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Porphyrins and Their Relation to the Metabolism of Blood Pigments

By CLAUDE RIMINGTON

(*National Institute for Medical Research, London*)

INTEREST in the physiological and pathological significance of the porphyrins has increased considerably during the last two decades, mainly as a result of the thorough chemical investigations of Hans Fischer and his collaborators, who have not only isolated and identified many of the naturally occurring porphyrins, but have also elucidated their constitution by analytic and synthetic methods. Whereas those rare metabolic anomalies characterized by enormously raised excretion of porphyrins, which were studied in the pioneer work of Garrod and Günther, could then be accounted little more than medical curiosities, the more exact chemical knowledge and quantitative technique developed during the last ten or fifteen years has shown that the porphyrins play a vital part both in normal and pathological pigment metabolism, and their study should help to throw light upon the mechanism of synthesis and utilization of blood pigment. We are still regrettably ignorant of the steps by which such an important substance as hæmoglobin is synthesized in the animal body; nevertheless, an important achievement was registered when Fischer (1929) completed a chemical synthesis of hæmatin, the iron complex of protoporphyrin, and succeeded in combining this in its reduced form with the protein globin, thereby producing hæmoglobin, similar in all respects to the natural substance.

CHEMISTRY OF THE PORPHYRINS

Chemically, all the pigments with which this discussion deals may be regarded as derived from porphin, a substance consisting of four nitrogen-containing pyrrole units joined together in a ring-like structure by means of four = CH — groups (fig. 1).

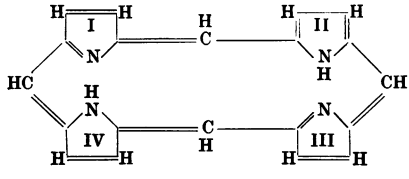


FIG. 1.—Porphin.

This ring system is very stable and imparts to the porphyrins their peculiar chemical and spectral properties. Each pyrrole group of porphin possesses two hydrogen atoms which may be replaced by other groups, for example, in ætioporphyrin there are four methyl groups and four ethyl groups attached at the eight corners of these rings in place of the H atoms of porphin. This substitution may, of course, be made in different ways according to the arrangement chosen. Thus there are four possible different ætioporphyrins which Fischer has named ætioporphyrins I, II, III, and IV, with the following constitutions,[1] and each is capable of giving rise to a potential series of porphyrin derivatives (fig. 2).

[1] Fischer has introduced a convenient abbreviation of the conventional formulæ. Only the four pyrrole rings are represented and these only by a bracket [ ] depicting the β β' carbon atoms with their attached groups. For example, see the formulæ for protoporphyrin (fig. 3).

Actually, only two of these four possible kinds of porphyrins are known in Nature, derivable from ætioporphyrins I and III respectively, and it has become customary to speak of the I and III series of porphyrins or pigments.

The majority of pigments such as hæmoglobin, myoglobin, cytochrome, catalase, bilirubin, urobilin, chlorophyll, &c., belong to the series III, while series I isomers are excreted in large quantities in certain pathological conditions. It is only recently

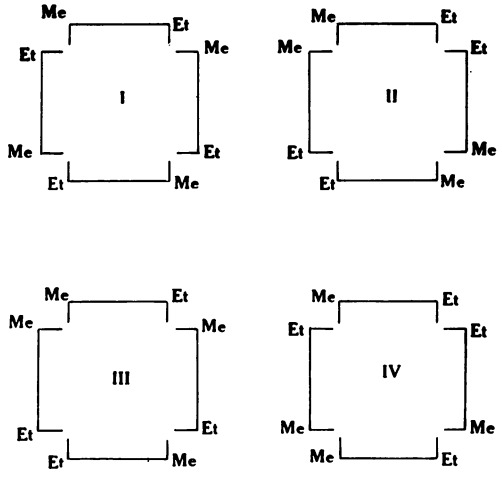


FIG. 2.—The four ætioporphyrins (abbreviated formula).

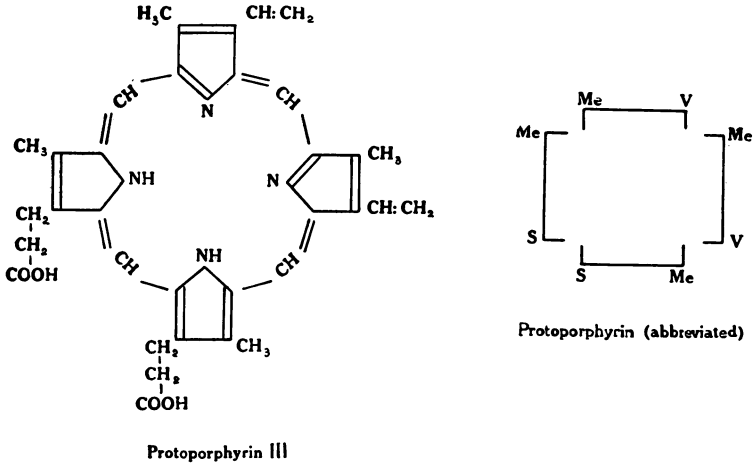


FIG. 3.—Protoporphyrin.

that investigators have realized adequately how important it is to ascertain to which isomeric series any pigment in question belongs.

Interconversion from one series to another is not possible *in vivo*, and therefore the excretion of a series I porphyrin demands a series I precursor, and demonstrates that it could not have been derived, for example, by breakdown, from a series III pigments like hæmoglobin.

Protoporphyrin is, from the biological point of view, the most important of the porphyrins, since when combined with iron it forms hæmatin, the pigment portion

of the hæmoglobin molecule and the respiratory enzymes, cytochrome, catalase, &c. Its constitution is as follows, from which it will be seen that it contains two carboxyl groups (fig. 3).

The coproporphyrins, next in complexity, contain 4 COOH groups and are designated coproporphyrin I and coproporphyrin III respectively, according to the arrangement of the substituents (figs. 4 and 5).

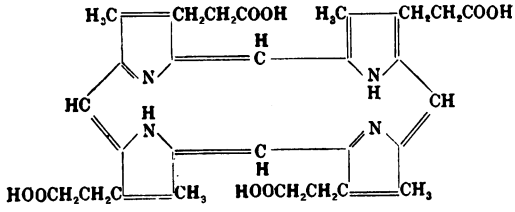


FIG. 4.—Coproporphyrin I.

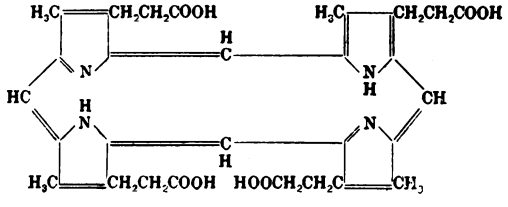


FIG. 5.—Coproporphyrin III.

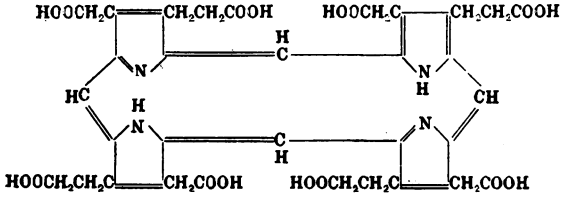


FIG. 6.—Uroporphyrin I (probable formula).

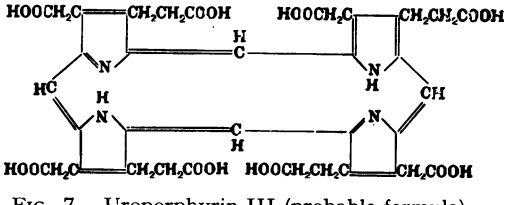
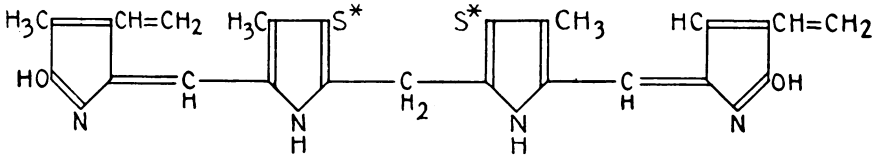


FIG. 7.—Uroporphyrin III (probable formula).

Similarly, uroporphyrins, both I and III, are known. These are porphyrins containing 8 COOH groups and their most probable formulæ are indicated by Fischer's representation as follows (figs. 6 and 7) :—

For comparison, the structure of bilirubin may be reproduced. It will be seen

that it is essentially similar to protoporphyrin, but the ring of four pyrrole groups has been broken with loss of one carbon atom and the molecule is now a chain of four pyrroles, connected by three carbon linkages (fig. 8).



bilirubin

FIG. 8. (S* = $CH_2.CH_2.COOH$).

The mechanism of the conversion of blood pigment into bilirubin and thence in the intestine into sterco- or urobilin will be discussed later.

DISTRIBUTION OF THE PORPHYRINS IN NATURE

Though present in small quantities, the porphyrins are widely distributed in both the animal and plant kingdoms, as are cytochrome, catalase, and the other hæmatin pigments. Grass, malt, and yeast, have been shown to contain coproporhyrin I and from yeast Fischer and Schwerdtel (1928) have also isolated hæmin (series III) identical in all respects with hæmin from hæmoglobin. Certain worms contain appreciable quantities of porphyrins (MacMunn, 1886 ; Fischer and Hilmer, 1926 ;) and a variety of bacteria have also been shown to be capable of synthesizing these pigments (Mallinckrodt-Haupt, 1938). Kämmerer (1924) found that a mixed inoculation of fæcal organisms was able to transform hæmoglobin into protoporphyrin and Jakob (1939) has recently shown that this capability is shared by a number of individual species in pure culture and is not, as Kämmerer thought, the outcome of bacterial synergism. The diphtheria bacillus, *C. diphtheriæ*, produces a relatively large amount of coproporphyrin whilst growing in suitable conditions, and Coulter and Stone (1931) have shown that porphyrin production and toxin formation are closely correlated.

The high porphyrin content of the fæces, following hæmorrhage into the alimentary canal (Boas, 1933 ; Snapper, 1919), which is utilized as a diagnostic sign, is unquestionably due to the breakdown into porphyrin of extruded blood pigment by the gut bacteria.

It may also be mentioned in passing that a group of light sensitive dermatoses described by Urbach (1938) were considered to be due to an abnormal intestinal flora rich in yeasts and producing excessive quantities of porphyrin.

The colouring matter of birds' egg shells consists largely of protoporphyrin (Fischer and Kögl, 1923, 1924), whilst a group of African birds, the plantain-eaters (*Turacus spp.*) are distinguished by the presence in the wing feathers of a very handsome red pigment, which, however, is readily soluble in slightly alkaline water and therefore easily washed out when the animals are kept in captivity. This pigment, named turacin, was first investigated by Church (1869, 1892), who found it to contain copper. Fischer and Hilger (1924) isolated uroporphyrin I and considered it to be the copper complex of this prophyrin—a rather remarkable circumstance considering the rarity of series I pigments in Nature—but the writer (Rimington, 1939*a*) in a recent reinvestigation of 11 different species of these birds, was able to find only uroporphyrin III. Völker (1939) has since described the occurrence of coproporphyrin III in the quills of several birds, particularly certain owls and bustards.

In the animal organism, the presence of small quantities of protoporphyrin in the erythrocytes of the blood was demonstrated by van den Bergh, Grotepass and Revers (1932), and the quantity has been shown by Schumm (1939) to lie normally between 9·4 and 19μg. According to Watson and Clarke (1937), it is the reticulocytes which contain this porphyrin, an observation in harmony with the finding of fluorescing erythrocytes (fluorecytes) in normal and pathological blood by Keller and Seggel (1934) and of porphyrin-containing erythroblasts in bone-marrow and fœtal blood by Borst and Königsdörfer (1929).

Small quantities of porphyrin are also present in bile, whilst van den Bergh, Grotepass and Revers (1932) found that protoporphyrin added to the perfused liver was transformed into coproporphyrin and excreted in the bile. The fæcal coproporphyrin may arise in part in this manner.

Normal urine contains approximately 20 to 50μg of coproporphyrin *per diem*. This was first isolated by Fink and Hoerburger (1934), who identified it as coproporphyrin I, but Grotepass (1938) has recently shown that the normal urinary porphyrin consists of a mixture of approximately equal parts of coproporphyrins I and III.

Since a study of the porphyrins may afford a clue as to the mechanism of synthesis of hæmoglobin, especial significance attaches to the distribution of these pigments in the fœtus. As already mentioned, Borst and Königsdörfer detected porphyrin in fœtal bones, bone-marrow, and blood. Fikentscher (1935), examining fœtal serum, found a maximum concentration of 8–10 μg per 100 c.c. at the 4th to 5th month and a slow decline to 1–3μg at the time of birth. According to Herold (1934), there is a marked excretion of porphyrin during the first five or six days of extra-uterine life, possibly to be correlated with the extensive breakdown of blood-cells which then occurs. Amniotic and allantoic fluids also contain porphyrin (Fikentscher, 1933), a finding which the writer has been able to confirm in the case of the sheep and ox. Meconium contains a relatively large amount of coproporphyrin I (about 2 mgm. per 100 grm. dry material), and the writer has found considerable quantities of both proto- and coproporphyrin in the tissues of a 7-months-old fœtal calf. All available evidence seems to point to a somewhat sudden increase in porphyrin distribution during the last month or so of fœtal life. Van den Bergh and Grotepass (1936) and Schønheyder (1938) have examined the porphyrin content of the hen's egg during incubation and have found that here also there is a progressive increase in porphyrin content during development.

Apart from the small quantities of porphyrin taken in with plant and animal foods, and that portion of the fæcal porphyrin attributable to bacterial activities and therefore, for the purposes of the argument, also of exogenous origin, the porphyrin of the excreta is derived from some internal process. In seeking to identify this process, one thinks first of the degradation of hæmoglobin, but as previously pointed out, a part of the urinary porphyrin is coproporphyrin I, and a series I pigment cannot be derived from one belonging to series III. Moreover, increased hæmolysis does not, under normal conditions, cause increase in porphyrin excretion. It would appear rather that the urinary coproporphyrin I is in the nature of a by-product, and the writer has put forward an hypothesis relating urinary porphyrin to erythropoietic activity (Rimington, 1938). Upon this view, the pyrrole precursors are capable of combining to give either I or III series pigments, but it is suggested that the synthesis is selectively catalysed, so that the latter predominate and the smaller quantity of series I by-product is excreted.

The mechanism of the breakdown of hæmoglobin to bile pigment has been studied by Lemberg (1935), who finds that, in all probability, the porphin ring is opened by oxidation (with loss of one carbon atom), whilst both iron and globin are intact.

Removal of the protein and rearrangement with loss of iron gives biliverdin, which is considered to be the bile pigment first formed. Bilirubin is formed from biliverdin by reduction. Lemberg pictures the whole process as follows (fig. 9).

At no stage in this transformation does a porphyrin appear, hence it is unlikely that the breakdown of hæmoglobin should increase porphyrin excretion. Certain experiments of Schreus and Carrié (1933), demonstrating an increase in urinary porphyrin following the injection of salvarsan, which is known to cause hæmolysis, appeared, however, to indicate that the two were closely related. An explanation of this discrepancy will probably be forthcoming as a result of some experiments which the writer still has in progress. They deal with the fate of hæmatin injected into the blood-stream. Fairley has shown that in man and monkeys this pigment combines with the serum albumin to form a substance which he has termed " methæmalbumin " ; but in other species it remains free. The object of the experiments was to ascertain in what form it was eliminated by the two groups of organisms, since Duesberg (1933–4) has reported that conversion of hæmatin into bilirubin does not take place *in vivo*. It was found that in both man, monkeys, and rabbits there is an increase in fæcal porphyrin following injection and a less marked increase in the urinary porphyrin level. This raised excretion is of longer duration in men and monkeys, which form methæmalbumin presumably owing to the slower rate at which the protein pigment complex is removed from the blood-stream. Concurrently it was found during an investigation of the porphyrinuria caused by the

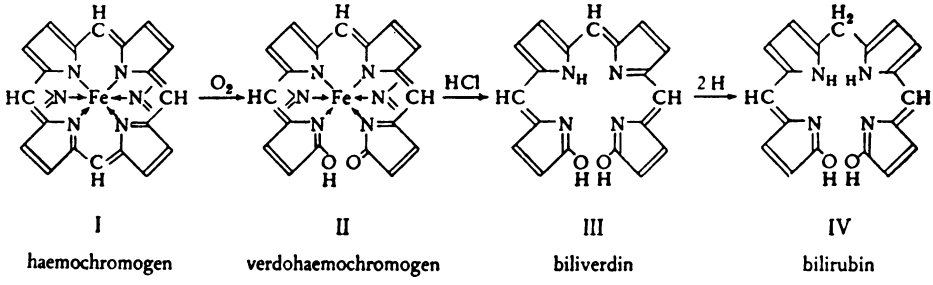


FIG. 9.—Mechanism of bilirubin formation (after Lemberg).

sulphonamide drugs that all those substances which caused increased porphyrin excretion were also capable of forming methæmoglobin *in vivo* (Rimington, 1939 *b*), and the suggestion is put forward that there is an alternative route of hæmoglobin breakdown by which the oxidized (ferric) or protein-free pigment is ultimately converted, not to bile pigment, but to porphyrin. Evidence in support of the existence of such a mechanism has also been adduced by Thomas (1938). Although it becomes of greater significance in pathological conditions, such a process may also be responsible for the small quantities of series III pigments excreted normally. It has been known for a long time that disturbances in the function of the liver may be accompanied by increased porphyrin excretion, and Dobriner (1937) has recently isolated the porphyrin from several such cases and characterized it as coproporphyrin III. Further, when a comparison is made of the literature records of " hæmatinæmia " and of increased porphyrin excretion, it is seen that there exists a close correspondence. These relationships and the cycle of events leading to porphyrin excretion may be represented by means of the following diagram. The implication of the equilateral triangle is that the factors indicated at the apices may be severally or jointly or interdependently responsible for the final manifestation of increased porphyrin excretion.

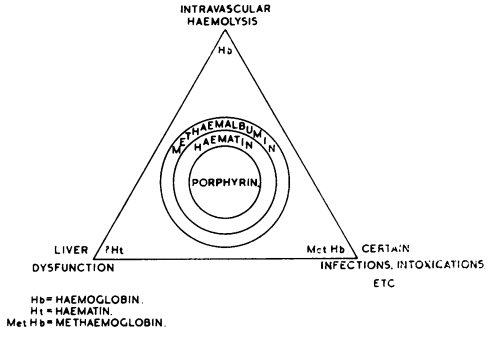


FIG. 10.—Hypothesis to explain increased Series III porphyrin excretion: diagrammatic representation.

PATHOLOGICAL CONDITIONS IN WHICH PORPHYRIN EXCRETION IS MARKEDLY INCREASED

In addition to the instances already discussed, there exist a group of pathological conditions in which porphyrin excretion is markedly raised above the normal level. Several schemes of classification of the porphyrinopathies have been proposed, based mainly upon the type of symptoms exhibited, abdominal, nervous, or cutaneous, or referring to the causative factor, i.e. " Porphyria congenita idiopathica " and " Porphyria acuta, var. idiopathica and var. toxica ". Waldenström (1937) prefers to reserve the term " Porphyry " for the constitutional diseases (congenital and acute), and consider the remaining cases with raised porphyrin excretion under the heading " Porphyrinuria ". This group would include the diseases with hepatic dysfunction, intoxication by drugs like sulphonal, the sulphonamides and antipyretics, plumbism, &c., the porphyrin excretion of which he regards as being purely symptomatic.

Congenital porphyry (Günther's " Porphyria congenita idiopathica " is a rare inherited metabolic anomaly, present, as the name implies, at birth, and characterized by continued excretion of relatively enormous quantities of coproporphyrins and uroporphyrins, both of the I and III series, but in which the I series isomers predominate. Post mortem, quantities of the same pigments are found in the various organs. Uroporphyrin is also deposited in the bones and teeth, imparting to them a deep brown colour, and the sufferers are markedly photosensitive. A condition identical with the human disease has been discovered and studied by the writer and collaborators (Fourie, 1936; Rimington, 1936, 1939*c*) in a herd of cattle in South Africa. The anomaly would appear to be inherited as a Mendelian recessive character. Apart from the discomforts attending light sensitivity, subjects with congenital porphyry do not seem to suffer any great inconvenience; in particular, there are no severe abdominal or nervous symptoms, and many patients have attained middle age and over.

Acute idiopathic porphyry, in contrast to the foregoing, is a disease which usually develops about the 30th year of life. There are repeated attacks (in women often associated with the menstrual cycle) of porphyrinuria, severe abdominal colic, and obstinate constipation. Later, nervous or neuromuscular symptoms develop, leading to progressive paralysis and death. Photosensitivity is absent, and the condition, according to Waldenström (1937) is inherited as a Mendelian dominant. There is no discoloration of the bones and neither can porphyrins be recovered from the visceral

organs post mortem (Mertens, 1937). Acute porphyry differs also from the congenital disease in that the porphyrins excreted in the urine are found to belong to the series III. This fact would render possible an origin from the blood pigment or myoglobin, and certain speculations have been made in this direction; however, the absence of porphyrins from the organs has always been a point difficult to understand. Recently, Waldenström, as a result of still unpublished experiments, has been led to the conclusion that the porphyrin excreted in acute porphyry arises secondarily from a precursor, probably of the dipyrromethene type, and it is this latter substance which is produced by the organism in such excess. Not only can it condense or polymerize to form substances of the cyclical uroporphyrin structure, but also urobilin-like pigments may arise by the union of two dipyrromethenes "end to end". The water-soluble, brown-red substances present in such urines and giving the Ehrlich aldehyde reaction, may thus be accounted for.

We are still far from understanding the nature of the constitutional metabolic anomalies responsible severally for the causation of these diseases, but it does seem that their chemical investigation is at last beginning to yield results of real value.

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