Advances in ENZYME REGULATION

Volume 3

GEORGE WEBER

Advances in
ENZYME REGULATION

Volume 3

Proceedings of the third symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Tissues held at Indiana University School of Medicine Indianapolis, Indiana October 5 and 6, 1964

Edited by
GEORGE WEBER

Indiana University School of Medicine
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Technical editor
Catherine E. Forrest Weber

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FOREWORD

Advances in Enzyme Regulation is now in its third volume. The appreciative reception of this new series reflected the need for such a source of information and inspiration.

Volume 3 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 and presents sections on unusual and unexpected aspects of blockers of enzyme synthesis and new concepts of oscillatory behavior in enzymatic control processes. With the maturing process in the development of insight into mechanisms of enzyme control, the Editor felt the time ripe for stimulating, provocative and authoritative reviews on key aspects of mammalian enzyme regulation. The review, Regulation of enzymes in nitrogen metabolism, was invited with this aim in mind and other reviews are planned for subsequent volumes.

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 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 GEORGE WEBER, *Editor*
1965

ACKNOWLEDGMENTS

THIS is the third in a series of Symposia dedicated entirely to problems and advances in 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 to gratefully acknowledge that Indiana University School of Medicine, the Damon Runyon Memorial Fund Inc., the American Cancer Society Institutional Fund, Burroughs Wellcome and Co., Merck Sharp & Dohme, and The Upjohn Company provided the financial support for this Meeting.

In the planning of the program, selection of participants and arrangements for the Symposium the advice of the following was invaluable: J. Ashmore, H. N. Christensen, C. F. Cori, O. Greengard, W. E. Knox, Sir H. A. Krebs, H. P. Morris, V. R. Potter and S. Weinhouse.

I am very obliged to Drs. Cori, Hastings, Lardy, Potter, Reich, Villee, and Weinhouse for serving as chairmen of the sessions, and to all contributing authors for their cooperation in the preparation of this volume.

At Indiana University School of Medicine in the local organization of the Symposium I had the kind assistance of Deans J. I. Nurnberger, A. D. Lautzenheiser and Doris H. Merritt. The efficient and competent help of R. Dault and J. P. Schall in accommodation arrangements and the expert assistance of J. Glore in the preparation of illustrations are much appreciated.

Thanks are due to Delores Cameron, Judy Hoeping, Freida Jones and Nancy B. Stamm, members of my staff who assisted in the local arrangements and in the typing of the manuscripts.

My highest appreciation is due to my wife, Catherine E. Forrest Weber, whose contribution in the role of Technical Editor was invaluable in the assembling of this volume.

GEORGE WEBER
Symposium Chairman

LIST OF PARTICIPANTS

JAMES ASHMORE, PH.D.
Department of Pharmacology
Indiana University School of Medicine
Indianapolis, Indiana

GEORGE E. BOXER, M.D., PH.D.
Merck Sharp & Dohme
Research Laboratories
Rahway, New Jersey

HELEN B. BURCH, PH.D.
Department of Pharmacology
Washington University
School of Medicine
St. Louis, Missouri

GEORGE F. CAHILL, JR., M.D.
Baker Clinic Research Laboratory
Harvard Medical School
Boston, Massachusetts

HALVOR N. CHRISTENSEN, M.D.
Department of Biological Chemistry
The University of Michigan
Medical School
Ann Arbor, Michigan

CARL F. CORI, M.D.
Department of Biological Chemistry
Washington University
School of Medicine
St. Louis, Missouri

ALBERT B. EISENSTEIN, M.D.
Washington University
School of Medicine and
Department of Medicine
Jewish Hospital of St. Louis
St. Louis, Missouri

EMMANUEL FARBER, M.D., PH.D.
Department of Pathology
University of Pittsburgh
School of Medicine
Pittsburgh, Pennsylvania

MURIEL FEIGELSON, PH.D.
Institute of Cancer Research
College of Physicians and Surgeons
Columbia University
New York, New York

BRIAN C. GOODWIN, PH.D.
Institute of Animal Genetics
University of Edinburgh
Edinburgh, Scotland

OLGA GREENGARD, PH.D.
Institute for Muscle Disease, Inc.
New York, New York

A. BAIRD HASTINGS, PH.D.
Scripps Clinic and Research
Foundation
La Jolla, California

ROBERT C. HAYNES, JR., M.D., PH.D.
Department of Pharmacology
Western Reserve University
Cleveland, Ohio

ERNST HELMREICH, M.D.
Department of Biological Chemistry
Washington University
School of Medicine
St. Louis, Missouri

TORE HULTIN, PH.D.
University of Stockholm
The Wenner-Gren Institute
Stockholm Va., Sweden

FRANCIS T. KENNEY, PH.D.
Biology Division
Oak Ridge National Laboratories
Oak Ridge, Tennessee

W. EUGENE KNOX, M.D.
Cancer Research Institute
New England Deaconess Hospital
Harvard Medical School
Boston, Massachusetts

HENRY A. LARDY, PH.D.
Institute for Enzyme Research
The University of Wisconsin
Madison, Wisconsin

ROBERT L. MCGEACHIN, PH.D.
Department of Biochemistry
University of Louisville
School of Medicine
Louisville, Kentucky

FLORENCE MOOG, PH.D.
Department of Zoology
Washington University
St. Louis, Missouri

HAROLD P. MORRIS, PH.D.
Nutrition & Carcinogenesis Section
Laboratory of Biochemistry
National Cancer Institute
Bethesda, Maryland

CHARLES A. NICHOL, PH.D.
Department of Experimental Therapeutics
Roswell Park Memorial Institute
Buffalo, New York

SHOSAKU NUMA, M.D.
Max-Planck-Institut für Zellchemie
Munich, Germany

HENRY C. PITOT, M.D., PH.D.
Department of Oncology
McArdle Memorial Laboratory
University of Wisconsin
The Medical School
Madison, Wisconsin

VAN R. POTTER, PH.D.
Department of Oncology
McArdle Memorial Laboratory
University of Wisconsin
The Medical School
Madison, Wisconsin

EDWARD REICH, M.D., PH.D.
Rockefeller Institute
New York, New York

JAY S. ROTH, PH.D.
Department of Biochemistry
The University of Connecticut
Storrs, Connecticut

HAROLD L. SEGAL, PH.D.
Department of Biology
State University of New York
Buffalo, New York

CLAUDE A. VILLEE, M.D.
Department of Biological Chemistry
Harvard Medical School
Boston, Massachusetts

DERYCK G. WALKER, PH.D.
Department of Biochemistry
University of Birmingham
Birmingham, England

GEORGE WEBER, M.D.
Department of Pharmacology
Indiana University School of Medicine
Indianapolis, Indiana

SIDNEY WEINHOUSE, PH.D.
Fels Research Institute
Temple University School of Medicine
Philadelphia, Pennsylvania

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RNA SYNTHESIS AND ENZYME INDUCTION BY HYDROCORTISONE*

F. T. KENNEY, D. L. GREENMAN,† W. D. WICKS,‡ and W. L. ALBRITTON§

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

INTRODUCTION

ENZYME induction in liver—when induction is defined as an increase in enzyme level—can be brought about by a variety of mechanisms, as is amply demonstrated elsewhere in this and in previous volumes of the *Advances in Enzyme Regulation* series. In some instances of induction, the mechanism whereby an enzyme level is increased is known, and such is the case in the inductions initiated by glucocorticoid hormones. Inductions of tyrosine transaminase,[1] glutamic-alanine transaminase,[2] and tryptophan pyrrolase[3,4] by glucocorticoid hormones have now been conclusively demonstrated to be due to an increased rate of enzyme synthesis. Thus analysis of the mechanism of hormonal induction can, in these instances, properly advance to the question: *How* do adrenal steroids increase the rate of synthesis of specific proteins? This question has led us to an analysis of hormonal effects on RNA metabolism, and the results of our investigations in this area are described below. These experiments have led, in turn, to a rephrasing of the question asked above, which we now choose to pose as: *Do* adrenal steroids increase the rate of synthesis of *specific* proteins?

RESULTS AND DISCUSSION

Any consideration of the possible mechanisms by which steroid hormones may influence hepatic enzyme synthesis must take into account the rapidity with which these hormones are removed from the liver. In Fig. 1, data are presented which demonstrate that hydrocortisone-induced enzyme synthesis reaches its maximal rate only after more than 90 per cent of an administered

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† National Cancer Institute Postdoctoral Fellow (Grant No. 6f2-CA-16, 375-01A1). Present address: McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland.

‡ American Cancer Society Postdoctoral Fellow.

§ Present address: University of Alabama Medical School, Birmingham, Alabama.

dose of ^{14}C-labeled steroid has been removed from the liver. While the possibility that the residual steroid may represent a particularly active form cannot be excluded, this result suggests that the primary hormonal effect involves cellular events which precede the actual formation of the polypeptide enzyme. Attention is thereby directed towards the nucleic acid components thought to be regulating enzyme synthesis.

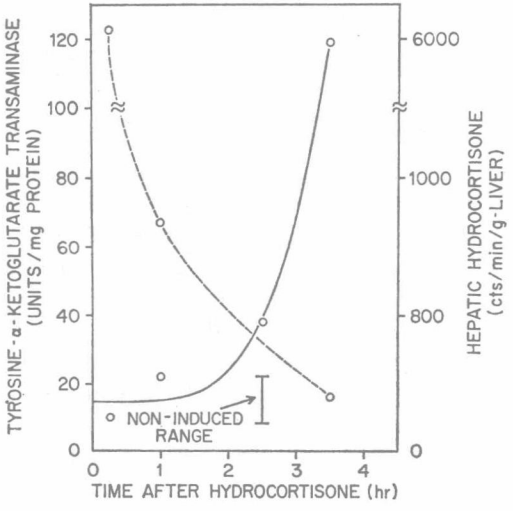

FIG. 1

Enzyme induction and retention of hydrocortisone.
Adapted from Kenney and Flora.[16]

Feigelson, Gross and Feigelson found that the turnover of RNA was increased in all the subcellular fractions of liver, following a single dose of cortisone.[5] By employing brief periods of exposure to ^{32}P ("pulse-labeling"), we later found[6] that the steroid effect is actually limited to nuclear RNA synthesis, which is dramatically increased by the hormone, while synthesis of cytoplasmic RNA is unchanged (Fig. 2). Increased synthesis of nuclear RNA precedes accumulation of the induced transaminase, a result kinetically consistent with the conclusion that the primary hormonal effect is on nuclear RNA synthesis, with the hormonally induced increase in this parameter resulting, in turn, in increased enzyme synthesis. Also consistent with this conclusion is the demonstration by Greengard, Smith and Acs[7] that actinomycin treatment prevents the hormonal elevation of transaminase activity, thus implicating DNA-directed RNA synthesis in this induction. When longer periods of exposure to ^{32}P were employed, a hormonal effect on cytoplasmic RNA labeling becomes apparent (Fig. 3), in agreement with the results of Feigelson *et al.*[5] The sequence of events following hormone

administration thus appears to be: (1) an increase in the rate of nuclear RNA synthesis, (2) passage of the newly synthesized RNA into the cytoplasm, and (3) utilization of this RNA in enzyme synthesis.

What is the *functional* nature of the RNA synthesized in response to hydrocortisone? In attempting to answer this question we were fortunate that

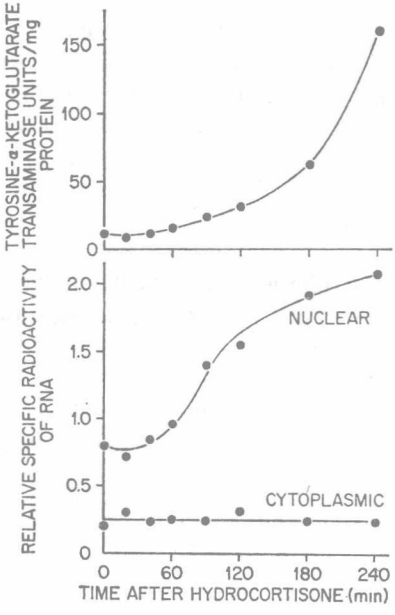

FIG. 2

Rates of synthesis of nuclear and cytoplasmic RNA during enzyme induction. From Kenney and Kull.[6]

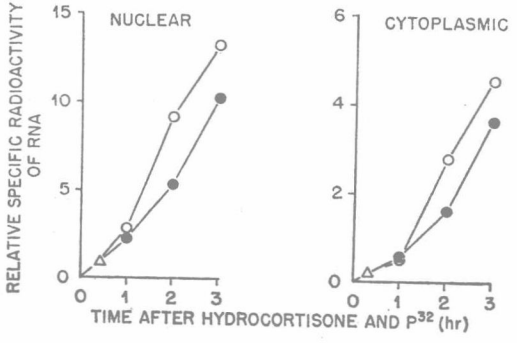

FIG. 3

Hydrocortisone effects on long-term ^{32}P-labeling of RNA. From Kenney and Kull.[6]

research in several laboratories has been directed toward learning more of the nature of the rapidly labeled RNA of animal tissues and cells. From these studies it is clear that the bulk of the rapidly labeled RNA is made up of two kinds of RNA. The first of these (pRNA†) is nucleolar in origin,[8] is of high molecular weight,[8–11] has a base composition like that of rRNA,[11] and under appropriate experimental conditions can be shown to move into the typical 28 and 18S (Svedberg units) components of rRNA.[8, 9, 11] These characteristics identify pRNA as a precursor form of rRNA. In addition to pRNA there is a second component (dRNA) which is synthesized in non-nucleolar regions of the nucleus,[8] is quite heterogeneous in size,[8–12] has a base composition like the cell DNA,[11] and which preferentially hybridizes with DNA.[13] These characteristics identify this RNA as dRNA, and it is possible that this is the cellular mRNA. However, positive identification of an RNA as mRNA must involve demonstration of messenger activity; i.e. the capacity to code for the synthesis of a particular polypeptide chain must be shown. While there have been numerous demonstrations of capacity to stimulate amino acid incorporation into protein in cell-free systems, there is as yet no firm proof of ability to code for the synthesis of a particular protein. Hence for the moment a positive functional designation of this RNA cannot be made, and we therefore prefer the operational designation dRNA.

TABLE 1

^{32}P Base Composition of Pulse-labeled Liver Nuclear RNA

Time after hydrocortisone (hr)	Per cent of total radioactivity				Ratio A + U/G + C
	CMP	GMP	AMP	UMP	
0	27	26	22	25	0.89
1	27	27	21	25	0.85
2	27	25	23	25	0.92
			Bulk liver RNA		0.63
			Liver DNA		1.34

Analysis of the base composition of the rapidly labeled nuclear RNA, and the finding that hormonal stimulation failed to alter this composition (Table 1), provided the first indication that synthesis of more than one kind of RNA is involved in hormonal enzyme induction. Composition of the newly synthesized RNA from the nuclei of adrenalectomized animals is like that of the total

† Abbreviations employed are: pRNA, nuclear precursor to ribosomal RNA; dRNA, DNA-like RNA; mRNA, messenger RNA; rRNA, ribosomal RNA; and tRNA, transfer RNA.

(unlabeled) nuclear RNA, and is indicative of a mixture of pRNA and dRNA. This result is in accord with the studies discussed above. Treatment with hydrocortisone failed to alter this composition, although the hormone effected a two- to threefold increase in the rate of labeling of RNA. We infer, then, that the hormonally induced RNA must be, like that synthesized in the absence of adrenal steroid, a mixture of pRNA and dRNA.

TABLE 2

Fractionation* of Pulse-labeled Nuclear RNA

Fraction no.	Extraction conditions	Per cent of total ^{32}P	Sedimentation peak of ^{32}P-RNA	Composition of ^{32}P-RNA A + U/G + C	Type of RNA
1	4°	6	4S	0.69	33 tRNA 67 ?
2	45°	25	~10S, disperse	0.72	90 pRNA 10 dRNA
3	65°	40	~20S, disperse	0.93	70 pRNA 30 dRNA
4	85° + SDS	28	~30S, disperse	1.09	40 pRNA 60 dRNA

* Modified from Georgiev *et al.*[11]

Direct evidence for this conclusion was sought, employing a slight modification of the thermal fractionation procedure introduced by Georgiev and his collaborators.[11] By this technique the ^{32}P-labeled nuclear RNA was separated into four fractions, containing RNA species differing in base composition and sedimentation characteristics (Table 2). Fraction 1 contains only low molecular weight ^{32}P-RNA and will be discussed separately below. Fractions 2, 3, and 4 contain the bulk of the RNA that becomes labeled in a brief exposure to ^{32}P. While our experience with this technique has not yielded the clear-cut separations of pRNA and dRNA reported by Georgiev *et al.*[11] the base composition of fraction 2 approaches that of rRNA, while the composition of fraction 4 approaches that of dRNA. From these differences in composition, we have calculated the amounts of the two types of RNA present in each fraction. Hormone treatment did not appreciably change the results of this fractionation, and it was therefore possible to determine whether or not hydrocortisone effects a selective increase in one or the other types of RNA. The results (Table 3) show clearly that the hormone effect is equivalent in all fractions, and thus we conclude that synthesis of both pRNA and dRNA is stimulated by the hormone.

TABLE 3

Hydrocortisone Effect on Pulse-labeling of High Molecular Weight RNA Fractions

Fraction no.	Total radioactivity		Fold increase
	−Hydrocortisone	+Hydrocortisone*	
2	55,800	102,500	1.8
3	91,700	185,000	2.0
4	54,500	133,000	2.1

* Hydrocortisone treatment was for 2 hr.

Whether tRNA synthesis is affected proved more difficult to resolve. The ^{32}P-RNA recovered in fraction 1 of the differential phenol extraction sedimented with a sharp peak over the 4S region known to contain tRNA. Liver cytoplasm similarly contains ^{32}P-RNA that sediments like tRNA. However, as we have shown,[14] most of this ^{32}P-RNA is not tRNA, but a mixture of low molecular weight species that probably reflects degradation of pRNA and/or dRNA. When tRNA is separated from this mixture by salt fractionation (Table 4) or by DEAE chromatography, it is clear that most of the ^{32}P-labeling of tRNA is due to turnover of the -pCpCpA terminus of the nucleotide chain. Labeling of the salt-insoluble RNA reflects *de novo* synthesis, and the synthesis of this RNA was affected by the hormone to the same extent as that of pRNA and dRNA. The effect of hormone on the labeling of tRNA (Table 5) was strong (two- to threefold) on the guanylic, adenylic, and uridylic residues, but limited (17 per cent) on the labeling of cytidylic acid. This indicates different mechanisms for the two types of labeling, that of cytidylic being due to end-group turnover, and that of the other nucleotides to *de novo* chain synthesis. Since the content of CMP in tRNA is roughly equivalent to that of GMP, we were able to correct the data for CMP for the extent of labeling due to chain synthesis. When this is done, it becomes apparent that

TABLE 4

Fractionation of Low Molecular Weight ^{32}P-RNA*

Fraction	Per cent of total	Acceptor activity	Sedimentation characteristics	^{32}P composition (per cent)			
				CMP	GMP	AMP	UMP
M NaCl soluble	33	+	4S	70	8	8	14
M NaCl insoluble	67	—	4S, disperse	29	20	27	24

* Modified from Greenman, Kenney, and Wicks.[14]