

**CHEMISTRY AND BIOLOGY  
OF PTERIDINES  
AND FOLATES**

# ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Editorial Board:

NATHAN BACK, *State University of New York at Buffalo*

IRUN R. COHEN, *The Weizmann Institute of Science*

DAVID KRITCHEVSKY, *Wistar Institute*

ABEL LAJTHA, *N. S. Kline Institute for Psychiatric Research*

RODOLFO PAOLETTI, *University of Milan*

---

## Recent Volumes in this Series

Volume 330

THE UNDERLYING MOLECULAR, CELLULAR, AND IMMUNOLOGICAL FACTORS IN  
CANCER AND AGING

Edited by Stringner Sue Yang and Huber R. Warner

Volume 331

FRONTIERS IN CEREBRAL VASCULAR BIOLOGY: Transport and Its Regulation

Edited by Lester R. Drewes and A. Lorrin Betz

Volume 332

MECHANISM OF MYOFILAMENT SLIDING IN MUSCLE CONTRACTION

Edited by Haruo Sugi and Gerald H. Pollack

Volume 333

OPTICAL IMAGING OF BRAIN FUNCTION AND METABOLISM

Edited by Ulrich Dirnagl, Arno Villringer, and Karl M. Einhäupl

Volume 334

NEW CONCEPTS IN THE PATHOGENESIS OF NIDDM

Edited by Claes Göran Östenson, Suad Efendic, and Mladen Vranic

Volume 335

DRUGS OF ABUSE, IMMUNITY, AND AIDS

Edited by Herman Friedman, Thomas W. Klein, and Steven Specter

Volume 336

ANCA-ASSOCIATED VASCULITIDES: Immunological and Clinical Aspects

Edited by Wolfgang L. Gross

Volume 337

NEUROBIOLOGY AND CELL PHYSIOLOGY OF CHEMORECEPTION

Edited by P. G. Data, H. Acker, and S. Lahiri

Volume 338

CHEMISTRY AND BIOLOGY OF PTERIDINES AND FOLATES

Edited by June E. Ayling, M. Gopal Nair, and Charles M. Baugh

---

A Continuation Order Plan is available for this series. A continuation order will bring delivery of each new volume immediately upon publication. Volumes are billed only upon actual shipment. For further information please contact the publisher.

# CHEMISTRY AND BIOLOGY OF PTERIDINES AND FOLATES

Edited by

**June E. Ayling**

**M. Gopal Nair**

**Charles M. Baugh**

University of South Alabama  
Mobile, Alabama

**SPRINGER SCIENCE+BUSINESS MEDIA, LLC**

---

Library of Congress Cataloging in Publication Data

Chemistry and biology of pteridines and folates / edited by June E. Ayling, M. Gopal Nair, Charles M. Baugh.

p. cm.—(Advances in experimental medicine and biology; v. 338)

“Proceedings of the Tenth International Symposium on Chemistry and Biology of Pteridines, held March 21–26, 1993, in Orange Beach, Alabama”—T.p. verso.

Includes bibliographical references and indexes.

ISBN 978-1-4613-6287-6 ISBN 978-1-4615-2960-6 (eBook)

DOI 10.1007/978-1-4615-2960-6

1. Pteridines—Congresses. 2. Folic acid—Congresses. 3. Prosthetic groups (Enzymes)—Congresses. I. Ayling, June E. II. Nair, M. Gopal. III. Baugh, Charles M. IV. International Symposium on Chemistry and Biology of Pteridines (10th: 1993: Orange Beach, Ala.) V. Series.

QP801.P69C47 1993

93-29401

599'.019'25—dc20

CIP

---

Proceedings of the Tenth International Symposium on Chemistry and Biology of Pteridines, held March 21–26, 1993, in Orange Beach, Alabama

ISBN 978-1-4613-6287-6

©1993 Springer Science+Business Media New York

Originally published by Plenum Press, New York in 1993

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

## PREFACE

The pteridines in their multitude of forms fulfill many roles in nature ranging from pigments to cofactors for numerous redox and one-carbon transfer reactions. This extraordinary diversity of function is unified by the unique chemistry of the pteridine heterocycle. The International Symposium on the Chemistry and Biology of Pteridines and Folates is a forum for presenting recent and exciting advances in this expanding field. In bringing together various disciplines, a synergy of ideas results that has often stimulated fresh approaches to major problems. The Tenth International Symposium held at Orange Beach, Alabama, March 21 - 23, 1993, proved no exception by providing new insights into folate enzymology, tetrahydrobiopterin and molybdopterin biosynthesis and function, enzyme synthesis and regulation, along with novel synthetic strategies for producing compounds that will expedite further study. The many outstanding scientific contributions found in the following chapters, which represent the work presented at the Symposium, are a reflection of the significant advances made since the Ninth International Symposium held in Zurich in 1989.

Since the 7th International Symposium in St. Andrews, Scotland, a tradition has evolved of honoring scientists who have made outstanding contributions to pteridine research with a Gowland Hopkins medal and lectureship. Sir Frederick Gowland Hopkins initiated the first investigation of what later proved to be pteridines in his studies of the yellow and white colors of butterflies. The selection of Edward Taylor and Wolfgang Pfleiderer for the 1993 Hopkins award seems especially appropriate in view of the impending 100th anniversary of the first synthesis of a pteridine (lumazine 6,7-dicarboxylic acid) by Kühling in 1894. Almost every chemist in the field, as well as many others, have greatly benefited from the careful and insightful work of these two scientists. Their many contributions are summarized on the following pages by Peter Beardsley, a former Ph.D. student of Edward Taylor, and Max Viscontini, still a very active pteridine chemist himself.

We would like to acknowledge members of the Advisory Committee who were of great assistance in various aspects of planning the Symposium. We would also like to thank the sponsors, listed on the Acknowledgments page, who made the meeting possible. The breadth of the support we have received from both governmental and corporate sponsors is an indication of the continuing recognition of the major role that pteridines play in biology and medicine. The efforts of members of the finance committee in helping to obtain this support are also greatly appreciated. Special thanks to Dusti Steelman and Judi Naylor for so cheerfully helping with the mailings, the preparation of the program and abstract book and with the registration before and during the meeting. Finally, we would like to acknowledge the invaluable involvement of Steven Bailey.

We were pleased to host this meeting in Alabama where significant and continuous contributions have been made to this field over the past 30 years. We look forward to the 11th International Symposium which will be organized by Wolfgang Pfeleiderer and Hartmut Rokos and held in Germany.

June Ayling  
Gopal Nair  
Charles Baugh

College of Medicine  
University of South Alabama

Mobile, April 1993

## GOWLAND HOPKINS LECTURESHIP

EDWARD C. TAYLOR



The choice of Ted Taylor as one of the two Gowland Hopkins Lecturers for this International Symposium could not be more appropriate. His contributions to the field of pteridine chemistry and biochemistry over a career spanning more than 45 years are equalled only by those of Wolfgang Pfeleiderer, the co-recipient of this honor. No one else even comes close!

While his work in general is so broad-based to perhaps defy specific characterization, yet through it all run the traits of exceptional cleverness, imagination and "elegant simplicity". He has a knack not only for designing and implementing novel synthetic approaches, but also for noticing the unexpected result and exploiting it in many variations to synthetic advantage. Taylor is famous for making the complex seem simple. His work is replete with examples of the synthesis of complex heterocyclic and other compounds in a short series of steps, many of which involve clever sequences of condensation, ring cleavage and rearrangement reactions, often occurring "in one pot".

Taylor's contributions to pteridine chemistry include a series of more than 125 papers on pteridines, aza- and deaza-pteridines, numerous patents, and the classic volume *Pteridine Chemistry*, co-edited with Pfeleiderer. These describe work which ranges from the basic chemistry of these ring systems, through development of synthetic methodology, to applications in the synthesis of natural products and the development of novel folate antimetabolites. The classic "Taylor Synthesis" involves elaboration of a pyrazine intermediate and allows unequivocal synthesis of the biologically important 6-substituted pteridines. He has applied this approach to the synthesis of natural pteridines including xanthopterin, folic acid, *L-erythro*-biopterin, neopterin, urothione, and Form A of the molybdenum cofactor - only a partial list.

Probably the most striking achievement recently has been the synthesis of a wide variety of deaza analogs of tetrahydrofolate. This work began with the synthesis nearly ten years ago of 5,10-dideazatetrahydrofolate (DDATHF, Lometrexol). This compound was shortly found to be a potent inhibitor of de novo purine biosynthesis. Broad spectrum antitumor activity was then found in pre-clinical models, and Lometrexol is now undergoing clinical trials with encouraging early results. The initial discovery of DDATHF has had a major impact on subsequent antifolate drug development. Not only was a new class of structures opened to further investigation, but added impetus was provided to the search for other novel inhibitors of previously unexplored enzymatic targets for antifolate attack. The result has been a major rekindling of interest in antifolate drug development, an area in which Taylor himself has been a major participant. This work has been full of new synthetic chemistry and biochemical surprises, and forms the basis for this Gowland Hopkins Lecture.

Many whose relationship to Taylor is through a common interest in pteridines may not realize that, despite the magnitude of his achievements in our field, this represents only a portion of the overall scope of his work. He has also made major contributions to basic chemistry and synthetic methodology in purines, pyrimidines, triazines and diazetidiones. In fact, he has contributed very significantly to our knowledge of virtually all heterocyclic systems. His contributions extend much further than heterocyclic chemistry and include elegant total syntheses of Chinchona alkaloids. His well known one step synthesis of tetrahydrocannabinol is a particularly illustrative example of his clever and imaginative approach to synthetic design. Finally, Taylor has made numerous major contributions to the whole field of synthetic organic chemistry. While there are many examples, particularly outstanding has been his pioneering and extensive work in the use of thallium reagents in organic syntheses. A long and highly productive collaboration between Taylor and Prof. Alexander McKillop has led to the development of extraordinarily versatile thallium reagents and reactions which have wide application in virtually every area of synthetic organic chemistry.

Taylor's role as a teacher and mentor may be almost as important as his own work. Those who hear his lectures and seminars are treated not only to exciting chemistry, but to presentations of extraordinary organization and clarity. To students and others who work with him, he transmits not only some of his profound understanding of how molecules work and how to assemble them but, perhaps more importantly, a considerable measure of his boundless enthusiasm and perpetual optimism. For Ted, it's never that a good idea "doesn't work", it's just that it "hasn't worked yet". Those who come to know him soon realize that these same qualities of vigor, enthusiasm and optimism apply to his whole approach to life. Those who have known him over the years realize that these are not diminishing, rather they are on a continuing upswing. We, therefore, look forward to a great deal more in the way of exciting new developments and contributions to pteridines and to chemistry in general.

G. Peter Beardsley  
Yale University School of Medicine



## GOWLAND HOPKINS LECTURESHIP

### WOLFGANG PFLEIDERER



The year 1955 was a milestone in pteridine chemistry. After four years of intense, exciting work, we had just published in *Helvetica Chimica Acta* three articles together with P. Karrer and E. Hadorn, in which we described the isolation of new compounds from the small fly *Drosophila melanogaster*, two of which had a deep sky blue (in German "Himmel Blau", H B) fluorescence and were therefore baptized  $HB_1$  and  $HB_2$  in our laboratory slang. We found out very quickly that  $HB_1$  corresponded to pterin, while the structure of  $HB_2$  gave us a lot of trouble. Experienced chemists like those of Stokstad's team would have immediately identified  $HB_2$  as L-biopterin. My lack of experience in pterin chemistry plunged me into an abyss of doubt, because I trusted blindly the elemental analyses, which, I must admit, in case of pterins, were far from being as accurate as they are today. To end a long story, our work remained more or less unnoticed - what were we looking for in these small flies, everybody wondered - especially because some weeks later Stokstad published the isolation of a growth factor, starting from a thousand liters of human urine, which he called L-biopterin, and determined its structure. Our work remained more or less overlooked, except in the eyes of a young chemist who recognized rapidly the importance of the research which was going on in our laboratory. In the beginning of 1956, a handsome looking man of good appearance, tall, with a determined face, missing the left arm, entered my office. A war-wounded man, I thought as I looked at him. I had in front of me Wolfgang Pfleiderer, not the Professor Pfleiderer we know today, but a young man. At that time he was 29 years old and well resolved to make his way in life.

"Professor Viscontini, I am a chemist at the University of Stuttgart, where in 1952 I gained my Ph.D. under the supervision of Professor Bredereck. My dissertation was devoted to the chemical and physicochemical properties of some purine derivatives. Now

I am writing my Habilitation about lumazines. Unfortunately, the topic does not really fill my supervisor with enthusiasm. As a consequence I am obliged to work by myself without people with whom I can discuss results, problems or difficulties. Recently I read your papers on pteridines isolated from *Drosophila melanogaster* and I was greatly impressed by the results you published on the research carried out on this fly. I have, therefore, ventured to come to Zurich in order to make contact with a person who shares my scientific interest. I would like to know, if I am correct or mistaken in involving myself so strongly in the field of pteridine chemistry". Here was finally somebody with whom I was in perfect communication! I felt that my work attracted him. I did my best to convince him how right he was, taking the way of hard research where results do not manifest themselves in kilograms of publications, but brings out the author's originality. I emphasized, that in my opinion, pteridines might play a great role in nature, that he had to go on in the direction he chose and that he would find later the benefit of it. In a short time we became close friends, with a very similar scientific philosophy. We separated, promising to meet again and to discuss as often as possible the results of our research. Some months later, in 1957 Pfleiderer gained his Habilitation and his appointment as Dozent at the University of Stuttgart. In 1958 he spent one year as Research Associate at the University of Princeton with Professor Edward Taylor.

In the meantime we went on with the isolation of three orange pigments, drosopterin, isodrosopterin, and neodrosopterin from *Drosophila*. In 1959 we published the first hypothetical structures that we proposed for these pigments. After one year of absence Pfleiderer did not hesitate to come to Zurich. "Sir, your structures are wrong. The UV and the VIS spectra do not match the proposed formulae". I understood perfectly his point of view, and suddenly it came to me: I felt isolated with my research, my results were not especially well appreciated, I was short of money and coworkers and now I had in front of me a promising young chemist, who, as I sensed, had a mind to get involved with the field of natural pterins. My decision was immediate: "Listen, Herr Pfleiderer, in my opinion, from the pteridines we just isolated there are two classes of compounds that seem important, the drosopterins and the L-biopterin. Both are difficult to study, but both will bring recognition to the chemist who elucidates their properties. I have confidence in you, devote yourself to the chemistry of drosopterins and I, for my part, will work on the chemistry of the L-biopterin".

From that day on I stopped working with drosopterin. In 1962 Professor Pfleiderer formulated his first ideas on their structure. I could have as easily proposed to Pfleiderer the study of L-biopterin and chosen the drosopterin for myself. Why did I choose L-biopterin? Don't ask me, I have no idea myself. Anyway, we never regretted our oral agreement, neither he nor me. We have remained close friends and loyal to our agreement. As it turned out, destiny and my choice made Professor Pfleiderer the oracle for drosopterins while I became the expert for tetrahydrobiopterins. Nevertheless, it is quite certain, that without my encouragement Professor Pfleiderer would have become famous, because of his personality. Some hundreds of communications and seven books published between 1953 and 1992 about pteridines and their derivatives bear witness to the wideness of the research he carried out, and the importance of the results he obtained. Let us cite succinctly: tautomeric structures of pteridines, photochemistry, electrochemistry, and most especially the chemistry of natural pteridines: structure of eugleniapterin, partial synthesis of tetrahydromethanopterin and, to my opinion the most important and the most admirable of all his works the structure and the synthesis of the drosopterin pigments.

Good luck for the future, dear Wolfgang!

Max Viscontini  
University of Zürich

## ACKNOWLEDGMENTS

Contributions from the following sponsors are gratefully acknowledged:

### **Major Corporate Sponsor**

Milupa AG., Germany

### **Other Corporate Sponsors**

Acme Business Products, Mobile  
American Cyanamid Company  
Beckman Instruments, Inc.  
Bristol-Myers Squibb  
Burroughs Wellcome Co.  
Ciba-Geigy Ltd., Switzerland  
Coca-Cola Company  
Dr. B. Schircks Laboratories, Switzerland  
Eli Lilly and Company  
F. Hoffmann-La Roche AG., Switzerland  
Henning Berlin GMBH, Germany  
ICI Pharmaceuticals, UK  
Marion Merrell Dow, Inc.  
Moravek Biochemicals  
Sankyo Corp., Japan  
SAPEC S.A., Switzerland  
Sigma Chemical Co.  
SmithKline Beecham  
Swiss Air  
The Upjohn Co.  
Warner-Lambert Co.  
Yamanouchi Pharmaceutical Co., Ltd., Japan

### **Organizations and Government Agencies**

International Union of Biochemistry and Molecular Biology  
National Institutes of Health  
    National Cancer Institute  
    National Institute of Allergy and Infectious Diseases  
National Science Foundation  
Ninth International Pteridine Symposium, Switzerland  
The Council for Tobacco Research  
Federation of American Societies of Experimental Biology  
American Chemical Society, Medicinal Chemistry Division

*and* South Alabama Medical Science Foundation and the  
College of Medicine of the University of South Alabama

## CONTENTS

### CHEMISTRY

GOWLAND HOPKINS LECTURE: Natural Pteridines - A Chemical Hobby . . . . .	1
W. Pfleiderer	
Properties and Reactions of Pteridines Carrying Functionalized Side Chains at the 3-Position . . . . .	17
P. Boyle and R. Camier	
N <sup>2</sup> -(N,N-Dimethylaminomethylene)-O <sup>4</sup> -(2-p-Nitrophenylethyl)-Biopterin: A Versatile Intermediate for a Glycosidation Reaction . . . . .	21
H. Yamamoto, T. Hanaya, K. Torigoe, and W. Pfleiderer	
The Synthesis of Fluorine-Containing Pterins . . . . .	25
C. Dunn, C.L. Gibson, C.J. Suckling, and W. Xing	
Formation of Fe <sup>III</sup> or Fe <sup>II</sup> Complexes with Acetylacetonate and 5,6,7,8- Tetrahydropterin as Ligands and Their Activation of Oxygen . . . . .	29
A. Schäfer, B. Fischer, R. Bosshard, M. Hesse, and M. Viscontini	
Stereoelectronic Effects in the Autooxidative Destruction of Reduced Folate Derivatives . . . . .	33
A.L. Fitzhugh	
MINDO/3 Molecular-Orbital Calculations on the 6R-BH <sub>4</sub> Molecule and Its Metabolic Precursors: Conformation and Activity . . . . .	39
S. Katoh, T. Sueoka, and T. Kurihara	
Identification, Stereoconfiguration, Chromatographic and Fluorescence Properties of Natural Pterins . . . . .	43
R. Klein	

### TETRAHYDROBIOPTERIN DEPENDENT HYDROXYLASES

The Mechanism of Cofactor Regeneration During Phenylalanine Hydroxylation . . . .	47
S.W. Bailey, S.R. Boerth, S.B. Dillard, and J.E. Ayling	

Structure-Function Studies of the Phenylalanine Hydroxylase Active Site and a Summary of Structural Features . . . . .	55
R.G.H. Cotton, D.W. Howells, J.A. Saleeba, I. Dianzani, P.M. Smooker, and I.G. Jennings	
Expression of Wild Type and Mutant Forms of Human Phenylalanine Hydroxylase in <i>E. coli</i> . . . . .	59
P.M. Knappskog, H.G. Eiken, A. Martinez, S. Olafsdottir, J. Haavik, T. Flatmark, and J. Apold	
A Re-examination of the Metal Requirement of <i>Chromobacterium violaceum</i> Phenylalanine Hydroxylase . . . . .	63
R.T. Carr and S.J. Benkovic	
Histidines 138 and 143 are Copper Binding Ligands in <i>Chromobacterium</i> <i>violaceum</i> Phenylalanine Hydroxylase . . . . .	67
S. Balasubramanian, R.T. Carr, C.J. Bender, J. Peisach, and S.J. Benkovic	
Characterization of the Iron Environment in Recombinant Human Tyrosine Hydroxylase, Using Mössbauer and EPR-Spectroscopy . . . . .	71
J. Haavik, E. Bill, M. Lengen, A. Martinez, T. Flatmark, and A.X. Trautwein	
Interaction of Substrate and Pterin Cofactor with the Metal of Human Tyrosine Hydroxylase as Determined by <sup>1</sup> H-NMR . . . . .	77
A. Martínez, C. Abeygunawardana, J. Haavik, T. Flatmark, and A.S. Mildvan	
Mechanistic Studies of Tyrosine Hydroxylase . . . . .	81
P.F. Fitzpatrick.	
Alleviation of Catecholamine Inhibition of Tyrosine Hydroxylase by Phosphorylation at Serine 40 . . . . .	87
S.C. Daubner and P.F. Fitzpatrick	
Glyceryl Ether Monooxygenase [EC 1.14.16.5]: Stoichiometry and Inhibition . . . . .	93
B. Kosar-Hashemi, H. Taguchi, and W.L.F. Armarego	

#### **TETRAHYDROBIOPTERIN REGENERATING ENZYMES**

The Isolation and Characterization of Clones of 4a-Hydroxytetrahydrobiopterin Dehydratase . . . . .	97
S. Kaufman, B.A. Citron, M. Davis, and S. Milstien	
Molecular Cloning and Recombinant Expression of the Human Liver Phenylalanine Hydroxylase Stimulating Factor Revealed Structural and Functional Identity to the Dimerization Cofactor for the Nuclear Transcription Factor HNF-1 $\alpha$ . . . . .	103
B. Thöny, F. Neuheiser, C.R. Hauer, and C.W. Heizmann	
Progress in the Study of Biosynthesis and Role of 7-Substituted Pterins: Function of Pterin 4a-Carbinolamine Dehydratase . . . . .	107
H.-Ch. Curtius, S. Ghisla, H. Hasegawa, N. Blau, and I. Rebrin	

Spectroscopic Characterization of Human Liver Pterin 4a-Carbinolamine Dehydratase . . . . .	111
I. Rebrin, H.Ch. Curtius, S. Ghisla, and F.H. Herrmann	
Is Dihydropteridine Reductase an Anomalous Dihydrofolate Reductase, a Flavin-Like Enzyme or a Short-Chain Dehydrogenase? . . . . .	115
J.M. Whiteley, N.H. Xuong, and K.I. Varughese	
Two Crystal Structures of Rat Liver Dihydropteridine Reductase . . . . .	123
K.I. Varughese, Y. Su, M.M. Skinner, N.H. Xuong, D.A. Matthews, and J.M. Whiteley	
New Inhibitors of Dihydropteridine Reductase (Human Brain) . . . . .	127
D. Randles, H. Taguchi, and W.L.F. Armarego	
CYS→SER Mutations in ch-Human Dihydropteridine Reductase . . . . .	131
C.M. Hardy, H. Averdunk, B. Paal, R.G.H. Cotton, and W.L.F. Armarego	
The Spectrum of Mutations in Dihydropteridine Reductase Deficiency . . . . .	135
P.M. Smooker, D.W. Howells, I. Dianzani, and R.G.H. Cotton	

### TETRAHYDROBIOPTERIN BIOSYNTHESIS

Cloning and Characterization of Genes Encoding Tetrahydrobiopterin Biosynthetic Enzymes . . . . .	139
R.A. Levine, J.C. States, P.Z. Anastasiadis, and D.M. Kuhn	
<i>Drosophila</i> GTP Cyclohydrolase: Multiple Isoform Products of a Single Gene Derive From Alternate Transcripts that are Developmentally Regulated and Functionally Specific . . . . .	147
J.M. O'Donnell, G. Ranganayakula, X. Chen, S. Krishnakumar, and W.S. Neckameyer	
Studies on GTP Cyclohydrolase I of <i>Escherichia coli</i> . . . . .	157
C. Schmid, W. Meining, S. Weinkauff, L. Bachmann, H. Ritz, S. Eberhardt, W. Gimbel, T. Werner, H-W. Lahm, H. Nar, and A. Bacher	
Partial Purification and Characterization of GTP Cyclohydrolase I from Spinach Leaves . . . . .	163
Y. Sohta, T. Ohta, and M. Masada	
Detection and Quantification of GTP Cyclohydrolase I mRNA . . . . .	167
M. Gütllich, K. Schott, T. Werner, A. Bacher, and I. Ziegler	
Localization of GTP Cyclohydrolase I mRNA in the Rat Brain by <i>In Situ</i> Hybridization . . . . .	171
S.I. Lentz, K. Hirayama, and G. Kapatos	
Expression of GTP Cyclohydrolase I mRNA in the Rat: Tissue Distribution and Effect of Reserpine . . . . .	175
K. Hirayama, S.I. Lentz, and G. Kapatos	

Regulation of Tetrahydrobiopterin Biosynthesis in Cultured Hypothalamic and Mesencephalic Neurons by Cyclic AMP-Dependent GTP Cyclohydrolase I Gene Expression . . . . .	179
K. Hirayama, M. Zhu, and G. Kapatós.	
Mycophenolic Acid Simultaneously Reduces Intracellular GTP and Tetrahydrobiopterin Levels in Neuro-2A Cells . . . . .	183
T. Harada, K. Hatakeyama, and H. Kagamiyama	
Human Liver 6-Pyruvoyl-Tetrahydropterin Synthase: Expression of the cDNA, Purification and Preliminary Characterization of the Recombinant Protein . .	187
B. Thöny, W. Leimbacher, N. Blau, C.W. Heizmann, and D. Bürgisser	
Enzymatic Properties of 6-Pyruvoyl-Tetrahydropterin Synthase Purified from Fat Bodies of Silkworm Larvae . . . . .	191
M. Masada	
Northern Blot Analysis of Sepiapterin Reductase mRNA In Mammalian Cell Lines and Tissues . . . . .	195
J. Maier, K. Schott, T. Werner, A. Bacher, and I. Ziegler	
Purification and Properties of Human Sepiapterin Reductase from Placenta . . . . .	199
J. Maier and I. Ziegler	

## TETRAHYDROBIOPTERIN REGULATION

Stimulation of Tetrahydrobiopterin Synthesis by Cytokines in Human and In Murine Cells . . . . .	203
E.R. Werner, G. Werner-Felmayer, G. Weiss, and H. Wachter	
Interferon- $\gamma$ and Kit-Ligand are Primary Regulators of GTP Cyclohydrolase Activity: Mechanisms and Implications . . . . .	211
I. Ziegler, K. Schott, and L. Hültner	
Differential Metabolism of Tetrahydrobiopterin in Monoamine Neurons: A Hypothesis Based Upon Clinical and Basic Research . . . . .	217
G. Kapatós, K. Hirayama, S.I. Lentz, M. Zhu, and S.L. Stegenga	
Tissue Distribution of Tetrahydrobiopterin Generating Enzymes . . . . .	223
M. Hoshiga, K. Hatakeyama, and H. Kagamiyama	
Co-Induction of Tetrahydrobiopterin Levels and Tyrosine Hydroxylase Activity in Cultured PC12 Cells . . . . .	227
P.Z. Anastasiadis, J.C. States, D.M. Kuhn, and R.A. Levine	
Long-Term Treatment of PC12 Pheochromocytoma with Dibutyryl Cyclic AMP Increases Biopterin Content in the Cells but Decreases that in the Medium . . . . .	231
N. Nakanishi, S. Onozawa, A. Isono, M. Hara, H. Hasegawa, and S. Yamada	

Nicotinic Cholinergic Regulation of Tetrahydrobiopterin Levels in Bovine Adrenal Chromaffin Cells . . . . .	235
J.C. Waymire, J.E. Ayling, and G.L. Craviso	
Inter-Relationships Between Pterins and Cytokines Produced During Allogeneic Immune Reactions and Possible Use as Early Markers of Immune Activation . . . . .	239
J.J. Rippin and D.C. Henderson	
An Example of the Detection of an Esophageal Carcinoma in Its Very Early Stage by Urinary Xanthopterin Determination . . . . .	243
T. Iino, H. Watanabe, W.L. Gyure, T. Mazda, H. Mieno, and M. Tsusué	
Neopterin in Subacute Sclerosing Panencephalitis . . . . .	247
H. Shintaku, R. Murata, H. Hattori, O. Matsuoka, T. Nakajima, T. Imamura, and Y. Sawada	
The 7-Deazaguanine Derivative, Queuine, Regulates Mammalian Cell Proliferation Depending on the Metabolic State . . . . .	251
W. Langgut, M. Haupt, and T. Reisser	

#### **TETRAHYDROBIOPTERIN DEFICIENCY**

Tetrahydrobiopterin Deficiency and an International Database of Patients . . . . .	255
N. Blau and J.-L. Dhondt	
Tetrahydrobiopterin Deficiency in Portugal: Results of the Screening for Hyperphenylalaninemia . . . . .	263
I.T. de Almeida, P.P. Leandro, R. Portela, A. Cabral, F. Eusébio, T. Tasso, A. Matasovic, and N. Blau	
A Microtitre Plate Method for Measuring Biopterin with Cryopreserved <i>Crithidia fasciculata</i> . . . . .	267
R.J. Leeming, S.K. Hall, H. Friday, P. Hurley, and A. Green	
Oral Administration of Liposomally Entrapped Tetrahydrobiopterin . . . . .	271
Y. Sawada, H. Shintaku, T. Nakajima, T. Imamura, Y. Tsubakio, C. Iwamura, G. Isshiki, and T. Oura	
Experimental Research on a Fetal Treatment for Tetrahydrobiopterin Deficiency . . .	273
H. Shintaku, T. Nakajima, T. Imamura, Y. Sawada, G. Isshiki, and T. Oura	
Experimental Research on a New Treatment for Maternal Phenylketonuria . . . . .	277
T. Imamura, H. Shintaku, T. Nakajima, Y. Sawada, G. Isshiki, and T. Oura	

#### **NITRIC OXIDE SYNTHASE**

Nitric Oxide Synthase: Function and Mechanism . . . . .	281
M.A. Marletta	



Macrophage Nitric Oxide Synthase: Tetrahydrobiopterin Decreases the NADPH Stoichiometry . . . . .	285
J.M. Hevel and M.A. Marletta	
Role of Tetrahydrobiopterin in Cytokine-Stimulated Metabolism of Tryptophan and Hydroxylation of Arginine . . . . .	289
S. Milstien, N. Sakai S. Kaufman, K. Saito, and M.P. Heyes	
Tetrahydrobiopterin Synthesis is Induced by LPS in Vascular Smooth Muscle and is Rate-Limiting for Nitric Oxide Production . . . . .	295
S.S. Gross, R. Levi, A. Madera, K.H. Park, J. Vane, and Y. Hattori	
6R-[ <sup>3</sup> H] Tetrahydrobiopterin Binding Activities in Rat Brain . . . . .	301
Yu. Watanabe, H. Morii, Y. Nemoto, B. Mayer, E.R. Werner, S. Miwa, and Ya. Watanabe	
Inducible Nitric Oxide Synthase Activity in Hepatocytes is Dependent on the Coinduction of Tetrahydrobiopterin Synthesis . . . . .	305
M. Di Silvio, D.A. Geller, S.S. Gross, A. Nussler, P. Freeswick, R.L. Simmons, and T.R. Billiar	
Modulation of Nitric Oxide Synthase Activity in Intact Cells by Intracellular Tetrahydrobiopterin Levels . . . . .	309
G. Werner-Felmayer, E.R. Werner, G. Weiss, and H. Wachter	

#### **OTHER FUNCTIONS OF TETRAHYDROPTERINS**

6R-L-Erythro-5,6,7,8-Tetrahydrobiopterin: A Regulator of Neurotransmitter Release . . . . .	313
K. Koshimura, T. Ohue, Ya. Watanabe, and S. Miwa	
Positron Emission Tomography Studies on Some Neurotransmitter Receptor Systems with 6R-Tetrahydrobiopterin Pretreatment . . . . .	321
Ya. Watanabe, Y. Tani, T. Kanai, P. Hartvig, O. Inoue, J. Andersson, A. Lilija, and B. Långström	
Positron Emission Tomography (PET) Study: The Effects of 6R-L-Erythro- 5,6,7,8-Tetrahydrobiopterin (R-THBP, SUN 0588) on the Central Dopamine D <sub>1</sub> , D <sub>2</sub> , and D <sub>3</sub> Receptors in Rhesus Monkey . . . . .	327
Y. Tani, T. Ishihara, T. Kanai, T. Ohno, H. Onoe, Ya. Watanabe, J. Andersson, A. Lilija, G. Westerberg, P. Hartvig, and B. Långström	
6R-L-Erythro-5,6,7,8-Tetrahydrobiopterin (R-THBP, SUN0588) Acts on the Brain Muscarinic and Nicotinic Cholinergic Receptors as Evaluated by Positron Emission Tomography (PET) Studies in Rhesus Monkey . . . . .	331
Y. Tani, T. Ishihara, T. Kanai, T. Ohno, Ya. Watanabe, J. Andersson, A. Lilija, G. Westerberg, P. Hartvig, and B. Långström	
Effect of 6R-Tetrahydrobiopterin on the Central Muscarinic Cholinergic Receptor as Evaluated by Positron Emission Tomography Studies Using Rhesus Monkey . . . . .	335
Ya. Watanabe, H. Onoe, M. Tanaka, K. Kobayashi, K. Suzuki, Y. Tani, S. Miwa, and O. Inoue	

Increase of Tetrahydropterins in Cell-Free Retinal Extracts in Response to Light Exposure . . . . .	339
G. Cremer-Bartels, H. Gerding, and K. Krause	
Effect of Triamterene on the Electroretinogram of Long Evans Rats . . . . .	343
G. Cremer-Bartels, H. Gerding, L. Hanneken, and K. Krause	
Immunoenzymatic Labeling of Biopterin and Neopterin in the Pigment Epithelium of Bovine Retina . . . . .	347
H. Gerding, E. Vollmer, G. Cremer-Bartels, H. Rokos, K. Krause, and H. Busse	
Reduced Pterins as Scavengers for Reactive Oxygen Species . . . . .	351
R. Shen and Y. Zhang	

### **MOLYBDOPTERIN COFACTORS**

Chemistry and Biology of the Molybdenum Cofactors . . . . .	355
K.V. Rajagopalan, J.L. Johnson, M.M. Wuebbens, D.M. Pitterle, J.C. Hilton, T.R. Zurick, and R.M. Garrett	
Studies on the Molybdenum Cofactor. Synthesis of (±)-Form B (Dephospho) . . . .	363
E.C. Taylor and I.S. Darwish	
Molybdenum-Pterin Complexes: A Functional and Structural Model for the Binding Site in the Enzyme Dimethyl Sulfoxide Reductase . . . . .	369
B. Fischer, H. Schmalle, E. Dubler, and M. Viscontini	
Human Molybdenum Cofactor Deficiency . . . . .	373
J.L. Johnson, K.V. Rajagopalan, and S.K. Wadman	
Molybdopterin Biosynthesis in Man. Properties of the Converting Factor in Liver Tissue from a Molybdenum Cofactor Deficient Patient . . . . .	379
J.L. Johnson and K.V. Rajagopalan	
Cloning of a Eukaryotic Molybdenum Cofactor Gene . . . . .	383
P. Kamdar, M.E. Shelton, and V. Finnerty	

### **SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTI-FOLATES**

#### **GOWLAND HOPKINS LECTURE:**

Design and Synthesis of Inhibitors of Folate-Dependent Enzymes as Antitumor Agents . . . . .	387
E.C. Taylor	
Synthesis and Antitumor Activity of LY288601, the 5,6-Dihydro Analog of LY231514 . . . . .	409
C.J. Barnett, T.M. Wilson, and G.B. Grindey	
Synthesis and Preliminary Biological Evaluation of Analogues of 5,8-Dideazaisofolic Acid and its 2-Desamino-2-Methyl Derivative Containing Fluorine at Position 5 . . . . .	413
J.B. Hynes, O.S. Fetzer, B. Shane, and J.H. Freisheim	

Synthesis and Biological Evaluation of Analogues of 5,8-Dideazaisofolic Acid (IAHQ) Modified at Positions 2, 4 and 9 . . . . .	417
J.B. Hynes, R.L. Hagen, B. Shane, and J. Freisheim	
New Thiophene Substituted 10-Deazaaminopterin: Synthesis and Biological Evaluation . . . . .	421
A. Abraham, J.J. McGuire, J. Galivan, B.R. Vishnuvajjala, and M.G. Nair	
Evaluation of the Anti-Arthritic Activity and an Alternate Synthesis of a Thiophene-Substituted 10-Deazaaminopterin . . . . .	425
A. Desai and M.G. Nair	
Lipophilic Antifolates as Candidates Against Opportunistic Infections . . . . .	429
J.R. Piper, C.A. Johnson, C.A. Hosmer, R.L. Carter, E.R. Pfefferkorn, S.E. Borotz, and S.F. Queener	
Analogues of Classical Antifolates Bearing Naphthoyl in Place of Benzoyl . . . . .	435
J.R. Piper, C.A. Johnson, J.A. Maddry, J.J. McGuire, G.M. Otter, and F.M. Sirotnak	
Synthesis and Biological Activity of Tricyclic, Conformationally Restricted Analogs of Lipophilic Pyrido[2,3- <i>d</i> ]Pyrimidine Antifolates . . . . .	441
A. Gangjee, F. Mavandadi, and S.F. Queener	
Novel 2,4-Diamino-5-Substituted Furo[2,3- <i>d</i> ]Pyrimidines as Potential Antifolates . . . . .	445
A. Gangjee, R. Devraj, S.F. Queener, and R.L. Kisliuk	
Bicyclic Conformationally Restricted Analogs of Nonclassical Pyrido[2,3- <i>d</i> ]Pyrimidines as Potential Inhibitors of Dihydrofolate Reductases . . . . .	449
A. Gangjee, A. Vasudevan, and S.F. Queener	
Synthesis, Structural and Biochemical Characterization of Cytostatic Methotrexate- $\gamma$ -Glutamyl-Glutathione Conjugates . . . . .	453
M. Kussmann, D. Wiehr, T. Knepper, and M. Przybylski	
Activation by Peptidases and Cytotoxicity of 2-(L- $\alpha$ -Aminoacyl) Prodrugs of Methotrexate . . . . .	457
H.T.A. Cheung, Z. Dong, L. Escoffier, M.A. Smal, and M.H.N. Tattersall	
Effect of a Novel Antifolate, N <sup><math>\alpha</math></sup> -(4-Amino-4-Deoxypteroyl)-N <sup><math>\delta</math></sup> -Hemiphthaloyl-L-Ornithine (PT523) on Growth of H35 Rat Hepatoma and HEPG2 Human Hepatoma Cells . . . . .	461
M.S. Rhee, J. Galivan, E.M. Tyobeka, M.L. Sherman, and A. Rosowsky	
Tubulin Binding Properties of Two Chiral Isomers with 1-Deaza-7,8-Dihydropteridine Structure . . . . .	465
D. Leynadier, V. Peyrot, M. Sarrazin, J.M. Andreu, C. Temple, G.A. Renner, and C. Briand	
Effects of Folic Acid on Pyrimethamine Teratogenesis in Rats . . . . .	469
G. Kudo, K. Tsunematsu, M. Shimoda, and E. Kokue	

## DIHYDROFOLATE REDUCTASE

Mutations of Human Dihydrofolate Reductase Causing Decreased Inhibition by Methotrexate . . . . .	473
R.L. Blakley, J.R. Appleman, S.K. Chunduru, T. Nakano, W.S. Lewis, and S.E. Harris	
Conformational Analysis of Human Dihydrofolate Reductase Inhibitor Complexes: Crystal Structure Determination of Wild Type and F31 Mutant Binary and Ternary Inhibitor Complexes . . . . .	481
V. Cody, A. Wojtczak, T.I. Kalman, J.H. Freisheim, and R.L. Blakley	
Computer-Aided Design of Mechanism-Based Pterin Analogues and MD/FEP Simulations of Their Binding to Dihydrofolate Reductase . . . . .	487
J.E. Gready, P.L. Cummins, and P. Wormell	
Does R67 Dihydrofolate Reductase Possess a Proton Donor? . . . . .	493
J.C. Holland, C.E. Linn, E. DiGiammarino, R.J. Nichols, and E.E. Howell	
Laser-Sensitized Tautomers in Dihydrofolate Reductase . . . . .	499
J.W. Ledbetter, W. Pfleiderer, and J.H. Freisheim	
Methotrexate-Insensitive Mutants of Human Dihydrofolate Reductase (hDHFR) Constructed by Site-Directed Mutagenesis at Phenylalanine 34 . . . . .	503
T. Nakano, J.R. Appleman, and R.L. Blakley	
Kinetic Investigation of Methotrexate Resistant Human Dihydrofolate Reductase (hDHFR) Mutants at Phenylalanine 31 . . . . .	507
S.K. Chunduru, J.R. Appleman, and R.L. Blakley	
The Effect of Codon 31 on the Relative Affinities for the Binding of Designed 8-Alkyl-Pterins to Dihydrofolate Reductase: A Statistical Perturbation Theory and Molecular Dynamics Simulation Study . . . . .	511
P.L. Cummins and J.E. Gready	
Effect of Codon 22 Mutations on Substrate and Inhibitor Binding for Human Dihydrofolate Reductase . . . . .	515
E. Ercikan, M. Waltham, A. Dicker, B.I. Schweitzer, and J.R. Bertino	
Thermodynamic Study of Folate Analogue Binding to Dihydrofolate Reductase from Different Species . . . . .	521
S. Sasso, R. Gilli, C. Lopez, J.C. Sari, and C. Briand	
Comparison of Binding and Activity of 8-Alkyl-Pterins and 8-Alkyl-N5- Deaza-Pterins with Dihydrofolate Reductase . . . . .	525
M.T.G. Ivery and J.E. Gready	
Development of a Spectrofluorimetric Method for Determining the $pK_a$ of Pterin-Analogue Ligands Bound to Dihydrofolate Reductase . . . . .	529
S-S. Jeong and J.E. Gready	