CIBA FOUNDATION SYMPOSIUM

ON

QUINONES IN ELECTRON TRANSPORT

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., M.R.C.P.

and

CECILIA M. O'CONNOR, B.Sc.

With 82 Illustrations

J. & A. CHURCHILL LTD.

104 Gloucester Place, London, W.1

1961

QUINONES IN ELECTRON TRANSPORT

Ciba Foundation Symposia

General Volumes

Mammalian Germ Cells	30s.
Preservation and Transplantation of Normal Tissues	25s.
Leukaemia Research	30s.
Chemistry and Biology of Pteridines	42s.
Porphyrin Biosynthesis and Metabolism	30s.
Histamine	50s.
Extrasensory Perception	27s. 6d.
Bone Structure and Metabolism	45s.
Ionizing Radiations and Cell Metabolism	45s.
The Nature of Viruses	42s.
Chemistry and Biology of Purines	48s.
Drug Resistance in Micro-organisms	50s.
Chemistry and Biology of Mucopolysaccharides	45s.
The Cerebrospinal Fluid	50s.
Amino Acids and Peptides with Antimetabolic Activity	45s.
Medical Biology and Etruscan Origins	45s.
Biosynthesis of Terpenes and Sterols	45s.
Carcinogenesis: Mechanisms of Action	48s.
Biochemistry of Human Genetics	50s.
Significant Trends in Medical Research	50s.
Cellular Aspects of Immunity	60s.
Congenital Malformations	45s.
Haemopoiesis: Cell Production and its Regulation	60s.
Adrenergic Mechanisms	70s.

A leaflet giving details of the Ciba Foundation Colloquia on Endocrinology, Colloquia on Ageing and Study Group volumes is available from the Publishers

THE Ciba Foundation, a unique international institution, owes its inception to the generosity of CIBA Limited, Basle. However, being established under British trust law, it enjoys complete independence in practice and policy.

Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, the proceedings of which are published in book form in the manner of the present volume. The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The purpose of the Ciba Foundation, which is to promote international co-operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a black sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the red sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

THE CIBA FOUNDATION

for the Promotion of International Co-operation in Medical and Chemical Research

41 PORTLAND PLACE, LONDON, W.1.

Trustees

THE RIGHT HON. LORD ADRIAN, O.M., F.R.S.
THE RT. HON. LORD BEVERIDGE, K.C.B., F.B.A.
SIR RUSSELL BRAIN, BT.
THE HON. SIR GEORGE LLOYD-JACOB
SIR RAYMOND NEEDHAM, Q.C., F.S.A.

Executive Council

SIR RUSSELL BRAIN, BT., *Chairman*
DR. F. GROSS
PROFESSOR A. HADDOW, F.R.S.
SIR ARTHUR VERE HARVEY, C.B.E., M.P.
THE HON. SIR GEORGE LLOYD-JACOB
PROFESSOR F. G. YOUNG, F.R.S.

Director, and Secretary to the Executive Council

DR. G. E. W. WOLSTENHOLME, O.B.E.

Deputy Director

MR. A. V. S. DE REUCK, M.Sc.

Assistant Secretary

MISS N. BLAND

Scientific Assistant and Librarian

MISS M. P. CAMERON, M.A.

Editorial Assistant

MISS MAEVE O'CONNOR, B.A.

Conference Assistant

MRS. NANCY G. SPUFFORD

PREFACE

It was late in 1958 that Dr. Karl Folkers proposed to the Director of the Ciba Foundation that a symposium should be organized to discuss some of the new quinones being discovered in animals, micro-organisms and higher plants, and which appeared to have important biochemical rôles.

In view of the Foundation's limited accommodation and, more especially, in order to allow every member a fair share of discussion within the three days of the meeting, conferences are restricted in size to about 30 people. This normally leads to a severe problem in selecting membership, primarily based on ability to contribute to the subject, but with some regard to international and inter-disciplinary exchanges. The pioneer work of Prof. R. A. Morton in Liverpool and of Prof. David E. Green in Wisconsin and their associates had already attracted other investigators to work on the quinones, but it seemed that for once the small group of workers would practically select themselves. By the time the meeting was held in 1960, however, interest in this line of research had increased so extensively that many active workers could not be included. It is hoped that this publication of the papers and discussions will be some compensation to them, of interest and value to those engaged in neighbouring research, and perhaps stimulate others to make fresh investigations of importance.

The Ciba Foundation is very much indebted to Dr. Karl Folkers for his initiative, his advice, his participation and his Chairmanship. The Director also wishes to record here, in the last major volume edited for the Ciba Foundation by Miss Cecilia O'Connor—now Mme. Oscar Mairlot—the profound gratitude to her of all concerned in the work, and especially the publications, of the Foundation. She has been a superb colleague whose

example will, it is hoped, exercise a lasting influence upon the technical standards and the human relationships involved in the production of books such as these.

Our thanks are also due to Mrs. Margarete Silverman and Miss M. P. Cameron who completed the preparation of this volume for the press; to Mr. J. and Mr. John Rivers, Directors of J. & A. Churchill Ltd., for their constant courtesy and helpfulness, and to Mr. William Hill for the rapid and skilful indexing of this and other volumes.

List of those participating in or attending the Symposium on "Quinones in Electron Transport'

11th-13th May, 1960

H. R. V. Arnstein	National Inst. for Medical Research, Mill Hill, London
A. J. Birch	Dept. of Organic Chemistry, University of Manchester
N. I. Bishop	Dept. of Biological Science, Fels Fund, The Florida State University, Tallahassee
A. F. Brodie	Dept. of Bacteriology and Immunology, Harvard Medical School, Boston
B. Chance	The Eldridge Reeves Johnson Foundation for Medical Physics, The School of Medicine, University of Pennsylvania, Philadelphia
J. B. Chappell	Dept. of Biochemistry, University of Cambridge
V. M. Clark	University Chemical Laboratory, Cambridge
F. L. Crane	Dept. of Biological Sciences, Purdue University, Lafayette
K. Folkers	Merck Sharp & Dohme Research Laboratories, Rahway
D. E. Green	Inst. for Enzyme Research, University of Wisconsin, Madison
J. Green	Walton Oaks Experimental Station, The Research Laboratories of Vitamins Limited, Tadworth, Surrey
R. Hill	Agricultural Research Council, London
O. Isler	F. Hoffmann-La Roche & Co., Ltd., Basle
S. K. Kon	The National Inst. for Research in Dairying, University of Reading, Shinfield
Sir Hans Krebs	Dept. of Biochemistry, University of Oxford
S. G. Laland	Inst. of Biochemistry, University of Oslo
R. L. Lester	Inst. for Enzyme Research, University of Wisconsin, Madison
F. Lynen	Max-Planck-Inst. für Zellchemie, München
C. Martius	Laboratorium für Biochemie, Eidg. Technische Hochschule, Zürich

T. Moore	Dunn Nutritional Laboratory, University of Cambridge
R. A. Morton	Biochemistry Dept., Johnston Laboratories, The University of Liverpool
R. E. Olson	Dept. of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh
E. R. Redfearn	Biochemistry Dept., Johnston Laboratories, The University of Liverpool
H. Rudney	Dept. of Biochemistry, School of Medicine, Western Reserve University, Cleveland
E. C. Slater	Laboratorium voor Fysiologische Chemie. Universiteit van Amsterdam
R. H. Thomson	Chemistry Dept., University of Aberdeen
Sir Alexander R. Todd	University Chemical Laboratory, Cambridge
L. W. Wattenberg	Dept. of Pathology, The Medical School, University of Minnesota, Minneapolis
O. Wiss	F. Hoffmann-La Roche & Co. Ltd., Basle

CONTENTS

PAGE

Chairman's opening remarks
by KARL FOLKERS 1

Isolation and characterization of ubiquinone (coenzyme Q) and ubichromenol
by K. A. Morton 5
Discussion: BRODIE, CHANCE, CLARK, CRANE, FOLKERS, J. GREEN, ISLER, KON, LESTER, MOORE, MORTON, OLSON, SLATER, TODD 25

Isolation and characterization of the coenzyme Q (ubiquinone) group and plastoquinone
by F. L. CRANE 36
Discussion: BRODIE, CRANE, FOLKERS, ISLER, MORTON, REDFEARN . . 75

Chemistry of ubiquinone and related compounds
by O. ISLER, R. RÜEGG, A. LANGEMANN, P. SCHUDEL, G. RYSER and J. WÜRSCH 79
Discussion: BRODIE, CRANE, FOLKERS, J. GREEN, ISLER, OLSON, TODD . 96

Coenzyme Q. XXIII. Organic and biological studies
by K. FOLKERS, C. H. SHUNK, B. O. LINN, N. R. TRENNER, D. E. WOLF, C. H. HOFFMAN, A. C. PAGE, Jr. and F. R. KONIUSZY . 100
Discussion: BIRCH, FOLKERS, J. GREEN, MOORE, MORTON, OLSON, THOMSON 126

Coenzyme Q and electron transport
by DAVID E. GREEN 130
Discussion: ARNSTEIN, CHANCE, D. E. GREEN 159

The oxidation of quinols by mitochondrial preparations
by E. C. SLATER, J. P. COLPA-BOONSTRA and J. LINKS . . . 161
Discussion: CHANCE, CHAPPELL, D. E. GREEN, LESTER, LYNEN, MARTIUS, REDFEARN, SLATER 185

***In vitro* phosphorylation involving oxidation of quinol phosphates**
by V. M. CLARK and SIR ALEXANDER TODD 190
Discussion: BRODIE, CHANCE, CHAPPELL, CLARK, D. E. GREEN, J. GREEN, KREBS, LESTER, LYNEN, MARTIUS, SLATER 200

Short communication:
Chroman formation and its rôle in oxidative phosphorylation
by P. J. RUSSELL, Jr. and A. F. BRODIE 205

Studies on the biosynthesis of the ubiquinone (coenzyme Q) series in animals and micro-organisms
by HARRY RUDNEY and TAKASHI SUGIMURA 211
Discussion: ARNSTEIN, J. GREEN, ISLER, LYNEN, MARTIUS, MOORE, MORTON, OLSON, RUDNEY, WISS 227

Biosynthesis of some monobenzenoid quinones
by A. J. BIRCH 233
Discussion: BIRCH, CRANE, J. GREEN, HILL, ISLER, LYNEN, OLSON, SLATER, THOMSON 239

PAGE

Studies on the biosynthesis of the terpenoid side chains of quinones
by F. LYNEN 244
Discussion: ISLER, LYNEN, OLSON 262

Biosynthesis of ubiquinones
by O. WISS, U. GLOOR and F. WEBER 264
Discussion: BISHOP, FOLKERS, D. E. GREEN, J. GREEN, ISLER, KON, LYNEN, MARTIUS, MOORE, MORTON, OLSON, SLATER, THOMSON, WISS . . 280

The biosynthesis of coenzyme Q in the rat
by ROBERT E. OLSON, G. HOSSEIN DIALAMEH and RONALD BENTLEY 284
Discussion: ARNSTEIN, BIRCH, BRODIE, CRANE, KON, MOORE, MORTON, OLSON, RUDNEY, WISS 302

Short communication:
Diet-induced changes in ubiquinone and ubichromenol levels in the rat
by J. GREEN 307
Discussion: CRANE, J. GREEN, OLSON, RUDNEY, SLATER 310

Recent investigations on the chemistry and function of vitamin K
by C. MARTIUS 312
Discussion: CHANCE, CRANE, FOLKERS, J. GREEN, ISLER, LESTER, LYNEN, MARTIUS, MOORE, OLSON, REDFEARN, RUDNEY, SLATER, THOMSON, WISS 321

Direct spectroscopic observations of the oxidation-reduction reactions of ubiquinone in heart and kidney mitochondria
by BRITTON CHANCE. 327
Discussion: CHANCE, CHAPPELL, D. E. GREEN, SLATER 340

The possible rôle of ubiquinone (coenzyme Q) in the respiratory chain
by E. R. REDFEARN 346
Discussion: BIRCH, BRODIE, CHANCE, CRANE, D. E. GREEN, LESTER, MORTON, OLSON, REDFEARN, SLATER 358

Histochemical studies of the effects of coenzyme Q_{10} and menadione on oxidative enzymes in normal and neoplastic cells
by LEE W WATTENBERG 367
Discussion: BRODIE, CHANCE, CRANE, D. E. GREEN, J. GREEN, HILL, LESTER, MOORE, MORTON, OLSON, RUDNEY, SLATER, WATTENBERG . 377

The possible rôle of plastoquinone (Q-254) in the electron transport system of photosynthesis
by NORMAN I. BISHOP 385
Discussion: BISHOP, BRODIE, CHANCE, CRANE, FOLKERS, HILL, LESTER, MORTON, REDFEARN, SLATER, THOMSON 404

General discussion: ARNSTEIN, BISHOP, BRODIE, CHANCE, CHAPPELL, CLARK, CRANE, FOLKERS, D. E. GREEN, J. GREEN, HILL, ISLER, KON, LYNEN, MOORE, MORTON, OLSON, RUDNEY, SLATER, THOMSON, WISS, WOLSTENHOLME 410

Chairman's closing remarks
by KARL FOLKERS 425

CHAIRMAN'S OPENING REMARKS

KARL FOLKERS

IT is somewhat common for important discoveries in science to stem from essentially accidental events of scientific experimentation. It can be said that in certain cases these new quinones—the ubiquinone or coenzyme Q series—which we shall discuss at this symposium, stemmed from fortuitous observations of absorption spectra, which disclosed the existence of these new quinones and led the observers to separate and reveal them in pure chemical form. In this way the opening pages of a new chapter in biochemistry were written in the scientific literature. Many other investigators decided to work in this new field of quinones, because of their evident importance and significance in natural biological processes.

This symposium is held at a time which is on an ascending curve of interest, and the published volume will constitute a comprehensive summary of most of the initial aspects of this new field.

In the late nineteen-twenties, Robert Hill in Cambridge had made milestone observations which showed that isolated chloroplasts and an electron donor could photochemically evolve molecular oxygen, and the techniques and the knowledge of photosynthesis were advanced. It is noteworthy that, in the nineteen-forties, T. Moore in Cambridge and M. Kofler in Basle recorded particular observations which bear upon our subject. Indeed, Kofler isolated one of these quinones in pure crystalline form. However, these Swiss and English observations apparently lay dormant in the literature for many years before new and independent observations in two more laboratories stimulated a series of international investigations. These researches are rapidly

progressing with an impetus that is leading to an ever-increasing file of reprints and photostats in the laboratories of the workers in this quinone field. I refer, of course, to the pioneering contributions from Richard Morton's laboratory at the University of Liverpool and David Green's laboratory at the Enzyme Institute of the University of Wisconsin. It is understandable that these two laboratories formulated the separate nomenclatures which we now know as the ubiquinone and the coenzyme Q series. I hope that Professors Morton, Green and Isler, and I, can initiate here a discussion of nomenclature to see whether a single system can be devised, and perhaps I can make a status report to you on the subject before the close of our symposium.

One aspect of this new quinone field which makes it so fascinating is that it embodies detailed studies of biochemical mechanisms and particularly of electron transport. Studies continually move from the somewhat empirical to the more mechanistic approaches. Concomitant investigations on oxidative phosphorylation augment the theoretical interest in these quinones and their related derivatives and other isoprenoid compounds. These quinones have already been found and biochemically studied in numerous phyla of living organisms, and the gaps offer research opportunities.

Any new concept in research acquires an enhanced interest when it bridges two fields, and the resulting synergism is to the mutual benefit of both fields. Nobody anticipated that the Liverpool studies on vitamin A deficiency in rats, and the Wisconsin studies on lipid extracts of beef heart mitochondria which function in the electron transport activities of certain particles, would build a bridge into the field of photosynthesis in higher plants. Crane's studies on coenzyme Q_{10} in mitochondrial electron transport led him and Lester and their associates to rediscover the related quinone (plastoquinone, Kofler's quinone), from plants, and to suggest that it may well have a corresponding rôle in photosynthetic electron transport. The experimental data

of Norman Bishop on this plant quinone have now led to the conclusion that it is actually an intermediate for electron transfer for photosynthesis.

The organic structural relationship between these new quinones and certain members of the fat-soluble vitamins, particularly vitamin K and E, have created bridges into these vitamin fields.

Although new discoveries are seemingly elucidated, they really only open up a never-ending expansion of new problems for innumerable investigators. The following questions may exemplify the expanding front in this research.

What are the precise mechanisms and inter-relationships at the molecular level?

What are the detailed structural steps of biosynthesis, in mammalian tissue and by micro-organisms?

What are the metabolic rôles and the inter-relationships which might be elucidated from intact animal studies and by microbial techniques?

Does coenzyme Q_{10} or ubiquinone, whether ingested in food or biosynthesized within the body by tissue or micro-organisms, have any nutritional significance for the welfare of man or lower animals? Or, more concisely, has ubiquinone or coenzyme Q_{10} a vitamin activity?

Would the so-called germ-free animal studies contribute significantly to our knowledge of biosynthesis?

If the dietary intake of quinone and/or the intrinsic biosynthesis of quinone are adequate, so that a primary nutritional inadequacy does not frequently occur, or does not occur, then can there be secondary or conditioned nutritional inadequacies? Conditioned deficiencies can result from man's various environmental conditions and bodily states that interfere with the ingestion, absorption or utilization of vitamins, or from other conditions which increase vitamin requirements or cause excessive destruction or abnormal excretion of the nutrients.

Are there areas of potential usefulness to man where research on

these quinones would be justified, such as in genetic diseases arising from "errors" of metabolism, in cancer, in infection, in postoperative metabolic recoveries, in pregnancy and in geriatrics?

Can these quinones be useful as laboratory tools to help elucidate unknown aetiology of disease? The data which will be presented by Wattenberg on neoplastic disease may indicate a trend.

It seems that this new field is so abundant in new research objectives and opportunities that one must use some discrimination as to what are the most significant items to pursue which fall within one's scope and facilities. I am confident that our formal presentations and our discussions at this symposium will generate new ideas and new priorities in the minds of all of us, and that we shall all return to our laboratories with anticipation of new experiments, and also with gratitude to the Ciba Foundation for this opportunity of sharing our research and forming new friendships.