

HANS FISCHER

## On haemin and the relationships between haemin and chlorophyll

*Nobel Lecture, December 11, 1930*

The Swedish Academy of Sciences has awarded to me the Nobel Prize for my investigations into blood and leaf pigments and for the synthetic preparation of haemin, and I therefore wish to report my results, in so far as this can be done in a brief lecture.

The blood pigment haemoglobin is a compound which can be split by diverse methods into its constituents, pigment and protein. Teichmann was the first to observe this cleavage under the microscope when he subjected blood to the effect of glacial acetic acid - common salt; Schälfejeff, Nencki, Piloty, Willstätter and others have used the method on a large scale, so that Teichmann's crystals are available by the kg. The Swedish scientist Mörner has also developed a method for effecting separation with the aid of alcohol - sulphuric acid, by means of which esters of haemin are obtained.

Haemin has the formula  $C_{34}H_{32}O_4N_4FeCl$ . Countless variations of this atomic arrangement are possible. By analytical methods of Nencki, Küster, Piloty, Willstätter, H. Fischer and their pupils, the pyrrole nature of the haemin was demonstrated, and much information was obtained about its constitution. When haemin is deprived of its iron, porphyrins are formed, which are substances having a sensitizing effect, as Hausmann has shown.

These porphyrins are widely distributed in Nature, and it was possible to assume as a working hypothesis from the start that there existed a relationship with the porphyrins of blood pigment; it was moreover to be expected that the determination of their constitution would also provide information about the constitution of haemin itself, just as the results of distintegration of bilirubin which is formed from blood pigment by biological means had stimulated determination of the constitution of blood pigment itself. This was the reason why I tackled systematically the investigation of natural porphyrins, and the biological degradation of blood pigment itself.

In cases of porphyria, a minor disease, the patient excretes large quantities of porphyrins. Previously, it was assumed that haematoporphyrin was involved; this view could be refuted. At least two porphyrins are excreted, uro-

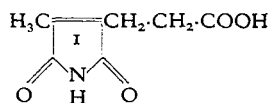
and coproporphyrin, both of which occur in the urine - one of the two, probably coproporphyrin, had been observed already by Hammarsten in a case of sulfonal poisoning -, although, quantitatively, most of the coproporphyrin occurs in the faeces. It is not always the same coproporphyrin that is present in the urine. Hijmans van den Bergh observed an isomeric coproporphyrin, admittedly only in a case of porphyria as yet. It is interesting to note that uroporphyrin also occurs in the pinions of African birds, in species of *Turacus*, in the form of a complex copper salt.

Closely related to uroporphyrin is conchoporphyrin which is present in seashells, probably in the form of calcium salt.

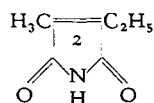
Coproporphyrin in particular is widespread; traces are present in normal urine, and also in yeast. In special cultures, yeast can be made to produce considerable quantities of coproporphyrin. The yeast is then brought to a condition which can be compared with porphyria in humans.

A porphyrin (ooporphyrin) is also contained in the spots on the eggs of birds incubating out of doors. Putrefaction of blood pigment produces a porphyrin identical with the above, Kämmerer's porphyrin; protracted putrefaction produces deuteroporphyrin.

In turning to a discussion of the determination of the constitution of haemin itself, I can of course only give a brief report of the most important results obtained in these investigations which extended over decades. The starting point was W. Küster's observation of the oxidative formation of haematinic acid (1) which, on decarboxylation, yielded methylethylmaleinimide (2) :



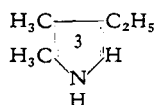
Haematinic acid



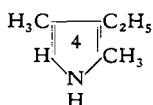
Methylethylmaleinimide

Reductive cleavage of the haemin yielded the following pyrrole derivatives of basic and acid nature:

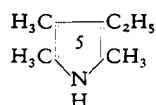
#### Haemopyrrole bases



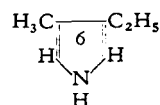
Haemopyrrole



Cryptopyrrole

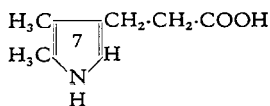


Phyllopyrrole

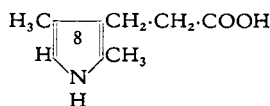


Opsopyrrole

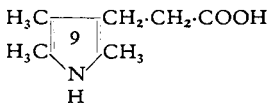
## Haemopyrrolic acids



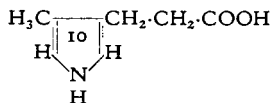
Haemopyrrole carboxylic acid



Cryptopyrrole carboxylic acid



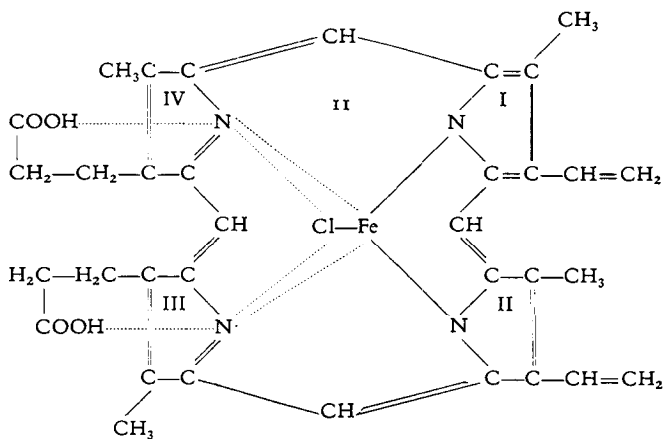
Phyllopyrrole carboxylic acid



Opsopyrrole carboxylic acid

During oxidation, the acid components yield Küster's haematinic acid, the basic constituents, methylethylmaleinimide. This established the principal features of their constitution ; exact proof is provided by syntheses which we shall not discuss in detail. These syntheses rendered these products available in any desired quantities. The yield of bases and acids from haemin is so substantial as to suggest that there are four pyrrole nuclei in the molecule, which agrees with molecular weight determination.

As early as 1912, W. Küster derived a constitutional formula for haemin which on the whole presents an accurate picture of haemin. We reproduce the formula as it has now been proved by synthesis; in reviewing the analytical results, we shall trace the logical, not the historical development.



Four pyrrole nuclei (more accurately : 1 pyrrole-, 1 maleinimide-, and 2 pyrroline-rings) are linked by four methin groups. The consecutive sequence

of simple and double bonds dictates the colour. The FeCl group has entered the molecule complex, substituting two NH groups.

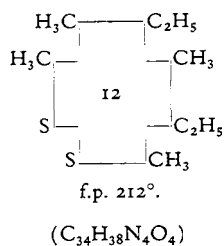
By reductive cleavage at the methin groups, a methyl residue and a free methin group are formed,



resulting in the mixture of pyrroles 3-10.

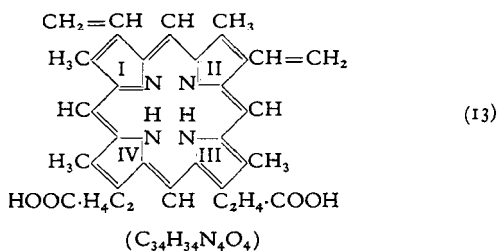
The methin groups are oxidized out by oxidative cleavage, and haematinic acid is obtained in a yield which corresponds to two pyrrole nuclei (III and IV). The base fraction (I and II) is evidently totally destroyed, which is explained by the vinyl groups at which oxidation is initiated.

If haemin is subjected to mild reduction with hydrogen iodide according to Nencki, we obtain mesoporphyrin (12). To simplify the extensive formula (see above) we omit the four methin groups and the lower half of the four pyrrole nuclei, indicate only the upper half of the pyrrole nucleus by a square bracket with the associated substituents; the propionic acids residue  $\text{CH}_2 \text{CH}_2 \text{COOH}$  we abbreviate by the Latin letter <<S>>.

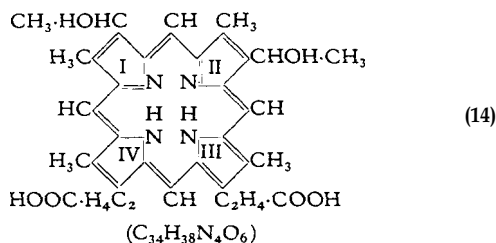


If we reduce haemin catalytically, we obtain mesohaemin, and in both cases the unsaturated side chains, i.e. the vinyl groups, are converted into ethyl residues, because, during oxidation, there is now formed methylethylmaleinimide, which we missed before, two molecules methylethylmaleinimide (2) and two molecules haematinic acid (i) being formed.

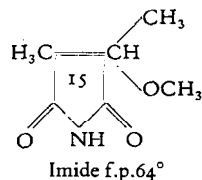
If haemin is deprived of its complexly bound iron, preferably with ferric formate we obtain proto- of Kimmerer's porphyrin:



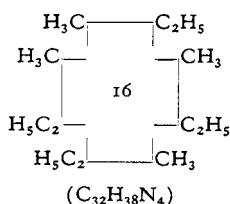
Only the complexly bound iron is removed, because, conversely, this porphyrin can again be converted into haemin. If, for iron separation according to Nencki, we use acetic acid - hydrogen bromide, hydrogen bromide will at first be added to the unsaturated side chains of haemin, as Willstätter observed, and hydrolysis occurs subsequently, so that haematoporphyrin is formed:



In this, two molecules water are added to the two vinyl groups (nucleus I and II) of the protoporphyrin, so that hydroxyethyl residues have formed. Again, on oxidation, haematoporphyrin only yields haematinic acid, because the basic fraction is destroyed, starting with the hydroxyethyl residues. If these hydroxyethyl residues are however stabilized by etherification, as Küster did in the case of tetramethylhaematoporphyrin, there are obtained, by oxidation, apart from two mol haematinic acid, two mol of the imide having the following constitution:



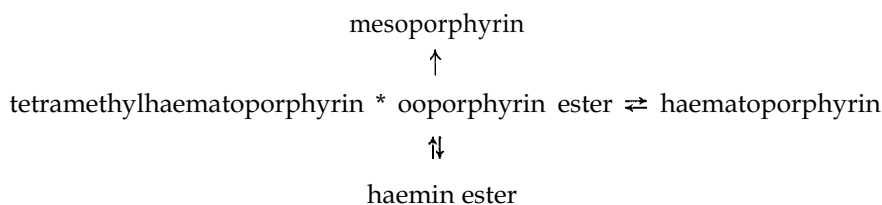
If we decarboxylate mesoporphyrin, we obtain, under evolution of carbon dioxide, the oxygen-free aetioporphyrin:



Chlorophyll can in the end be broken down into two aetioporphyryns, pyrroporphyrin ( $\text{C}_{30}\text{H}_{34}\text{N}_4$ ) and phylloaetioporphyryn ( $\text{C}_{31}\text{H}_{36}\text{N}_4$ ) which cannot be obtained from blood pigment, but both of which represent secondary conversion products of chlorophyll.

*On the constitution of natural porphyrins*

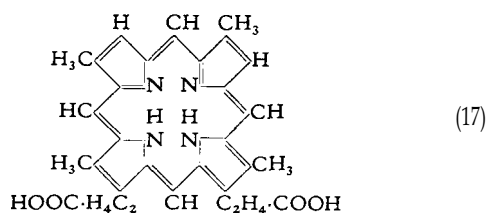
Ooporphyrin is identical with protoporphyrin or Kammerer's porphyrin. If we introduce iron complexly into ooporphyrin, we obtain haemin. If we reduce ooporphyrin ester with hydrogen iodide, we obtain mesoporphyrin, and the ester mentioned can also be converted into tetramethylhaematoporphyrin and haematoporphyrin. The following diagram illustrates these conversions which are in part reversible:



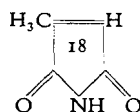
Ooporphyrin therefore is haemin without the iron.

These reactions with ooporphyrin from egg-shells constituted partial synthesis of haemin and provided much information about its constitution. The haemin synthesis which was later carried out was based on these observations.

In short-term haemoglobin putrefaction, it is only the complexly bound iron which is separated apart from globin, and Kammerer's porphyrin forms, which can be reconverted into haemin. In protracted putrefaction of haemoglobin for several months with sodium carbonate, the unsaturated side chains of haemin are separated, and deuterohaemin forms. The complexly bound iron is therefore substantially preserved. From deuterohaemin, we obtain the iron-free deuteroporphyrin by splitting off the iron.



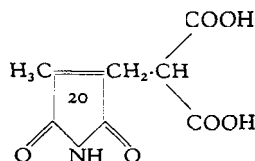
This is proved by isolation of citraconimide (18)



by oxidation apart from haematinic acid, further by bromination, which yields dibromodeuteroporphyrin, and by oxidation of dibromodeuteroporphyrin which leads to the formation of bromocitraconimide (19) apart from haematinic acid:

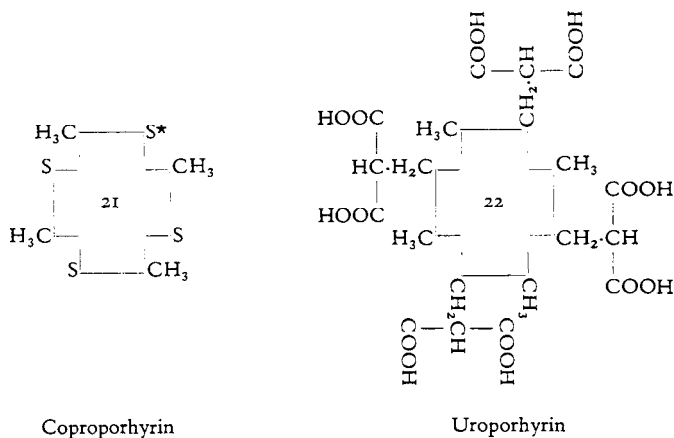


On oxidation, uroporphyrin yields carboxylated haematinic acid (20), coproporphyrin yields haematinic acid (1).



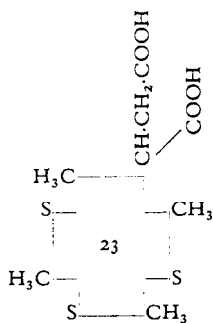
On reduction, coproporphyrin yields exclusively haemopyrrolecarboxylic acid (7).

The base fraction is absent, so that coproporphyrin most probably represents a mesoporphyrin containing propionic acid residues in the place of the two ethyl residues, whereas uroporphyrin would then represent the corresponding "malonic acid". The following formulae correspond to these relationships:



This is in harmony with the decarboxylation of the two pigments which leads to aetioporphyrin; from this we can draw conclusions as to the presence of four methyl and four ethyl groups in aetioporphyrin. The decarboxylation reaction in the natural porphyrins was exceptionally smooth; for the first time we obtained an aetioporphyrin in the characteristic butterfly crystal shapes, which determined the establishment of the relationships between the synthetic aetioporphyrins, to be described later, and the analytical aetioporphyrin.

In mother-of-pearl shells, conchoporphyrin was discovered, which is a monocarboxylated coproporphyrin which yields a pentamethylester, and which probably has the following constitutional formula:



This formula contains a succinic acid residue as a side chain. Recent synthetic results suggest that uroporphyrin also contains two succinic acid residues, because a synthetically prepared porphin-octacarboxylic acid was found to be non-identical with uroporphyrin.

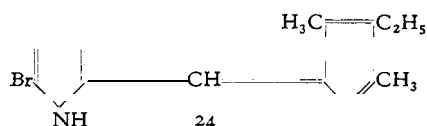
\*  $S = CH_2 \cdot CH_2 \cdot COOH$ .



*Synthetic results*

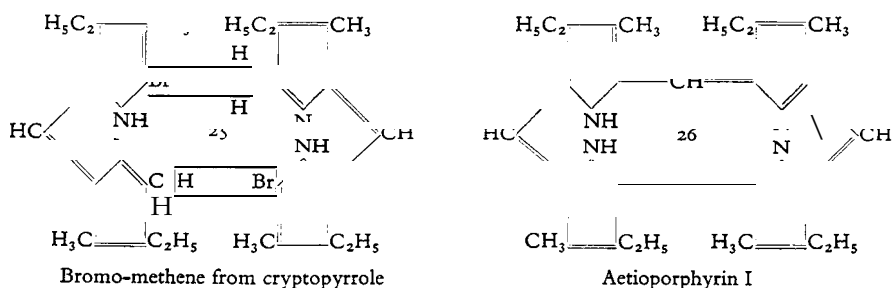
Different views have been put forward about the constitution of haemin and aetioporphyrin. Apart from Küster's view, it was above all the constitution of a tetrapyrrolethylene, as put forward by Willstätter (see Willstätter's Nobel Lecture on June 3, 1920, p. 9: *Les Prix Nobel en 1914--1918*), and the possibility of combining the two formulae, which were under discussion. The methods of synthesis had to be applied systematically in this field; this was however opposed by the relatively slight development of pyrrole chemistry, only cryptopyrrole having been prepared synthetically among the reductive cleavage products of blood pigment and bile pigment, and the yields were so small that the systematic development of pyrrole pigments was out of the question. One after another, all the cleavage products of blood pigment were however prepared synthetically, and the methods were perfected so that these pyrroles are now available for synthetic purposes in any desired quantity.

*Inter alia*, the action of bromine on cryptopyrrole leads to a magnificent brominated methene which possesses the following constitutional formula:

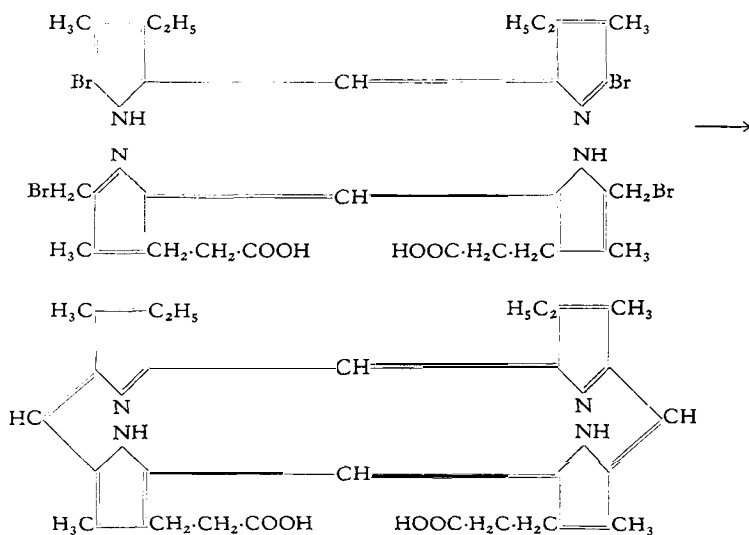


This formula has been established by synthesis, and it has become fairly common to convert tri-substituted pyrroles into methenes of this type by means of bromine. (Frequently, bromine also enters in the  $\alpha'$ -methyl group. These methenes are distinguished by exceptionally easy conversion into porphyrins.)

On being treated with acids, in particular on boiling with formic acid, the above methene yielded aetioporphyrin. The reaction is due to the combination of two molecules of the methene, separation of hydrogen bromide, and dehydrogenation according to the following formula :

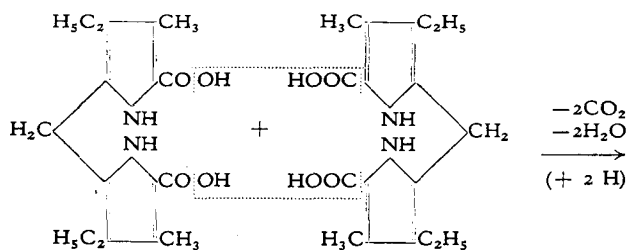


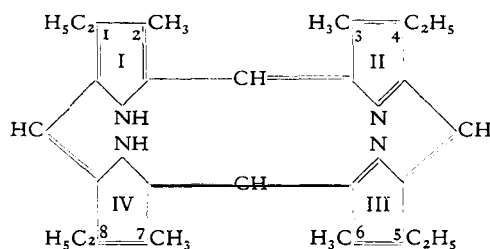
From the point of view of preparation, particular significance attaches to the synthesis from dinuclear-brominated dipyrromethenes and methylbrominated dipyrromethenes, which synthesis is represented by the following diagram:



which also supports the view of the constitution of aetioporphyrin and of the porphyrins in general in accordance with Küster's old formula.

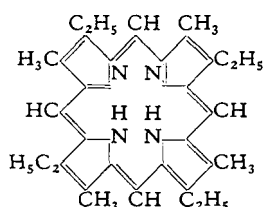
Porphyrin synthesis from mononuclear-brominated methenes by condensation of two molecules can also be applied to diverse methenes, and, in one of the two methenes, a free  $\alpha$ -methin group may take the place of nuclear bromine. This synthesis has proved itself in particular for porphinmonopropionic acids. With a good yield, porphyrin synthesis can also be effected from methane dicarboxylic acids, preferably by heating with formic acid, when the leuco compound will first form at  $40^\circ$  with vigorous carbon dioxide evolution, and will then be converted into porphyrin.



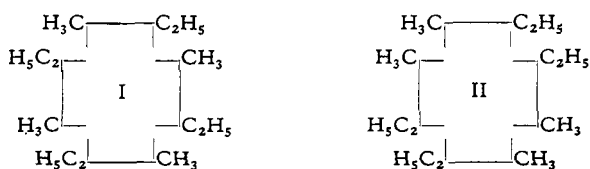


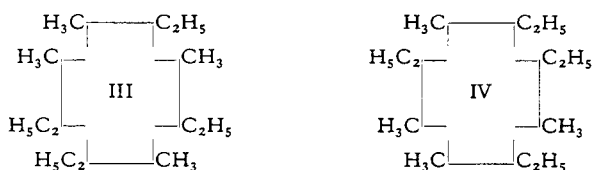
The blood pigment cleavage products opsopyrrole 6 and opsopyrrole carboxylic acid 10, which possess two free methin groups were converted into porphyrins under the effect of formic acid and formaldehyde (methyl chloride in ether is also very suitable), and this synthesis again proves the presence of four methin groups in the porphyrins and consequently in haemin.

These syntheses prove the correctness of Küster's formula; there are four methin groups which link four pyrrole nuclei, and aetioporphyrin is expressed by the following formula:

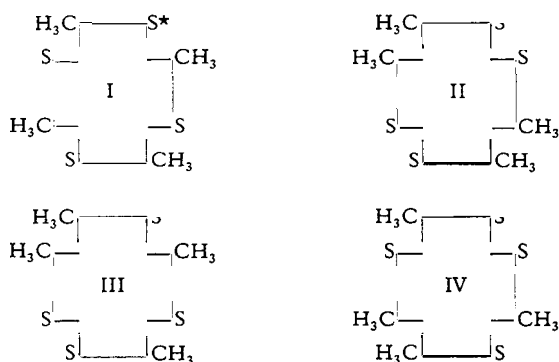


If, in this formula, we imagine the methyl and ethyl residues to be replaced by hydrogen, we obtain *porphin* on which all porphyrins are based. If we introduce four methyl and four ethyl residues into this system, we obtain, conversely, "aetioporphyrin", but it is evident that the introduction of the four methyl and four ethyl residues can be effected in four different ways, or in other words that four different isomers are possible depending on the relative position of the methyl and ethyl residues, as follows:





These four aetioporphyrins have been synthesized by different methods and have been found to differ. Mixed crystallization of I and II produced the typical butterfly crystal shape of aetio-uroporphyrin (see p. 172), so that a preparation identical with "natural aetioporphyrin" was obtained for the first time. The identification of the aetioporphyrins is however difficult, because they all possess high melting points. With the coproporphyrins, the situation is simpler. If we replace the ethyl residues in the aetioporphyrin by propionic acid residues, four tetramethyl-tetrapropionic acid porphins are formed, theoretically, which represent the four theoretically possible coproporphyrins:



All four coproporphyrins, which are spectroscopically identical, were in fact produced synthetically; but whereas the four aetioporphyrins melt at about 400°, the esters of the coproporphyrins are characterized by sharp melting points, so that difference or identity can easily be established. Coproporphyrin I was found to be identical with the coproporphyrin from cases of porphyria, with the coproporphyrin from yeast, and the coproporphyrin from uro- and conchoporphyrin. This proves formula No. 21 on p. 172.

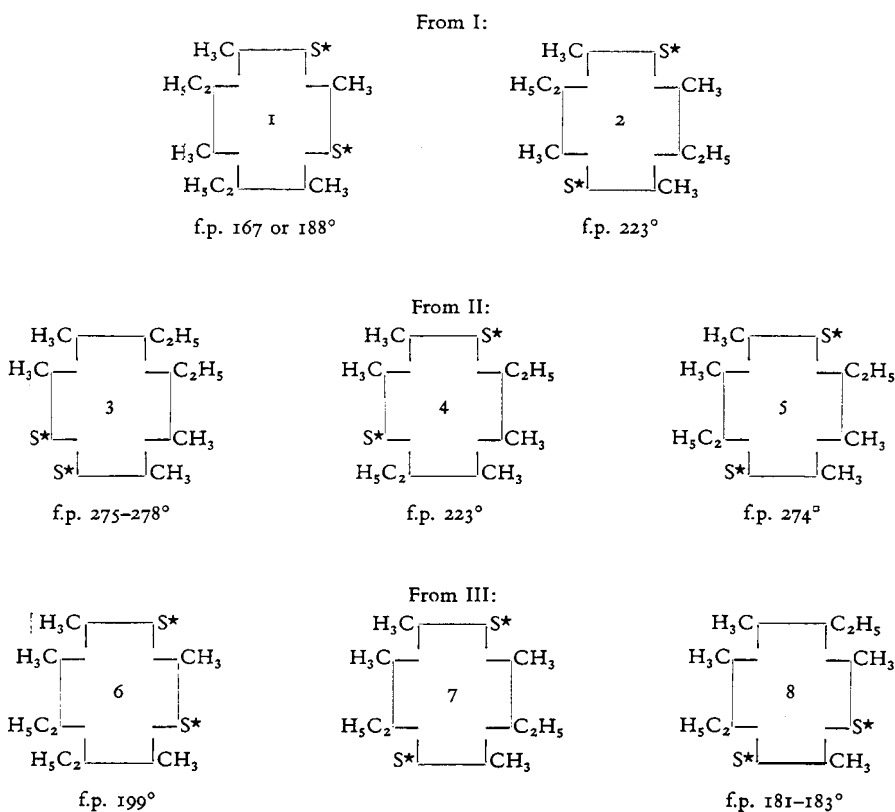
On the other hand, the coproporphyrin of Hijmans van den Bergh (p. 166) was identical with coproporphyrin III (see above).

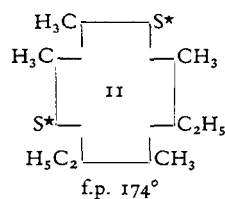
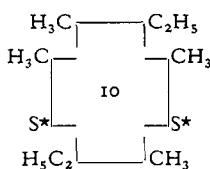
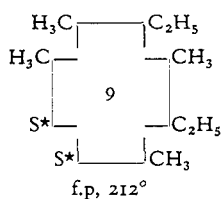
The dualism of the porphyrins which had first been recognized biologically from a study of porphyria, in particular that of yeast, had therefore been exactly demonstrated. Nature works on two different systems of reactions.

\*  $\text{S} = \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$ .

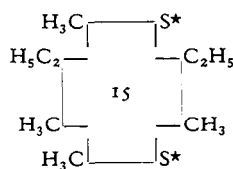
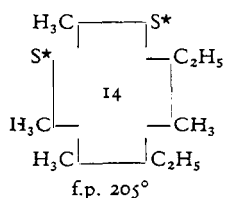
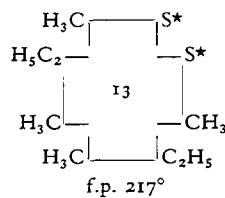
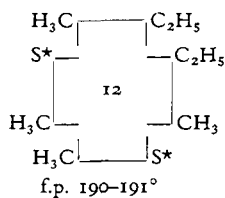
It is important to effect identification of coproporphyrin (and of course of uroporphyrin where necessary) in every case of porphyria by isolation as ester and by melting point. Whether the coproporphyrin of normal urine is III or I, remains undecided. II and IV are highly improbable.

The most important problem now was the determination of the sequence for haemin. Since haemin contains two unsaturated side chains, elucidation was exceptionally difficult, and we therefore turned to the synthesis of the mesoporphyrins, where conditions were much simpler, in particular because mesoporphyrin is a very stable substance. Analysis showed that mesoporphyrin is a tetramethyldiethyl-dipropionic acid-porphin, and the synthesis of such porphyrins presented no difficulties; theoretically, there were however a large number of possible isomers, which are derived from the 4 aetioporphyrins in that propionic acid residues (S) replace two ethyl residues. It will be seen that two variations are possible at I, three at II, six at III, and four at IV, 15 isomers in all, which are shown in the following table



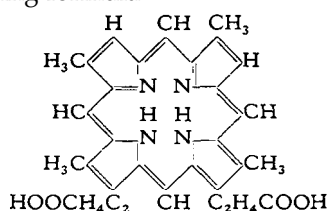


From IV:



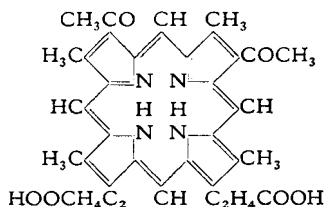
Of these fifteen mesoporphyrins, 12 were synthesized. Their dimethyl ester melting points are stated in the formulae. Mesoporphyrin 9 (see above) was found to be identical with mesoporphyrin from haemin. Since mesoporphyrin 9 is derived from aetioporphyrin III (see table), the structure of blood pigment corresponds to aetioporphyrin III. This proved the mode of arrangement of the side chains in haemin, and it became possible to embark upon systematic synthesis.

Haemin synthesis was carried out as follows. In protracted putrefaction of haemoglobin, we have repeatedly mentioned deuterohaemin or deuteroporphyrin, which lacks the unsaturated side chains of haemin. Since the unsaturated side chains of haemin are converted into ethyl residues on transition to mesoporphyrin, we obtain the formula of deuteroporphyrin if we replace the two ethyl residues in mesoporphyrin 9 by hydrogen. Deuteroporphyrin has therefore the following formula

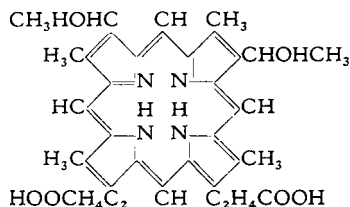


\*  $\text{S} = \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$ .

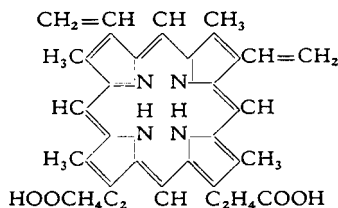
The synthesis of this substance was carried out and it was proved to be identical with deuteroporphyrin. Into its iron salt, deuterohaemin, we succeeded in introducing two acetyl residues, to form diacetyl-deuterohaemin or diacetyldeuteroporphyrin:



which on partial reduction yielded haematoporphyrin, which therefore had the following formula:



Two molecules of water could be removed from the haematoporphyrin; protoporphyrin was thus formed, which was converted into haemin by introducing iron:



According to the crystallographic investigation by Prof. Steinmetz, this haemin was shown to be wholly identical with haemin.

Proof of constitution was further complemented by synthesis of tetramethyl-haematoporphyrin which on oxidation yielded imide 15 (see p. 169) with a melting point of 64°.

Once the constitution of haemin had been elucidated and it had been identified as a derivative of aetioporphyrin III, considerable interest attached to the

determination of the arrangement of the substituents in chlorophyll, to discover whether relationships existed with aetioporphyrin I as in the majority of the natural porphyrins, or to aetioporphyrin III, or whether an entirely different parent substance was involved. I can only mention in passing the fundamental investigations by Willstätter (cf. his Nobel Lecture and the book on chlorophyll by Willstätter and Stoll, Verlag W. Springer, Berlin 1913); I shall select from Willstätter's important results only those which contributed to the solution of the problems mentioned above or which made such a solution possible. During energetic degradation of chlorophyll with alkali, phyllo-, pyrro-, and rhodo-porphyrin are formed, from which Willstätter obtained, by pyro-reaction, an aetioporphyrin which he considered to be identical with the aetioporphyrin from blood pigment. We resumed investigation and were able to isolate from rhodo- and pyrro-porphyrin a common aetioporphyrin, which had one ethyl residue less than the aetioporphyrin from mesoporphyrin. Phylloporphyrin yielded an aetioporphyrin which differed from the above spectroscopically, also carried a free methin group and could be converted into pyrro-aetioporphyrin by alcoholate degradation, under loss of one C-atom. The constitution of the latter compound, and of the three porphyrins mentioned, was subsequently practically completely clarified by synthetic means.

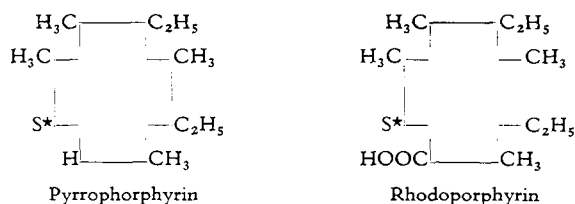
Pyrroporphyrin is a tetramethyl-diethyl-monopropionic acid-porphin. Closely related to it is rhodoporphyrin, which has a carboxyl group at the free methin group of the pyrroporphyrin.

The isomer problem was particularly complicated in the case of these porphyrins, because 24 in all exist for each of the porphyrins.

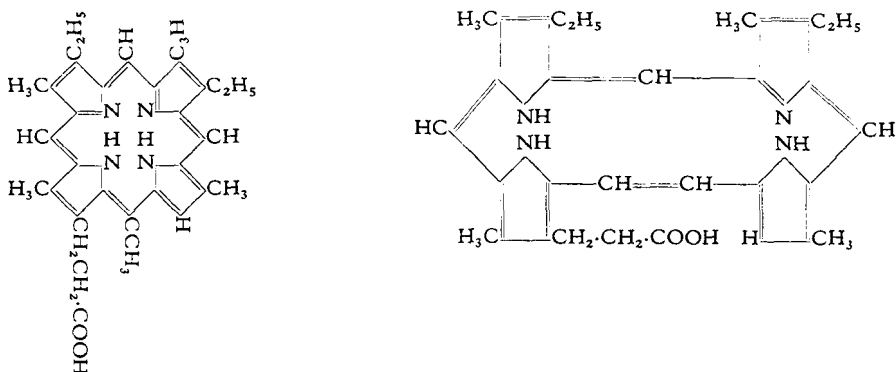
The principle of constitution determination was as follows: there are 8 isomers of tetramethyl-triethyl-monopropionic acid-porphins: all 8 were synthesized. If we remove from these triethylporphins one ethyl residue, we obtained the pyrroporphyrin type, of which 24 isomers exist, because one ethyl group each can be removed from the 8 isomeric triethylporphins. By indirect means, we reintroduced the ethyl residue into the natural pyrroporphyrin, and obtain 1,3,5,8-tetramethyl-2,4,6-triethylporphin-7-propionic acid, which is derived from aetioporphyrin III. This proved the derivation of pyrroporphyrin from aetioporphyrin III, but not the position of the free methin group, which could be present in 2-, 4-, or 6-position. The 6-position was proved by synthesis of 1,3,5,8-tetramethyl-2,4-diethyl-7-propionic acid-6-carboxylic acid-porphin, which thus carries the carboxyl group at 6, and was proved to be identical with the natural rhodoporphyrin. At the same time, the synthesis



was effected of 1,3,5,8-tetramethyl-2,4-diethyl-7-propionic acid-porphin, which was found to be identical with the "natural pyrrorporphyrin". This proved the o-position of the characteristic groups of the principal chlorophyll-porphyrins and also that of phylloporphyrin, because phylloporphyrin can be converted into pyrrorporphyrin with the assistance of alcoholate. Pyrro- and rhodoporphyrin therefore have the following constitutional formulae:



Phylloporphyrin is represented by one of the following diagrams:



as was proved by synthesis; the position of the bonds, as different from the blood pigment porphyrin, has not been clearly established as yet.

*What has however been established is the relative position of the substituents of rhodo-, pyrro-, and phyllo-porphyrin, which agrees in principle with those of the blood pigment, a proof which has recently been confirmed by conversion of chlorophyll-pyrrorporphyrin into mesoporphyrin IX.*

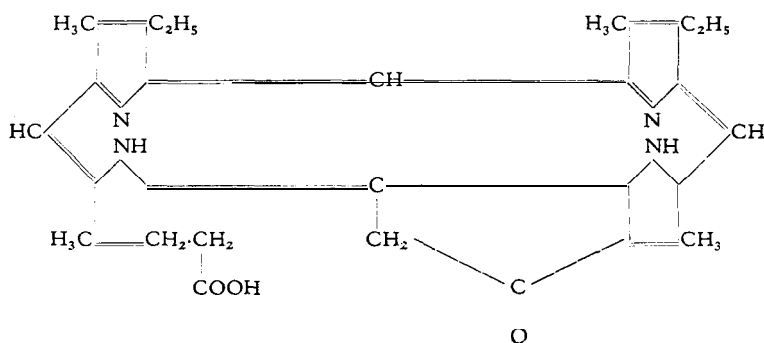
The chlorophyllporphyrins mentioned are isolated by energetic degradation with alcoholate, and the question that remained to be solved was whether chlorophyll is in fact based on the porphin nucleus.

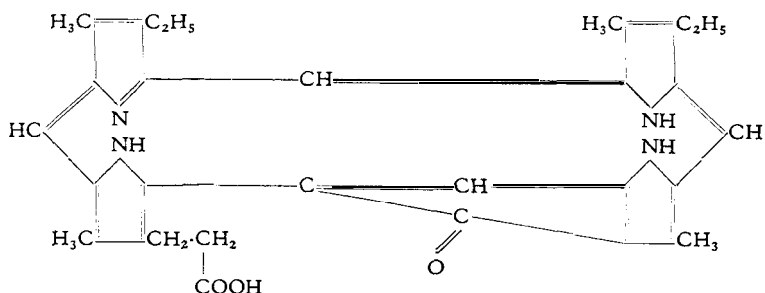
Mild degradation of chlorophyll derivatives with hydrogen iodide, formic acid, and other reagents led to numerous porphyrins, which we shall not discuss in detail.

If we consider further that the phylloerythrin isolated by Lobisch and Fischler, and also by Marchlewski, must be considered to be a porphyrin on the strength of spectroscopic analysis, and that this substance has recently been produced by purely chemical means from phaeoporphyrin  $a_6$  and  $a_5$  by exposure to glacial acetic acid - hydrogen bromide at  $45^\circ$ , there can hardly be any doubt that chlorophyll is not based on the porphin nucleus itself, but on a system closely related to it. It should be noted that substances spectroscopically analogous with chlorophyllchlorines and -rhodines are relatively easily obtained also by synthesis.

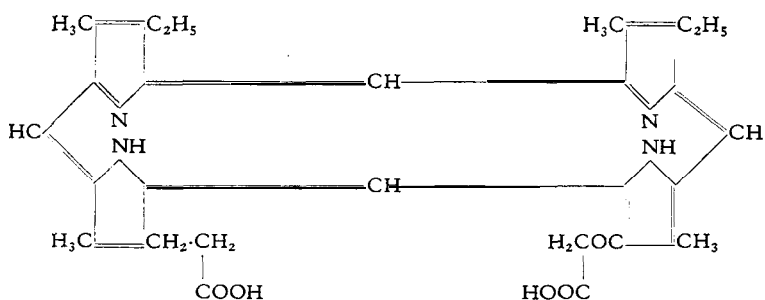
Dehydration of porphinpropionic acids leads to rhodines; reduction of porphyrins with alcoholates, in particular of iron salt, to chlorines; as does oxidation of the porphyrins with sulphuric acid and hydrogen peroxide. The chlorine spectrum can however also be produced by mere heating of the porphyrins in inert solvents, and exposure of coproporphyrin to light leads to chlorine.

Phylloerythrin is produced biologically from chlorophyll in the gastrointestinal tract of ruminants, where fermentation processes above all take place, so that we can assume at least as a working hypothesis that phylloerythrin in principle is close to chlorophyll in structure. The constitution of phylloerythrin has recently been determined with some probability, but, as in the case of phylloporphyrin, the mode of linkage of two pyrrole nuclei has not been definitely established. The following two formulae for phylloerythrin are therefore under discussion:

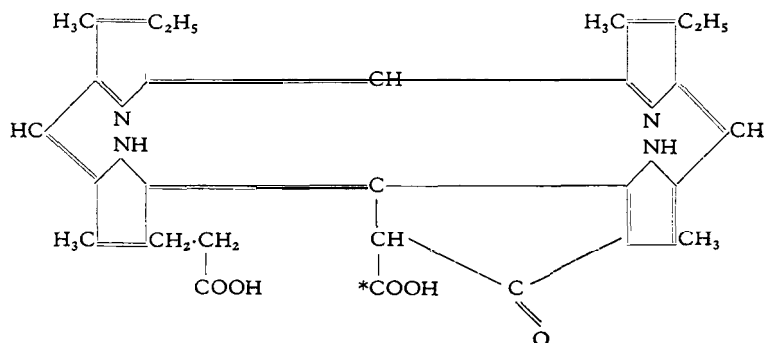




According to this, the relationships between chlorophyll and blood pigment are closer than has been assumed hitherto, if, as is most probable, the first of these formulae for phylloerythrin is the correct one, because phylloerythrin will then be relatively closely related to mesoporphyrin. If we imagine oxidation in this in the  $\beta$ -position at a propionic acid residue, as has been demonstrated by Knoop and others for the oxidative degradation of fatty acid, we arrive at a porphyrin-ketopropionic acid having the following formula:



If we assume dehydrating combination of the reactive methylene group in the keto side chain with a methin group in the  $\alpha$ -position, we obtain the following formula :



whose close relationships to phylloerythrin are evident, and whose carboxyl group marked with an asterisk\* must have the instability which characterises a carboxyl group in chlorophyll itself and in derivatives close to it. Under energetic alkali action, the carboxyl group is separated and the intermediate phylloerythrin is converted into phyllo- and rhodo-porphyrin by hydrolytic or reductive oxidative disruption of the  $\text{CH}_2\text{-CO}$  link, as has been established by experiment. Evidence of the above relationships still remains to be produced, as indeed the chlorophyll molecule still presents many riddles whose solution will present difficulties considering the instability of the molecule, in connection with which we must stress in particular the spectroscopic difference of the derivatives closest to chlorophyll and of the porphyrins. This difficulty has however largely been overcome by analytical and synthetic experiments. Mild reduction leads to porphyrins, and Nature herself brings about porphyrin formation by phylloerythrin formation in the organism of ruminants. Thus difference in constitution between the porphyrins and chlorophyll cannot be very great, as is also proved by the synthetic production of chlorines. Chlorines can be produced by reductive and by oxidative means from porphyrins; the chlorine spectrum even occurs under mere heating of the porphyrins in inert solvents, and on exposure of porphyrins to light. Numerous methods are available for porphyrin syntheses. Those close to chlorophyll must therefore also be amenable to synthesis. Further work is required on reconversion of chlorophyll porphyrins obtained analytically into chlorines, and on their biological behaviour, but an even larger number of investigations will be needed for clarifying the constitution of chlorophyll.

Since the sequence of the substituents in chlorophyll is known, the way is open on principle for systematic synthetic research, and because porphyrins occur in plants, we would seem to be justified in assuming a common evolutionary origin of the two pigments necessary for the life of animals and plants, a hypothesis which well merits experimental treatment.