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Volume 1

Proceedings of a symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Liver held at Indiana University School of Medicine Indianapolis, Indiana October 1 and 2, 1962

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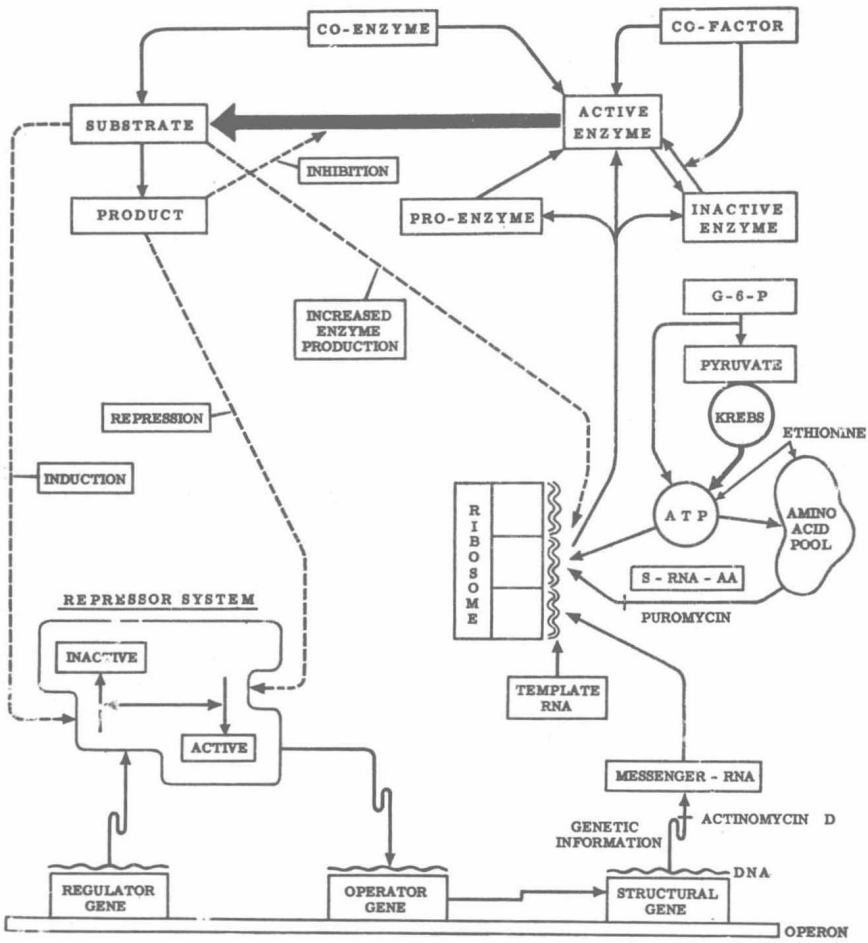
FACTORS INVOLVED IN REGULATION OF ENZYME ACTIVITY AND SYNTHESIS



FOREWORD

Advances in Enzyme Regulation is a new series which will be devoted to the elucidation of factors which regulate enzyme activity and synthesis in mammalian tissues under physiological and pathological conditions. It is hoped that these volumes will record the important progress made on this strategic area of metabolic regulation at the molecular level as well as serve as guideposts and beacons for investigators in the planning of research and the teaching of students in both basic and clinical fields of endeavor.

Indiana University
1963

GEORGE WEBER, *Editor*

ACKNOWLEDGMENTS

THIS IS the first Symposium 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 the United States Public Health Service provided the financial support for this Meeting.

In the planning of the program, selection of participants and arrangements for the Meeting the advice of the following was invaluable: B. B. Brodie, O. Greengard, W. E. Knox, H. C. Pitot, V. R. Potter, S. Weinhouse and J. W. Wilson. I am most obliged to Drs. Knox, Potter, Weinhouse and Wilson for serving as chairmen of the sessions, and to the 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. D. VanNuys, A. Lautzenheiser and Doris H. Merritt. The efficient and competent help of Mr. R. Dault in accommodation arrangements and the highly skilled assistance of Mr. J. Glore in the preparation of illustrations are appreciated.

Particular thanks are due to members of my staff who were in attendance or assisted in other ways in the local arrangements for this Meeting: Keren Call, Freida Jones, Donna J. Lee, Carlyanna Lindberg, Patricia Olsen, and R. L. Singhal and I am especially grateful for the many-sided and talented assistance of Nancy B. Stamm.

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

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OPENING ADDRESS

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SPECIAL SYMPOSIUM LECTURE

SYMPOSIUM CHAIRMAN'S INTRODUCTION

STUDY AND EVALUATION OF REGULATION OF ENZYME ACTIVITY AND SYNTHESIS IN MAMMALIAN LIVER*

GEORGE WEBER

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BACKGROUND AND PURPOSE OF THE SYMPOSIUM

There has been an acute need for those who work on enzyme regulation and synthesis in mammalian liver to get together to exchange ideas and clarify the many problems unique to mammalian organisms. Enzyme synthesis in microorganisms has been the subject of Symposia; however, the concepts developed regarding unicellular organisms are not necessarily transferrable to the mammalian. Since there never had been a Symposium on Regulation of Liver Enzyme Synthesis, it was felt that the realization of such a meeting would add an important impetus to the further development of this field.

This Symposium aims at a synthesis of available data and concepts by combining critical discussions on the biochemistry, physiology and pathology of regulation of enzyme activity and synthesis in liver with evaluation of experimental approaches in influencing enzymes in neoplastic liver for the purpose of achieving chemotherapeutic effects.

THE CENTRAL THEME OF THE SYMPOSIUM

The mammalian organism is kept in a dynamic steady state by a delicate coordination of innumerable metabolic reactions. As a result of its ability to adapt to altered conditions, an efficiently functioning organism is maintained in homeostatic balance. The crucial point in the maintenance of homeostasis is the capacity of the organism to increase or decrease the rate of various reactions. This is accomplished by supplying or cutting off

*The following abbreviations were used in the text, tables and figures: G-6-P, glucose-6-phosphate; FDP, fructose-l,6-diphosphate; G-6-Pase, glucose-6-phosphatase; FDPase, fructose-l,6-diphosphatase; PGM, phosphoglucomutase; PHI, phosphohexose isomerase; LDH, lactic dehydrogenase; G-6-P DH, glucose-6-phosphate dehydrogenase; 6-PG DH, 6-phosphogluconate dehydrogenase; SN, supernatant nitrogen; HN, homogenate nitrogen; HYX, hypophysectomized.

the production or availability of metabolites and hormones. At the molecular level this ability of the organism to increase or decrease reaction rates is based on the activities of enzyme systems.

When the development and operation of strategic enzyme systems proceed smoothly and the metabolic functions are properly coordinated by the regulatory mechanisms of the body, a state of health exists. However, when there are faults in the genetic influences, in the development, function or coordination of enzyme systems, resulting in absence or alterations of enzyme activities or synthesis, the dynamic equilibrium of the whole system may be disturbed. Then, depending on the localization and severity of such lesions, pathological conditions arise.

To study the regulatory factors governing the activity and synthesis of mammalian enzyme systems, various approaches have been designed. In order to examine the various points of regulatory attack, simplified "blueprints" are presented which illustrate certain critical factors influencing enzyme activity and synthesis (Figs. 1 and 2) and outline the possible role of enzymes in homeostasis (Fig. 3).

The central task of this Symposium is to examine evidence for the existence and operation of these tentative concepts in mammalian liver. It is hoped that many of the present views may be clarified and put in sharper focus and evaluation as a result of this Meeting. It is also expected that with the advancing steps of our understanding these preliminary concepts will be improved so that eventually such blueprints can serve as a firm basis for the design of treatment of disease by Enzyme Pharmacology.

CONCEPTS OF REGULATION OF ENZYME ACTIVITY AND SYNTHESIS IN LIVER

First of all, we must make a clear-cut differentiation between enzyme activity and enzyme amount in order to separate the factors which may selectively influence activity or amount of enzyme involved. In the following the concepts outlined in Fig. 1 are discussed.

Factors Influencing the Size of Enzyme Population (Enzyme Amount or Concentration)

The term "enzyme population" means the sum total of the individual molecules of a certain enzyme at the tissue level.[1] This concept is more meaningful than its synonyms, enzyme concentration or enzyme amount, because it focuses attention on the dynamic aspects of the maintenance of a number of enzyme molecules by a continuous process of renewal and destruction in the tissues of the organism. The word "population" emphasizes the possible heterogeneity of the enzyme molecules which may be in various

stages of synthesis and breakdown. It also underlines the possibility, which may be of significance in pathology, that certain of the enzyme molecules may be deficient, malformed or inactive since in the process of continuous replication a certain distortion of pattern can occur.[2] The concept of enzyme population also implies the possible presence of a number of enzyme molecules of slightly different biophysical properties involved in the catalysis of the same reaction and thus contributing to the basis of heterogeneity of the population of a single enzyme. This aspect of the present concept emphasized what has become known as the phenomenon of isozymes.[3]

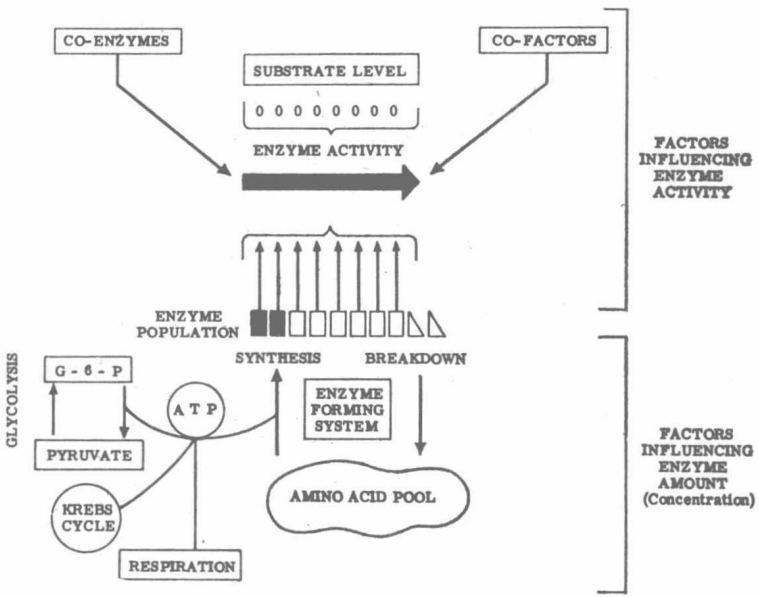

FIG. 1
Factors influencing enzyme activity and enzyme population (amount or concentration).

It is generally assumed that when linear kinetic enzyme assay conditions are used the enzymatic activity measured is a reflection of the amount of enzyme protein present or, in other words, it is an indicator of the size of the enzyme population. Thus, under classical enzymological conditions, the existing enzyme population is one of the chief determinants of the enzyme activity.

However, there are other crucial factors which are in turn responsible for the size and behavior of the enzyme population. The enzyme population is in a dynamic steady state in which, under normal physiological circumstances, a remarkable constancy of composition is maintained in face of a continuous turnover of the numerous components of the system.

The synthesis of the enzyme molecules depends on the presence of genetic directives, DNA-directed transfer of information to messenger RNA, the existence of functioning template RNA on available ribosomal sites, and the presence of soluble RNA-amino acid complexes. In short, there is operational an Enzyme Forming System, on the existence of which only little information is available, especially in mammalian organism.

For the optimal functioning of the complicated and many-faceted Enzyme Forming System a balanced amino acid pool is necessary. The maintenance of an enzyme population also continuously depends on

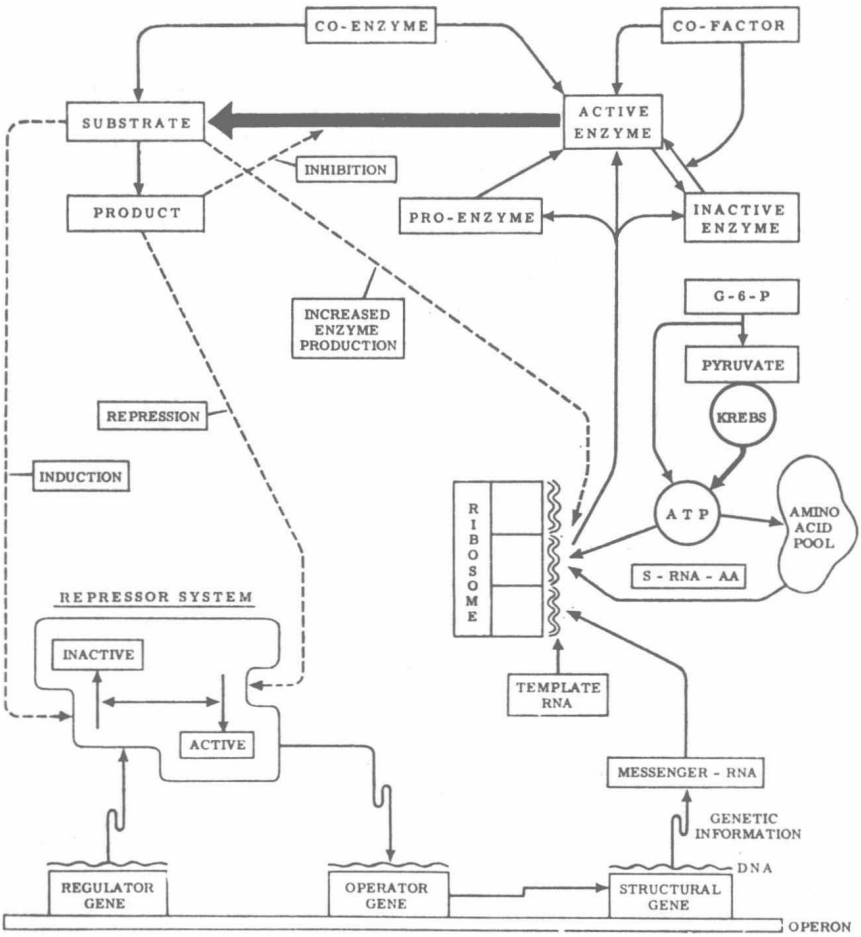

FIG. 2
Factors involved in regulation of enzyme activity and synthesis. The various components, some proved to operate in mammalian conditions and some, postulated (e.g. repressor systems), are indicated.

availability of energy which may be derived from glycolysis and/or respiration and must be coupled to the specific Enzyme Forming System in question. The presence in adequate concentration and the ready availability of the different components of this machinery and the coordinating and regulating influence of substrates, products, repressors and hormones acting on various attacking points of this complex system together result in the maintenance of specific enzyme populations in living tissues. A simplified picture of these concepts is presented in Fig. 1 and a more detailed illustration of the factors discussed is shown in Fig. 2.

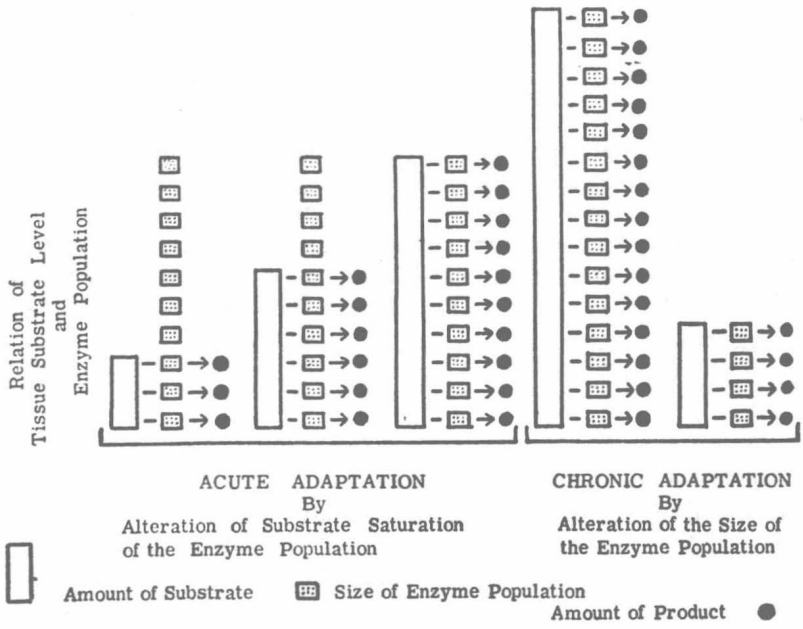

FIG. 3
Role of enzymes in homeostasis. The function of enzyme systems in the maintenance of homeostasis by acute and chronic adaptation is illustrated.

Factors Regulating Enzyme Activity

The activity of an enzyme at the tissue level depends in addition to the size of the enzyme population also on the existing biophysical and biochemical milieu such as pH and temperature and is particularly influenced by the available levels of cofactors, coenzymes, substrate and product concentrations (Figs. 1, 2). It has been suggested that the basic unit which determines the rate of metabolic processes at the cellular level is the