GROWTH, NUTRITION, AND METABOLISM OF CELLS IN CULTURE

EDITED

George H. Rothblat and Vincent J. Cristofalo

VOLUME III

GROWTH, NUTRITION, AND METABOLISM OF CELLS IN CULTURE

EDITED BY

George H. Rothblat and Vincent J. Cristofalo

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PENNSYLVANIA

VOLUME III

1977

ACADEMIC PRESS New York San Francisco London

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

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

Library of Congress Cataloging in Publication Data

Rothblat, George H
Growth, nutrition, and metabolism of cells in culture.

Includes bibliographies.
1. Cell physiology. 2. Cell metabolism. 3. Cell culture. I. Cristofalo, Vincent J., Date joint author. II. Title.
QH631.R66 574.8'761 71-182625
ISBN 0-12-598303-4

PRINTED IN THE UNITED STATES OF AMERICA

GROWTH, NUTRITION, AND METABOLISM OF CELLS IN CULTURE

Volume III

CONTRIBUTORS

Ho Sam Ahn
William M. Baird
Carlo M. Croce
Leila Diamond
Gerald L. Forrest
Alan F. Horwitz
Robert R. Klevecz
K. S. Koch
H. L. Leffert
John B. Little
Maynard H. Makman
George M. Martin
Liselotte Mezger-Freed
Stephen A. Morris
Roland M. Nardone
Charles E. Ogburn
George P. Studzinski
Jerry R. Williams

LIST OF CONTRIBUTORS

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

Ho Sam Ahn, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York (295)

William M. Baird, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania (421)

Carlo M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania (83)

Leila Diamond, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania (421)

Gerald L. Forrest, Department of Cell Biology, Division of Biology, City of Hope National Medical Center, Duarte, California (149)

Alan F. Horwitz, Departments of Biochemistry and Biophysics and Human Genetics, University of Pennsylvania Medical School, Philadelphia, Pennsylvania (109)

Robert R. Klevecz, Department of Cell Biology, Division of Biology, City of Hope National Medical Center, Duarte, California (149)

K. S. Koch, Molecular Biology Laboratory, Salk Institute, La Jolla, California (225)

H. L. Leffert, Cell Biology Laboratory, Salk Institute, La Jolla, California (225)

John B. Little, Laboratory of Radiobiology, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts (197)

Maynard H. Makman, Departments of Biochemistry and Pharmacology, Albert Einstein College of Medicine, Bronx, New York (295)

George M. Martin, Department of Pathology, University of Washington, Seattle, Washington (1)

Liselotte Mezger-Freed, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania (57)

Stephen A. Morris, Department of Pharmacology, Albert Einstein College of Medicine, Bronx, New York (295)

Roland M. Nardone, Department of Biology, Catholic University of America, Washington, D.C. (471)

Charles E. Ogburn, Department of Pathology, University of Washington, Seattle, Washington (1)

George P. Studzinski, Department of Pathology, New Jersey Medical School, Newark, New Jersey (355)

Jerry R. Williams, Laboratory of Radiobiology, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts (197)

PREFACE

The first two volumes of this treatise dealt with the uptake, synthesis, and degradation of biologically important compounds and with the techniques of mammalian, plant, and invertebrate cell culture systems. Since the publication of Volumes I and II there has been rapid development in the techniques of cell and tissue culture and in our understanding of the factors underlying many aspects of cell behavior. Analysis and critical evaluation of these developments are fundamental to the realization of their potential in research. In this volume, we have attempted to focus on a number of specific, timely areas of research that make use of cell and tissue culture.

The major theme of this volume is growth and its regulation in animal cells. The authors have covered many different facets of this topic. For example, Drs. H. L. Leffert and K. S. Koch have critically discussed the role of growth factors in cell culture systems, while Drs. Makman, Morris, and Ahn have analyzed the effects of cyclic nucleotides in cell proliferation in culture. Metabolic regulation during the cell cycle has been discussed by Drs. R. R. Klevecz and G. L. Forrest, and the role of the cell surface in growth and metabolic regulation is considered by Dr. A. F. Horwitz.

Aspects of abnormal cell growth and metabolism are detailed in a chapter on carcinogenesis *in vitro* by Drs. L. Diamond and W. M. Baird and in a chapter on viral transformation by Dr. G. P. Studzinski. The subject of DNA repair is reviewed by Drs. J. R. Williams and J. B. Little, while the principal approach to genetic analysis using cell fusion techniques is discussed by Dr. C. M. Croce.

The growth of vascular cells in culture has provided significant impetus to atherosclerosis research, while the culture of haploid vertebrate cells holds great potential for genetic analysis of cell function. These areas are discussed by Drs. G. M. Martin and C. E. Ogburn in their chapter on cultures of blood vessels; data on haploid cell culture are reviewed by Dr. L. Mezger-Freed. The value of using cell cultures to test for the possible toxicity of various pharmacologic

agents is just being realized. The fundamental principles of this powerful tool are discussed by Dr. R. M. Nardone.

We wish to thank the authors of this volume for their care and efforts. We would also like to acknowledge Margaret McGee for her help with many aspects of our editorial work. Finally we acknowledge the support of N.I.H. Grants AG00378 and HL20608.

George H. Rothblat
Vincent J. Cristofalo

CONTENTS OF OTHER VOLUMES

Volume I

Volume II

CONTENTS

1

CELL, TISSUE, AND ORGANOID CULTURES OF BLOOD VESSELS

George M. Martin and Charles E. Ogburn

I. Introduction

A. Historical Background

Inspired by the pioneering work of Ross G. Harrison (1907) on cultivated neural tissue, many laboratories around the world quickly began to culture a variety of embryonic and adult tissues, including vascular tissues. The father of organoid culture of vascular tissue was probably a Frenchman (Fleig, 1910); he employed a very modern bioassay—arterial transplantation! Carrel and Burrows

(1910) appear to have been the first investigators concerned with cells migrating and proliferating from vascular explants. As far as one can tell, they grew cells from the adventitia of an artery ("arterial sheath"), noting that "vegetation was very weak and stopped entirely after a few days." Interestingly, one can find only a rare report since that time (for example, Fisher-Dzoga *et al.*, 1973) in which specific attention has been devoted to adventitial cell proliferation; to this day little is known concerning the nature of the cell type(s) which preferentially grow out from such perivascular tissues.

A selection of the literature of this "primordial" period and of the subsequent decades (the "classical" period) of refinements in tissue culture media and in light microscopy is reviewed in Pollak's useful monograph (1969), in Murray's discussion of smooth muscle culture (1965), and in her comprehensive bibliography of research in tissue culture (Murray and Kopech, 1953). The Murray references are especially useful in that they include many foreign language publications not referenced by Pollak. It is doubtful, however, that a detailed review of this earlier literature would be productive (at least with respect to cell cultures) as there is so much confusion concerning what cell types were actually being described. We shall therefore concentrate on the "modern period," which we shall loosely define as those researches which deal with rather more quantitative aspects of cell culture and/or which utilize biochemical, immunological, or fine structure techniques for the elucidation of highly characteristic or specific cell markers. This latter period is very modern indeed, beginning essentially in the 1970's. Nevertheless, the field has been sufficiently active to result in several current reviews on various aspects of the subject (Fedoroff, 1973; Gimbrone, 1976; Ross and Kariya, 1976). The reader should be aware, however, that major uncertainties remain concerning the morphological, biochemical, and genetic properties of these systems. It seems likely that we will require up-dated reviews within two or three years.

B. Significance

Clearly, much of the current flurry of research on *in vitro* studies of vascular tissues is motivated by the growing recognition that, in developing societies, vascular diseases account for an enormously high proportion of morbidity and mortality. We see from Table I, for example, that in 1973, about 55% of all United States death certificates implicated disease categories with major cardiovascular components as the cause of death. About a third of all deaths were attributed to ischemic heart disease, the principal cause of which is coronary artery atherosclerosis and thrombosis. Of course, individuals who die of cancer, the second leading general category of mortality, are generally elderly, and typically also suffer from one or more types of arteriosclerosis, especially