A Ciba Foundation Symposium

PORPHYRIN BIOSYNTHESIS AND METABOLISM

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., B.Ch.

and

ELAINE C. P. MILLAR, A.H.W.C., A.R.I.C.

With 70 Illustrations

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PORPHYRIN BIOSYNTHESIS AND METABOLISM

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PREFACE

FROM the time of the Ciba Foundation's Symposium on "Isotopes in Biochemistry" in 1951, the Director of the Foundation had had in mind the possibility of a conference to follow up the work then described by Dr. D. Shemin on porphyrin biosynthesis. He therefore fell in very readily with a suggestion by Professor C. Rimington for such a symposium in 1955, especially as Prof. Rimington willingly accepted much of the responsibility for the selection of members and the construction of the programme. Dr. J. E. Falk also gave considerable help in these matters. At the Symposium Prof. Rimington appropriately occupied the Chair throughout the meeting, for which both members and the Foundation have cause to be very grateful.

To those to whom this book serves as an introduction to the activities of the Ciba Foundation it should be explained that it is an international centre, which is established as an educational and scientific charity under the laws of England. It owes its inception and support to its founder, CIBA Ltd., of Switzerland, but is administered independently and exclusively by its distinguished British Trustees.

The Foundation provides accommodation for scientific workers who visit London from abroad, organizes and holds international conferences, conducts (in conjunction with the Institut National d'Hygiène) a post-graduate medical exchange scheme between England and France, arranges informal meetings for discussions, awards an annual lectureship, has initiated a scheme to encourage basic research relevant to the problems of ageing, assists international congresses and scientific societies, is building up a library service in special fields, and generally endeavours to give aid in all matters that may promote international co-operation in scientific research.

Leading research workers from different countries and in different disciplines are invited to attend the symposia or colloquia. The size of the group is, however, very strictly limited in order to obtain a free conversational manner of discussion—although the basic timetable of the programme is strictly observed. The smallness of the groups means the exclusion of many workers active and interested in the subjects discussed, and therefore the proceedings of these conferences are published and made available throughout the world.

It is hoped that the papers and discussions in this book will prove not only informative and stimulating, but will also give to readers a sense of participation in an informal and friendly occasion.

List of those participating in or attending the Symposium on "The Biosynthesis of Porphyrins and Porphyrin Metabolism," 8th–10th February 1955

K. ALTMAN	School of Medicine and Dentistry, University of Rochester, New York
J. CANIVET	Faculty of Medicine, Paris
D. L. DRABKIN	Dept. of Biochemistry, Graduate School of Medicine, University of Pennsylvania, Philadelphia
ELISABETH I. B. DRESEL	Dept. of Chemical Pathology, University College Hospital Medical School, London
L. ERIKSEN	Universitetets Fysiologiske Institutt, Oslo
J. E. FALK	Dept. of Chemical Pathology, University College Hospital Medical School, London
P. FORMIJNE	Kliniek voor Inwendige Ziekten, University of Amsterdam
A. GAJDOS	Clinique Médicale de l'Hotel-Dieu, Paris
MME. GAJDOS-TÖRÖK	Clinique Médicale de l'Hotel-Dieu, Paris
K. D. GIBSON	National Institute for Medical Research, London
S. GRANICK	Rockefeller Institute for Medical Research, New York
C. H. GRAY	Dept. of Chemical Pathology, King's College Hospital, London
JUNE LASCELLES	Microbiology Unit, Dept. of Biochemistry, University of Oxford
S. F. MACDONALD	National Research Council, Ottawa
A. NEUBERGER	National Institute for Medical Research, London
C. RIMINGTON	Dept. of Chemical Pathology, University College Hospital Medical School, London
G. SCHAPIRA	Laboratoire de Recherches de Biochimie Médicale, Université de Paris
R. SCHMID	Univ. of Minnesota Medical School, and Dept. of Biochemistry, Columbia University, New York
S. SCHWARTZ	Dept. of Internal Medicine, University of Minnesota
R. SCHWYZER	CIBA Limited, Basle
J. J. SCOTT	National Institute for Medical Research, London

D. SHEMIN	Dept. of Biochemistry, Columbia University, New York
W. STICH	Dept. of Internal Medicine, University of Munich
B. THORELL	Dept. of Pathology, Karolinska Sjukhuset, Stockholm
A. VANNOTTI	Clinique Médicale Universitaire, Lausanne
J. WALDENSTRÖM	Medical Clinic, Malmö Allmänna Sjukhus, Malmö, Sweden

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Chairman: C. RIMINGTON

CHAIRMAN'S OPENING REMARKS

C. RIMINGTON

THIS is, as far as I know, the first Conference devoted entirely to porphyrin biochemistry, and it struck me as being of no little interest that it happens to fall almost exactly 25 years after the *in vitro* synthesis of haemin by Fischer and Zeiler, and nearly 10 years after the demonstration of the incorporation of labelled glycine into the haem of circulating blood, by Shemin and Rittenberg. Prof. Drabkin has reminded me of another early date which is of great historical interest, the discovery of the first porphyrin, when in 1867 Thudichum prepared in an unequivocal manner haematoporphyrin (which he called cruentine) by the action of concentrated acid on haemoglobin, thus anticipating Hoppe-Seyler by several years. I have Thudichum's original preparations and specimens of cruentine and also some of his preparations of the bile pigments, bilirubin and biliverdin, and these are being displayed during the Symposium as an exhibit in the old conference room. Beside them I have also placed two volumes of reprints of early papers on porphyrins and related pigments which were collected by Sir Archibald Garrod and contain many of the classics, such as the paper by Stokvis, and which are closely annotated in Garrod's own hand. I hope these treasures from the past will prove of interest to you.

You will note with interest that the reference to Thudichum's historic paper is a rather obscure one; it is in the report of the medical officer to the Privy Council for 1867, Vol. X., Appendix No. 7. Out of the medical committee of the Privy Council arose the Medical Research Council and so one might say perhaps that Thudichum was in fact the first porphyrin chemist associated with the Medical Research Council.

Prof. Waldenström has also very kindly contributed for

exhibition a most striking map showing the distribution of known porphyria families in Sweden.

To return to the present occasion, it is truly astounding what rapid developments have taken place in our knowledge of porphyrin biosynthesis since the last time the subject was discussed within these walls at the Ciba Foundation's conference on " Isotopes in Biochemistry " in 1951. In arranging the present meeting the difficulty was not so much to find sufficient material to warrant a three-day conference as that of exercising a rigid selection, so that discussion could be intensive on a chosen theme rather than diffuse over the whole wide field. Porphyrin and haem biosynthesis seemed the obvious choice and porphyria diseases (with the exception of limited reference to experimental porphyria) have been deliberately excluded. This has meant the unavoidable exclusion also of many colleagues working on porphyria whom we would otherwise have liked to invite.

I feel that we have now come to a stage in the investigation of porphyrin biosynthesis when every step has to be scrutinized with the utmost critical intensity to ensure that we are following reliable signposts towards full understanding of the biosynthetic process. My own group has been especially concerned with the position of uroporphyrin, coproporphyrin and protoporphyrin in the biosynthetic route towards haem, but I am sure there will be other equally good examples brought forward during the conference.

From your programmes you will see that we have twenty communications and these have been arranged so that they fall roughly into three main groups, although considerable overlap is inevitable. First we consider the early stages in biosynthesis, pre-pyrrole, if you like, and porphobilinogen; then more particularly the rôle of porphyrins and the binding of iron. In session four are brought together communications relating to the use of Sedormid and allyl*iso*propylacetamide in causing deranged porphyrin metabolism. These studies may eventually throw further light upon the normal biosynthetic process and it is in this aspect which we hope now to consider

them rather than in the light of their relation to the natural porphyria diseases. We shall not enter into long discussions on porphyria as such as it is our intention if possible to try and concentrate intensively on the theme of normal biosynthesis.

I should like, in opening this Conference, to follow the very fine example of the President of the United States in Council and our own Parliament when it comes together in session and to ask for God's guidance in our deliberations and a successful outcome of our meeting.

THE SUCCINATE-GLYCINE CYCLE; THE RÔLE OF δ-AMINOLEVULINIC ACID IN PORPHYRIN SYNTHESIS*

DAVID SHEMIN, PH.D.

The Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y.

THE problem of the biosynthesis of porphyrins, in the formation of the initial intermediates, can be considered, from a more general point of view, to be merely one aspect of the metabolism of glycine. Many overall aspects of glycine metabolism are now known, and especially the metabolic pattern of the α-carbon atom of glycine in its utilization for the synthesis of other compounds has been established. The α-carbon atom of glycine, no longer attached to the carboxyl group, is utilized for the synthesis of porphyrins (Altman, Casarett, Masters, Noonan and Salomon, 1948; Muir and Neuberger, 1950; Radin, Rittenberg and Shemin, 1950; Wittenberg and Shemin, 1950), the ureido groups of purines (Karlsson and Barker, 1949), the β-carbon atom of serine (Sakami, 1949; Winnick, Moring-Claesson and Greenberg, 1948) and for methyl groups (Arnstein, 1950; Weissbach, Elwyn and Sprinson, 1950). This metabolic pattern is similar to that of the so-called "C_1" compounds, with the exception that the latter cannot substitute for glycine in porphyrin synthesis. It would seem therefore that these apparently unrelated compounds (porphyrins, purines, serine and methyl groups) have one common feature, namely the participation of the α-carbon atom of glycine for their synthesis.

It would appear reasonable, from a unitarian approach,

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therefore, to consider the possibility of glycine being metabolized via a pathway in which intermediates are produced which then can be utilized for the synthesis of these different compounds. In an attempt to unify the reactions of glycine we have postulated a series of reactions, called the succinate-glycine cycle (Fig. 1). This pathway for glycine metabolism suggested itself from a study of the mechanism of porphyrin

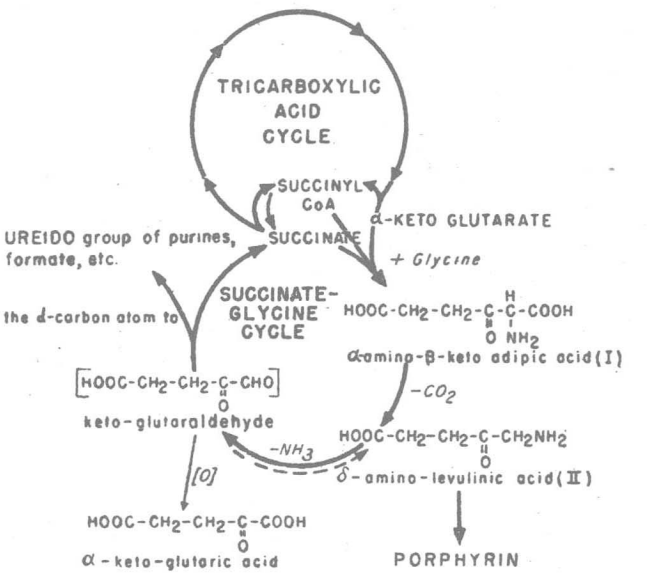

FIG. 1. Succinate-glycine cycle: a pathway for the metabolism of glycine.

synthesis. In this pathway it is postulated that "active" succinate condenses on the α-carbon atom of glycine to give rise to α-amino-β-ketoadipic acid. This β-keto acid decarboxylates to give rise to δ-aminolevulinic acid, which can be utilized for porphyrin synthesis or be further metabolized in such a manner that its δ-carbon atom (originally the α-carbon atom of glycine) is utilized for the synthesis of the ureido groups of purines, the β-carbon atom of serine, and for methyl groups, while the remaining four-carbon atom residue is