The Biosynthesis of Porphyrins and Porphyrin Metabolism


The emphasis at this symposium was on the biosynthesis and intermediary metabolism of porphyrins. Clinical problems and investigations were not discussed, but one of the 5 half-day sessions was devoted to experimental porphyria in animals. The discussions were held under the chairmanship of Prof. C. Rimington of London. Six of the 26 panel members were from the United States.

The symposium was opened by Shemin (Columbia, New York) who discussed the metabolic pathway of porphyrin biosynthesis via the succinate-glycine cycle. "Active" succinate derived from the tricarboxylic acid cycle, condenses with glycine to form α-amino-β-keto adipic acid, which on decarboxylation yields δ-aminolevulinic acid. Both compounds are obligatory intermediates in porphyrin biosynthesis. Two moles of δ-aminolevulinic acid condense to form porphobilinogen, which in turn gives rise to porphyrin and heme formation. A highly purified water soluble enzyme preparation has been isolated from duck erythrocytes, which catalyzes the condensation of δ-aminolevulinic acid to porphobilinogen in vitro. Gibson (Mill Hill, London) has been able to isolate a similar enzyme preparation from an acetone powder of ox liver. The enzyme, which tentatively has been termed δ-aminolevulinic acid dehydrase, is activated by glutathione, but inhibited by versene. δ-Aminolevulinic acid dehydrase activity has been found in various tissues of the rabbit. Liver is by far the most active tissue, followed by bone marrow, kidney, spleen, blood, brain and myocardium in this order. In experimental Sedormid porphyria, the enzyme activity is significantly increased in liver and kidney, whereas in phenylhydrazine anaemia, a considerable increase in activity is found in blood and spleen.

The in vivo metabolism of δ-aminolevulinic acid was discussed by Scott (Mill Hill, London). Isotopically labeled δ-aminolevulinic acid was administered to normal human subjects and to patients with porphyria. The rate of excretion of this compound in the urine is very rapid, which renders the interpretation of the data obtained rather difficult. For example, the degree of labeling of porphobilinogen and porphyrins arising from labeled δ-aminolevulinic acid could not be used for measuring the "pool" of these heme precursors in the body. In the course of these studies it was noted that in human subjects parenteral administration of δ-aminolevulinic acid with subsequent exposure to light resulted in generalized erythema and edema. In the rat, 55 per cent of the administered labeled δ-aminolevulinic acid was excreted unchanged in the urine; in addition 3-5 per cent appeared as porphobilinogen in the urine, and 7 per cent as protoporphyrin and 1 per cent as coproporphyrin in the bile. Of particular interest was the finding that in the intestine protoporphyrin and stercobilin were labeled to the same extent. The radioactivity of both compounds was considerably higher than that of the circulating heme.

Falk (Univ. College Hosp., London) showed that in a system using hemolyzed chicken erythrocytes, porphobilinogen and δ-aminolevulinic acid are equivalent as substrates for the formation of protoporphyrin. Significant amounts of copro- and uroporphyrin were formed only when these substrates had been present in large excess. The addition of ferrous ions to the system decreased the amount of protoporphyrin formed, but increased heme synthesis.

Dresel (Univ. College Hosp., London) used dilution-type experiments to demonstrate that δ-aminolevulinic acid and porphobilinogen are true intermediates in heme biosynthesis. On the other hand, her data suggested that a variety of porphyrins, including uroporphyrins, coproporphyrin III, hematoporphyrin IX and protoporphyrin IX play no role as intermediates in the formation of heme. The finding that free protoporphyrin IX may not be a true precursor of heme is contrary to results obtained by others using more direct experiments, in which labeled protoporphyrin IX was found to be readily incorporated into heme. As to the porphyrins with more than two carboxyl groups, however, data reported by
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other members of the panel seemed to support Dresel's concept, that these porphyrins are not intermediates in heme biosynthesis, but rather side products. Shemin had failed to observe incorporation of labeled coproporphyrin III into heme in the hemolyzed duck erythrocyte system. Similarly Schwartz (Minneapolis) was unable to demonstrate in vitro conversion of C14-labeled uroporphyrin III to protoporphyrin IX using a variety of systems. In the course of these experiments he has observed that radioactivity which originally seemed to indicate new formation of protoporphyrin actually was due to contamination of the protoporphyrin fraction with several green porphyrins which were radioactive. The significance of these green pigments, which can be purified by chromatographic methods, is not known.

The evidences derived from these studies seemed to indicate that copro- and uroporphyrin are probably not direct precursors of heme, but that the true intermediates are structurally closely related to them. This concept was challenged by Eriksen (Oslo), who reported experiments employing paper chromatographic and radioautographic methods, the results of which were believed to demonstrate the conversion of porphyrins with 3 to 8 carboxyl groups to free protoporphyrin and to heme. Eriksen's proposed scheme of heme biosynthesis, which involves a stepwise decarboxylation of uroporphyrin to form, ultimately, protoporphyrin and heme, is in agreement with an earlier report by Altman (Rochester, N. Y.) who claimed to have observed the utilization of uroporphyrin III for heme synthesis. Finally studies with chlorella mutants reported by Granick (Rockefeller Inst., N. Y.) seemed to suggest that hematoporphyrin and a tricarboxylic monovinylporphyrin may be possible precursors of protoporphyrin in red blood cells. However, Altman showed that in the hemolyzed duck red cell system, labeled hematoporphyrin IX, which he has prepared from protoporphyrin, failed to be utilized for heme synthesis.

The panel spent considerable time discussing the various concepts bearing on the relationship of the free porphyrins to heme synthesis in vivo and in vitro. The problem is complex, and its solution appears to require complete elucidation of the mechanism by which 4 moles of porphobilinogen condense to form the porphyrin ring. Only then will it be possible to determine the exact relationship of the naturally occurring porphyrins to heme formation.

Drabkin (Univ. of Penn., Philadelphia) reviewed some of his more recent studies on the metabolism of cytochrome c. Of particular interest were experiments which were designed to determine the rate of cytochrome c synthesis in yeast adapting and adapted to oxygen. With the use of glycine-2-C14, it could be shown that in the course of adaptation to aerobic life cytochrome c formation in yeast cells is greatly increased as compared to the formation of "non-specific" proteins. Schapira (Paris) reported a difference in the rate of incorporation of Fe3+ and glycine-2-C14 between the alkali-resistant and the alkali-sensitive fractions of rabbit hemoglobin. Similar observations were made in human cord blood.

In the session devoted to experimental porphyria, Schmid (Minneapolis and New York) showed that the production of Sedormid porphyria in rabbits and rats is accompanied by a precipitous fall in liver catalase activity. The relationship between the decrease in catalase, which appears to be the result of a block in enzyme synthesis, and the excessive porphyrin formation in the liver is not clear. In similar experiments with Sedormid, Vannotti (Lausanne) observed, in addition to the rapid fall in catalase, a slight decrease in cytochrome c concentration in the liver. Rimington referred to studies done in his laboratories which showed that allylisopropylacetamid has properties essentially similar to those of Sedormid except for the fact that the former has only a minor hypnotic effect upon the animal. Other aspects of experimental porphyria were discussed by Schwartz, Gajdos (Paris), Formijne (Amsterdam) and Stich (Munich).

The last speaker on the formal program was MacDonald (Nat. Research Council, Ottawa) who reported the successful synthesis from dipyrromethenes of pure uroporphyrin II and uroporphyrin IV. The availability of these two porphyrins as reference compounds will help to facilitate the identification of naturally occurring uroporphyrins.

The Ciba Symposium on Porphyrin Metabolism has given the workers in this field in various countries a welcome opportunity to compare the results of their studies and to discuss differences in opinion and interpretation. This is probably one of the most important aspects of the conferences which the Ciba Foundation is sponsoring in various fields of the medical sciences.—Rudi Schmid, Columbia University, College of Physicians and Surgeons.