



Polyamines in Normal and Neoplastic Growth

Edited by
Diane H. Russell

A NATIONAL CANCER INSTITUTE SYMPOSIUM VOLUME

Polyamines in Normal and Neoplastic Growth

*Proceedings of a Symposium of the
National Cancer Institute, U.S.A.*

Edited by

Diane H. Russell, Ph.D.

*National Cancer Institute
Baltimore Cancer Research Center
Baltimore, Maryland, U.S.A.*

Raven Press, Publishers ■ New York

Distributed in the Eastern Hemisphere by

North-Holland Publishing Company ■ Amsterdam

© 1973 by Raven Press Books, Ltd. All rights reserved. This book is protected by copyright. No part of it may be duplicated or reproduced in any manner without written permission from the publisher.

The following contributors to this book are employees of the United States Government; their contributions are therefore not subject to the above-mentioned prohibition on duplication:

Robert C. Gallo, Olle Heby, Carl C. Levy, Laurence J. Marton, Seymour M. Perry, Diane H. Russell, Stephen C. Schimpff, Richard M. Simon, T. Phillip Waalkes, and Kwang B. Woo.

Made in the United States of America

International Standard Book Number 0-911216-44-8
Library of Congress Catalog Card Number 72-96336

POLYAMINES IN NORMAL
AND NEOPLASTIC GROWTH

List of Contributors

Juhani Ahonen

*Department of Medical Chemistry,
University of Turku, Turku, Finland*

Uriel Bachrach

*Department of Molecular Biology,
Hebrew University, Hadassah Medical
School, Jerusalem, Israel*

William T. Beck

*Department of Pharmacology, Yale
University School of Medicine, New
Haven, Connecticut 06510*

Miriam Ben-Joseph

*Department of Molecular Biology, Hebrew
University, Hadassah Medical School,
Jerusalem, Israel*

Anne Blackledge

*Biochemistry Group, University of
Sussex, School of Biological Sciences,
Falmer, Brighton, Sussex, England*

Craig V. Byus

*Department of Biochemistry, University
of New Hampshire, Durham, New
Hampshire 03824*

E. S. Canellakis

*Department of Pharmacology, Yale
University School of Medicine, New
Haven, Connecticut 06510*

Seymour S. Cohen

*Department of Microbiology, University
of Colorado Medical Center, Denver,
Colorado 80220*

Gordon L. Coppoc

*Department of Veterinary Physiology and
Biochemistry, Purdue University,
Lafayette, Indiana 47907*

E. Delain

*Laboratoire de Microscopie Electronique,
Institut Gustave Roussy, 94, Villejuif,
France*

M. Drue Denton

Department of Internal Medicine,

*University of Cincinnati College of
Medicine, Cincinnati, Ohio 45229*

Arnold S. Dion

*Molecular Biology Section, Institute for
Medical Research, Camden, New Jersey
08103*

Robert H. Fillingame

*Department of Biochemistry, University
of Washington, School of Medicine,
Seattle, Washington 98195*

Robert C. Gallo

*Laboratory of Tumor Cell Biology,
National Cancer Institute, National
Institutes of Health, Bethesda, Maryland
20014*

Charles W. Gehrke

*Department of Agricultural Chemistry,
University of Missouri, Columbia, Missouri
65201*

Eduard Gfeller

*Department of Anatomy, Johns Hopkins
University School of Medicine, Baltimore,
Maryland 21205*

Wade Gibson

*The University of Chicago, Chicago,
Illinois 60637*

Helen S. Glazer

*Department of Internal Medicine,
University of Cincinnati College of
Medicine, Cincinnati, Ohio 45229*

Kenneth D. Graziano

*Section of Immunology and Cell Biology,
Baltimore Cancer Research Center,
Baltimore, Maryland 21211*

Luce Gresland

*Unité de Biologie Moléculaire, Groupe
de Recherche No. 8 du C.N.R.S., Institut
Gustave Roussy, 94, Villejuif, France*

Bruce Hacker

*Division of Oncology, Albany Medical
College, Albany, New York 12208*

Pekka Hannonen

*Department of Medical Chemistry,
University of Helsinki, Helsinki, Finland*

Sami I. Harik

*Department of Pharmacology, Johns
Hopkins University School of Medicine,
Baltimore, Maryland 21205*

Inez A. Hawk

*Section on Enzymology and Drug
Metabolism, Laboratory of Pharmacology,
Baltimore Cancer Research Center,
National Cancer Institute, Baltimore,
Maryland 21211*

Olle Heby

*Baltimore Cancer Research Center,
National Cancer Institute, Baltimore,
Maryland 21211*

Edward J. Herbst

*Department of Biochemistry, University of
New Hampshire, Durham, New Hampshire
03824*

Brigid L. M. Hogan

*Biochemistry Group, School of Biological
Sciences, University of Sussex, Falmer,
Brighton, Sussex, England*

Erkki Hölttä

*Department of Medical Chemistry,
University of Helsinki, Helsinki, Finland*

J. Huppert

*Unité de Biologie Moléculaire, Groupe de
Recherche No. 8 du C.N.R.S., Institut
Gustave Roussy, 94, Villejuif, France*

Juhani Jänne

*Department of Medical Chemistry,
University of Helsinki, Helsinki, Finland*

Leon T. Kremzner

*Department of Neurology, College of
Physicians & Surgeons of Columbia
University, New York, New York 10032*

Kenneth C. Kuo

*Department of Agricultural Chemistry,
University of Missouri, Columbia, Missouri
65201*

Phoebe S. Leboy

*Department of Biochemistry, School of
Dental Medicine, University of
Pennsylvania, Philadelphia, Pennsylvania
19104*

Carl C. Levy

*Laboratory of Pharmacology, Baltimore
Cancer Research Center, National
Institutes of Health, Baltimore, Maryland
21211*

Carol-Ann Manen

*Department of Zoology, University of
Maine, Orono, Maine 04473*

Michael R. Mardiney, Jr.

*Section of Immunology and Cell Biology,
Baltimore Cancer Research Center,
Baltimore, Maryland 21211*

Laurence J. Marton

*Laboratory of Pharmacology, Baltimore
Cancer Research Center, Baltimore,
Maryland 21211*

William E. Mitch

*Laboratory of Pharmacology, Baltimore
Cancer Research Center, Baltimore,
Maryland 21211*

Dan H. Moore

*Department of Cytological Biophysics,
Institute for Medical Research, Camden,
New Jersey 08103*

David R. Morris

*Department of Biochemistry, University
of Washington, School of Medicine,
Seattle, Washington 98195*

Susan Murden

*Biochemistry Group, University of Sussex,
School of Biological Sciences, Falmer,
Brighton, Sussex, England*

Donald L. Nuss

*Department of Biochemistry, University
of New Hampshire, Durham, New
Hampshire 03824*

Gavril W. Pasternak

Department of Pharmacology, Johns

*Hopkins University School of Medicine,
Baltimore, Maryland 21205*

Pamela Piester

*Department of Biochemistry, School of
Dental Medicine, University of
Pennsylvania, Philadelphia, Pennsylvania
19104*

G rard A. Quash

*Biochemistry Department, University of
the West Indies, Kingston, Jamaica*

Aarne Raina

*Department of Biochemistry, University
of Kuopio, Kuopio, Finland*

Bernard Roizman

*Departments of Microbiology and
Biophysics, The University of Chicago,
Chicago, Illinois 60637*

Diane H. Russell

*Laboratory of Pharmacology, Baltimore
Cancer Research Center, National Cancer
Institute, Baltimore, Maryland 21211*

Ted T. Sakai

*Department of Microbiology, University of
Colorado Medical Center, Denver,
Colorado 80220*

Amelia Schenone

*Ben May Laboratory, The University of
Chicago, Chicago, Illinois 60637*

Stephen C. Schimpff

*Medicine Section, Baltimore Cancer
Research Center, National Cancer
Institute, Baltimore, Maryland 21211*

Morton Schmukler

*Laboratory of Pharmacology, Baltimore
Cancer Research Center, Baltimore,
Maryland 21211*

Nikolaus Seiler

*Max-Planck-Institut f r Hirnforschung,
Arbeitsgruppe Neurochemie, 6000
Frankfurt, Germany*

Edward G. Shaskan

Section of Neurosciences, Division of

*Biological and Medical Sciences, Brown
University, Providence, Rhode Island
02912*

Richard M. Simon

*Division of Cancer Treatment, National
Cancer Institute, Bethesda, Maryland
20014*

Frank G. Smith

*Department of Internal Medicine,
University of Cincinnati College of
Medicine, Cincinnati, Ohio 45229*

Solomon H. Snyder

*Departments of Pharmacology and
Psychiatry, Johns Hopkins University
School of Medicine, Baltimore, Maryland
21205*

Ching-Hsiang Su

*Department of Microbiology, University
of Pennsylvania, Philadelphia,
Pennsylvania 19104*

James G. Vaughn

*Amino Acid Analyzer Applications
Division, Beckman Instruments,
Incorporated, Palo Alto, California*

T. Phillip Waalkes

*Department of Health, Education and
Welfare, National Institutes of Health,
National Cancer Institute, Bethesda,
Maryland 20014*

Thomas Walle

*Department of Pharmacology, Medical
University of South Carolina, Charleston,
South Carolina 29401*

George Weber

*Department of Pharmacology, Indiana
University School of Medicine,
Indianapolis, Indiana 46202*

H. G. Williams-Ashman

*Ben May Laboratory, The University of
Chicago, Chicago, Illinois 60637*

Kwang B. Woo

*Division of Cancer Treatment, National
Cancer Institute, Bethesda, Maryland
20014*

Christopher C. Wylie
*Department of Anatomy, University
College London, London, England*

David C. Zellner
Department of Internal Medicine,

*University of Cincinnati College of
Medicine, Cincinnati, Ohio 45229*

Robert W. Zumwalt
*Department of Agricultural Chemistry,
University of Missouri, Columbia,
Missouri 65201*

Contents

- 1 Polyamines in Growth—Normal and Neoplastic
Diane H. Russell
- 15 Tumor Cells, Polyamines, and Polyamine Derivatives
Uriel Bachrach and Miriam Ben-Joseph
- 27 Polyamine Metabolism in Normal and Neoplastic Neural Tissue
Leon T. Kremzner
- 41 Cations and the Reactivity of Thiouridine in *Escherichia coli* tRNA
Ted T. Sakai and Seymour S. Cohen
- 55 The Role of Polyamines During tRNA Processing in Leukemic Cells
Bruce Hacker
- 71 The Stimulation of RNA Synthesis by Spermidine: Studies with *Drosophila* Larvae and RNA Polymerase
Edward J. Herbst, Craig V. Byus, and Donald L. Nuss
- 91 Influence of Polyamines on the Hydrolysis of Polynucleotides by *Citrobacter* Ribonuclease
Carl C. Levy, William E. Mitch, and Morton Schmukler
- 103 *In Vitro* Studies of RNA Methylation in the Presence of Polyamines
Phoebe S. Leboy and Pamela Piester
- 111 RNA and Protein Synthesis in a Polyamine-Requiring Mutant of *Escherichia coli*
David R. Morris
- 123 The Structural and Metabolic Involvement of Polyamines with Herpes Simplex Virus
Wade Gibson and Bernard Roizman
- 137 Polyamine Metabolism in the Brain
N. Seiler

- 157 Anti-Polyamine Antibodies and Growth
G. Quash, Luce Gresland, E. Delain, and J. Huppert
- 167 Polyamine-Synthesizing Enzymes in Regenerating Liver and in Experimental Granuloma
Aarne Raina, Juhani Jänne, Pekka Hannonen, Erkki Hölttä, and Juhani Ahonen
- 181 Aspects of Polyamine Biosynthesis in Normal and Malignant Eukaryotic Cells
H. G. Williams-Ashman, G. L. Coppoc, Amelia Schenone, and George Weber
- 199 Polyamine Disposition in the Central Nervous System
Solomon H. Snyder, Edward G. Shaskan, and Sami I. Harik
- 215 Specific Increases in Polyamines in Mixed Lymphocyte Reactions
Laurence J. Marton, Kenneth D. Graziano, Michael R. Mardiney, Jr., and Diane H. Russell
- 221 Changes in Polyamine Metabolism in Tumor Cells and Host Tissues During Tumor Growth and After Treatment with Various Anticancer Agents
Olle Heby and Diane H. Russell
- 239 The Effect of Growth Conditions on the Synthesis and Degradation of Ornithine Decarboxylase in Cultured Hepatoma Cells
Brigid L. M. Hogan, Susan Murden, and Anne Blackledge
- 249 Accumulation of Polyamines and Its Inhibition by Methyl Glyoxal Bis-(Guanyldrazone) During Lymphocyte Transformation
Robert H. Fillingame and David R. Morris
- 261 The *In Vivo* Chemical Stimulation of Hepatic Ornithine Decarboxylase Activity: Modifications of Activity at the Transcriptional and Post-Transcriptional Levels of Protein Synthesis
William T. Beck and E. S. Canellakis
- 277 Polyamines in Marine Invertebrates
Carol-Ann Manen and Diane H. Russell

- 289 The Stimulation of RNA Synthesis in Mature Amphibian Oocytes by the Micro-Injection of Putrescine
C. C. Wylie and Diane H. Russell
- 299 Interrelations of S-Adenosylmethionine and Polyamines in *Escherichia coli* K12
Ching-Hsiang Su and Seymour S. Cohen
- 307 Putrescine: A Sensitive Assay and Blockade of Its Synthesis by α -Hydrazino Ornithine
Sami I. Harik, Gavril W. Pasternak, and Solomon H. Snyder
- 323 Cation Requirement for RNA and DNA-Templated DNA Polymerase Activities of B-Type Oncogenic RNA Viruses (MuMTV)
Arnold S. Dion and Dan H. Moore
- 335 Structural and Biochemical Changes in the Nucleolus in Response to Polyamines
Eduard Gfeller, Carl C. Levy, and Diane H. Russell
- 343 The Determination of Polyamines in Urine by Gas-Liquid Chromatography
Charles W. Gehrke, Kenneth C. Kuo, Robert W. Zumwalt, and T. Phillip Waalkes
- 355 Gas Chromatography-Mass Spectrometry of Di- and Polyamines in Human Urine: Identification of Monoacetylspermidine as a Major Metabolic Product of Spermidine in a Patient with Acute Myelocytic Leukemia
Thomas Walle
- 367 Elevated Polyamine Levels in Serum and Urine of Cancer Patients: Detection by a Rapid Automated Technique Utilizing an Amino Acid Analyzer
Laurence J. Marton, James G. Vaughn, Inez A. Hawk, Carl C. Levy, and Diane H. Russell
- 373 Clinical Application of New Methods of Polyamine Analysis
M. Drue Denton, Helen S. Glazer, Thomas Walle, David C. Zellner, and Frank G. Smith

- 381 A Quantitative Model for Relating Tumor Cell Number to Polyamine Concentrations
Kwang B. Woo and Richard M. Simon
- 395 Polyamines—Potential Roles in the Diagnosis, Prognosis, and Therapy of Patients with Cancer
Stephen C. Schimpff, Carl C. Levy, Inez A. Hawk, and Diane H. Russell
- 405 Some Recent Observations on the Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Leukemia
Robert C. Gallo
- 415 Index

Polyamines in Growth— Normal and Neoplastic

Diane H. Russell

*Laboratory of Pharmacology, Baltimore Cancer Research Center,
National Cancer Institute, Baltimore, Maryland 21211*

It is indeed the fulfillment of much hope and effort that brings us together today. We are beginning a two-day program concerning polyamines, small organic cations which are prime candidates for many regulatory roles in the control of the growth process. This is a rather lofty position for the polyamines. These compounds are the most maligned of amines, as they bear names such as putrescine, spermidine, and spermine. These symbols immediately invoke two images, one being putrefaction, the other being male genital function. The early work on polyamines left many biochemists with the impressions that these cations were the end product of a degradative pathway and that the instances of polyamine occurrence in mammalian systems were keyed to bacterial decay or to excretion into seminal fluid. The early efforts of Celia and Herbert Tabor of the National Institutes of Health in elucidating the biosynthetic pathway in bacteria and the work of Seymour Cohen and his group in linking polyamine biosynthesis to RNA metabolism have provided the backbone for the expansion of polyamine research into the mammalian system (1, 2). This expansion was further catalyzed by an article by Dykstra and Herbst (3) expressing the relationship between spermidine synthesis and RNA synthesis in regenerating rat liver. Somehow the stigma of polyamines being involved in decay began to fall away as Dykstra and Herbst showed that the uptake of putrescine and its conversion into spermidine in partially hepatectomized rats was a major event. The large accumulation of spermine in seminal fluid has never been explained and remains one of the unanswered questions.

My own interest in polyamine research occurred during my collaboration with Dr. Solomon Snyder. We were intrigued by the role of histidine decarboxylase in the rapid growth process as postulated by Kahlson (4). In discussion, we postulated that if histidine decarboxylase, an enzyme which

forms histamine, a diamine, is important in rapid growth, this should be greatly enhanced in all rapid-growth systems. However, studies had indicated that this was not true. Could it be that histamine serves a function in certain rapidly growing tissues which could be served in other tissues by polyamines? Therefore, we looked at the first enzyme in the polyamine biosynthetic pathway in regenerating rat liver. In a pilot experiment, we assayed ornithine decarboxylase activity in the liver of sham-hepatectomized rats and in the liver of rats that had undergone a partial hepatectomy 24 hr prior to sacrifice. Results were rewarding. It appeared that ornithine decarboxylase activity was very low in the liver of normal rats: the counts were in the range of 200 to 400 cpm. However, the counts for the first 24-hr regenerating liver sample were around 20,000.

After finding this dramatic increase in ornithine decarboxylase activity in regenerating rat liver (5), which is rather unusual since mammalian enzymes usually fluctuate a few-fold and rarely 25-fold such as found for ornithine decarboxylase, I was astounded to find in the literature how widely polyamines had been implicated in cell regulation. The quotation that comes to mind appears in Seymour Cohen's book *The Introduction to the Polyamines* (2) at the beginning of Chapter 1: "All this has been said before—but since nobody listened, it must be said again" (André Gide). It seemed reasonable to suppose that early increases in ornithine decarboxylase activity which lead to such dramatic increases in the putrescine and spermidine pools in growing tissues had to be of great importance. First, polyamines had been implicated in growth processes. Herbst and his collaborators had found that certain bacterial mutants exhibit absolute requirements for polyamines (6). Further, polyamines were implicated by Seymour Cohen and others in the regulation of RNA synthesis (7, 8).

To summarize, then, we found that increased ornithine decarboxylase activity was one of the earliest, marked events that occurs after partial hepatectomy in the rat. Its increased activity appears to parallel the early increase in RNA synthesis, and precedes by many hours the maximal DNA synthesis that occurs in regenerating rat liver (9). We also found a close relationship between ornithine decarboxylase activity and the initiation of rapid growth in chick embryos and tumors (5).

The ability of ornithine decarboxylase activity to fluctuate rapidly in response to the introduction or withdrawal of stimuli suggests that putrescine synthesis is under strict modulation. The rapid turnover rate of hepatic ornithine decarboxylase is the most striking example of this modulation.

We found that ornithine decarboxylase activity declined rapidly in unoperated rats or in hepatectomized rats after cycloheximide administration. The decline had an estimated half-life of 11 min (10). To my knowledge, this

is the most rapidly turning over mammalian enzyme known. Further, estimating the half-life of ornithine decarboxylase after growth hormone stimulation and decline, without any inhibitors, led to a similar estimation of a half-life of less than 20 min (11). The only evidence lacking, of course, was evidence of the turnover rate of the purified enzyme. Since ornithine decarboxylase has not been purified to homogeneity, it was impossible to do studies on the purified enzyme. This rapid turnover rate is of great importance because the synthesis of most mammalian enzymes is a linear function of time, whereas enzyme degradation is an exponential function of time. Therefore, rates of change of enzyme levels from one steady state to another are determined solely by the degradation rate of the enzyme. The very high degradative rate of ornithine decarboxylase suggests that its activity changes rapidly in response to stimuli for new synthesis. Taken together, these data suggest that polyamine synthesis is a finely modulated process. Moreover, they suggest that this kind of sensitive regulation of synthesis would be necessary only to control the level of compounds important in the cell stimulatory system. This is of further importance when you consider that in most mammalian tissues there are not known enzymes that degrade or metabolize the polyamines. Therefore, an overproduction of the polyamines could lead to an elevated growth rate for a particular tissue or organ. This could be catastrophic in an adult mammal, since most of the tissues and organs are in dynamic equilibrium, and are not growing *per se*. In the mammalian organism, exceptions to this generalization are proliferating surfaces such as the gut, secreting organs such as the pancreas, and abnormal growths such as cancers (discussed in detail later).

I. HORMONAL REGULATION OF POLYAMINE BIOSYNTHESIS

If polyamine biosynthesis is necessary for growth processes to occur, it should be expected that this biosynthesis would be affected by hormones that regulate growth. Indeed, this is true. Castration in the rat results in a rapid decrease in both ornithine decarboxylase activity and S-adenosyl-L-methionine decarboxylase activity in the rat ventral prostate. When testosterone is administered to the castrated rat, there is a rapid increase in the activities of both ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase (12). In young rats, ornithine decarboxylase activity exhibits an early dramatic induction after growth hormone administration, followed later by a substantial increase in the level of S-adenosyl-L-methionine decarboxylase activity (11, 13, 14). *De novo* synthesis appears to be involved in the enzyme inductions since the administration of RNA and protein inhibitors suggest that both protein synthesis and DNA-dependent

RNA synthesis are necessary for these elevations to occur. Enhancements in the biosyntheses of putrescine, spermidine, and spermine can be shown also in the castrated rat uterus after estradiol administration (15–17). Further, it has been reported that cortisone has an effect on hepatic ornithine decarboxylase activity (18). The mammary gland, which is under strict hormonal control and which can be cycled through growth, lactation, and involution, exhibits intensive polyamine biosynthesis and accumulation during pregnancy and lactation, with the concentration of spermidine reaching levels above 5 mM during midlactation (19). If the number of suckling young is decreased by removing them from the mother, the amount of spermidine drops precipitously and is concomitant with dramatic drops in the amount of RNA present. To my knowledge, there are no growth processes that occur without prior stimulation of polyamine biosynthesis.

II. EMBRYONIC SYSTEMS

One of the compelling reasons to study polyamine biosynthesis and accumulation in embryonic systems is to assess polyamine metabolism in a maximally responding system. The other compelling reason, however, is to understand the growth process *per se*, which is best exemplified here. Further, the embryonic system, considered a normal growth system, most nearly parallels tumor systems. That is, “Resemblance of hepatoma to fetal tissues indicates some resemblance of all tumors to all fetal tissues, a general tendency that can be called the fetalism of tumors” (20). It appears that tumors and fetal systems resemble each other because both fetal tissues and tumors tend to be undifferentiated and therefore exhibit very similar enzyme patterns. Knox (20) states that “undifferentiated tumors are very similar to each other, as similar as some fetal tissues are to one another.” Organ-specific components and great diversity disappear in undifferentiated tumors, and are only present in the highly differentiated tumors which are rather rare. These concepts which stress the similarities of tumors and fetal systems are gaining more acceptance from the scientific community. A recent report indicated that several human tumors contain the fetal form of thymidine kinase in contrast to other human tissues which have only a postnatal thymidine kinase (21). Therefore, the finding that polyamine biosynthesis and accumulation is an early marked event in all types of embryos [chick (22, 23), toad (24, 25), rat (26), and sea urchin (27, 28)] has implications for the understanding and the control of the cancer process. The same rapid synthesis and accumulation of polyamines that is exhibited by embryos is also exhibited by tumors in early growth stages (29). An effective inhibitor of putrescine synthesis or spermidine synthesis would

appear to be an ideal cancer chemotherapeutic agent. If the levels of polyamines of a particular system could be lowered, it should decrease the viability of the tumor substantially.

We have screened a large number of analogues of ornithine *in vitro* for their ability to inhibit ornithine decarboxylase activity from 24-hr regenerating rat liver. Most of these analogues contained ring structures attached to the delta amino group. Only two were even moderately good inhibitors *in vitro*, α -methyl ornithine (Table 1) and *n*-methyl ornithine. At 10^{-3} M, *n*-methyl ornithine resulted in a 40% inhibition of ornithine decarboxylase. Neither of these analogues changed the ornithine decarboxylase activity during the course of L1210 leukemia in mice nor resulted in an increased survival rate for those leukemic mice receiving the drug(s).

TABLE 1. *Effect of an ornithine analogue on ornithine decarboxylase activity of 24-hr regenerating rat liver*

Inhibitor	Concentration of inhibitor (M)	% Inhibition
α -Methyl ornithine	0	0
	10^{-7}	5.3
	10^{-6}	13.3
	10^{-4}	40.5
	10^{-3}	64.6

III. PHYSIOLOGICAL SIGNIFICANCE OF POLYAMINES

It has been stated before that the accumulation of polyamines is concomitant with RNA synthesis and, of course, with protein synthesis. The correlation of polyamine synthesis with RNA synthesis is parallel in so many systems that it is hard to believe at this point that there is not a cause and effect relationship. Probably because of the tight relationship between RNA synthesis and DNA synthesis in certain systems, there appears at times to be a relationship between polyamine concentrations and DNA synthesis. However, there are systems in which you can uncouple RNA synthesis and DNA synthesis, such as in the heart undergoing hypertrophy after constriction of either the aortic or the pulmonary artery; in this case there is extensive polyamine accumulation which again correlates with RNA synthesis, but there is no concomitant DNA synthesis (30, 31).

The first clue that has come from our work as to one possible physiological role for the polyamines comes from work on developing *Xenopus laevis*. There is an anucleolate mutant of *X. laevis* which is unable to make riboso-