Metabolic Pathways

edited by
David M. Greenberg

Volume 4

nucleic acids,
protein synthesis, & coenzymes

3rd edition

Metabolic Pathways

THIRD EDITION

EDITED BY

David M. Greenberg

University of California
San Francisco Medical Center
San Francisco, California

VOLUME IV

Nucleic Acids, Protein Synthesis, and Coenzymes

ACADEMIC PRESS New York and London 1970

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

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
Berkeley Square House, London W1X 6BA

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 67-23160

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE

This volume concludes the survey of the knowledge available on the metabolic pathways of the major biological constituents of living organisms. In the sixteen years since publication of the first edition of this work, in 1954, the amount of knowledge has increased at a fantastic rate. There now remains only a few important biological compounds in which the details of biosynthesis and dissimilation are lacking. In addition to knowledge of the individual steps in each of the reaction sequences of the various metabolic pathways, most of the enzymes have now been identified and, in many instances, purified and characterized. In the last few years there has been a change in emphasis in the nature of the research on metabolism to the study of control and regulation.

Most of this volume is devoted to nucleic acids and protein synthesis. The development of knowledge of control and regulation depends to a large extent on the advances made in these subjects. Many important discoveries have been made on the nature of the genetic code, the mechanism of DNA and RNA syntheses, the fractionation of the several important types of ribonucleic acids, and their roles in initiating and controlling the various steps in the process of protein synthesis. Even so, these subjects are still in their infancy and very much remains to be learned. However, there is no lack of eager searchers, and this field now dominates the whole of biochemical research so that in a few years this too may become a well-mapped discipline in spite of its great complexity.

I must express my sincere thanks and appreciation to the numerous coauthors who have worked hard and devotedly with me in the preparation of the several editions of "Metabolic Pathways."

San Francisco, California
June, 1970

DAVID M. GREENBERG

List of Contributors

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

C. BAGLIONI, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts (277)

GENE M. BROWN, Division of Biochemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts (369, 383)

B. COLOMBO, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts (277)

T. W. GOODWIN, Department of Biochemistry, Johnston Laboratories, University of Liverpool, Liverpool, England (353)

STANDISH C. HARTMAN, Department of Chemistry, Boston University, Boston, Massachusetts (1)

SAUL KIT, Division of Biochemical Virology, Baylor University College of Medicine, Houston, Texas (69)

VICTOR W. RODWELL, Department of Biochemistry, Purdue University, Lafayette, Indiana (411)

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Purines and Pyrimidines

Standish C. Hartman

GLOSSARY

The following abbreviations are used:

PRPP	5-Phosphoribosyl l-pyrophosphate
PRA	5-Phosphoribosylamine
GAR	Glycinamide ribonucleotide

FGAR	Formylglycinamide ribonucleotide
FGAM	Formylglycinamidine ribonucleotide
AIRP	5-Aminoimidazole ribonucleotide
Carboxy-AIRP	5-Amino-4-imidazolecarboxylic acid ribonucleotide
AIC	4(5)-Amino-5(4)-imidazolecarboxamide
AICRP	5-Amino-4-imidazolecarboxamide ribonucleotide
Succino-AICRP	5-Amino-4-imidazole-*N*-succinocarboxamide ribonucleotide
Formyl-AICRP	5-Formamido-4-imidazolecarboxamide ribonucleotide
P_i	Inorganic orthophosphate
PP_i	Inorganic pyrophosphate
FH_4	5,6,7,8-Tetrahydrofolic acid

Other abbreviations follow the conventions of the *Journal of Biological Chemistry*.

The following are the proper chemical and corresponding trivial names, respectively, used for the intermediates of purine biosynthesis:

2-Amino-*N*-ribosylacetamide 5′-phosphate	Glycinamide ribonucleotide
2-Formamido-*N*-ribosyl acetamide 5′-phosphate	Formylglycinamide ribonucleotide
2-Formamido-*N*-ribosylacetamidine 5′-phosphate	Formylglycinamidine ribonucleotide
5-Amino-1-ribosylimidazole 5′-phosphate	5-Aminoimidazole ribonucleotide
5-Amino-1-ribosyl-4-imidazolecarboxylic acid 5′-phosphate	5-Amino-4-imidazolecarboxylic acid ribonucleotide
N-(5-Amino-1-ribosyl-4-imidazolylcarbonyl)-L-aspartic acid 5′-phosphate	5-Amino-4-imidazole-*N*-succinocarboxamide ribonucleotide
4(5)-Amino-5(4)-imidazolecarboxamide	5-Amino-4-imidazolecarboxamide

5-Amino-1-ribosyl-4-imidazolecarboxamide 5′-phosphate	5-Amino-4-imidazolecarboxamide ribonucleotide
5-Formamido-1-ribosyl-4-imidazolecarboxamide 5′-phosphate	5-Formamido-4-imidazolecarboxamide ribonucleotide
Inosine 5′-phosphate	Inosinic acid
Adenosine 5′-phosphate	Adenylic acid
Xanthosine 5′-phosphate	Xanthylic acid
Guanosine 5′-phosphate	Guanylic acid

I. INTRODUCTION

As components of all biological systems, purines and pyrimidines have drawn the attention of biological chemists for nearly 200 years, beginning with the discovery of uric acid by Scheele (*1*), through the isolation of nucleic acids by Miescher (*2*) and Kossel (*3*), the recognition of nucleotides as coenzymes and metabolic intermediates, and leading to our present knowledge of the genetic and chemical properties of nucleic acids and the role of nucleotides in metabolic regulation. A natural part of the interest in these compounds has been concerned with their metabolism–biosynthesis, degradation, and interrelationships with other cellular processes. These studies have in large part achieved their present success through application of the powerful techniques of modern biochemistry: isolation of discrete enzyme systems, use of isotopic tracers, chromatography, and biochemical genetics. When the chapter on this topic was prepared for the previous edition of *Metabolic Pathways*, the individual steps in the synthesis and degradation of purines and pyrimidines compounds had been established. Other summaries have appeared describing techniques for the isolation and assay of the intermediates and enzymes involved (*4*). Recent developments in this area of metabolism have centered about questions of metabolic control and enzymology. This chapter will reflect the emphasis on these topics.

II. BIOSYNTHESIS OF INOSINIC ACID

The principal naturally occurring purines are shown in Fig. 1. Although the two substances, adenine and guanine, of this group

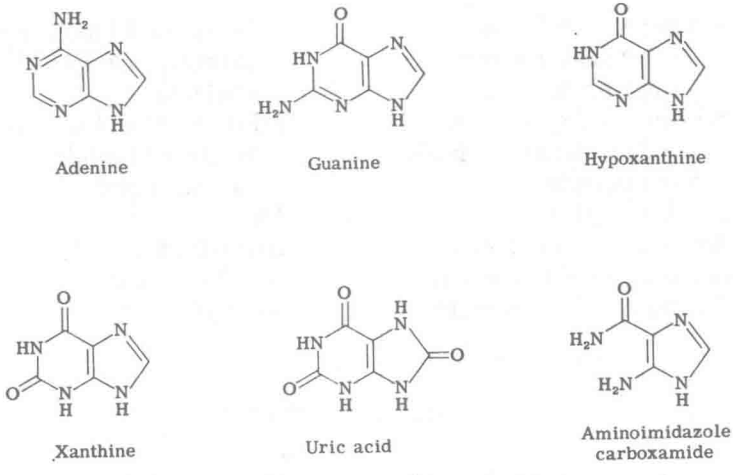

FIG. 1. Structures of important purines and related compounds.

characteristically found in nucleic acids are often of primary interest, systems which produce another purine, uric acid, have been of particular value in studies on this biosynthetic pathway. Birds, especially, have been useful objects of study because of the quantitative significance of this pathway as the primary means for eliminating excess dietary nitrogen in the form of uric acid. The early studies of Krebs and co-workers (*5,6*) demonstrated that pigeon liver slices accumulate a purine precursor of uric acid which they identified as hypoxanthine. These experiments revealed both the organ system of importance and the nature of the principal end product. Further progress in this area awaited the development and application of isotope tracer methodology in order to establish the metabolic origins of the various atoms of the purine ring system.

A. Precursors of the Purine Ring System

Studies on the incorporation of various labeled precursors into the purine ring system initially involved administration of tracers to intact animals, isolation of uric acid, and degradation of this product chemically to component parts. Thus, Shemin and Rittenberg (7) showed that the amino group of glycine was a direct precursor of the N-7 of uric acid in man (see Fig. 2). After feeding ^{13}C-labeled compounds to pigeons and analyzing the excreted uric acid, Buchanan's group concluded that formate served as the origin of C-2 and C-8, that carbon dioxide labeled position 6, and that glycine entered positions 4, 5, and 7 as an intact molecule (*8,9*). The latter conclusion was strengthened by the finding of

Karlsson and Barker (*10*) that the α-carbon of glycine labeled position 5 of uric acid. The observation that formate could act in a biosynthetic capacity was a significant first step in the recognition of the metabolism of C_1 compounds, and it led directly, together with other observations, to the identification of the function of folate compounds in biochemistry. The α-carbon of glycine (*11,12*) and the β-carbon of serine (*12,13*) were also able to provide C-2 and C-8 of the purine ring by reactions involving derivatives of tetrahydrofolic acid which are now well understood (*14*).

While earlier studies had shown that ^{15}N-labeled ammonium ions (*15*) and aspartic acid (*16*) were incorporated into uric acid *in vivo*, the likelihood of randomization of the ^{15}N throughout the metabolic nitrogen pool made it impossible to establish the immediate sources of the remaining nitrogen atoms by these methods. Identification of the origins of atoms 1, 3, and 9 required the development of a cell-free system competent to carry out the whole biosynthetic sequence. Greenberg soon found that homogenates of pigeon liver could effect purine synthesis *de novo* (*17*). Soluble enzyme fractions from pigeon liver and ethanol precipitates thereof were also found to possess all of the requisite enzymes (*18,19*). With such preparations and with the use of ^{15}N-labeled substrates, the α-amino group of aspartic acid was found to contribute N-1 of the purine ring (*20*), while the amide nitrogen of glutamine was shown to be the donor of both positions 3 and 9 (*19*).

The origins of each of the positions of the purine ring are summarized in Fig. 2. It should be pointed out that these assignments were fully corroborated by subsequent studies on the synthetic pathway, described below. Observations from many laboratories have established that the heterocyclic system of the nucleic acid purines, adenine and guanine, is derived from the same key precursors as is the case for the excretory

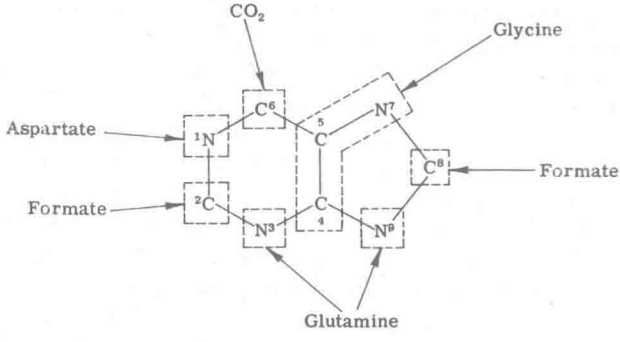

FIG. 2. Precursors of the purine ring system.

purine, uric acid, facts providing presumptive evidence for a common route of synthesis. Thus, in the rat (*21*), in yeast (*22*), and in bacteria (*23*), the incorporation of labeled precursors into adenine and guanine compounds occurs as anticipated according to Fig. 2, and, in fact, the uptake of ^{14}C-glycine or ^{14}C-formate into purines has been the conventional method for assessing synthesis *de novo* of these compounds. One possible exception to this generalization, treated below, is that ammonia, rather than glutamine, may provide N-9 in certain circumstances.

B. General Features of the Synthetic Pathway

1. Importance of Phosphoribosyl Intermediates

The availability of soluble enzyme preparations from pigeon liver capable of effecting the rapid synthesis of purines from small molecule precursors soon led to clarification of certain key features of the pathway. Of primary importance were the results of Greenberg (*24*), and subsequently, of Schulman and Buchanan (*25*), showing that the initial purine compound formed by such systems was neither hypoxanthine nor its riboside, inosine, but rather was the nucleotide inosinic acid (IMP). The possibility that intermediates in the pathway were 5-phosphoribosyl derivatives was thus clearly indicated. Furthermore, the direct derivation of the metabolically functional nucleotide forms of adenine and guanine, i.e., AMP and GMP, from IMP appeared highly likely.

When ^{14}C-glycine or formate and a complete system of reactants, including ribose 5-phosphate, glutamine, aspartate, bicarbonate, and an ATP-regenerating source were incubated with soluble enzyme fractions from pigeon liver, maximal rates of incorporation of precursors into IMP were observed (*24,26*). A major contribution was made by Goldthwait, Peabody, and Greenberg, who isolated glycinamide ribonucleotide (GAR) and formylglycinamide ribonucleotide (FGAR) from reaction mixtures such as this, but from which the bicarbonate and aspartate were omitted (*27,28*). The identities of early intermediates in the pathway were thus established for the first time and, significantly, these compounds were phosphoribosyl derivatives. The agent contributing the phosphoribosyl unit in these reactions was soon found to be phosphoribosyl pyrophosphate (*29,30*), a compound previously identified as a participant in the direct formation of pyrimidine and purine nucleotides from the respective bases (see Section III). Recognition of other intermediates in the pathway leading to formation of IMP was aided by

fractionation of the crude enzyme system and by the use of isolated intermediates, such as FGAR, as substrates for further enzymic conversions.

2. PURINES AND C_1 METABOLISM

An interesting facet in the development of this topic centers around the compound, 5-amino-4-imidazolecarboxamide (AIC), shown in Fig. 1. This substance was initially detected as a diazotizable amine in cultures of *Escherichia coli* poisoned with sulfonamides (*31*), and its structure was determined by Shive *et al.* (*32*). Its structural relationship to hypoxanthine is evident; AIC lacks only C-2 to complete the bicyclic ring system. The antifolate compound, aminopterin, also causes excretion of AIC in animals (*33*) as well as in bacteria (*33,34*). The fact that purines can partially overcome the inhibition of bacterial growth produced by sulfonamides provides a substantive connection between AIC and purine synthesis (*35*), but of particular significance was the observation of Gots that a purine-requiring mutant of *E. coli* excreted substantial quantities of this amine and its riboside (*36*). [The nature of another link in the chain of intermediates leading to purines was indicated by the identification of aminoimidazole riboside in the culture medium of another purine auxotroph of *E. coli* (*37*).] *In vivo*, ^{14}C-AIC is converted to purines by pigeons (*38*), rats (*39*), yeast (*40*), and isolated erythrocytes (*41*). It became apparent from isotope dilution studies in pigeon liver homogenates that AIC was not on the main path of purine synthesis from glycine. Rather, it seemed likely that the ribonucleotide form of the amine (AICRP) was the more immediate precursor of IMP (*38*), an expectation borne out when this substance became available via condensation of the base with PRPP (*42*). An initial indication of the direct relationship between AICRP and IMP was the observation that pigeon liver extracts catalyze an exchange between the 2 position of IMP and ^{14}C-formate (*43*). This process could be visualized as the opening of the purine ring to release formate and the imidazole derivative, the reverse of which would comprise the terminal steps in the synthesis *de novo* of IMP. The observation that 5-formyltetrahydrofolate significantly stimulated this exchange reaction provided the first experimental demonstration of the proposed action of folate compounds as transformylation cofactors.

Recognition of the direct action of tetrahydrofolate in the terminal steps of purine synthesis provided the rationale for the accumulation of AIC and its derivatives in states of folate deficiency induced mutationally, nutritionally, or by drugs. What is not so obvious is how the imida-