BIOGENETIC ORIGIN OF THE PYRROLE PIGMENTS

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THE whole subject of the metabolic pathways adopted by living cells in the synthesis of their multitudinous and often exceedingly complex products is bound up with the most difficult and recondite problems in the fields of biochemistry and physiology. In every case most of the essential information, and especially the final proof, can come only from biochemical studies, by the application of their typical techniques, but the purely organic chemist has already made, and can still make, an important contribution.

The distinctive methods used in the biochemical approach have been well described by M. Thomas [1] and involve the search for intermediate metabolites, fixation of intermediates by chemical combination with added reagents, differential inhibition or activation of enzymes, and feeding and privative experiments. The introduction of isotopic tracers during the last few years has completely altered the whole picture, and great advances have been made. There is no doubt also that, if the special technique developed by T. Caspersson [2] for identifying nucleic acids and proteins in the actual living cell by microspectrography could be extended to other simpler cell substances, results of very great importance would follow.

The organic approach has so far been confined mainly to the field of plant products and to the work of two authors. R. Robinson [3] in England from 1917 to the present day has made outstanding contributions to the whole field, and C. Schöpf [4] in Germany since 1932 to the alkaloid field in particular. Two distinct methods have been developed. The first method, initiated during 1900—10 by some early ideas of Pictet, Willstätter, and Winterstein and Trier on alkaloids, was widely elaborated and extended with impressive results by Robinson over the whole field. The method entails first a careful examination of the main architectural framework of the individual members of each class, such as, for example, sugars, fats, anthocyanins, steroids, alkaloids ; then, by dissecting the molecules and applying a wide knowledge of organic reactions and common cell metabolites, the detection of a repeating feature which occurs intact in more than one form of combination and is so prominent that it justifies valid deduction as a building unit. The second method, initiated by Robinson with his elegant synthesis of tropinone in 1917, and widely developed by Schöpf in a striking and very successful

[1] " Plant Physiology ", 3rd Edn., J. & A. Churchill, London, 1947, pp. 213—219 ; for a review of the biochemical approach to the particular problem of alkaloid biogenesis, see R. F. Dawson, *Adv. Enzymol.*, 1948, **8**, 203.

[2] Symposia of the Society for Experimental Biology, I, 1947, p. 127.

[3] (*a*) *J.*, 1917, **111**, 762 ; (*b*) *ibid.*, p. 876 ; (*c*) Madrid Lecture, IX Congreso Internacional de Química Pura y Aplicada 1934 ; (*d*) *J.*, 1936, 1079 ; (*e*) *J. Roy. Soc. Arts*, 1948, **96**, 795.

[4] (*a*) *Angew. Chem.*, 1937, **50**, 779, 797 ; (*b*) FIAT Review of German Science 1939—1946, Preparative Organic Chemistry, Part II, p. 117.

manner, has come to be known as *synthesis under physiological or cell-possible* conditions. The starting materials used are reactive substances, which are either known (or presumed) to be plant-cell intermediates; the reactions are carried out in aqueous solution at room temperature, the reagents used must be mild (Robinson) and the pH conditions especially must be carefully controlled (Schöpf).

From the biogenetic point of view the general methods of organic chemistry are much too drastic to be of assistance, and there is no doubt that a broad development of this type of synthesis under physiological conditions would be of great value, both for the contribution it would make to general synthetic methods, and for the bearing it would have on problems of biogenesis. Although the biochemical approach will always be the more important, the chemical approach is considered to be necessary, since the two are complementary, information gained from the one side suggesting new approaches from the other.

The Review which follows has been confined to the one topic of the origin of the pyrrole pigments.* After a detailed account of the evidence which has accumulated from biochemical investigations on both plant and animal pyrrole pigments, the chemical methods of synthesis are considered in juxtaposition in order to see whether any clues may be obtained from this side, and, finally, the review is summarised and some conclusions are drawn and speculations made.

General.—The basic skeleton of the pyrrole pigments is formed from four pyrrole nuclei, joined together by four methene bridge carbon atoms as shown (I) to give a large inner ring of sixteen atoms. The molecule is flat, and its great stability and aromatic character show that it must be stabilised by resonance. Only one of the many possible arrangements of the double bonds is shown in (I).

The nomenclature in the series is in an unsatisfactory state.[5] The parent substance (I) is called porphin, but the derivatives are called either porphins or porphyrins. These derivatives are often found free in small amount in living material, but when they are co-ordinated with a metal such as iron or magnesium and combined with a protein they become substances of outstanding physiological importance. Thus the chlorophylls, the green colouring matters of plants, are protein–magnesium dihydroporphyrins containing an extra ring; hæmoglobin, the red pigment in blood cells, is an iron porphyrin combined with the protein globin; and the enzymes peroxidase, catalase, and the widely-occurring cytochromes are essentially of the same type as hæmoglobin.

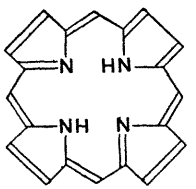
It should be noted that in all the naturally occurring porphyrin deriva-

[5] A. D. Mitchell, " British Chemical Nomenclature ", Ed. Arnold, London, 1948, pp. 124, 131.

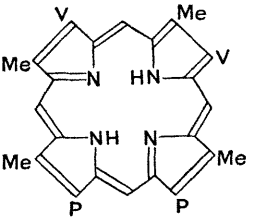
* Since the present review was completed, the problem has been discussed by R. Bentley (*Ann. Reports*, 1948, **45**, 253). There is some overlap in the references quoted, but Bentley's article was of much wider scope and his treatment of the present subject was necessarily much briefer. The problem has also been briefly considered by C. Rimington in " Hæmoglobin " (Symposium), Butterworth's Scientific Publications, London, 1949, p. 241.

tives there are no ring-carbon atoms carrying hydrogens ; * all are substituted or involved in ring formation. The predominating side-chains found are $\cdot CH_3$, $\cdot CH{:}CH_2$, $\cdot CH_2 \cdot CH_2 \cdot CO_2H$, and, less often, $\cdot C_2H_5$, $\cdot CHO$, $\cdot CH_2 \cdot CO_2H$: the groups $\cdot CO \cdot CH_3$ and $\cdot CH(SR) \cdot CH_3$ (= vinyl + RSH) occur once only.[6]

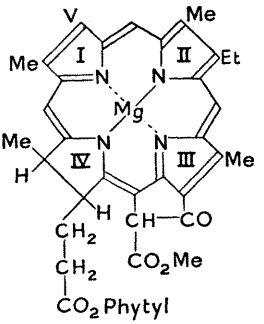
One of the most widely distributed porphyrins is protoporphyrin 9 (II), which contains methyl, vinyl, and propionic acid groups. Co-ordination of this porphyrin with iron gives hæmin, the prosthetic group of hæmoglobin ; co-ordination with magnesium, addition of two hydrogen atoms, formation of an additional 5-membered ring with one of the propionic acid residues,



(I.)



(II.)



V = CH : CH_2

P = $CH_2 \cdot CH_2 \cdot CO_2H$

(III.)

and esterification of one carboxylic acid group with methyl alcohol and the other with phytyl alcohol give one of the protein-free chlorophylls (III).

The attack on the intricate problem of the biosynthetic origin of the pyrrole pigments has been in progress for a great many years. The end is not yet in sight, but great advances have been made. The problem has been approached from both the plant- and the blood-pigment side, with the latter greatly predominating. There is as yet no evidence that both the plant and the animal cells adopt the same mechanism for synthesising the porphyrin nucleus ; but, to take one example only, in view of the fact that

[6] S. Granick and H. Gilder, *Adv. Enzymol.*, 1947, **7**, 305.

* Unless the ring is reduced, as in (III).

the porphyrin-containing cytochromes have been shown by D. Keilin [7] to occur in such a very wide variety of living cells, it would not be unreasonable to assume that the route employed is essentially the same.

Information from the Plant Side.—The information from the plant side will be considered first. In 1915 and 1920 B. Oddo and G. Pollacci [8] conducted experiments with seedlings which showed that chlorophyll formation could take place in the absence of iron salts, but only if the nutrient media contained an assimilable pyrrole derivative. If no pyrrole derivative was present, iron was an indispensable element. The conclusion was therefore drawn that iron was concerned only in the formation of the pyrrole nucleus, and that it played no further part in the synthesis of chlorophyll. This result has been questioned by other workers who, while recognising that absence of iron always causes chlorophyll deficiency (chlorosis), do not agree that it can be cured by addition of pyrrole derivatives.[9] Although Oddo's results were confirmed by A. Lodoletti [10] in 1938, the most recent work, summarised by H. Burström,[11] indicates that the whole problem of chlorosis is much more complex than was at first thought. Thus the ability of the plant cell to build up chlorophyll from a nutrient containing preformed pyrrole units is still an open question.

In 1929 H. Emde [12] reviewed the constitution of hæmin, which had just been synthesised by H. Fischer and K. Zeile,[13] and considered the question of its biogenesis. Assuming the origin of the vinyl groups to be by loss of carbon dioxide from an acid, Emde suggested that the main structure of hæmin could be dissected into four normal hexose chains and four triose chains, thus requiring originally six hexose molecules. He suggested that the pyrrole nuclei arose by substitution of oxygen by nitrogen in original furan rings. It must be pointed out that, on chemical grounds, the latter suggestion is very improbable because the conversion of furan into pyrrole derivatives requires vigorous conditions.[14]

No further suggestions regarding the biosynthesis of chlorophyll were made until 1940, when the subject was reviewed by G. Mackinney.[15] This author refers to the great difficulty of chlorophyll investigations, owing to the ease with which chlorophyll can be formed in the leaf from colourless precursors and the speed with which it can disappear in dying leaves. He is of opinion that it is somewhat unimaginative to assume that proline (IV), hydroxyproline (V), or a pyrrole derivative is the probable precursor in the living leaf, and mentions the very important fact that at no stage have the presence of dipyrrylmethenes (VI) or bilin (VII) pigments been detected in higher plants.

[7] See E. Baldwin, " Dynamic Aspects of Biochemistry ", Cambridge Univ. Press, 1947, pp. 112 *et seq.*

[8] *Atti R. Accad. Lincei*, 1915, [v], **24**, ii, 37 ; *Gazzetta*, 1920, **50**, i, 54.

[9] (*a*) G. Mackinney, *Ann. Rev. Biochem.*, 1940, **9**, 470 ; (*b*) H. H. Strain, *ibid.*, 1944, **13**, 598.

[10] *Boll. Chim. farm.*, 1938, **77**, 609 ; *Chem. Abstr.*, 1939, **33**, 7843.

[11] *Ann. Rev. Biochem.*, 1948, **17**, 593.

[12] *Pharm. Acta Helvet.*, 1929, **4**, 121.

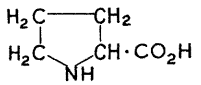
[13] *Annalen*, 1929, **468**, 114.

[14] L. N. Owen, *Ann. Reports*, 1945, **42**, 169.

[15] Ref. 9 (*a*).

In fact, nothing is known either about the route taken in chlorophyll destruction or about the fragments resulting,[16] so that no clue concerning the reverse process, the biosynthesis, has yet been obtained from this direction.

No light on the biosynthesis of chlorophyll has resulted from the large number of investigations carried out on the supposed chlorophyll precursor known as protochlorophyll, which has been isolated from plants grown in the dark. A description of the confusing results obtained has been given by E. I. Rabinowitch (1945) in his comprehensive book on photosynthesis,[17] and the conclusion reached by this author, after careful survey of all the evidence, is that the whole problem of chlorophyll development in seedlings is greatly in need of further study. The most important chemical evidence is that of H. Fischer *et al.*,[18] who in 1939 identified a product from protochlorophyll, which supported the view that protochlorophyll itself was simply chlorophyll less the two hydrogen atoms on ring IV. If proto-



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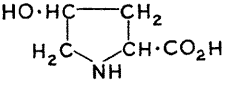

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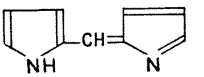

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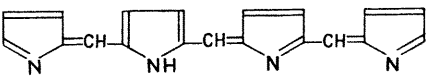


(VII.)

chlorophyll is the true precursor of chlorophyll, the final stage, produced by the action of light, is therefore a reduction. This observation, if correct, would dispose of all theories based on the final reaction being an oxidation. Some evidence that this final stage is more than a simple photochemical transformation has recently been obtained by J. H. C. Smith.[19]

A different type of approach to the whole problem, and one which takes us to what must be the final stages of the biosynthesis, was started in 1948 by S. Granick.[20] An *X*-ray-induced mutant of *Chlorella* was found to produce, instead of the normal green cells, brown cells which on extraction gave only protoporphyrin 9 (II). Thus the mutant appeared to have lost the power to complete the synthesis of the chlorophyll molecule (III). A mutant was also obtained which produced only magnesium protoporphyrin 9, thus suggesting that introduction of magnesium is the next stage. This leaves the final stages of suitable oxidation, reduction, ring closure, and esterification to be completed. Earlier, S. Granick and

[16] Confirmed also by K. Egle, *Bot. Arch.*, 1944, **45**, 93.

[17] " Photosynthesis ", Interscience, New York, 1945, Vol. I, pp. 404, 431, 445.

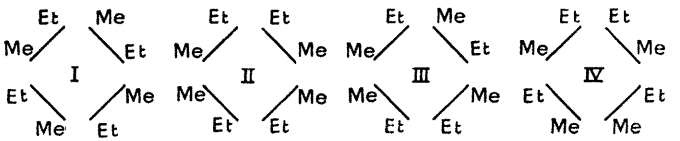
[18] H. Fischer, H. Mittenzwei, and A. Oestreicher, *Z. physiol. Chem.*, 1939, **257**, IV.

[19] *J. Amer. Chem. Soc.*, 1947, **69**, 1492 ; *Arch. Biochem.*, 1948, **19**, 449 ; see also V. M. Koski and J. H. C. Smith, *J. Amer. Chem. Soc.*, 1948, **70**, 3558.

[20] *J. Biol. Chem.*, 1948, **172**, 717 ; 1948, **175**, 333.

H. Gilder [21] had shown that in the analogous case of the biological introduction of iron into a porphyrin, the presence of the vinyl side-chain was essential.

Information from the Blood-pigment Side.—Work from the blood-pigment side was summarised in 1940 in two valuable reviews, by K. Dobriner and C. P. Rhoads [22] and by W. J. Turner.[23] In order to appreciate these contributions, it will be necessary to mention first the reference substances, the ætioporphyrins, used by Hans Fischer in his outstanding investigations in this field.[24] If a fully substituted porphyrin be built up with a methyl and an ethyl group in each ring, only four combinations are theoretically possible. These are given below, an abbreviated formula being used for simplicity.



None of these ætioporphyrins (I—IV) occurs in Nature, but all four were prepared synthetically by Fischer. Any porphyrin under investigation could then be converted by suitable methods into an ætioporphyrin, and the type thus determined by comparison with the four standard ætioporphyrins of known constitution. The important discovery was made that all naturally occurring porphyrins are derived from ætioporphyrins I or III, the chlorophylls from type III only but the blood pigments from both types; and the existence of these two types in Nature was referred to by Fischer as the *dualism* of the porphyrins.[25] Inspection of the two formulæ concerned reveals that III could be derived from I simply by the reversal of one pyrrole nucleus. The significance of this will be discussed later.

The Theory of Dobriner and Rhoads.—Dobriner and Rhoads have put forward a theory to account for the formation in Nature of both type-I and type-III pigments, as illustrated by coproporphyrin I and hæmin.* They postulate the formation of the tetrapyrrole by the linking together, through two carbon atoms of unspecified origin, of two planar dipyrrylmethene building units *A* and *B*, as shown in formulæ on facing page.

If two *A*'s unite, type-I porphyrins result (the discrepancy here will be

[21] *J. Gen. Physiol.*, 1946, **30**, 1.

[22] *Physiol. Rev.*, 1940, **20**, 416.

[23] *J. Lab. Clin. Med.*, 1940, **26**, 323.

[24] H. Fischer and H. Orth, " Die Chemie des Pyrrols ", Akademische Verlagsgesellschaft, Leipzig, 1934, 1937, 1940.

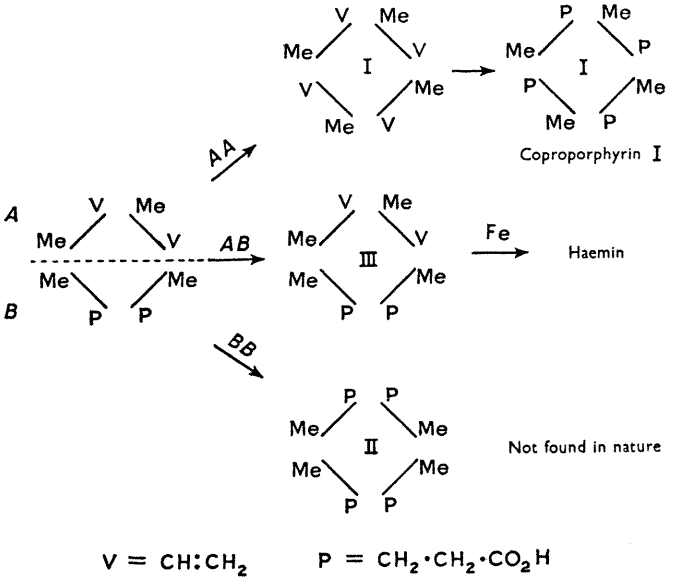
[25] Ref. 24, Band II, 1, 507; the dualism of the blood hæmins reported by H. Fischer, *Z. physiol. Chem.*, 1939, **259**, 1, was later withdrawn by H. Fischer and C. G. Schröder, *Annalen*, 1939, **541**, 196; see C. Rimington, *Ann. Rev. Biochem.*, 1943, **12**, 442.

* A theory on the same general lines was suggested independently by C. Rimington, but as it was published in relatively inaccessible journals and inadequately abstracted it is not generally known; see *Onderstepoort J. Vet. Sci. Animal Ind.*, 1936, **7**, 567; *Compt. rend. Trav. Lab. Carlsberg, Sér. chim.*, 1938, **22**, 454,

discussed later). For formation of coproporphyrin I on this theory, the vinyl groups after linking up become $\cdot CH_2 \cdot CH_2 \cdot CO_2H$. If *A* unites with *B*, a type-III porphyrin is formed ; and if two *B*'s unite, a type-II compound results. The latter has not been found in Nature.

The medical evidence, obtained by studying normal and pathological body conditions, indicates that, normally, type-III porphyrin formation greatly predominates over that of type I, and, abnormally, the reverse.[26]

It will be seen that this theory of Dobriner and Rhoads, requiring as it does the final linking of two dipyrrylmethenes, is based on Fischer's successful synthetic methods, which started from such units. It must be stressed that there is practically no experimental evidence for or against the theory.



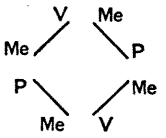
Only one claim has been made in connection with the isolation of a dipyrrylmethene from a natural source.[27] This concerns the substance known as porphobilinogen, which occurs in the urine of certain porphyria cases and is thought to be a mixture of two dipyrrylmethenecarboxylic acids. The evidence presented so far, however, is not sufficient to characterise the substance definitely. It has been obtained only in aqueous solution, and the molecular weight of about 350 was found by a diffusion method. The colourless aqueous solution on storage or when boiled with hydrochloric acid gives probably two porphyrins, one of which was identified through its methyl ester as uroporphyrin III (abbreviated formula below). Since

[26] D. L. Drabkin, *Ann. Rev. Biochem.*, 1942, **11**, 532 ; see also ref. 33 and R. R. McSwiney, R. E. H. Nicholas, and F. T. G. Prunty, *Biochem. J.*, 1949, **44**, xx.

[27] J. Waldenström and B. Vahlquist, *Z. physiol. Chem.*, 1939, **260**, 189 ; see also F. T. G. Prunty, *Biochem. J.*, 1945, **39**, 446.

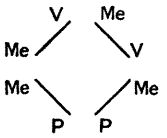
uroporphyrin III requires a condensation of the *A–B* type, porphobilinogen is thought to be a mixture of two methenes.

The Theories of Turner.—The second review mentioned above, by Turner,[23] will now be discussed in detail as it is considered to be a very stimulating contribution to the main problem. Turner commences his analysis by drawing attention to the fact that any complete theory of



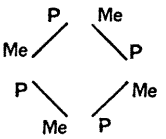
Protoporphyrin 2

(Type I)

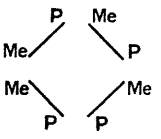


Protoporphyrin 9

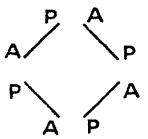
(Type III)



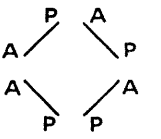
Coproporphyrin I



Coproporphyrin III



Uroporphyrin I



Uroporphyrin III

P= $CH_2 \cdot CH_2 \cdot CO_2H$ A = $CH_2 \cdot CO_2H$ V = $CH{:}CH_2$

porphyrinogenesis in animals must explain the following experimental observations: (*a*) the occurrence of the following porphyrins: protoporphyrins 2 and 9, coproporphyrins I and III, uroporphyrins I and III; (*b*) the normally great predominance of protoporphyrin 9; (*c*) the normal appearance of small and equal amounts of the two coproporphyrin isomers; and (*d*) the extremely rare occurrence of the uroporphyrin isomers.

Although the protoporphyrins are numbered 2 and 9 after Fischer, to distinguish them from the numerous other isomers, it will be seen that, like

the two coproporphyrins and the two uroporphyrins, they are derived from the basic type ætioporphyrins I and III.

On careful examination of these six formulæ Turner detects that they can all be built up by suitable choice from three primary units, pyrroles 1, 2, and 3.

Pyrrole 1

Pyrrole 2

Pyrrole 3

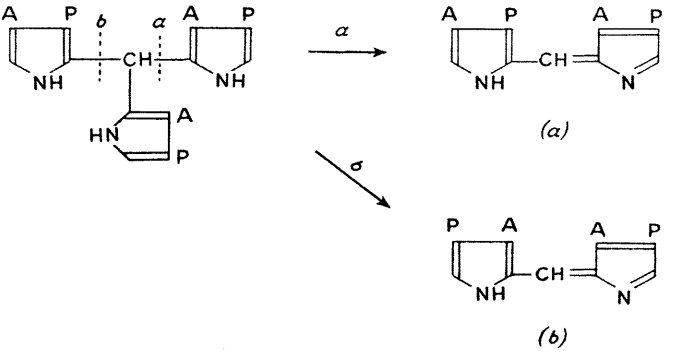
$A = CH_2 \cdot CO_2H$ $P = CH_2 \cdot CH_2 \cdot CO_2H$ $V = CH{:}CH_2$

It is obvious that pyrrole 2 could be derived from pyrrole 1 by decarboxylation of the acetic acid residue, and pyrrole 3 from pyrrole 2 by dehydrogenation and decarboxylation. Although Turner states that these are well-known biological reactions, he must be speaking generally, because, while the analogy of the well-known succinic $\rightarrow$ fumaric acid dehydrogenation would apply to the first stage, biological decarboxylation has so far only been demonstrated in the systems $\cdot CO \cdot CO_2H$, $\cdot CO \cdot CH_2 \cdot CO_2H$ and $\cdot CH(NH_2) \cdot CO_2H$. On the other hand H. Fischer *et al.*[28] have stated that the few acetic pyrroles known are chemically unstable and lose carbon dioxide readily. Turner therefore puts forward pyrrole 1 as the primary building stone of the proto-, copro-, and uro-porphyrins. In order to show how these arise from pyrrole 1, he assumes the formation of a dipyrrylmethene through the usual aldehyde synthesis, that is, introduction of an aldehyde group into one pyrrole nucleus and condensation with another in presence of acid to give the dipyrrylmethene, normally isolated as a salt; but A. H. Corwin *et al.*[29] have shown that this type of synthesis is not so simple or straightforward as was at first thought, but proceeds through the intermediate formation of a tripyrrylmethane, which then undergoes fission at points *a* or *b* as shown and, when the units involved are dissimilar, gives rise to several dipyrrylmethenes. In the case of pyrrole 1, only one nucleus is involved and, according to Turner, only two dipyrrylmethenes (*a* and *b* below) result. This statement, however, is correct only if certain assumptions are made, namely, that in both the condensations necessary to give the tripyrrylmethane, the linking takes place under the A (acetic acid) group and not under the P (propionic acid) group. Since pyrrole 1 itself has not yet been synthesised, the preferred point of attachment is still in doubt. Support for the tripyrrylmethane mechanism operating in Nature comes from one source only—the isolation from *Bacillus prodigiosus*, by

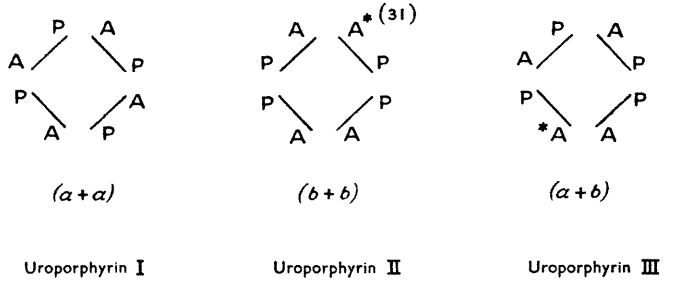
[28] H. Fischer and H.-J. Hofmann, *Z. physiol. Chem.*, 1937, **246**, 23; H. Fischer and A. Müller, *ibid.*, p. 31; see also H. Fischer and E. Elhardt, *ibid.*, 1938—39, **257**, 61; H. Fischer, W. Neumann, and J. Hirschbeck, *ibid.*, 1943, **279**, 1.

[29] A. H. Corwin and J. S. Andrews, *J. Amer. Chem. Soc.*, 1936, **58**, 1086; 1937, **59**, 1973; J. Paden, A. H. Corwin, and W. A. Bailey, *ibid.*, 1940, **62**, 418.

F. Wrede *et al.*,[30] of the red pigment prodigiosin, which has been shown to be a tripyrrylmethene derivative. If tetrapyrroles are now made as shown, by combining the planar dipyrrylmethene units $a + a$, $b + b$, $a + b$ through two carbon atoms of unspecified origin, uroporphyrins I, II, and III result.



A = $CH_2 \cdot CO_2H$ P = $CH_2 \cdot CH_2 \cdot CO_2H$



Uroporphyrin I Uroporphyrin II Uroporphyrin III

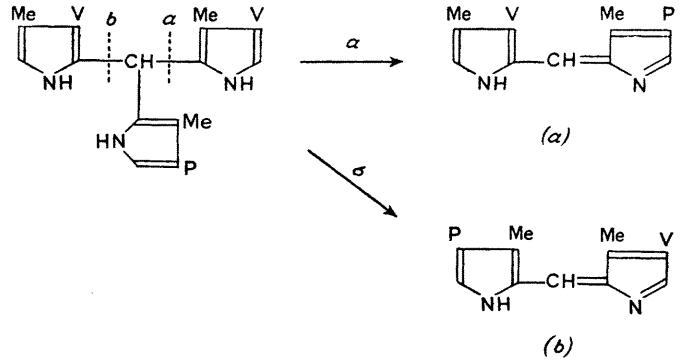
By a similar process but using pyrrole 2 in place of pyrrole 1, Turner derives coproporphyrin I, II, and III (above formulæ with Me replacing A throughout).

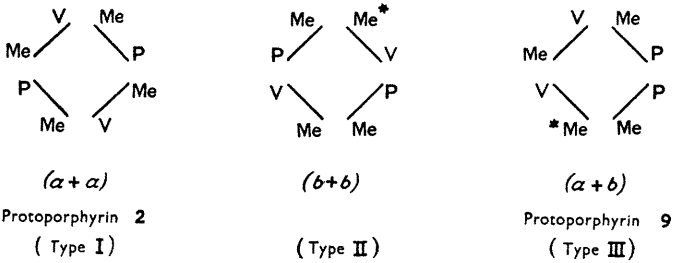
For protoporphyrins 2 and 9 the initial units must be two of pyrrole 3 and one of pyrrole 2 and (again not stated by Turner) condensation under the Me groups must be assumed. In the case of the methyl pyrrole propionic

[30] For refs. see ref. 24, Band II, 1, 153; see also H. Fischer and W. Siedel, FIAT Review of German Science, 1939—46, Biochemistry, Part I, p. 116.

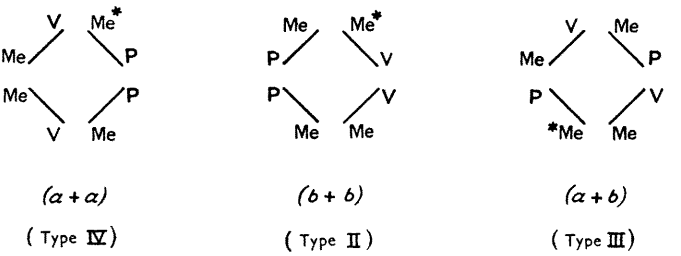
[31] The purpose of the asterisk on the formulæ is to aid identification of the particular type, when compared with the four standard ætioporphyrins. When turned round in the plane of paper, on the marked group, so that this group becomes the top left-hand corner Me of the ætioporphyrin, comparison should be assisted.

acid, it is known that condensation does actually take place preferentially under the methyl,[32] but for the methyl-vinyl derivatives no experimental evidence exists to indicate how the condensation would proceed.





Additional possibilities :



The error mentioned in discussing Dobriner and Rhoads's review, and repeated by D. L. Drabkin,[26] is also made by Turner. In none of these reviews is attention drawn to the fact that, in the case of the combination,

[32] A. H. Corwin, Gilman's " Organic Chemistry ", John Wiley & Sons, New York, 1943, Vol. II, 1275.

through two carbon atoms of unknown origin, of two dipyrrylmethene units containing unlike first and fourth groups, a second porphyrin can result by reversal of one of the dipyrrylmethene units. Thus in the case of protoporphyrin there are three additional possibilities—types IV, II, and III as shown—and, in the case of uroporphyrin and coproporphyrin, one additional possibility each—type IV—by reversal of one of the units in the $a + a$ combination (see p. 55).

To explain the normally great predominance of protoporphyrin 9, Turner supposes that this is due to the production of pyrrole 3 in excess, for a reason unknown but perhaps because of its greater stability compared with the other two. The normal appearance of small and equal amounts of the two coproporphyrin isomers would require pyrrole 2 to be next in stability, and the extremely rare occurrence of the uroporphyrin isomers would be caused by the normally great instability of pyrrole 1, an observation which receives experimental support as regards the $\cdot CH_2 \cdot CO_2H$ group from the chemical investigation by Fischer.[28]

On examination of Turner's contribution as a whole, it is considered that, within the limitations indicated, it offers a very reasonable explanation of the experimental facts. It also has the great merit that it suggests new experimental approaches, such as a further search for dipyrrylmethene intermediates and for types-II and -IV isomers, and also biological investigations using pyrroles 1, 2, and 3, and dipyrrylmethenes, preferably all marked isotopically, in feeding experiments.*

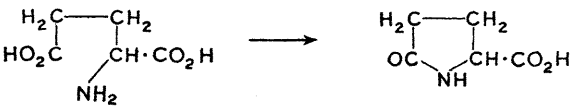
In the latest contribution from the medical side, C. J. Watson and E. A. Larson,[33] in a comprehensive review mainly of the urinary coproporphyrins in health and disease, state that the production of uroporphyrin I appears to be characteristic of the metabolic error known as porphyria, but that the position of uroporphyrin III is less understood. On paper, the interconversion of the uro- and copro-porphyrins appears to be simple, involving as it does the gain or loss of two carbon dioxide molecules, but there is no proof that these interconversions take place in the body. In spite of all the careful investigations which have been carried out, the authors' final conclusion is that the site, mode of formation, and physiological role of the coproporphyrins still remain in doubt.

Recent Work with Tracers.—Turning now to the consideration of the most recent work in the general field, we find very important results achieved from 1945 onwards by the use of isotopic tracers. K. Bloch and D. Ritten-

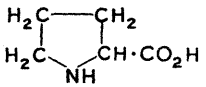
[33] *Physiol. Rev.*, 1947, **27**, 478.

* Added in Proof. An impressive new theory of porphyrinogenesis has been put forward by R. Lemberg and J. W. Legge, " Hematin Compounds and Bile Pigments ", Interscience, New York, December 1949, pp. 632—645. It is postulated that, from simple cell constituents such as α-ketoglutaric acid and ammonia, a pyrrole precursor is given, similar to that proposed by Turner, containing acetic and propionic acid residues, but with the significant addition of α-carbon substituent. This unit is held to produce *five* dipyrrolic precursors only, which give uroporphyrins I and III, coproporphyrins I and III, and protoporphyrin 9 (type III). The non-occurrence of type-II porphyrins is accounted for, and finally it is shown mathematically that the new theory can be used to predict correctly the ratio of the coproporphyrin isomerides I and III produced under various pathological conditions.

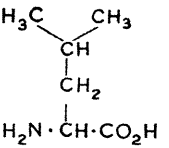
berg [34] administered deuteroacetate orally to rats and found deuterium in the hæmin isolated from the blood. They drew attention to the possible significance of this, in view of the known biological conversion of acetate into acetoacetate [35] and the well-known Knorr synthesis of pyrroles from acetoacetic ester (and compounds of similar type) and ammonia.[36] This was followed by some results of outstanding importance obtained by D. Shemin and D. Rittenberg.[37] They fed a man, and also rats, with glycine labelled with ^{15}N and proved that this glycine-nitrogen was the precursor of the nitrogen in the protoporphyrin of hæmoglobin in the man and the rat. (The utilisation was later [38] proved to be very rapid in the case of man.) They also fed labelled glutamic acid (VIII), proline (IX), leucine (X), and ammonia (as ammonium citrate) to rats. The choice of



(VIII.)



(IX.)



(X.)

these particular substances was significant. Proline already possesses the pyrrole structure and glutamic acid can obtain it by dehydration, and both of these substances have often been suggested as precursors; leucine was chosen as a representative amino-acid which with its branched chain was most unlikely to take part in pyrrole ring formation; and ammonia was selected in case the nitrogen source was simply ammonia obtained by deamination of any amino-acid. The results were conclusive. The isotopic nitrogen of glycine appeared in such concentrations in the hæmin as to suggest direct utilisation of glycine in the synthesis, while all the other compounds gave concentrations of only one-thirteenth to one-fifth of the glycine value, which supported an indirect route. Although this result

[34] *J. Biol. Chem.*, 1945, **159**, 45 (footnote); L. Ponticorvo, D. Rittenberg, and K. Bloch, *ibid.*, 1949, **179**, 839.

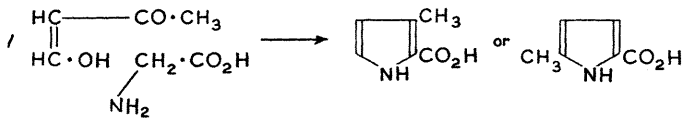
[35] E. Stotz, *Adv. Enzymol.*, 1945, **5**, 149.

[36] A. A. Morton, "The Chemistry of Heterocyclic Compounds", McGraw-Hill Book Co., New York and London, 1946, p. 57.

[37] *J. Biol. Chem.*, 1945, **159**, 567; *ibid.*, 1946, **166**, 621, 627; summarised by E. Lederer, *Ann. Rev. Biochem.*, 1948, **17**, 496.

[38] I. M. London, D. Shemin, R. West, and D. Rittenberg, *J. Biol. Chem.*, 1949, **179**, 463.

only supports the direct use of the nitrogen of the glycine molecule, Shemin and Rittenberg suggested that perhaps the two carbon atoms were also involved. In support of this they drew attention to the purely chemical experiment reported by H. Fischer and E. Fink [39] in 1944. This consisted of mixing aqueous solutions of formylacetone (in the form of its sodium salt or its acetal) and glycine at room temperature and pH 8, and after a short time obtaining a positive Ehrlich test for the presence of a pyrrole. It must be emphasised that the theory which follows is built up, on the chemical side, on this colour test alone, for no solid was isolated and the work, which was to be continued, was interrupted by Fischer's death. Since the Ehrlich test usually indicates a free α-position, although some pyrroles with only a free β-position respond to it,[40] the condensation could have proceeded as shown (XI, XII). Condensation with the formylacetone in the reverse position would give a pyrrole with both α-positions substituted. Fischer and Fink suggested that this type of mild condensation might be the method employed in Nature for the synthesis of pyrrole derivatives. Consideration of this work in conjunction with the results obtained with ^{15}N and with deuterium led Shemin and Rittenberg to suggest that the



(XI.) (XII.)

pyrrole nuclei in porphyrins might originate from the nitrogen and α-carbon atom of glycine and some carbon unit derived from acetate ; but, since all naturally occurring metallic porphyrin derivatives contain completely substituted pyrrole rings, the deuterohæmin in the first experiments must have the deuterium in the side chains or on the methene bridge carbons, and therefore no real clue concerning the source of the remaining three carbon atoms of the ring can be obtained from deuterium experiments. Further work on blood obtained from human subjects suffering from sickle-cell anæmia, on isolated duck's blood,[41] and on dog's blood [42] confirmed the synthesis of hæmin from glycine labelled with ^{15}N. The discovery involving isolated duck's blood is of special interest and importance, since it provides a readily accessible biological system for further study *in vitro*.

The next step was to test Shemin and Rittenberg's theory that, in addition to the nitrogen, both carbon atoms of glycine were involved in hæmin synthesis. The experiments to determine the position of the α-carbon were carried out by K. I. Altman *et al.*[43] who fed rats with glycine

[39] *Z. physiol. Chem.*, 1944, **280**, 123. [40] Ref. 24, Band I, 66.

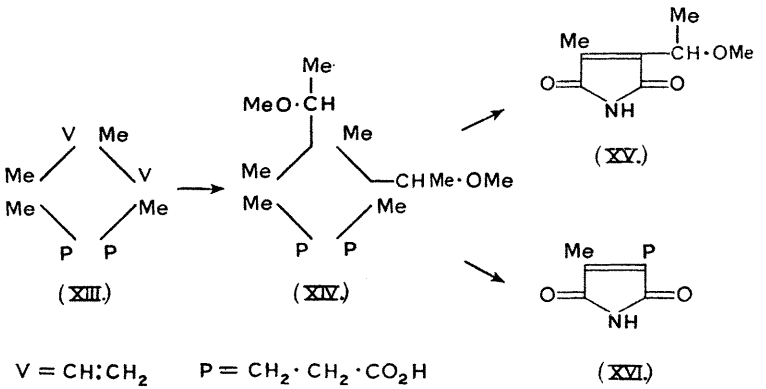
[41] D. Shemin, I. M. London, and D. Rittenberg, *J. Biol. Chem.*, 1948, **173**, 797, 799.

[42] M. Grinstein, M. D. Kamen, and C. V. Moore, *J. Lab. Clin. Med.*, 1948, **33**, 1478.

[43] K. I. Altman, G. W. Casarett, R. E. Masters, T. R. Noonan, and K. Salomon, *Fed. Proc.*, 1948, **7**, 2 ; *J. Biol. Chem.*, 1948, **176**, 319 ; see also K. I. Altman, K. Salomon, T. R. Noonan, *ibid.*, 1949, **177**, 489.

labelled on the methylene carbon atom, and obtained results which indicated that this carbon atom was incorporated into hæmin. The exact point is still to be determined by degradative experiments, but it is reasonable from all the other evidence to suggest that it appears in the pyrrole nuclei. The fate of the carboxyl carbon atom of glycine was examined by M. Grinstein, M. D. Kamen, and C. V. Moore [44] by giving glycine labelled on the carboxyl-carbon atom to a dog and a rat, and isolating crystalline protoporphyrin methyl ester and globin from the blood. Their results showed that the carboxyl carbon atom was not utilised in porphyrin formation but appeared in the globin.

That glycine is incorporated into both types of substituted pyrrole found in protoporphyrin 9 (XIII) (namely, the methyl-vinyl- and the methyl-propionic acid substituted nuclei) was then shown by J. Wittenberg and D. Shemin.[45] Hæmin isolated from the blood of ducks, and also from a man, after they had been fed with ^{15}N-labelled glycine, was chemically



treated to convert the vinyl groups into ·CHMe·OMe, thus giving hæmatoporphyrin dimethyl ether (XIV), which was then oxidised. The ^{15}N concentrations found in the porphyrin and the resulting two maleinimides (XV and XVI) were all equal, thus proving that glycine was the precursor for both types of pyrroles. Although it is not mentioned by Wittenberg and Shemin, this result would seem to support the view of Turner, described above, that the pyrrole building units originate from a common pyrrole precursor. The most recent work with ^{15}N-glycine on a human subject [46] suggests that coproporphyrin 1, uroporphyrin 1, protoporphyrin 9 from hæmin, and stercobilin (an open-chain tetrapyrrole) all derive from a common pyrrole precursor.

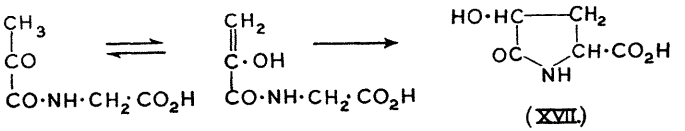
Since the work of Fischer and Fink, described above, on a mild method

[44] *J. Biol. Chem.*, 1948, **174**, 767; 1949, **179**, 359.

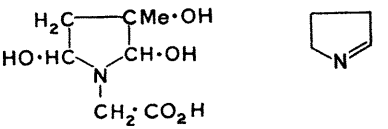
[45] *Ibid.*, 1949, **178**, 47; the same conclusion was reached independently by H. M. Muir and A. Neuberger using a similar isolation procedure; *Biochem. J.*, 1948, **43**, lx; 1949, **45**, 163.

[46] M. Grinstein, R. A. Aldrich, V. Hawkinson, and C. J. Watson, *J. Biol. Chem.*, 1949, **179**, 984; see also C. H. Gray and A. Neuberger, *Biochem. J.*, 1949, **44**, xlv.

of forming the pyrrole nucleus, two further contributions have been made. M. Errera and J. P. Greenstein [47] examined the effect of pH on the ultraviolet absorption of pyruvoylglycine. At pH < 10 two characteristic maxima were obtained, but these disappeared irreversibly at pH > 10. From this evidence, and from the fact that the ionisation constant of the crude material obtained was similar to that of the known pyrrolidonecarboxylic acid, the authors tentatively suggest that a pyrrolidone derivative (XVII) is formed from pyruvoylglycine at pH > 10. A. M. Kuzin and A. R. Guseva,[48] from the condensation of glycine and pyruvic acid at pH 5—6, have isolated, as a calcium salt, a compound thought to be the pyrrolidine derivative (XVIII) containing only the glycine-nitrogen in the ring, but the evidence produced so far is anything but conclusive.



(XVII.)



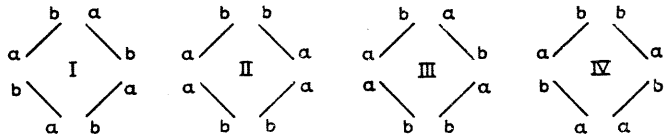
(XVIII.)

The Contribution of Granick and Gilder.—In 1947, a very valuable contribution to the whole subject was made in a review by S. Granick and H. Gilder.[6] This review forms a very comprehensive background to the whole question of the biosynthesis, for it deals with the distribution, structure, and function of the naturally occurring tetrapyrroles (porphyrins), the functions of the porphyrin side-chains, porphyrin synthesis, decomposition of the porphyrins and iron porphyrins, and the physical properties of the tetrapyrroles. The section on porphyrin synthesis is very brief, and refers simply to the reviews by Dobriner and Rhoads and by Turner, already discussed above, but one point of interest is made. They draw attention to the fact that the reduced ring IV in chlorophyll and bacteriochlorophyll is usually thought of as having arisen by addition of two hydrogen atoms to the pyrrole ring IV; but they suggest that, in fact, the pyrrole rings in porphyrins might originate from dihydro- or even tetrahydro-pyrroles by oxidation. In this connection, the recent demonstration by C. Schöpf [49] of the great reactivity of the CH=N linking in the dihydropyridine ring of some compounds, in tetrahydropyridine itself (Δ^1-piperideine) and in the

[47] *Arch. Biochem.*, 1947, **14**, 477; **15**, 445; *J. Nat. Cancer Inst.*, 1947, **8**, 39.
[48] *Biochimia*, 1948, **13**, 27.
[49] FIAT Review of German Science, 1939—46, Preparative Organic Chemistry, Part II, p. 117; see also E. Herzog, *Chimia*, 1948, **2**, 206.

newly isolated but insufficiently characterised Δ^1-pyrroline may be of importance in the linking of the pyrrole units in the α-position to give the porphyrins, although so far the reactions studied with Δ^1-pyrroline involve the addition of a further ring across the CH=N linking, embracing the nitrogen as well as the α-carbon atom.[49a]

Robinson's Theory.—Robinson has applied his method (cf. p. 45) to the problem of the pyrrole pigments.[50] To simplify the discussion he deals with Fischer's four standard ætioporphyrin types (I—IV):



The methyl and ethyl groups, or their equivalents, are represented by *a* and *b*. The problem is to find an explanation of the occurrence of the natural pigments in two types only, I and III, instead of the four theoretically possible. Robinson refers to Fischer's surprising discovery of the "dualism" of the natural porphyrins in which the two types produced are so closely similar, except for the reversal of one pyrrole nucleus. As already mentioned, Robinson's approach is to search for an intact unit which occurs linked in more than one way. If this can be detected, it is a very strong argument that the whole unit itself is used in the biosynthesis. In the above case, the complete reversal of a pyrrole nucleus identifies it immediately as a structural unit. On this evidence a reasonable dissection of the porphyrin molecule is into four pyrrole nuclei joined by four carbon atoms, of unknown origin. In the condensations necessary to build up the ring, the linkings C–*ab* and C–*ba* will not be accomplished with equal ease. Robinson selects the union C–*ab* as the favoured combination, although the argument would apply equally well to C–*ba*. The four types could then result as shown below. It will be seen that the exact stage at which the C atoms are added is very important, and, where necessary, each C atom is therefore numbered to indicate this sequence.

Starting with four preformed C–*a*–*b* units, condensation can only lead to the formation of type I.

Starting with two *b*–*a*–C–*a*–*b* units, formation of type II only is possible.

Regular cyclic condensation starting with four *a*–*b* units and four C atoms, with the stipulation of preferred condensation of C under *a* whenever possible, gives type III.

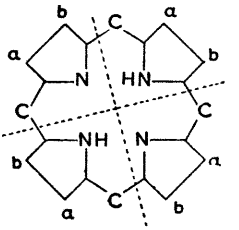
Less regular cyclic condensation, starting as in type III, but adding the last unit to the starting end and then completing the ring with the fourth C, gives type IV.

[49a] Cf. E. Anet, G. K. Hughes, and E. Ritchie, *Nature*, 1949, **163**, 289 and **164**, 501, who have shown that the open-chain 3-methylaminobutyraldehyde, and also 4-amino- and 4-methylamino-valeraldehyde, condense with acetoacetic acid or acetonedicarboxylic acid at mild pH to give naturally-occurring 2-substituted pyrrolidine and piperidine compounds.

[50] Ref. 3 (*e*), p. 799.

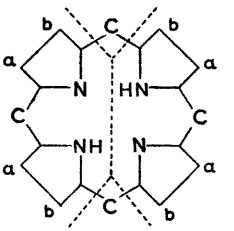
The important conclusion reached by Robinson is that the formation of type I would result from the presence of an excess of the preformed unit C–*a*–*b*, while in the absence of such an excess regular cyclic condensation of the independent units *a*–*b* and C as indicated would give type III.

Four C -a-b



Type I

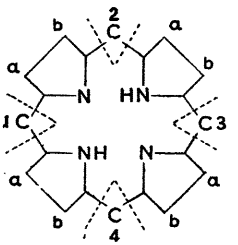
Two b-a- C -a-b
And two C



Type II

Four a-b and four C

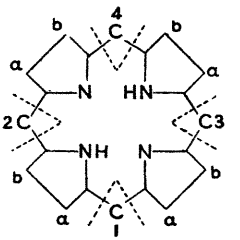
b-a-C-a-b-C-a-b-C-a-b
1 2 3
C
4



Type III

Four a-b and four C

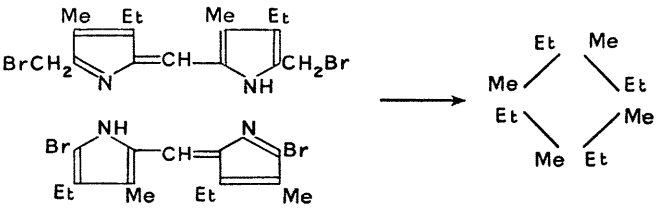
b-a-C-a-b-C-a-b
1 2
C-a-b-C
3 4



Type IV

Chemical Methods of Synthesis.—The chemical methods of synthesis of the porphyrin ring system will now be considered, in order to see whether they can throw any light on the mode of biosynthesis. During evolution and development of these syntheses this biochemical aspect appears not to have received attention, and purely chemical considerations have pre-

vailed throughout. The methods employed have been discussed fully by Fischer and Orth in their book,[51] and the most widely used procedures have been summarised by Morton.[52] They consist essentially of joining two suitably substituted dipyrrylmethanes, or the unsaturated dipyrrylmethenes (as salts), under drastic conditions, such as treatment with concentrated sulphuric acid or boiling formic acid, or heating with a reducing organic acid melt at 180—190°. One example illustrates the method (XIX → XX).



(XIX.) (XX.)

These unusual synthetic methods, which have yielded such fruitful and far-reaching results in the porphyrin field, were discovered quite accidentally by Fischer, when he was trying to proceed from one dipyrrylmethene (or methane) to another. With different substituents filling the vacant positions in the pyrrole nuclei, and in the vigorous conditions of the condensations, many isomers result and the yields are normally poor ; it was only by prodigious efforts that Fischer and his collaborators were able to isolate pure products at all.

Fischer discovered a milder method for performing this type of condensation, but used it only occasionally. This consisted of dissolving a dipyrrylmethane in 90% formic acid, and at room temperature or 40° drawing air through the solution for several days.[53] The condensation of one dipyrrylmethane with two free α-positions with another dipyrrylmethane containing two aldehyde groups, a synthesis which at first sight would appear to offer the advantage of milder reaction conditions, was tried once by Fischer [54] with little success and does not seem to have been pursued further. The preparation of a porphyrin by step-wise addition of each pyrrole nucleus, and final closure of the resulting linear tetrapyrrole, has many attractions from the constitutional point of view. Fischer, although he made several of these linear tetrapyrroles,[55] appears not to have examined this route, but A. H. Corwin and S. R. Buc,[56] after some preliminary experiments, have been able to delimit the conditions necessary for success.

The synthesis of porphyrins starting with a single-nucleus pyrrole derivative was used by Fischer only on rare occasions, owing to its obvious

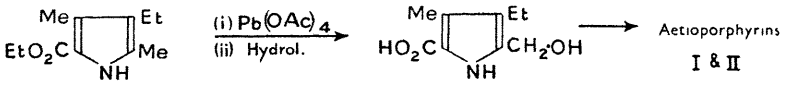
[51] Ref. 24, Band II, 1, pp. 160—173. [52] Ref. 36, p. 86.

[53] H. Fischer and H. Andersag, *Annalen*, 1926, **450**, 217 ; H. Fischer, P. Halbig, and B. Walach, *ibid.*, 1927, **452**, 284.

[54] H. Fischer and P. Halbig, *ibid.*, 1926, **447**, 128.

[55] Ref. 24, Band II, 1, p. 619. [56] *J. Amer. Chem. Soc.*, 1944, **66**, 1151.

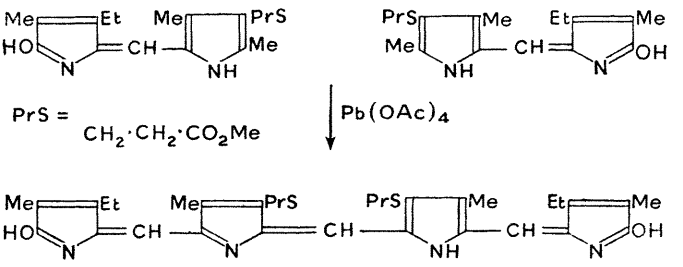
limitation to the simplest symmetrical compounds.[57] With a pure synthetic pyrrole derivative, treatment with formic acid at 100° gave no porphyrin, and heating in a sealed tube to 160° was necessary. This was explained by postulating that a source of hydrogen was necessary, and that this hydrogen



(XXI.) (XXII.)

was obtained at the higher temperature by decomposition of the formic acid. By direct addition of a hydrogen donor, such as formaldehyde, to the formic acid, the porphyrin was obtained at 100°. The simple pyrrole derivatives isolated by degradation of porphyrins usually contained impurities which acted as hydrogen donors, and the porphyrin reaction with derivatives of such origin proceeded at 100°.[58] Other conditions used were treatment of a pyrrole with glyoxal tetramethyl acetal and concentrated hydrochloric acid at 100°, or with di(chloromethyl) ether in ethereal solution at room temperature.[59]

The most recent synthesis of this single-nucleus type was discovered by W. Siedel and F. Winkler [60] while examining the properties of the pyrrole derivative (XXII), prepared by oxidation of (XXI) with lead tetra-acetate. A mixture of ætioporphyrins I and II, probably formed through their reduced (porphyrinogen) forms, was obtained from this single-nucleus carbinol by heating it alone at 160—170° (49% yield), by boiling its solution in methyl-alcoholic hydrobromic acid in a stream of air (36·4% yield), or



(XXIII.)

simply by setting it aside in methyl alcohol alone for several days exposed to air (yield less than above). The last method in particular is very surprising, and demonstrates the great tendency for the large, stable, 16-membered porphyrin ring to be formed. This tendency is connected with the

[57] Ref. 24, Band II, 1, p. 165.

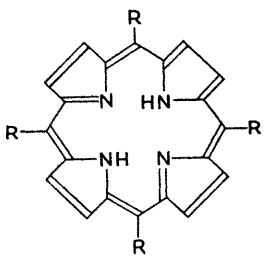
[58] H. Fischer, E. Sturm, and H. Friedrich, *Annalen*, 1928, **461**, 247 ; H. Fischer and A. Treibs, *ibid.*, 1926, **450**, 144.

[59] Ref. 24, Band II, 1, 192.

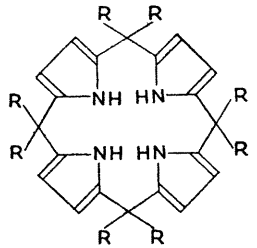
[60] *Annalen*, 1943, **554**, 165, 186.

presence of substituent groups in the β-positions. Removal of one or both substituents produced a carbinol-acid, which gave no porphyrin even when heated with reagents in a sealed tube.

An entirely new method of joining two dipyrrylmethenes together, to give the open-chain bilin derivative (XXIII) in 60—66% yield has been described by W. Siedel and E. Grams.[61] The reagent was lead tetra-acetate and the conditions were very slow addition of the oxidising agent in the cold to the substance dissolved in acetic acid, followed by a final warming on the water-bath. It is possible that this method could be extended to the preparation of a porphyrin by oxidising a dipyrrylmethene, or even a single nucleus pyrrole, containing two α-methyl groups. This oxidation would probably require a reagent other than lead tetra-acetate, which with some porphyrins has already been shown by Fischer [62] to give xanthoporphinogens (porphyrins + 4 oxygen atoms, of unknown constitution), and would have more force if it succeeded with a biological oxidising agent.



(XXIV.)



(XXV.)

The parent substance of the porphyrins, porphin (XXIV ; R = H) and symmetrical porphins containing substituents on the methene-bridge carbon atoms only (XXIV ; R = alkyl or aryl) were made by P. Rothemund [63] by treating various aldehydes with pyrrole itself in presence of basic catalysts ; but although over 25 aldehydes are stated to have given porphins (presumably identified spectroscopically), experimental details and analysis have been given only for porphin itself and for tetraphenylporphin, and for their metallic salts [together with some evidence for the existence of "*iso*-porphins", which are very probably dihydroporphins (chlorins)].[64] Thus pyrrole with formaldehyde in methyl alcohol in presence of pyridine in a sealed tube at 90—95° gave, in 30 hours, porphin in 0·1% yield. Rothemund states that these condensations proceed at room temperature during several weeks, but no details are given, and in the latest preparation of the deep-blue crystalline tetraphenylporphin, obtained in about 10% yield

[61] *Z. physiol. Chem.*, 1940, **267**, 49.

[62] Ref. 24, Band II, 2, p. 423.

[63] *J. Amer. Chem. Soc.*, 1935, **57**, 2010 ; 1936, **58**, 625 ; 1939, **61**, 2912 ; P. Rothemund and A. R. Menotti, *ibid.*, 1941, **63**, 267 ; 1948, **70**, 1808.

[64] S. Aronoff and M. Calvin, *J. Org. Chem.*, 1943, **8**, 205 ; M. Calvin, R. H. Ball, S. Aronoff, *J. Amer. Chem. Soc.*, 1943, **65**, 2259 ; R. H. Ball, G. D. Dorough, and M. Calvin, *ibid.*, 1946, **68**, 2278.

from benzaldehyde and pyrrole in presence of pyridine, the conditions involved heating in a sealed tube for 48 hours at 220°.

Porphin itself was also synthesised by H. Fischer and W. Gleim [65] in about 0·1% yield contemporaneously with Rothemund, but the method used by them was to heat pyrrole-2-aldehyde in alcohol and formic acid for 36 hours.

Brief consideration will now be given to the properties and conditions necessary for the formation of the partially reduced porphyrins known as porphyrinogens (porphinogens) (XXV). Fischer prepared some of these (XXV; R = H and ring positions substituted) by mild reduction of the corresponding porphyrins [66] and showed that they were colourless crystalline substances which, when impure, were re-oxidised in air quantitatively in one day to the original porphyrin, but, when pure, were stable in air for several weeks. A. Vannotti [67] has summarised the few experimentally observed colour changes which have suggested the natural formation, in certain body conditions, of porphyrins through the porphyrinogen forms. In the new synthesis of ætioporphyrins I and II mentioned above,[60] Siedel and Winkler postulated the mixed ætioporphyrinogens as intermediates in the condensation and, after shorter treatment of the pyrrylcarbinol with boiling methyl-alcoholic hydrobromic acid, actually isolated crystalline porphyrinogens. On long storage in air the colourless crystals gradually gave the mixed porphyrins. Several stable porphyrinogens (XXV; R = alkyl) with fully substituted methylene-bridge carbon atoms have been made by condensing some aliphatic ketones with pyrrole.[68] For example, when acetone and pyrrole in alcohol are warmed with a trace of concentrated hydrochloric acid, a vigorous reaction takes place and the colourless crystalline octamethylporphyrinogen is soon deposited in good yield. A small yield was also obtained [69] from a homogeneous solution of pyrrole, excess of acetone, and water in presence of a trace of hydrochloric acid, added at room temperature. The solution became warm (to 37°) during the reaction. Porphyrinogens with two aryl groups on the methylene bridges, or with only one hydrogen on the bridges substituted by any type of group, are unknown.

These colourless porphyrinogens are of fundamentally different character from the coloured porphyrins. Those containing unsubstituted methylene bridges are unstable, and this must be caused primarily by the impossibility of resonance between the pyrrole nuclei. Stability can be achieved either by loss of hydrogen to give the corresponding porphyrins, or by substitution of all the methylene hydrogen atoms by alkyl groups.

Summary, Conclusions, and Some Speculations.—With so much chlorophyll being synthesised and degraded every year in readily accessible leaves of all types, it seems strange that no clue at all to the biosynthesis of the main structure itself has yet been obtained from this source. By the application of the newer and more delicate techniques of chromatography

[65] *Annalen*, 1935, **521**, 157.
[66] Ref. 24, Band II, 2, p. 420.
[67] "Porphyrin und Porphyrinkrankheiten," Springer, Berlin, 1937, p. 20.
[68] Ref. 24, Band I, pp. 393—395.
[69] V. V. Tschelincev and B. V. Tronov, *J. Russian Phys. Chem. Soc.*, 1916, **48**, 105.

and partition chromatography, and by further development of tracer experiments with plants, it is probable that the great difficulties encountered from this side will soon be overcome.

The success so far achieved regarding the final stages of the chlorophyll biosyntheses, by the method of approach using *X*-ray induced mutants of plant cells, is encouraging, and valuable advances may be obtained by extension of this method.

The experimental observations from the medical side are being added to rapidly. The theory of the formation of the porphyrin ring by condensation of dipyrrylmethene units is an ingenious interpretation of the experimental facts. Its weakness lies in the fact that many more types could result than are actually observed, although this could be explained by enzymic direction along the correct channels.

The work with tracers has only just begun, and the results of outstanding importance already obtained with glycine augur well for the future development of this fundamental method.

Several lines of investigation have converged to indicate that the four pyrrole nuclei are all derived from a common pyrrole precursor, and therefore that in the biosynthesis they are added as complete units.

With regard to the chemical methods of synthesis of the porphyrin skeleton, the general impression obtained is one of very drastic, typically organic methods, relieved by glimpses of very mild methods which might well have biological importance. The great tendency for the large ring to be formed from a well-substituted, reactive, single nucleus pyrrole simply by storage in a solvent with access to air, and the joining together of two dipyrrylmethene molecules, admittedly only at one end so far, by oxidation of the two α-methyl groups may be of great significance biologically. The formation of the final aromatic ring system *via* the partly reduced porphyrinogen forms is quite possible, since these forms are chemically unstable and change into the stable aromatic form even on storage in air. In connection with the possible formation by way of the completely reduced system, there is no evidence here to act as a guide, since such a substance has not yet been prepared. Preliminary condensation of partly reduced pyrrole units, such as the reactive Δ^1-pyrrolines, to form the large 16-membered ring with later aromatisation, should also receive consideration. With regard to the side-chains, it would be unlikely from a chemical standpoint for these to be added after completion of the main skeleton. No suggestion appears yet to have been entertained that the final ring system is formed by the collapse of a 20-membered ring containing 8 CO groups, suitably placed for conversion into pyrrole rings by treatment with ammonia, on the lines of the well-known synthesis of 2 : 5-dimethylpyrrole from acetonylacetone. On the present evidence such a route is unlikely.

The final picture which emerges from all the evidence is of a common preformed pyrrole precursor for all the rings, built up from the glycine-nitrogen and probably the α-carbon atom, the side-chains in the two β-positions being possibly $\cdot CH_2 \cdot CO_2H$ and $\cdot CH_2 \cdot CH_2 \cdot CO_2H$; this pyrrole derivative then condenses, with adjustment of the side chains at some stage,

by means of four carbon atoms, whose origin is yet to be discovered,* in a manner which leads on the chlorophyll side to a type-III protoporphyrin, with subsequent formation of the additional ring, and on the blood-pigment side to both type-I and type-III pigments. Thus although there are many important gaps to be filled, the successes achieved up to the present offer much encouragement for the prosecution of further studies, and the final solution of the problem seems to be approaching within reach.

* Possibly the methylene carbon atom of glycine. See H. M. Muir and A. Neuberger, *Biochem. J.*, 1949, **45**, xxxiv.