Molecular Parasitology

EDITED BY

J. Thomas August

Department of Pharmacology and
Experimental Therapeutics
The Johns Hopkins University
School of Medicine
Baltimore, Maryland

1984

ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)

ORLANDO SAN DIEGO NEW YORK LONDON
TORONTO MONTREAL SYDNEY TOKYO

Academic Press Rapid Manuscript Reproduction

Proceedings of the Third John Jacob Abel Symposium on Drug Development Held at The Johns Hopkins University School of Medicine, Baltimore, Maryland, June 13–15, 1983

COPYRIGHT © 1984, BY ACADEMIC PRESS, INC.
ALL RIGHTS RESERVED.
NO PART OF THIS PUBLICATION MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM OR BY ANY MEANS, ELECTRONIC OR MECHANICAL, INCLUDING PHOTOCOPY, RECORDING, OR ANY INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT PERMISSION IN WRITING FROM THE PUBLISHER.

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 84-45875

ISBN 0-12-068060-2

PRINTED IN THE UNITED STATES OF AMERICA

84 85 86 87 9 8 7 6 5 4 3 2 1

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Stephen B. Aley (89), *Malaria Section, Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

Maria L. Cardoso de Almeida (19), *MRC Biochemical Parasitology Unit, The Molteno Institute, University of Cambridge, Cambridge, England*

Robin F. Anders (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia*

William S. Aronstein (185), *Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

John W. Barnwell (89), *Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

Stephen M. Beverly (231), *Department of Biological Sciences, Stanford University, Stanford, California 94305*

Graham V. Brown (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia*

Ernest Bueding (283), *Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205*

Clint E. Carter (167), *Departments of General Biology, Vanderbilt University, Nashville, Tennessee 37235*

Jeffrey A. Coderre (231), *Departments of Biochemistry and Biophysics and of Pharmaceutical Chemistry, University of California, School of Pharmacy, San Francisco, California 94143*

Ross L. Coppel (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia*

Alan F. Cowman (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria Australia*

John E. Donelson (33), *Department of Biochemistry, Basic Science Building, University of Iowa, Iowa City 52242*

Joan Ellis (127), *Biochemistry Department, New York University Medical Center, New York, New York 10016*

Paul T. Englund (53), *Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Robert R. Freeman (79), *Department of Molecular Biology, Wellcome Research Laboratories, Beckenham, Kent, BR3, 3BS, England*

G. Nigel Godson (127), *Biochemistry Department, New York University Medical Center, New York, New York 10016*

Stephen L. Hajduk (53), *Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Donald A. Harn (155), *Division of Tropical Medicine, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts 02115*

D. Janiece Harrison (167), *Department of General Biology, Vanderbilt University, Nashville, Tennessee 37235*

David T. Hart (63), *Research Unit for Tropical Diseases, International Institute of Cellular and Molecular Pathology, Brussels, Belgium*

Julia Hawkins (283), *Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205*

Anthony A. Holder (79) *Department of Molecular Biology, Wellcome Research Laboratories, Beckenham, Kent, BR3, 3BS, England*

Russell J. Howard (89), *Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

David J. Kemp (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia*

Matty Knight (177), *Division of Parasitology, National Institute for Medical Research, Mill Hill, London NW7, 1AA, England*

Richard W. F. Le Page (19), *Department of Pathology, University of Cambridge, Cambridge, England*

Samuel A. Lewis (185), *Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Klaus R. Lingelbach (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia*

James R. Lupski (127), *Biochemistry Department, New York University Medical Center, New York, New York 10016*

J. Joseph Marr (205), *Division of Infectious Diseases, Department of Medicine and Microbiology, University of Colorado Health Sciences Center, Denver, Colorado 80262*

Onno Misset (63), *Research Unit for Tropical Diseases, International Institute of Cellular and Molecular Pathology, Brussels, Belgium*

Graham F. Mitchell (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia*

Theodore E. Nash (145), *Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

Amy P. Norden (185), *Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Ruth S. Nussenzweig (117), *Department of Pathology and Division of Parasitology, Department of Microbiology, New York University Medical Center, New York, New York 10016*

Victor Nussenzweig (117), *Department of Pathology and Division of Parasitology, Department of Microbiology, New York University Medical Center, New York, New York 10016*

Fred R. Opperdoes (63), *Research Unit for Tropical Diseases, International Institute of Cellular and Molecular Pathology, Brussels, Belgium*

Luiz S. Ozaki (127), *Biochemistry Department, New York University Medical Center, New York, New York 10016*

Gilberto Payares (177), *Division of Parasitology, National Institute for Medical Research, Mill Hill, London NW7 1AA, England*

Richard E. Reeves (267), *Departments of Biochemistry and of Tropical Medicine and Medical Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112*

Robert B. Saint (103), *The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia*

Daniel V. Santi (231), *Departments of Biochemistry and Biophysics and of Pharmaceutical Chemistry, University of California, School of Pharmacy, San Francisco, California 94143*

Howard J. Saz (251), *Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556*

Leonard W. Scheibel (275), *Department of Preventive Medicine, Uniformed Services, University of the Health Sciences, Bethesda, Maryland 20814*

Robert T. Schimke (231), *Department of Biological Sciences, Stanford University, Stanford, California 94305*

此为试读,需要完整PDF请访问: www.ertongbook.com

Andrew J. G. Simpson (177), *Division of Parasitology, National Institute for Medical Research, Mill Hill, London NW7 1AA, England*

S. Ronald Smithers (177), *Division of Parasitology, National Institute for Medical Research, Mill Hill, London NW7 1AA, England*

Mette Strand (185), *Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Pamela Svec (127), *Biochemistry Department, New York University Medical Center, New York, New York 10016*

Mervyn J. Turner (19), *MRC Biochemical Parasitology Unit, The Molteno Institute, University of Cambridge, Cambridge, England*

Keith Vickerman (3), *Department of Zoology, University of Glasgow, Scotland*

Ching C. Wang (217), *Department of Pharmaceutical Chemistry, University of California, School of Pharmacy, San Francisco, California 94143*

Judith B. Weiss (185), *Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

H. Guy Williams-Ashman (237), *Ben May Laboratory for Cancer Research and Departments of Biochemistry and of Pharmacological and Physiological Sciences, University of Chicago, Chicago, Illinois 60637*

Preface

John Jacob Abel was the first professor of pharmacology and experimental therapeutics in what was then called The Johns Hopkins Medical School. A pioneer in many fields of biochemical research and teaching, Professor Abel was the first to crystallize insulin at a time when proteins were not expected to exhibit such a defined structure or hormonal action. He was also the first to introduce, for what he called "vividiffusion," an artificial kidney from which today's hemodialysis machines were directly developed.

The rapidly developing field of molecular parasitology provides an exciting focus for the Third John Jacob Abel Symposium on Drug Development. The topic is especially fitting because the chemotherapy of trypanosomiasis was another of Professor Abel's particular interests. The trivalent thioglycollate salts of antimony which he developed and tested in rats, rabbits, and a celebrated donkey were a mainstay of the antiparasitic armamentarium for many years.

Professor Abel described his work in the 1910 volume of the Journal of Pharmacology and Experimental Therapeutics; a warm and amusing account of the donkey experiments was published by his collaborator, Professor Leonard G. Rowntree, in the Bulletin of the Johns Hopkins Hospital almost 50 years later. The donkey, called Maud, was experimentally infected and treated by intrajugular injections. Treatment was complicated because she bucked and struggled every time Professor Abel signaled by saying "Now" that he was ready to inject. One day, after a long series of futile attempts over a period of about half an hour, Professor Rowntree discovered that the stable boy took Professor Abel's cue as a signal to jab a spike into Maud's belly.

The trivalent antimonials were tried in a variety of conditions and were widely used to treat several serious parasitic infections, including schistosomiasis. The drug's mechanism of action against schistosomes, inhibition of the parasite's phosphofructokinase to interfere with its energy metabolism was demonstrated decades

after Professor Abel's original work by Professor Ernest Bueding, whom we are fortunate to include as one of the participants in the present Symposium.

Like Professor Abel, Professor Bueding is a pioneer in several areas of biochemical research. He was among the first to successfully attack the difficult problems of parasite metabolism and has made important contributions to unraveling the mechanisms by which certain drugs exert carcinogenic side effects. He has also introduced several methods by which these carcinogenic effects can be prevented without compromising the drug effects that are desired.

We are pleased and proud to dedicate this present book to the honor of Professor Ernest Bueding, and to the memory of Professor John Jacob Abel.

J. THOMAS AUGUST

Acknowledgments

This symposium has been possible only because of the generous support from the following sponsors:

Supported by major funding from
Hoffmann–La Roche, Inc.
Merrell Dow Research Center

Additional contributions from
Abbott Laboratories
A. H. Robins Company
Burroughs Wellcome Company
E. I. Du Pont de Nemours and Company (Experimental Station)
McNeil Pharmaceutical
Merck Sharp and Dohme Research Laboratories
Monsanto Company
Ortho Pharmaceutical Corporation
Pfizer Central Research
Smith Kline and French Laboratories
Stuart Pharmaceuticals (Division of ICI Americas, Inc.)

I wish to acknowledge the excellent support provided by Mrs. Sue Orefice for coordinating both the symposium and the proceedings and to thank Mrs. Donna Williamson and Mrs. Susan Maurizi for the typing and editing of the manuscripts.

J. THOMAS AUGUST

Contents

Contributors ix
Preface xiii
Acknowledgments xv

SESSION I BIOCHEMISTRY AND MOLECULAR BIOLOGY OF TRYPANOSOMES
Chairman: **Dr. Paul Englund**

1. Trypanosome Survival Mechanisms. Biological Basis of Trypanosome Antigenic Variation 3
Keith Vickerman
2. The Release of Variant Surface Glycoproteins of *Trypanosoma brucei* 19
Maria L. Cardoso de Almeida, Richard W. F. Le Page, and Mervyn J. Turner
3. The Genetics of Antigenic Variation in Trypanosomes 33
John E. Donelson
4. The Replication of Kinetoplast DNA 53
Stephen L. Hajduk and Paul T. Englund
5. Metabolic Pathways Associated with the Glycosomes (Microbodies) of the Trypanosomatidae 63
Fred R. Opperdoes, Onno Misset, and David T. Hart

SESSION II MOLECULAR BIOLOGY AND IMMUNOCHEMISTRY OF MALARIA

Chairwoman: **Dr. Ruth Nussenzweig**

6. Blood Stage *Plasmodium yoelii* and *Plasmodium falciparum* Antigen Characterization 79
Anthony A. Holder and Robert R. Freeman

7. Antigenic Variation of a Malarial Protein on the Surface of *Plasmodium knowlesi* Infected Erythrocytes 89
Russell J. Howard, John W. Barnwell, and Stephen B. Aley

8. Detection and Analysis of *Plasmodium falciparum* Antigens Expressed in *Escherichia coli* 103
Ross L. Coppel, Robin F. Anders, Graham V. Brown, Alan F. Cowman, Klaus R. Lingelbach, Robert B. Saint, Graham F. Mitchell, and David J. Kemp

9. Protective Immunity to Malaria Sporozoites 117
Victor Nussenzweig and Ruth S. Nussenzweig

10. Molecular Biology of *Plasmodium knowlesi* Sporozoites: Cloning and Expression of the Surface Antigen Gene 127
G. Nigel Godson, Joan Ellis, James R. Lupski, Luiz S. Ozaki, and Pamela Svec

SESSION III MOLECULAR BIOLOGY AND IMMUNOCHEMISTRY OF SCHISTOSOMES

Chairwoman: **Dr. Mette Strand**

11. Fate of Two Excretory–Secretory Antigens in Schistosomiasis 145
Theodore E. Nash

12. *Schistosoma mansoni:* Passive Transfer of Immunity by an Anti-Egg Monoclonal Antibody 155
Donald A. Harn

13. Schistosome Soluble Egg Antigens 167
Clint E. Carter and D. Janiece Harrison

14. Identification and Biosynthesis of Schistosome Surface Antigens 177
Andrew J. G. Simpson, Matty Knight, Gilberto Payares, and S. Ronald Smithers

此为试读,需要完整PDF请访问: www.ertongbook.com

15. *Schistosoma* Worms Proteins: Composition and Immunoreactivity 185
Mette Strand, William S. Aronstein, Amy P. Norden, Samuel A. Lewis, and Judith B. Weiss

SESSION IV CHEMOTHERAPY OR PARASITIC DISEASES–I
Chairman: **Dr. Paul Talalay**

16. Pyrazolopyrimidines as Antagonists of Purine Metabolism in Protozoans 205
J. Joseph Marr
17. Purine and Pyrimidine Metabolism in *Trichomonadidae* and *Giardia* 217
Ching C. Wang
18. Methotrexate Resistant *Leishmania tropica*. A Model for Drug Resistance in Pathogenic Protozoa 231
Daniel V. Santi, Jeffrey A. Coderre, Stephen M. Beverley, and Robert T. Schimke
19. Comparative Aspects of Polyamine Metabolism: Implications for Chemotherapy 237
H. Guy Williams-Ashman

SESSION V CHEMOTHERAPY OF PARASITIC DISEASES–II
Chairman: **Dr. Ernest Bueding**

20. Anaerobic Energy Yielding Reactions 251
Howard J. Saz
21. Pyrophosphate-Energy-Conservation in a Parasite and Its Suppression by Chemical Agents 267
Richard E. Reeves
22. Chelating Agents in the Chemotherapy of Malaria 275
Leonard W. Scheibel
23. Globinases and the Antischistosomal Actions of Cyclosporin A 283
Ernest Bueding and Julia Hawkins

Index 289

I. Biochemistry and Molecular Biology of Trypanosomes

TRYPANOSOME SURVIVAL MECHANISMS. BIOLOGICAL BASIS OF TRYPANOSOME ANTIGENIC VARIATION

Keith Vickerman

Department of Zoology
University of Glasgow
Scotland

Trypanosomes lead extremely hazardous lives. There can be few unicellular eukaryotes which have to cope with such dramatic changes in their environment and respond with such feats of endurance as these tiny flagellates. In recent years we have learned a lot about how the African trypanosomes, in particular, survive the dangers that assail them as they pursue a life cycle through two different hosts--a mammal and a tsetse fly (Glossina spp).

Trypanosoma brucei, the species about which we know most, naturally infects a variety of mammalian hosts including antelopes and other game animals, domestic animals, and man. Only certain genetic variants can infect man, however, and these are given separate sub-specific names according to the character of the disease, sleeping sickness, that they induce--T. b. gambiense for the chronic West African illness, T. b. rhodesiense for the more acute East African disease. T. b. brucei refers to non-man-infecting stocks of the species. All three subspecies live in the blood, lymphatics, and connective tissues of their mammalian hosts. They can traverse the walls of blood and lymph capillary vessels and at a later stage of infection cross the choroid plexus into the brain and cerebrospinal fluid. Multiplication by binary fission occurs in long slender forms of the parasite in all sites, but non-dividing short stumpy forms arise in the blood and lymph, and these appear to be the forms which survive when ingested by the tsetse fly vector. The complicated cycle of development in the vector is shown in Fig. 1. After multiplication in the midgut as the so-called procyclic form, a fortunate few (and it may be only one) take a marathon swim back through the mouth parts and up the salivary duct to the salivary glands. Here a further bout of multiplication while attached to the gland epithelium takes place with the release of the mammal-infective, metacyclic stage into the lumen of the gland for discharge with the fly's saliva when it bites another mammal.

In addition to T. brucei, two other tsetse-transmitted trypanosome species are of importance in Africa, T. congolense and T. vivax. Apart from their initial multiplication at the site of fly bite, these trypanosomes are intravascular parasites, T. congolense multiplying while attached to the capillary endothelia of its host, T. vivax free in the blood. Their developmental cycle in the fly is simpler as their final metacyclic-generating phase is in the proboscis of the vector, not

Copyright © 1984 by Academic Press, Inc.
All rights of reproduction in any form reserved.
ISBN 0-12-068060-2

the salivary glands, and T. vivax manages to avoid the initial round of development in its vector's midgut. We know far less about these trypanosomes than about T. brucei because satisfactory replication of their life cycle in the laboratory is more difficult than it is for that species.

Combatting Trypanosome Survival Mechanisms

An understanding of the trypanosome's survival mechanisms is, of course, relevant to the design of means of destroying the parasite by chemotherapy or immunoprophylaxis. The most obvious advantages of trypanosomes over more sluggish parasites are their capacity for speedy movement and ability to penetrate endothelia to invade new environments. Both depend upon flagellar activity and the maintenance of a streamlined shape which, in turn, depend upon the organisms' ability to construct cytoplasmic microtubules. These structures reinforce the cortex of the trypanosome, maintaining its elongated shape; they also form the doublets in the flagellar axoneme, and flagellar bending results from the sliding of adjacent microtubule doublets in relation to one another. Both α- and β-tubulins which compose the microtubules have now been characterized for trypanosomatids and have been found to differ significantly in their drug-binding properties (1). Suitable microtubule inhibitors might prevent movement or division and upset the critical surface area/volume ratio of the parasite, and the affinity of benzimidazole derivatives for unicellular eukaryote tubulins may provide a possible chemotherapeutic lead (2).

Although the trypanosome's swimming activity can be vital for completion of its migration in the fly or its escape into the tissues from the bloodstream, the question whether its incessant movement in circulating blood represents a wanton wastage of energy has been justifiably raised. The same query might be raised over the flagellar beating of both T. brucei attached in the tsetse salivary glands and T. congolense attached to the capillary vessel walls. It is likely, however, that the flagellar beating, in addition to providing propulsive force, may also serve to circulate the surrounding medium (as in the crowded vector midgut or salivary gland) or make for a throughput of medium in the pocket at the base of the flagellum. It is from this pocket that serum proteins are pinocytosed (3) and into it that the remains of parasite authophagic processes are ejected (4), so frequent replacement of its contents through the rotary pump-like action of the flagellum in the pocket is probably necessary. The principal drug used in treating human sleeping sickness--Suramin--enters the parasite by pinocytosis bound to serum albumin (5). It would be worth investigating the action of anti-flagellar spermicides with a view to their use in the chemotherapy of African trypanosomiasis on account of the importance of flagellar activity in trypanosome survival. The design

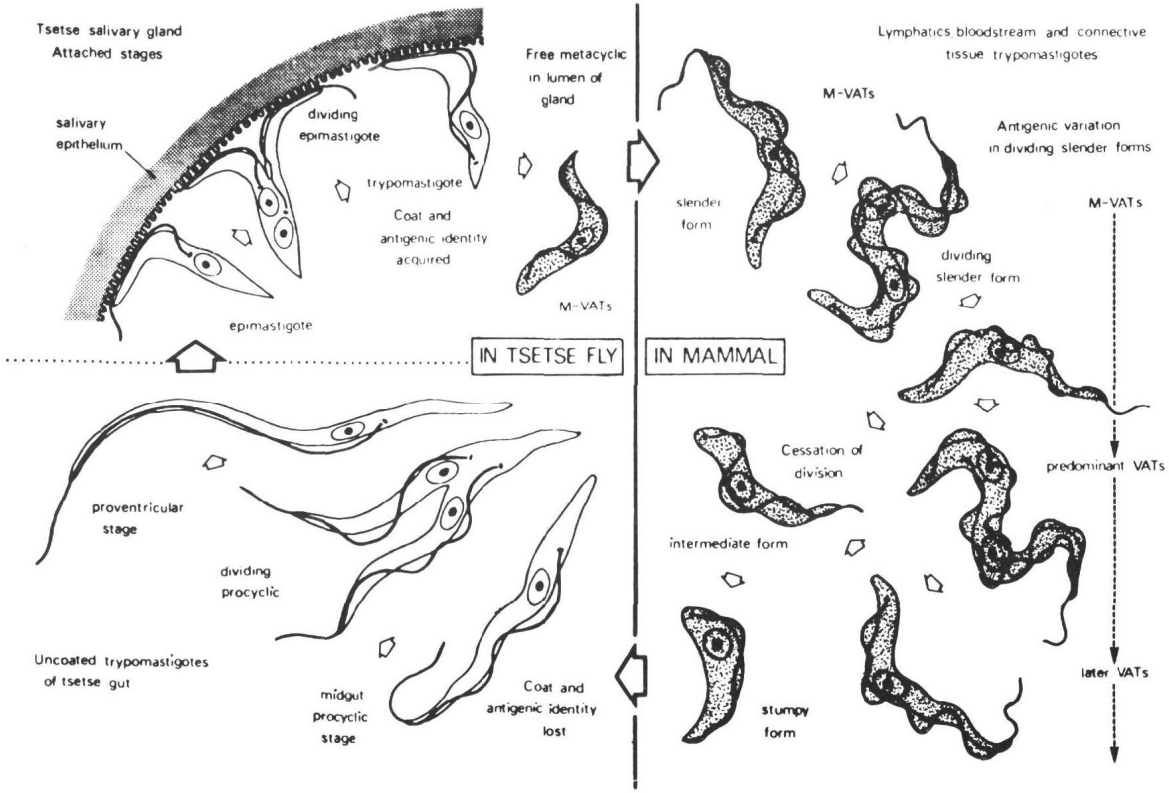

Fig. 1. Diagram of life cycle Trypanosoma brucei. Stages possessing the variable antigen-containing surface coat are shaded. Modified from "Immunology of Parasitic Infections," Sidney Cullen and Kenneth Warren, eds. (1982). Blackwell Scientific Publications, Oxford, England.

此为试读，需要完整PDF请访问：www.ertongbook.com