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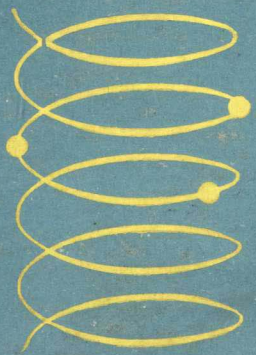
Outlines of Enzyme Chemistry



Second Edition

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Enzyme Chemistry

With a chapter on the Synthesis of Enzymes

by

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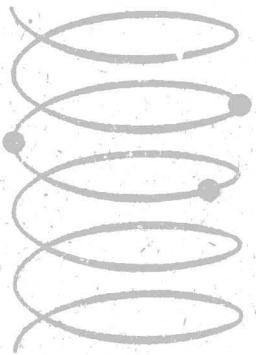

Revised & Enlarged

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Outlines of Enzyme Chemistry

Preface to the Second Edition

The widespread acceptance of the proposition that enzyme chemistry can be presented as a general discipline, without reference to the detailed properties and characteristics of each individual enzyme, has been a source of some satisfaction to the authors of *Outlines of Enzyme Chemistry*. Thus the first edition of the book has found some value as a class text in this country and has been translated into the Italian and Japanese languages.

In spite of this measure of success, the authors have been keenly aware that the book suffered certain serious deficiencies with respect to both content and emphasis. Some of the deficiencies were perhaps unavoidable; who, for example, could have predicted in 1954 the vast strides about to be made in the mechanism of synthesis of the polynucleotides? And who could have predicted the hitherto unsuspected presence of metal ions in purified enzymes or the presence of a cofactor such as pyridoxal phosphate in crystalline phosphorylase?

In this second edition the authors have attempted to incorporate some such recent developments in enzymology. And in order to indicate the current trend in enzyme research, new chapters which deal with metal ion bonding, the mechanism of enzyme action, and the dynamics of nucleotide transformations, have been added.

To increase the usefulness of the book as a tool in the research laboratory we have appended a catalogue of over 500 enzymes. This list, though necessarily incomplete, provides some very general characteristics of each enzyme and gives a reference to the research literature.

The authors wish to take this opportunity to again express their appreciation to those individuals who helped with the preparation of the first edition. In connection with the second edition, special thanks must be tendered our colleagues in the Biochemistry and Virus Laboratory, particularly Dr. H. Fraenkel-Conrat.

J. B. Neilands
P. K. Stumpf

Berkeley, California
July, 1958

Preface to the First Edition

This book is designed to serve the following twofold purpose: (*a*) to introduce senior undergraduate and beginning graduate students to the general subject of enzyme chemistry, and (*b*) to provide background material for research workers in fields other than enzymology. Although many excellent advanced treatises on enzymes have already appeared, they are useful only to specialists, and, furthermore, their cost renders them prohibitive for class use.

The book is based on lecture material presented in a one-semester course given in the Department of Biochemistry of the University of California. This course is designed for students who have had a thorough grounding in chemistry and physiology as well as in biochemistry.

Over half of the book has been devoted to the general properties of enzymes. Brief sections have been included on specific coenzymes, enzymes, and enzyme systems. The study of metabolic problems at the enzyme level has also received attention. However, because there are too many individual enzymes to permit a discussion of each of them, we have studiously avoided writing a "catalogue of enzymes." We have, instead, attempted to treat the subject matter in outline form in order to bring into focus the entire field of enzyme chemistry.

Photographs of several eminent enzyme chemists appear in three chapters. It is obvious, however, that these few are by no means the only individuals who have made outstanding contributions to the field of enzymology. For practical reasons it has been possible to include photographs of only a small number of workers from representative fields of research.

We are deeply indebted to the many reviewers and others who helped in various ways to make this book a reality. We are particularly grateful to R. A. Alberty, P. D. Boyer, B. Chance, D. E. Green, A. C. Griffin, F. M. Huennekens, B. L. Horecker, M. J. Johnson, E. P. Kennedy, H. A. Lardy, H. Neurath, and E. R. Stadtman.

J. B. Neilands
P. K. Stumpf

Berkeley, California
January, 1955

Nomenclature

Physical Constants

k	rate constant
k^0	zero-order rate constant
k^1	first-order rate constant
K_{eq}	equilibrium constant
K_{app}	apparent equilibrium constant
Ka	acidic dissociation constant
pKa	log $1/Ka$
pKa'	apparent pKa
A	ligand
pA	log 1/A
K_s	stability constant
n	complexation number
C	total concentration
M	metal ion
v	velocity
V	maximum velocity
K_m	Michaelis constant
T.N.	turnover number
Ki	enzyme-inhibitor dissociation constant
$\Delta F°$	standard free-energy change
$\Delta F'$	apparent free-energy change
E_0'	normal oxidation-reduction potential
F	faraday
$M.W.$	molecular weight
s	sedimentation coefficient
D	diffusion coefficient
$\bar{V}$	partial specific volume
M_{sD}	molecular weight by sedimentation and diffusion
u	electrophoretic mobility
pI	isoelectric point
O.D.	optical density
ϵ	molecular extinction coefficient

Coenzymes

DPN+	oxidized diphosphopyridine nucleotide
DPNH	reduced diphosphopyridine nucleotide
TPN+	oxidized triphosphopyridine nucleotide
TPNH	reduced triphosphopyridine nucleotide
NMN+	nicotinamide mononucleotide
PALPO	pyridoxal phosphate
PAMPO	pyridoxamine phosphate
TPP	thiamine pyrophosphate
AMP, ADP, ATP OMP, ODP, CTP GMP, GDP, GTP UMP, UDP, UTP	mono-, di-, and triphosphates of adenosine, cytidine, guanosine, and uridine
FMN	flavin mononucleotide
FAD	flavin adenine dinucleotide
CoA	coenzyme A (acetylation)

Enzymes

En	enzyme
EnS	enzyme-substrate compound
EnI	enzyme-inhibitor compound
ADH	alcohol dehydrogenase
LDH	lactic dehydrogenase

Metabolites

GSH	glutathione, reduced
GSSG	glutathione, oxidized
PEP	phosphoenolpyruvate
PGA	phosphoglyceric acid
GAP	glyceraldehyde phosphate
DAP	dihydroxyacetone phosphate
OAA	oxaloacetic acid

Miscellaneous

Pi	inorganic phosphate
PP	pyrophosphate
DNA	deoxyribonucleic acid
RNA	ribonucleic acid

Contents

Photographs of Enzymologists

Part 1

GENERAL PRINCIPLES

1

Introduction and History

1. Fermentation

The world's first biochemists were those primitive peoples who concerned themselves with the seemingly miraculous transformation of juices into alcoholic beverages. Such "biochemists" were at once the first enzymologists, since the chemical transformations wrought by them were catalyzed by enzymes contained within the living yeast cell.

During the 17th century several attempts were made to solve the over-all chemical nature of the fermentation process. These efforts culminated some one hundred years later in the experiments of Lavoisier (1) who showed by means of balance sheets that the sugar was converted to carbon dioxide and alcohol.

The 19th century witnessed both the identification of fermentation as a physiological act of the yeast cell and the introduction of the somewhat dogmatic view of Pasteur (2) that life and fermentation were inseparable. Many attempts were made in this period to extract the fermentation enzyme from yeast, but for one reason or another all of these yielded negative results. As is so often the case in science, the great discovery was made purely by accident. In 1897 E. Buchner (3) required a quantity of purified protein for therapeutic purposes. He ground yeast with sand, filtered off the broken cells, and added a large amount of sugar to the filtrate as a preservative. He was astonished to find that the sugar was rapidly fermented by the cell-free extract.

Harden and Young (4) made an important contribution in 1905 when they showed that Buchner's dialyzed zymase required the addition of a heat-stable cofactor or coenzyme. Thus within a single decade two fundamental discoveries—solubilization of zymase activity and introduction of the coenzyme concept—opened the route to the

eventual isolation and identification of all of the large number of individual enzymes and cofactors making up the zymase system.

2. Chemical Nature of Enzymes

Unfortunately mere solubilization of the fermentation enzymes provided little information about their chemical nature. In 1926 James B. Sumner (5) obtained a crystalline protein from the jack bean and showed that urease activity was intimately associated with the crystals. J. H. Northrop soon achieved the crystallization of a large number of proteolytic enzymes and prepared a monograph on this subject (6). Thus the protein nature of enzymes became firmly established. So far as is now known, all enzymes are proteins, and, indeed, most of the protein of metabolically active tissue is enzyme protein.

In the 1930 to 1940 era Warburg (7) and his school were able to crystallize or purify a large number of respiratory enzymes. Theorell (8), working in Warburg's institute, reversibly dissociated the "old yellow enzyme" into a protein part (apoenzyme) and a cofactor (prosthetic group). The separated components were inactive. This experiment opened up the possibility of studying the mode of binding of the cofactor to the protein.

3. Energetics and Metabolic Cycles

Harden and Young (4) had found in 1905 that inorganic phosphate as well as co-zymase caused a stimulation of carbohydrate oxidation in yeast juice. Many of the intermediates in carbohydrate oxidation were soon found to be sugar phosphates, and it became for a time the practice to assign such compounds the name of its discoverer, e.g., fructose-1,6-diphosphate = Harden and Young ester. However, the fundamental role of phosphate in these processes came from studies of muscle biochemistry. Lundsgaard (9) noted that a phosphagen, phosphocreatine, vanished from a contracting muscle in which the carbohydrate metabolism had been blocked by iodoacetate. Lohman (10), Meyerhof (11), Lipmann (12), and others brought cellular energetics to the enzyme level when they showed that the phosphate of certain of the esters produced as a result of carbohydrate oxidation existed in equilibrium with the phosphate of adenosine triphosphate and the phosphagens. These compounds were said to contain the "high-energy phosphate bond" (~P) and were characterized by a very large $-\Delta F^\circ$ of hydrolysis. In a brilliant review article Lipmann (12) suggested that such phosphorylated intermediates represent the means whereby the cell is able to trap the chemical energy of carbo-

hydrate, energy that is ultimately to be used in payment for the expensive synthetic reactions of life.

The studies on carbohydrate metabolism described above were carried out mainly with yeast and muscle. They did much to strengthen the concept of the unanimity of biological processes. As early as 1932 Harden (13) was able to write: "This mechanism for the decomposition of the sugar molecule is not confined to the yeast cell, for it has been found that the same processes are involved in the conversion of carbohydrate into lactic acid in muscle and in the decomposition of carbohydrates by bacteria, moulds, and the higher plants."

After a sufficient number of the individual steps in carbohydrate metabolism had been studied it became clear that certain reactions could be grouped into a metabolic cycle. Thus Szent-Györgyi 14) found evidence for the interconversion of the four-carbon dicarboxylic acids; Krebs (15) showed this to be part of a cyclic scheme now variously known as the citric acid, tricarboxylic, or Krebs cycle.

The existence of metabolic schemes and cycles naturally introduced the question of the subcellular organization of enzymes. For example, the entire fatty-acid oxidation scheme as well as various other systems was found to be associated with the particulate components of the cell (16).

4. Future of Enzymology

Consideration of the above brief discussion of the historical aspects of enzymology renders it apparent that this discipline has developed mainly along two routes.

There are those who have regarded the enzyme molecule simply as a catalytic protein. Such studies were preceded by the development of elaborate apparatus and techniques for obtaining the enzyme in a pure state. Research in this field has provided molecular and kinetic data for a number of crystallized enzymes and has led to general acceptance of the enzyme-substrate compound theory first advanced by Michaelis and Menten in 1913 (17).

On the other side of the ledger are those who have worked with the enzyme simply as a cog in the complicated metabolic machinery of the cell. Such investigators have cared little for the purity of their preparations; by the judicious use of inhibitors and by other devices they have "isolated" the activity of the individual enzymes. Their research has been rewarded by the establishment of a multitude of metabolic cycles and has given a more fundamental understanding of cellular energetics.

There is every reason to suppose that future research in enzymology

will be vigorously pursued along the two main lines already pioneered. The organic and physical structure of the enzyme active site will be subjected to detailed investigation. Alternate pathways of metabolism will be uncovered as the tissues of a wider variety of species are scrutinized with analytical methods of increasing sensitivity. Research into the metabolism and synthesis of protein and nucleic acids will balance the already impressive fund of knowledge existing in the area of carbohydrate and fat metabolism.

It is to be hoped that these basic and fundamental studies in enzymology will one day find important application in the diverse fields of industry, agriculture, and medicine.

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