MOLECULAR BIOCHEMISTRY

EDWARD M. KOSOWER

MOLECULAR BIOCHEMISTRY

EDWARD M. KOSOWER

Department of Chemistry
State University of New York
Long Island Medical Center
Stony Brook, New York

New York
San Francisco
Toronto
London

McGRAW-HILL BOOK COMPANY, INC. 1962

MOLECULAR BIOCHEMISTRY

 Library of Congress Catalog Card Number 61-18728

35320

II

PREFACE

One of the most challenging frontiers to human inquiry lies within the domain of molecular biology, the explanation of biological processes and phenomena in molecular terms. In areas as diverse as genetics, medicine, and pharmacology, the relationship between molecular occurrence and visible result is being eagerly sought.

In many cases, the molecular occurrences are or result from chemical transformations. For example, the medically identified condition of galactosemia has been traced to a deficiency of the enzyme galactose-1-phosphate uridyl transferase, which catalyzes the conversion of UDP-galactose to UDP-glucose.

During the last fifty years, enormous effort on the part of biochemists has led to a fairly complete understanding of the stoichiometry of intermediary metabolism, that is, a description of the sequences of compounds through which a molecule of, say, glucose is converted into carbon dioxide and water.

Over the same time period, vigorous research into the mechanisms of reaction of organic compounds (physical-organic chemistry) had led to a considerable clarification of the pathways by which one organic compound is converted into another.

In the past few years, a new research area has emerged from the application of the physical-organic approach to the problems in chemical transformation found in biochemistry. We choose to call this area *molecular biochemistry*, and delimit it as the study of the detailed chemical mechanisms of the chemical transformations in biology, usually as they are described in biochemistry.

The present book is an attempt to survey and organize the area in such a way as to indicate the numerous lacunas which exist as well as to

point out some of the exciting new avenues of research with implications for molecular biochemistry. The level at which the book is written is roughly that of a second-year graduate student in chemistry or biochemistry, but it could be read with profit by research workers in both fields, or by younger students with a good background in organic mechanisms.

The book is divided into three rather unequal parts: the first, a survey and classification of many of the reactions of intermediary metabolism; the second, a discussion of the mechanisms of many of these reactions, with the relationship to the enzymatic reaction shown where possible; and the third, a brief discussion of one of the central problems of molecular biochemistry, the "active site." To a large extent, the distribution of topics has been dictated by such objective factors as the frequency of occurrence of certain reaction types as outlined in the first part, and the availability of material which could be discussed within the context of this book. In some cases, e.g., decarboxylation, an organization of the subject matter from organic chemistry was deemed worthwhile, and in others, i.e., ester and amide hydrolysis, the discussion is shortened because of the extensive review material which can be readily obtained.

The special interests of the author are no doubt visible in the section on pyridine nucleotides, but it should be emphasized that detailed chemical inquiries into biologically important reactions of molecules, apart from their intrinsic chemical value, often have serendipitous consequences.

A number of simplifying conventions have been adopted in the presentation of the figures, as follows: Enzymatic reactions are almost always written as proceeding in one direction, whereas they are, in fact, reversible. Formulas are written with full bonds, even though these do not always reflect the correct charge distributions (as in carboxylate, phosphate, etc.). The state of ionization is usually that which probably exists at physiological pH.

The author is indebted to the Alfred P. Sloan Foundation for a fellowship which released him to a great extent from the burden of other duties and accelerated the completion of the book. He also owes a considerable amount to friendly critics, especially Prof. Edward L. King, of the Department of Chemistry, and Prof. Helmut Beinert, of the Enzyme Institute, both of the University of Wisconsin. For stimulating conversations on many aspects of the subject, he would like to thank Dr. Leonard Peller and Dr. David Lemal, of the Department of Chemistry, University of Wisconsin, and many other students and colleagues, among whom might be mentioned Sue Brown; Jon Brodie; Prof. S. Wakil, of Duke University; Prof. Y. Hatefi, Prof. D. E. Green; Prof. H. Khorana; Dr. W. Lee, of the Enzyme Institute; and Dr. H. Abrash.

Edward M. Kosower

ACKNOWLEDGMENTS

The author is grateful for permission to publish excerpts from the following:

"The Dissolution," by John Donne, in "The Complete Poetry and Selected Prose of John Donne and the Complete Poetry of William Blake," p. 44. Copyright 1941 by Random House, Inc., New York.

"Écoutez la Chanson Bien Douce," by Paul Verlaine, in "Verlaine et Les Poètes Symbolistes," with notes by A. Micha, p. 29. Copyright by Editions Messein, Paris.

"Tartuffe," by Molière, p. 30. Copyright 1941 by Librairie Larousse, Paris.

"Die Lorelei," by Heinrich Heine, in "The Penguin Book of German Verse," introduced and edited by Leonard Forster, p. 329. Copyright by Penguin Books, Ltd., London.

"Du Maître D'Astres et de Navigation" in "Amers," by St.-John Perse, p. 40. Copyright 1958 by Bollingen Foundation, Inc., New York. Reproduced by permission of Bollingen Foundation, New York.

"The Waste Land, I. The Burial of the Dead" in "Collected Poems 1909–1935 by T. S. Eliot." Copyright 1936 by Harcourt, Brace, and World, Inc., New York.

"La Cantatrice Chauve," by E. Ionesco. Copyright 1954 by Librairie Gallimard, Paris.

"Ciba Foundation Symposium on the Biosynthesis of Terpenes and Sterols," 1959. Copyright 1959 by J. and A. Churchill, Ltd., London.

"Veglia," by Guiseppi Ungaretti, in "Penguin Book of Italian Verse," edited by George Kay, p. 366. Copyright by Arnoldo Mondadori Editore, Milan.,

"Antoine et Cléopâtre" by José-María de Heredia, in "Penguin Book of French Verse," vol. 3, "The Nineteenth Century," p. 184. Copyright by Penguin Books, Ltd., London.

"Gulliver's Travels," by Jonathan Swift. Copyright 1940 by Heritage Press, New York.

"Klage," by Rainer Maria Rilke, in "Selected Poems" by R. M. Rilke. Copyright 1941 by Insel-Verlag, Wiesbaden.

"The Backbone Flute," by Vladimir Mayakovsky, in "The Bedbug and Selected Poetry," edited by Patricia Blake. Copyright 1960 by Meridian Books, Inc., New York.

"Japanese Haiku," by Shusen, p. 21. Copyright 1955–1956 by Peter Pauper Press, Mount Vernon, N.Y.

"Expectation," by Thomas Stanley, in "The Centuries' Poetry 2: Donne to Dryden," p. 132. Copyright by Penguin Books, Ltd., London.

"Bitter Lemons," by Lawrence Durrell, p. 38. Copyright by E. P. Dutton and Co., Inc., New York.

"The Old Familiar Faces," by Charles Lamb, in "The Centuries' Poetry 3." Copyright by Penguin Books, Ltd., London.

"Sueño," by Juan Ramón Jiménez, in "The Penguin Book of Spanish Verse," edited by J. M. Cohen, p. 354. Copyright by Penguin Books, Ltd., London.

"Canto notturno di un pastore errante dell'Asia," by G. Leopardi, in "The Penguin Book of Italian Verse," edited by G. R. Kay, p. 279. Copyright by Penguin Books, Ltd., London.

"Hard, Ain't It Hard," words and music by Woody Guthrie. Copyright 1952 by Ludlow Music, Inc., New York, N.Y. Used by permission.

CONTENTS

PART 1

BIOCHEMICAL PATTERNS

1.0. INTRODUCTION

Shee is dead; And all which die
To their first elements resolve. . . .
John Donne

Life is the resultant of a complex set of chemical and physical processes. Driven by urges with both idealistic and practical roots, man is now engaged in a vigorous effort to understand the molecular basis of life. One should perhaps be a bit shy of repeating an optimistic statement of the early part of the century, "Nothing indicates, however, at present that the artificial production of living matter is beyond the possibilities of science . . . ,"[1] but few scientists would assert that such an accomplishment is impossible.

The complexity of physical phenomena has led to fragmentation of scientific studies. To appreciate the relationship of molecular biochemistry to other disciplines concerned with life, a hierarchy of sciences can be constructed, in which it can be remarked that particle size is a determinant factor in position (Table 1.0-1).

Sciences in the same or neighboring levels are those which are most enriched by interdisciplinary cooperation, because of the ease in hybridizing intellectual and experimental techniques. Thus, biochemistry and organic chemistry possess a relationship appropriate to the

[1] J. Loeb, "The Mechanistic Conception of Life," p. 5, University of Chicago Press, Chicago, 1912.

exchange of ideas and experience in the form of the area of *molecular biochemistry.*

The ultimate source of energy for life on this planet is the radiation of the sun. Systems present in certain varieties of life can utilize radiation to convert one set of molecules into another (*photosynthesis*), and by this means a fair proportion of the incident radiation within a certain wavelength range is trapped. The most important sequence of this type results in the formation of carbohydrates and oxygen from carbon dioxide

TABLE 1.0-1. HIERARCHY OF SCIENCES

Level	*Science*
Abstract	Mathematics
Subnucleonic	High-energy physics
Subatomic	Nuclear physics
Atomic	Spectroscopy
Polyatomic (low number)	Theoretical chemistry
	Molecular, solid-state physics
	Physical chemistry
	Organic chemistry
Polyatomic (high number)	MOLECULAR·BIOCHEMISTRY
	Polymer chemistry
	Biochemistry
Subcellular	Colloids
	Genetics
	Virology
	Immunology
Cellular	Cytology
	Bacteriology
Polycellular	Physiology
	Neurology
Organism	Medicine
Polyorganism	Psychology
	Sociology

and water. Evolution has also led to mobile organisms that consume energy at a higher rate than would be available through photosynthesis, and these depend parasitically upon the organisms which utilize radiation directly.

Early in the evolutionary process, a large number of intermediate steps were introduced into the pathways from stored energy to oxidation products so as to gain the most effective use of the energy available. The basic reaction sequences are common to many kinds of living systems and are summarized in a general way in Fig. 1.0.

The primary nutrients for highly organized living systems are carbohydrate, lipid, and protein; these are converted through many simpler compounds to carbon dioxide, water, and nitrogen compounds by means

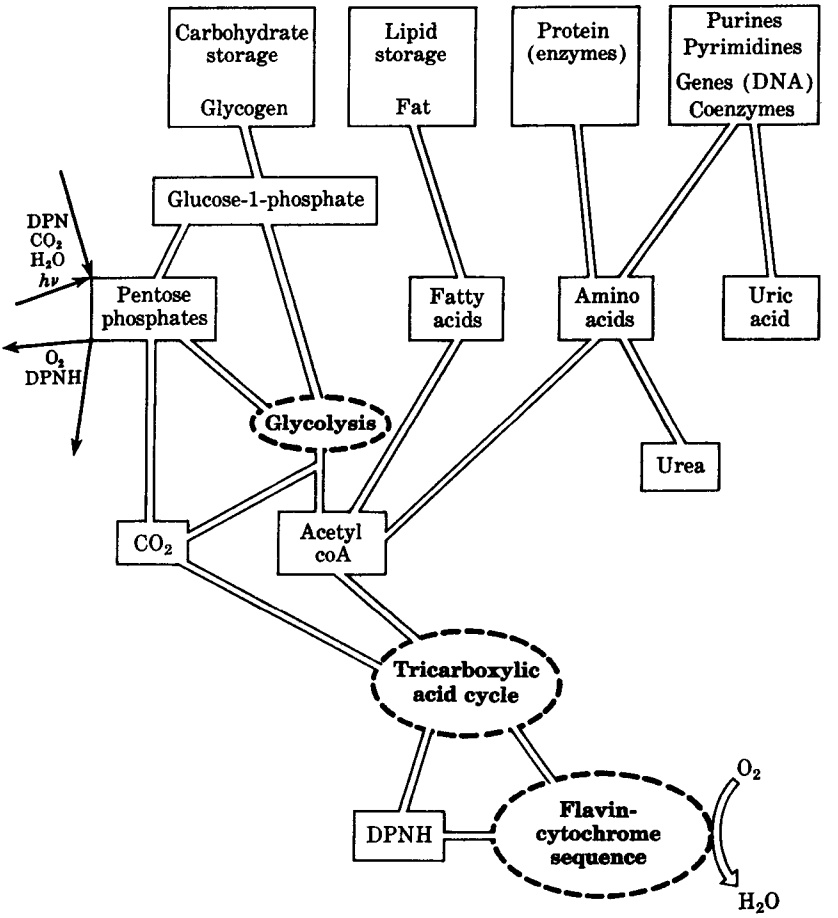


FIG. 1.0. Basic pathways in intermediary metabolism.

of reactions catalyzed by enzymes. Much of the metabolic "traffic" proceeds through the intermediate, acetyl coenzyme A, which is then introduced into the "tricarboxylic acid cycle." A reduction product of that "cycle," DPNH (reduced diphosphopyridine nucleotide), is reoxidized by the "terminal oxidation sequence," which, in turn, reduces oxygen to water. These relationships, illustrated in Fig. 1.0, provide the background for the detailed discussions of Part 1 of this book.

1.1. GLYCOLYSIS

Écoutez la chanson bien douce
Qui ne pleure que pour vous plaire,
Elle est discrète, elle est légère:
Un frisson d'eau sur de la mousse!
Paul Verlaine

It will be noted in this section and others that the arrows in the reaction schemes are accompanied by symbols. These are intended to indicate the reaction type or types occurring in the transformation. For example, GH signifies that a carbon-hydrogen bond has been formed or broken,

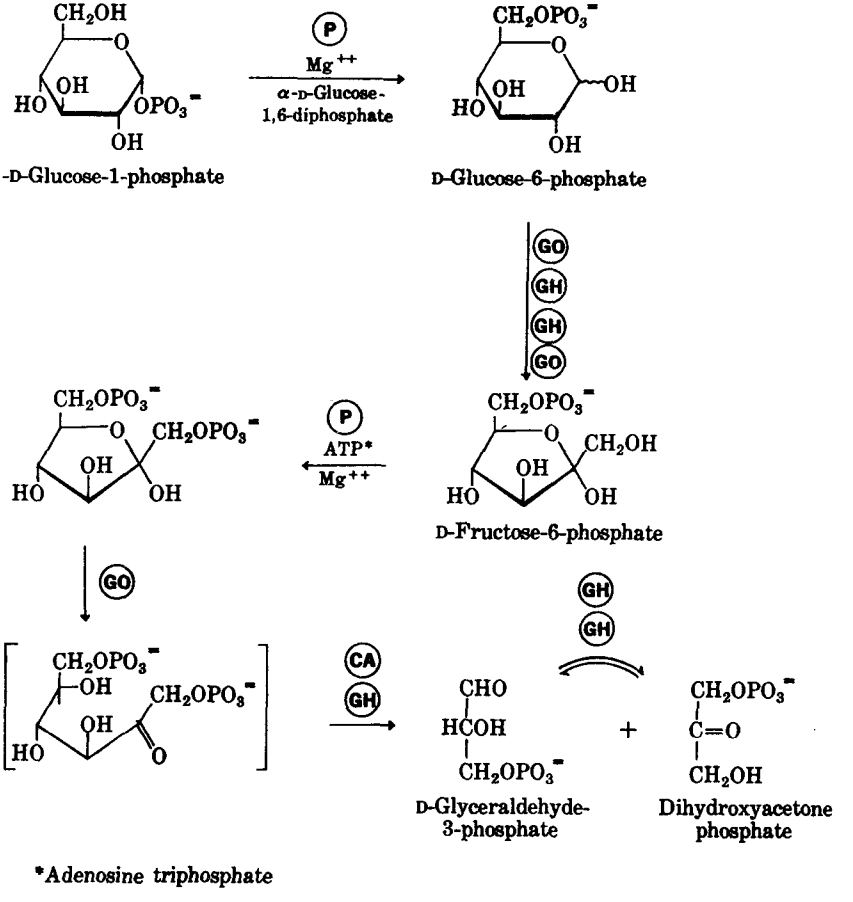


FIG. 1.1. Transformation of α-D-glucose-1-phosphate into triose phosphates.

without implying a mechanism for the process. The classification along these lines aided in the selection of reactions for scrutiny in Part 2 (cf. Sec. 1.9) and should be of some help in focusing attention on the mechanistic problems to be solved in molecular biochemistry.

The degradation of sugar stored within a biological system as a polymer such as glycogen (a derivative of poly-α-1,4-glucose) probably takes place by two major pathways and one minor route. The most important sequence involves the transformation of α-D-glucose-1-phosphate into the triose phosphates (Fig. 1.1) and then into pyruvic acid (Fig. 1.2). The initial step for each route is the conversion of glycogen into α-D-glucose-1-phosphate.

The discussion of reaction sequences will not include, for the most part, identification of the enzymes which catalyze the reactions. The names and other pertinent information may be found in standard works.[2,3]

1.1-1. α-D-Glucose-1-phosphate is converted into glucose-6-phosphate by a reaction in which the 1-phosphate group is transferred to the enzyme and a phosphate group from the enzyme moves to the 6-hydroxyl group. The coenzyme for the reaction, glucose-1,6-diphosphate, apparently serves to produce phosphoenzyme, that is, a phosphate derivative of the enzyme. (The enzyme is phosphoglucomutase in this case.[4])

The formation of D-fructose-6-phosphate from D-glucose-6-phosphate requires opening of the pyranose ring, enolization by loss of a proton, ketonization by gain of a proton, and closing of the furanose ring. It is highly probable that the enzyme catalyzes *both* ring opening and proton

[2] J. B. Neilands and P. K. Stumpf, "Enzyme Chemistry," John Wiley & Sons, Inc., 2d ed., New York, 1958.

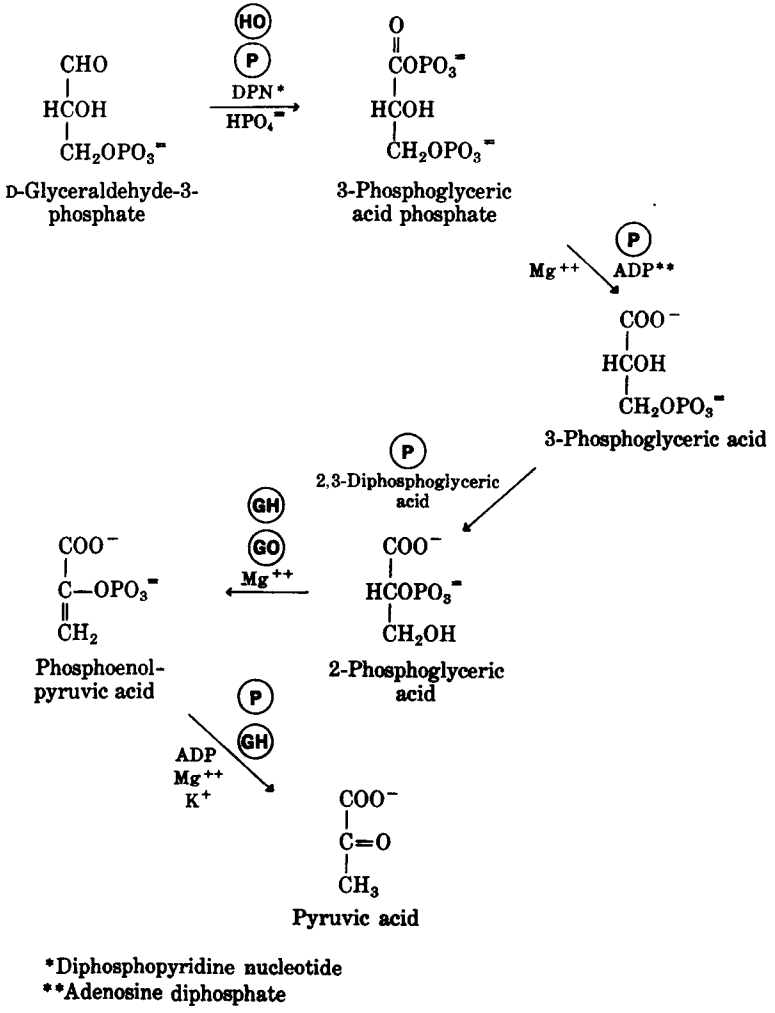
[3] "The Enzymes," vols. 1–7, ed. by P. D. Boyer, H. A. Lardy, and K. Myrbäck, Academic Press, Inc., New York, 1959–1962.

[4] It is a matter of some interest that the phosphate of the phosphoenzyme has been found bonded to a serine hydroxyl group[5] (cf. Sec. 3.1C). It was suggested that the identity of the hexapeptide sequence which included the serine phosphate with a hexapeptide derived by degradation of phosphorylated chymotrypsin implied that enzymes with similar functions have similar sequences at the "active site."[5] However, sequences from other hydrolytic enzymes which include a serine phosphate and are also derived by degradation of a phosphorylated enzyme (e.g., pseudocholinesterase and aliesterase[6]) permit only the statement that "the common sequence" is aspartic (or glutamic) acid–serine–glycine. In addition, some doubt has been cast upon the sequence in the hexapeptide derived from phosphoglucomutase.[7] Although the suggestion is interesting, there seems no compelling reason for its correctness on theoretical grounds, and no further discussion of this point appears in Sec. 3.1, except for an allusion to the presence of aspartic (or glutamic) acid in Table 3.1-1.

[5] D. E. Koshland, Jr., and M. J. Erwin, *J. Am. Chem. Soc.*, 79, 2657 (1957).

[6] H. S. Jansz, D. Brons, and M. A. P. J. Warringa, *Biochim. et Biophys. Acta*, 34, 575 (1959).

[7] F. Sanger, *J. Polymer Sci.*, 49, 10 (1961).



*Diphosphopyridine nucleotide
**Adenosine diphosphate

FIG. 1.2. Conversion of D-glyceraldehyde-3-phosphate into pyruvic acid.

transfer since the reported rate of ring opening of D-glucose to the aldehyde (less than 10^{-2} sec^{-1}, cf. Table 2.3-5) is considerably less than the over-all rate of isomerization reported for human erythrocyte phosphoglucose isomerase[8] (perhaps 600 to 1000 sec^{-1}), given the assumption that the enzyme has a molecular weight between 100,000 and 150,000. The four steps are illustrated in Fig. 1.3. It is known that the proton transfers are highly stereospecific.[8]

[8] Y. J. Topper, in "The Enzymes," vol. 5, chap. 26, Academic Press, Inc., New York, 1961.

D-Fructose-6-phosphate is converted to D-fructose-1,6-diphosphate by adenosine triphosphate (ATP). The diphosphate is rearranged to the acyclic ketose form, which undergoes dealdolization to an equal mixture of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. The latter two (the "triose phosphates") are interconvertible by proton loss to the enol, followed by gain of a proton.

D-Glyceraldehyde-3-phosphate is oxidized by diphosphopyridine nucleotide (DPN) and triose phosphate dehydrogenase (TPD) to

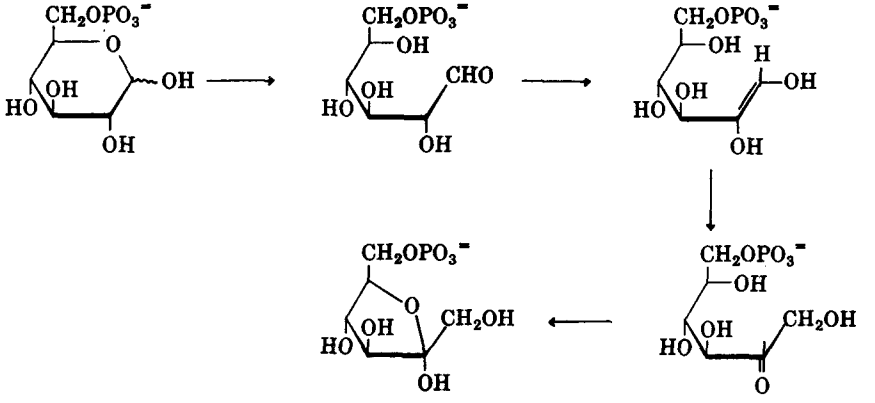
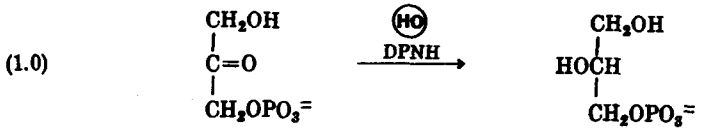


FIG. 1.3. Isomerization of D-glucose-6-phosphate to D-fructose-6-phosphate.

3-phosphoglyceryl-TPD (an acyl enzyme), and the latter reacts with phosphate ion to yield 3-phosphoglyceric acid phosphate and the enzyme (Fig. 1.2). The acyl phosphate reacts with adenosine diphosphate (ADP) to form 3-phosphoglyceric acid and ATP. An isomerization involving 2,3-diphosphoglyceric acid as a coenzyme produces 2-phosphoglyceric acid from the 3-phosphoglyceric acid. A dehydration of 2-phosphoglyceric acid leads to phosphoenolpyruvic acid, and the latter reacts with ADP to give pyruvic acid and ATP (Fig. 1.2).

The interconversion of dihydroxyacetone phosphate and L-glycerol-1-phosphate catalyzed by DPN is of importance in connection with the biosynthesis of fats and lecithin [Eq. (1.0)].

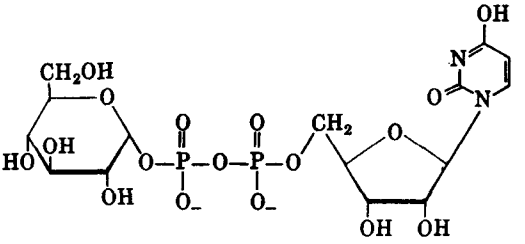


(1.0)

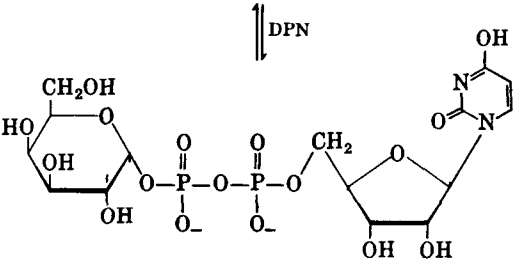
An apparent Walden inversion occurs in the transformation of uridine diphosphate galactose to uridine diphosphate glucose [Eq. (1.1)]. The

reaction is, however, catalyzed by an oxidation-reduction coenzyme, DPN, in addition to the enzyme galactowaldenase.

(1.1)



Uridine diphosphate glucose



Uridine diphosphate galactose

1.1-2. The occurrence of pentose phosphates in metabolizing systems, the results of experiments concerned with the way in which C^{14} is distributed in degradation products from an initial location in glucose, and a requirement in certain systems for triphosphopyridine nucleotide (TPN) rather than DPN has led to the construction of a second scheme of glucose degradation called the pentose phosphate pathway. It is conveniently considered in two sections, the first being the TPN-dependent oxidation of glucose-6-phosphate to ribulose-5-phosphate, the second a moderately complicated set of interconversions between pentoses, trioses, tetroses, hexoses, and heptoses.

D-Glucose-6-phosphate is oxidized by TPN to 6-phospho-δ-gluconolactone which is hydrolyzed to 6-phosphogluconic acid. Although it is possible that the acid reacts with TPN in a concerted decarboxylation-oxidation, it is more likely that oxidation with TPN forms a 3-keto acid, which subsequently decarboxylates to an enolate ion, the latter acquiring a proton to yield ribulose-5-phosphate (Fig. 1.4).

D-Ribulose-5-phosphate is converted by enolization and ketonization into two isomeric pentose phosphates, D-ribose-5-phosphate and D-xylulose-5-phosphate. A two-carbon fragment is removed from the xylulose phosphate by addition of thiamin to the carbonyl group followed by dealdolization, then transferred to the ribose phosphate in an aldol