Advances in
ENZYME REGULATION

Volume 14

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Proceedings of the Fourteenth Symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Tissues held at Indiana University School of Medicine Indianapolis, Indiana October 6 and 7, 1975

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FOREWORD

Advances in Enzyme Regulation is now in its fourteenth volume. The appreciative reception of this series reflected the need for such a source of information, inspiration, and laboratory and teaching companion.

Volume 14 concentrates on subjects which have reached the stage of productive summarization and critical evaluation in the light of extensive new results. This book also lives up to its goal of advancing a few steps ahead of the general front of mammalian enzyme regulation studies.

It has been my editorial policy to impose as few restrictions as possible, emphasizing, however, the objectives of excellence of contribution, perfection in presentation, and penetration and scope in interpretation. This principle gives a wide range of freedom to the participants to express their concepts. Thus, the responsibility for detail – accuracy of reporting, preciseness of references, allocations of priority, expressions of judgment and evaluation – lies with the individual authors.

The Editor, who enjoyed the advice of leaders in the field, has been organizing the Symposia and selecting new topics and speakers on the basis of immediate and long-range significance of the scientific contributions. It is hoped that the comments and suggestions of investigators and teachers in this field will continue to come to the Editor's office and contribute to shaping the course of forthcoming conferences and volumes.

Indiana University 1975 GEORGE WEBER, *Editor*

ACKNOWLEDGEMENTS

This is the fourteenth in a series of Symposia dedicated to problems and advances in the regulation of enzyme activity and synthesis in mammalian systems.

I take great pleasure in expressing appreciation for the support and assistance I received in organizing and conducting this Conference. I wish gratefully to acknowledge that Indiana University School of Medicine, Burroughs Wellcome and Co., Hoffman–LaRoche, Eli Lilly and Co., The Squibb Institute for Medical Research, The Upjohn Co., and U.S.V. Pharmaceutical Corporation provided the financial support for this Meeting.

In the planning of the program, selection of the participants and arrangements for the Symposium the advice of the following was invaluable: Donald S. Coffey (U.S.A.), Benno Hess (Germany), Helmut Holzer (Germany), Frank M. Huennekens (U.S.A.), Nobuhiko Katunuma (Japan), Yoshiaki Miura (Japan), S. E. Severin (U.S.S.R.), Paul Talalay (U.S.A.), Sidney Weinhouse (U.S.A.), and William J. Whelan (U.S.A.).

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At Indiana University School of Medicine in the local organization of the Symposium I had the kind assistance of Dean Steven C. Beering. The efficient and competent help of David M. Paul in accommodation arrangements and the expert assistance of Carol Eitzen in the preparation of illustrations are very much appreciated.

Thanks are due to members of my secretarial staff, Maureen H. Senour, LaVerne Mulvey, and Charlotte M. Oda, who assisted in local arrangements and in the typing of the manuscripts.

My highest appreciation is due to my wife, Catherine E. Forrest Weber, whose contribution to the format and English style has been most valuable in the assembling of this volume.

George Weber
Symposium Chairman

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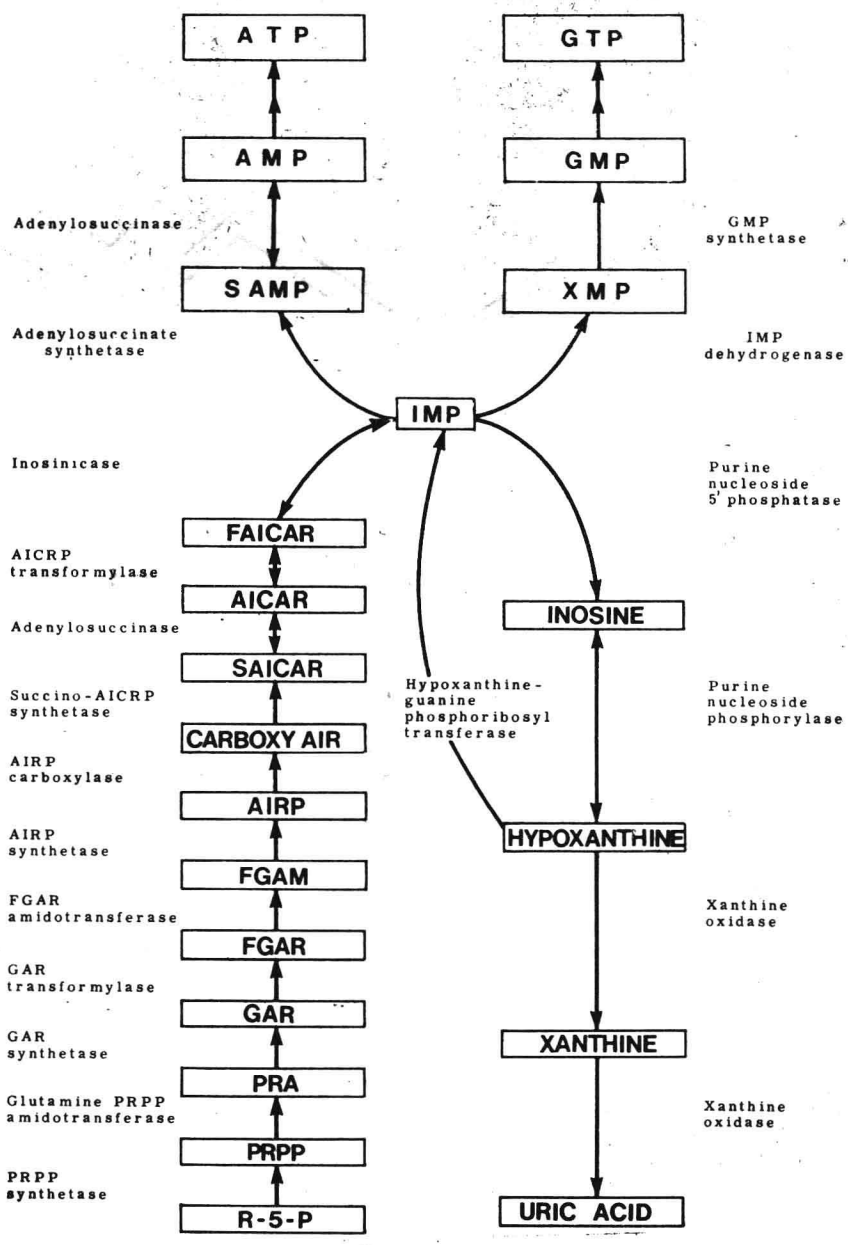
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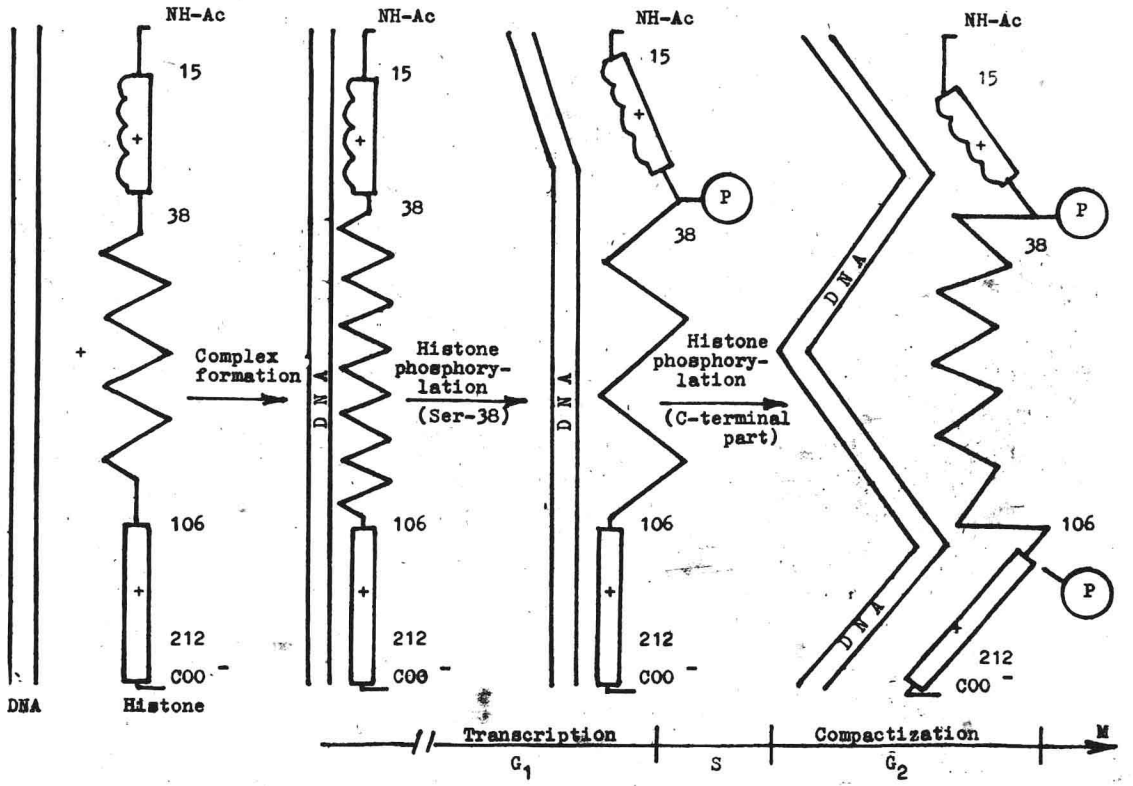
Adenylosuccinase
GMP synthetase
SAMP
XMP
Adenylosuccinate synthetase
IMP dehydrogenase
IMP
Inosinicase
Purine nucleoside 5' phosphatase
FAICAR
AICRP transformylase
AICAR
INOSINE
Adenylosuccinase
SAICAR
Hypoxanthine-guanine phosphoribosyl transferase
Purine nucleoside phosphorylase
Succino-AICRP synthetase
CARBOXY AIR
AIRP carboxylase
AIRP
HYPOXANTHINE
AIRP synthetase
FGAM
Xanthine oxidase
FGAR amidotransferase
FGAR
GAR transformylase
GAR
XANTHINE
GAR synthetase
PRA
Glutamine PRPP amidotransferase
Xanthine oxidase
PRPP
PRPP synthetase
R-5-P
URIC ACID


NH-Ac
15
38
106
212
COO -
DNA
Histone
Complex formation
DNA
Histone phosphory-lation (Ser-38)
P
Histone phosphory-lation (C-terminal part)
Transcription
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G1
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SESSION I

REGULATION OF PURINE METABOLISM

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KEY ENZYMES OF IMP METABOLISM: TRANSFORMATION- AND PROLIFERATION-LINKED ALTERATIONS IN GENE EXPRESSION

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INTRODUCTION

Previous work in this Laboratory elucidated the pattern of enzymatic and biochemical imbalance in a series of hepatomas exhibiting vastly different growth rates. These studies are part of a program designed to provide solutions for the following questions. Is there an ordered pattern of metabolic alterations in neoplastic cells? If there is a definite pattern, can a linking with the neoplastic transformation and with the growth rate and malignancy be demonstrated? What are the principles that characterize the transformation- and malignant-progression linked alterations in the biochemical aspects of gene expression?

From these studies the following answers have emerged. In the biological model system that provides tumors of different malignancy, both transformation-linked and progression-linked alterations can be pinpointed. The transformation-linked alterations occur in all tumors, whereas the progression-linked alterations parallel the growth rate and malignancy of the different tumor lines.

The identification and elucidation of the linking with malignancy of the biochemical alterations was made possible by introduction of the concept of key enzymes as indicators of alterations in gene expression through which modulation of reprogramming of gene expression is governed. A systematic analysis that evaluated the behavior of opposing pathways and key enzymes and their ratios led to the discovery that there was an ordered pattern in neoplastic cells that exhibited a transformation- and progression-linked metabolic imbalance. Previous studies elucidated the main principles of cancer enzymology and biochemistry on the basis of studies in carbohydrate, pyrimidine, ornithine and membrane cAMP metabolism. Current work in this Laboratory was designed to test the applicability of these approaches and concepts to purine metabolism. For this reason a careful evaluation of purine metabolism was made which considered this metabolic area in the following fashion. For the purpose of

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identifying and selecting the enzymes and opposing and competing metabolic pathways, purine metabolism was viewed as consisting of four main areas of metabolic pattern: (1) the synthetic pathways of IMP* that include the *de novo* and recycling reactions; (2) the catabolic pathways of IMP that oppose the synthetic pathways and in man end in uric acid and in the rat in allantoin; (3) the synthetic utilization of IMP leading to biosynthesis of GMP, GDP and GTP; (4) the synthetic utilization of IMP channeling into the production of AMP, ADP and ATP. A schematic summary of the overall reactions and the enzymes involved is provided in Figure 1. The series of synthetic reactions is under a delicate feedback balance exerted by the end products of these pathways.

Purine biosynthetic steps provide important targets in the design of chemotherapeutic weapons against neoplastic diseases. Because of theoretical and practical interest a systematic program was started to gain insight into the pattern of reprogramming of gene expression as manifested in this pathway in normal, differentiating, proliferating and neoplastic tissues.

To gain information on the pattern of gene expression enzymes were chosen for study that appeared to be keys to an understanding of the anabolism and catabolism of IMP and its synthetic utilization. For this reason glutamine PRPP amidotransferase, the first enzyme committed to *de novo* purine biosynthesis, and xanthine oxidase, the rate-limiting enzyme of IMP degradation, were selected as possible indicators of the capacity for IMP synthesis and degradation. Uricase, that governs the final step in purine degradation in the rat, was also determined. The enzymes directly involved in IMP utilization, IMP dehydrogenase that produces XMP and adenylosuccinate synthase that leads to S-AMP, were examined as possible indicators of the potential for synthetic utilization of IMP. In addition, the subsequent two enzymes, GMP synthetase that produces GMP and adenylosuccinase that yields AMP, were studied.

MATERIALS AND METHODS

Biological Systems

Inbred strains of male Buffalo and ACI/N rats of 180–300 g of weight and male Wistar rats of different age groups were maintained in individual cages with Purina Laboratory Chow and water available *ad libitum* unless stated otherwise.

*The following abbreviations are used in this paper: amidotransferase, glutamine PRPP amidotransferase (amidophosphoribosyltransferase), EC 2.4.2.14; G-6-P DH, glucose-6-phosphate dehydrogenase, EC 1.1.1.49; IMP dehydrogenase, inosinic acid (inosine 5′-phosphate) dehydrogenase, EC 1.2.1.14; SAMP synth, adenylosuccinate synthetase, EC 6.3.4.4; TA, transaldolase, EC 2.2.1.2; UDP kinase, (nucleosidediphosphate kinase); ATP:UDP phosphotransferase, EC 2.7.4.6.