BRITISH MEDICAL JOURNAL

LONDON SATURDAY DECEMBER 16 1939

CLINICAL PORPHYRINURIA WITH REPORT OF A CASE OF THE ACUTE IDIOPATHIC TYPE

BY

F. G. CHANDLER, M.D., F.R.C.P.

G. A. HARRISON, M.D.

AND

C. RIMINGTON, M.A., Ph.D., B.Sc.

(From St. Bartholomew's Hospital and the National Institute for Medical Research)

Porphyria of such a degree that the abnormal colour leads to examination of the urine is rare (see below). Study of cases, however, may be of considerable importance in furthering our knowledge of the building up of haemoglobin and of the metabolism of pigments in the human body. Similar studies are clearly indicated in lesser grades of porphyria which are not suspected from the colour of the urine but which require for their recognition quantitative determinations of, or special qualitative tests for, the porphyrins. Indeed, the separation of "clinical" or "frank" porphyria from the lesser grades is obviously arbitrary, but has clinical value in two respects: first, the urinary colour itself calls for special investigation; and, secondly, it seldom happens that the porphyria is clinically of paramount importance unless the urine is abnormal in colour. For these reasons discussion is here limited to clinical porphyria.

Probably relatively few physicians in this country are familiar with the brilliant work of Hans Fischer and his colleagues on the chemistry of the porphyrins. Since studies of porphyria and all hypotheses of the causes of porphyria must be subject to this knowledge, it is essential to give at least a brief summary of it. Owing to the almost complete absence of a reasonable account of acute idiopathic porphyria from British textbooks, our clinical experience of the case reported below should be of value.

Nomenclature and Summary of Chemistry of Porphyrins

Haemoglobin belongs to the group of chromoproteins and consists of globin united with a complex pigment, haematin. Haematin is an iron derivative of porphyrin. The porphyrins themselves contain no iron and are not proteins. Haemin is the hydrochloride of haematin. It has been known for many years that when iron is removed from haemin by the action of acids a porphyrin is formed, called "haematoporphyrin" by Nencki, its discoverer. It was for long assumed that the porphyrins in urine were the same, hence the employment of the term "haematoporphyrinuria," which is even now sometimes seen in clinical literature. Later it was shown that

only by the use of mild reagents such as acetic acid and hydrazine upon blood or haemin is it possible to separate the corresponding porphyrin without alteration. This natural porphyrin is designated "protoporphyrin" ($C_{34}H_{34}N_4O_4$). Haematoporphyrin ($C_{34}H_{38}N_4O_6$), which results when a drastic reagent such as concentrated sulphuric acid is used, is a chemical artefact and not a naturally occurring substance. Hans Fischer showed that the main porphyrin in the urine in congenital porphyria was different from protoporphyrin, and called it "uroporphyrin." The chief porphyrin in the faeces in the same disease was different again, and Fischer called it "coproporphyrin" (*syn.*, stercoporphyrin).

It has long been known that even in health the urine contains minute traces of a porphyrin. By working up enormous volumes this has been isolated and shown to be coproporphyrin. Nowadays, therefore, the term "porphyria" is employed to denote an excretion of urinary porphyrin in excess of the normal amounts, and the porphyrins present may be coproporphyrin or uroporphyrin, or both. Similarly, the faeces may contain a mixture of porphyrins, but in this excrement the list is extended; in addition to coproporphyrin and uroporphyrin there may be protoporphyrin (by degradation of haemoglobin ingested or from haemorrhage into the gut), deuteroporphyrin (by degradation of haemoglobin or myohaemoglobin), and others.

Hans Fischer has synthesized haemin, which is easily prepared from blood in crystalline form (Teichmann's crystals), and has shown that it is $C_{34}H_{32}N_4O_4FeCl$, with the following structural formula:

FIG. 1.—Structure of haemin.

Fischer and his colleagues have established the formulae of many of the porphyrins, some of which are given in

the table below. The parent substance of them all is porphin, $C_{20}H_{14}N_4$ or $C_{20}H_{16}N_4.H_8$, with the following structure:

FIG. 2.—Structure of porphin.

The different porphyrins are obtained by substitution of different groups (methyl, ethyl, vinyl, acetic, propionic) for the hydrogens in the β , β' position in the four pyrrole rings of porphin. The corresponding haemins are obtained by attachment of $Fe^{+++}Cl$ to the nitrogens of the four pyrrole rings (see formula of protohaemin above). In the corresponding haematins (oxyhaematins) $Fe^{+++}OH$, and in the reduced haematins Fe^{+} (ferrous), is in the same position.

The following table summarizes the formulae of the substances which have been or are about to be mentioned:

Substance	Ring-structure	Side-chain Groups
Porphin	$C_{20}H_{14}N_4$	8H
Aetioporphyrin ..	$C_{20}H_{14}N_4$	4(CH ₃) and 4(C ₂ H ₅)
Protoporphyrin ..	$C_{20}H_{16}N_4$	4(CH ₃); 2(CH ₂ CH ₂ COOH) and 2(CH:CH ₂)
Haemin (protohaemin)	$C_{20}H_4FeClN_4$	4(CH ₃); 2(CH ₂ CH ₂ COOH) and 2(CH:CH ₂)
Coproporphyrin ..	$C_{20}H_{16}N_4$	4(CH ₃) and 4(CH ₂ CH ₂ COOH)
Uroporphyrin ..	$C_{20}H_{16}N_4$	4(CH ₂ COOH) and 4(CH ₂ CH ₂ COOH)
Deuteroporphyrin ..	$C_{20}H_{16}N_4$	4(CH ₃), 2(CH ₂ CH ₂ COOH) and 2H
Bilirubin	Porphin ring opened	

This is complicated, but becomes more so when the fact is considered that numerous isomers of these compounds are possible, depending upon the order of arrangement of the substituent groups upon the pyrrole rings; Fischer *et al.* have synthesized many of these. There are fifteen possible isomers of protoporphyrin, but by suitable treatment a simpler porphyrin—aetioporphyrin—is obtained, of which there are only four possible isomers, designated, for convenience, aetioporphyrins I, II, III, and IV.

FIG. 3.—The four aetioporphyrins. Fischer's abbreviated formulae, showing positions of substituents in β , β' positions in pyrrole rings.

These may be regarded as the starting-point of four series of derived pigments, I, II, III, and IV. So far, in

Nature porphyrins of the I and III series alone have been encountered, and the difference between these will be seen to lie only in the exchange of positions of two neighbouring groups in one of the rings. The structures of the naturally occurring coproporphyrins and uroporphyrins are reproduced in Figs. 4 to 7.

FIG. 4.—Coproporphyrin I: $C_{36}H_{38}N_4O_6$.

FIG. 5.—Coproporphyrin III.

FIG. 6.—Uroporphyrin I: $C_{40}H_{38}N_4O_{10}$.

FIG. 7.—Uroporphyrin III.

Fischer *et al.* showed that haemoglobin and chlorophyll belong to series III, whereas the porphyrins in the excreta in disease belong mainly to series I. This is important, because it excludes the possibility of the porphyrins excreted in disease being derived by breakdown from haemoglobin. Passage from series III to series I would necessitate breakdown into the constituent pyrrole units and rebuilding into the new configuration—a sufficiently unlikely occurrence.

In addition to deciding whether the porphyrins in urine are coproporphyrin or uroporphyrin, or both, it is now necessary to show whether they belong to series I or III, or both, if data are to be obtained on which sound hypotheses can be built as to their origin.

In the methods employed in this work advantage is taken, as usual, of differences in solubility, crystalline form, melting-point, mixed melting-point, spectroscopic properties, etc. Since the free porphyrins do not possess sharp melting-points, they are converted into their crystalline methyl esters, which have well-defined melting-points for identification. So far as possible the investigations are carried out quantitatively; but they are laborious, and require considerable experience owing to the small amounts of the porphyrins and the complexity of biological fluids and tissues.

The melting-points of the methyl esters are: coproporphyrin I, 252° C.; coproporphyrin III, 145° C. (remelt 172° C.); uroporphyrin I, 302° C.; uroporphyrin III, 261° C.

FIG. 8.—Crystalline porphyrin esters. The uroporphyrin I ester was from bones (Rimington (1936), Fig. 13), the other three esters from the urine of our case of acute idiopathic porphyrinuria.

Wave-lengths ($m\mu$) of Absorption Bands of Porphyrins

Acid Spectra (25 per cent. HCl):

Proto-	602	557
Copro-	594	551
Uro-	597	554

Alkaline Spectra (N/10 alkali):

Proto-	645	591	540	?
Copro-	618	569	539	504
Uro-	612	561	539	504

Neutral Spectra (in ether):

Neutral Spectra (in ether):						
Proto-	633	585	576	537	502	
Copro-	624	597	578	568	529	498
Uro-	Insoluble in ether.					

Classification, Frequency, and Literature of Clinical Porphyrinuria

Adhering to the definition already given, there are three types of clinical porphyrinuria: (1) congenital (less than thirty cases published); (2) due to drugs, such as sulphonal, trional, etc. (about 100 cases published); (3) acute idiopathic, of unknown cause—about fifty cases recorded in the older literature; recently 103 from Sweden by Waldenström (1937). Several schemes of classification have been proposed, but need not be considered in detail here. Waldenström (1937) has recently made the suggestion that the term "porphyry" should be reserved to embrace conditions 1 and 3, in which the constitutional element predominates, while all other types of increased porphyrin excretion should be grouped under the heading "porphyrinuria."

The literature dealing with porphyrins and porphyrinuria is enormous, and in this brief summary only a few reviews and long articles can be mentioned, from which, however, the reader will have no difficulty in obtaining an introduction to it. Congenital porphyrinuria is dealt with by the

late Sir Archibald Garrod (1923) in his *Inborn Errors of Metabolism*. Reference should also be made to Mackey and Garrod (1922, 1926) and to Garrod (1936). For the German work on the case of Petry, see Fischer, Hilmer, Lindner, and Pützer (1925) and Borst and Königsdörffer (1929). For congenital porphyrinuria in cattle, see Fourie (1936), Rimington (1936), and Fourie and Rimington (1938). Porphyrinuria due to drugs is reviewed by Günther (1922). Papers by Garrod and Hopkins (1894-6) and Drigalski (1937) contain interesting points. Increased porphyrin excretion in pellagra has been dealt with by Beckh, Ellinger, and Spies (1937). Acute idiopathic porphyrinuria is reviewed by Günther (1922). See also Mason, Courville, and Ziskind (1933), Waldenström (1934, 1939), Mertens (1937), Turner (1938), and Geissler (1939). For general reviews consult Fischer and Zerweck (1924), Fischer (1930), Carrié (1936), Vannotti (1937), Waldenström (1937), Hernando (1938), and Watson (1938).

Chief Signs and Symptoms of Clinical Porphyrinuria

Only the salient features are summarized in the following table. Further details concerning the acute idiopathic form are given below.

Type of Porphyrinuria	Congenital	Due to Drugs	Acute Idiopathic
Red urine	From birth	After much drug	Usually in adult life
Photosensitivity ..	Severe	Absent	Absent or very mild
Pigmentation of bones and teeth	Present	Absent	Absent
History of drugs ..	No	Yes	No
Abdominal and other toxic symptoms	None	Present	Present
Sex incidence.. ..	Mostly males	Usually females	Mostly females

A Case of Acute Idiopathic Porphyrinuria

The patient was a male insurance clerk aged 28, and was referred to St. Bartholomew's Hospital by Dr. K. R. Bali on September 19, 1938. He was under the care of one of us, complaining of severe abdominal pain, constipation, vomiting, loss of weight, occasional frontal headache, and insomnia.

HISTORY OF PATIENT

He had had measles and scarlet fever in childhood. In 1929 or 1930 he had pain over the whole abdomen, in occasional attacks, usually coming on ten to thirty minutes after a meal and frequently relieved by an alkaline powder. These attacks, each lasting about a fortnight, were separated by intervals of three or four weeks and became slowly more severe, until in 1937 he started to vomit. In 1937 his appendix was removed at Old Church Hospital, Romford; it was said to be chronically inflamed. Following this he had pneumonia and a left-sided pleural effusion. He left Romford much improved, but was admitted to St. Bartholomew's Hospital because the pleural effusion persisted. A skiagram of the chest revealed some dense calcareous foci in the right upper lobe, and the erythrocyte sedimentation rate was 49 mm. in the first hour. He was sent to Ventnor Sanatorium for six months, at the end of which period he was free from symptoms. There was never any specific evidence of tuberculosis, and never any haematemesis or melaena.

The patient believed it was in 1935 that he first noticed that his urine would sometimes darken on standing; darkening of the urine was not observed at Ventnor. In March, 1938, he had a bad attack of abdominal pain and vomiting, and his urine was red. There was no history of drug-taking, apart from the alkalis mentioned above, and six Dr. Cassell's tablets just before his final admission on September 19, 1938. His father suffered from disease of the kidneys, and passed urine the colour of blood. Two sisters and three brothers are living, but there is no history of any of them passing abnormally coloured urine. One sister died from pulmonary tuberculosis. There is no consanguinity between the parents.

The final attack started two weeks before his admission to St. Bartholomew's Hospital. He had been unable to keep anything down, and had no desire for food. He had lost a stone in weight in three weeks. On examination he was thin and sallow-looking. His whole body was a little brown, and

this was most noticeable on his face, on which a few pigmented spots were present, but no scars. There was no patchy bronzing and no hypertrichosis. On the front of both knees there were six or seven round scars 1/4 to 1/2 inch in diameter, believed to be due to falls as a boy, but there were none on the elbows; he had a few septic spots on the trunk, each about 3 mm. in diameter. There was no definite evidence of photosensitivity. The eyes were normal, the tongue was furred, the teeth sound and not stained, and the mucous membranes were normal in colour. The temperature was occasionally 99°, but generally normal; the pulse was about 100, and the respiration rate normal.

EXAMINATION

Nothing abnormal was detected in the heart, and the blood pressure was 120/96. There were no physical signs in the chest, but a skiagram again showed the calcareous foci mentioned above; tubercle bacilli were not found in the sputum, which yielded a mixed growth of organisms in which pneumococci predominated. The erythrocyte sedimentation rate was 5 mm. in the first hour (September 21), and the Wassermann and sigma reactions were negative. There was generalized tenderness over the abdomen, but no mass was felt at first, and the liver and spleen were not enlarged. A barium meal on September 22 revealed nothing abnormal, and another on September 28 showed that though evacuation took a considerable time there was no evidence of obstruction. Tests for occult blood in the faeces were negative. Apart from brisk reflexes, nothing unusual was found in the nervous system.

The urine was normal in colour immediately after it was passed, but rapidly darkened, becoming deep brown or reddish brown in a few hours. Thus a sample received in the laboratory on September 21 was of a deep port-wine colour and acid in reaction; spectroscopically there were two absorption bands in the green very much like those of oxyhaemoglobin, and a band at the junction of the green and blue. Microscopically there were no red cells. Chemically there was a minute trace of protein, but the pyramidon test for blood was negative; Schlesinger's test for urobilin was strongly positive. By means of the tests given later it was shown without any doubt that the oxyhaemoglobin-like spectrum was due to a metallo-porphyrin. Subsequently many samples of urine were examined by two of us, and all showed the same behaviour, though of course the concentration of metallo-porphyrin and of urobilin varied. The effect of heat was as described under "Urinary Tests." One of us (for preliminary communication see Rimington, 1938a) separated the porphyrins from the urine and faeces by the methods outlined later.

The serum calcium was 9.3 mg. (October 24), the blood urea 52 mg. per 100 c.cm. (October 4), and no abnormal pigments were detected spectroscopically in the plasma or in the laked red cells (October 24).

CLINICAL HISTORY

The abdominal symptoms became worse, and occasionally the patient complained of pain in the arms, legs, and neck. By October 2 the cramp-like abdominal pain was so severe and the vomiting so frequent and distressing that he begged for something to be done. At this time a hard mass was felt in the epigastrium, which was slightly movable. A surgeon was called in, and agreed to operate, though very reluctantly, on the grounds that the lump was probably a malignant mass and the case hopeless. In spite of the fact that we knew there is intense spasm of the intestinal tract in acute idiopathic porphyria, the one of us who was responsible for the patient's welfare urged operation because of the pain, the mass in the abdomen, and the fact that in many books porphyria is described as associated with certain diseases of the stomach. It is possible that the last clinical point is not sound, and that there is no conclusive evidence of the association of organic disease of the stomach with clinical porphyria; in that case the statement repeated in one textbook after another should be deleted.

A laparotomy was performed on October 12. The stomach was in extreme contraction, suggesting at first a leather-bottle stomach, this producing the apparent tumour. Otherwise nothing abnormal was found. The patient recovered quickly, and for some unknown reason was entirely free from symptoms for several days, and there was much less porphyrin in his urine. On October 17 the pain returned, and thereafter the porphyria increased.

On October 19 Dr. Denny-Brown kindly made a neurological examination. He found no definite sensory changes, no convincing focal wasting, and only a general weakness.

All the reflexes were brisk; there was no tenderness of muscles and no loss of vibration or position sense. He did not think there was a true peripheral neuritis, and felt certain that the pains were not neuritic.

The patient's condition rapidly deteriorated, and he begged for more and more opium. There is little doubt that he suffered acute and intolerable pain, and it would have been kinder to increase the dose of omnopon, which was kept within reasonable limits in the hope of recovery. Not obtaining the complete relief from pain for which he asked, the patient wished to be discharged, and on October 24 was transferred to the care of his own doctor. On October 25 he was unable to use his arms, and there was great weakness in the hands and legs. On October 27 his temperature was still normal, but he developed a cough. Increasing asthenia led to his death on October 28. A necropsy was performed, but unfortunately the pathologist was not aware of the findings in hospital, so there was no special study of the bones or central nervous system and no material was kept for chemical investigation. We do not consider, therefore, that a report of the findings would be of any value. (For post-mortem histology, cf. Geissler, 1939.)

During the patient's stay in hospital vigorous symptomatic treatment was instituted, which need not be detailed. Eight intravenous injections, each of 10 c.cm. of 10 per cent. calcium gluconate, were given, but afforded no relief.

Simple Urinary Tests in Porphyrinuria

The colour of the urine varies from brown to red, and is often described as "port-wine." Sometimes the fresh urine is more or less normal in colour, but slowly darkens so that in a few hours it is obviously abnormal, and ultimately may become deep reddish brown or almost black. This is due to the excretion of porphyrinogens and other chromogens of unknown constitution which are slowly changed in light and air to the corresponding porphyrins and pigments. It has been recognized for many years that the pigmentation is due mainly to the unknown pigments. To produce the change rapidly the urine may be heated at 100° C. for half an hour; the darkening starts in a few minutes and, so far as our limited experience goes, is complete in half an hour. Potassium permanganate and other oxidizing agents have been recommended to produce the change, but in our hands, though they might cause darkening, they did not produce porphyrins judging from spectroscopic examination. We therefore recommend that in routine examinations the urine be heated at 100° C. for half an hour before it is concluded that there is no gross porphyria.

It is, of course, the examination with the spectroscope that establishes the diagnosis of porphyria. In a typical case the four-banded spectrum of alkaline porphyrin is seen when the urine is examined in an ordinary boiling-tube (about 1 inch in diameter). The alkaline spectrum is seen at all natural reactions of urine; the acid spectrum is produced only by the addition of a mineral acid. Obviously the concentration of porphyrins varies, and it is necessary to have a working rule as to how thick a layer of urine shall be viewed before declaring a negative result. We suggest that clinical porphyria can safely be excluded if a 4-inch or 5-inch layer* of urine does not show the absorption bands (compare Günther, 1922.) The effect of heat should, however, be tried as mentioned above; in highly pigmented urines it is impossible to see through so thick a layer, and the simple extraction method given below must be tried before concluding that there is no porphyrin.

A second important point in the spectroscopic examination is that occasionally the porphyrin is excreted as a metal complex, and the urine shows two absorption bands in almost the same positions as the bands of oxyhaemoglobin, instead of the typical four-banded spectrum. Careful observation reveals the fact that the β band is the more intense, whereas with oxyhaemoglobin it is the α band which is the darker. Wave-length measurements may be used to differentiate, but to make this conclusive it is necessary to use a reversion spectroscope (see later). Since the porphyrins contain no iron and are not proteins, negative chemical catalytic tests for blood (guaiac, pyramidon, reduced phenolphthalein, etc.), and

* For a convenient method of varying the thickness, see *Biochem. J.*, 1938, 32, 933.

negative tests for proteins, rule out haemoglobin; but, naturally, positive tests do not exclude a metallo-porphyrin. Confirmation of the presence of a porphyrin or metallo-porphyrin is easily obtained by adding a few drops of concentrated HCl or H_2SO_4 to about 10 c.cm. of urine, when the acid spectrum is obtained.

As mentioned above, when the porphyrins are dilute or the urine is highly pigmented it is necessary to extract them for certain recognition. A simple method is to acidify about 15 c.cm. of urine with a few drops of glacial acetic acid and then to add about 5 c.cm. of ethyl acetate or of amyl alcohol. After shaking thoroughly, the mixture is centrifuged and the upper layer is transferred to another tube and examined spectroscopically, if necessary in a thick layer (cf. previous footnote). Shaking the extract with one drop of HCl produces the acid spectrum. This method does not give positive results with normal urines, but will detect slight increases in porphyrin excretion.

Some Special Investigations of the Urine and Faeces

By spectroscopic means it is impossible to differentiate between the series I and the series III isomers of the same porphyrin; indeed, the data already given show that careful measurements of wave-lengths are required to distinguish between the uroporphyrins and coproporphyrins, for the maximum difference between corresponding absorption bands is only of the same order as that between the bands of oxyhaemoglobin and of carboxyhaemoglobin respectively (about 60 Å.U.). A differential estimation of the isomers, therefore, necessitates first a separation of the porphyrins from the urine.

Several methods have been proposed for this purpose. Nebelthau (1899) found that the addition of acetic acid to urines containing much porphyrin caused the formation of a slowly settling precipitate in which nearly all the porphyrin was included. To Garrod (1894-5) we owe one of the earliest procedures of a roughly quantitative nature. To 100 c.cm. of urine was added 20 c.cm. of a 10 per cent. solution of sodium hydroxide. This produced a precipitate of the earthy phosphates, upon which much of the porphyrin was adsorbed. After filtration the precipitate was washed off the filter paper by water and sufficient acid and alcohol added to dissolve it completely. The solution then showed the acid porphyrin spectrum. A calcium precipitate was used to carry down the bulk of the porphyrin as an adsorbate by both Salkowski (1890-1) and Dobriner (1936). Barium precipitates have been used for a similar purpose, and were also investigated by Salkowski. In our own case four volumes of 10 per cent. barium chloride added to ten volumes of urine produced a precipitate containing almost all the porphyrin, but it is probable that irregularities would be observed were this method to be tried upon a number of different urines (see below). Fink (1937), in his work on the isolation of the normal urinary coproporphyrin, employed with success a thick layer of paper pulp to adsorb porphyrin from the large bulk of urine acidified with acetic acid, and Grotepass (1938) similarly filtered large quantities of urine through infusorial earth in order to obtain enough pigment for chemical identification.

By far the most convenient and reliable technique, however, is that of chromatographic adsorption, using the Tswett column, and first applied to the particular case of the extraction of porphyrins from urine by Waldenström (1935); this is the method we have employed in the investigation of the present case and is described below in full.

As has already been pointed out, pathological urines may contain both uroporphyrins and coproporphyrins. A fundamental difference in physical properties exists between these two pigments—namely, the coproporphyrins may be extracted by ether from an aqueous solution solidified with acetic acid, while the uroporphyrins do not pass up into the ether phase under these conditions. A preliminary separation may thus easily be effected. A further difference exists between uroporphyrins I and III, in that the latter, as discovered by Waldenström, Fink, and Hoerbüger (1935), may be removed from a carefully acidified solution by repeated extraction with ethyl acetate. Uroporphyrin III was thus obtained in pure form

for the first time from the urine of acute idiopathic porphyrinurias. Fischer's (1926) method for the isolation of the coproporphyrins from urine, faeces, serum, etc., is based upon the acetic acid-ether solubility discovered by Sallett (1896); a similar technique has been used by Schumm. Briefly, the material is strongly acidified by acetic acid and then shaken with successive quantities of ether until all the porphyrin is extracted. The combined ether extracts are washed with water to remove most of the acetic acid, and the porphyrins recovered by shaking with dilute hydrochloric acid. According to the concentrations of acid used, a further separation may be achieved of the different ether-soluble porphyrins present, since, for example, coproporphyrin can be extracted completely by 0.5 per cent. HCl but protoporphyrin not until an acid concentration of 3 or 4 per cent. is used.

In the following description of the methods by which we isolated the individual porphyrins from the urine and faeces of our patient these methods find illustration.

Preliminary Tests upon the Urine

When viewed by a direct-vision spectroscope the red-brown urine voided by this patient exhibited two well-defined absorption bands at approximately 580 and 542 $\text{m}\mu$. (Using the same instrument, the readings for oxyhaemoglobin were 577; 540 $\text{m}\mu$.) The addition of a reducing agent caused no change, but upon acidification with a few drops of concentrated hydrochloric acid an immediate shift was seen to approximately 600 and 556 $\text{m}\mu$. There was strong continuous absorption in the blue end of the spectrum and also in the far red region. The two-banded spectrum is closely similar to that of the zinc complex of uroporphyrin.

Isolation of Coproporphyrins and Chromatographic Separation applied to the Urine Residue

Two litres of urine (collected October 4 to 10) were acidified with 100 c.cm. of glacial acetic acid and shaken twice with 1-litre portions of ether. The ether phase was washed well with water and the porphyrin then removed by shaking into 1 per cent. hydrochloric acid. This solution showed absorption bands at 595 and 549 $\text{m}\mu$. Spectrophotometric comparison with a standard coproporphyrin solution gave the yield as 0.306 mg. By addition of potassium acetate and shaking with ether the porphyrin was transferred back again to the organic solvent, which exhibited the neutral four-banded spectrum of coproporphyrin 625, 570, 532, 498. The solution was evaporated to dryness, esterified by methyl alcoholic hydrochloric acid, and the dry ester treated with anhydrous ether, in which coproporphyrin III ester is soluble but coproporphyrin I ester insoluble. All dissolved except a small unweighable quantity, which was, however, crystallized from hot chloroform-methyl alcohol and obtained in the form typical of coproporphyrin I methyl ester, melting-point 230° to 240° C. (Fig. 8). The ether solution was concentrated to low volume in a weighed tube and then stoppered and set aside in the ice-chest. The crystals which formed were washed sparingly with dry ether and then with petrol: weight, 0.3 mg.; melting-point, 172° to 174° C. (Fig. 8). The coproporphyrin fraction of the urine thus consists principally of coproporphyrin III (0.15 mg. per litre), together with a trace of coproporphyrin I.

The residual urine, after removal of the ether-soluble porphyrins, was allowed to filter slowly under gentle suction through a column of alumina (Merck's "nach Brockmann") in a vertical glass tube measuring 2×30 cm. The first filtrate was yellow, but a brownish-red pigment followed which showed no absorption bands in the red or green, but the broad band of urobilin in the blue region of the spectrum. When all the urine had passed through the column, the chromatogram was washed by percolation with 1 litre of 5 per cent. acetic acid, which extracted the last of the reddish-brown pigment. Glacial acetic acid now removed a small quantity of pigment, migrating as a narrow zone and emerging as a mahogany-red solution with two faint bands at 580-555 and 530. On standing in the ice-chest the liquid deposited the substance responsible for this spectrum, which proved to be uroporphyrin metal complex.

After washing out the acetic acid from the column with water, dilute ammonia (50 c.cm. of specific gravity 0.88 to 200 c.cm. of water) removed the porphyrins. First came a deeply coloured zone emerging as a dark red solution consisting mainly of the metal complex (two-band spectrum), and following this the main bulk of the material in a bright red solution in which the four bands of the alkaline spectrum of uroporphyrin were very sharply defined. Since any metal in complex combination with the porphyrin is eliminated by the strong acid used during the process of esterification, the above solution and the fraction consisting mainly of metal complex (which was put once more through the chromatographic process to remove some accompanying brown material) were combined and acidified by acid to pH 3.7. On standing, the porphyrin material was precipitated completely. It was centrifuged off, washed with acetate buffer solution of pH 3.7, and dried *in vacuo*, then esterified and the ester crystallized from hot chloroform-methyl alcohol. It separated in the form of very fine hair-like needles usually in aggregates (Fig. 8) and having a melting-point of 258° C., not depressed by admixture with uroporphyrin III methyl ester. The total yield was 42 mg., corresponding to 21 mg. per litre of urine. Uroporphyrin I could not be detected.

	Yields of Methyl Esters (mg. from 2,000 c.cm.)	Melting-points of Methyl Esters
Coproporphyrin I	minute trace	230° to 240° C.
Coproporphyrin III	0.3	172° to 174° C.
Uroporphyrin I	nil	—
Uroporphyrin III	42.0	258° C.

Identification of the Metal Present in Complex Combination

Using a calibrated reversion spectroscope the wave-lengths of the bands due to the metallo-porphyrin were 5,782; 5,406 A.U. (oxyhaemoglobin in the same instrument was 5,769; 5,389). Messrs. Adam Hilger, Ltd., kindly took spectrograms of the materials absorbed on a barium precipitate of the patient's urine and of a normal urine, and concluded that no doubt there was a greater concentration of zinc in the patient's sample.

The acid liquids from the esterification process, containing the metal which had been removed from its combination with the porphyrin, were evaporated to dryness, taken up in a little water, and filtered. Spot tests applied to this solution were positive for Zn, Fe, and Cu. It is thus possible that more than one porphyrin metal complex was present, although the observed absorption spectrum corresponded most nearly to that of uroporphyrin zinc complex, for which Mertens (1937) gives 571.5; 536 mμ. (For the coproporphyrin zinc complex Kapp (1939) gives 575; 539. Kapp and Coburn (1936), however, mention that higher dilutions gave 578; 541.) Zinc in complex combination with porphyrins has been observed by other workers, among whom may be mentioned Turner (1938) and Derrien and Cristol (1930). There is no doubt that the metal complex was present in the urine as voided, since a specimen obtained immediately after micturition still exhibited the characteristic two-banded spectrum. On the other hand the chromatographic results make it quite clear, also, that some of the uroporphyrin was not so combined. All the urine was collected in porcelain or glass, and never came into contact with metal.

Isolation and Identification of Porphyrins from the Stools

There was available 275 grammes of material (October 14 to 18) having a moisture content of 64.9 per cent. The mass was ground up with about 1 litre of acetone and, after standing for some time, this solvent was filtered off and replaced by a further 1 litre of acetone. The defatted residue was then ground with 100 c.cm. of glacial acetic acid, 1 litre of ether added, and the mixture shaken in a stoppered jar for one and a half hours. After decantation the extraction of the residue was repeated, using 50 c.cm. of acetic acid and 1 litre of ether. The combined ether extracts were filtered through a sintered glass filter-funnel into a large separating funnel and washed several times with water. Shaking with six 100-c.cm. portions of 5 per cent. HCl removed the por-

phyrins. Since there was some emulsification, the extract was cautiously warmed on a water bath and stirred until the ether had been removed. There remained a clear deep red liquid containing only a little suspended matter which could be easily filtered off. The porphyrin was returned to ether with the aid of potassium acetate, and the ether solution, after washing, extracted first with 0.5 per cent. (coproporphyrin fraction) and then with 5 per cent. hydrochloric acid (uroporphyrin fraction).

The 0.5 per cent. acid extract was freed from some urobilin, etc., by shaking with chloroform, and the quantity of porphyrin determined spectrophotometrically: yield 2 mg. The uroporphyrin fraction (5 per cent. acid), after a little further purification, gave a solution containing 2.4 mg. of porphyrin. The total yield thus corresponded to 4.56 mg. per 100 grammes of dried faeces, or 1.6 mg. per 100 grammes of undried stool, and approximately half was coproporphyrin.

By esterification and separation with anhydrous ether as described above it was shown that there was only a trace of material crystallizing like coproporphyrin III, the main fraction (1 mg. from 1.1 mg. of crude esters) being identified as coproporphyrin I with ester melting-point 245° to 247° C. Examination of the stool residue for uroporphyrin failed to reveal any trace of this pigment.

Recovery and Purification of Urinary Porphyrin from a Barium Chloride Precipitate

The chromatographic process can be relied upon, but demands much time and attention. Our results with barium chloride precipitation followed by certain innovations are considered, therefore, to be worthy of record, because pure uroporphyrin ester was readily obtained in good yield. To 7.3 litres of urine was added 2.2 litres of 12 per cent. barium chloride solution, and the fine precipitate collected upon a large Buchner funnel, washed by water and then by methyl alcohol. It was then suspended in methyl alcohol containing 2 per cent. by weight of sulphuric acid and the mixture refluxed on the water bath for some hours. After filtering off the deeply coloured liquid by suction, the residue was boiled with a fresh lot of this mixture, the process being repeated yet a third time. The three extracts were combined and placed in a large separating funnel with about 300 c.cm. of chloroform. Much water was then added and, after shaking, the chloroform layer was run off. Extraction with further smaller quantities of chloroform removed all the porphyrin ester from the acid aqueous layer. This crude chloroform solution was brownish red in colour and quite clearly contained much pigment in addition to porphyrin ester. To it was added twice the volume of petroleum ether, and the mixture was shaken repeatedly with 1 per cent. hydrochloric acid until no further brown material was either extracted or precipitated at the interface. This amorphous brown impurity was filtered off, and the remaining chloroform solution shaken repeatedly with 10 per cent. hydrochloric acid, which removed the porphyrin quantitatively, affording a clear deep red solution (1.25 litres). It was allowed to stand overnight at room temperature for complete hydrolysis to take place. 100 c.cm. of 50 per cent. sodium hydroxide was added and enough saturated potassium acetate solution (150 c.cm.) to bring the pH to about 3.7, when there was an immediate precipitation of the free porphyrin. This was centrifuged off, washed by acetate buffer solution of pH 3.7, then with water, and dried *in vacuo*. Re-esterification and crystallization from hot chloroform-methyl alcohol gave 64.5 mg. of well-crystallized uroporphyrin III methyl ester, melting sharply at 260° C.—that is, approximately 9 mg. per litre of urine.

Discussion

Acute idiopathic porphyrinuria is clearly recognized in Germany and elsewhere on the Continent; it is being increasingly reported in America, but even to-day it is scarcely mentioned in British literature. Yet some of the early cases were published in this country, under such titles as "Haematoporphyrinuria not due to Sulphonal"—

for example, Calvert and Garrod (1901), Garrod (1904), Brown and Williams (1909). Possibly the negative form of title was partly responsible for lack of interest; or perhaps the fact that it was noted that not all cases of porphyria were accompanied by grave symptoms led to the conclusion that the porphyria was incidental and of little importance clinically; then again, sulphonal had just been recognized as a cause of porphyria, and it was not always certain that the patient had not taken this or a similar drug; also, several patients were diagnosed as suffering from abdominal diseases and the porphyria was regarded as secondary.

The modern attitude is that, if a patient has abdominal pain, constipation, and vomiting, with a brown or red urine containing porphyrin which can be detected without special methods of separation and concentration, the possibility of "acute idiopathic porphyria" should be considered. A difficulty is that in certain patients the symptoms and porphyria may not be striking at first—hence some authors would make a fourth group of "chronic porphyria"—and there may be several mild attacks before one which is grave establishes the diagnosis. None the less, there can be no doubt of the existence of a separate clinical group of porphyrias of the acute idiopathic form. The diagnosis is important in practice because it prevents unnecessary operations: in our patient there was no clear-cut history of the urine being red at the time of the appendicectomy in 1937; but the laparotomy in 1938 was unnecessary, as now we all agree, although, as noted previously, he was temporarily better after it. The decision whether to operate or not will remain difficult in future cases, for no physician wishes to be responsible for stopping an operation which could have done good. In the presence of grave abdominal symptoms it is the significance attached to the porphyria which settles the issue: we repeat that, if the urine is brown or red and contains porphyrins that can be detected without special methods of separation and concentration, the diagnosis of acute idiopathic porphyria may be made. But the responsible physician may still hesitate, for he knows that the condition is rare, and justifiably may refuse to be guided largely by a laboratory report. He will be influenced much more if the tests for porphyrins are quantitative: in acute porphyria several milligrammes are excreted in the twenty-four hours' urine, whereas in health 0 to 50 μ g. (1 μ g. or 1 = 1/1000 mg.) and in the lesser grades (see below) of porphyria only fractions of a milligramme are present.

It is interesting that there are important differences chemically as well as clinically between the three types of porphyria. In the acute idiopathic form the urinary porphyrins belong mainly to series III, whereas in congenital porphyria they are largely series I compounds.

	Copro I	Copro III	Uro I	Uro III
Health	Very slight trace	Very slight trace	Nil	Nil
Congenital porphyria	+	Trace	+++	Trace
Drug porphyria (sulphonal, etc.)	+	? Trace	+++	? Trace
Acute idiopathic .. .	Very slight trace	+	Nil	+++
Lead poisoning .. .	? Slight trace	+	Nil	Nil

Congenital porphyria is distinctive in several respects; there are marked photosensitization and pigmentation of bones and teeth, neither of which is present in the other types (see under heading "Chief Signs").

Were it not that series I porphyrins apparently predominate in sulphonal porphyria too (Fischer and Duesberg, 1932; Fischer and Libowitzky, 1936), it might have been suggested that the distinctive features of the congenital form were due to the series I isomers, which are known to be more active as photosensitizers. It is curious that in sulphonal porphyria uroporphyrin I, whereas in the acute idiopathic form uroporphyrin III, predominates, according to Fischer and his collaborators, for otherwise the two groups have almost everything in common; indeed, they are often classified as subgroups of the same "toxic" group. In contradistinction to the congenital form, both show in the advanced stages similar abdominal and nervous symptoms.

One point emerges quite clearly, however, as indicated in our section on the chemistry of the porphyrins. It is inconceivable that the excreted series I porphyrins can be derived from the breakdown of haemoglobin or other series III compounds. It is possible that where series III isomers are excreted these may have had such an origin, although in modern literature the tendency is to assume that it is at some stage in the building up of haemoglobin, etc., that an error or toxic effect has occurred which causes an excessive excretion of these porphyrins also. Experiments by one of us at present in progress may help to throw light upon the origin of the series III porphyrins in various pathological conditions (cf. Rimington, 1938, b; 1939).

Summary

"Clinical porphyria" is defined; it includes the congenital form, that due to drugs such as sulphonal, and the acute idiopathic type, of which a case is described because it is not well recognized in British literature.

A summary is given of the chemistry of the porphyrins, for upon that depend not only the simple tests used in clinical laboratories but also the special investigations being made at several centres in the hope of throwing light on the synthesis of haemoglobin and the metabolism of related pigments.

The urine may contain uroporphyrin or coproporphyrin or both, and two isomers of each (I and III) have to be considered; methods for the separation and identification of these are described; in our case of acute idiopathic porphyria, uroporphyrin III accounted for about 99 per cent. of the total urinary porphyrins.

REFERENCES

- Beckh, W., Ellinger, P., and Spies, T. D. (1937). *Quart. J. Med.*, n.s. **6**, 305.
 Borst, M., and Königsdörffer, H. (1929). *Untersuchungen über Porphyrie*, Leipzig.
 Brown, W. Langdon, and Williams, H. O. (1909). *Lancet*, **1**, 1105.
 Calvert, J., and Garrod, A. E. (1901). *Clin. Soc. Trans.*, **34**, 41.
 Carrié, C. (1936). *Die Porphyrie*, Leipzig.
 Derrien, E., and Cristol, P. (1930). *C. r. Soc. Biol.*, Paris, **103**, 126.
 Dobriner, K. (1936). *J. biol. Chem.*, **113**, 1.
 Drigalski, W. von (1937). *Klin. Wschr.*, **16**, 1779.
 Fink, H. (1937). *Ber. Deut. Chem. Ges.*, **70**, 1477.
 Fischer, H. (1915). *Hoppe-Seyl. Z.*, **95**, 34.
 — (1926). *Abderhalden's Handb. biol. Arbeitsmethoden*, Abt. I, Teil II, p. 169.
 — (1930). *Naturwiss.*, **18**, 1026.
 — and Duesberg, R. (1932). *Arch. exp. Path. Pharmacol.*, **166**, 95.
 — Hilmer, H., Lindner, F., and Pützer, B. (1925). *Hoppe-Seyl. Z.*, **150**, 44.
 — and Libowitzky, H. (1936). *Ibid.*, **241**, 220.
 — and Zerweck, W. (1924). *Ibid.*, **137**, 176.
 Fourie, P. J. J. (1936). *Onderstepoort J. vet. Sci.*, **7**, 535.
 — and Rimington, C. (1938). *Ibid.*, **10**, 431.
 Garrod, A. E. (1894-5). *J. Physiol.*, **17**, 349.
 — (1904). *Trans. path. Soc. Lond.*, **55**, 142.
 — (1923). *Inborn Errors of Metabolism*, 2nd ed., London.
 — (1936). *Quart. J. Med.*, n.s. **5**, 473.
 — and Hopkins, F. G. (1894-6). *J. Path. Bact.*, **3**, 434.

- Geissler, J. (1939). *Klin. Wschr.*, **18**, 378.
 Grotepass, W. (1938). *Hoppe-Seyl. Z.*, **253**, 276.
 Günther, H. (1922). *Ergebn. allg. Path. path. Anat.*, **20**, i, 608.
 Hernando, T. (1938). *Biol. méd.*, Paris, **28**, 293.
 Kapp, E. M. (1939). *Brit. J. exp. Path.*, **20**, 33.
 — and Coburn, A. F. (1936). *Ibid.*, **17**, 255.
 Mackey, L., and Garrod, A. E. (1922). *Quart. J. Med.*, **15**, 319.
 — (1926). *Ibid.*, **19**, 357.
 Mason, V. R., Courville, C., and Ziskind, E. (1933). *Medicine*, **12**, 355.
 Mertens, E. (1937). *Hoppe-Seyl. Z.*, **250**, 57.
 Nebelthau, E. (1899). *Ibid.*, **27**, 324.
 Rimington, C. (1936). *Onderstepoort J. vet. Sci.*, **7**, 567.
 — (1938a). *Chem. and Ind.*, **16**, 1110.
 — (1938b). *C. r. Lab. Carlsberg*, **22**, 454.
 — (1939). *Proc. roy. Soc. Med.*, **32**, 1268.
 Saillet (1896). *Rev. Médecine*, **21**, 542.
 Salkowski, E. (1890-1). *Hoppe-Seyl. Z.*, **15**, 286.
 Turner, W. J. (1938). *Arch. intern. Med.*, **61**, 762.
 Vannotti, A. (1937). *Porphyrie und Porphyrinrankheiten*, Berlin.
 Waldenström, J. (1934). *Acta med. scand.*, **83**, 281.
 — (1935). *Dtsch. Arch. klin. Med.*, **178**, 38.
 — (1937). *Acta med. scand.*, Suppl. 82, pp. 1-254.
 — Fink, H., and Hoerbuerger, W. (1935). *Hoppe-Seyl. Z.*, **233**, 1.
 — and Vahlquist, B. (1939). *Ibid.*, **260**, 189.
 Watson, C. J. (1938). *Downey's Handbook of Haematology*, vol. 4, p. 2445, H. Hamilton, London.

CAUSES OF POST-PARTUM HAEMORRHAGE*

BY

FARQUHAR MURRAY, M.D., F.R.C.S., F.R.C.O.G.

*Professor of Midwifery and Gynaecology, University of
Durham College of Medicine*

The causes of post-partum haemorrhage can be divided into two main groups. The first and larger consists of cases where an otherwise normal uterus has been treated unkindly by Nature or other agencies; the second includes cases in which there is some pathology or possibly a misplaced placental site.

Group I: The Normal Uterus

Many primiparae after a spontaneous delivery have cervical lacerations, a few of which extend above the level of the vaginal vault or have some degree of perineal laceration. It is a rarity for such cervical or perineal lacerations to give rise to alarming or dangerous bleeding. If, however, an incompletely dilated os is forcibly stretched by the premature application of forceps or an injection of pituitary extract, or by an accelerated breech delivery, the cervical lacerations are considerably increased, and indeed may assume the nature of a minor degree of rupture of the uterus. Too rapid stretching of the perineum by forceps or pituitary extract or by imperfect control of the head during a spontaneous delivery also results in severe trauma. It is surprising how seldom severe bleeding occurs even in these cases, although there may be considerable shock.

The most important bleeding point is the placental site. Prolonged or obstructed labours exhaust the uterine muscle and predispose to an imperfect third stage as regards the mechanism and control of bleeding. Mismanagement of an otherwise perfectly normal third stage is a prolific cause of difficulty and bleeding at this time. The uterus if well treated performs its work satisfactorily. At the Tarnier Clinic in Paris I noted that the women were delivered on their backs, and the resident doctor left them alone after delivery during the third stage. He returned from time to time to palpate the uterus. This procedure struck me as somewhat casual, and as different

from what is the general rule in this country, where the patient is usually delivered on her left side and may remain in this position for some time before she is turned on her back to await the separation of the placenta, and where massage of the uterus is often performed.

A study of some hundreds of cases of incomplete B.B.A.s on the hospital district, where the baby had been born but the placenta had not come away by the time the nurse arrived, showed that in only one instance was a slight post-partum haemorrhage reported. These women had no skilled attention before the arrival of the nurse or district doctor.

A series of cases injected with 1 c.cm. of pituitary extract immediately after the delivery of the baby, with a view to studying the effect on the third stage and the amount of blood loss, revealed that the only cases in which there was any trouble were those which had been allowed to remain unduly long on their side after the injection was given or in which the uterus had been massaged. It is well known that a uterus which is kept out of the pelvis either at laparotomy or by means of a full bladder tends to bleed. If a patient lies for long on her side before the placenta comes away the uterus tends to fall up into the abdomen, with disturbance of its neuro-muscular mechanism.

With the rarest possible exceptions I deliver patients in the lithotomy and cross-bed position aided by a Clover's crutch. Unless there has been a long labour and much anaesthetic has been used I do not allow anyone to touch the uterus, but proceed to repair any laceration or episiotomy wound which may be present and merely lightly palpate the fundus from time to time to determine if the placenta has separated. The third stage is uniformly most satisfactory, and there is a minimum of blood loss. The time factor is a most important one, especially at night. The time of delivery should be noted, and at least ten to fifteen minutes allowed to elapse—unless there is bleeding—before deciding whether the placenta is ready for expulsion or not.

Occasionally one meets with post-partum haemorrhage of an insidious nature if the placental separation is not going according to plan. This is most likely to be found if the patient is nervous or has had a long and tiring labour or a lot of anaesthetic. The placenta in these cases has left the fundus but has not yet been completely detached from the lower part of its site. The physical signs are misleading. An attempt at expression fails to deliver the placenta, but a mass of blood clot the size of a small orange is squeezed out. There is no active continuous bleeding, but merely expulsion of a mass of blood clot each time an attempt is made to express the placenta. Each clot represents several ounces of blood and the patient slowly deteriorates. It is a sound rule to remove these placentae manually while the patient's condition is good.

I have never seen sepsis arise where a manual removal of the placenta followed by an intra-uterine antiseptic douche was done while the patient was in fairly good condition. Delay in doing this owing to a dread of causing sepsis or because the blood loss is intermittent and slight, and not continuous and severe, is responsible for many bad results.

Group II: The Pathological Uterus

In the other group of cases there is some intrinsic mischief in the uterus. One such, a multipara, was referred to me as her two previous confinements had been

* Contribution to a discussion in the Section of Obstetrics and Gynaecology at the Annual Meeting of the British Medical Association, Aberdeen, 1939.