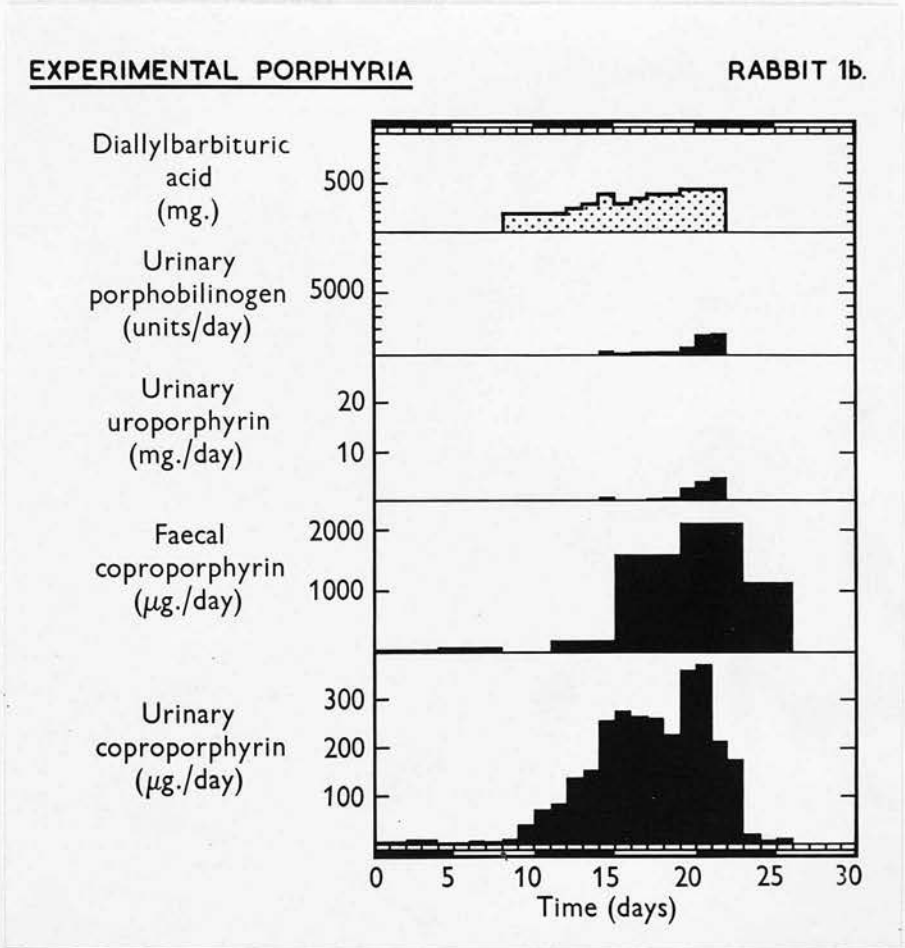


Fig. 1. Effects of diallylbarbituric acid on the urinary uroporphyrin, coproporphyrin and porphobilinogen and faecal coproporphyrin excretion of rabbit 1b.

Rabbit No.	Total uroporphyrin (μg./day)		Porphobilinogen (units*/day)	
	Average	Maximum	Average	Maximum
1b	1720	4750	456	1607
2a	360	800	78	161
3	250	340	78	109

* Vahlquist (1939)

Table 2. Daily urinary uroporphyrin and porphobilinogen excretion in rabbits receiving diallylbarbituric acid.

daily average of 38 μg./day to a daily average of 1495 μg./day.

Tissue porphyrins Table 3 summarizes the tissue analyses for porphyrin of four normal rabbits, and five rabbits which had died from barbiturate intoxication at a stage when they were excreting increased urinary coproporphyrin. There was an increase of copro- and proto-porphyrin concentrations in the bile and liver in intoxicated rabbits, but the levels in the bone marrow showed no abnormality. The liver of rabbit 10 gave a faint but definitely positive reaction for porphobilinogen. Uroporphyrin was never found in any tissue.

Blood examination Haemoglobin determinations, erythrocyte counts and reticulocyte counts were made immediately before, during and

Table 3. *Tissue porphyrin determinations on four normal rabbits and five rabbits treated with barbiturates*

The five rabbits died while excreting excessive urinary coproporphyrin. Figures represent μg./ml. (bile) or μg./g. (liver and marrow).

Rabbit no.	Bile		Liver				Bone marrow			
	Copro-porphyrin	Proto-porphyrin	Copro-porphyrin	Proto-porphyrin	Porpho-bilinogen	Uro-porphyrin	Copro-porphyrin	Proto-porphyrin	Porpho-bilinogen	Uro-porphyrin
Normal										
Control I	0·15	Nil	0·10	0·10	Nil	Nil	0·10	Nil	Nil	Nil
Control II	0·38	0·34	0·18	0·12	Nil	Nil	0·20	0·15	Nil	Nil
Control III	0·74	0·41	0·18	0·51	Nil	Nil	0·22	0·24	Nil	Nil
Control IV	Insufficient obtained		0·02	0·23	Nil	Nil	0·18	0·16	Nil	Nil
Diallylbarbituric acid										
8	Insufficient obtained		5·5	49	Nil	Nil	0·10	0·12	Nil	Nil
7	25	172	11·8	30·9	Nil	Nil	0·12	0·14	Nil	Nil
5	5·2	84·0	3·2	4·66	Nil	Nil	0·15	0·22	Nil	Nil
Sodium allyl(1-methylbutyl)barbiturate										
10	0·78	12·68	4·76	17	Faint +	Nil	0·10	0·24	Nil	Nil
9	Insufficient obtained		1·91	3·84	Nil	Nil	0·30	0·31	Nil	Nil

after administration of the drug to three rabbits treated with diallylbarbituric acid and one treated with allylisopropyl barbituric acid. No significant change was observed in any of the erythrocyte counts or reticulocyte counts. In two of these rabbits (2b and 3), a slight fall (about 2 g.%) in the haemoglobin level was observed, while two rabbits (1b and 7) showed no significant change.

Urinary amino acids In seven rabbits, in which a rise of urinary coproporphyrin was obtained, the urinary amino acid pattern was no different before and at the end of barbiturate administration.

Identification of porphyrins The ether-soluble porphyrins obtained from the urines of rabbits treated with allylisopropyl-barbituric acid or sodium allyl(1-methylbutyl)barbiturate consisted entirely of coproporphyrin III. The ester m.p.'s and the results of paper chromatograms are shown in Table 4.

Table 4. *Identification of urinary porphyrins. Summary of results*

	Free porphyrin	Porphyrin ester				
		Paper chromatography				
	Paper chromatography (Nicholas & Rimington, 1951)	α Band	(*a*) Chu *et al.* 1951	(*b*) Falk & Benson, 1953	M.p. of crystals (°)	Summary
Diallylbarbituric acid	8 COOH	625·1	—	Mainly series III	274	Uroporphyrin III
	6 COOH	623·6	—	—	—	Hexacarboxylic porphyrin
	5 COOH	622·5	Series III	—	—	Pentacarboxylic porphyrin III
	4 COOH	621·1	Series III	—	—	Coproporphyrin III
Sodium allyl(1-methyl-butyl)barbiturate	4 COOH	621·3	Mainly series III	—	154, remelt. 172–4	Coproporphyrin III
Allyl*iso*propyl-barbituric acid	4 COOH	621·7	Mainly series III	—	150, remelt. 160	Coproporphyrin III

The porphyrin esters obtained from the urine of rabbits treated with diallylbarbituric acid were fractionated on a magnesium oxide column. This separated first an octacarboxylic porphyrin with an α band 625.0 mμ.; then two intermediate fractions with α bands 623.0 and 621.6 mμ., respectively; and finally a tetracarboxylic porphyrin with α band 621.1 mμ. The first fraction was rechromatographed on a magnesium oxide column and yielded a single porphyrin zone. The appearance of the crystals obtained from this zone was suggestive of uroporphyrin III. The m.p. (274°) and paper chromatography (Falk and Benson, 1953) showed the series III isomer to be that mainly present (see Table 4). The two intermediate fractions were further chromatographed on an aluminium oxide column. This allowed the separation of a main portion with α band 622.5 mμ. and a lesser portion with α band 623.5 mμ., which behaved as pentacarboxylic and hexacarboxylic porphyrins, respectively, when examined by paper chromatography (Nicholas and Rimington, 1951). On a paper chromatogram (Chu et al. 1951), the pentacarboxylic porphyrin had about the same mobility as coproporphyrin III. The final fraction was identified as coproporphyrin III by melting point and paper chromatography (see Table 4).

DISCUSSION

This work has shown that of the nine different barbiturates examined, six were capable of producing some effect on the porphyrin metabolism of rabbits. Of seventeen rabbits used, eight were given more than one barbiturate in separate courses. This was considered as a possible factor influencing results. However,

another barbiturate was never started until the urinary coproporphyrin level had returned to its normal from the previous course, and the results in Table 1 show that the same drug, used on different animals, whether it was their first or subsequent barbiturate, generally gave corresponding results in porphyrin excretion. For the more effective barbiturates, sufficient numbers of rabbits were used, on a primary or subsequent barbiturate course, to demonstrate the unimportance of previous intoxication. Difference in effect upon porphyrin excretion must depend, among other things, upon the chemical constitution of the drug, the daily dosage employed and its duration, and also, possibly, upon the normal level of porphyrin excretion of the individual animal.

The results in Table 1 show that the barbiturates used may be classified in four groups according to their effectiveness (Fig. 2).

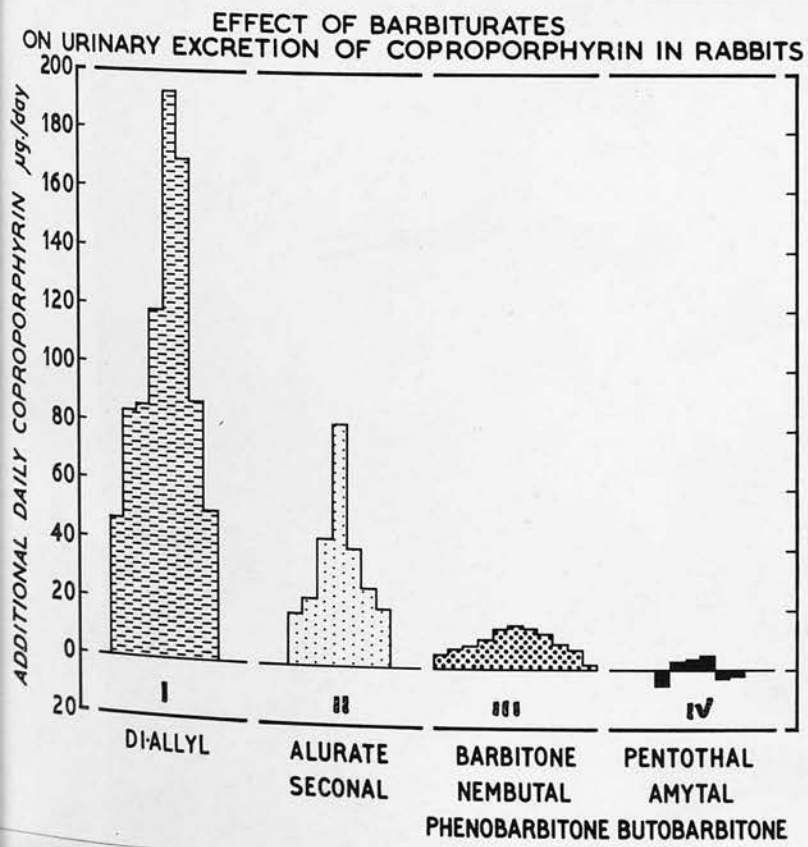


Fig. 2. Each vertical segment represents the average daily coproporphyrin output in a single rabbit during barbiturate administration in excess of (or less than) the normal average daily coproporphyrin excretion. I, diallylbarbituric acid; II, allylisopropylbarbituric acid and sodium allyl(1-methylbutyl) barbiturate. III, sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)barbiturate, and sodium ethylphenylbarbiturate. IV, sodium ethyl(1-methylbutyl) thiobarbiturate, sodium isoamylethylbarbiturate, and sodium butylethylbarbiturate.

(1) Diallylbarbituric acid, which caused a considerable rise of urinary coproporphyrin in each of eight rabbits and, in three of these, the excretion also of uroporphyrin and porphobilinogen.

(2) Allylisopropylbarbituric acid and sodium ally(1-methylbutyl)barbiturate, which caused a moderate rise of urinary coproporphyrin.

(3) Sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)barbiturate, and sodium ethylphenylbarbiturate, which caused only a slight increase of coproporphyrin in the urine.

(4) Sodium ethyl(1-methylbutyl)thiobarbiturate, sodium isoamylethylbarbiturate, and sodium butylethylbarbiturate, which did not alter significantly the urinary coproporphyrin excretion.

The possession of the allyl group thus appeared to make the barbiturate more effective in producing a rise of porphyrin in the urine. These drugs also caused deeper and more prolonged hypnosis in rabbits. Drugs possessing an allyl group have been known to give rise to pathological and metabolic changes in humans and in experimental animals. Popper (1936) and Jürgens (1951) have shown that allylformate and N-(diallylacetyl)urea, respectively give rise to hepatic cellular degeneration in experimental animals. N-(Allylisopropylacetyl)urea may cause thrombocytopenic purpura in humans (Ackroyd, 1949), may influence the course of human acute porphyria (Duesberg, 1932), and gives rise to the hepatic type of experimental porphyria in rabbits with the excretion of large amounts of uroporphyrin and porphobilinogen (Schmid and Schwartz, 1952). Diallylbarbituric acid

has for some time been known to be one of the most toxic of the barbiturates and for this reason has generally been abandoned as an hypnotic in humans.

The finding in post-mortem tissue analysis that the liver and not the bone marrow contained excessive copro- and proto-porphyrin (and in one instance porphobilinogen) recalls the finding in the liver of porphobilinogen (Prunty, 1945) and uroporphyrin (Watson, Schwartz and Hawkinson, 1945) in human acute porphyria and of these two substances in experimental porphyria in rabbits (Schmid and Schwartz, 1952). It also may be related to the known hepatotoxic effect of other allyl derivatives in experimental animals. It is of interest, too, that the coproporphyrin and uro-porphyrin produced in excess were the series III isomers. Rimington (1952) has proposed a scheme of possible enzymic derangements, occurring in the porphyrias and the toxic porphyrinurias. It is not possible from the present results to interpret the precise mechanism occurring in the barbiturate porphyrinurias, but a reasonable inference from them might be that some of the barbiturates, especially those containing the allyl group, may interfere with the enzymes which decarboxylate uroporphyrin III to coproporphyrin III and protoporphyrin and, perhaps, with those responsible for the transformation of protoporphyrin to haem. Diallylbarbituric acid may also block the formation of tetrapyrroles from porphobilinogen. The main site of this interference may possibly be the liver.

The present findings raise the questions of the bearing of these animal experiments upon barbiturate medication in human beings and of the relation, if any, which exists between human

acute porphyria and the disturbances of pigment metabolism caused by barbiturates in rabbits. The doses of barbiturates used in the animal experiments were proportionately (weight for weight) greatly in excess of those normally taken by humans. The rabbits, however, generally tolerated these daily doses well and were mostly able to survive the period of drug administration. Humans if given a proportionate amount of barbiturate almost certainly could not survive. A weight-for-weight comparison of the effect of the barbiturates on human and rabbit porphyrin metabolism would therefore be misleading. From these experiments, we may possibly infer that barbiturates in proportionately smaller dosage may effect the abnormally sensitive porphyrin metabolism in human acute porphyrias; the clinical impression that this does in fact take place may receive some substantiation from these results (Section 3). The differing degree of effectiveness of the barbiturates in the experimental animal may also explain the divergence of opinion on this clinical impression, which was based on the observation of different barbiturates. It is of great interest that diallylbarbituric acid administered to a normal animal causes the excretion of porphobilinogen and uroporphyrin.

A further point of inference may be important. Barbiturates have been shown to inhibit some of the oxidative processes of brain tissue (Quastel and Wheatley, 1933), the degree of inhibition being directly proportional to the hypnotic activity of the drugs. These authors also emphasized the importance of the allyl group in this connexion. Changes in nerve and brain

tissue occurring in acute porphyria (Mason, Courville and Ziskind, 1933; Denny-Brown and Sciarra, 1945; Gibson and Goldberg, 1956) may be the most important lesions responsible for the clinical results of this disease. It is possible that barbiturates might aggravate this upset in brain and nerve metabolism, as distinct from their effect on porphyrin metabolism. This might explain why some barbiturates, producing no marked effect on the porphyrin metabolism of rabbits, have been among those suspected of adversely affecting the course of acute porphyria. (see Section 8).

SUMMARY

1. Barbiturates have been suspected of adversely affecting the clinical course of acute porphyria. To assess this clinical impression objectively, the effect of nine barbiturates on the porphyrin metabolism of rabbits has been observed.
2. These barbiturates may be classified into four groups.
(i) Diallylbarbituric acid, which caused a considerable rise of urinary coproporphyrin III in each of eight experimental rabbits and in three of these the excretion also of uroporphyrin III and porphobilinogen. (ii) Allylisopropylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate, which caused a moderate rise of urinary coproporphyrin III.
(iii) Sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)-barbiturate and sodium ethylphenylbarbiturate, which caused only a slight coproporphyrin increase in the urine.
(iv) Sodium ethyl(1-methylbutyl)thiobarbiturate, sodium isoamylethylbarbiturate and sodium butylethylbarbiturate, which

did not alter significantly the urinary coproporphyrin excretion.

3. The effect of the presence of an allyl group in the barbiturate is discussed.

4. The significance of these results in animals is considered in relation to human acute porphyria.

6

EXPERIMENTALLY PRODUCED PORPHYRIA IN ANIMALS

6. EXPERIMENTALLY PRODUCED PORPHYRIA IN ANIMALS

Introduction

Stokvis, who made the first observation on the "sulphonal haematoporphyrinuria" in man, also produced the first experimental coproporphyrinuria, by the administration of sulphonal to rabbits and dogs (Stokvis, 1895). These experiments were later confirmed by other workers. In 1939 Waldenström and Wendt attempted unsuccessfully to produce an experimental porphyrinuria by the non-hypnotic derivative of sulphonal, dimethyl sulphone dimethyl methane. Rimington and Hemmings (1938) found that sulphonilamide caused a high excretion of coproporphyrin in rats, while Dent and Rimington (1947) observed a marked coproporphyrinuria in rats fed on a diet including oxidised casein. The pattern of porphyrin excretion in the urine of rabbits given lead was investigated by Weatherall and Comfort in 1952. Schwartz, Keprios, and Schmid (1952) caused the excretion of uroporphyrin, increased coproporphyrin and porphobilinogen in the urine of rabbits treated with phenylhydrazine, lead and exposure to unfiltered light from a mercury arc lamp. Schmid and Schwartz (1952) described an experimental porphyria in rabbits induced by sedormid (allyl-isopropyl-acetylurea) in which they noted the urinary excretion of large amounts of uroporphyrin and porphobilinogen and in many of their animals the development of 'transient paralysis of the hind legs and bladder and functional gastrointestinal disturbances', reminiscent of human acute porphyria. The chemical findings of these authors were confirmed, but the difficulty in making a

clinical assessment of rabbits treated with sedormid, which is a profound hypnotic, led me to investigate related drugs, one of which might produce the same chemical features, but yet be non-hypnotic. Such a drug is allyl-isopropylacetamide (Goldberg, 1953) (henceforward called A.I.A.). This drug has given a clearer view of what happens to rabbits affected by a pigment dyscrasia, apparently the same as in human acute porphyria.

Comparison of the effects of these related drugs on the porphyrin metabolism of rabbits, together with the similar study of certain barbiturates (Section 5), has also allowed the definition of a chemical structure which may interfere with normal porphyrin formation. Some rabbits receiving sedormid or A.I.A. excreted large amounts of porphobilinogen and uroporphyrin, while others excreted only small amounts of these materials. A study of this difference in individual animals and its relation to corresponding differences in the pathological histology of the liver and urinary amino-acid excretion has confirmed the importance of the liver in porphyrin metabolism (Prunty 1945; Watson, Schwartz and Hawkinson 1945).

The occasionally beneficial effect of splenectomy in congenital porphyria (Aldrich, Hawkinson, Grinstein and Watson 1951) suggested a trial of the effect on a rabbit of splenectomy between two separate courses of sedormid.

The type of cells in which porphyrins are normally fabricated is unknown, but the liver is probably of great importance. An attempt has been made to investigate the role of the reticulo-endothelial cells of the liver in the porphyrin metabolism

of experimental porphyria by means of the R.E. blockading substance thorium dioxide (Gottlieb 1934), although it is doubtful if a complete blockade can be achieved.

For some time the barbiturates have been considered to have an adverse effect on patients with acute porphyria. Waldenström (1939) considered that they might precipitate neurological manifestations in patients who might otherwise suffer only the abdominal symptoms of this disease. For this reason the effect was observed of a barbiturate given to an A.I.A.-intoxicated rabbit already excreting large quantities of porphobilinogen and porphyrins. Further to explore any possible relationship between experimental porphyria, paralysis and barbiturates, an experimental porphyria, with and without the addition of a barbiturate, was induced in fowls, animals known to be susceptible to metabolic derangement of their nervous system. An experimental porphyria has also been induced in rats by means of A.I.A.

METHODS AND MATERIALS

Rabbits (2 to 4 kg. body weight) were kept in metabolism cages, allowing the separate collection of urine and faeces. In general, porphyrin and porphobilinogen determinations were carried out on 24 hr collections of urines; in a few cases, 48 hr collections were used. Where faecal porphyrins were determined, 2-, 3- or 4-day specimens were pooled, having been collected daily and stored at 2°C. In most cases, a 4- to 7-day base-line period was allowed before the drug was given. Oral drugs were administered once daily by gastric intubation.

Urinary, faecal and tissue porphyrin and porphobilinogen determinations were done as in Section 5. Uroporphyrin in tissues was determined, after extraction of ether-soluble porphyrins, by shaking repeatedly with N-ammonium hydroxide until extracts no longer exhibited red fluorescence when brought to 0.5N in HCl. The dissolved uroporphyrin was determined spectrophotometrically as in the case of urine. Identification of porphyrins by chromatographic methods, melting-point and spectrophotometric determinations and haematological measurements were carried out as in Section 5. Platelet counts were done by the direct method using Rees-Ecker solution (Wintrobe 1951).

The isolation of crystalline porphobilinogen and its identification by paper chromatography were carried out by the methods described by Westall (1952) or Cookson and Rimington (1954).

Blood pressure in the rabbits was determined by the method of Grant and Rothschild (1934). Four consecutive readings were taken under their recommended conditions at the same time each morning for several days before the drug was given and then similarly during the course of its administration.

All rabbits' urines were examined initially, and at the end of the drug course for amino-acids by the method of Datta, Dent and Harris (1950). Urines found to contain many amino-acids were then further examined by the method of Dent (1948).

The thorium dioxide used was in a 25% colloidal suspension ('thorotrast' - Heyden).

RESULTS

Porphyrins

Urine Table 1 summarizes the urinary uroporphyrin * and porphobilinogen excretion of twenty-three rabbits, intoxicated with allyl-isopropyl-acetamide (A.I.A.) (nine rabbits), sedormid (six rabbits), propyl-isopropyl-acetamide (three rabbits) and allyl-isopropyl-acetic acid (five rabbits). For comparison, the results of a similar series, using diallyl barbituric acid (Section 5), have been added to the present group. A.I.A. and sedormid caused an immediate increase above normal in the level of urinary coproporphyrin, mounting rapidly stepwise with continued administration of the drug until the 4th to 7th day, when porphobilinogen and uroporphyrin were noted in the urine (see figures 1 and 2). From this point, the urinary coproporphyrin excretion remained approximately constant, but the porphobilinogen and uroporphyrin mounted to reach maximum levels 2 to 3 days later. These drugs were continued in every case except two (Nos. 5 and 12) until the rabbit died. In rabbits 5 and 12, withdrawal of the drug led to a rapid (within 48 hr.) cessation

* Urines were heated with 2N acetate buffer pH 4.2 for 20 min. at 100°C to convert porphobilinogen present into uroporphyrin and the total uroporphyrin then determined spectrophotometrically (see Methods). The term 'urinary uroporphyrin' is used throughout this paper to indicate the total uroporphyrin so measured.

TABLE 1. EFFECTS OF DRUGS ON URINARY PORPHYRIN AND PORPHOBILINOGEN EXCRETION OF RABBITS

rabbit no.	route*	dose (mg/kg/day)	duration (days)	uroporphyrin (mg/day) mean	uroporphyrin (mg/day) max.	porphobilinogen (mg/day) mean	porphobilinogen (mg/day) max.	copro-porphyrin (μg/day) additional to normal
				A.I.A.				
1	I.G.	187	14	1·52	2·62	5·3	11·2	289
2	I.G.	226	11	0·43	1·48	2·7	11·0	264
3	I.G.	217	8	1·47	3·87	6·3	20·1	272
4	I.G.	170	13	10·21	18·33	53·7	101·8	292
5	I.G.	182	23	6·32	15·85	36·8	70·4	342
6	I.G.	176	8	7·37	12·52	19·8	34·5	203
7	I.G.	181	12	10·26	17·7	63·6	116·2	188
8	I.G.	183	5	2·46	7·66	18·81	62·4	217
9	I.G.	204	13	3·85	13·44	9·05	24·4	125
				sedormid				
10	I.G.	257	9	1·53	5·5	5·4	11·5	120
11	I.G.	230	9	6·6	19·0	7·4	18·2	182
12	I.G.	217	11	11·29	27·0	38·0	64·5	94
13	I.G.	187	8	8·8	15·2	not done		279
14	I.G.	212	14	1·78	3·8	3·7	9·0	104
15	I.G.	187	7	2·5	3·2	not done		126
				P.I.A.				
16	I.G.	236	6	—	—	—	—	nil
17	I.G.	256	12	—	—	—	—	5
18	I.G.	143	7	—	—	—	—	nil
				A.I.Ac. acid				
19	I.G.	372	5	—	—	—	—	5
20	I.G.	154	12	—	—	—	—	16
21	I.M.	193	6	—	—	—	—	10
22	I.M.	267	9	—	—	—	—	9
23	I.G.	128	9	—	—	—	—	2
				dial				
24	I.M. or I.G.	113	15	0·36	0·80	0·6	1·24	194
25	I.M. or I.G.	103	35	1·72	4·75	3·6	12·4	169
26	I.M.	100	17	0·25	0·34	0·6	0·84	119
27	I.M. or I.G.	128	12	—	—	—	—	88
28	I.M.	102	7	—	—	—	—	86
29	I.M.	125	9	—	—	—	—	84
30	I.M.	114	7	—	—	—	—	51
31	I.M.	148	7	—	—	—	—	47

* I.G. = intragastric; I.M. = intramuscular; A.I.A. = allyl-*iso*propyl-acetamide; P.I.A. = *n*-propyl-*iso*propyl-acetamide; A.I.Ac. acid = allyl-*iso*propyl-acetic acid; dial = diallyl-barbituric acid.

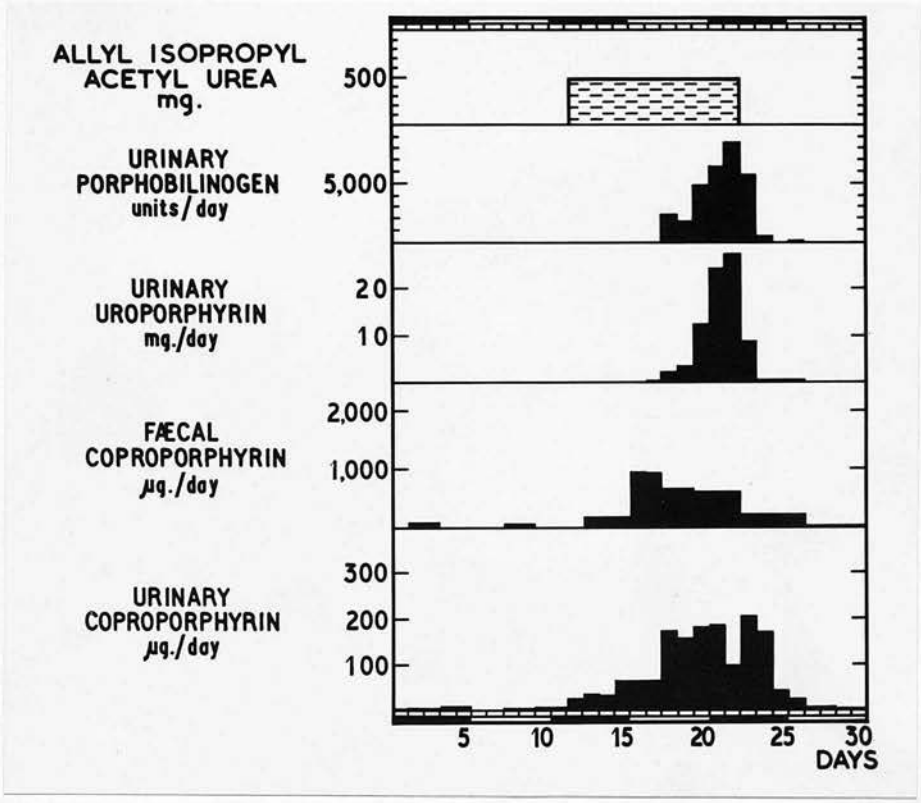


Figure 1. Effect of sedormid in a rabbit on its excretion of urinary porphobilinogen and coproporphyrin and faecal coproporphyrin. 130 units of porphobilinogen equal 1 mg. of porphobilinogen (Westall 1952).

of urinary porphyrin and porphobilinogen excretion and a more gradual lessening of coproporphyrin excretion until the former normal levels were regained in 4 days' time. No significant difference was noted when comparing the pattern of porphyrin and porphobilinogen excretion of the sedormid rabbits with that of theA.I.A. rabbits, except that one A.I.A.-intoxicated rabbit (No. 5) continued to excrete large amounts of these materials for 3 weeks, a duration not found possible with sedormid rabbits. Some rabbits (Nos. 4, 5, 6, 7, 11 and 12) excreted large amounts

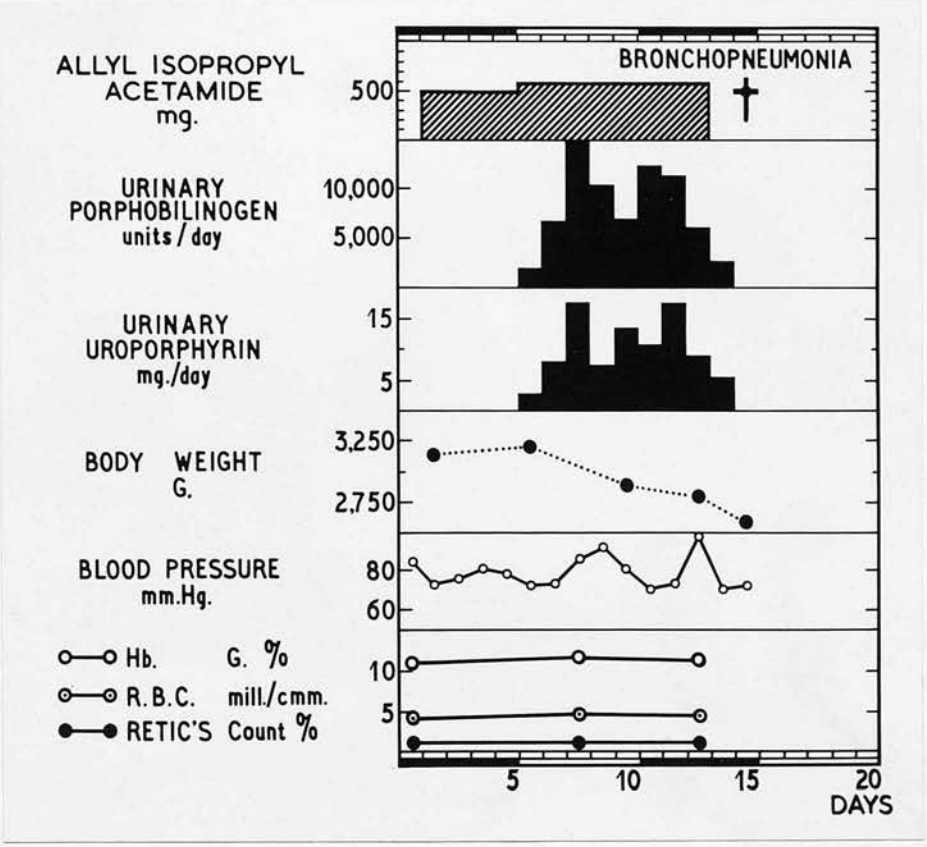


Figure 2. Effect of allyl-isopropyl-acetamide in a rabbit on its excretion of urinary porphobilinogen and on its body weight, systolic blood pressure and haematological values. 130 units of porphobilinogen equal 1 mg. of porphobilinogen (Westall 1952).

of porphobilinogen and uroporphyrin and continued to do so for as long as the drug was given (Nos. 5 and 12), or until some intercurrent cause of death intervened, e.g. bronchopneumonia in rabbits 7 and 12 or uraemia in rabbit 4. Other rabbits (Nos. 1, 2, 3 and 10) reached maximum excretions of uroporphyrin and porphobilinogen at much lower levels and ceased excreting these substances, although still maintaining a coproporphyrinuria, 1 or 2 days before death from severe hepatic damage.

The chemical identification of urinary porphyrins is summarized in Table 2.

n-Propyl-isopropyl-acetamide showed no effect on the porphyrin excretion of rabbits, while allyl-isopropyl-acetic acid caused slight increases in coproporphyrin excretion above normal (more than 5 μg/day) in three out of five rabbits tested.

Faeces Faecal coproporphyrin was determined in six rabbits (Nos. 5, 11, 12, 13, 14 and 15) whose daily excretion rose from normal (pre-drug) levels of 254, 44, 70, 64, 6 and 61 μg to levels of 495, 315, 942, 473, 1060 and 221 μg respectively. In the two animals which survived (Nos. 5 and 12), faecal coproporphyrin levels were determined after the cessation of the drug. In No. 5 the average daily coproporphyrin excretion for the first 8 days of this period was 3659 μg. This animal was severely constipated during the drug course, but excreted a normal quantity of faeces as soon as the drug was withdrawn. The large rise from 495 to 3659 μg/day was thus probably

TABLE 2. IDENTIFICATION OF PORPHYRINS. SUMMARY OF RESULTS

drug	material	free porphyrin no. of carboxyl groups	α band in $CHCl_3$ (mμ)	porphyrin methyl ester isomeric series by paper chromatography	m.p. of crystals (°C)	summary
sedormid	urine	8	625·3	mainly III	254–258	uroporphyrin III
		5	622·4	—	—	—
		4	621·2	III	—	coproporphyrin III
A.I.A.	urine	8	625·6	mainly III	247–261	uroporphyrin III
	plasma*	8	625·0	I and III	—	uroporphyrin III and I
	bile	8	—	III	—	uroporphyrin III
		4	—	III	—	coproporphyrin III
		2	630·2	—	—	protoporphyrin

* Ether-insoluble fraction only.

due to rapid evacuation of the overloaded bowel. In rabbit No. 12 constipation was less marked, and the faecal coproporphyrin fell from a mean of 602 μg/day on the last 3 days of the drug course to 202 μg/day on the 3 days following the cessation of dosage.

In the course of these determinations it became evident that the faecal protoporphyrin was considerably raised. The quantitative extraction of this porphyrin was rendered unsatisfactory by the presence of large amounts of chlorophyll-type pigments in the stool.

Bile Increased coproporphyrin and protoporphyrin levels were found in the bile of all rabbits showing an increased urinary coproporphyrin excretion (see Table 3). In six rabbits intoxicated with either A.I.A. or sedormid, an ether-insoluble porphyrin was obtained. On spectrophotometric examination in 0.5 N-HCl this porphyrin had a maximal absorption at 405 mμ. It was further investigated by chromatographic methods and shown to be uroporphyrin III. The coproporphyrin was identified by paper chromatographic methods as of the series III type (see Table 2).

Plasma In three rabbits (Nos. 4, 7 and 8) the plasma or serum at post-mortem showed red fluorescence. In one of these (No. 4) this was found, by further investigation, to be due to coproporphyrin and uroporphyrin III with some uroporphyrin I (see Table 2).

Tissues (see Table 3) The coproporphyrin and protoporphyrin of liver and bile showed increases above normal,

TABLE 3. SUMMARY OF TISSUE PORPHYRIN AND PORPHOBILINOGEN DETERMINATIONS

(The results are expressed as μg/g or ml. of tissue or bile respectively.)

	liver				bile*			spleen†		marrow‡		kidney			
rabbit no.	copro.	proto.	pbg.	uro.	copro.	proto.	uro.	copro.	proto.	copro.	proto.	copro.	proto.	pbg.	uro.
							controls								
A	0·10	0·10	nil	nil	0·15	nil	nil	0·15	0·10	0·10	nil	0·11	0·03	nil	nil
B	0·18	0·12	nil	nil	0·38	0·34	nil	insufficient		0·20	0·15	0·03	0·02	nil	nil
C	0·18	0·51	nil	nil	0·74	0·41	nil	insufficient		0·22	0·24	0·24	0·07	nil	nil
D	0·02	0·23	nil	nil		insufficient		0·08	0·16	0·18	0·16	0·10	0·03	nil	nil
							A.I.A.								
1	4·67	3·96	nil	nil		insufficient		2·61	1·41	1·14	0·74	4·67	3·96	nil	nil
2	3·94	17·48	nil	nil		insufficient		insufficient		0·16	0·43	—	—	—	—
3	0·52	1·60	nil	nil	69·5	165·00	152·00	0·24	0·24	1·31	0·69	—	—	—	—
4	5·50	32·30	36·0	2·58	4·9	12·00	10·00	0·90	1·70	0·90	2·00	0·50	0·47	+	2·20
6	2·75	50·00	+ + +	nil	159·0	231·00	22·50	0·60	1·15	0·13	0·19	3·24	1·31	+	2·29
7	4·62	73·00	nil	nil	115·0	224·00	nil	—	—	trace	trace	—	—	—	—
8	2·55	7·91	nil	85·00	51·0	308·00	97·00	0·54	nil	0·84	0·69	—	—	—	—
9	2·70	14·85	nil	nil	57·5	93·00	nil	0·44	0·56	0·12	0·17	—	—	—	—
							sedormid								
10	0·54	26·00	nil	nil	—	—	—	—	—	—	—	—	—	—	—
11	5·65	3·00	2·9	nil	—	—	—	—	—	—	—	—	—	—	—
12	8·00	12·80	6·0	nil	33·0	219·00	128·00	—	—	—	—	—	—	—	—
13	0·30	3·12	nil	nil	13·6	44·20	37·00	—	—	—	—	—	—	—	—
14	4·62	7·39	nil	nil	—	—	—	—	—	—	—	—	—	+	—
15	2·86	4·68	26·0	nil	—	—	—	—	—	nil	nil	1·30	1·10	nil	—
							P.I.A.								
16	0·21	0·26	nil	nil		insufficient		—	—	0·05	0·26	—	—	—	—
							A.I.Ac. acid								
19	0·45	0·52	nil	nil	1·1	1·99	nil	0·13	0·18	0·10	0·10	—	—	—	—
21	0·40	1·00	nil	nil	0·43	1·07	nil	—	—	0·16	0·14	—	—	—	—
22	0·53	2·82	nil	nil	1·18	1·73	nil	—	—	1·18	1·73	—	—	—	—

A.I.A. = allyl-*iso*propyl-acetamide; P.I.A. = *n*-propyl-*iso*propyl-acetamide; A.I.Ac. acid = allyl-*iso*propyl-acetic acid; copro. = coproporphyrin; uro. = uroporphyrin; proto. = protoporphyrin; pbg. = porphobilinogen.

* Bile porphobilinogen *nil* in every case.

† Spleen porphobilinogen a *trace* in no. 8, otherwise *nil*. Spleen uroporphyrin 1·84 in no. 4, otherwise *nil*.

‡ Marrow porphobilinogen a *trace* in no. 8, otherwise *nil*. Marrow uroporphyrin 0·7 in no. 4, otherwise *nil*.

proportional to the degree of porphyrin increase in the urine. In A.I.A.- and sedormid-intoxicated rabbits, the spleen, marrow and kidney coproporphyrin and protoporphyrin were slightly raised above normal, but not to the same degree as in liver and bile. In rabbit 4, portions of skeletal muscle tissue showed marked porphyrin fluorescence. Two upper incisor teeth which had accidentally been broken at the beginning of the experiment grew to their previous length in 1 week. At post-mortem examination, the roots of these teeth had porphyrin fluorescence. Rabbit 16 (n-propyl-isopropyl-acetamide) had no porphyrin increase in any tissue. Rabbits 19, 21 and 22 (allyl-isopropyl-acetic acid) had small increases in liver and bile coproporphyrin and protoporphyrin, while marrow coproporphyrin and protoporphyrin were also slightly raised in rabbit 22.

Porphobilinogen

Urine Porphobilinogen was isolated from rabbit urine by the method of Westall (1952) or that of Cookson and Rimington (1954) and found to be identical in all respects with porphobilinogen from acute porphyria urine.

Plasma In four rabbits (Nos. 1, 4, 7 and 8) the blood plasma tested qualitatively for porphobilinogen (Vahlquist 1939) at the height of the induced attack, or at post-mortem, gave a positive result. In the case of rabbit 8, a spectrophotometric extinction curve was also obtained showing maxima at 527 and 556 mμ, characteristic of the product of porphobilinogen and Ehrlich's aldehyde reagent. The concentration of plasma porphobilinogen in rabbit 4 post-mortem was 105 μg/ml. (for quantitative

determination of porphobilinogen see Westall (1952)).

Tissues In five rabbits there was a positive porphobilinogen reaction in the liver; in three of these it was also positive in the kidney. Faeces, brain, bile, spleen and marrow were negative for porphobilinogen, with the exception that in rabbit 8 traces of porphobilinogen were present in spleen and marrow. In this animal the plasma porphobilinogen was strongly positive, and these trace reactions could have been derived from the blood which its spleen and marrow contained.

Urinary amino-acids

These were identified in ten rabbits (A.I.A.- or sedormid-intoxicated) by means of paper chromatography. Normal rabbit urine (10 to 15 μl. in method of Datta et al. (1950) and 15 to 25 μl. in method of Dent (1948)) shows only a glycine spot. In rabbit 14 no abnormality was detected at the end of the drug course. In five rabbits, all high excretors of uroporphyrin and porphobilinogen (Nos. 4, 5, 6, 7 and 12), the glycine spot at the end of the course was markedly reduced in intensity from that before the drug was given. In rabbits 4 and 6, this diminution was established by an approximately quantitative experiment. Volumes of urine, from before and at the end of the drug course, corresponding to the same fraction of the total daily urine output, were placed on to the same paper chromatogram and developed by either phenol or butanol acetic acid respectively, in a one-dimensional run. The dilution of the stronger urine necessary to match the weaker was assessed and confirmed experimentally by further chromatographic runs. This experiment showed administration of

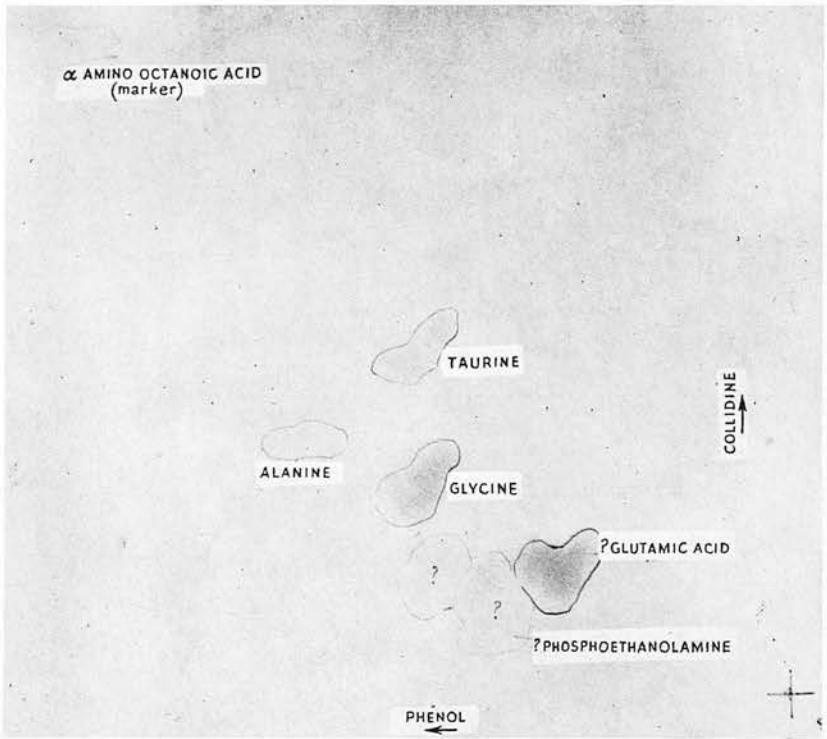


FIGURE 3. Urinary amino-acids of rabbit 1 on last day of experiment.

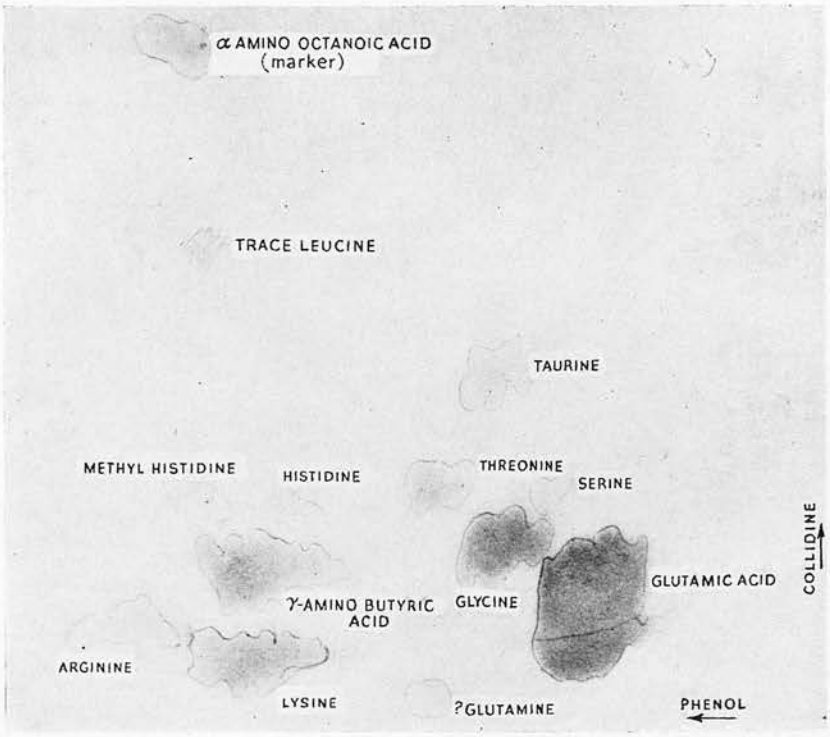


FIGURE 4. Urinary amino-acids of rabbit 10 on last day of experiment.

the drug in both animals to have caused at least fourfold reduction in urinary excretion of glycine. Rabbits 1, 10 and 12 (all poor excretors of uroporphyrin and porphobilinogen) voided many amino-acids at the end of the drug course, the day before death, viz:-

Rabbit 1 excreted taurine, alanine, glutamic acid, glutamine, ethanolamine phosphate and glycine (see figure 3).

Rabbit 10 excreted glutamic acid, glycine, α-amino butyric acid, lysine, threonine, taurine, small amounts of histidine, methyl histidine, arginine, serine and a trace of leucine (see figure 4).

Rabbit 12 excreted glutamic acid, glycine, alanine, taurine, threonine, β-alanine, histidine, trace valine, trace tyrosine, leucine and some aspartic acid.

CLINICAL FINDINGS OF A.I.A.- AND SEDORMID-INTOXICATED RABBITS

Neurological

Rabbits intoxicated with sedormid differed from those treated with A.I.A. Sedormid proved to be a profound hypnotic, the animals remaining unconscious for about 10 hours after each dose of the drug. Those treated with A.I.A. never lost consciousness, but a few appeared slightly dazed for about 1 to 1.5 hours after the administration of the drug. It was thus very difficult to evaluate the claim of Schmid and Schwartz (1952) that sedormid-treated rabbits exhibited paralysis or paresis. Using A.I.A. alone, limb paralyses or pareses never occurred, and these rabbits remained normally mobile and active even though excreting amounts of uroporphyrin as high as 7 mg/day or more for

many days; rabbit No. 5 maintained an excretion near this level during an entire 3 weeks' course of the drug.

Gastro-intestinal

Appetite and weight Both groups quickly developed a relative anorexia; they left most of their pellet (M.R.C. No. 18, Bruce and Parkes 1946) diet, but usually ate cabbage and drank sufficient water to maintain their normal urinary volume. The rabbits lost weight steadily; mean daily losses for those on A.I.A. being 52 g (six rabbits tested), and for those on sedormid 36 g (five rabbits tested). In the case of rabbit 5, normal appetite returned the day following cessation of the drug (A.I.A.), and the body weight gradually rose to its previous level.

Faeces Rabbits of both groups became quickly constipated. Thus average daily faecal weights were recorded of 87 g before and 17 g during the administration of A.I.A., and of 60 g before and 25 g during the administration of sedormid. In most rabbits this drop took place the day following the first dose of the drug. The stool weight of rabbit 5 rose from an average level of 4 g/day during the period of drug administration to 89 g/day on the second day after withdrawal of the drug.

Straight X-ray of the abdomen was done on two rabbits before and at weekly intervals during A.I.A. administration. In one (No. 1) no difference was observed, while in the other (No. 5), a progressively increasing gaseous distention of loops of bowel was revealed. This appearance gradually returned to normal on withdrawal of the drug.

Blood pressure

Table 4 summarizes the results of systolic blood-pressure readings of four rabbits intoxicated with A.I.A. It is difficult to presume any significant change of blood pressure consequent on drug administration. The systolic blood pressure of rabbit 10 maintained a steady level, averaging 73 mm.Hg in the first 6 days of its sedormid course, during which there was a mounting excretion of coproporphyrin and later of uroporphyrin and porphobilinogen. On the 7th, 8th, 9th and 10th days, the blood pressure fell to 38, 42, 40 and 14 mm.Hg respectively, during which time there was a fall of porphobilinogen and uroporphyrin excretion, although the drug was maintained at the same dose. The rabbit died on the 10th day; post-mortem examination revealed hepatic necrosis.

TABLE 4. SYSTOLIC BLOOD PRESSURE OF RABBITS WITH EXPERIMENTAL PORPHYRIA

rabbit no.	mean uroporphyrin excretion (mg/day)	mean daily B.P. (systolic) (mm Hg)	
		before drug	during drug
1	1·52	78	85
3	1·47	64	69
5	6·32	81	74
7	10·26	83	78

Haematological

The results of serial haematological determinations in five rabbits are summarized in Table 5, together with total loss of porphyrin in the urine during the course of the experiment. One animal (No. 5) provided data for porphyrin loss in both urine and faeces. The determinations of Hb., R.B.C. and reticulocytes

TABLE 5. COMPARISON OF HAEMATOLOGICAL RESULTS AND TOTAL PORPHYRIN EXCRETIONS ADDITIONAL TO NORMAL, OF 5 RABBITS TREATED WITH ALLYL-*ISO*-PROPYL-ACETAMIDE (NOS. 1, 4, 5 AND 7) AND SEDORMID (NO. 10)

rabbit no.	duration of drug (days)		Hb. (g %)	R.B.C. (10^6/mm^3)	reticulocytes (%)	total uroporphyrin loss (mg)	total coproporphyrin loss additional to normal (mg)	faecal coproporphyrin loss additional to normal (mg)
1	14	before	10·64	4·10	2·5	21·24	4·05	—
		after	11·77	4·25	1			
4	13	before	13·61	4·90	1	122·5	3·66	—
		after	14·28	4·77	1			
5	23	before	13·31	5·77	2·5	139	7·87	28·65
		after	13·77	5·05	1			
7	12	before	10·88	4·55	1	92·2	2·58	—
		after	11·42	4·71	1			
10	9	before	13·2	5·29	0·75	10·71	1·36	—
		after	13·2	5·19	1			

showed no significant variation. Determinations of blood platelets and clot retraction on two rabbits before and during sedormid administration also showed no significant change.

Dermatological

The rabbits showed no evidence of photosensitivity although in a normally bright room. The sedormid skin patch test of Ackroyd (1949) was applied for 48 hours to the shaved skin of the inferior dorsal area of rabbit 11, during its course of sedormid. There was no skin reaction to this test.

Effect of barbiturate

The effect was observed of a small dose of a barbiturate on a rabbit (No. 4; 3.3 kg) already excreting high levels of uroporphyrin and porphobilinogen under the influence of A.I.A. intoxication. 100 mg of sodium allyl-(1-methyl butyl)-barbiturate was injected intramuscularly on the 14th day of the drug

course. The rabbit remained unconscious for the next 24 hours. In normal rabbits the same dose relative to body weight of this barbiturate caused only slight transient sedation. By the next day the rabbit had recovered and seemed alert, but the day following it lapsed into a weak paretic state, described more fully below.

Mode of death

Histopathology of tissues In rabbits 1, 3, 10 and 13, post-mortem examination of tissues was normal apart from hepatic necrosis. Rabbit 2 at post-mortem had mid-zone degenerative changes, such as vacuolation and haemorrhage, in the liver. These five rabbits had become obviously weaker in the 2 days before death, and in rabbit 10 this was accompanied by a fall in systolic blood pressure. Rabbits 2 and 3 were found dead in their cages, but rabbits 1, 10 and 13 showed a similar mode of exitus. They all had tremor of limbs and violent convulsions, and in rabbit 13 there was marked opisthotonos lasting 15 to 30 min. before death.

Rabbits 7 and 12 became weak and dyspnoeic and developed a purulent nasal discharge the day before death. At post-mortem examination, bronchopneumonia was noted and confirmed microscopically. Rabbit 8 was apparently normal until the day of death, when it became weak, with drooping head, and with a mucoid discharge from eyes and nose. This rabbit died during intubation. At post-mortem the liver and kidneys were pale, and histological examination revealed vacuolation of the peripheral cells of the liver lobules and of the convoluted tubular epithelium of the

kidneys. The lungs showed a patchy bronchitis with foci of collapse and pulmonary oedema. The clinical diagnosis of rabbit 'snuffles' (Pasteurella septica) was not confirmed bacteriologically.

Rabbit 4, which had been given an injection of barbiturate (see above), became very weak with paresis of neck and limb muscles 2 days following this injection, after having recovered from its hypnotic effect. The urinary volume had decreased, but the urine contained no protein and merely an occasional erythrocyte on microscopic examination. There was absence of pain sensation in the right hind leg, but electromyography of the limb muscles by Dr. O. Lippold, University College, failed to reveal any abnormality. The rabbit's condition deteriorated and 2 days after the onset of this state it was sacrificed, bled and immediately perfused with 10% formol saline to allow investigation of the nervous system. Dr. J.C.B. Fenton, Dept. of Pathology, University College Hospital, examined these tissues. Myelin changes were observed in the peripheral nerves of this animal, but they were considered to be atypical of true myelin degeneration and could be attributed to causes other than the action of the drug, e.g. fasting and dehydration. The blood plasma was markedly opalescent. Table 6 shows the results of blood and plasma examinations, for which I am indebted to Dr. F.V. Flynn, Department of Clinical Pathology, University College Hospital. A lower nephron nephrosis (with consequent uraemia) was indicated by the microscopic finding of tubular distension with flattened epithelium and fatty vacuolation of some of the convoluted tubules

of the kidneys. The liver showed minor periportal fatty changes without evidence of necrosis.

TABLE 6. BLOOD ANALYSES OF RABBIT 4 (A.I.A. + BARBITURATE) COMPARED WITH NORMAL LEVELS

	rabbit 4	normal rabbit
plasma cholestrol (mg %)	410	60–100 (34)
blood urea (mg %)	308	45 (4)
plasma potassium (m.eq/l.)	6·34	4·5–5·5 (20)
plasma sodium (m.eq/l.)	140	140 (20)

Of the remaining five rabbits, Nos. 6, 11 and 15 died within half an hour after intubation, and post-mortem examination failed to show any histological abnormality. Rabbit 9 became weak, with deep, laboured respiration on the day before its death. Histology of its tissues was normal. Rabbit 14 did not recover from the hypnotic effect of its dose of sedormid. Again, post-mortem histological examination was normal.

SPLENECTOMY

In rabbit 12, a period of 3 weeks rest was allowed after a course of sedormid lasting 11 days. A splenectomy was then performed, and the following day the rabbit was started on a second course of sedormid with the same dose as in the initial course. Porphobilinogen and uroporphyrin were noted, in addition to increased coproporphyrin, on the 2nd day of drug administration and rose gradually until a daily level of 9.5 and 2.2 mg. of porphobilinogen and uroporphyrin, respectively, were obtained after 1 week. The rabbit then died and was found at autopsy to have bronchopneumonia.

EFFECT OF RETICULO-ENDOTHELIAL BLOCKADE

Table 7 summarizes the results of sedormid intoxication in the rabbits (Nos. 32, 33 and 34) which had a simultaneous reticulo-endothelial blockade by thorium dioxide. In two of these rabbits a splenectomy had been performed before the blockade, in order to increase the concentration of thorium dioxide in the liver.

Rabbit 32 showed no change in blood haemoglobin concentration, erythrocyte, reticulocyte and platelet counts, but did develop a leucocytosis, from 3000/mm^3 to 13,400/mm^3, and a diminished clot retraction at the end of the experiment. This rabbit also developed an abnormal urinary amino-acid pattern, from a solitary glycine spot before the experiment to an excretion of glutamic acid, glycine, taurine, glutamine and histidine before death. At post-mortem examination there was marked hepatic necrosis. Rabbit 33 survived the full course of the experiment, but 20 days later, when the same dose of sedormid was repeated, the rabbit went into profound coma from which it did not recover. The liver parenchyma at post-mortem examination appeared healthy. Rabbit 34 was found dead in its cage; there had been some infection of the splenectomy wound. Microscopic examination of the livers of rabbits 32 and 33 showed the presence of thorium dioxide mainly in the Kupffer cells, but also in small amounts throughout the hepatic cells.

A further experiment was carried out on a normal rabbit in which an external bile fistula had been performed. Administration of thorium dioxide in similar dosage to the above

TABLE 7. EFFECTS OF RETICULO-ENDOTHELIAL BLOCKADE IN EXPERIMENTAL PORPHYRIA

rabbit no.	total dose thorium dioxide (ml/kg)	dose sedormid (mg/kg/day)	duration of sedormid course (days)	urinary porphobilinogen (mg/day) mean	urinary porphobilinogen (mg/day) max.	urinary uroporphyrin (mg/day) mean	urinary uroporphyrin (mg/day) max.	urinary coproporphyrin additional to normal (μg/day)	faecal coproporphyrin additional to normal (μg/day)
32	18·1	232	13	4·05	7·05	1·58	3·23	121	571
33*	13·4	218	13	1·68	3	0·69	1·63	134	—
34*	10·5	238	10	2·12	2·46	0·04	0·06	100	—

* These rabbits had their spleen removed before thorium dioxide was given.

TABLE 8. PORPHYRINS AND PORPHOBILINOGEN IN TISSUES AND EXCRETA OF 2 FOWLS TREATED WITH A.I.A.

The values in liver, bile and marrow are expressed as μg/g or μg/ml. Those in excreta refer to the mean daily output (μg/day). The coproporphyrin and protoporphyrin figures in the excreta are those additional to mean normal daily levels. The bile and bone marrow contained no porphobilinogen or uroporphyrin.

	liver				bile		marrow		excreta			
chicken	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin	copro-porphyrin	proto-porphyrin	copro-porphyrin	proto-porphyrin	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin
1	10·60	32·43	6	6·32	—	—	0·25	1·34	788	757	104	1,610
2	1	44	0	1·13	451	366	—	—	761	1,165	16	791

experiment did not cause any fall in the levels of bile porphyrins and urinary porphyrins.

EXPERIMENTS ON FOWLS

A.I.A. in gelatin capsules was administered to two Rhode Island Red chickens, each weighing 2 kg. No. 1 received the drug for 18 days and No. 2 for 17 days. Chicken 2 was also given injections of sodium allyl-(1-methylbutyl)-barbiturate (seconal) in a mean daily dosage of 37 mg. during the final 7 days of its drug course.

Porphyrin and porphobilinogen excretion In both animals there was an immediate rise in the coproporphyrin and protoporphyrin content of the excreta. Porphobilinogen and uroporphyrin were noted on the 4th and 7th day respectively (see Table 8). Chicken 1 excreted greater amounts of porphobilinogen and uroporphyrin than chicken 2. The porphobilinogen was identified by means of paper chromatography (Westall 1952) as being identical with that isolated from the urine of a case of acute porphyria. Among the porphyrins contained in the excreta, uroporphyrin III and coproporphyrin III were identified by means of paper chromatography (see Methods).

Clinical and pathological results Both chickens were active throughout the course of this experiment, although they lost an average of 30 g. weight daily. The weight of their excreta progressively diminished. Their Hb. and R.B.C. levels did not alter throughout the experiment. Towards the end of the experiment, both chickens became weak and lethargic, although frank paralysis was not seen. They were sacrificed by means of

nembutal intravenously, bled and infused through the aorta with 10% formol saline. The examination of their nervous system by Dr. J.C.B.Fenton did not show myelin degeneration. Histological examination of the kidneys of both these fowls showed subnuclear vacuolation in the convoluted tubules. The liver of chicken No. 1 was normal and contained porphobilinogen, but that of No. 2 showed cellular necrosis and contained no porphobilinogen.

EXPERIMENTS ON RATS

Nine Wistar strain albino rats (175 to 204 g) were placed in metabolism cages, allowing the separate collection of urine and faeces. After a preliminary base-line period of 3 days, they were given A.I.A. by means of gastric intubation through a blunted metal needle (size: internal diameter 18 s.w.g. (0.048 in.), external diameter 15 s.w.g. (0.072 in.)) attached to a glass syringe. The A.I.A. powder had previously been thoroughly ground up in a mortar and suspended in 'cellophas' (I.C.I.) 4% (1 part) and water (4 parts). A useful concentration of the drug was 100 mg/ml. of suspension. It was found that the appropriate dose in these rats was about 100 mg/day (400 to 500 mg/kg.).

Porphyrin and porphobilinogen excretion (see Table 9). The nine rats showed an immediate rise in urinary coproporphyrin. In rat 6 faecal porphyrins were also determined. The increase in ether-soluble porphyrins in the faeces greatly exceeded that in the urine, e.g. rat 6 had a mean daily increase of urinary coproporphyrin of 54 μg, the rises in daily stool coproporphyrin and protoporphyrin being 115 and 436 μg respectively. Seven rats excreted porphobilinogen, on the 2nd (rats 1, 2, 3 and 4), 4th (rat

TABLE 9. MEAN DAILY URINARY PORPHOBILINOGEN AND COPROPORPHYRIN EXCRETION OF RATS TREATED WITH A.I.A. THE COPROPORPHYRIN IS THAT ADDITIONAL TO THE MEAN NORMAL LEVEL IN EACH RAT

rat no.	A.I.A. dose (mg/kg/day)	duration (days)	porphobilinogen (mg/day)	coproporphyrin (μg/day)
1	455	18	2·64	37
2	405	22	5·72	57
3	258	13	0·39	33
4	286	7	0·042	18
5	490	15	1·27	52
6	500	13	1·27	54
7	470	8	0	30
8	490	15	2·945	36
9	350	6	0	30

TABLE 10. PORPHYRINS AND PORPHOBILINOGEN (μg/g) IN TISSUES OF RATS TREATED WITH A.I.A.

	liver				kidney				spleen			
rat no.	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin	copro-porphyrin	proto-porphyrin	porpho-bilinogen	uro-porphyrin
2	0·1	0·3	316	5·3	1·42	1·13	59	15	0·32	1·15	0	2·48
5	2·38	1·2	28·4	4·4	2·23	0·79	33	12·4	0·13	0·85	0	0
6	0·85	0·55	7	0·87	0·79	0·06	0	2·67	0·1	0	0	0
7	0·27	0·43	0	0	0·23	0·15	0	0	—	—	0	—
8	0·58	0·40	145	0·91	0·77	0·91	49	0·62	—	—	0	—
9	0·26	0·30	0	0	0·48	0·31	0	0	—	—	0	—
control	0·02	0·16	0	0	0·08	0·07	0	0	0·05	0·29	0	0

5), 6th (rat 6) or 11th (rat 8) day of drug administration. The mean level of porphobilinogen excretion was about 2 mg/day, but rat 2 had persistently high levels reaching a maximum of 15.2 mg/day. Freshly voided urine, containing porphobilinogen, was normally coloured and had no porphyrin fluorescence. Spectrophotometric examination of this fresh urine showed no evidence of any preformed uroporphyrin. It darkened on standing and became fluorescent, although these changes were not so marked as in the rabbit urines. Small quantities of porphobilinogen were found in the rat's stools.

Two rats (Nos. 7 and 9) excreted no porphobilinogen, although there was a rise in urinary coproporphyrin. Throughout the experiment they became progressively weaker, and by the 6th and 8th day, respectively, were on the point of death, when they were sacrificed (see below).

The porphyrin excreted in the urine was defined as coproporphyrin series III by means of paper chromatography (see Methods). Porphobilinogen was isolated from the urine as characteristic crystals using the method of Cookson and Rimington (1954).

Clinical and pathological results All rats showed some loss of weight (mean loss 2.5 g/day) and diminished appetite, although not as severely as did rabbits. They did not have the profound constipation noted in most rabbits, although in some rats there was a diminution in stool weight from a normal mean 5 g/day to a mean of 3.1 g/day. They appeared to be more dazed than the rabbits for several hours after A.I.A. administration.

There was no clinical evidence of paralysis. During the course of the experiment, rats 1 and 2 were each exposed to a carbon arc lamp at 1 ft. distance on two separate occasions, lasting 15 and 30 min. respectively. Neither rat showed any sign of photosensitivity. The clinical course of rats 7 and 9 has been described. The other seven rats remained mobile and alert. They were sacrificed at the end of the experiment. Chemical analysis of tissues is summarized in Table 10. Those rats excreting porphobilinogen in the urine had porphobilinogen in the liver, while rats 7 and 9 had porphobilinogen neither in their urines nor livers.

Histology of the livers in those rats excreting porphobilinogen was normal. The liver of rat 7 showed fatty degeneration, but that of No. 9 was histologically normal. Amino-acid chromatograms of the urines, before and at the end of the experiment, were done in rats 1, 2, 3, 4, 5, 7 and 10. No change in the amino-acid pattern was noted. Histological examination of the nervous system of rats 1, 2, 3 and 4 by Dr. J.C.B. Fenton did not show myelin degeneration.

DISCUSSION

A question, important to our understanding of human acute porphyria, is whether or not the known porphyrins or their precursors, or unknown substances, released by the pigment dyscrasia, cause the clinical symptoms of this disease. The work on the pharmacological action of carefully purified porphyrins and porphobilinogen (Section 4) afforded no evidence for any direct action of these substances, and the finding that porphobilinogen is probably a normal precursor of porphyrins and haem

(Falk, Dresel and Rimington 1953) renders less likely the former assumption that the symptomatic disturbances in acute porphyria were due to porphobilinogen. The work of Schmid and Schwartz (1952) suggested a direct association between the chemical disturbance induced by sedormid and the gastro-intestinal and neurological symptoms which they observed in rabbits. In the present observations, however, the hypnotic action of sedormid obscured the clinical features of the rabbit porphyria. Allyl-isopropyl-acetamide allows an experimental porphyria without hypnosis. The separation of these two properties, excess porphyrin and porphobilinogen production and hypnosis, is in itself noteworthy, since some of the most potent drugs disturbing porphyrin metabolism, e.g. sulphonal, trional, sedormid and certain barbiturates, are hypnotics.

Using either sedormid or A.I.A., I have confirmed the association of the chemical and gastro-intestinal features of experimental porphyria, but have failed to observe any paralyses, with one exception (rabbit 4) where a barbiturate was given in addition to A.I.A. The clinical and histo-pathological pictures were here complicated by uraemia. In order to study this point more fully, experiments were carried out on fowls, animals particularly susceptible to metabolic derangement of the nervous system. Fowl 2 was given a barbiturate in addition to A.I.A. In these animals there was no evidence, clinical or histopathological, of any selective damage to nervous tissues. The experiments in which rats were intoxicated with A.I.A. showed that, in spite of high levels of porphobilinogen and porphyrin excretion in the

urine, the animals remained normally mobile and alert, and histopathological examination of the nervous system revealed no lesion. Another point of dissimilarity between rabbit experimental porphyria and human acute porphyria is the absence of hypertension, although this is a common finding in acute porphyria. The question may be asked, how relevant to the human disease are the results of the experimentally produced porphyria in animals? It can be argued that if an animal is exposed to a chemical porphyria for little more than 3 weeks, this may not be sufficient time to produce the clinical manifestations of the human disease, since in man the metabolic abnormality may be of long standing before the onset of symptoms. Sulphonal, however, may precipitate an acute porphyria in man within 5 - 20 days (Hearder, 1896; Erbslöh, 1903; Pförtner, 1914).

The haematological data agree with the normal blood levels obtained in active phases of human acute porphyria. It must be emphasized that these figures are concentration levels, and blood volumes, which might alter in the progressive wasting states of these rabbits, were not measured. Courtice and Gunton (1949) have noted an increase in the plasma volume of rabbits in which cabbage was added to the normal diet. On the other hand, Henschel, Mickelson, Taylor and Keys (1947) have shown that the absolute plasma volume <u>increased</u> slightly in a group of thirty-two men undergoing a period of semi-starvation in which about one-quarter of the body weight was lost, approximately the same proportion as lost by the rabbits studied. If the latter findings are applicable to the present experiments, they would suggest that

the undiminished haemoglobin concentration levels in the rabbits, implied, at least, maintenance of the total amount of haemoglobin throughout a period during which large quantities of porphyrins were excreted. Table 11 attempts to estimate the ratio of daily porphyrin loss to normal daily protoporphyrin formation in three of these rabbits (Nos. 4, 5 and 7). This ratio is about 1 to 2. It is suggested that if the porphyrin excretion represented a deviation of porphyrin from normal haem production, this would have resulted in a recognizable anaemia. Drabkin (1951) has suggested that the porphyrins excreted in the human porphyrias might be the result of an 'under-utilization' owing to diminished globin supply, rather than of an 'overproduction' of these pigments. The findings in this rabbit porphyria would point to an 'overproduction' rather than an 'under-utilization'. Another factor strengthening this view is the diminished urinary glycine excretion noted by paper chromatography in those rabbits (Nos. 4, 5, 6, 7 and 12) with the high total porphyrin output of 126, 147, 38, 95 and 88 mg, respectively, during the periods under consideration.

TABLE 11. ESTIMATED RATIO (EXPRESSED AS A PERCENTAGE) OF DAILY PORPHYRIN EXCRETION TO NORMAL DAILY PROTOPORPHYRIN FORMATION IN 3 RABBITS TREATED WITH A.I.A.

The normal daily protoporphyrin formed was calculated from the following data: (*a*) blood volume in the normal rabbit is 70 ml/kg (6) and (*b*) average life span of the rabbit erythrocyte is about 68 days (22). (*c*) haemoglobin level as determined at commencement of experiment (see table 5).

rabbit no.	body weight (kg)	probable blood volume	estimated normal proto-porphyrin formed (mg/day)	determined porphyrin loss (mg/day)	$\frac{\text{porphyrin loss}}{\text{normal porphyrin formation}}$ (%)
4	4·12	288	19·6	9·8	50·0
5	3·69	258	17·7	7·65	43·3
7	3·14	220	15·0	7·9	52·7

Such a diminution of glycine excretion was not observed in other rabbits treated with the same drugs, nor in rabbits treated with certain barbiturates (Section 5) where the total porphyrin excretion was much less. This suggests that in the high porphyrin excretors there was some encroachment on the glycine pool; but there is, however, still the possibility (in rabbit 4) that the diminished glycine excretion is due to renal failure, for it is known that in the human, amino-acid excretion in the urine decreases in renal failure (Dent 1954). Glycine is known to be a precursor of porphyrin (Shemin and Rittenberg 1946). Amino-acid chromatography was carried out on the urines of 2 patients in the active phase of acute porphyria. No abnormality was detected. It is of interest that one patient (Case No. 4) had a diminished hippuric acid synthesis. It is possible that this was caused by a lowered level of the glycine pool.

Table 12 groups together and compares six 'good porphyrin excretors' (above 6 mg uroporphyrin/day) and four 'poor porphyrin excretors' (below 2 mg uroporphyrin/day). These ten animals, out of a series of fifteen treated with A.I.A. or sedormid, clearly fell into two groups. The comparison of these groups emphasizes the role of the liver in the production of porphobilinogen. All poor excretors had pathological evidence of severe hepatic damage and porphobilinogen was not present in the livers. The pronounced amino-aciduria might be an expression of this hepatic damage. On the other hand, the livers of good excretors showed either normal parenchymatous tissue or minor changes, with porphobilinogen present in four out of five examined.

TABLE 12. COMPARISON OF 'GOOD' AND 'POOR' EXCRETORS

rabbit no.	mean uroporphyrin excretion (mg/day)	final urinary amino-acids	liver	
			porphobilinogen	histology
4	10·21	glycine diminished	+ + +	minor periportal fatty change
5	6·32	glycine diminished	animal still on drug after 23 days	
6	11·62	glycine diminished	+ + +	some fatty change, no necrosis
7	10·26	glycine diminished	0*	parenchyma normal
11	6·6	not determined	+ + +	parenchyma normal
12	11·29	glycine diminished	+ + +	parenchyma normal
1	1·52	many present	0	cellular necrosis
2	0·43	not determined	0	mid-zone degenerative changes
3	1·47	many present	0	cellular necrosis
10	1·53	many present	0	cellular necrosis

* No drug given during last 2 days of life. Died from bronchopneumonia.

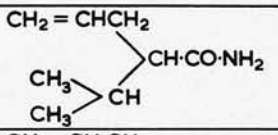
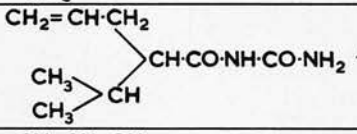
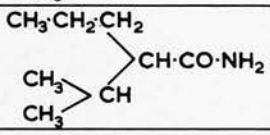
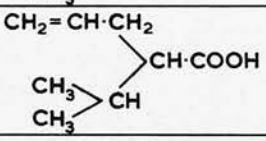
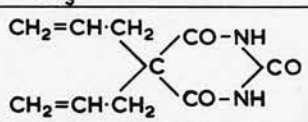
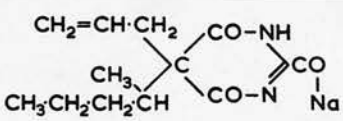
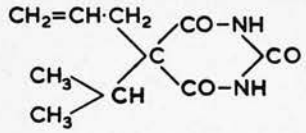
The significance of the diminished urinary glycine spot in these good excretors has been discussed. The importance of the liver in porphyrin metabolism has been emphasized previously (Prunty 1945; Watson et al. 1945). The marked fall in the urinary excretion of porphobilinogen and porphyrin 1 to 2 days before death of the 'poor excretors' suggests a failure of synthetic activity in the liver due to progressive intoxication. These findings also suggest that the difference between good and poor excretors lies in the inherent ability of the liver of an individual rabbit to deal effectively with the drug (A.I.A. or sedormid). One might speculate upon the possibility of this difference being genetically determined, and draw a comparison with human acute porphyria in which a genetically determined aberration of metabolism (probably affecting the liver) is known to exist.

Of the remaining five rabbits, three could not be classed

as 'good' or 'poor' excretors owing to lack of adequate data (Nos. 9, 14 and 15); one (No. 8) died from an infection before maximum porphyrin levels were reached, and the remaining rabbit (No. 13) would have been classified as a good excretor (8.8 mg uroporphyrin/day) had it not died with hepatic necrosis 6 days after the cessation of sedormid administration.

In attempting a comparison between human acute porphyria and experimental porphyria in the rabbit, emphasis has so far been laid on the difference between these two states. There are, however, certain similarities, viz. the chemical similarity of the porphyrins and porphobilinogen excreted, the relatively undisturbed blood picture and the constipation, the gaseous distension seen radiologically (Mason, Courville and Ziskind 1933), although it cannot be excluded that the gastro-intestinal symptoms in the rabbit might be a direct result of the drug employed. The histological changes in the liver, where any such have occurred, are more severe than those usually described for human acute porphyria. On the other hand, several rabbit livers were histologically normal, just as in many cases of human acute porphyria. Certain drugs containing allyl groups are known to be hepatoxic (Popper 1936; Section 5), and this must be considered in relation to the pathological changes seen in some of the rabbits. The histological changes in the renal tubules of rabbits 4 and 8 are reminiscent of the lower nephron nephrosis described by Prunty (1946) in a human case of acute porphyria. Bronchopneumonia, common as a final cause of death in acute porphyria, occurred in two rabbits.

TABLE 13 Chemical Structure and Effect of Certain Drugs on The Porphyrin Metabolism of Rabbits

Drug	Structure	Effect on urinary porphyrin excretion
Allyl-isopropyl-acetamide (A.I.A.)		+++
Allyl-isopropyl-acetyl-urea. (Sedormid)		+++
n-Propyl-isopropyl-acetamide.		—
Allyl-isopropyl-acetic acid.		±
Diallyl-barbituric acid. (Dial)		++
Sodium allyl (1-methyl butyl)-barbiturate. (Seconal)		+
Allyl-isopropyl-barbituric acid.		+

+ + + Very marked effect (much coproporphyrin, porphobilinogen and uroporphyrin).
+ + Marked effect (much coproporphyrin, some porphobilinogen and uroporphyrin in three out of eight rabbits).
+ Moderate effect (coproporphyrin only).
± Slight coproporphyrin rise only.
- - - No effect.

Table 13 presents the chemical structures of the drugs used and their effects on the porphyrin metabolism of rabbits. From this summary it may be concluded that at least one allyl group,

together with an acid amide, ureide or a cyclic ureide, as in the barbiturate series, is the constant chemical structure in the compounds found to be effective.

The results of the experiments using reticulo-endothelial blockade by means of thorium dioxide agree with the work of Vannotti (1937), who found that a preliminary reticulo-endothelial blockade did not diminish porphyrin excretion in normal rabbits, but decreased porphyrin formation in lead-intoxicated rabbits. The three rabbits in the present experiment behaved as 'poor excretors'. This might be due to the effect of thorium dioxide on the Kupffer cells or the hepatic cells. It is possible, however, that all three of these rabbits would have been 'poor excretors' even without thorium dioxide.

The results of splenectomy in rabbit 12 indicate that porphobilinogen, uroporphyrin and coproporphyrin can be formed in the absence of the spleen.

The porphyria induced by A.I.A. in rats showed a certain similarity to that in the rabbits, although points of difference were also noted. The distinction between 'good excretors' and 'poor excretors' was more clearly evident in rats than in rabbits. The rats tolerated a relatively higher dose of A.I.A., were much less constipated than the rabbits and showed no change in their urinary amino-acid patterns. Both the rat and rabbit illustrate that, as in the normal animal, the main route of excretion of the ether-soluble porphyrins is in the faeces. The main route of excretion of porphobilinogen appears to be the urine, although small amounts are excreted in the stool.

All the drugs used in this investigation (with the exception of the barbiturates) were most generously provided by the Hofmann La Roche Co., Basle.

SUMMARY

The chemical findings of Schmid and Schwartz (1952) in experimental porphyria of rabbits induced by sedormid have been confirmed.

Since sedormid is hypnotic, a group of related drugs has been tested to find one which might produce the chemical picture in animals without hypnosis. Such a drug is allyl-isopropyl-acetamide (A.I.A.). In this investigation, the constant chemical structure affecting porphyrin metabolism was found to be CH_2=CH-CH_2-CHR-CO-NH- . Some rabbits excrete large amounts of porphobilinogen and uroporphyrin when given either sedormid or A.I.A., others produce little. It is suggested that the cause of this difference is related to a variability of the individual rabbit liver to deal effectively with these drugs.

Rabbits, intoxicated with either drug, became constipated, had poor appetite and lost weight. They did not become paralyzed, nor show any change in systolic blood pressure or in their haematological values. Two fowls, one also given a barbiturate, and nine rats were intoxicated with allyl-isopropyl-acetamide. Although these animals excreted relatively high levels of porphobilinogen and porphyrins, they did not develop paralysis.

The experimentally induced porphyria in animals is compared with human acute porphyria. The effects are described of reticulo-endothelial blockade, splenectomy and barbiturate administration on porphyria induced experimentally in rabbits.

Experimental porphyria appears to be due to an over-production of porphyrins, rather than to an under-utilization of porphyrin pigments.

7

THE RENAL EXCRETION OF PORPHOBILINOGEN IN MAN AND ANIMALS

7. THE RENAL EXCRETION OF PORPHOBILINOGEN IN MAN AND ANIMALS

Introduction

Porphobilinogen is excreted in the urine in large quantities in acute porphyria and is always found in the livers of fatal cases of this disease. In experimental porphyria in animals induced by sedormid (allylisopropylacetylurea) or allylisopropylacetamide, porphobilinogen is likewise found in the urine and liver.

Westall (1952) isolated porphobilinogen in crystalline form, an achievement which allowed an adequate experimental study of this substance. The fate of porphobilinogen administered to the rat was recorded, and this led to observations on the renal clearance of endogenous porphobilinogen in patients with acute and latent porphyria. These studies suggested that in both experimental porphyria and in acute porphyria, the liver is the site of formation of the porphobilinogen excreted in the urine.

7A. FATE OF PORPHOBILINOGEN, ADMINISTERED ENTERALLY OR PARENTERALLY, IN THE RAT

Previous reports on the behaviour of porphobilinogen when administered to experimental animals are conflicting, probably because the porphobilinogen used was only partially purified or was available in amounts too small for adequate investigation. Thus Waldenström and Wendt (1939) injected partially purified porphobilinogen into rabbits and found it to be excreted in the urine, while Prunty (1945) failed to find any in the urine under similar conditions.

The following experiments with porphobilinogen were done in order to clarify its mode of excretion in an experimental animal. It was found that porphobilinogen, when administered parenterally to the rat, is excreted into the urine mainly by glomerular filtration. If it is given enterally or parenterally, it is not found in the livers of rats subsequently killed, at a time when the plasma still contains porphobilinogen. These findings suggest that the porphobilinogen, found in the livers of rats with experimental porphyria, has been formed there and has not been transported to the liver from an extrahepatic site.

Falk, Dresel and Rimington (1953) showed that porphobilinogen behaved as a precursor of certain porphyrins in a haemolysed chicken-erythrocyte system. In the present work, a small but significant conversion of porphobilinogen into coproporphyrin III and uroporphyrin III has been detected in the rat.

METHODS

General

The Wistar-strain rats used weighed between 150 and 214 g. with the exception of rat No. 7 which weighed 112 g. Rats Nos. 9 and 13 were female. They were placed in metabolism cages. In studies lasting 2 weeks, separate daily urinary and faecal specimens were analysed every second day for porphyrins, the specimens not determined on the day of excretion being stored at 2°. In experiments where urine was collected at frequent intervals, e.g. half-hourly or hourly, micturition was induced by a 'tail-pulling' procedure similar to that described by

Hutschenreuter (1933). In collecting urine from metabolism cages, the cage was washed with water, until the washings were free from porphobilinogen (see below). In the experiment on rat No. 9, where hourly urine specimens were taken for 20 hours, the interior of the metabolism cage was washed 4 times at the end of each hour with a total vol. (each hour) of 200 ml. water.

The porphobilinogen used had been isolated in the crystalline form (Westall, 1952; Cookson and Rimington, 1954) and was administered as the hydrochloride in 0.9% (w/v) NaCl, using 0.5 ml. for parenteral injection and 1 ml. for enteral administration. Intravenous injections were given into a tail vein, subcutaneous into the dorsal area of the rat. All enteral administration was carried out by gastric intubation.

Haematological values were determined as described in Section 5.

Isolation and determination of porphyrins

Urine Coproporphyrin was extracted by the method of Schwartz, Zieve and Watson (1951). Uroporphyrin from the same specimen of urine was obtained by combining the aqueous and sodium acetate washings from the coproporphyrin extraction, and bringing these to pH3.0-3.2. The washings were then shaken with ethyl acetate, the aqueous layer was discarded and uroporphyrin extracted with 0.6N-HCl.

Faeces Coproporphyrin and protoporphyrin were extracted by the method of Schwartz and Wikoff (1952) with the following modifications. The ethyl acetate was shaken with 20-30 ml. 0.005% aqueous I_2 immediately after extraction of porphyrins with

3N-HCl. The aqueous layer was discarded and a further extraction with 3N-HCl carried out, in order to obtain any porphyrin precursor. This was not found in any specimen. The final coproporphyrin extraction in 0.1N-HCl was not shaken with $CHCl_3$. Uroporphyrin was obtained from the sodium acetate washings of the ethyl acetate layer during the above procedure. These acetate washings were dealt with in the same way as described for urinary uroporphyrin.

The porphyrins, so extracted from urine and faeces, were determined by the method of Rimington and Sveinsson (1950) using a Beckman spectrophotometer, model D.U.

Paper chromatography of porphyrins This was carried out as described in Section 5.

Isolation and determination of porphobilinogen

Urine and tissues Porphobilinogen was determined as described in Section 5. It was assumed that 1 mg. of porphobilinogen is equivalent to 130 Vahlquist (1939) units (Westall, 1952).

Faeces To a weighed amount of faeces in a centrifuge tube, sufficient water was added to give a thin paste. Clarification was effected by adding 10% (w/v) lead acetate in 3% (w/v) acetic acid, in a volume one-tenth of that of the diluted faeces, mixing and then centrifuging. The supernatant was decanted and excess Pb^{2+} was precipitated with a solution containing NaH_2PO_4 (123 g./l.) and K_2HPO_4 (138 g./l.). The mixture was centrifuged and the supernatate analysed for porphobilinogen as described for urine.

Plasma Protein was precipitated by adding 0.33 vol. of 20% /w/v) trichloroacetic acid to centrifuged plasma as obtained post-mortem. The mixture was then centrifuged and the supernatant tested for porphobilinogen. In the living rat 0.1 ml. of blood from the tail was sucked into a 0.1 ml. pipette, previously rinsed with heparin. The blood was discharged into 1.5 ml. of 5% (w/v) trichloroacetic acid contained in a centrifuge tube. This was thoroughly shaken, centrifuged and the supernatant tested for porphobilinogen. The observation that porphobilinogen does not enter erythrocytes (Section 6) was confirmed in the present study. The blood haematocrit for the rat was taken to be 40 (Wintrobe, 1951) and plasma porphobilinogen was calculated on this basis.

Paper chromatography This was done as described by Westall (1952) and Cookson and Rimington (1953).

RESULTS

Intravenous injection of porphobilinogen

Excretion pattern of porphobilinogen and porphyrins Rats Nos. 1 and 2 were given intravenous injections of 9.04 and 9.4 mg., respectively, of porphobilinogen after a preliminary period of 8 and 7 days, respectively, during which daily faecal and urinary porphyrins were determined. These determinations were continued during the succeeding 8 days. Porphobilinogen was rapidly excreted in the urine of both rats. It appeared in the urine within 10 min. of injection and was detectable until 8 hours later. In rat No. 1 specimens of urine were collected every 2 hours during the 12 hour period after injection. The results of porphobilinogen,

uroporphyrin and coproporphyrin determinations on these specimens are illustrated in Fig. 1. This shows that nearly half of the total porphobilinogen excreted in the urine (6.07 mg.) was passed in the first 2 hours after injection, while the excretion of the small quantities of uroporphyrin (4.59 μg.) and coproporphyrin (6.85 μg.) was more protracted. A smaller yield of porphobilinogen was obtained in the urine of rat No. 2, viz. 2.85 mg., of which 2.6 mg. were excreted in the first 3 hours after injection. This rat excreted a total of 4.95 μg. of uroporphyrin and 6.0 μg. of coproporphyrin in the 24 hours following injection.

Paper chromatography of these porphyrins showed that they behaved as uroporphyrin III and coproporphyrin III, respectively. The porphobilinogen excreted in the urine was indistinguishable from crystalline porphobilinogen by paper chromatography, using butanol-acetic acid (Westall, 1952). Furthermore, the lactam derivatives (Cookson and Rimington, 1953) of the porphobilinogen excreted in the urine and of crystalline porphobilinogen, behaved identically on a paper chromatogram.

Table 1 summarizes the mean daily faecal coproporphyrin and protoporphyrin values before and after administration of porphobilinogen in rats Nos. 1 and 2. These figures are similar in each rat. Paper chromatography of these porphyrins both before and after porphobilinogen administration demonstrated that some dicarboxylic porphyrin (similar in properties to protoporphyrin) as well as coproporphyrin had been extracted by 0.1N-HCl and thus the coproporphyrin determinations in Table I are too high. However, an amount of dicarboxylic porphyrin similar to that of

Table 1. *Urinary and faecal porphyrin determinations made with rats nos.* 1, 2 *and* 3, *before and after porphobilinogen* (*Pbg.*) *administration, by the intravenous* (*i.v.*) *or enteral* (*g.i.*) *routes*

Urinary and faecal porphobilinogen were recovered after its administration. The durations of the 'before' periods for rats nos. 1, 2 and 3 were 8, 7 and 6 days respectively; those for 'after' were 3 days in each case.

Rat no.	Weight (g.)	Dose of Pbg. (mg.)	Mean urinary coproporphyrin (μg./day)		Mean faecal coproporphyrin (μg./day)		Mean faecal protoporphyrin (μg./day)		Pbg. recovered		
			Before	After	Before	After	Before	After	Faeces (mg.)	Urine (mg.)	% (urine + faeces)
1	266	9·04 (i.v.)	1·7 (±0·6)	3·7 (±2·2)	134 (±33)	78 (±16)	135 (±39)	130 (±31)	0·097	6·07	68
2	172	9·4 (i.v.)	1·3 (±0·4)	3·7 (±1·9)	53 (±10·7)	42 (±9·1)	98 (±25)	122 (±36)	0·055	2·85	31
3	214	3·68 (g.i.)	1·6 (±0·6)	2·1 (±1·4)	39 (±12·6)	27 (±6·7)	66 (±15·2)	79 (±29·2)	1·676	0	45

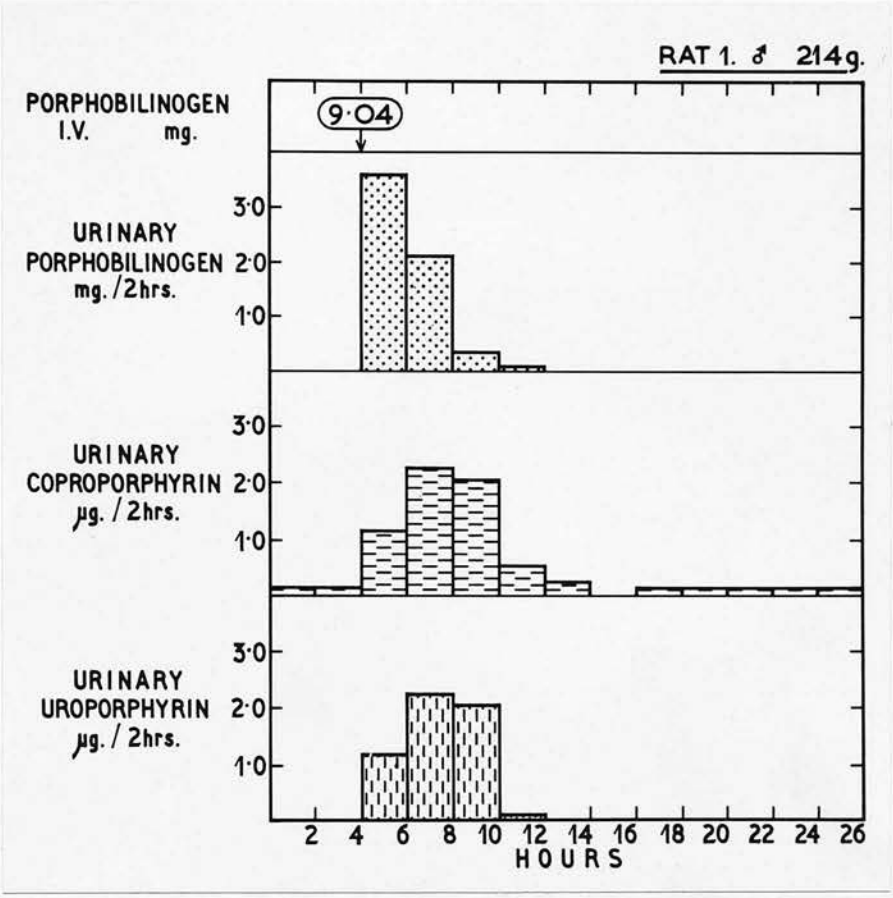


Fig. 1. Urinary excretion of porphobilinogen and porphyrins after the intravenous injection of porphobilinogen in rat No. 1.

coproporphyrin (as judged by fluorescence on paper chromatograms) was extracted by 0.1N-HCl in each case. It was therefore considered that no significant change had occurred in faecal porphyrins as a result of porphobilinogen administration.

Neither rat showed any abnormal symptoms during the 8-day period following the porphobilinogen injection. Haemoglobin contents, red cell numbers and reticulocyte counts were determined in both rats, before and at the end of the experiment and one day before and one day after the injection. No significant changes in these haematological values were observed.

Rats killed within 30 min. Porphobilinogen (2.2, 2.0 and 2.14 mg.) was injected by vein into rats Nos. 4-6 and these were killed after 30, 30 and 10 min., respectively. The porphobilinogen contents of the tissues of these rats are summarized in Table 2. The urine, plasma and kidneys always contained porphobilinogen, while liver, spleen, brain, bone marrow and Harderian glands gave consistently negative results. Subcutaneous fat and lungs contained porphobilinogen, while skeletal and cardiac muscles (thoroughly washed free of blood) of rat No. 6 contained porphobilinogen. The plasma porphobilinogen level of rat No. 6 was approximately 4 times that of rats Nos. 4 and 5 (killed at 30 min.). Small amounts of porphobilinogen were found in the gut contents.

Subcutaneous injection of porphobilinogen

Rats killed within 30 min. after single injection. Porphobilinogen (2.0 and 2.62 mg.) was injected into rats Nos. 7 and 8, respectively, and the animals were killed 30 min. after the injection. The pattern of urinary and tissue porphobilinogen (Table 2) was similar to that found after intravenous injection. The plasma porphobilinogen level was higher than that found in rats Nos. 4 and 5.

Rats killed 20.25 hr. after half-hourly injections, maintained for 20 hr. In experimental porphyria in rats induced by allylisopropylacetamide, porphobilinogen is consistently found in the liver in high concentration, also in the kidney and urine, and sometimes in the plasma. This hepatic porphobilinogen could have been formed in the liver or at an extrahepatic site, e.g. the bone marrow, and transported by the blood stream to the liver.

Table 2. *Tissue porphobilinogen (Pbg.) determinations of rats* (4–11), *given porphobilinogen, intravenously* (*i.v.*) *subcutaneously* (*s.c.*) *or by gastric intubation* (*g.i.*)

Rat no. 12 was given 50 mg. of allyl*iso*propylacetamide (A.I.A.). Duration refers to period between porphobilinogen or A.I.A. administration and killing of animals. Porphobilinogen results are expressed as μg. total porphobilinogen (urine and gut contents) or as μg./g. or μg./ml. (the remaining tissues): ' + ' indicates a trace.

				Porphobilinogen										
													Pbg. recovered	
Rat no.	Body wt. (g.)	Dose Pbg. (mg.)	Duration (min.)	Urine (total)	Gut (total)	Kidney	Plasma	Liver	Skeletal muscle	Fat	Lungs	Heart muscle	(μg.)	(%)
4	150	2·2 (i.v.)	30	854	+	50	11·3	0	*	*	*	*	976	43
5	174	2·0 (i.v.)	30	805	2·5	42·5	8·3	0	0	11·3	9·1	*	966	48
6	160	2·14 (i.v.)	10	4	+	124	38	0	10·6	9·3	8	0	1 305	61
7	112	2·0 (s.c.)	30	645	+	75	27·6	0	*	+	+	8	1 186	59
8	151	2·62 (s.c.)	30	719	10	100	26·2	0	5	16·5	10·6	0	1 021	39
9	150	40·0 (s.c.)	1215	30 160	280	69·5	27·6	0	0	7·3	6·9	0	31 734	79
10	190	3·4 (g.i.)	450	168	2122	0	0	0	0	0	0	0	2 290	67
11	197	4·85 (g.i.)	120	87	1750	0	2·1	0	0	0	0	0	1 955	40
12	174	0† (g.i.)	1200	515	9·8	10·7	0	28·6	0	0	0	0	806	—
13	142	Normal control	—	0	0	0	0	0	0	0	0	0	—	—

* Not tested. † 50 mg. A.I.A.

The very rapid excretion of porphobilinogen in the urine and the absence of porphobilinogen from the liver in the above experiments, when the plasma still contained porphobilinogen, made such an extrahepatic site unlikely. However, it was still possible that the liver might take up porphobilinogen from an extrahepatic site if porphobilinogen were present in the plasma for any length of time. To determine the approximate length of the period during which significant plasma levels are maintained in experimental porphyria, rat No. 12 was given allylisopropylacetamide by gastric intubation. Within 20 hours this rat had excreted porphobilinogen in the urine. It was killed and porphobilinogen was found in the liver and kidney (Table 2).

In order to mimic the effects of continuous production of porphobilinogen by a possible extrahepatic site throughout this period, 1 mg. of porphobilinogen was injected subcutaneously into rat No. 9 every 30 min. for 20 hours. The initial two doses were given together at the beginning of the experiment. Urinary excretion of porphobilinogen was determined hourly and urinary uroporphyrin and coproporphyrin, as well as plasma porphobilinogen were determined every 2 hours (Fig. 2). The rat was killed 15 min. after the final injection and tissues were analysed for porphobilinogen and porphyrins (Tables 2 and 3). The liver did not contain porphobilinogen, while the gut contents showed a significant amount of porphobilinogen, presumably from passage through the bile ducts. During the 20 hour period of the experiment the rat showed no pathological symptoms.

Inspection of Fig. 2 shows that during the latter 10 hours

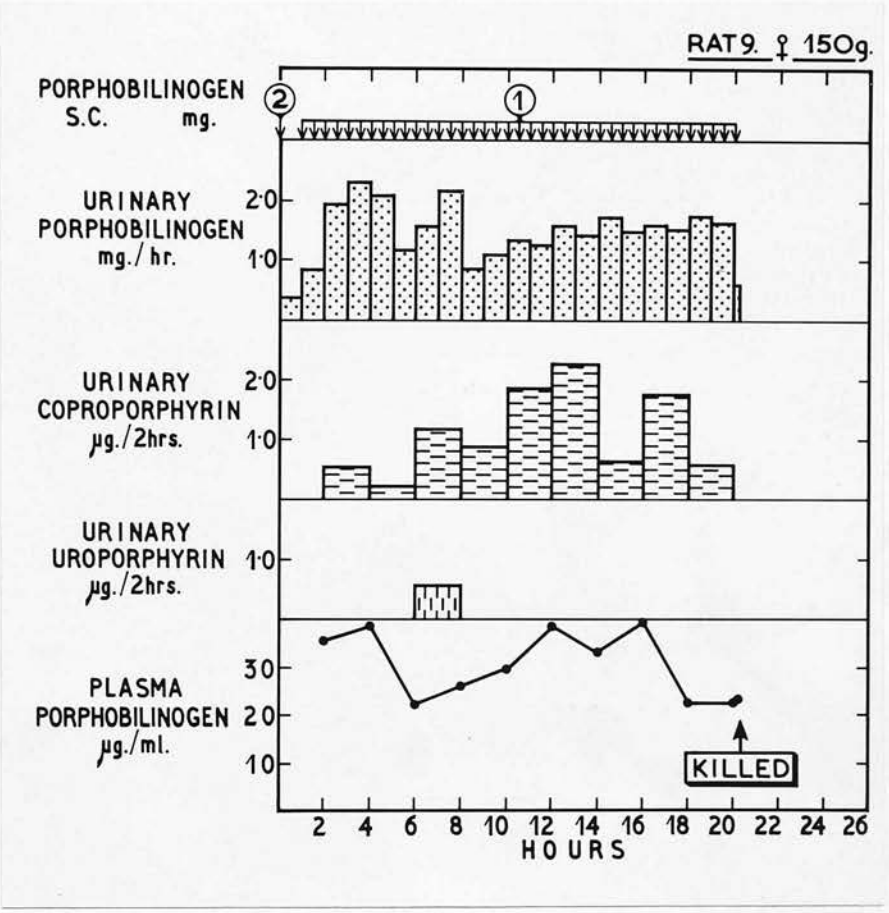


Fig. 2. Effects of repeated subcutaneous injections of porphobilinogen for 20 hr. on urinary porphobilinogen and porphyrin excretion and on plasma porphobilinogen. For experimental details see text.

Table 3. *Porphyrin determinations (µg./g. or µg./ml.) in tissues of rats which had been given porphobilinogen*

Uroporphyrin was not found in any of the tissues of these rats.

Rat no.	Liver		Kidney		Blood	
	Copro-porphyrin	Proto-porphyrin	Copro-porphyrin	Proto-porphyrin	Copro-porphyrin	Proto-porphyrin
4	0·04	0·02	0·01	0·04	0	0·06
6	0·23	0·16	0·12	0·13	0·02	0·26
7	0·09	0·11	0	0·25	0	0
9	0·02	0	0·31	0·57	0	0
13 (control)	0·02	0·16	0·08	0·07	0·02	0·49

of this experiment the hourly urinary excretion levels of porphobilinogen were remarkably constant - a mean of 1530 ± 151 μg. (S.D.)/hr. The mean plasma porphobilinogen level during this period was 31 ± 7.4 μg. (S.D.)/ml. Thus the renal clearance for porphobilinogen in this rat was 0.82 ml./min. or 0.55 ml./min./100 g. of body weight. This figure is in good agreement with inulin clearance found in the rat (Smith, 1951) and would therefore suggest that porphobilinogen is filtered by the glomeruli and not reabsorbed to a significant extent.

Table 3 summarizes the liver, kidney, and blood porphyrin determinations of rats Nos. 4, 6, 7 and 9, which had been given porphobilinogen parenterally. There is no significant elevation of the porphyrin levels.

Enteral administration of porphobilinogen

Excretion pattern of porphobilinogen and porphyrins

A study similar to that described for rats Nos. 1 and 2, was carried out in rat No. 3. After a preliminary period of 6 days, 3.68 mg. of porphobilinogen were given by gastric intubation. Daily urinary and faecal porphobilinogen and porphyrin contents were determined during the base-line period and in the 7 days following porphobilinogen administration (Table 1). There was no significant change in the porphyrin levels in the urine or faeces. No porphobilinogen appeared in the urine of this rat (cf. rats Nos. 10 and 11, below), but 45% of the porphobilinogen administered was recovered in the faeces. Traces of porphobilinogen were present in the faeces up to the sixth day after porphobilinogen administration.

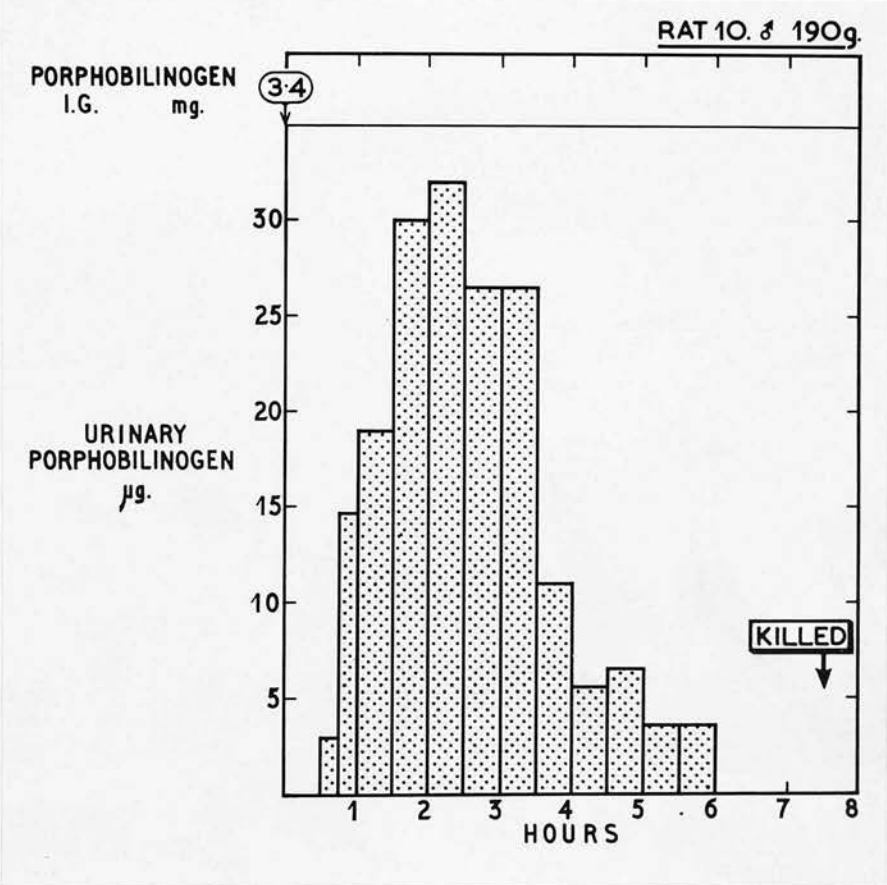


Fig. 3. Urinary excretion of porphobilinogen after intragastric intubation of 3.4 mg. of porphobilinogen.

Rat killed after 7.5 hr. Rat No. 10 was given 3.4 mg. of porphobilinogen by gastric intubation. Urinary porphobilinogen determinations were done every 30 min. (Fig. 3). Porphobilinogen was detected in the urine within the second half-hour and was excreted in small amounts until 6 hours after its administration. Maximum excretion in the urine occurred between 2 and 2.5 hours. Between 6 and 7.5 hours, when the rat was killed, no porphobilinogen

was detected in the urine. After death 2.12 mg. of porphobilinogen was recovered from the intestinal contents. It was notable that in spite of this large quantity of porphobilinogen in the gut, it was not excreted in the urine in the last 1.5 hr. and this would suggest that porphobilinogen is only absorbed in the upper part of the rat intestine. Porphobilinogen was not found in the other tissues (Table 2).

Rat killed after 2 hr. Rat No. 11 was given 4.85 mg. of porphobilinogen by gastric intubation and killed 2 hours later in order to see whether porphobilinogen could be detected in the liver during a phase of maximal absorption of porphobilinogen from the gut (see above). Urinary porphobilinogen determinations, as in rat No. 10, showed the excretion of 11.3, 30 and 46 μg. of porphobilinogen in the second, third and fourth half-hour periods, respectively, after administration. The rat was then killed and 1.75 mg. of porphobilinogen were recovered from the intestinal contents. The plasma contained 2.1 μg. of porphobilinogen/ml., whilst 0.104 mg. of porphobilinogen was recovered from gut tissues after they had been thoroughly washed with normal saline. Porphobilinogen was not present in liver, kidney, muscle, fat, Harderian gland or brain (Table 2). No porphyrins were detectable in the liver.

In vitro incubation of porphobilinogen with gastro-intestinal contents of a rat

This experiment was carried out to investigate further the absence of conversion of porphobilinogen into porphyrins in the gut, which was demonstrated in rat No. 3. A rat was killed and

its gastro-intestinal contents were mixed with sterile normal saline to give a total vol. of 23 ml. 4.5 ml. of this were pipetted into each of four 50 ml. conical flasks. Flasks Nos. 1 and 2 were immersed in boiling water for 5 minutes and 1 mg. of porphobilinogen in 0.56 ml. of sterile normal saline was added to flasks Nos. 2 and 4. The pH of each of the contents of these flasks was about 6.6. The flasks were then plugged with cotton wool and incubated for 4 hours with shaking at 90-100 oscillations/min. at 37°. The contents were then analysed for porphobilinogen and porphyrins (Table 4).

The results show that a 59 and 52% recovery of porphobilinogen was achieved from flasks Nos. 2 and 4, but there was no significant change in the porphyrin content in any flask, with the exception of a small quantity of uroporphyrin (0.71 μg) found in flask No. 2.

Table 4. *Incubation of porphobilinogen with gastro-intestinal contents of a rat*

An equal volume of a mixture of gastro-intestinal contents in sterile 0·9% (w/v) NaCl was added to each of flasks nos. 1–4. Flasks nos. 1 and 2 were immersed in boiling water for 5 min. before incubation.

Flask no.	Porphobilinogen added before incubation (mg.)	Porphobilinogen recovered after incubation (mg.)	Uroporphyrin (μg.)	Coproporphyrin (μg.)	Protoporphyrin (μg.)
1	0	0	0	0·16	4·10
2	1	0·59	0·71	0·36	2·90
3	0	0	0	0·29	6·25
4	1	0·52	0	0·26	2·75

DISCUSSION

The results show that most of the porphobilinogen administered parenterally is excreted in the urine and that this excretion is rapid; small amounts, however, are recovered in the faeces. Given, however, by the enteral route, it is mainly excreted

unchanged in the faeces, although small amounts, (in the case of rat No. 10, one-twentieth of the porphobilinogen given) may be excreted in the urine. The excretion behaviour of the naturally occurring porphyrins (uroporphyrin, coproporphyrin and protoporphyrin), after enteral and parenteral administration to experimental animals, has been studied previously. Fischer (1916) and Günther (1922) found that uroporphyrin given parenterally to mice and rabbits, respectively, is mainly excreted in the urine, while Fischer (1915) found that uroporphyrin, when taken by himself by mouth, was passed unchanged in the stool. On the other hand, coproporphyrin III, given orally or parenterally to rats, is not passed in the urine, but recovered in the stool (Hoffbauer, Watson and Schwartz, 1953). Protoporphyrin injected parenterally into dogs with a bile-renal fistula was not accounted for in the porphyrins excreted thereafter, apart from traces of material resembling deuteroporphyrin (Watson, Pass and Schwartz, 1941). The excretion of porphobilinogen thus resembles more that of uroporphyrin than that of coproporphyrin or protoporphyrin. The mean percentage recovery of the porphobilinogen administered, parenterally or enterally, was 53 $\pm$ 14. This compares with the 50% recovery of coproporphyrin III injected parenterally into rats by Hoffbauer et al. (1953).

The absence of abnormal symptoms in any of the rats used in the present work confirms the results of the pharmacological study of porphobilinogen in Section 4. It is of some interest that a small, though significant, rise of coproporphyrin III occurred in the urine of rats Nos. 1, 2 and 9, besides some excretion of

uroporphyrin III. This is in accordance with the demonstration by Falk et al. (1953) using a haemolysed chicken erythrocyte system, that porphobilinogen is a precursor of uroporphyrin, coproporphyrin and protoporphyrin.

The site of the excessive porphobilinogen formation in acute porphyria is of importance. Prunty (1945) showed that porphobilinogen is found in high concentration in the liver at autopsy of these cases, and this observation has been repeatedly confirmed. Thus Schmid, Schwartz and Watson (1954) include this disease in their 'porphyria hepatica' group. Porphobilinogen is also found in the kidney and urine and sometimes in the bile and plasma, but not in other tissues. In experimental animals drugged with sedormid or allylisopropylacetamide, these same tissues also contain porphobilinogen and in the same relative concentrations. In fact Schmid and Schwartz (1952) refer to the experimental porphyria produced by sedormid as belonging to the 'hepatic type'. This suggested but did not prove, that in acute porphyria and in experimental porphyria of animals, porphobilinogen is formed in the liver. It did not exclude the possibility that porphobilinogen is formed at an extrahepatic site, e.g. the bone marrow, and is transported by the blood stream to the liver. The present experiments indicate that porphobilinogen is mainly excreted by glomerular filtration in the rat. Further, they show that porphobilinogen is not found in the liver after enteral or parenteral administration. The plasma porphobilinogen levels in these experiments were high, higher than those found in an attack of acute porphyria. In rat No. 9 this plasma level was maintained

for 20 hours, a period which had been found sufficient to induce an experimental porphyria by means of allylisopropylacetamide, with accumulation of porphobilinogen in the liver in rat No. 12, (Fig. 4). These facts would suggest that extrahepatic porphobilinogen formation in experimental porphyria in the rat is improbable. It is also of interest that there was no significant increase of either coproporphyrin or protoporphyrin in the livers of rats given porphobilinogen. In the livers of animals with experimental porphyria such an increase is present.

7B. RENAL CLEARANCE OF ENDOGENOUS PORPHOBILINOGEN IN MAN

There is some evidence that the mechanism of porphobilinogen excretion in the rat may be relevant to human acute porphyria. In this disease, the high porphobilinogen concentrations in the urine and the persistently low concentrations in the plasma, even at the height of an attack, suggest a rapid excretion of that substance. Furthermore, a sharp rise of porphobilinogen excretion in the urine often accompanies and may even precede the onset of clinical symptoms of porphyria, which would also suggest rapid elimination. Finally, acute porphyria urine, which is freshly passed and has not lain in the bladder for long, contains mainly porphobilinogen with only traces of coproporphyrin and uroporphyrin. This is the same pattern of porphobilinogen and porphyrin excretion as is found in the urine of a rat, injected parenterally with porphobilinogen. A greater conversion of porphobilinogen into porphyrins would be expected, if porphobilinogen were not rapidly

RAT 9 RAT 12

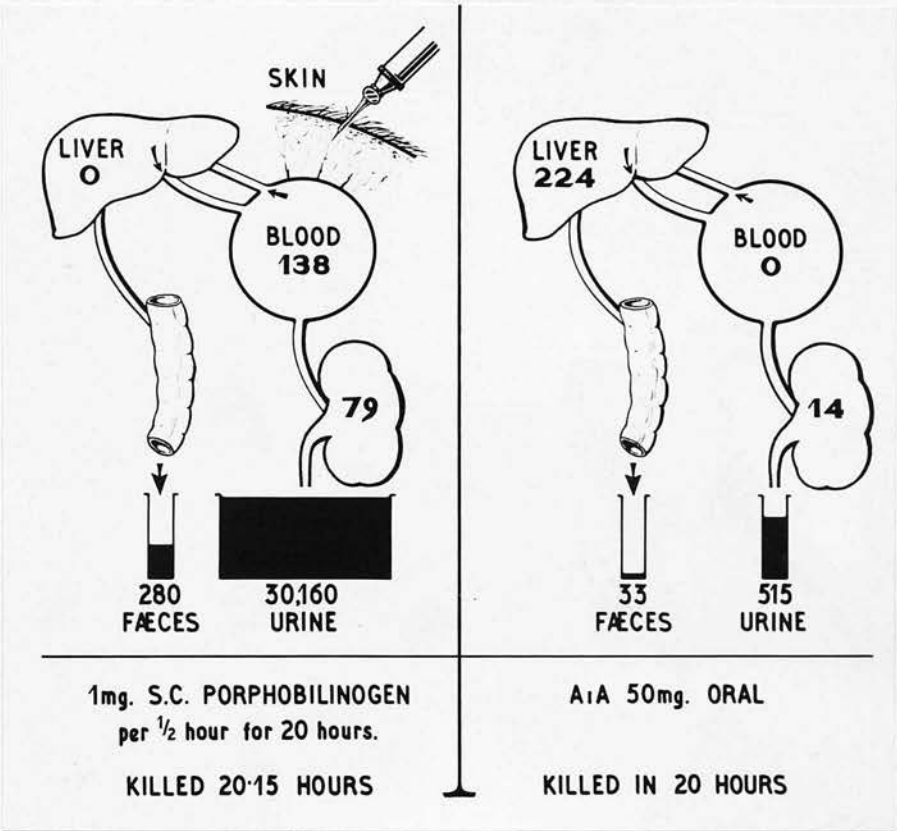


Fig. 4. Porphobilinogen (μg.) in tissues of rats 9 and 12. Rat 9 received repeated subcutaneous (S.C.) injections of porphobilinogen. Rat 12 was given a single oral dose of allyl-isopropyl-acetamide (A.I.A.). No porphobilinogen has accumulated in the liver of rat 9, despite maintained high blood-level, whereas the liver of rat 12 contains much porphobilinogen.

eliminated in acute porphyria. The probability of such a rapid elimination would explain the absence of significant photosensitivity in this disease, since this symptom is caused by an excessive amount of formed porphyrins in skin. These findings would suggest

that the renal excretion of porphobilinogen in the rat might be similar to that in human acute porphyria. For these reasons studies were carried out to determine the renal clearance of endogenous porphobilinogen in three cases of acute intermittent porphyria (two during an acute attack and one during a remission) and in one case of latent porphyria. The results suggest that in these patients endogenous porphobilinogen was in fact excreted by glomerular filtration with little or no tubular reabsorption.

METHODS AND MATERIALS

Clinical

The Cases

Case 1. (Mrs. I.B. aged 21. Case No. 39 in Appendix). Height 155 cm., weight 43.6 kg. Acute intermittent porphyria. This was her first attack. She had had recurrent bouts of abdominal pain, transient hypertension, and tachycardia for 6 weeks. There was weakness of her arms and legs. The experiment was done during the acute phase on a day when she was co-operative and had little abdominal pain. She excreted 215 mg. of porphobilinogen in her urine on that day.

Case 2. (Mrs. J.M. aged 23. Case No. 38 in Appendix). Height 162 cm., weight 55.5 kg. Acute intermittent porphyria. This was her first attack. She had had severe abdominal pain and vomiting for 3 weeks and had difficulty in passing urine. On the day of the experiment her symptoms had greatly improved and she excreted 52 mg. of porphobilinogen in her urine.

Case 3. (Mr. C., aged 29). Height 193 cm., weight 74 kg. Latent porphyria. Situs inversus totalis. Bronchiectasis.

Traumatic epilepsy. He has never had symptoms suggesting acute intermittent porphyria, yet he excretes about 100 mg. of porphobilinogen in his urine each day. (Patient of Dr. Donald Hunter, London Hospital).

Case 4. (Mr. L.F. aged 24. Case No. 14 in Appendix). Height 175 cm., weight 65.5 kg. Acute intermittent porphyria in remission. A year before he had a very severe attack with quadriplegia. He still had wasting of the small muscles of his hands. He excreted about 120 mg. of porphobilinogen in his urine daily. Two separate experiments were made in this case: (a) clearance of endogenous porphobilinogen, and (b) simultaneous clearance of endogenous porphobilinogen and of inulin.

All the patients were recumbent in bed throughout each experiment, except Case 3, in whom the effect of postural change on the renal clearance of porphobilinogen, creatinine, and urea was investigated.

Food and Fluids

Each patient was given breakfast before the start of the experiment. Cases 1 and 4(a) in addition had a light lunch during the experiments. Cases 2, 3, and 4(b) fasted throughout the experiments. The patients were encouraged to drink water and orange-juice from an hour before till the end of the clearance periods.

Collection of Urine and Plasma

Urine was passed voluntarily by all the patients except Case 2, who had to be catheterised at the end of the clearance period. The minimum of urine passed in any single clearance period

was 230 ml. The mean of all such volumes was 544 ± 260 ml.

The experiments in every case were made between 7 a.m. and 5 p.m. In Case 1 the urine excreted in 6 hours (from 11 a.m. till 5 p.m.) was collected, and plasma specimens were taken at 1, 3 and 5 hours after the start of the experiment. Case 2 emptied her bladder at 7 a.m. and was catheterised 4¼ hours later. Plasma specimens were taken at 1 and 3 hours after the start of the experiment. In cases 3 and 4(a) the patients had four and five consecutive 2-hour clearance periods beginning at 7 a.m. and 9 a.m. respectively. Plasma specimens were taken an hour after the start of each clearance period. In Case 4(b) the clearance of endogenous porphobilinogen was studied for 2 hours (from noon to 2 p.m.). An intravenous inulin drip had been set up at 11 a.m., and inulin clearance was determined for the period 12.30 - 2.0 p.m. The inulin clearance recorded represents the average of three separate half-hourly inulin clearances recorded in this 1½ hours. The minimum of urine passed during each half-hourly period was 194 ml., and the mean volume for the three periods was 240 ± 35 ml.

Urea and creatinine clearances were measured in addition to porphobilinogen clearances in certain cases (see table).

Biochemical Determinations

Porphobilinogen was determined in urine and plasma by methods described in Section 5. In every case 1.5 ml. of 20% (w/v) trichloracetic acid was added to 6 ml. of plasma.

Weighed quantities of crystalline porphobilinogen were added to distilled water and normal human plasma, sufficient to

TABLE 5

RENAL CLEARANCES IN PORPHYRIA

Case no.	Duration		Posture	Clearance of (ml. per min.)			
	From	To		Creatinine	Urea	Endogenous porphobilinogen	Inulin
1	11 A.M.	5 P.M.	R	79	42	117	..
2	7 A.M.	11.15 P.M.	R	..	31	93	..
3	9 A.M.	11 A.M.	S	77	51	70	..
	11 A.M.	1 P.M.	R	92	69	105	..
	1 P.M.	3 P.M.	S	82·5	53	97	..
	3 P.M.	5 P.M.	R	91	73	104	..
4(*a*)	7 A.M.	9 A.M.	R	79	52	84	..
	9 A.M.	11 A.M.	R	64	54	85	..
	11 A.M.	1 P.M.	R	91	67	89	..
	1 P.M.	3 P.M.	R	87	54	89	..
	3 P.M.	5 P.M.	R	80	54	82	..
				Mean 80 ± 9·2	56 ± 5·5	86 ± 2·8	..
(*b*)	Noon	2 P.M.	R	..	..	90	..
	12.30 P.M.	2 P.M.	R	..	..	..	94

In case 4(*b*) the endogenous porphobilinogen clearance and the inulin clearance were measured simultaneously.
R, recumbent, S, standing.

give concentrations in the same range as that of the specimens of urine and plasma to be tested. The densities of these standard aqueous and plasma solutions when treated with Ehrlich's reagent were measured at 552 mμ on a 'Unicam' spectrophotometer (S.P.500), and calibration curves were drawn, the spectrophotometric readings being plotted against known concentrations of porphobilinogen in water and plasma.

The urine in each case was diluted 25 - 100 times with distilled water. It was therefore considered permissible to measure the porphobilinogen content of this very diluted urine by the calibration curve of the standard aqueous porphobilinogen solutions. The porphobilinogen contents of patients' plasma were likewise calculated from the calibration curve of the standard plasma-porphobilinogen solutions.

The specimens of urine and plasma were examined for their porphobilinogen content within half an hour of collection; before the determination they were stored at 2°C.

* Urea was determined in blood by the method of Van Slyke and Cullen (Hawk et al. 1947). Urinary urea was determined by the hypobromite method (Harrison 1947).

* Creatinine The methods described by Hawk et al. (1947) were used for plasma and urinary creatinine.

Inulin clearance The method used for this was essentially that described by Higashi and Peters (1950). I must thank Dr. E. J. Ross for guidance and assistance in this procedure.

Results

The results of these experiments are summarised in the table. The mean plasma-porphobilinogen concentrations in Cases 1, 2, 3, and 4(a) were 1.0 ± 0, 1.0 ± 0.1, 0.6 ± 0.1, and 1.3 ± 0.2 μg. per ml. respectively.

The mean endogenous porphobilinogen clearances of all the patients when recumbent, corrected in each case to 1.73 sq. m. of body-surface area, was 106 ± 28.4 ml. per min.

Discussion

The results suggest that, in these patients, endogenous porphobilinogen was filtered by the glomeruli and not excreted or reabsorbed by the tubules to any significant extent. Creatinine and inulin are known to be entirely excreted by glomerular filtration. In Cases 3 and 4(a) the simultaneous renal clearances of creatinine and endogenous porphobilinogen were similar. This similarity was less obvious in Case 1. The simultaneous renal

* I must thank the department of Clinical Pathology, University College Hospital, London, for these determinations.

clearances of inulin and endogenous porphobilinogen in case 4(b) were similar. The mean of the corrected porphobilinogen clearance of all the patients when recumbent (106 ± 28.4 ml. per min.) compared favourably with the figure recorded by Smith (1951) for glomerular filtration-rates obtained in mixed groups of males and females (116 ± 28.1 ml. per min.).

The results of the influence of posture on the renal clearance of creatinine, urea, and porphobilinogen in Case 3 require comment. Brun et al. (1945) recorded that glomerular filtration, measured by inulin clearance, and renal plasma flow, measured by diodone clearance, became lower when the reclining posture was changed to the passive erect posture, and then rose to their former levels when the patient resumed the reclining posture. These changes took place within 15 - 30 minutes of the change of posture. White and Rolf (1948) and Epstein et al. (1951) confirmed this influence of posture on inulin clearance, and Ni and Rehberg (1931) and White and Rolf (1948) showed a similar effect on the renal clearance of creatinine. On the other hand, Viar et al. (1951) did not find any influence of posture on creatinine clearance.

The renal clearance of endogenous porphobilinogen in Case 3 was somewhat higher in the reclining position than in the erect position, and the simultaneous urea and creatinine clearances showed a similar effect. These observations do not clarify fully the role, if any, of the renal tubules in the excretion of endogenous porphobilinogen, since the clearance of urea, which is slightly reabsorbed by the tubules, and of diodone, which is excreted by the

tubules, are also influenced by changes of posture. However, the close correspondence between the changes in porphobilinogen and creatinine output suggest a closely related mechanism of excretion.

It has been suggested that, in experimental porphyria in the rat, porphobilinogen is formed in the liver and not at an extrahepatic site. In acute porphyria in man porphobilinogen is found in the same tissues as in experimental porphyria in the rat - i.e. liver, kidney, and plasma. In addition the mechanism of the renal clearance of porphobilinogen in acute porphyria in man appears to be the same as in the rat. This suggests that the reasoning that porphobilinogen is formed in the liver in experimental porphyria in the rat is valid for acute porphyria in man.

Porphobilinogen is a substance of low molecular weight (226) and is pharmacologically inactive; it can be easily and accurately measured in very small concentrations in urine and plasma. After its injection into rats a maximal recovery of 79% was obtained, of which 75% was found in the urine, while the slight increase of urinary porphyrins reflected a 0.1% conversion of the injected porphobilinogen. Possibly this substance or one closely allied to it might provide a suitable tool for the determination of the glomerular filtration-rate.

The fact that some patients with latent porphyria and acute porphyria in remission may excrete large quantities of porphobilinogen in the urine while they have a determinable plasma-porphobilinogen level should be remembered when an attempt is being made to assess the progress of a case of acute porphyria. It is also clear that clinical interpretations of changes in urinary

output of porphobilinogen must take into account changes in renal function as well as changes in the production of porphobilinogen. For example, a decreased output of porphobilinogen can mean either that the patient is getting over his acute attack of porphyria or that his condition is worsening because of renal failure, with, of course, a lowered glomerular filtration-rate. Plasma-porphobilinogen levels and clearance determinations can be used to distinguish between these two possibilities.

SUMMARY

1. Porphobilinogen has been administered parenterally and enterally to rats. After parenteral injection, porphobilinogen was rapidly and mainly excreted in the urine. Small amounts were excreted in the faeces. After enteral administration, only small amounts were slowly excreted in the urine. Most of it was passed unchanged in the faeces. The porphobilinogen excreted in urine was identical chromatographically with that administered.

2. A calculation of renal clearance of porphobilinogen in the rat suggests that porphobilinogen is mainly excreted by glomerular filtration without significant reabsorption.

3. A small but significant rise of coproporphyrin III, as well as the excretion of some uroporphyrin III, was noted in rat urine after parenteral injection of porphobilinogen. There was no significant change in faecal porphyrin excretion after enteral or parenteral administration of porphobilinogen.

4. Aerobic incubation in vitro at 37° of porphobilinogen with gastro-intestinal contents of a rat showed no evidence of

conversion of porphobilinogen into porphyrins.

5. Porphobilinogen was not found in the liver when rats were killed shortly after its enteral or parenteral administration, nor was it found in the liver of a rat after subcutaneous injections of porphobilinogen every half-hour for 20 hours, during which time a high plasma porphobilinogen concentration was maintained. These findings suggest that the porphobilinogen found in the liver of a rat with experimental porphyria is in fact formed there and is not transported by the blood stream to the liver from an extra-hepatic site.

6. The relevance of these results, obtained in the rat, to human acute porphyria, is discussed.

7. Renal clearance has been studied in three cases of acute porphyria and in one case of latent porphyria.

8. The results suggest that endogenous porphobilinogen is excreted in man by glomerular filtration without appreciable tubular reabsorption. The significance of this finding is discussed.

8

PATHOGENESIS AND ORIGIN OF CLINICAL MANIFESTATIONS

8. PATHOGENESIS AND ORIGIN OF CLINICAL MANIFESTATIONS

Since the early descriptions of acute porphyria, there has been speculation on its pathogenesis and the mechanism of the causation of symptoms. Harley (1890) considered that there was an auto-intoxication due to faulty oxidation of a urinary pigment. In his discussion on sulphonal-induced porphyria, Stokvis (1895) postulated that the drug caused haemorrhages into the mucous membranes of the alimentary canal and that the blood pigment, so released, was converted to porphyrins. On the other hand, Oswald (1895) suggested that sulphonal affected the adrenals, which at that time were thought to be concerned with the degradation of haemoglobin. This effect, he believed, led to such an accumulation of the decomposition products of haemoglobin, which he considered to be porphyrins, as to seriously affect the nervous system and cause death. Later, Barker and Estes (1912), on the basis of a post-mortem examination, postulated that gastro-intestinal dilatation was the primary condition which caused a toxaemia. Snapper (1922) performed 2 autopsies on cases of acute porphyria and found tuberculosis of the retroperitoneal lymph glands in one and an infected hydronephrosis in the other. He then suggested that the colic and paralysis were caused primarily by a chronic poisoning of the retroperitoneal nerve plexuses. Garrod (1923) considered that patients with congenital porphyria lack an enzyme which is responsible for one of the stages in the conversion of blood pigment into bile pigment. Several authors claimed that porphyrins may influence isolated strips of animal intestine or

uterus (Gunther 1922, Supniewski 1927, Reitlinger and Klee, 1928, Simici, 1938, and Vannotti 1954). These pharmacological studies seemed convincing proof (Carrie, 1936) that the substances, causative of symptoms in the human disease, were the porphyrins. Berg (1945) suggested that the porphyrins produced a block in neuromuscular transmission. Waldenström however (1937, 1939) cast doubt on the view that symptoms were caused by porphyrins but stressed the importance of vasospasm. He also suggested that certain pyridine derivatives, possibly formed from the excessive pyrroles, affected the nervous system. Lowry et al. (1950) suspected that porphobilinogen or some closely related substance was the cause of the clinical manifestations, while Denny-Brown and Sciarra (1945) considered that the pathological changes in the nervous system might be caused by an intermittent ischaemia, probably due to a circulating vasoconstrictor substance.

The work reported in Section 4 suggested that purified porphyrins and porphobilinogen are pharmacologically inactive and also rendered unlikely the suggestion that a circulating vasoconstrictor substance exists in this disease. This interpretation was strengthened by the production of an experimental porphyria in animals by the non-hypnotic substance allyl-isopropylacetamide (Section 6). In this condition the animals excreted large amounts of porphobilinogen, but yet showed no clinical similarity to human acute porphyria, apart from constipation and loss of weight. Furthermore, porphobilinogen, not porphyrins, is the main excretion product in acute porphyria. Freshly voided urine contains little formed porphyrins. This may explain the absence of gross photo-

sensitivity of skin in acute porphyria, since photosensitivity is found in other types of porphyria in which formed porphyrins are excreted. The darkening of the skin noted in several cases of the present series may be caused by the simultaneous excretion of δ-aminolaevulic acid (Granick and Schrieck, 1955), which has some slight photosensitising action (Scott, 1955; Jarrett et al. 1956).

The views on the origin of the clinical manifestations have therefore been contradictory, but there has been an increasing agreement that the liver is the seat of the abnormal porphyrin metabolism. Thus Garrod (1900), Neubauer (1900) and Mason, Courville and Ziskind (1933) supported this view and in 1945, Prunty found porphobilinogen in the liver of a case of acute porphyria. This was confirmed by Gray (1950) and Schmid et al. (1954). The latter classified acute porphyria as being of the hepatic type (Section 1). The work of the author suggested that the porphobilinogen found in the liver is made there and is not transported from an extrahepatic site (Section 7).

In 1956, Gibson and Goldberg confirmed the importance of primary demyelination in this disease. The general distribution of this pathological change throughout the nervous system (Table 4 Appendix) suggests that the clinical features may be explained entirely on a neurogenic basis.

The neurological clinical manifestations are clearly related to the neuropathological findings. Thus the demyelination of peripheral nerves, from post-mortem or biopsy tissues, explains the limb paralysis. The lesions in the phrenic nerve in Case 32 are related to the respiratory paralysis which occurred in that

patient. In Case 24 ataxia and nystagmus occurred and foci of demyelination were found in the white matter of the cerebellum.

Some mental changes were observed in Cases 2, 24, 32 and 43 and in each of these perivascular foci of demyelination were found in the cerebral white matter. It is also possible that an underlying mental instability was brought out by an attack (Günther, 1922). Spillane (1947) noted that when cerebral demyelination occurs in subacute combined degeneration, it is usually found in patients who have had psychotic manifestations.

Gastro-intestinal symptoms consist primarily of pain, constipation, vomiting and diarrhoea. Intestinal spasms alternating with atonia and dilatation, varying in location, but predominantly in the upper tract, have occasionally been observed at laparotomy of these patients. They have also been demonstrated both by intragastric balloon (Berg, 1945) and by radiography (Berlin and Cotton, 1950; Calvy and Dundon, 1952). Such effects may have been caused by patchy demyelination of the preganglionic motor fibres that innervate the viscera. These fibres have their cell stations in the spinal cord and medulla. Retrograde degenerative changes were found in these nuclei. Demyelination of the Vagus nerves (Cases 2 and 32) and of fibres of the sympathetic chain (Case 32) were also noted. The demyelinating lesions in these nerves were disseminated patchily and this may have resulted in an irregular motor innervation of the bowel, which could cause the symptoms described above.

Berlin and Cotton (1950) have put forward a similar neurogenic hypothesis for the gastro-intestinal features of this disease.

They point out the similarity of the effects of porphyria and vagotomy upon gastro-intestinal motility and consider that the greater involvement of para-sympathetic innervation in porphyria may be responsible for dilatation and atony as the predominant functional disturbance. Watson (1954) reported marked relief of abdominal pain in 1 case after bilateral splanchnicectomy.

Non-abdominal pain in the limbs and elsewhere may be due to sensory-fibre damage in the peripheral nerves. The changes in the posterior spinal root ganglia in the cases of this series were generally slight and inconstant, but such changes have been described by Bostroem (1920), Mason, Courville and Ziskind (1933) and by Denny-Brown and Sciarra (1945).

Hypertension and Tachycardia The sino-aortic regulation of blood pressure and pulse rate is an important buffer mechanism. Pulsatile expansion of the carotid sinus and aorta stimulates the passage of impulses along the afferent pathways of the medullated carotid sinus and aortic nerves which join the glossopharyngeal and vagus nerves respectively. They end in the reticular substance and the nucleus of the tractus solitarius. From the adjacent dorsal nucleus of the vagus nerve, inhibitory impulses are distributed to neighbouring vasomotor centres and also to the hypothalamic circulatory centres (Kezdi 1954).

It is clear that interruption at any point in this pathway may block the buffer mechanism and give rise to hypertension and tachycardia. Changes in the vagus nerves, nucleus solitarius, the dorsal vagal nucleus and reticular substance have been observed in Cases 2, 32 and 43 (Table 4 Appendix). Kezdi (1954) has

investigated this buffer mechanism in a case of acute porphyria by observing the effect of stimulation (digital pressure) or block (procaine) of the carotid sinus region on blood pressure and pulse rate. He noted an absence of response in active phases of the disease and postulated a disruption of the mechanism and a neurogenic cause for hypertension and tachycardia.

Thus it is possible to explain all the main clinical features on a neurological basis. In view of the pharmacological, pathological and experimental evidence, such an explanation would seem more rational than the others which have been reviewed. It is suggested therefore that in acute porphyria there is a fundamental disturbance of pyrrole pigment metabolism which has its site in the liver. Associated with this there may be a related disturbance in the metabolism of the nervous system, of which demyelination is the pathological expression. The exact nature of this association is not clear. The recent elucidation of the porphyrin-like structure of Vitamin B_{12} (Bonnet et al. 1955) may have some relevance, particularly when it is realized that demyelination of the nervous system may take place in pernicious anaemia (Spillane, 1947). This hypothesis is illustrated in Fig. 1. A substance X has been postulated, of which porphobilinogen is a precursor and which is essential for the nutrition of the myelin of the nervous system. A metabolic "block" to the formation of this substance in the liver would lead to an excessive production of porphobilinogen and δ-aminolaevulic acid and also to demyelination of the nervous system. It is possible that a genetically-determined deficiency in a specific enzyme,

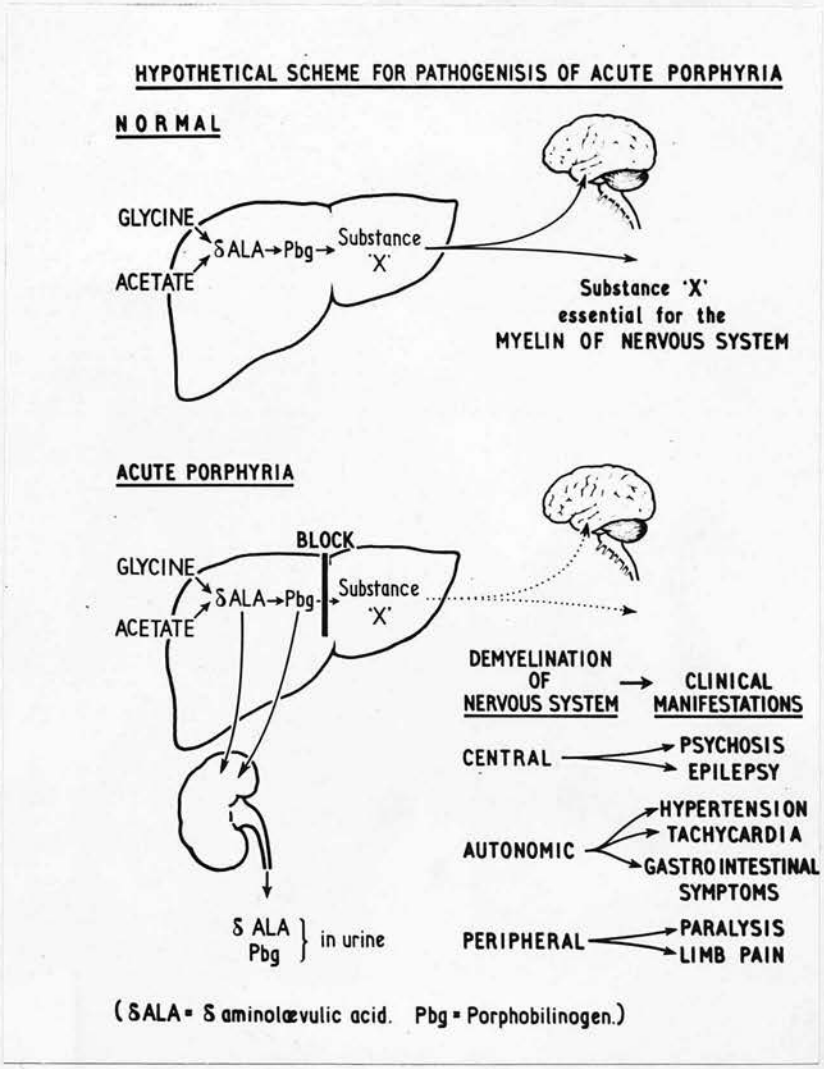


Fig.1. Section 8

A substance X has been postulated of which porphobilinogen is a precursor and which is essential for the nutrition of myelin. A metabolic block to the formation of this substance in the liver would lead to an excessive production of porphobilinogen and also to demyelination of the nervous system.

variable in the expression of its severity, might explain the "block". The intermittent nature of the disease might be related to the effect of other factors, e.g. endocrine factors, infection, barbiturates etc., on this abnormally sensitive pathway. In experimental porphyria in animals neither paralysis nor demyelination of nervous tissue was found, although much porphobilinogen accumulated in the liver. This suggests that the fundamental biochemical lesions in human acute porphyria and experimental porphyria are not identical.

The role of the barbiturates in this hypothesis requires particular mention. They appear to be associated with the onset or the aggravation of the neurological phase of the disease and they may precipitate an attack. Barbiturates have their hypnotic action by inhibiting the oxidative mechanisms of brain tissue and some of them, at least, influence pyrrole pigment metabolism in animals. Barbiturates may obtain their adverse effect in acute porphyria not only by inhibition of the formation of substance X but also by a direct depression of the oxidation of brain tissue.

The varied clinical features of acute porphyria, as well as its pathological chemistry, could be explained on the basis of this general hypothesis.

APPENDIX

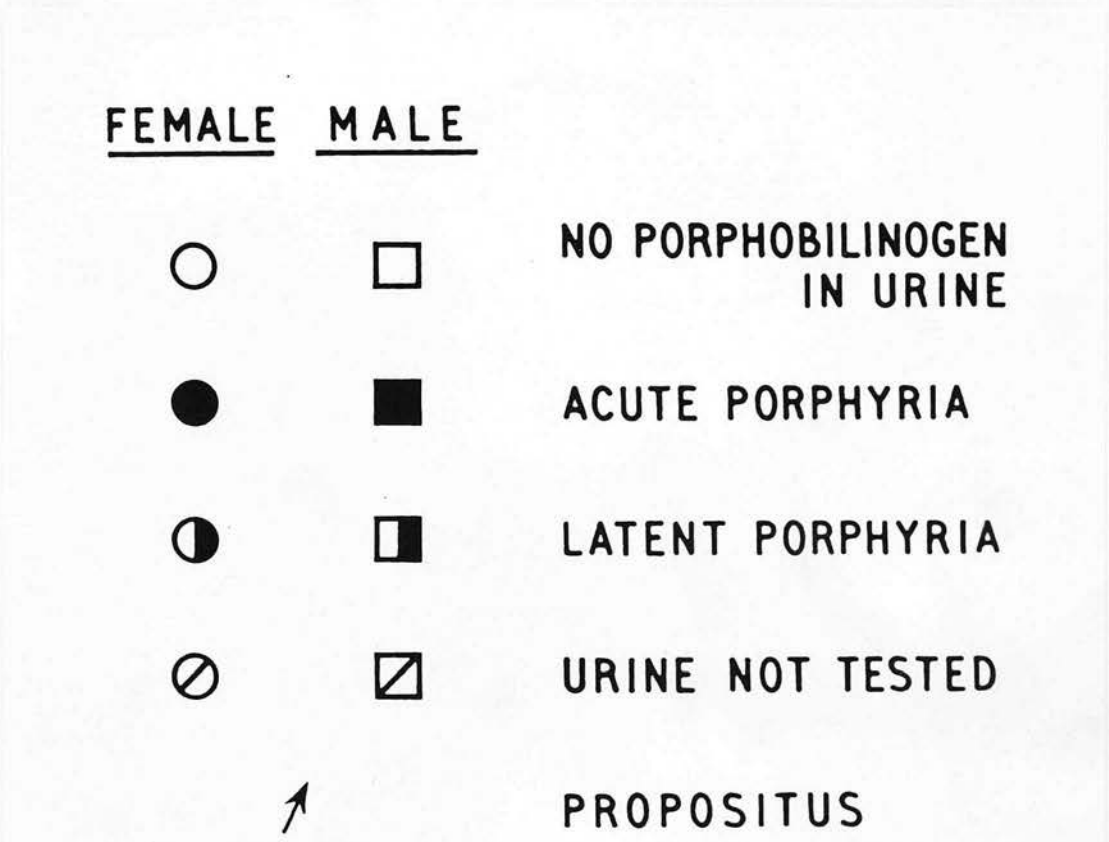
APPENDIX

Abbreviations and Notes:

P.R.	Pulse Rate.
B.P.	Blood Pressure.
Hb.	Haemoglobin concentration.
R.B.C.	Red blood cell count.
W.B.C.	White blood cell count.
E.S.R.	Erythrocyte sedimentation rate.
C.S.F.	Cerebrospinal fluid.

The year (in brackets) and age given at the beginning of each case history, refer to the year in which symptoms commenced and the age of the patient in that year.

The methods for the determination of porphyrins and porphobilinogen in urine and tissues have been described in Section 5. It should be noted that the characteristic excretion product in acute porphyria urine is porphobilinogen. The urinary uroporphyrin concentrations, recorded in some of these cases, were estimated after heating the urine for 20 minutes with a pH 4.2 acetate buffer, which converts much of the porphobilinogen to uroporphyrin.



Symbols used for family trees

Mrs. E.H. Age 24 years (1953) Filler in fireworks factory

Case No.1

Five days after the birth of her first child, in December 1952, this woman had an attack of severe colicky abdominal pain lasting a few hours. The child died one month later from bronchopneumonia. In May 1953 she had an attack of severe abdominal pain, diarrhoea and vomiting, with aching pains in her limbs and a feeling of weakness. This lasted for 3 days. She was well until 27/10/53 when she developed a chill and with this she had colicky abdominal pain, diarrhoea and vomiting; she was sleepless and nervous. She was admitted to Livingstone Hospital on 5/11/53 when phenobarbitone gr. $\frac{1}{2}$ t.d.s. and sodium amytal gr. 3 was given daily for the next 5 days. During this period she developed weakness of arms and legs and numbness of forearms and hands. On 10/11/53 she was transferred to Joyce Green Hospital, Dartford, Kent, where red urine was noted for the first time. On examination she was afebrile. P.R. 110-120/minute. B.P. $\frac{150}{90}$. There was marked loss of power in all muscle groups, especially proximals, hypo-algesia and hypo-aesthesia from fingers to middle of upper arms and from toes to middle of thighs, bilateral facial nerve weakness, difficulty in swallowing and coughing. W.B.C. 24,000/cmm. Hb. 13 G%. C.S.F. normal. Serum electrolytes showed low serum sodium and chlorides and slightly raised blood urea (Table 1). Liver function tests (Table 2). Urine porphobilinogen +++ She became incontinent of urine and faeces, was semi-conscious and had Cheyne-Stokes respiration. Died 11/11/53. P.M. (Dr. N.P. Orchard). Early

bronchopneumonia of lower lobes. Recti abdominis showed area of degeneration and foci of round cells. Marked degeneration of Purkinje cells of cerebellum. The anterior horn cells at all levels of the spinal cord showed degenerative changes. Chemical pathology of tissues (Table 3).

Summary 3 attacks of increasing severity following birth of first child. In the final fatal attack she was given barbiturates and developed neurological manifestations. Note diarrhoea, not constipation, as accompaniment of abdominal pain.

Mrs. L.B. Age 52 years (1953) Housewife Case No. 2

This patient was admitted to Mile End Hospital, London, on 4/6/53 because of morning vomiting and general nervousness of 6 weeks' duration. Examination on admission showed only hypertension (B.P. $\frac{250}{140}$) and obesity. She was given phenobarbitone gr. $\frac{1}{2}$ b.d. on a few occasions. 12 days after admission (16/6/53) she began to complain of severe epigastric pain and vomiting. There was some tenderness in the epigastrium but no rigidity. The same day an intravenous pyelogram was carried out ("pyeloselectan" 20 ml.) and after this she felt much worse. Seconal gr. 6 was given in a B.P. sedation test and the same dose of this barbiturate was repeated next day. At this time she was noisy and was behaving strangely. She continued to have pains in the abdomen and also in the back and upper thighs. She became very constipated and was incontinent of urine at times.

Plate I. (Appendix).

Gall stone of Case 2.
This fluoresced markedly and contained coproporphyrin III and a trace of uroporphyrin.

On 27/6/53 she passed dark red urine, containing porphobilinogen and uroporphyrin, determined later as the series III isomer type. Examination on that day showed absence of ankle and knee tendon reflexes and generalized loss of pin-prick sensation. She had a flaccid quadriparesis and generalized numbness. Her B.P. fell to $\frac{150}{100}$ and her P.R. rose from about 80/minute to 100/minute. She remained afebrile. W.B.C. 7,100/cmm. E.S.R. 8mm/hour. Hb. 13 G%. Blood urea rose from 38 mg.% on admission to 92 mg.%. Urine contained no protein. She became dyspnoeic with bubbling respirations and died on 30/6/53.

P.M. showed left-sided heart failure. Pulmonary haemorrhagic oedema. The gall-bladder contained a large pigment stone, the shape of a walnut (see Plate 1) 3.2 x 2.5 cm., 12.2 g. in weight. Chemical analysis of the stone showed the following: Bilirubin 15% net weight; calcium 0.1% net weight; porphyrin 0.05% net weight. The porphyrin content was almost entirely copro-porphyrin series III, although a trace of uroporphyrin was discovered by means of paper chromatography. The chemical pathology of post-mortem tissues is described in Table 3.

Summary Solitary fatal attack occurring in a woman of 52 with marked hypertension, possibly essential hypertension. Two seconal sedation tests may have precipitated the onset of neurological symptoms.

Miss M.M. Age 25 years (1952) Bakery Assistant Case No. 3

This patient was first admitted to Stobhill General Hospital, Glasgow, on 1/12/52 with a history of severe colicky abdominal pain and vomiting of 4 days' duration. The pain started in the epigastrium and moved to the right iliac fossa. She described it "as if someone was tearing my inside out". Examination showed tenderness in the right iliac fossa. The pain continued intermittently for 5 weeks and she discharged herself from hospital on 9/1/53 although she felt then that her (R) arm was becoming weaker. During this admission she received sodium amytal on at least one occasion. She was re-admitted on 15/1/53 with abdominal pain and severe pain in her knees, hips and shoulders. There was marked weakness of her arms and legs and slurring of speech. Porphobilinogen and uroporphyrin (15 mg./24 hours at maximum) were then found in the urine. She was given a course of A.C.T.H. from 26/1/53 to 12/2/53 (total of 340 mg. A.C.T.H.). She improved during this period but it was difficult to assess the influence of A.C.T.H. since daily uroporphyrin determinations had shown a downward trend, even before A.C.T.H. was commenced. In the next few months there was a marked improvement in the power of her limbs and by 18/5/53 they had almost recovered, apart from some persistence of wrist drop. During this time she had occasional pain in the scapular region and in the abdomen. On 30/7/53 she was discharged to a convalescent home where she continued to get better.

She was repeatedly re-admitted to hospital for 2-3 weeks each time from September to December 1953 because of abdominal

pain and vomiting. Finally on 12/7/54 she was re-admitted with abdominal pain, vomiting and slight weakness of her legs. She was found to be 7½ months pregnant. She was given pethidine in large doses for her pain and gave birth precipitately to a live male infant on 15/7/54. Several hours later she had a generalized epileptic convulsion lasting 4 minutes. In the next 5 days she had 1-4 fits each day. She developed a hypochloraemic alkalosis (Table 1) and her blood urea rose to 268 mg.% before death despite replacement therapy. She developed hypertension (B.P. $\frac{175}{135}$) for the first time since her illness. P.R. rose to 100/ minute. Urine contained protein. Mentally she was apathetic and withdrawn. She remained afebrile but died 27/7/54.

P.M. showed basal consolidation of both lungs. Serum electrolytes during final admission (see Table 1). Liver function tests (see Table 2). For chemical pathology of tissues see Table 3.

Family History Her sister has porphobilinogen in her urine. The infant passed porphobilinogen in his urine for 2 days after birth.

Summary A series of attacks lasting 20 months, the final fatal outcome occurring 10 days after the birth of a 7½ months foetus. Epilepsy, hypertension followed the birth of the child.

Mr. R.G. Age 22 years (1944) Labourer Case No. 4

He was first admitted to hospital, while in the R.A.F. in 1944, because of severe colicky abdominal pain and constipation. While in hospital he developed pains in the legs and paraesthesiae of feet and also became hallucinated, e.g. he said that there were birds in the bed and thousands of them in the ward. His legs, and later his arms, became weak but there was never any sensory loss. He was discharged from the R.A.F. and between 1945 and 1948 he had many attacks requiring 11 admissions to the Ayr County Hospital and 6 admissions to the Western Infirmary, Glasgow. In these hospitals the same kind of attack as above was recorded. In most attacks vomiting occurred. In the severest phase of abdominal colic his abdomen was noted to be rigid. Marked tenderness of thigh and calf muscles was found on several occasions and the knee tendon reflexes were transiently diminished. Mental confusion and hallucinations were repeatedly present. Skin infections were common and in one episode (September, 1948) a (R) lower lobe pneumonia occurred. The attacks usually lasted a few days to one month. There was always marked loss of weight during his attacks. His last major one was in September 1950. He was seen in 1952 when he stated that he still got mild attacks of abdominal discomfort lasting a few hours, every 3-4 months. Afebrile during all attacks except when he had pneumonia. B.P. in severe attacks rose to $\frac{170}{130}$ and pulse rate to 130-150/minute. E.C.G. normal. Hb. 15.5 G%. R.B.C. 5.46 million/cmm. Reticulocyte count 2%. W.B.C. 5,400/cmm. Liver function tests (see Table 2).

Treated with I.V. calcium gluconate, saline or glucose saline. Eucortone or DOCA and Vitamin B therapy had no apparent benefit.

Summary A series of severe attacks with abdominal and neuro-psychiatric manifestations over a period of 6 years.

Mrs. G.W. Age 24 years (1955) Housewife Case No. 5

This young woman was admitted to Grantham and Kesteven General Hospital on 29/3/55 because of weakness, constipation, vomiting and lower abdominal pain of 10 days' duration. The pain was usually a dull ache in the (R) iliac fossa, sometimes radiating to the (L) iliac fossa. She had lost some weight in the past few months and had been admitted to this hospital 4 years previously with pain in the (R) iliac fossa. B.P. $\frac{148}{100}$. P.R. 99-100/minute. Temperature 99°F. She was discharged after 1 week.

She was re-admitted 6 weeks later, on 25/5/55, because of the recurrence of symptoms in the previous 3 weeks. There was tenderness on deep pressure low in the (R) iliac fossa. W.B.C. 19,000/cmm. She was given sodium amytal on 2 occasions and later complained of numbness of her legs, abdomen and shoulders. There was diminished pain sensation on the skin of the abdomen and lower limbs. Started on cortisone 25 mg./6 hours with marked improvement. Discharged 28/7/55. Re-admitted 10/9/55 with recurrence of pain 5 days previously, as well as marked frontal headaches, associated with photophobia.

Given soneryl on night of admission and for 3 nights thereafter and started on cortisone, 25 mg. b.d. next day. 3 days after admission reddish urine was noted and a diagnosis of acute porphyria was made. She was given atropine, belladonna and pethidine. She was also given neostigmine 2 days before death.

Her doctor felt that the atropine relieved her pain. She developed attacks of tachypnoea (40/minute) on 9/9/55. W.B.C. 45,300/cmm. There was some moist râles in her chest. 2 days later marked muscle weakness and wasting was noted, particularly in the extensors of the wrist. The tendon reflexes of arms and legs were absent. There was a deterioration in her general condition on 22/9/55. She was breathing rapidly and was slightly cyanosed and later that day she died.

The patient was "emotional" during her last admission and became disorientated prior to death.

She was given cortisone 25 mg. q.i.d. 13-15/9/55. Her husband thought her symptoms became worse at the time of menstruation. She had delivered a normal child 2 years prior to her final illness. Urine:protein negative, micro. N.A.D. E.S.R. 8 mm/hour. Hb. 105%. Urine porphobilinogen +++ .

Family History None of note.

Summary 3 increasingly severe attacks within 6 months occurring in a young woman of 24 years. In the first attack gastro-intestinal symptoms only occurred; in the second paraesthesiae, and in the third and fatal attack paralysis occurred. The second and third attacks were associated with barbiturate administration. Normal pregnancy and delivery 2 years previously.

Mrs. J.S. Age 23 years (1954) Canteen Assistant Case No. 6

This woman was admitted 22/9/54 to the General Infirmary, Salisbury, because of abdominal pain and vomiting. She had had amenorrhoea for 3 months prior to this and she had been given sulphonamides and butobarbitone as treatment for 1 week before admission. In hospital she was nervous and in pain. B.P. $\frac{135}{90}$ P.R. 76/min. Temperature normal. Hb. 14 G%. W.B.C. 6,900/cmm. E.S.R. 26 mm./hour. (Westergren). Urine porphobilinogen 120 mg./litre. Urine: protein negative. The pain gradually settled and it was noted that she had a mass in her (R) fornix. A diagnosis of tuberculous endometritis was made on histological evidence and she was later treated with streptomycin and P.A.S. (para-amino-salicylic acid). She did not develop neurological manifestations and she had no further symptoms for 16 months after the attack, although she continued to excrete 26 mg. porphobilinogen/litre urine during remission.

Previous History Appendicectomy at 16 years. At 21 years while in the Middle East she developed malaria and reported to her doctor that she could not feel her (L) leg. He could find no clinical abnormality and the limb soon returned to normal.

Family History Not known.

Summary A solitary attack associated with tuberculous endometritis in a young woman of 23 years.

Mr. W.J.S. 38 years (1950) Brewery Traveller Case No. 7

Since 1950 he has had 4 attacks of abdominal pain, vomiting and neuropsychiatric symptoms, each attack following an alcoholic bout.

1950 Nervous, irritable, numb feelings at back of head, hands and legs, anorexic, colicky abdominal pain and vomiting. Passed red urine. On examination epigastric tenderness, sluggish reflexes.

May 1953 He had a similar attack as well as lumbar pain radiating to shoulders, following an excess of gin drinking. On this occasion he complained of great muscle weakness in all his limbs, particularly in the upper limbs. P.R. 100-120/min. Tendon reflexes barely elicited. No sensory loss. C.S.F. normal. Hysterical throughout admission. Given seconal, sodium amytal and soneryl on various occasions throughout stay in hospital. Given aneuryn HCl. and nicotinamide. Discharged July 1953.

April 1954 He had improved since his last admission to hospital but 3 weeks before this admission he had drunk a good deal. A similar attack occurred with the additional symptoms of thick speech, diplopia and diarrhoea as well as constipation. Examination showed absence of tendon reflexes except biceps; nystagmus in all directions. B.P. $\frac{180}{126}$. Grade II retinopathy. C.S.F. normal. Urine gave a strong Ehrlich's aldehyde reaction. Childlike mental behaviour. Memory loss, disorientation. He became very much improved in 2-3 weeks. During this period he was given seconal, membutal and veronal and at the time of his

discharge he continued to have seconal gr. 1½ nocte.

Treatment as before.

November 1955 He remained well until 4 weeks before this 4th admission to hospital. He had again been drinking to excess and had started taking sodium amytal nightly, and noted that his urine had become red. Marked difficulty in walking. Unable to eat unaided. Limbs hypotonic. Tendon jerks absent. Coarse nystagmus. P.R. 100-120/min. Emotionally labile, hysterical behaviour. Porphobilinogen discovered in his urine and diagnosis of acute porphyria established. Treated with Vit. B therapy as before. Barbiturates discontinued. Gradual improvement after about 2 weeks. Muscular power improved. B.P. returned to normal, fundi normal and his mental condition greatly improved.

On an examination in April 1956 he was completely normal apart from residual weakness of both wrists and small muscles of his hands.

Liver function tests on his several admissions showed some abnormalities, e.g. thymol turbidity 3-4 units, cephalin flocculation + to +++ (Table 2). W.B.C. usually about 6,000/cmm. E.S.R. 5 mm./hour.

Family History Nil of note.

Summary Four attacks of abdominal pain, vomiting and neuro-psychiatric symptoms of increasing severity associated with alcoholism and to some extent with barbiturate intake.

Mrs. P.I.A. 30 years (1953) Housewife Case No. 8

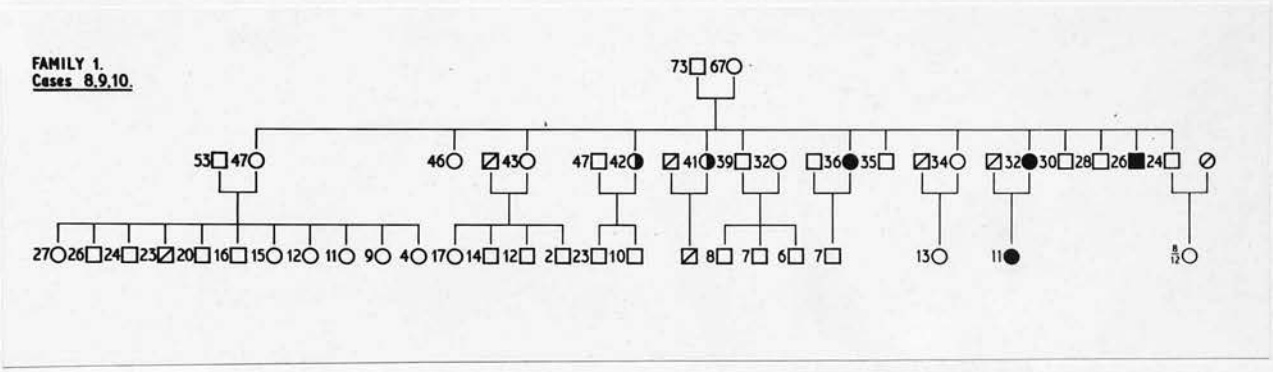
This woman was admitted in December 1954 to Lincoln County Hospital with periumbilical pain, recurrent vomiting and headaches of about 1 week's duration. She was constipated for 4 days before admission. B.P. $\frac{180}{128}$. P.R. 130/min. E.S.R. 11 mm (Westergren). Hb. 15.5 G%. W.B.C. 11,600/cmm. Urine prophobilinogen 113 mg./24 hours. Serum cholesterol 322 mg.%. She was apathetic, drowsy and in pain for 1-2 weeks but rapidly recovered. During the acute attack she had a series of epileptiform attacks without focal onset. B.P. returned to $\frac{140}{90}$. Discharged 7.1.55. Treated with pethidine, atropine and morphia with beneficial results. Since then until May 1956 she has had no symptoms.

Family History See Family 1 and cases 9 and 10.

Summary Solitary attack with abdominal symptoms, hypertension and epilepsy.

Mr. D.C. 26 years (1955) Driver Case No. 9

Admitted to Lincoln County Hospital 4.1.55. because of severe abdominal pain, nausea, flatulance, vomiting and constipation of 3 days' duration. The pain was dull and aching, mainly periumbilical, but going through to both loins and back. No loss of weight or paraesthesiae. Slight tenderness over right rectus abdominis muscle and in both loins. B.P. $\frac{145}{105}$ (normal in remission $\frac{120}{80}$). P.R. 70/minute. Urine contained trace of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protein. Haematological examination, normal except W.B.C. 12,600/cmm. Liver function tests, normal (Table 2). Urine contained much porphobilinogen. No neurological signs. Skin was unusually pigmented. Symptoms gradually settled in 8 days and he was discharged from hospital on 20.1.55. Treated with pethidine and atropine gr. $\frac{1}{100}$, both of which helped him. He was well until 26.7.55. when he was admitted to hospital for a tendon and digital nerve repair operation following an arm injury. As an anaesthetic he was given I.V. thiopentone, gas and oxygen. Two days later he had severe periumbilical pain, vomiting, general malaise and constipation. There was some tingling in the (L) calf, and occasional difficulty in starting micturition but no neurological signs. This cleared up in a few days and he was discharged 2.8.55. He has been well since then.

Family History See Family 1 and Cases 8 and 10.

Summary One attack with abdominal pain and hypertension. A second attack followed an operation, for which I.V. thiopentone was given.

Miss R.A. Age 11 years (1955) Schoolgirl Case No. 10

She was seen in November, 1955, because of a family history of acute porphyria. She had been complaining at intervals of slight pains in the loins, for about 1 year. In October 1955 she had some subcostal pain - slight and quite transient. She had never had any really acute abdominal pain or

vomiting, headache or constipation. Menstruation had not commenced. Urine porphobilinogen 29 mg./litre. Urine 3½ years previously contained no porphobilinogen.

Family History See Family 1 and cases 8 and 9.

Summary Transient loin and subcostal pain and porphobilinogenuria in a child of 11 years.

Mr. W.F.A. Age 23 years (1954) Porter Case No. 11

This man was first admitted to the North Middlesex Hospital, London, on 15/1/54 with central abdominal colicky pain, loss of appetite, nausea and vomiting of 24 hours' duration. There was some tenderness in the lower abdomen particularly on the right but no guarding or rigidity and some rectal tenderness on pressure to the right anteriorly. B.P. $\frac{140}{90}$. A laparotomy was performed and a normal appendix was removed. He continued to have pain and also complained of weakness of his abdominal muscles and lower limbs and became nervous and irritable. He was treated with ultra-violet light and improved. In June 1954 he developed nausea, anorexia, vomiting and complained of pains in his limbs. He was anxious and miserable. He was given phenobarbitone gr. ½ on 5 occasions while at home. On 15/6/54 he was admitted to Claybury Hospital with general weakness and pains in his limb muscles. Temperature 100°F. P.R. 95-120/minute. B.P. $\frac{180}{115}$. There was a peculiar pigmentation of the abdomen and flexures of his arms. He was given sodium amytal

gr. 3 on 12 occasions. For a short time he had sugar in the urine and a mild diabetic glucose tolerance curve. He became incontinent of urine and unruly, developed nystagmus, analgesia of the (R) arm and leg, and diminished (R) knee and ankle tendon reflexes. There was fine tremor of all his muscles and his speech became slurred. His behaviour became childlike. Urine (8/7/54) contained protein and granular casts. Blood urea 144 mg.%.

He was re-admitted to the North Middlesex Hospital on 15/7/54. In view of the marked muscle weakness, including bilateral wrist-drop, and myasthenic appearance, he was given a course of prostigmine but this had no obvious effect. He made slow but steady improvement and became more co-operative. By 3/8/54 he could walk unsupported and he insisted on taking his discharge. His urine contained large quantities of porphobilinogen. Hb. 13 G%. R.B.C. 5 million/cmm. W.B.C. 10,000/cmm.

Summary 2 attacks in a man of 23. In the first, with gastro-intestinal symptoms, an appendicectomy was performed. At the onset of the second he was given barbiturates and then developed severe neuropsychiatric manifestations. Myasthenic appearance but no response to prostigmine.

Mrs. S.C. Age 28 years (1951) Housewife Case No. 12

Between August, 1951, and March, 1953, this woman has had about 11 attacks of abdominal pain, vomiting, constipation, pains in the neck and thighs, often with loss of power of hands and arms and sometimes of legs and voice. These attacks came on several days before her menstrual period and would only alleviate 1-2 weeks after her period. Her longest remission was between May, 1952, and January, 1953, during which time she was on methyl testosterone. Her normal B.P. is $\frac{125}{85}$ but with onset of abdominal pain there was usually a rise, e.g. $\frac{160}{120}$. Afebrile. Mentally, she showed no abnormality apart from slight depression during phases of paralysis. There was no history of taking barbiturates. Since March 1953 she has made considerable progress. There has been no further attack since then. The urine contained much porphobilinogen and uroporphyrin, during these attacks. The urinary uroporphyrin on 16/3/53 was 7.8 mg./day.

Further records - Hb. 13.1 g.%. W.B.C. 6,400/cmm. E.S.R. 16mm/hour (Westergren). Given courses of cortisone and A.C.T.H. without obvious benefit.

Family History Her sister had a similar attack 4 years previously in the early months of her pregnancy. The urine was not tested.

Summary Repeated attacks for 2 years with gastro-intestinal and neurological symptoms. No history of barbiturate intoxication. Attacks associated with onset of menses. Improvement with methyl testosterone administration. Cortisone therapy without benefit.

Miss J.M. Age 32 years (1953) Storekeeper Case No. 13

This woman was admitted to the Southern General Hospital, Glasgow, on 11/3/53. 3 weeks before admission she developed pain and numbness with swelling on the (L) side of her face. 3 days before her admission she suffered severe pain in the lower abdomen, vomited and was constipated. During the first 3 weeks of her stay in hospital she became weaker and wasted. She first complained of weakness of the (R) arm then also of the (L) arm and legs. Examination showed muscular wasting, around the shoulder joints, hips and thighs. There was absence of pin-prick sensation over the trunk and proximal portion of the limbs. Afebrile. B.P. $\frac{115-125}{70-80}$ throughout attack. P.R. 120/minute. There was slight tenderness in the abdomen, but no guarding. There was no mental abnormality. A course of cortisone was given to her from 3-21/4/53. During this time she improved greatly and she was discharged on 7/5/53 in a fit state. Information from this patient on 4/8/54 states that she has kept very well, and has no abdominal pain or muscle weakness.

Further records - the urine contained much porphobilinogen and porphyrins. Hb. 13 G%. R.B.C. 4.8 million/cmm. W.B.C. 6,800/cmm. There was no history of taking any drugs apart from occasional veganin before admission to hospital.

Summary A neurological form of the disease with no history of barbiturate ingestion. Improvement associated with cortisone therapy.

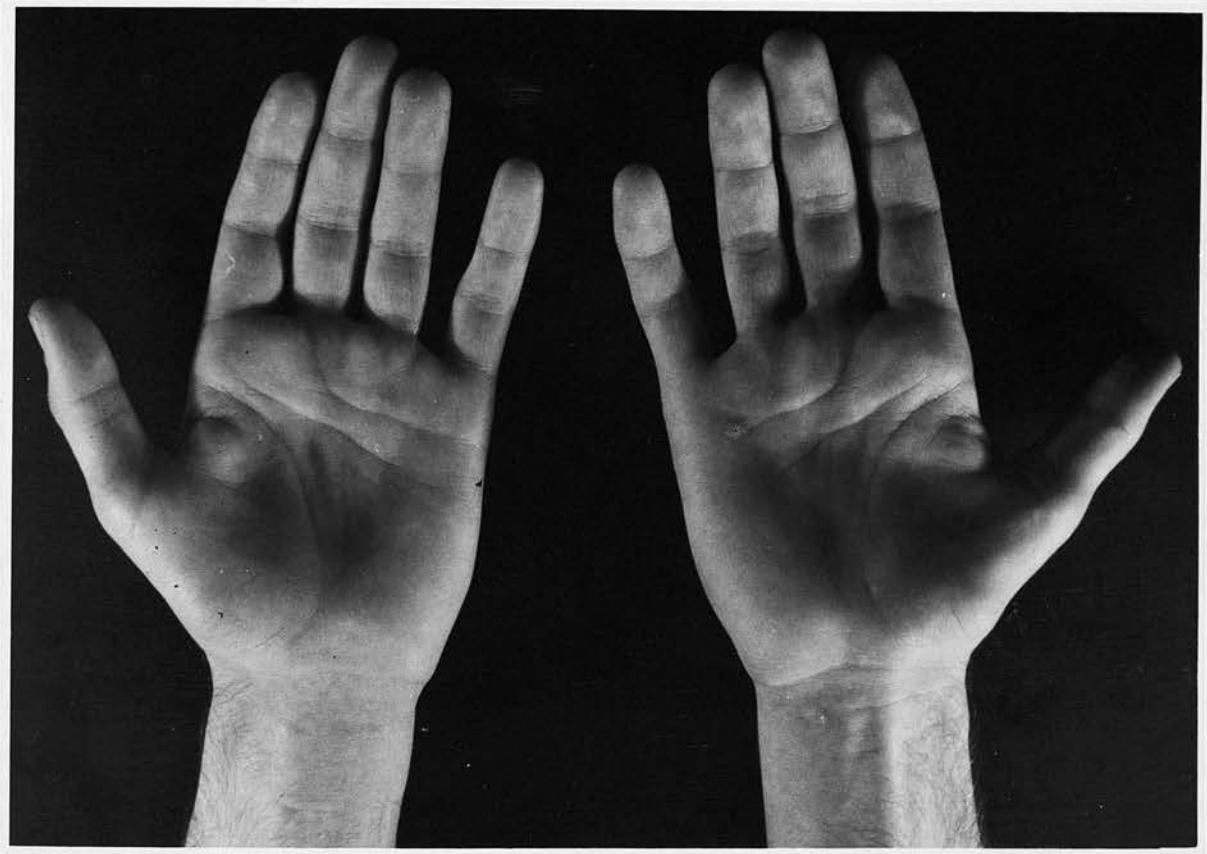


Plate 2(a). Appendix.

Case No. 14. Wasting of hand muscles.
Thenar muscles are obviously affected.

Mr. L.F. Age 24 years (1953) Post Office Technician Case No. 14

This young man was admitted to Wanstead Hospital, London, in April 1953 because of colicky abdominal pain constantly present for 3 weeks. Shortly before the onset of the pain he had a boil on (R) forearm, abscesses of (L) elbow and gum and a poisoned finger. His own doctor had given him 2 tablets of sonalgin each night for about 2 - 3 weeks before his admission to hospital in order to make him sleep. A diagnosis of acute porphyria was made since the urine contained much porphobilinogen. His symptoms subsided and he was discharged. He was re-admitted on 1/6/53 with marked weakness of upper and lower limbs, abdominal pain, vomiting and constipation. His legs and arms became paralysed with absence of all tendon reflexes and pin- and pinprick sensation. This state continued for several months but he gradually improved and was able to walk and use his arms. He was discharged on 21/12/53. When seen on 27/7/54 he still had some degree of wrist drop and foot drop and wasting of thenar, hypothenar and interossei muscles of both hands (Plates 2, a and b). In August 1954 he still had occasional cramps in his calf muscles and still felt that the skin of his legs was very sensitive. On 27/7/54 he excreted 193 mg. porphobilinogen/litre of urine. There was no mental abnormality during his attack, although he admits that he felt like committing suicide when the pain was very severe.

Further records - B.P. $\frac{150}{90}$. P.R. 90/minute. Hb. 16 G%. R.B.C. 5.5 million/cmm. Reticulocyte count 0.5%. Urine - protein negative.

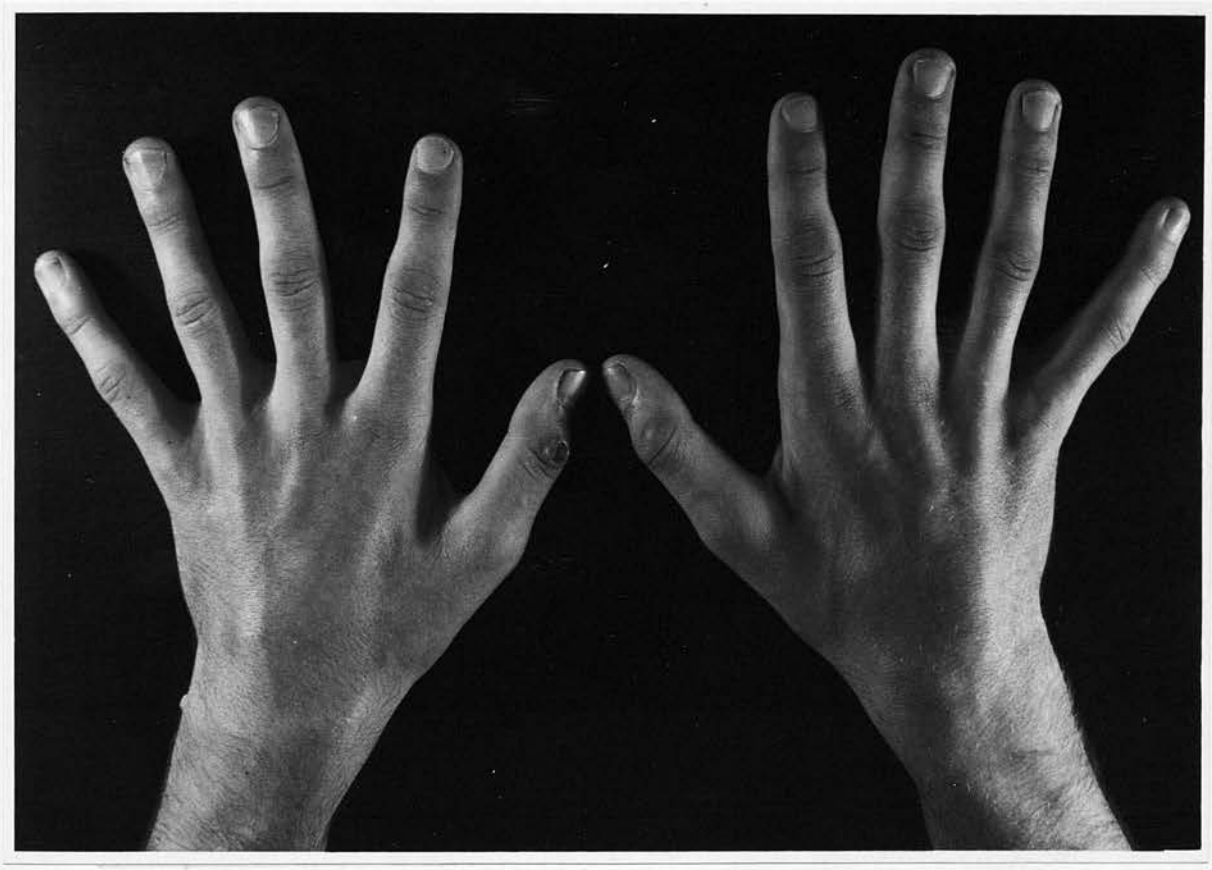


Plate 2(b). Appendix.

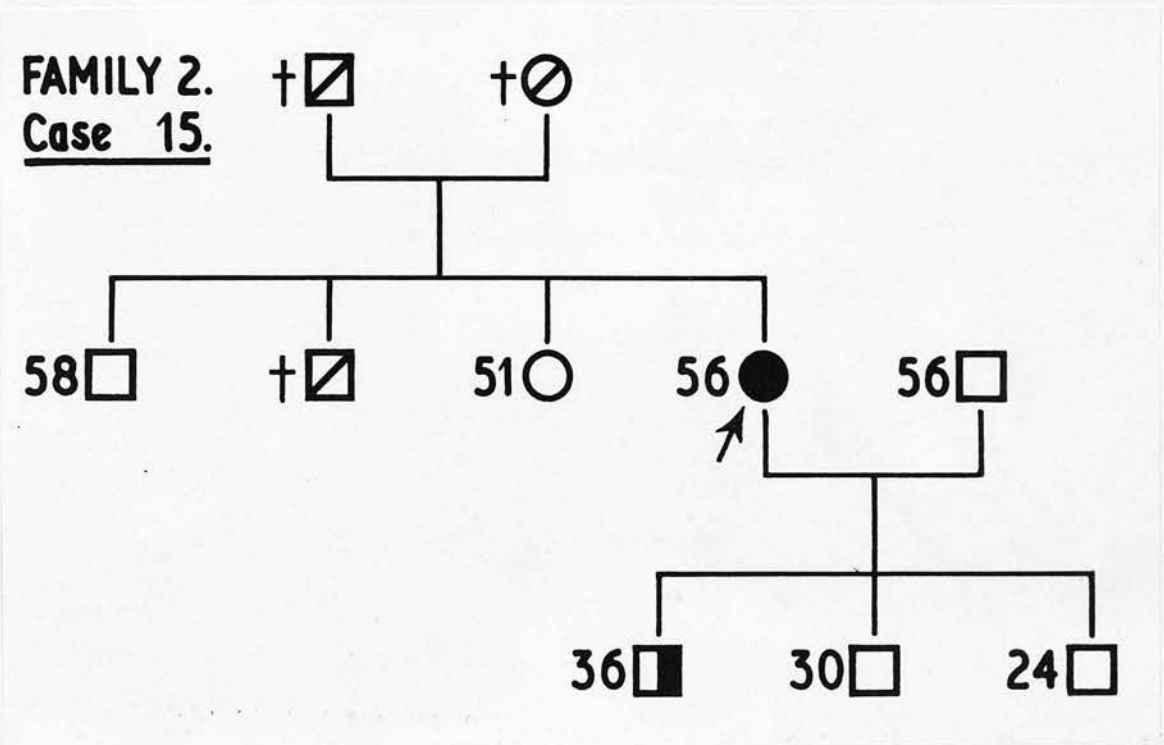
Case No. 14. Wasting of hand muscles.
Interossei notably affected.

Summary Multiple skin infections and barbiturate administration followed by gastro-intestinal symptoms. Severe neurological phase of the disease ensued later. Almost complete recovery of motor power within 1 year.

Mrs. M.B. Age 56 years (1952) Housewife Case No. 15

This woman was admitted to University College Hospital, London, on 6/7/52 with severe pain in the back and legs and tiredness and lassitude for 4 weeks. In the previous 2-3 weeks her appetite had become poor and she was constipated. For 10 days she had had attacks of mid-abdominal pain and vomiting and noted that her urine was dark. She had been taking phenobarbitone gr. ½ b.d. for the past 12-18 months and sodium amytal gr. 2 or 3 nightly for 10 days before admission.

Examination showed that she was co-operative but rather drowsy and slow in response to questions. Temperature 98.4°F. E.S.R. 5 mm/hour. P.R. 100-120/minute. B.P. $\frac{165}{115}$. Apart from diminished pin-prick sensation and weakness in both legs, the examination of the nervous system was normal. The abdomen was flaccid and there was tenderness in the (R) hypochondrium and in both renal angles. The urine contained much porphobilinogen and 4.36 mg. uroporphyrin/day. The pain was relieved by pethidine and in a few days she became brighter and very co-operative. Her legs became stronger and her pulse rate fell to 90/minute. In 1 week's time her urinary uroporphyrin had fallen to 0.77 mg./day. She was discharged on 19/7/52 and warned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Case 15.
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against taking barbiturates.

She reported on 29/7/54, 2 years later, that she was feeling much better and had had no symptoms. Her urine then did not contain porphobilinogen.

Further records - Hb. 14 G%. W.B.C. 8,600/cmm. Blood urea 27 mg.%. Urine contained a trace of protein. Liver function tests (see Table 2). Stool porphyrin - Coproporphyrin 150 μg./g. dry weight. Protoporphyrin 285 μg./g dry weight. Total blood porphyrin 27 μg./100 ml. of blood (normal).

Family History Her eldest son had porphobilinogen in his urine. This was confirmed by paper chromatography in 1954. There was no porphobilinogen in his urine 2 years later. See Family 2.

Summary Onset of an attack after prolonged ingestion of phenobarbitone and sodium amytal in a woman of 56 years. She had no porphobilinogen in her urine 2 and 4 years after her attack. Her son is a latent case.

Mr. S.G.H. Age 59 years (1951) Foreman cleaner Case No. 16

This man was admitted to Whittington Hospital, London, on 10/4/51. For 4 days he had complained of persistent colicky epigastric pain, vomiting and constipation. The day before his admission he had become incoherent and repetitious in his speech and could only see dimly. While he was being admitted to hospital he had a sudden epileptic fit, Jacksonian in type. This began with clonic movements of the (L) arm, then (L) leg

and finally both sides of the body. The clonic movements increased in violence for several minutes and he then settled into unconsciousness. Within 5 minutes he again became restless and violent and was given 0.3 g. pentothal I.V. He had been taking phenobarbitone and codeine every night for 2 years.

Examination showed that he was afebrile. P.R. 70/minute. B.P. $\frac{150}{100}$. W.B.C. 11,000/cmm. Hb. 18 G%. R.B.C. 6 million/cmm. The abdomen was soft and there was no tenderness. There was no abnormality in the nervous system except extensor plantar responses immediately after the fit. C.S.F. normal. Serum electrolytes normal (Table 1). Liver function tests (Table 2). Urine contained much porphobilinogen and 16.4 mg. of uroporphyrin/day.

He had no further epileptic fits and his vision returned to normal within 2 days. Within 1 week his abdominal pain had ceased and normal bowel motions had commenced. Mentally he was rather depressed. He was discharged from hospital on 25/4/51. He still felt very weak for the next 3 months and remained in bed. He reported that he slept badly and had difficulty in walking. After this he steadily improved. He reported on 29/7/54 that he had been free from symptoms, apart from slight head pains, in the past 3 years. He had scrupulously avoided barbiturates. On 4/8/54 his urine contained 130 mg. porphobilinogen/litre and 204 μg. coproporphyrin/litre.

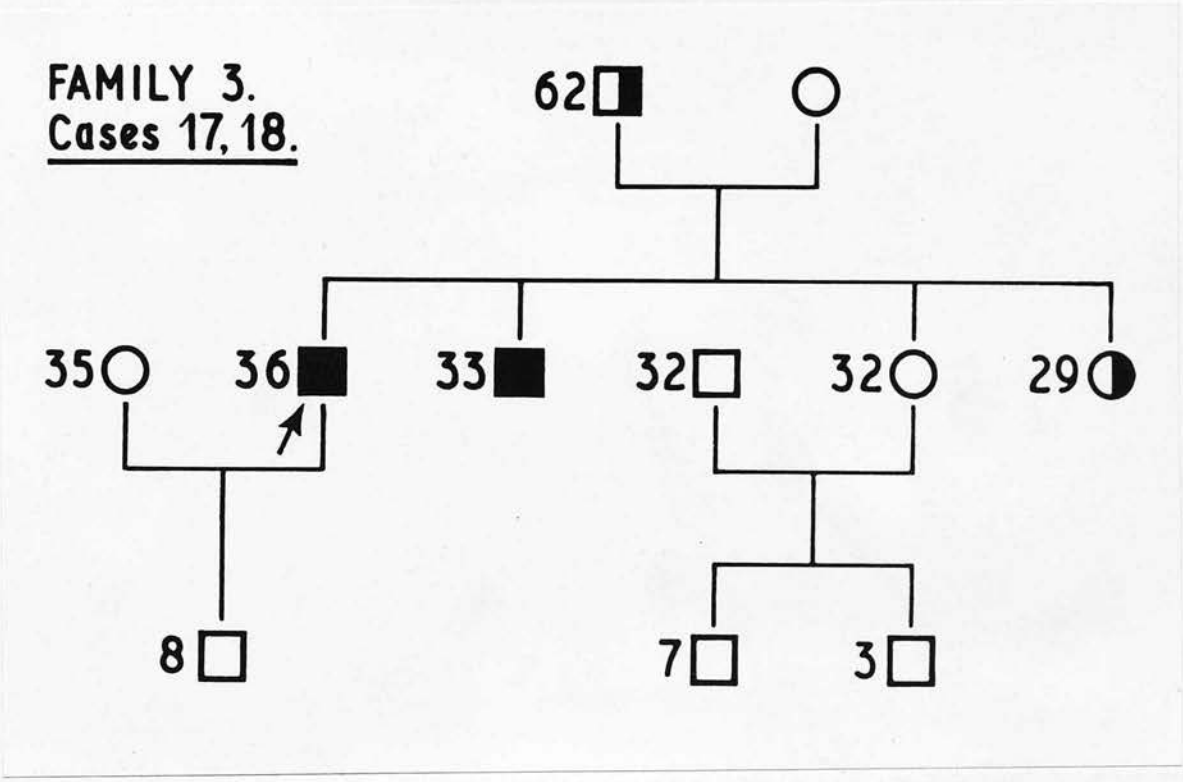
Summary Onset of acute porphyria after 2 years of nightly phenobarbitone ingestion. Presented to hospital as a case of Jacksonian epilepsy. No further attack after 3 years but still

passes porphobilinogen in his urine.

Mr. D.H.W. Age 30 years (1950) Farm labourer Case No. 17

This man was admitted to Bedford General Hospital on 15/12/50. He had developed acute tonsillitis 2 weeks previously and with it he had abdominal pain, constipation and vomiting. Just before he was admitted he had a convulsive seizure. While sitting, his eyes had turned up, his limbs moved jerkily for 10 minutes and there was slight frothing at the mouth. He remained in coma for 1 hour after this. He had been having epigastric pain intermittently for about 1 year.

Examination on admission showed no abnormality in the nervous system. B.P. $\frac{164}{110}$. P.R. 100-110/minute. Afebrile. W.B.C. 21,000. E.S.R. 8mm./hour. He was put on phenobarbitone gr. $\frac{1}{2}$ t.d.s. and butobarbitone gr. 3 nocte p.r.n. 10 days after admission he developed a bilateral flaccid paralysis of the upper limb and shoulder girdle muscles. Later he developed a bilateral lower motor neurone facial palsy. He improved after this until March 1951 when his voice became husky and he had a bovine cough. At this time, too, a bilateral flaccid paralysis and complete loss of superficial sensation in his lower limbs occurred. A lumbar puncture showed a normal C.S.F. He was transferred to the National Hospital, Queen Square, on 9/4/51 where it was noted that he had gross generalized involvement of motor power, including paresis of the 7th, 9th, 10th, 11th and 12th cranial


FAMILY 3.
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nerves, as well as extensive sensory impairment. An E.E.G. was normal. The urine was found to contain much porphobilinogen and 51.15 mg. of uroporphyrin/day. He improved slightly and was discharged on 27/9/51 to Manfield Hospital. With physiotherapy his hand power improved, his legs were only little improved. By September 1952 he had managed to walk a little with crutches. 2 years later, September 1954, he was able to walk but he still felt his hands and legs somewhat weak. He had no abdominal pain. In 1956 he still had some flexion deformity of his fingers and had to wear an orthopoedic support in his shoes.

Further records - Liver function tests (Table 2).

Family History Brother is Case 18. See Family 3.

Summary Attack commencing with gastro-intestinal symptoms and an epileptic seizure. In hospital he received barbiturates and a very severe neurological involvement occurred. It is of interest that his brother who was never given barbiturates had a much less severe attack (Case No. 18).

Mr. C.W. Age 30 years (1953) Farm labourer Case No. 18

This man is a younger brother of D.H.W. (Case No. 17). In 1951 his urine contained porphobilinogen and 2.645 mg./litre of uroporphyrin. His urine had been tested routinely, since his brother was an established case of acute porphyria. He had no symptoms, however, until 5 days before his admission to Bedford General Hospital in 13/4/53. He then complained of severe,

continuous, gnawing pain in the (L) lumbar region, passing down to the left iliac fossa, vomiting and slight diarrhoea. He was sleepless and passed dark urine. There was no history of barbiturate ingestion.

Examination on admission showed that he was anxious. There was no abdominal guarding or rigidity but a deep tenderness about the umbilicus. There was nothing abnormal in the nervous system. B.P. $\frac{146}{94}$. P.R. 100/minute. Afebrile. Hb. 13 G%. R.B.C. 4.41 million/cmm. W.B.C. 10,000/cmm. The urine contained much porphobilinogen and uroporphyrin but no protein. Pethidine was used successfully for the relief of pain.

Within 7 days of his admission his pain had gone and he was discharged on 25/4/53. He was seen again on 18/7/53. He had had a slight backache associated with the passage of darker urine than normal but was otherwise well. He was well in July 1956 but still excreted 92 mg./litre porphobilinogen in his urine.

Family History See Family 3.

Summary Short, sharp attack with lumbar pain and diarrhoea. Note same age of onset as brother (Case No. 17).

Mr. J.L.S. Age 37 years (1951) Engineer Case No. 19

This man was admitted to Whittington Hospital, London, on 26/5/51 with the sudden onset of anterior central chest pain and shortness of breath of 1 day's duration. The pain lasted several hours and was relieved by an injection of morphia. P.R. 100/minute. Temperature 99-100°F. W.B.C. 5,000/cmm. Hb. 15.5 G%.

Urine contained much porphobilinogen. There was recurrence of substernal pain and breathlessness the next day but he eventually settled down and was discharged on 6/6/51. For about 1 year before this admission he had been given a small white tablet b.d. which made him "dopey" and for 7 days before admission he had been given daily injections into his arm which also made him sleepy. These drugs have been ascertained as phenobarbitone and sodium amytal during the first 7 months of this period but it has not been possible to make sure what kind of drug was given thereafter. There was a previous history since 1942 of abdominal pain coming on ½ hour after meals and sometimes eased by medicines. In 1945 he was discharged from the Army because of a 'duodenal ulcer' and in 1948 he had an appendicectomy, at which a normal appendix was removed.

After his discharge from hospital he continued to have severe pressing, upper abdominal pain, sometimes relieved by food. He was re-admitted in March 1953 when a partial gastrectomy was performed. The pathologist reported a simple ulcer of the lesser curvature of the stomach.

He was last seen on 11/1/54 when he reported that he had had no abdominal pain since his operation.

Further records - his urine on 21/1/53 contained porphobilinogen and 0.6 mg./litre of uroporphyrin.

Summary The association of porphobilinogenuria with a gastric ulcer. Onset of his "attack" unusual - anterior chest pain and breathlessness but no other cause for these symptoms could be found. Had taken sleeping tablets for 1 year prior to onset.

Mrs. B.S. Age 23 years (1953) Housewife Case No. 20

This patient was admitted to the Royal Infirmary, Sheffield, in March 1953 with a history of abdominal pain, constipation and paraesthesia of her feet. This occurred at the time of her period, which was not suppressed. Examination showed generalised abdominal tenderness. There was no neurological abnormality. B.P. $\frac{160}{90}$. Temperature 99-100°F. P.R. 100-110/minute. W.B.C. 5-10,000/cmm. E.S.R. 6mm/hour (Westergren). Her symptoms settled in a few weeks and she was discharged 19/6/53. She was re-admitted 2 months later with a second, milder attack of abdominal pain. On this occasion her urine contained much porphobilinogen and 7.3 mg./litre uroporphyrin.

She was seen in April 1954, when she had become pregnant and had had no further episodes of pain since her last normal period. She remained symptom-free during her pregnancy but had a return of abdominal pain after delivery for a short time. She was last seen in April 1956 and had had no further symptoms since then.

Summary 2 mild attacks. Pregnancy without incident but exacerbation immediately after delivery.

Mr. R.P. Age 43 years (1952) Motor mechanic Case No. 21

This man had occasional abdominal pain with constipation from the age of 35. He was admitted to Birkenhead General Hospital aged 43 years in May, 1952, with a 4 day history of

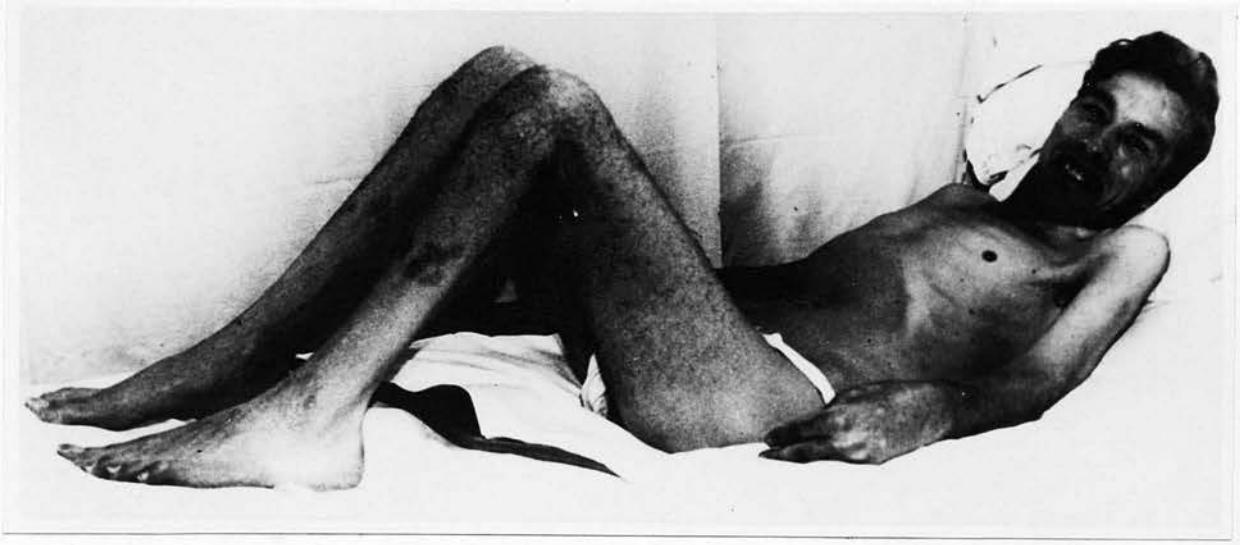


Plate 3(a) Appendix.

Case No. 21.

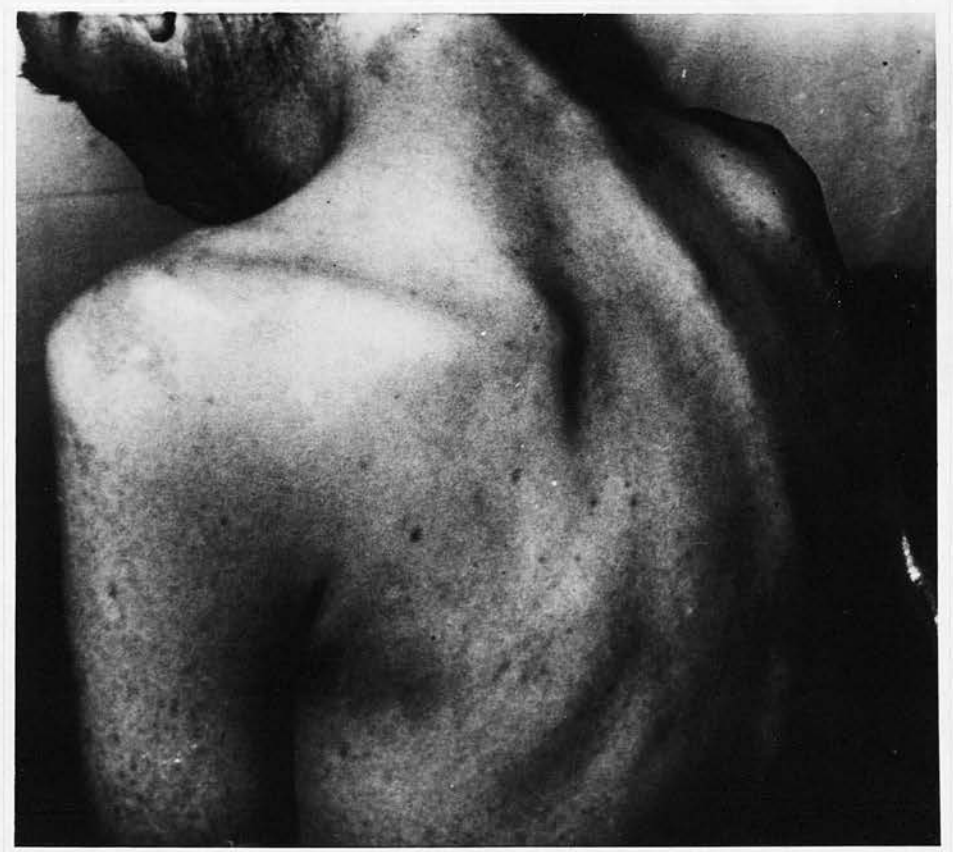
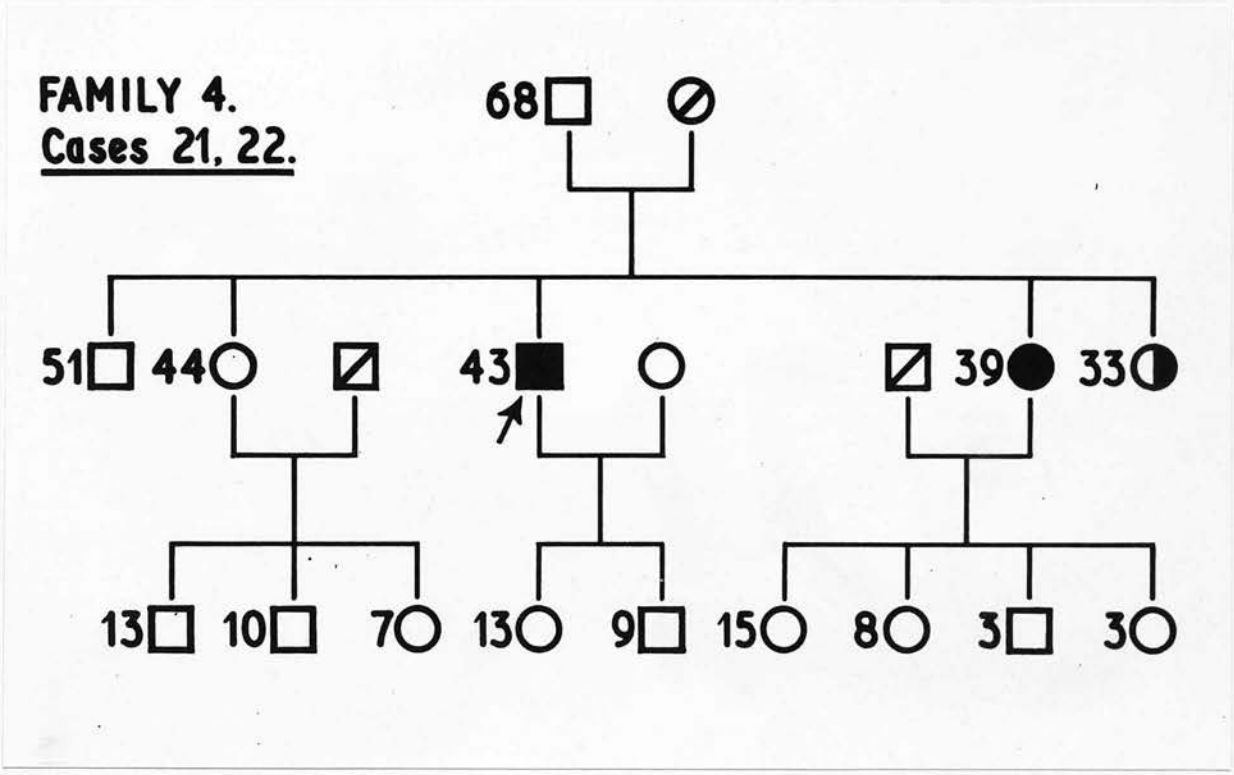


Plate 3(b) Appendix.

Case No. 21.

colicky abdominal pain beginning in the (R) iliac fossa, anorexia, vomiting and slight difficulty in micturition. There was tenderness and some rigidity in the (R) iliac fossa. A laparotomy was performed and a normal appendix was removed. Intravenous pentothal was given at operation. Barbiturates (type unknown) had been given by his own doctor at home between 1950-1952. He was re-admitted to hospital in June 1952, with general weakness and loss of weight. On this occasion he was given seconal and nembutal. He was re-admitted once again in July 1952, and on this occasion porphobilinogen was discovered in his urine. He was generally weak and there was wasting and tenderness in the muscles of the shoulder girdle, hips and face (Plates 3 a and b). There was slight impairment of superficial sensation over cervical 5 and 6 dermatomes, numbness of posterior aspect of thighs and tenderness and hyperalgesia of the scrotal area. He was constipated on admission, but had diarrhoea for several days after admission. There was loss of sensation in the bladder with difficulty in starting micturition and he had to be catheterised for some days. His condition deteriorated for several weeks following admission and he became mentally confused and irrational with lucid intervals. The muscular weakness progressed from proximal to distal muscles and he developed wrist drop with flexion at metacarpo-phalangeal joints with extension at interphalangeal joints (Plates 3 a and b). His speech became slurred, he had difficulty in swallowing; there was intercostal breathing and almost complete paralysis of his legs. Throughout this time he had intermittent attacks of severe colicky pains with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marked perspiration. After this regression his condition remained stationary for about 1 month and he then made steady progress. He was discharged in December, 1952, not completely recovered, but he gained strength at home. He was seen again in 1954. Although he still gets occasional cramplike pain in the lower abdomen and legs, he has returned to work and leads a normal life. His urine still becomes dark on standing. In August 1956 he had completely recovered, but continued to excrete porphobilinogen in the urine.

Further records He was afebrile throughout his illness apart from one occasion when he had an upper respiratory infection. His pulse rate remained at 105-120/minute during his severe phase. B.P. $\frac{110}{70}$, but this was only taken when he was recovering. E.C.G., C.S.F. - normal. Hb. 13.6 G%, R.B.C. 5.2 million/cmm. W.B.C. 9,000/cmm. Urine contained no protein.

Liver function tests (Table 2). Serum electrolytes (Table 1).

Family History He has 2 sisters, both of whom excrete porphobilinogen in the urine. One of these (Mrs. D.K., Case No. 22) has had symptoms. See Family 4.

Summary A prolonged attack with quadriparalysis and cranial nerve involvement. Barbiturates, including seconal given. Complete recovery.

Mrs. D.K. Age 36 years (1949) Housewife Case No. 22

This woman is a sister of Case No. 21. On routine examination in 1952, much porphobilinogen was found in her urine. In 1949, 2 weeks after the birth of twins, she developed an "acute tonsillitis". Shortly after this she complained of abdominal pain, constipation, pains in the legs, blurring of vision and headaches. Admitted to hospital 3-9-49; noted to be disorientated and restless, tendon reflexes sluggish, C.S.F. normal, tachycardia, B.P. $\frac{170}{100}$. She gradually became rational, but still complained of generalised pains. One month after admission she was discharged, although she complained of leg pains. The patient remembered that her urine became very dark during this illness.

In 1950 she was admitted to hospital because of chest pains. Since then (until 1956) there have been no further symptoms, suggestive of acute porphyria. Before the birth of her twins, she had 2 other children in 1937 and 1944.

Family History See Family 4 and Case No. 21.

Summary One attack in a woman aged 36 years, commencing 2 weeks after the birth of twins.

Miss J.F. Age 17 years (1952) Schoolgirl Case No. 23

This young girl died after an explosive first attack of acute porphyria. About 2 months before her death she became restless and sleepless at night. 8 days before her death she began to vomit and have frequent cramp-like abdominal pain, first on one side then on the other. There was constipation and some abdominal distension. She was given 2 tablets of soneryl (gr. 1½) on 3 or 4 nights for her sleeplessness. Her mother noted that she passed dark urine, which stained her pyjamas brown. Four days before her death she developed retention of urine, paraesthesiae in the genital area and had difficulty in focusing her eyes. Her speech became nasal and slurred, she had difficulty in swallowing and her legs became weak. Her breathing was rapid. She was admitted to the London Clinic where her general weakness increased. While there she was given phenobarbitone and soneryl. She was admitted on 8.9.52., 2 days before her death, to the National Hospital for Nervous Diseases, Queen Square, London.

On examination there, she was anxious, restless, agitated and confused. Her pupils did not react to light. There was well-marked palatal weakness and fluid regurgitated through her nose. The power of her limbs and trunk muscles was diminished and the lower limb reflexes were absent. There was a doubtful (R) extensor plantar response. Her intercostal muscles were weak. There was a vague level, mid-thoracic, below which all forms of sensation were impaired. B.P. $\frac{150}{100}$. Pulse 120/minute. Afebrile. Respiration 30/minute. She was given hexamethonium 30 mg. 3 hourly which brought her B.P. down to $\frac{90}{60}$ for a short

period after each injection. Two days after admission her breathing became irregular and jerky and despite artificial respiration with the Bragg-Paul apparatus she died on 10.9.52.

Further records Urine - Porphobilinogen 248mg/litre.
Uroporphyrin 96.8mg/litre.

Paper chromatographic investigation of the urinary porphyrin showed it to be entirely uroporphyrin series III. Chemical pathology of P.M. liver - Table 3.

Summary An explosive fatal attack in a girl of 17 years. Onset of restlessness and insomnia; severe neurological manifestations; barbiturates given on several occasions.

Mr. M.C.B.W. Age 33 years (1953) Lorry driver Case No. 24

This man was admitted to Whancliffe Hospital, Sheffield, with a history of pain in both shoulders (R) knee and sacral region of 1 week's duration. He also complained of constipation and numbness in the upper part of thighs and genitalia. In 1944 he had similar pains and they had been diagnosed as being caused by rheumatic fever, but he remembered that he then passed red urine. On examination it was noted that he was hysterical, there was muscular inco-ordination of his limbs, diminished pain and light touch sensation in the 'bathing trunks' area and a nystagmus to the left. There was no abdominal tenderness. B.P. $\frac{130}{70}$. Before he was diagnosed as a case of acute porphyria he was given seconal gr. 3 each night from 27.2.53. to 4.3.53.

He became generally weak, was unable to swallow and there was difficulty in coughing. He became incontinent, rambling and noisy and died on 8.3.53.

Further records He remained afebrile throughout this period except during the day before his death when his temperature rose to 100°F. Pulse Rate 100-120/minute. Hb. 14.8 G%. E.S.R. 6mm./hour. W.B.C. 12,000/cmm. Blood urea 48 mg%. Urine (7.3.53.) contained 19.6 mg/litre of porphobilinogen and 12 mg./litre uroporphyrin, identified later as uroporphyrin series III. Post-mortem showed fatty degeneration of the liver, gross gastric petechial haemorrhages and pulmonary and cerebral congestion. Foci of demyelination were found in the white matter of the cerebellum. (See table 4). Chemical pathology of P.M. tissues - see table 3.

There was no family history of note. His 2 children did not excrete porphobilinogen in their urines.

Summary An explosive, fatal attack onset by articular pain. Death from general paresis 4 days' after administration of seconal.

Miss W.E. Age 21 years (1951) Whisky-bond worker. Case No. 25

This patient was admitted to the Western Infirmary, Glasgow, 19.1.51., with a history of lower abdominal pain, nausea and vomiting of 2 days' duration. The abdominal pain was constantly present, but more severe and colicky at times and she also had

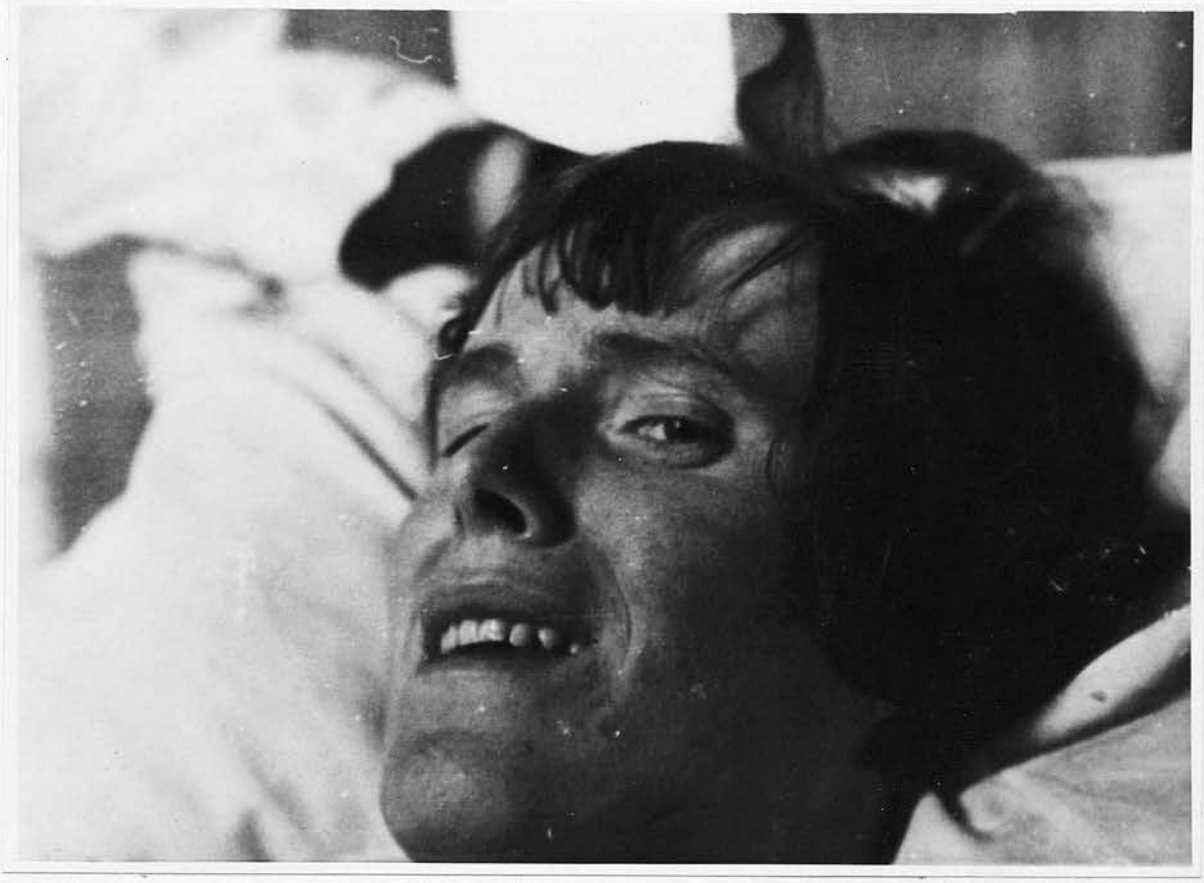


Plate 4. Appendix.

Case No. 25. At this time she often screamed, had visual hallucinations and threatened to commit suicide.

pains in the medial aspect of her thighs. Her mother had noted that she was thinner and less active since 2 months previously. On examination it was noted that she was anxious, crying and cyanosed. Temperature 102°F., Pulse Rate 140/minute and B.P. $\frac{150}{115}$. W.B.C. 8,200/cmm. There was slight abdominal tenderness, but no rigidity. The urine was claret-coloured and contained much porphobilinogen. She menstruated 3 days after her admission, the abdominal pains lessened and the B.P. fell to $\frac{115}{80}$.

One month after her admission (19.2.51) she commenced a prolonged attack lasting 6 weeks with almost constant abdominal pain, tachycardia and amenorrhoea with loss of weight. She often screamed, had visual hallucinations and threatened suicide (Plate 4). By 27.3.51. she became weak, hypotensive (B.P. $\frac{70}{54}$), somewhat dehydrated and was still in considerable pain. She was given 850 ml. of normal saline by subcutaneous drip using 9 mg. hyaluronides and started on a course of A.C.T.H. 12.5 mg 6 H. intramuscularly (50 mg/day) lasting 1 week (Fig.1). Her general condition improved greatly within 1-2 days. Her pain diminished, B.P. rose to $\frac{120}{100}$ and she became somewhat euphoric. She then proceeded to a remission lasting for 1 year. Her periods returned in July 1951, she put on weight, but mentally she was quieter and "different".

She was re-admitted to hospital on 26.1.52. and remained there for the next 4 months with an attack similar to her previous one. She was given nembutal gr. $1\frac{1}{2}$ in error, and this coincided with an aggravation of her condition. She was put on a short course

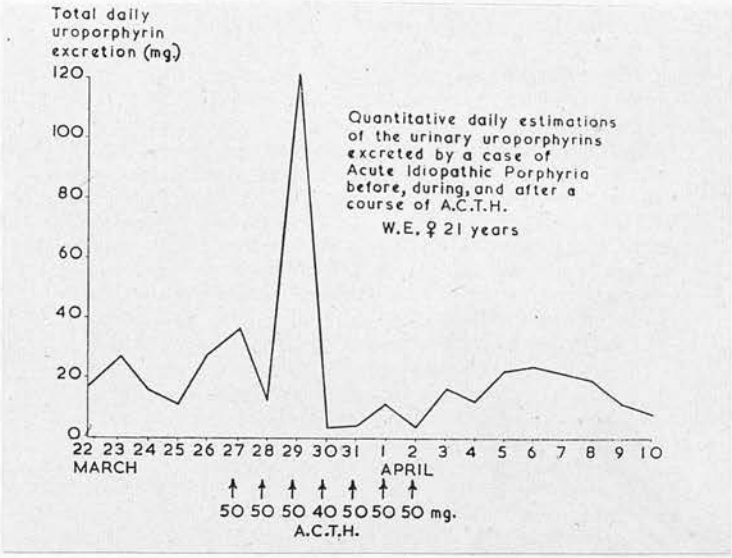


Fig. 1. Appendix.

Case No. 25.

of A.C.T.H. and developed an erysipelas-like rash of her leg and a temperature of 103°F. She was very weak at this stage, her serum chlorides were diminished (Table 1), and on 26.2.52., after a period of overbreathing (respirations 46/minute), she developed a tetanic spasm of her hands which were in the position of main d'accoucheur. During this spasm her serum calcium was 9.0 mg% and CO_2 combining power 67 vols%. She gradually improved after antibiotic therapy, but there was considerable generalised muscular weakness for some time. There were no abnormal neurological signs apart from this muscular weakness and absent knee tendon reflexes. During this phase she had complained of abdominal pains and shooting pains in her legs, but these, too, diminished. She was discharged from hospital on 14.4.52., since when she has had no further attacks. She reported in August 1954, that she was working on a farm and feeling very fit.

Further records Liver function tests (Table 2) normal. Hb. 13 G%. R.B.C. 4.49 million/cmm. Reticulocytes 1%. Urine contained no protein. There was no relevant family history.

Summary 2 severe attacks, one in 1951, the other in 1952. Severe abdominal pain and psychotic changes occurred in both attacks. In the first attack administration of A.C.T.H. was associated with improvement. In the second attack she was given nembutal gr. 1½ in error and developed generalised muscular weakness. She made a complete recovery.

Mrs. J.G. Age 19 years (1949) Housewife Case No. 26

This girl was admitted to Whittington Hospital, London, 28.12.49. because of severe generalised abdominal pain, vomiting and constipation. These symptoms had been coming on gradually for a few months, but had become much more severe just before admission. On 29.12.49. her appendix was removed. A few days later she complained of weakness of the back, arms and thighs. She was discharged on 7.1.50. While in this hospital, she had been given soneryl gr. 3 on two occasions. At home she still felt unwell; she shivered, coughed and there was increasing weakness of her muscles. She was admitted to Coppetts Wood Hospital, London, as a case of poliomyelitis.

On examination, it was noted that there was weakness of the face, limbs and trunk. Afebrile. Pulse rate 110/minute. W.B.C. 12,000/cmm. Hb. 12.6 G%. R.B.C. 6.06 million/cmm. C.S.F. was normal. Urine was pink in colour and contained much porphobilinogen and 48 mg/litre of uroporphyrin (19.1.50); it also contained a trace of protein. She became much weaker. First her arms and then her legs became paralysed. There was no sensory loss. She was mentally clouded and could not speak. There was amenorrhoea and loss of 3 stones in weight. She gradually improved and after 3 months she was transferred to the Metropolitan Hospital, where she remained for 2 months. She put on weight, her periods returned and her legs and arms recovered their power. Since August 1950, she has been well although she continues to pass porphobilinogen in her urine. She had her first baby in June 1952, and there was no trouble during pregnancy.

She reported in July 1954 that she was well, had had no abdominal pain and was 5 months pregnant.

Family History Her 2 cousins are said to have died from a similar condition.

Summary A very severe attack with marked neurological signs, commencing as a simple attack with gastro-intestinal symptoms during which barbiturates were given. 2 normal pregnancies since her complete recovery.

Mrs. B.E. Age 23 years (1953) Hairdresser Case No. 27

This young woman had been healthy until 4 days before her admission to the Western Infirmary, Glasgow, on 1.7.53., because of pain, nausea, vomiting and constipation. The pain started across the lumbar region and radiated anteriorly and downwards into both groins and thighs; it was constant though varying in severity. She had never been given barbiturates. B.P. $\frac{130}{75}$. Pulse rate 140/minute. Afebrile. E.S.R. 6mm/hour (Westergren). W.B.C. 7,200/cmm. Hb. 14 G%. R.B.C. 4.8 mill/cmm. Urine contained much porphobilinogen and porphyrins. There were no abnormal neurological signs. The pain settled about 6 days after her admission and she was discharged shortly afterwards. She was well when seen 1 year later.

Further records Liver function tests - Table 2.

Summary Single short attack. No paralysis or hypertension. Site of pain unusual.

Mrs. M.S. Age 23 years (1953) Civil Servant & Housewife Case No. 28

This young woman had been suffering from intermittent attacks of abdominal pain and vomiting lasting about 12 hours for 3 months before her first admission to the North Middlesex Hospital, London, 7.10.53. For one week before her admission she had severe, constant, epigastric pain and insomnia, for which she was given sodium amytal and phenobarbitone. One day before her admission she had a generalised epileptic attack and this was the presenting cause of her admission. Her husband had noted a slight personality change in her - she had become quieter, more subdued and annoyed by small things. She never had had fits previously nor was there epilepsy in the family.

On admission she was given intramuscular phenobarbitone gr. 3 on 2 occasions and phenobarbitone gr. $\frac{1}{2}$ t.d.s. by mouth. A series of generalised clonic convulsions followed during the next 2 days and ceased when barbiturates were terminated on the diagnosis of acute porphyria. She continued to have intermittent abdominal pain for a few weeks, but this gradually lessened and she was discharged on 30.10.53. Her B.P. had risen during her attack to $\frac{159}{110}$ and fell to a basal level of $\frac{146}{94}$. Pulse rate rose to 120/minute. Afebrile. The urine contained much porphobilinogen and 9.33 mg/day of uroporphyrin. Hb. 12 G%. Liver Function Tests - normal (Table 2). Urine had a trace of protein.

She was re-admitted to hospital on 23.2.54. and again on 25.6.54. for further attacks of abdominal pain, constipation and vomiting with hypertension. There were no convulsions on these

occasions. These attacks were associated with increases in porphobilinogen output. On each occasion the pain settled and she was able to be discharged in 1-2 weeks.

All 3 attacks commenced at the time of her menstrual periods which were delayed by 5-10 days.

Family History Nothing of note except that her mother has migraine.

Summary This woman was admitted to hospital because of epilepsy, for which barbiturates were given. Epileptic fits ceased when the barbiturates were discontinued.

Mrs. E.F.A. Age 46 years (1951) Housewife Case No. 29

This woman was admitted to the National Hospital, Queen Square, London, on 23.1.51. 6 weeks before her admission she developed a "herpetic" rash on the upper sternum and (L) side of her neck. She slept badly at this time and was given sleeping tablets and injections. 1 week later she started to vomit and continued to do so for about 2 weeks. 4 weeks before her admission she had a sudden acute abdominal pain in the region of the umbilicus. The pain lessened but was followed by a dull abdominal ache and pain across the middle of the chest. These pains lessened in the next few weeks. She also became nervous and melancholic and was afraid that she might commit suicide. She developed pains in the soles of her feet and was unable to put her weight on them. She was seen by a psychiatrist and was admitted to Shenley Mental Hospital. While there she became

weaker and her abdominal pain continued. She had temporary diplopia. It was noted that she had nystagmus, slurred speech, dysdiadokokinesia, and tremor of her tongue. She was then transferred to the National Hospital, Queen Square, London, as a case of post-herpetic encephalitis.

On examination at that hospital it was noted that she was co-operative, slow in action and speech and cried easily. There was a (R) ptosis, general muscular weakness and tenderness, and absent knee and ankle tendon reflexes. There was no sensory loss, except a possible hypoalgesia of cervical 3-5 dermatomes on the (L) side. B.P. $\frac{140}{90}$. Pulse rate 120/minute. Temperature 101°F on admission, but this settled to normal in 3 days. E.S.R. 19 mm/1 hour. Hb. 13 G%. R.B.C. 4.42 million/cmm. W.B.C. 4,100/cmm. The urine contained much porphobilinogen and porphyrins and a trace of protein.

Her condition gradually improved; the abdominal pain lessened and she became stronger. This improvement was marred by a thrombophlebitis but she was well enough to be discharged on 7.3.51. Mentally she returned to normal. She herself states that she felt much more stable when she realised that her doctors had found some cause for her symptoms and did not attribute them to 'nerves'.

She reported on 3.8.54. that she was very well. She had had no pain for over 3 years. Her urine on that date contained no porphobilinogen. In 1956 she had had no recurrence of her disease.

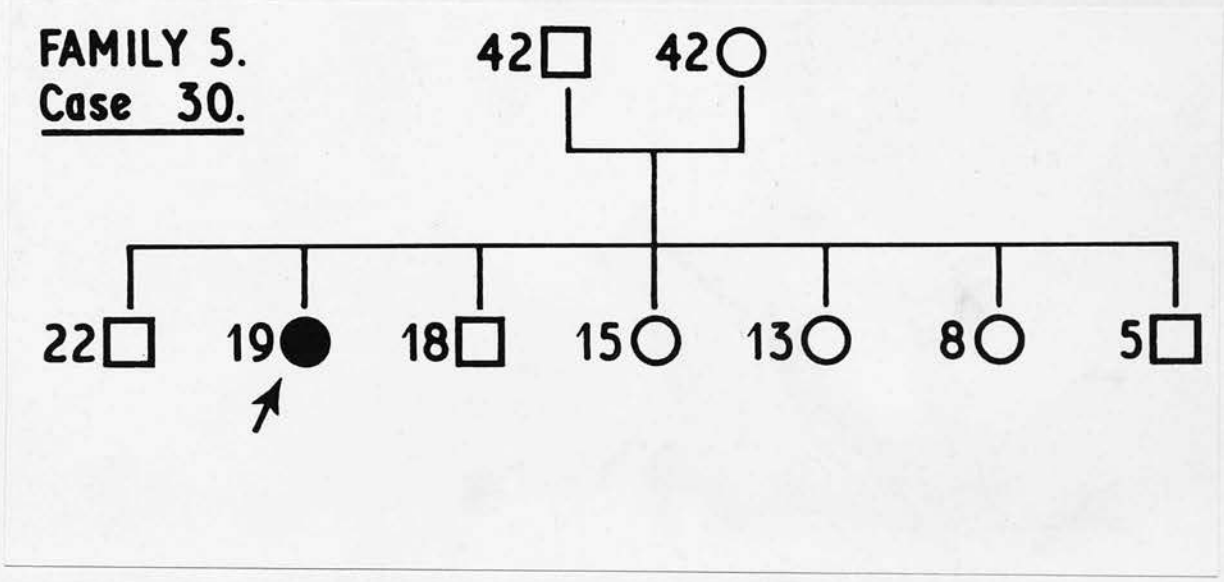
Further records Liver function tests - Table 2. E.E.G. was 'slightly abnormal' (p. 30).

Family History Her grandmother had an episode of paralysis. Her paternal uncle had depression and committed suicide.

Summary An attack following herpes zoster and the administration of "sleeping tablets". Neuropsychiatric manifestations.

Mrs. D.J. Age 19 years (1955) Housewife Case No. 30

This patient was admitted to Maelor General Hospital, Wrexham, on 13/6/55, during the first month of her pregnancy, because of abdominal pain and vomiting of 48 hours duration. She was also constipated and complained of generalised trunk and limb pains. Before the diagnosis was suspected she was given nembutal gr. 1½ and this was repeated 4 times in the next 5 days. 10 days after admission she complained of extreme generalised muscular tenderness and about 1 week later there was marked weakness of her arms and legs with wasting of limb muscles, impairment of pin-prick sensation over the front of her thighs and legs and absent upper limb and weak (R) knee tendon reflexes. Her B.P. on admission was $\frac{120}{90}$ but rose to $\frac{150}{110}$ with the onset of limb weakness. There was marked retinal artery spasm. Her P.R. was usually about 110-120/minute. She had one episode of pyrexia (101°F) which resolved in 3 days and this was possibly due to a mild urinary infection. During her attacks she was very miserable and unco-operative. Hb. 14.8 G%. W.B.C. 4,400/cmm. Reticulocyte count 1.5%. She was treated with pethidine and amidone for her pain. Priscol, chlorpromazine and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prostigmine were given without benefit. 'Ansolysen' retard I.M. did control the hypertension but had no effect on pain. She developed intercostal muscle weakness but did not require an artificial respirator. There was difficulty in taking food and fluids and she had to be fed by intragastric drip from 12/7/55 to 3/9/55. Her condition gradually improved although she complained of pain in her back and lower abdomen. She gave birth spontaneously to a live 7 month old infant on 13/12/55. One hour after delivery her pulse rose to 160/minute. She was transfused with blood but after less than ½ pint had been given she collapsed and died. The infant died 11 hours after birth. Urine porphobilinogen 127 mg/litre (24/6/55), 80 mg/litre (13/9/55).

Family History Nil of note. See family 5.

Summary A severe attack in a girl of 19 years commencing in the first month of pregnancy and terminating fatally a few hours after the premature birth of her child, who also died. Neurological manifestations associated with barbiturate administration.

Mr. E.E. Age 34 years (1949) Clerk Case No. 31

In July 1949, this man was admitted to hospital because of a purulent (R) maxillary sinusitis, following a tooth extraction 1 month previously. He was also sleepless and constipated. He was given penicillin injections and also phenobarbitone daily and discharged in 10 days. Towards the end of this period he had severe epigastric pain and flatulence and felt

very unwell. These symptoms continued while he was at home, but lessened and disappeared after 14 days.

He was well until May 1953, when he complained of pain in the (R) temple for which he was given sodium amytal and phenobarbitone gr. ½ daily for 5 days. He then developed considerable weakness of his legs, severe epigastric pain, violent vomiting and insomnia. He was admitted to Croydon General Hospital where porphobilinogen was found in his urine. He was depressed and lacrimose and later became somnolent and unco-operative. He had marked general weakness, especially of hands and legs. B.P. $\frac{160}{100}$. Pulse rate 90-110/minute. Temperature 98.4°F.- 100°F. E.S.R. 6mm/1 hour. Blood urea 90 mg% urea clearance 46% of average normal. Urine - trace protein.

He remained very weak for 3 weeks; 10 days after his admission he developed a (L) femoral vein thrombosis. His legs regained their power and he was discharged 17.7.53., 5 weeks after admission. His fingers still remained weak for the next 6 months but improved rapidly after treatment with wax baths.

When seen on 20.8.54. he was very well. There was no porphobilinogen in his urine.

Family record The urines of his mother, father, son and daughter contained no porphobilinogen.

Summary 2 attacks in 1949 and 1953; neurological symptoms in 1953; a history of barbiturate administration in both 1949 and 1953.

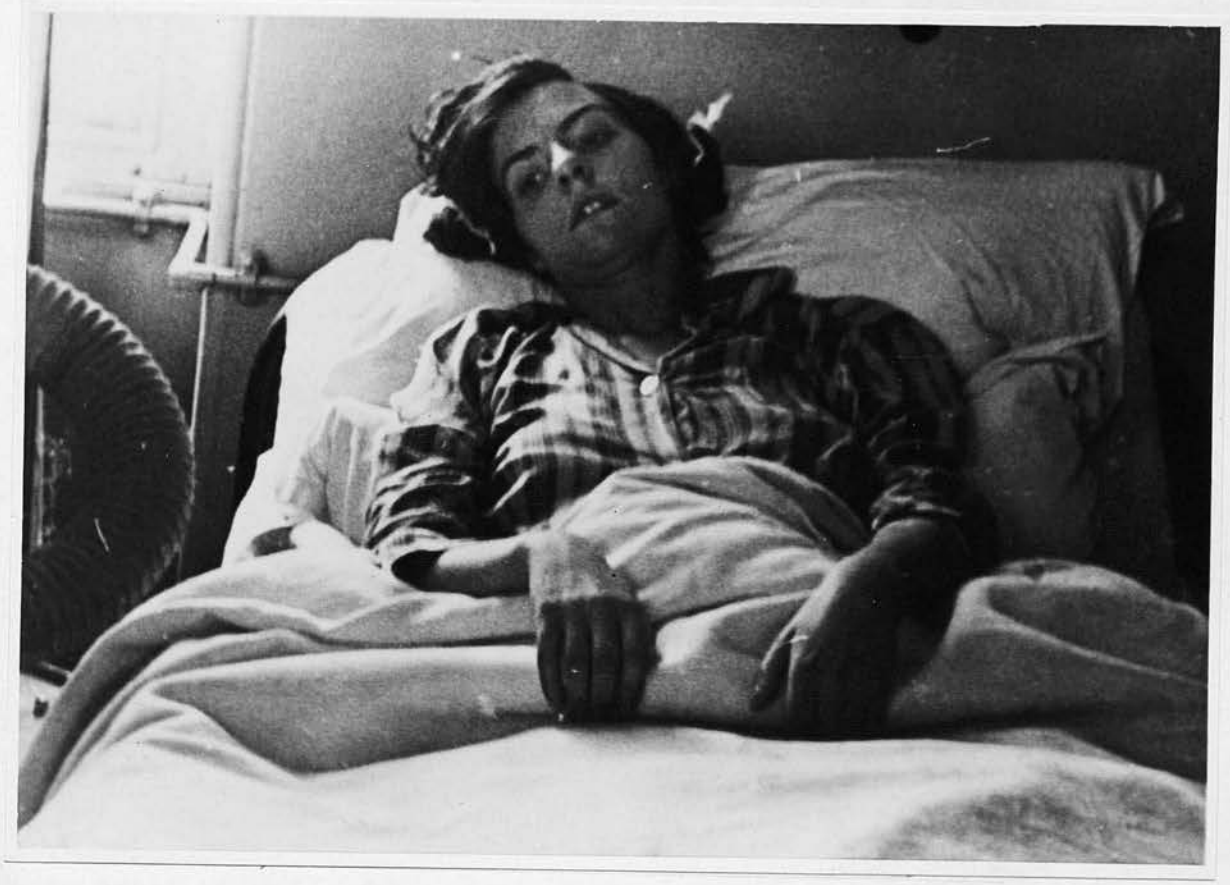


Plate 5. Appendix.

Case No. 32.

Miss A.W. Age 20 years (1952) Employed at home. Case No. 32

This girl was quite well until 10 weeks before her death on 14.3.52. She first complained of lower abdominal pain, constipation, vomiting and dysuria. She was admitted to the Deaconess Hospital, Edinburgh, where extensor plantar responses were noted on examination and a mild urinary infection was treated with streptomycin. She was also given phenobarbitone gr. $\frac{1}{2}$ t.d.s. for 3 weeks since she was difficult to control and very nervous. On one or two occasions she received sodium luminal when her pain was severe. She was discharged after 1 month, during which period she had lost 1 stone in weight. She then went on holiday to Glasgow, but while there she developed weakness of arms and legs. She became markedly hysterical and was admitted to Glasgow Royal Mental Hospital, where porphobilinogen in large amounts was found in the urine. She was transferred to the Western Infirmary, Glasgow, on 11.3.52. and died 3 days later. During this short period her muscular weakness had progressed to a complete flaccid quadriplegia with absence of tendon reflexes and plantar reflexes (Plate 5). Sensation was normal. There was nystagmus and difficulty in swallowing, micturition and breathing. She died in a Drinker Respirator.

Further records (11-14.3.52.) Pulse rate 110-120/minute. B.P. $\frac{128}{86}$. E.S.R. Wintrobe - 2mm/1 hour. W.B.C. 12,300/cmm (Neutrophil polymorphs 82%. Lymphocytes 10%. Monocytes 8%). Hb. 15.5 G%. R.B.C. 5.62 million/cmm. Volume of packed red cells 55,50,50%. Urine had trace of protein and large quantities of porphobilinogen. Serum sodium and potassium normal,

chlorides diminished (Table 1). Empirical liver function tests - normal (Table 2).

P.M. Report (Dr. J.B. Gibson) This was not remarkable with the exception of the lungs and nervous system. In the lungs there were patches of collapse and haemorrhage, apparently due to respiratory embarrassment. The pathological findings in the nervous system are summarised in Table 4.

Family History Nil except her brother had infantile paralysis.

Summary A fatal first attack in a girl of 20 years. Phenobarbitone and sodium amytal given. Quadriparalysis, respiratory paralysis, death.

K.B. Age 14 years (1953) Schoolboy Case No. 33

This boy had a fatal attack of acute porphyria in 1953 lasting 7 weeks. For about 1 year before this he had occasional attacks of colicky abdominal pain about every 2 months and lasting 1 day.

On 18.7.53. he started to have abdominal pain once again and this continued for 5 days, when he had a generalised epileptiform convulsion lasting 5-10 minutes, with jerking of all limbs, tongue-biting, and incontinence. He was admitted to Peterborough Memorial Hospital (23.7.53), where phenobarbitone was given in large doses for 1 week to control his fits. While there he developed a flaccid quadriparalysis, papilloedema and a B.P. of $\frac{220}{150}$. On 21.8.53. he was transferred to the National

Hospital for Nervous Diseases, Queen Square, London. He was noted to be a wasted ill-looking boy who was drowsy but co-operative. There was slight neck stiffness and early papilloedema of the (L) fundus and irregular narrowing of the retinal arteries. All limb muscles were wasted and tender. He could not sit up unaided. The knee tendon reflexes were absent. There was a feeble (R) extensor plantar response and hypoalgesia over his shins. Afebrile. P.R. 106/minute. B.P. $\frac{180}{145}$. He continued to have fits, became restless and noisy. He was put on hexamethonium bromide 25 mg. 3 hourly for 36 hours, but this was only effective in reducing the B.P. for short periods.

He became more restless and inaccessible with lucid intervals. On 3.9.53. he became dyspnoeic, had 3 small fits and died next morning in coma.

Further records W.B.C. 6,800/cmm. Hb. 16G%. R.B.C. 5.68 million/cmm. E.S.R. 3mm/1 hour. Liver function tests - Table (2). Serum electrolytes Table (1). The urine contained much porphobilinogen. C.S.F. - normal. E.E.G. abnormal (p.29).

Family History His married sister, age 25 years, developed abdominal pain and vomiting in October 1952, followed by fits and paralysis of both legs. She died after 5 weeks. The urine of this patient was not tested for porphobilinogen. His parents and 5 remaining brothers and sisters are well and have no porphobilinogen in their urines.

Summary A fatal attack, lasting 7 weeks in a boy of 14 years with abdominal pain, epileptic fits, papilloedema, hypertension and gross paralysis of limbs and respiration. Intermittent

bouts of abdominal pain for 1 year before this. His sister died of a similar condition, also with fits 1 year previously. Barbiturates given to the boy before onset of paralysis.

Miss J.D. Age 33 years (1951) Hairdresser Case No. 34

This woman has had 6 attacks of abdominal pain and vomiting from 1951 to 1953. In October, 1951, she had acute abdominal pain, constipation, vomiting and frequency of micturition for which a laparotomy was performed in Cumberland Infirmary, Carlisle. No abnormality was found. The symptoms continued for 3 weeks after operation. On 28.2.52. she had another attack of severe abdominal pain and vomiting, and although excess porphyrins were then found in the urine, a second operation was done because of the fear of a possible intestinal obstruction. She continued to vomit after operation and burst her operation wound as a result of this. On 16.8.52. she was admitted to the Western Infirmary, Glasgow, with a similar attack lasting 18 hours. There had also been some numbness and weakness of her legs. It was noted then that she was restless and anxious. Afebrile. B.P. $\frac{150}{90}$ (in a previous attack this had risen to $\frac{182}{100}$). Pulse rate 110-120/minute. There was some generalised abdominal tenderness, but no rigidity. Her legs were weak, but there were no other abnormal neurological signs. She was given morphia, pethidine and aspirin for her pain, which gradually settled down in a few weeks. She had a further similar attack in November 1952, for which

A.C.T.H. was given for 2 weeks without apparent influence on symptoms. There were 2 further attacks in April and May 1953. Since then, until August 1954, she has had mild attacks of abdominal pain only. All her previous attacks had been pre-menstrual and her physician had suppressed her periods with testosterone since April, 1953.

Further records Basal B.P. $\frac{110}{70}$. Liver function tests - normal (Table 2). The urine contained much porphobilinogen. She had not taken barbiturates since 1951.

Summary Recurrent attacks of abdominal pain, vomiting and constipation for 2 years in a woman of 33. No history of barbiturates. Numbness and weakness of legs on 1 occasion.

Mrs. A.W. Age 21 years (1953) Housewife Case No. 35

This woman began to have abdominal pain and nausea for 2 weeks after a missed menstrual period. She was thought to be pregnant, possibly to have a tubal pregnancy, and was admitted to Stobhill General Hospital, Glasgow, on 18.10.53. Examination showed that there was loss of power in the muscles of her back and arms. There was no sensory loss. B.P. $\frac{120}{80}$. Pulse rate 100-130/minute. Afebrile. The urine contained much porphobilinogen and 22mg/litre of uroporphyrin.

Her abdominal pain persisted for several weeks in hospital, but this gradually lessened and her muscle power improved. Mentally she was truculent and of low intelligence. When she was

discharged she attended the antenatal clinic for several months and her pregnancy was confirmed. She continued to excrete large amounts of porphobilinogen in her urine without symptoms. Before the end of her pregnancy she defaulted from the antenatal clinic and has not been able to be traced.

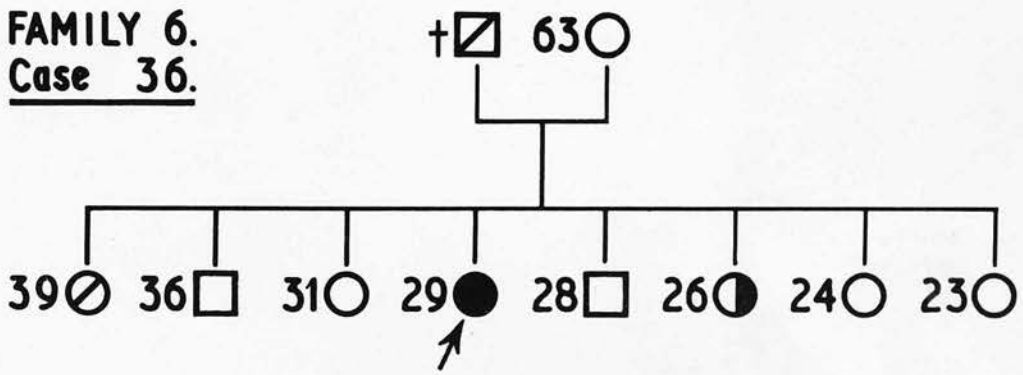
There was no history of barbiturates.

Summary A first attack occurring in very early pregnancy in a woman of 21 years. Abdominal pain and muscular paresis. Tachycardia but no hypertension. The attack settled and she continued her pregnancy.

Miss I.M. Age 28 years (1950) Nurse Case No. 36

This young woman was well until December, 1950, when she started to have intermittent attacks of lower abdominal pain, constipation, vomiting and pain in the lower legs and feet, with the passage of reddish urine. This went on for 1 year during which she was admitted on 2 occasions (2.8.51. and 10.12.51.) to Hackney Hospital, London. While there it was noted that her B.P. was intermittently raised $\frac{180}{110}$ - $\frac{170}{110}$) but sometimes returning to normal ($\frac{140}{80}$). Pulse rate was 130/minute during severe phases. She was given barbiturates on several occasions during her stay in hospital, e.g. phenobarbitone gr. 3 p.r.n., and nembutal gr. 3 p.r.n. nocte. She was afebrile. She became very depressed, was considered to be hysterical and was admitted to a mental hospital 27.11.51. for a short time for electric convulsion

FAMILY 6.
Case 36.



treatment. After this she developed a quadriparesis, lateral nystagmus, slight (R) ptosis, diffuse loss of pin-prick sensation, incontinence, aphonia and dysphagia.

In this critical state, she was admitted to the Metabolic Ward at University College Hospital, London, under Dr. Blake-Pritchard and Dr. C.E. Dent. B.P. $\frac{160}{90}$. There was a persistent tachycardia (110/min.). The urine contained much porphobilinogen and uroporphyrin (maximal value 66mg/day). She was treated with hexamethonium bromide 40 mg. 3 hourly, to keep the systolic B.P. at 120 and prophylactic chloramphenicol. She improved gradually over several weeks - her voice first, then swallowing, then her limbs. Her urinary uroporphyrin fell to 5mg/day. She had repeated B. coli urinary infections which caused slight relapses in which the urinary uroporphyrin level rose to 15mg/day. A course of cortisone was given for 1 week without much effect. She became severely emaciated and her calorie intake was reinforced by the addition of 70 ml. absolute alcohol to her diet. She was also given isonicotinic acid hydrazide and methyl androstenediol to improve her appetite and nutritional state. Her physical condition improved gradually during the next 12 months and she became able to walk and use her hands, but there was an insidious degeneration of her mental condition throughout 1953 and she was placed (August, 1954) in a mental home as a certified patient. She was hallucinated, delusional and unable to work. In 1955 she made a remarkable physical and mental recovery and is now (1956) normal apart from the excretion of porphobilinogen in the urine. This improvement

was associated with the administration of neostigmine.

Family History Sister is a latent porphyria. See Family 6.

Summary A very severe prolonged attack with marked neurological symptoms. Barbiturates were given before onset of paralyses. Gradual physical improvement but insidious mental deterioration. Patient was certified in a mental home, but is now completely recovered.

Mrs. K.M. Age 23 years (1951) Housewife Case No. 37

This woman was admitted to University College Hospital, London, as a surgical emergency in January, 1952, because of severe colicky abdominal pain, vomiting and constipation of 10 days' duration with the passage of dark urine. A similar less severe episode had occurred 1 year previously. On examination it was noted that her temperature was 100°F. Pulse rate 100-120/minute. She had an acute tonsillitis and culture of a throat swab gave a growth of haemolytic streptococcus. Her urine contained much porphobilinogen and 28.28 mg/litre of uroporphyrin on the day after her admission. B.P. $\frac{150}{120}$. Retinal artery constriction with small waves of spasm, was noted. Her condition settled after a course of penicillin and her urinary uroporphyrin was 0.87 mg/litre 16 days later.

She was re-admitted on 14.9.52. with a sore throat, constipation, headache and nausea, but no abdominal pain. She had an acute tonsillitis and her temperature was 103°F.

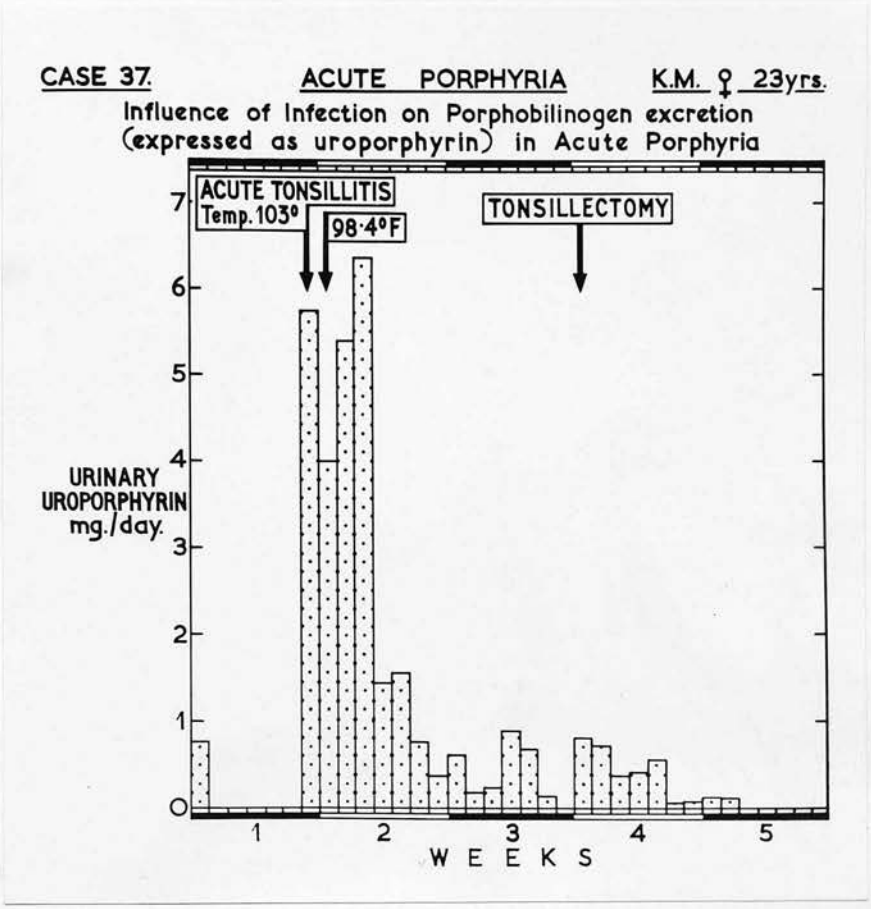


Fig. 2. Appendix.

Case No. 37.

Hb. 15 G%. W.B.C. 9,100/cmm. There was no tachycardia or hypertension on this occasion. She was again treated with penicillin and her temperature rapidly fell and her condition improved. A tonsillectomy was performed on 29.9.52. There was no untoward reaction from this. The daily porphobilinogen levels (expressed as uroporphyrin) throughout this period are noted in Fig. 2. They show the rise of porphobilinogen associated with this infection and its fall when the infection was controlled. She has remained very well for the past 4 years. Her urine on 8.2.53. did not contain porphobilinogen.

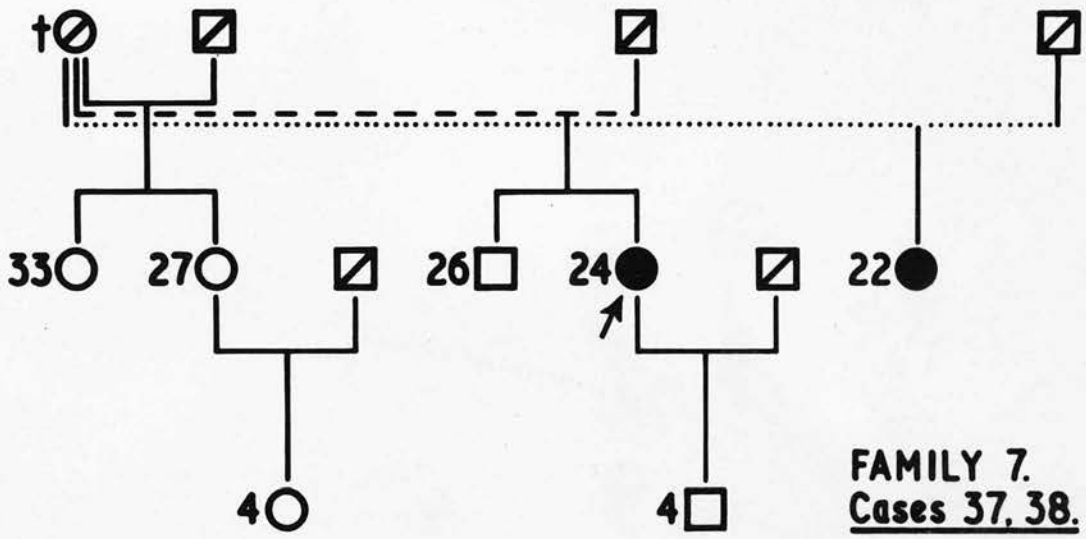
Family history See Family 7.

Summary 2 attacks of acute porphyria, each associated with acute tonsillitis. Remission after tonsillectomy.

Case No. 38

Mrs. J.M. Age 23 (1954) Packer in cigarette factory & housewife

This patient, a sister of Case No. 37, was well until 4.8.54. when she complained of sharp, gripping, lower abdominal pain and pains in the lateral aspects of her thighs. There was vomiting associated with the pain. She had given birth to a baby girl 4 months previously. In view of this, she was admitted to the obstetric wing of University College Hospital, London, as a possible case of postpartum salpingitis and treated with penicillin and sulphonamides. The pains diminished, but they returned with the onset of her period 4 days later, and it was then noted that her urine contained much porphobilinogen. She had



FAMILY 7.
Cases 37, 38.

became constipated throughout this period and also complained of backache. On 16.8.54. she was transferred to the Medical Unit, U.C.H., London.

On examination it was noted that she still had severe abdominal pain. There was generalised abdominal tenderness, but no rigidity. She was mentally alert and intelligent. She had difficulty in passing urine and required to be catheterised for several days. There were no abnormal neurological signs. B.P. $\frac{150}{95}$. Pulse rate 80/90/minute. Afebrile. Her pain and vomiting settled after about 1 week. She developed a symptomless urinary infection which was treated successfully with streptomycin.

On 13.10.55. she delivered a normal child after an uneventful pregnancy.

Further records Plasma electrolytes (18.8.54) showed diminished chlorides (Table 1). Liver function tests (18.8.54) (Table 2). Hb. 15 G%. W.B.C. 6,800/cmm. E.S.R. 20mm/1 hour. Her urine throughout her stay in hospital contained 50-100mg/day of porphobilinogen.

Family history See family 7.

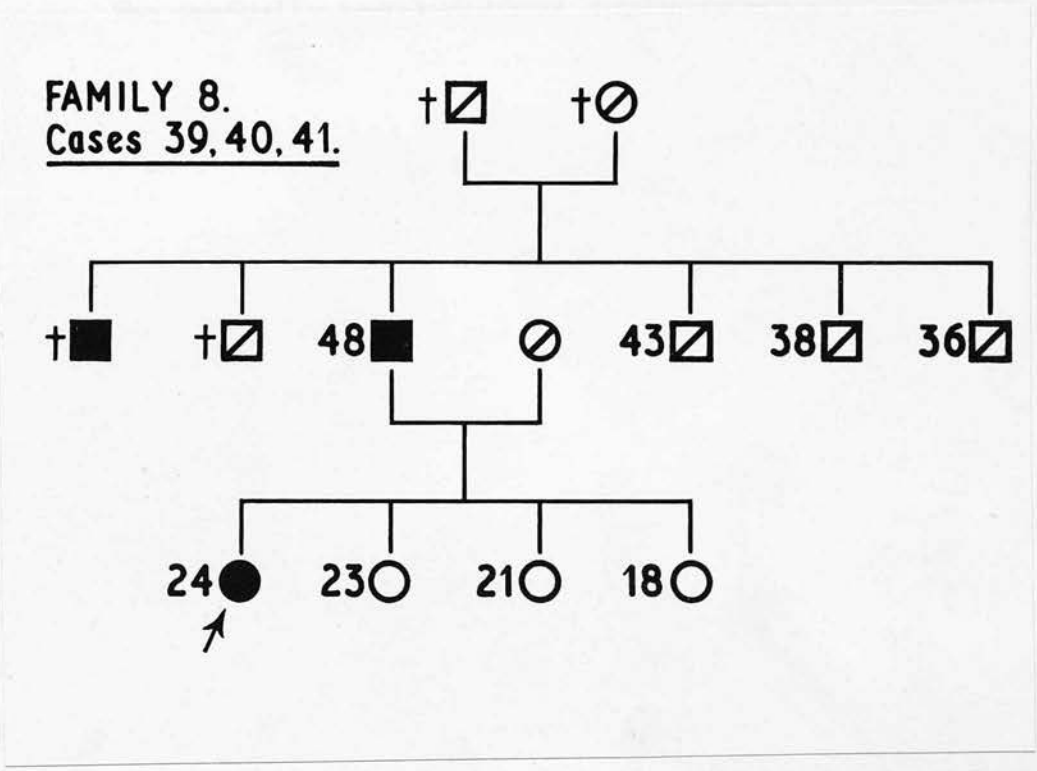
Summary A first attack in a woman of 23, 4 months after the birth of her child. No neurological signs but retention of urine for several days. Normal pregnancy one year later.

Mrs. I.B. Age 21 years (1954) Housewife Case No. 39

This young woman was admitted to Luton and Dunstable Hospital on 4.7.54. with a history of severe continuous abdominal pain, mainly in the epigastrium, with vomiting, constipation and loss of weight of 2 weeks' duration. She had given birth to a child 2 weeks before the onset of symptoms. She also had some dysuria and frequency for which her doctor had given her sulphatriad. He also gave her codeine and butobarbitone. There was a previous history of abortion after a 6 months' pregnancy in March 1953. In June 1953 she had cramp-like epigastric and leg pains.

In Luton and Dunstable Hospital she appeared very ill and mentally disturbed. There was epigastric tenderness but no rigidity, paresis of legs and arms but no sensory loss. She was transferred to University College Hospital, London, on 14.7.54. and it was noted that she was in considerable pain. Limb paresis was still present. Only the (L) knee and (R) arm tendon reflexes could be obtained. B.P. $\frac{160}{120}$. Pulse rate 130/minute. Urine contained 150-200 mg. porphobilinogen daily.

She was treated with pethidine for her pain. She was also given probanthine 15mg 4H for 7 days and tetra ethyl ammonium chloride 100mg t.d.s., for 14 days. There was no dramatic improvement of symptoms from both of these drugs. On 21.8.54. she developed a temperature of 101°F. This was due to a (R) lower lobe pneumonia, caused by a penicillin-resistant staphylococcus pyogenes. This was successfully treated with aureomycin. The infection must have taken place after the onset


FAMILY 8.
Cases 39, 40, 41.
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of her illness because her chest X-ray in Luton and Dunstable Hospital was normal.

She gradually improved after the treatment of her pneumonia. Her abdominal pains lessened, the muscular power and tendon reflexes returned and the B.P. and pulse rate returned to normal. She was discharged on 22.8.54.

Further records 4-14.7.54. Hb. 11.8 G%. R.B.C. 4 million/cmm. W.B.C. 11,600/cmm. E.S.R. 17mm/1 hour. Liver function tests - normal (Table 2). C.S.F. normal.

Family history Her father is Case No. 40. Her paternal uncle is Case No. 41. See family 8.

Summary A moderately severe attack in a girl of 21, starting 2 weeks after the birth of her child. Mild neurological signs. Butobarbitone given. Intercurrent pneumonia treated successfully.

Mr. R.A. Age 29 years (1935) Shipyard holder Case No. 40

This man is the father of Case No. 39. His urine was examined when his daughter was unwell and found to contain porphobilinogen. His past history is very suggestive of a prolonged attack of acute porphyria. He had always noted that his urine was amber coloured - 'the colour of whisky'. On 16.6.35. he had a sudden (L) subcostal, gnawing, intermittent pain with vomiting and constipation. These symptoms recurred for the next 2 years, during which time he was admitted, on 2 occasions, to the Royal Infirmary, Greenock. Pulse rate 70-90/minute. Afebrile.

Barium meal was negative. No abdominal rigidity. He remembers that his legs and arms became very weak and his wife relates how he became changed mentally. He would not recognise her. On one occasion he jumped over the stair railings and tried to set things on fire while he was in hospital. He remembers that he was getting injections and tablets which made him sleepy. Examination of the records shows that these injections were mainly morphia and atropine. He did however have alonal gr. 2 on 25.6.35. and medinal gr. 7.5 on 26.6.35. The hospital authorities wished to have him certified, but his father took him home and he gradually recovered his strength and normal mental state. On 15.1.37. a laparotomy was carried out at Broadstone Hospital, Port Glasgow, because of his persistant abdominal pain and a retrocaecal, non-inflamed appendix was removed. His wife states that it was 1940 before he was quite normal mentally and till then she felt afraid to be alone with him in the house.

He has been well from that time until 1954, except for a period of 5 weeks in hospital in 1948 when he had generalised oedema, which quickly settled. Examination in August, 1954, showed no physical abnormality except a B.P. of $\frac{170}{110}$. Urine contained 72mg/litre porphobilinogen and 58μg/litre copro-porphyrin.

Family history See family 8.

Summary Acute porphyria with onset 19 years ago. Marked mental change with gradual recovery to normal. Still excretes porphobilinogen in his urine.

Mr. J.A. Age 31 years (1933) Shipyard worker Case No. 41

This man, a brother of Case No. 40, began to be troubled with recurrent attacks of abdominal pain in 1933, with constipation. A laparotomy was done in November 1933, and his appendix was removed. At operation areas of spasticity of the large bowel were seen. The pain continued after operation. In January, 1934, he found that he could not extend his fingers properly, nor his (R) wrist. 1 month later he complained of pain in his chest, which persisted and was worse on exertion. He was admitted to the Victoria Infirmary, Glasgow, on 7.3.34. and was noted to be emaciated, pale, sad and depressed. Movements of arms were weak. Bilateral wrist drop was present. There was weakness of the legs, but there was no foot drop. Tendon reflexes of the limbs were absent. There was no sensory loss. His urine was noted to be dark amber in colour. He was discharged from this hospital on 6.7.34., "very much improved". His pain continued after his discharge.

On 3.5.35. he was admitted to the Royal Infirmary, Greenock, with a complaint of severe abdominal pain. On examination he was found to be in a debilitated condition with bilateral wrist drop and a flaccid quadriparesis. B.P. $\frac{125}{85}$. Pulse rate 80-100/minute. Afebrile. C.S.F. normal. On 10.5.35. he developed a carbuncle of his back and sugar was found in his urine. He was given medinal 2 tablets on 8.5.35. His condition deteriorated rapidly and he died on 15.5.35.

Family history See family 8.

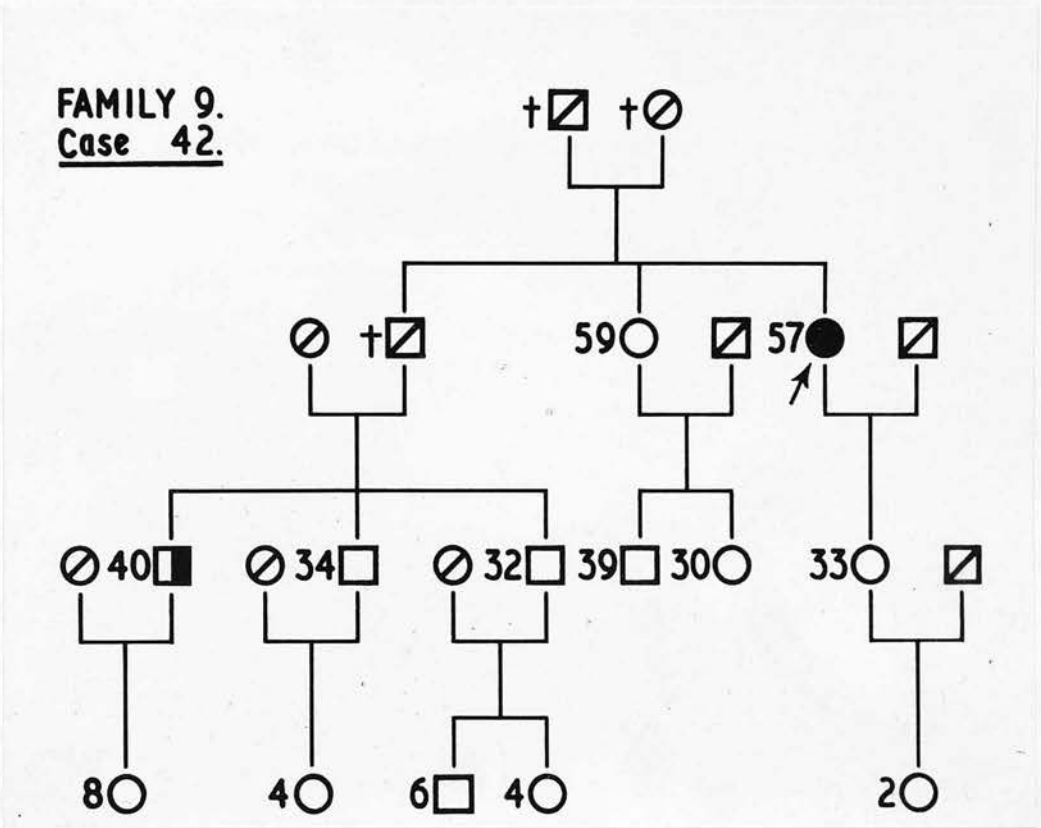
Summary The history here is very suggestive of acute porphyria,

although the urine, noted to be dark, was not investigated for the presence of porphobilinogen. The subsequent family history which unfolded - proved acute porphyria in his brother and niece - makes such a diagnosis very likely.

Mrs. L.W. Age 55 years (1952) Housewife Case No. 42

This woman was admitted to the London Hospital in May, 1954. In the previous 2 years she had bouts of diarrhoea coming on in the early morning, lasting 2-3 days and occurring every 2 to 3 weeks. She had passed mucus during these attacks, never blood. She had hypogastric pain during the attacks and sometimes she vomited. Between attacks she was constipated. Her last attack occurred 4 weeks before her admission to hospital. For the past 8 weeks she had noted that she had passed dark urine. Past History 18 years before, she had abdominal pain and vomiting. She was then admitted to the London Hospital. A laparotomy was performed and a retrocaecal fibrosed appendix was removed. She has had headaches, sometimes with vomiting, in the past 15 years. In recent years she has felt very lethargic and has never wanted to go out of the house, particularly since her husband died 4 years ago.

On examination it was noted that she was an obese middle-aged woman. There was no physical abnormality, except that the palate elevated to the (R). B.P. $\frac{150}{90}$. Pulse rate 65-95/ minute. Afebrile. The urine contained 78.3 mg. porphobilinogen/ litre.


FAMILY 9.
Case 42.
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While in hospital she had a severe attack of constant generalised abdominal pain with persistent vomiting and constipation, lasting one week. This was the most severe attack of abdominal pain that she had suffered. She was discharged from hospital 1 month after admission. Since then she still has recurrent attacks of diarrhoea and also has had severe headaches.

Barbiturates have not been given to this patient for several years.

Family history See family 9. Her nephew, age 40 years, has porphobilinogen in his urine. He is very healthy. The specimens of the remaining relatives were negative for porphobilinogen.

Summary A middle-aged woman with recurrent attacks of diarrhoea, vomiting and some abdominal pain. She passes porphobilinogen in her urine. Long history of migraine. Nephew is a latent case.

Mr. J.W. Age 18 years (1952) Case No. 43

This young man was well until February, 1952, since when he had intermittent attacks of nausea, vomiting, constipation, abdominal and leg pains until his death in December 1953. He required to be admitted to the Royal Infirmary, Glasgow, on 5 occasions throughout this 22 months' period. In some of these attacks he had transient hypertension (B.P. $\frac{170}{100}$, $\frac{190}{140}$), tachycardia (100-120/minute) and mental disorientation. In his final attack he was re-admitted to hospital because of headache and pains

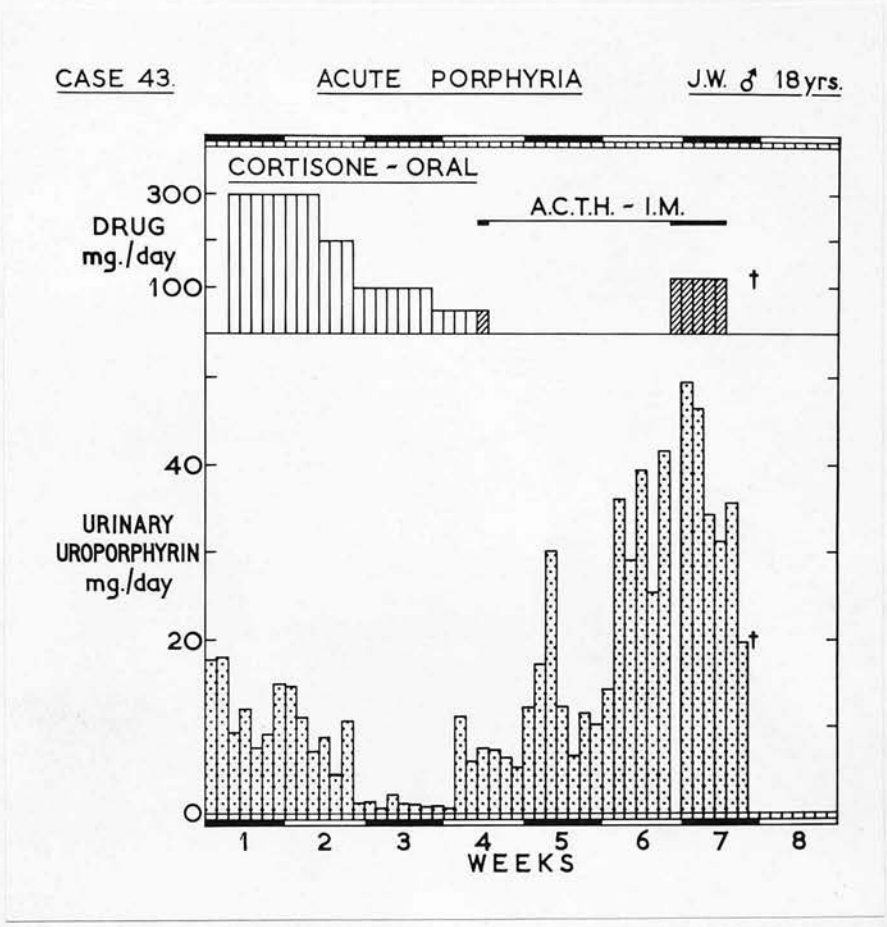
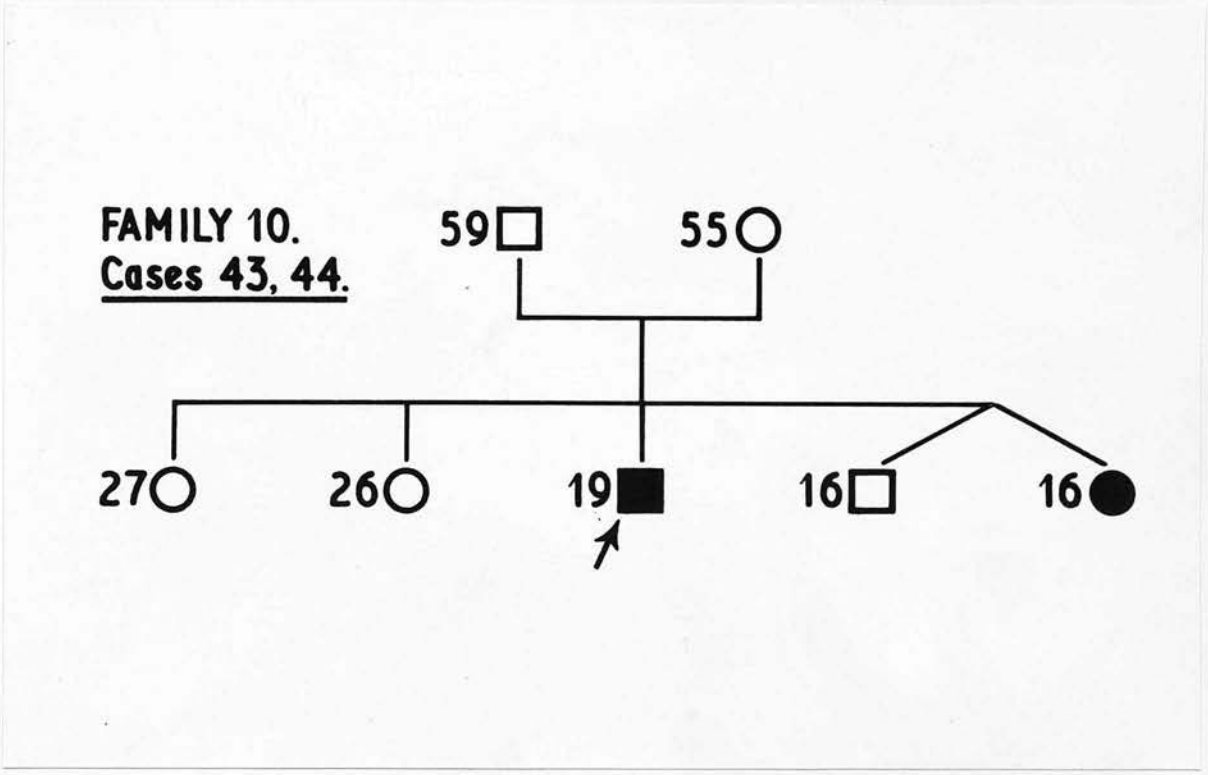


Fig. 3. Appendix.

in his back and limbs. He later developed tremor and weakness of his arms and legs. This limb weakness progressively increased for 2 months until his death on 14.12.53. from bronchopneumonia. He was given a prolonged course of cortisone and a short course of A.C.T.H. in his final illness, but these did not seem to improve him. He had never been given barbiturates.
Further records Hb. 14.6 - 15.4 G%. Liver function tests - normal (Table 2). E.S.R. 9mm/1 hour (Westergran).
Throughout his illness he excreted much porphobilinogen in his urine. P.M. tissues - Chemical analysis - Table 3.
Family History His sister is Case No. 44. See family 10.
Summary Recurrent attacks for nearly 2 years, final fatal one with slow development of limb weakness. Barbiturates not given.

Miss M.W. Age 15 years (1951) Case No. 44

This girl is the sister of Case No. 43. Her mother stated that she had intermittent attacks of abdominal pain and vomiting since early childhood. In 1951 she was admitted to an infectious diseases hospital because of abdominal pain, diarrhoea and pain and stiffness in the legs. She was diagnosed there as a case of acute porphyria, but before the diagnosis was made she was given barbiturates. In May to June, 1952, and again in November, 1952, she was under the care of Professor L.J. Davis at the Glasgow Royal Infirmary, with similar symptoms. There was an intermittent low-grade pyrexia and tachycardia of 120/minute.


FAMILY 10.
Cases 43, 44.
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W.B.C. 8-10,000/cmm. She was re-admitted on 4.2.53. with the same pattern of symptoms. The B.P. was $\frac{160}{120}$, the first occasion on which hypertension was noted. She was dehydrated and her abdomen was tender. Both her legs were weak and there was increase of muscle tone and a (L) extensor plantar response. Her legs were hyperaesthetic but otherwise there was no sensory abnormality. Her condition improved within a few days. As in other attacks, her initial diarrhoea gave place to constipation.

After her discharge from hospital she remained well until 28.6.54. when she was re-admitted with abdominal and muscular pain, vomiting and constipation of 2 days' duration. There was tenderness and weakness of her legs but no sensory changes. B.P. $\frac{160}{100}$. She improved on symptomatic treatment during the following 10 days and was finally discharged on 3.8.54. She has remained well since then. The urine has contained large amounts of porphobilinogen during attacks.

Family history See family 10. Her maternal aunt died in 1938 after a prolonged illness ending in mental disturbance. Her twin brother complained of intermittent abdominal and leg pain in 1955 and required to be admitted to hospital. Porphobilinogen was never detected in his urine and the porphyrin contents of several specimens were normal. It was considered that this was an hysterical simulation of acute porphyria.

Summary Repeated attacks of acute porphyria for 3 years. Diarrhoea a recurrent feature. Minimal neurological signs. Barbiturates not given since 1951.

Mr. D.W. Age 20 years (1940) Leather manufacturer Case No. 45

This man was admitted to University College Hospital, London, on 1.5.54. because of severe vomiting and prostration which occurred at the end of, and following a journey by sea from the U.S.A. He started to vomit while on board ship and continued to do so in the few days between his arrival in this country and his admission to hospital. He had taken dramamine on his boat journey, though the sea was calm. No barbiturates were taken. He had 4 such attacks of severe vomiting in the past - in 1940, 1946, 1949 and 1951, the first three associated with stormy air trips. In his first attack in 1940 he also had tingling of his extremities, weakness of his muscles and aphonia, but these symptoms passed off in 24 hours. The attack in 1951 came on after an injection of pentothal. There has never been any abdominal pain.

Past History He had asthma and flexural eczema as a child. He still gets asthmatic attacks. He had jaundice in 1945 in Sao Paulo, but this was not associated with vomiting. There is no skin photosensitivity. On examination it was noted that he was dehydrated and mentally clouded. Afebrile. Pulse rate 70-80/minute. B.P. $\frac{115}{70}$. Urine (3.5.54.) contained 41mg/litre of porphobilinogen and 7.58mg/litre of ether soluble porphyrin, later shown by paper chromatography to be Coproporphyrin III with some hexacarboxylic and/or heptacarboxylic porphyrin. There was only a faint trace of uroporphyrin. The urine also gave a moderately strong acetone reaction. The stool porphyrin determination was - coporporphyrin 15.42mg/g dry wt., proto-

phyrin and deuteroporphyrin 3.5mg/g dry wt. Paper chromatographic analysis of the coproporphyrin fraction showed the same pattern of porphyrins as in the urine.

The patient was treated with 5% dextrose in normal saline and 'avomine'. He stopped vomiting and became very much better in a few days. He was discharged on 8.5.54.

Further records 21.5.54. Urine Porphobilinogen - faintly present (confirmed by paper chromatography). Coproporphyrin 1.57mg/litre. Stool Coproporphyrin 2.23mg/g. dry wt. Protoporphyrin and deuteroporphyrin 0.06mg/g dry wt. Blood (Plasma + erythrocytes) - Protoporphyrin 32.8 µg/100 ml. Coproporphyrin 1.33µg/100 ml.

Family history There is a family history of asthma and skin allergy. One brother died from Leukaemia. There is no other history suggestive of acute porphyria. His son excreted normal quantities of porphyrin in urine and stool.

Summary Recurrent attacks of severe vomiting and prostration associated with travel in the past 14 years. No abdominal pain, tachycardia or hypertension. In the 1 attack during which he was observed, he excreted porphobilinogen and much coproporphyrin III in the urine. When the attack had subsided the porphobilinogen and coproporphyrin excretions in the urine had greatly decreased, although they were still abnormally high.

Mr. B.J.P. Age 25 years (1952) Physicist Case No. 46

This man has been perfectly healthy apart from intermittent pain in the right iliac fossa in the past 2 years (1952-1954). The pain comes on in the morning soon after rising from bed and is more often present when he is worried. It is not accompanied by vomiting, nor associated with eating food. In 1948 his appendix was removed because of bilious attacks. There is no skin photosensitivity.

The fact that his porphyrin excretion was abnormal was first noted by Dr. F.W. Darby, who was, at the time, doing a series of normal human urinary coproporphyrin studies. The urinary coproporphyrin of this man was abnormally high. Clinical examination revealed no other abnormality.

Examination of his urine on July 1954 showed porphobilinogen (confirmed qualitatively by paper chromatography), coproporphyrin 1.524mg/litre.

Stool Coproporphyrin 259.2µg/g. dry weight. Protoporphyrin - trace only. Paper chromatography of these porphyrins showed the urinary porphyrin to consist of mainly coproporphyrin III, with some uroporphyrin and some hexacarboxylic porphyrin, while the stool porphyrin was entirely coproporphyrin III.

Family History Of his relatives, only his mother had recurrent abdominal pain. His father had died from syringomyelia in 1951. Urine and stool specimens were obtained from his mother, maternal aunt and his own baby daughter. His mother's stool showed some increase in porphyrin excretion (coproporphyrin 162µg/g. dry weight). The remaining specimens were normal.

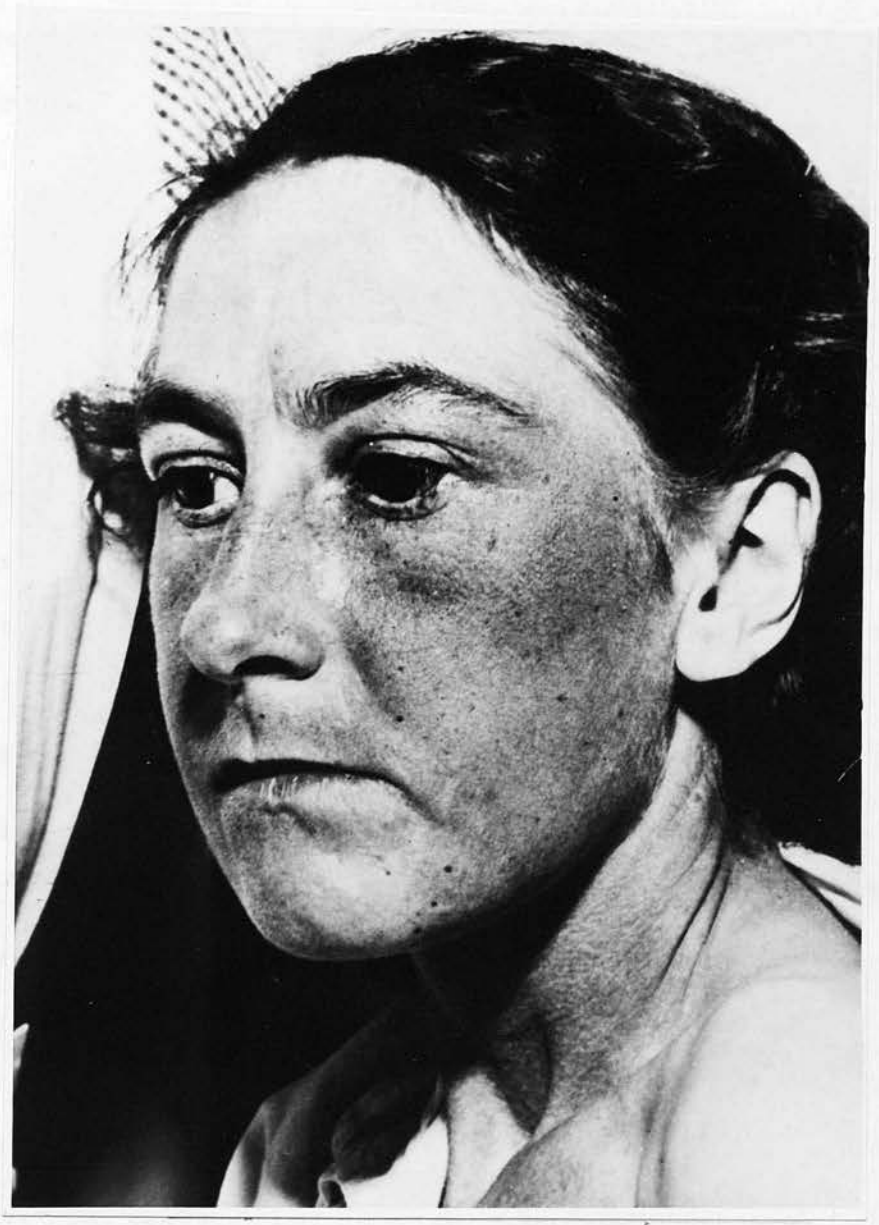


Plate 6a. Appendix.

Case No. 47. Residual pigmentation on cheeks and bridge of nose.

Summary A healthy young man, with only slight recurrent abdominal pain, excretes porphobilinogen in the urine and much coproporphyrin III in the urine and stool.

Miss S.W. Age 28 years (1950) Machinist Case No. 47

This young woman was admitted to the Western Infirmary, Glasgow, on 25.1.52. because of constant colicky lower abdominal pain, vomiting, constipation and the passage of dark urine for 2 days. There was also difficulty in micturition. 9 days before admission she developed a red rash on the bridge of her nose and on both cheeks for which she was given white tablets, probably sulphonamides.

Past History In 1950 she was admitted to the Royal Infirmary, Glasgow, because of pain in the right iliac fossa and the passage of dark urine. A laparotomy was performed and a normal appendix was removed. There was no history of photosensitivity. On examination in January, 1952, it was noted that she was very thin, weak and that her face was pigmented with oedema and vesiculation of her cheeks (plate 6a). Pulse rate 100-120/ minute. Afebrile. B.P. $\frac{120}{80}$ on admission, but she later developed a diastolic hypertension (B.P. $\frac{130}{110}$). Her arms and legs became weak, although she could always move them feebly. There was a selective weakness of the extensors of the wrist and fingers giving the 'tricorne' hand (plate 6b). There was no sensory loss or loss of muscular co-ordination, as far as this could be examined. At one period bilateral extensor plantar

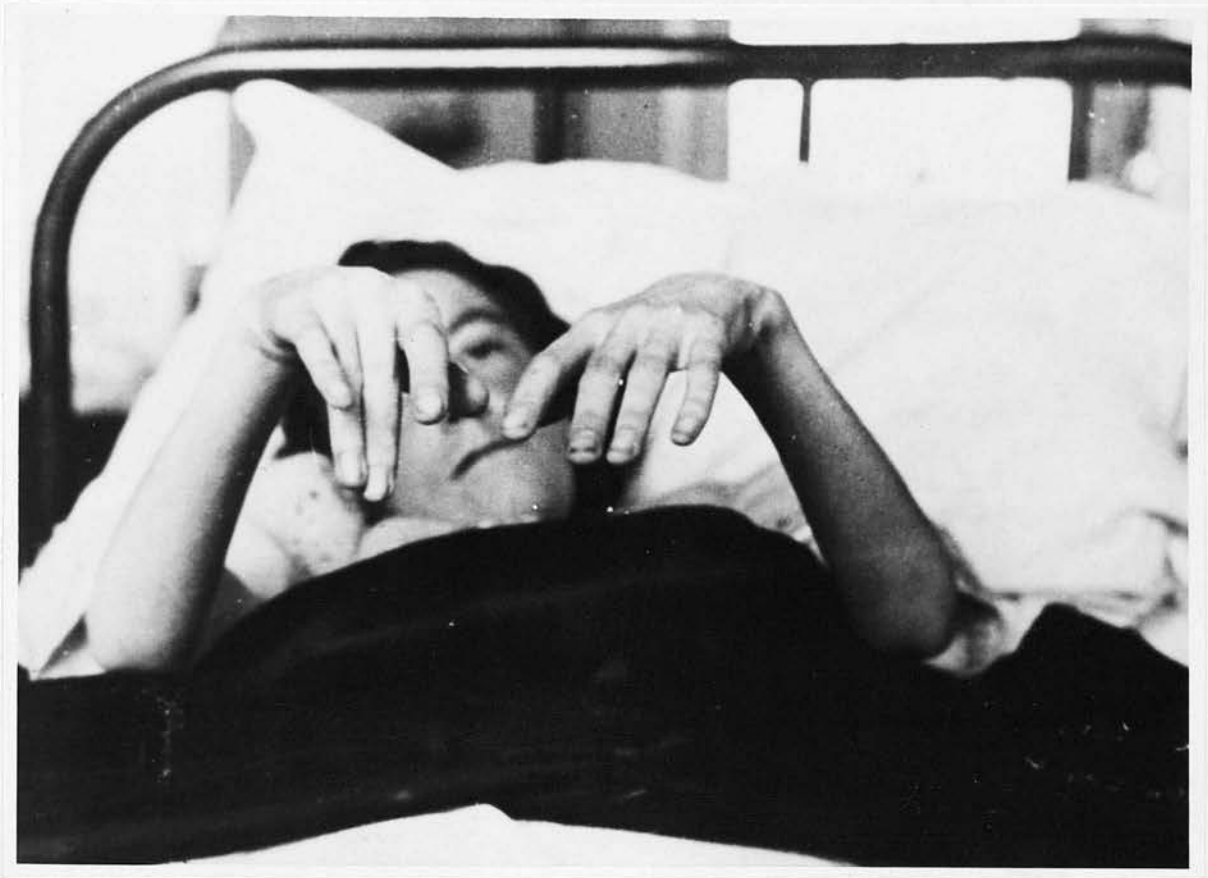


Plate 6b. Appendix.

Case No. 47.

The "tricorne" hand.

responses could be obtained and there were increased ankle and knee tendon reflexes present with ankle clonus. Her abdominal pain continued for several weeks, but latterly she complained of numbness of her abdomen and pains in the legs and scapular region. Her vomiting stopped about 4 weeks after her admission and she was given 0.5 n. saline orally and parenteral normal saline in order to raise her persistently lowered serum chloride. The administration of 100 mg. A.C.T.H. per day (15-21.2.52.) brought about a prompt and sustained restoration of the normal level. During this period her volume of packed red cells fell from 51% (31.1.52.) to 42% (16.2.52.). There was a gradual improvement in her condition after 6 weeks and she was eventually discharged on 4.4.52. to a convalescent home.

There were no psychological changes throughout her illness apart from some reasonable anxiety over her condition.

Throughout her illness she had amenorrhoea which continued till May, 1952. She has reported regularly since then till September, 1954, and during all this time she has remained very well and has gained over 1 stone in weight.

Further records Her urine throughout her illness contained much porphobilinogen. The maximum uroporphyrin concentration was 34mg/litre.

Serum electrolytes (Table 1) - before and after A.C.T.H.

Liver function tests (Table 2). Plasma did not fluoresce.

Examination of her fundus on 13.2.52. when B.P. was $\frac{124}{104}$ showed a "Fundus Hypertonicus", with spasm of the retinal arteries (Plate 7). The fundus became normal in appearance when her B.P. returned to normal.

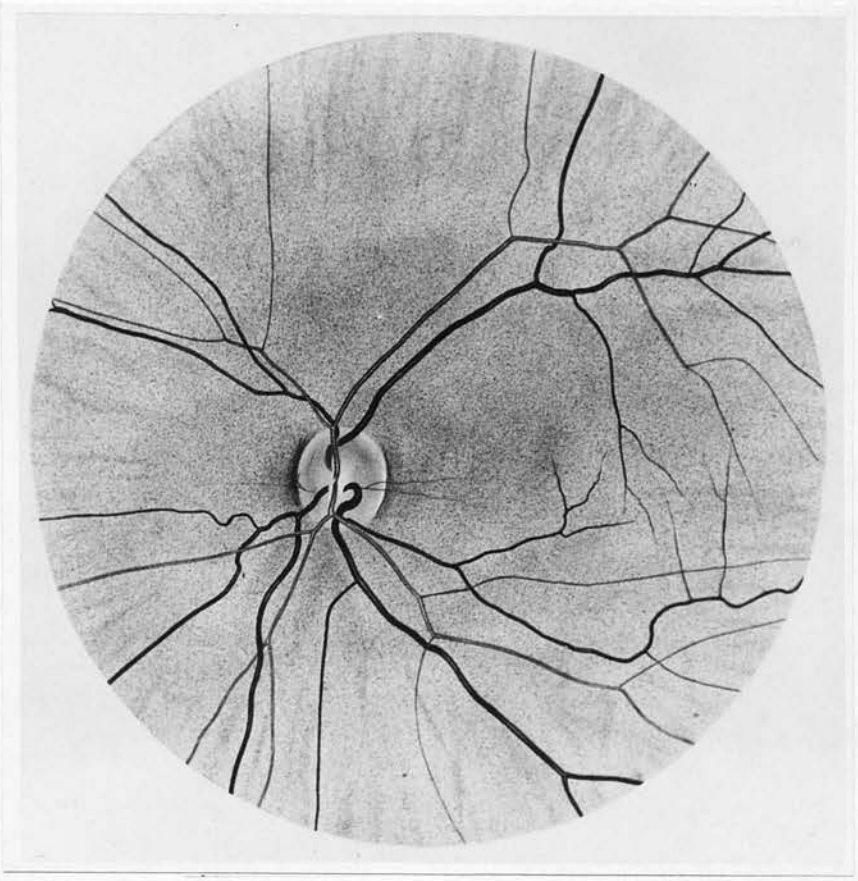


Plate 7. Appendix.

Case No. 47. Fundus Hypertonicus.

I wish to thank Mr. Donald, Ophthalmology department Western Infirmary, Glasgow, for this painting.

Date	Stool		Urine		
	Copro-porphyrin µg/g. dry	Proto-porphyrin µg/g. dry	Copro-porphyrin µg/litre	Uro-porphyrin mg/l.	Porpho-bilinogen
6.3.52. (active phase)	161	644		18.41	+ + +
¤25.4.52.	340	401	58.5	0	0
¤10.8.54.	404	203	484	0	0

¤ phase of remission

Summary The general history of this girl is characteristic of acute porphyria. The skin lesion in the beginning of the 1952 attack is puzzling, since there was no photosensitivity either before or after this occasion. The urine and stool in remission contained increased amounts of porphyrins.

Miss E.R. Age 15 years (1951) Schoolgirl Case No. 48

This girl was well until 17.5.51. when she developed a sore throat. She was treated with penicillin and sulphonilamide. The next day she complained of pain in the lower abdomen and in both hips. The pain was constantly present and associated with nausea, vomiting and constipation. On 20.5.51. she had headache with epistaxes and was said to have had two "convulsions" with

stiffness of her body, but without loss of consciousness. She was admitted next day to the Western Infirmary, Glasgow. The pain persisted for about 1 week and she was discharged shortly after this. B.P. $\frac{130}{80}$. Pulse rate 120/minute. Afebrile. Her urine contained much porphobilinogen.

She was well until September, 1952, when she had a 'cold' and this was treated with aspirin powders. Shortly afterwards she complained of lower abdominal pain radiating down both thighs, and also pain in the lumbar region. There was no vomiting or constipation. She was re-admitted to the Western Infirmary, Glasgow, on 24.9.52. B.P. $\frac{140}{88}$. Pulse rate was high throughout this attack and maximally 160/minute. There was a low grade pyrexia with a maximum of 101.2°F. W.B.C. 7,200/cmm. Hb. 12.5 G%. Her pain continued for about 15 days after her admission to hospital. On 29.9.52. she had a sudden partial loss of vision, lasting a few hours. About 5 days later she became confused, hallucinated and hysterical and almost unmanageable for some time, but settled down in a few days. She became so homesick and unhappy that she was allowed to go home. She remained unwell and was given 300,000 units daily of distaquaine penicillin for 6 days, as well as phenobarbitone gr. $\frac{1}{2}$ nightly. Her condition deteriorated throughout this period and she was then admitted to Ayrshire Central Hospital, where she died with respiratory paralysis, after having been placed in an artificial respirator for 10 days.

<u>Further records</u> Becosyn 50 mg. t.d.s. and priscol 20 mg. t.d.s. given throughout second attack without apparent effect. There

is no family history of note. She had not menstruated up to the time of her first admission.

Summary 2 attacks, the second fatal, in a girl of 15 years. The respiratory paralysis before death was preceded by barbiturate administration.

Mr. W.B. Age 36 years (1954) Minister Case No. 49

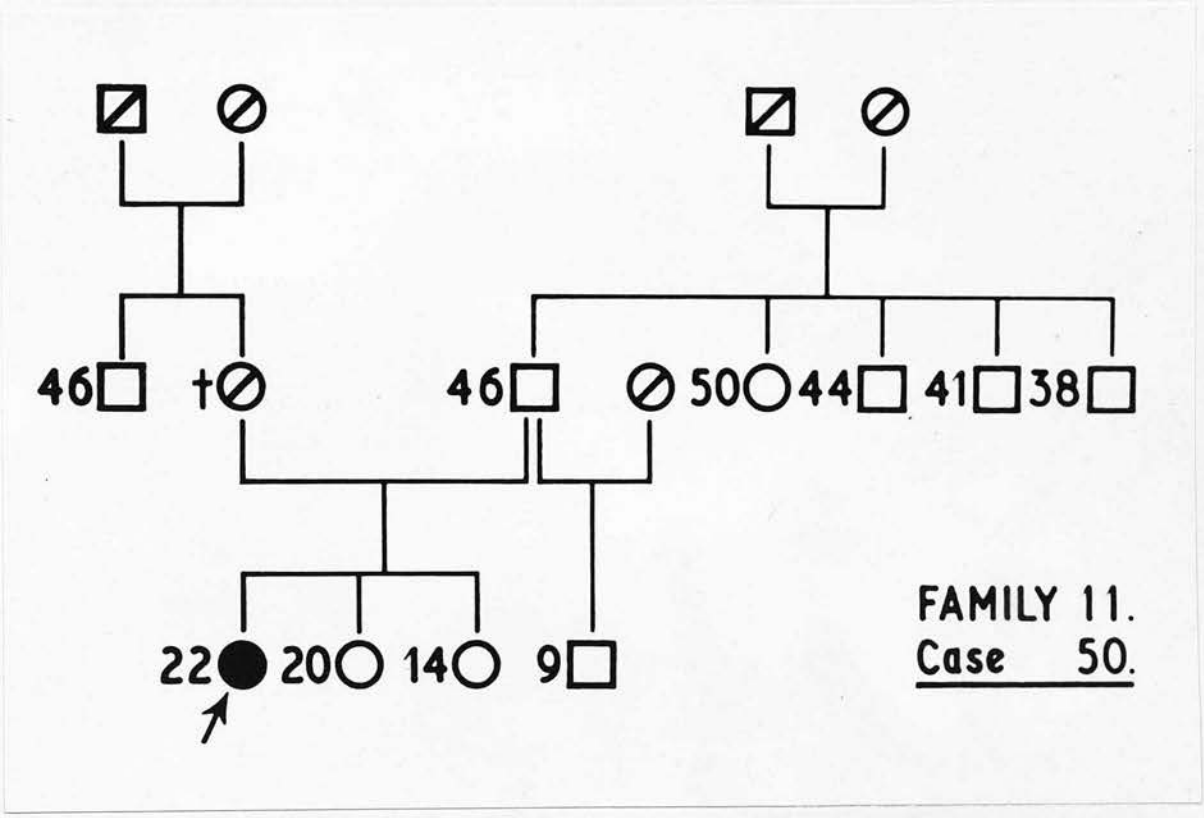
This man was well until September 1954, when he developed a sore throat for which he was given sulphonamides. Shortly after this (3/10/54) he complained of colicky epigastric pain at first constant and later intermittent, lasting for 2-3 weeks during which time he was also constipated. No cause for the pain could be found. He was seen by a psychiatrist because he was a difficult patient. He had no neurological features, apart from bilateral nystagmus. B.P. $\frac{160}{110}$. P.R. 95/minute. Temperature 97.2°F. E.S.R. 17 mm/hour. Hb. 14.4 G%. W.B.C. 4,400/cmm. Intermittent proteinuria. He gradually recovered and had no further attack for $1\frac{1}{2}$ years. He was given barbiturates after the pain started but did not develop paralysis. Urine porphobilinogen 68 mg/litre. Coproporphyrin 317 µg/litre.

Family History His only child is an epileptic. There is no porphobilinogen in the urine of this child.

Summary Solitary attack following a sore throat and sulphonamides. Barbiturates given during an attack but paralysis did not develop.

Mrs. M.B. Age 22 years (1954) Housewife Case No. 50

This young woman developed migraine in February 1953 and was put on phenobarbitone gr.$\frac{1}{2}$ b.d. or t.d.s. Although this made her feel giddy, sickly and dazed, she continued to take the drug until May 1954. She became progressively more depressed and after a peculiar "stiff" turn in which she felt depressed and cried, she was sent to the Maudsley Hospital for treatment. An E.E.G., performed there, was normal and she was put on sodium amytal nocte which was continued until 1st September, 1954. In August 1954 she complained of vague, intermittent abdominal pain, lethargy and depression. She then went on holiday to the Isle of Wight. The pain got more severe, radiating from the loins down both sides to the lower abdomen. It was constantly present, though it varied in intensity, and was associated with vomiting. She was admitted to hospital. Since she also had albuminuria, a diagnosis of pyelitis was made and she was treated with phenobarbitone and sulphonamides. Five days later she developed twitching movements in the (R) side of the face and loss of consciousness and subsequently had 4 further epileptiform attacks. When she recovered from these she was confused and drowsy and was transferred to a mental hospital. She still had abdominal pain, vomiting, anorexia and complained of some numbness from the waist downwards. It was noted that she passed dark urine and a diagnosis of acute porphyria was established. She was then transferred to University College Hospital, London (22.9.54.). She was drowsy, apathetic and still complained of abdominal pain and numbness of lower limbs.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FAMILY 11.
Case 50.

B.P. $\frac{140}{100}$. P.R. 116/minute. There was weakness on straight leg raising and absent knee jerks. Slight pyuria and bacilluria (B. coli). Fundi normal. Urine porphobilinogen 7.7 mg/litre. Blood urea 27 mg.%. Hb. 15 G%. W.B.C. 7,400/cmm. She had lost over 1 stone in weight since the onset of her illness and had to be persuaded to take an adequate diet. The abdominal pain continued and was now associated with diarrhoea. The pain gradually eased and her mental condition improved. She was discharged 11/10/54. Since June 1954(until 1956) she has been very well, entirely free of symptoms.

Previous illnesses Nocturnal enuresis since childhood. She is liable to hay-fever in summer.

Family history 9 other members of family tested for porphobilinogen in urine and all found negative (see family No. 11).

Summary Prolonged barbiturate administration for migraine followed by depression and later epilepsy and neurological symptoms. Porphobilinogen in urine.

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The medical superintendents of the Victoria Infirmary Glasgow and Broadstone Hospital, Port Glasgow.

TABLE 1 (Appendix)

Serum or Plasma Electrolytes

Case No.	Date.	Sodium (m.eq.l.)	Potassium (m.eq.l)	Chloride (m.eq.l.)	Bicarbonate (m.eq.l.)	Urea mg.%
Normal Values.		(134-148)	(3.5-5.5)	(97-107)	(24-31)	(20-40)
1	10.11.53. (died 11.11.53)	121	5.3	80	27.3	58
3	12. 7.54.		4.1	98	35.2	75
	16. 7.54.		3.1	78	39.6	99
	18. 7.54.	130	3.15	88	33	67
	24. 7.54. (died 25.7.54)	148	4.5	90	40	268
21		134	3.7	100		
25	29. 1.52.	130	4.85	101		
	20. 2.52.	136	3.7	87		
	21. 2.52.	139	4.7	93		
	25.2.52.	135	4.5	91		
	27.2.52.	137	5.1	95		
	3.3.52.	139	4.6	103		
	5.3.52.	147	5.1	103		
32	12.3.52.	154	4.6	93		
33	21.8.53.	153	4.6	100		
38	18.8.54.	142	4.55	88	25	
47	29.1.52.	114	4.6	68		
	1.2.52.			67		
	5.2.52.			71		
	12.2.52.			64		
	18.2.52.	142	3.84	93		
	19.2.52.	152	4.1	94		
	21.2.52.	143	3.58	99		
	23.2.52.	146	3.84	99		
	25.2.52.	139	4.23	95		
	28.2.52.	146	6.66	100		
	1.3.52.	142	5.38	96		
	3.3.52.	145	4.23	100		
	11.3.52.	139	4.48	96		
	17.3.52.	139	4.36	104		
	27.3.52.	135	5.38	96		
	24.4.52.	156	4.87	98		

A.C.T.H.100mg/d. 15-21.2.52.

TABLE 2

Empirical Liver Function Tests

Case No.	PLASMA Protein			Alkaline Phosphatase (King Armstrong units)	Thymol Turbidity (units)	Thymol Flocculation	Cephalin Flocculation	Bilirubin (mg%)
	Total g%	Albumen g%	Globulin g%					
1	6.6	4.1	2.5	6	1			0.2
3				26	2			0.7
4	6.85	3.22	3.30					
7					3		+ + +	
9	6.2	3.8	2.4	8	0			
15	6.4	4.1	2.3		2			
17				13	1			0.2
21	5.3	2.9	2.4	10	2			
25	6.85	4.57	2.0	8	2	0		0.7
27	7.4	5.8	1.6	6	5			
30				6	4	14		0.2
32	7.4	5	2.4	6	1	0		0.3
33	7.12	3.9	1.3	25	2			0.5
34	4.5	3.1	1.4	4	1	0		
38					3			0.7
47	8.0	4.9	3.1	8	3			0.3
49	7.1							0.5
50	6.6	3.5	2.8					

Table 3. POST-MORTEM TISSUE ANALYSIS

Results expressed as ug/G of wet tissue.
A blank indicates that analysis was not done.

CASE	LIVER				KIDNEY				BONE MARROW				SPLEEN			
	Pbg	Uro	Copro	Proto	Pbg	Uro	Copro	Proto	Pbg	Uro	Copro	Proto	Pbg	Uro	Copro	Proto
Control I 50 years CORONARY THROMBOSIS	0	0	1.1	1.0	0	0	0.3	1.9	0	0	1.1	0.9	0	0	0.8	0.7
Control II 78 years CARDIAC FAILURE PNEUMONIA	0	0	0.2	1.3	0	0	0.3	0.7	0	0	0.6	0.9	0	0	0.4	0.5
No. 1	50	1.9	1.8	3.3					0	0	0.2	0.8	0	0	0.3	0.8
No. 2	36	8.5	1.2	0.6					0	0	0.2	0.2	0	0	0.2	0.2
No. 3	19				51				0				0			
No. 23	18	9.6	0.9	0.8												
No. 24	17		0.4	0.7					0							
No. 32	+++								0				0			
No. 43*	31	2.9	0.7	0.5	49	14	7.7	2.4	0	0	0.5	0.2	0	0	0.4	0.5

* Heart, lung, spinal cord, skeletal muscle, thyroid, adrenal, pancreas, did not contain porphobilinogen

Pbg = Porphobilinogen
Uro = Uroporphyrin
Copro = Coproporphyrin
Proto = Protoporphyrin

TABLE 4

Summary of Neuropathology (Gibson and Goldberg, 1956)

Case No.	Peripheral Nerves	Autonomic Nervous System	Spinal Posterior Root Ganglia	Spinal Cord	Medulla	Cerebellum	Cerebrum
2	Demyelination e.g. Sciatic Nerve	Demyelination of Vagus Nerve	Changes Generally Slight and Inconstant	Chromatolysis of Anterior Horn Cells and of Lateral Horn cells.	Chromatolysis of all Nuclei, particularly Dorsal Vagal Nucleus, Nucleus Solitarius Nucleus Ambiguus. Reticular Substance(Cases 32 & 43)	Foci of Demyelination in White Matter	Perivascular, foci of demyelination in parietal, frontal & occipital lobes, mainly in parietal lobes. Slight oedema. Slight chromatolysis of some cortical cells.
24	Demyelination e.g. Sciatic Nerve						
32	Demyelination e.g. Phrenic Nerve	Demyelination Vagus Nerve and Sympathetic Chain					
43	Demyelination e.g.Brachial Plexus. Musculo-Cutaneous N.						
36	*BIOPSY Demyelination Dorsal Interosseus Nerve of Foot						

* Drury, 1956.

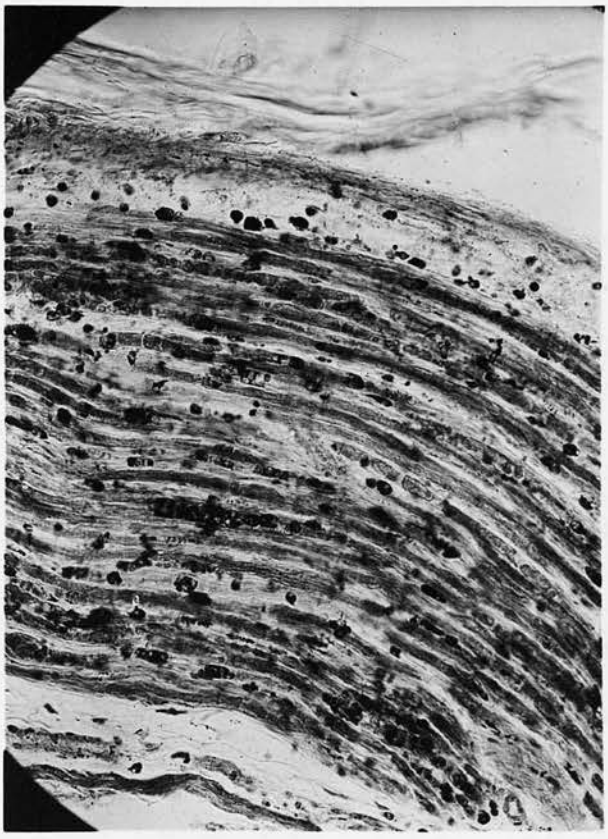

Plate 8. Appendix.

Case No. 32. Right phrenic nerve undergoing demyelination, with phagocytosis of released lipoid. Free macrophages are seen in oedematous spaces beneath the endoneurium.
Sudan IV X 120.

I am indebted to Dr. James B. Gibson for his permission to include Plates 8, 9, and 10.

Plate 10. Appendix.

Case No. 43. Parietal white matter, showing a small perivascular focus of demyelination.
Spielmeyer. X30.

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