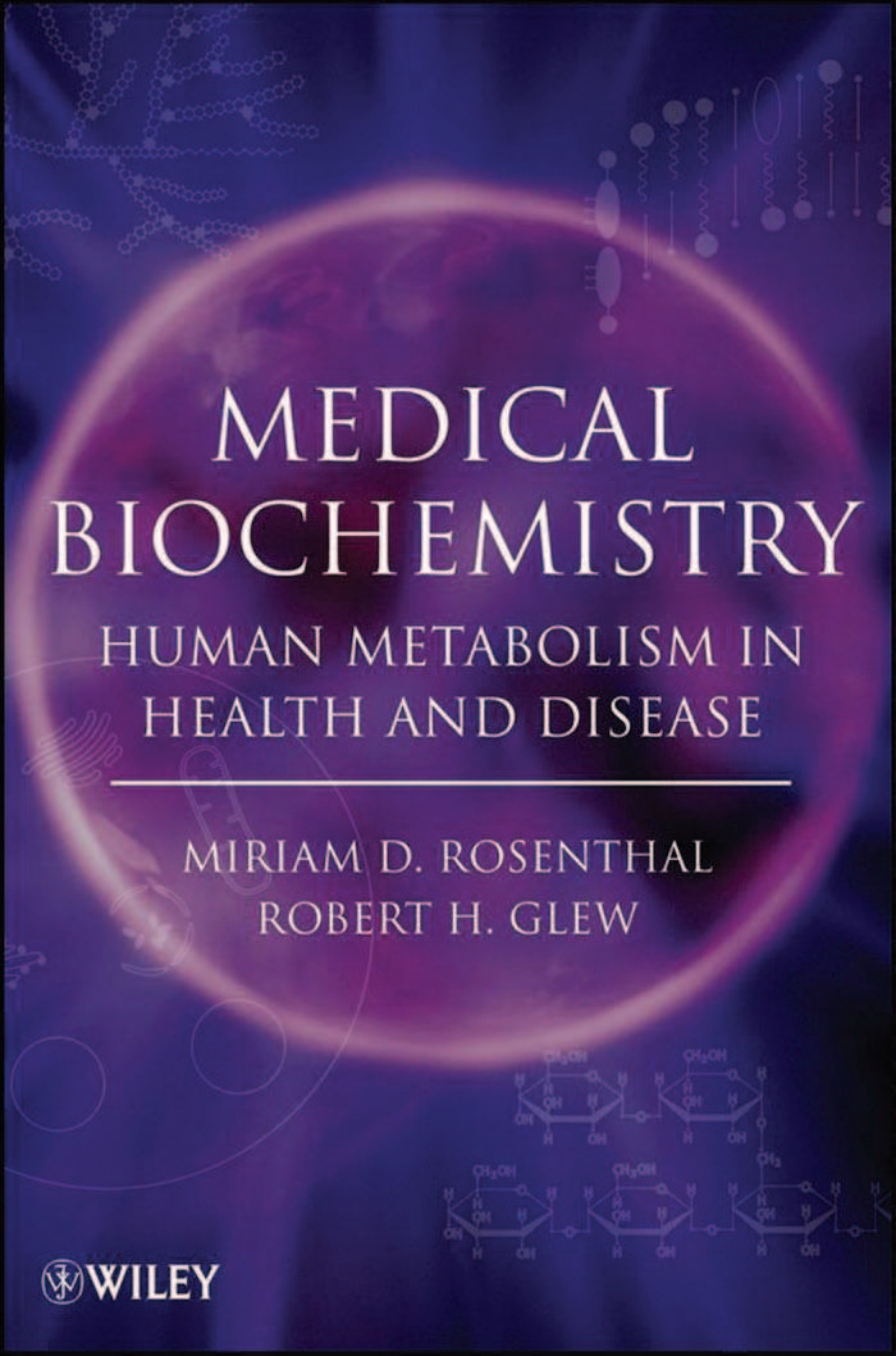
MEDICAL
BIOCHEMISTRY
HUMAN METABOLISM IN
HEALTH AND DISEASE
MIRIAM D. ROSENTHAL
ROBERT H. GLEW
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MEDICAL BIOCHEMISTRY

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Human Metabolism in Health and Disease

MIRIAM D. ROSENTHAL
ROBERT H. GLEW

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PREFACE

Human metabolism is a key component of the basic science knowledge that underlies the practice of medicine and allied health professions. It is fundamental to understanding how the body adapts to physiologic stress, how defects in metabolism result in disease, and why data from the clinical chemistry laboratory are useful to diagnose disease and monitor the efficacy of treatment. Over the more than three decades that each of the authors has been teaching biochemistry to medical students, we have found students increasingly overwhelmed with details that tend to obscure rather than elucidate principles of human metabolism.

Our main aim in writing this book was to provide students in the health professions with a concise resource that will help them understand and appreciate the functions, constituent reactions, and regulatory aspects of the core pathways that constitute human metabolism and which are responsible for maintaining homeostasis and well-being in humans. We have tried to accomplish this by emphasizing function, regulation, and disease processes, while minimizing discussion of reaction mechanisms and details of enzyme structure.

Each chapter is organized in a consistent manner beginning with an explanation of the main functions of the pathway under discussion. Next comes a brief accounting of the cells, tissues, and organs in which the pathway is expressed and the conditions under which the normal function of the pathway is especially important. The bulk of each chapter is devoted to the reactions that account for the function of the pathway, with emphasis on key steps in the pathway. The next section of each chapter discusses the ways in which the activity of the pathway is regulated by hormones, genetic factors, or changes in the intracellular concentration of key metabolites. Each chapter concludes with a discussion of the more common and illustrative diseases that result from defects in or derangements of regulation of the pathway.

This volume is deliberately modest in size. Instead of providing exhaustive coverage of all the reactions that human cells and tissues are capable of executing, we have chosen examples that illustrate the physiologic and pathophysiologic significance of the topic. The authors' expectation is that each chapter will be read for comprehension rather than to provide abundant fact and detail. During their subsequent education and professional careers, the readers will undoubtably have need for more information on many topics discussed in this book. We hope that this book will provide them with the tools to comprehend and appreciate the detailed resources—both print and electronic—that contain the ever-expanding body of knowledge on human metabolism in health and disease.

MIRIAM D. ROSENTHAL
ROBERT H. GLEW

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Drs. Rosenthal and Glew previously coedited *Clinical Studies in Medical Biochemistry* (3rd ed., 2006, Oxford University Press, New York). The book uses case presentations to develop the contextual basis of human metabolism, nutrition, and the molecular bases of disease.

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CHAPTER 1

INTRODUCTION TO METABOLISM

1.1 INTRODUCTION

Intermediary metabolism is the name given to the sequences of biochemical reactions that degrade, synthesize, or interconvert small molecules inside living cells. Knowledge of the core metabolic pathways and their interrelations is critical to understanding both normal function and the metabolic basis of most human diseases. Rational interpretation and application of data from the clinical chemistry laboratory also requires a sound grasp of the major metabolic pathways. Furthermore, knowledge of key biochemical reactions in the two dozen or so core metabolic pathways in humans is essential for an understanding of the molecular basis of drug action, drug interactions, and the many genetic diseases that are caused by the absence of the activity of a particular protein or enzyme.

1.1.1 Metabolic Pathways

Metabolism occurs in small discrete steps, each of which is catalyzed by an enzyme. The term *metabolic pathway* refers to a particular set of reactions that carries out a certain function or functions. The pathway of gluconeogenesis or glucose synthesis, for example, operates mainly during a period of fasting, and its primary function is to maintain the concentration of glucose in the circulation at levels that are required by glucose-dependent tissues such as the brain and red blood cells. Another example of a metabolic pathway is the tricarboxylic acid (TCA) cycle, which oxidizes the two

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carbons of acetyl-coenzyme A (acetyl-CoA) to CO_2 and water, thus completing the catabolism of carbohydrates, fats (fatty acids), and proteins (amino acids).

1.1.2 Metabolic Intermediates

Biochemical pathways are comprised of organic compounds called *metabolic intermediates*, all of which contain carbon, hydrogen, and oxygen. Some metabolic intermediates also contain nitrogen or sulfur. In most instances, these compounds themselves have no function. An exception would be a compound such as citric acid, which is both an intermediate in the TCA cycle and a key regulator of other pathways, including oxidation of glucose (glycolysis) and gluconeogenesis.

1.1.3 Homeostasis

Homeostasis refers to an organism's tendency or drive to maintain the normalcy of its internal environment, including maintaining the concentration of nutrients and metabolites within relatively strict limits. A good example is glucose homeostasis. In the face of widely varying physiological conditions, such as fasting or exercise, both of which tend to lower blood glucose, or following the consumption of a carbohydrate meal that raises the blood glucose concentration, the human body activates hormonal mechanisms that operate to maintain blood glucose within rather narrow limits, 80 to 100 mg/dL (Fig. 1-1). Hypoglycemia (low blood glucose) stimulates the release of gluconeogenic hormones such as glucagon and hydrocortisone, which promote the breakdown of liver glycogen and the synthesis of glucose in the liver (gluconeogenesis), followed by the release of glucose into the blood. On the other hand, hyperglycemia (elevated blood glucose) stimulates the release of insulin, which promotes the uptake of glucose and its utilization, storage as glycogen, and conversion to fat.

Maintenance of the blood calcium concentration between strict limits is another example of homeostasis. The normal total plasma calcium concentration is in the range 8.0 to 9.5 mg/dL. If the calcium concentration remains above the upper limit of normal for an extended period of time, calcium may deposit, with pathological consequences in soft tissues such as the heart and pancreas. Hypocalcemia (a.k.a. tetany) can result in muscle paralysis, convulsions, and even death; chronic hypocalcemia causes rickets in children and osteomalacia in adults. The body uses vitamin D and certain hormones (e.g., parathyroid hormone, calcitonin) to maintain calcium homeostasis.

1.2 WHAT DO METABOLIC PATHWAYS ACCOMPLISH?

1.2.1 Generation of Energy

The primary dietary fuels for human beings are carbohydrates and fats (triacylglycerols). The human body also obtains energy from dietary protein and—for some

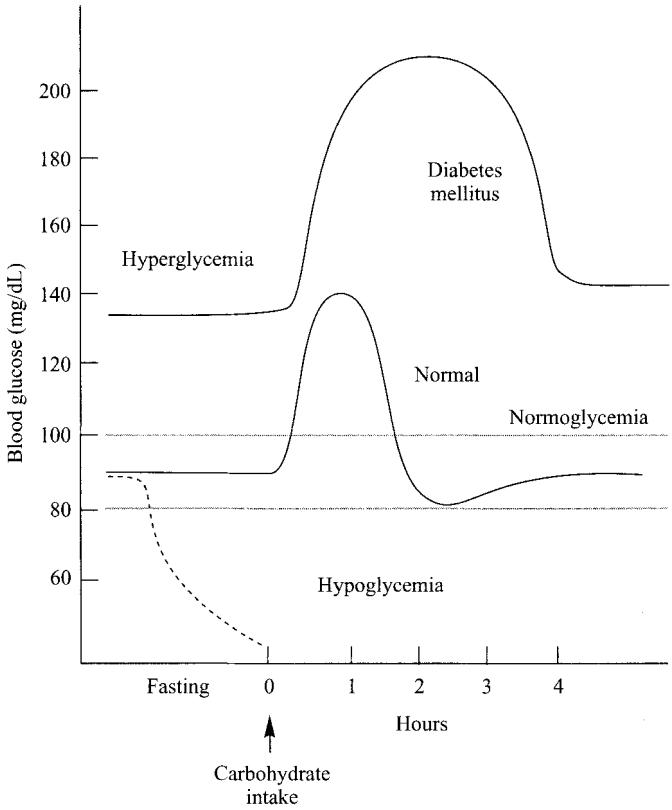

FIGURE 1-1 Changes that occur in the blood glucose concentration in a healthy adult, a person with type II diabetes mellitus, and a person experiencing fasting hypoglycemia. Following ingestion of a carbohydrate-containing meal, there are three features that distinguish the glucose vs. time curve for the person with type II diabetes relative to the healthy adult: (1) the initial blood glucose concentration is higher (approx. 135 vs. 90 mg/dL), (2) the rise in in the glucose level following the meal is greater; and (3) it takes longer for the glucose concentration to return to the fasting glucose level.

people—ethanol. Metabolism of these fuels generates energy, much of which is captured as the high-energy molecule adenosine triphosphate (ATP) (Fig. 1-2). The ATP can be used for biosynthetic processes (e.g., protein synthesis), muscle contraction, and active transport of ions and other solutes across membranes.

1.2.2 Degradation or Catabolism of Organic Molecules

Catabolic pathways usually involve cleavage of C–O, C–N, or C–C bonds. Most intracellular catabolic pathways are oxidative and involve transfer of reducing equivalents (hydrogen atoms) to nicotinamide-adenine dinucleotide (NAD^+) or flavine-adenine dinucleotide (FAD). The reducing equivalents in the resulting NADH or

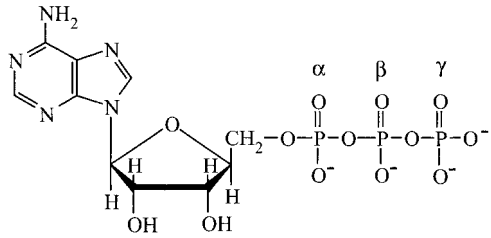

FIGURE 1-2 Structure of adenosine triphosphate.

$FADH_2$ can then be used in biosynthetic reactions or transferred to the mitochondrial electron-transport chain for generation of ATP.

1.2.2.1 Digestion. Before dietary fuels can be absorbed into the body, they must be broken down into simpler molecules. Thus, starch is hydrolyzed to yield glucose, and proteins are hydrolyzed to their constituent amino acids.

1.2.2.2 Glycolysis. Glycolysis is the oxidation of glucose into the three-carbon compound pyruvic acid.

1.2.2.3 Fatty Acid Oxidation. The major route of fatty acid degradation is β-oxidation, which accomplishes stepwise two-carbon cleavage of fatty acids into acetyl-CoA.

1.2.2.4 Amino Acid Catabolism. Breakdown of most of the 20 common amino acids is initiated by removal of the α-amino group of the amino acid via transamination. The resulting carbon skeletons are then further catabolized to generate energy or are used to synthesize other molecules (e.g., glucose, ketones). The nitrogen atoms of amino acids can be utilized for the synthesis of other nitrogenous compounds, such as heme, purines, and pyrimidines. Excess nitrogen is excreted in the form of urea.

1.2.3 Synthesis of Cellular Building Blocks and Precursors of Macromolecules

1.2.3.1 Gluconeogenesis: Synthesis of Glucose. This pathway produces glucose from glycerol, pyruvate, lactate, and the carbon skeletons of certain (glucogenic) amino acids. Gluconeogenesis is crucial to maintaining an adequate supply of glucose to the brain during fasting and starvation.

1.2.3.2 Synthesis of Fatty Acids. Excess dietary carbohydrates and the carbon skeletons of ketogenic amino acids are catabolized to acetyl-CoA, which is then utilized for the synthesis of long-chain (C16 and C18) fatty acids. Storage of these fatty acids as adipocyte triacylglycerols provides the major fuel source during the fasted state.

1.2.3.3 Synthesis of Heme. Heme is a component of the oxygen-binding proteins hemoglobin and myoglobin. Heme also functions as part of cytochromes, both in the mitochondrial electron transport chain involved in respiration-dependent ATP synthesis and in certain oxidation–reduction enzymes, such as the microsomal mixed-function oxygenases (e.g., cytochrome P450). Although most heme synthesis occurs in hemopoietic tissues (e.g., bone marrow), nearly all cells of the body synthesize heme for their own cytochromes and heme-containing enzymes.

1.2.4 Storage of Energy

Cells have only a modest ability to accumulate ATP, the major high-energy molecule in human metabolism. The human body can store energy in various forms, described below.

1.2.4.1 Creatine Phosphate. Most cells, especially muscle, can store a limited amount of energy in the form of creatine phosphate. This is accomplished by a reversible process catalyzed by creatine kinase:

$$\text{ATP} + \text{creatine} \rightleftharpoons \text{creatine phosphate} + \text{ADP}$$

When a cell's need for energy is at a minimum, the reaction tends toward the right. By contrast, when the cell requires ATP for mechanical work, ion pumping, or as substrate in one biosynthetic pathway or another, the reaction tends to the left, thereby making ATP available.

1.2.4.2 Glycogen. Glycogen is the polymeric, storage form of glucose. Nearly all of the body's glycogen is contained in muscle (approximately 600 g) and liver (approximately 300 g), with small amounts in brain and type II alveolar cells in the lung. Glycogen serves two very different functions in muscle and liver. Liver glycogen is utilized to maintain a constant supply of glucose in the blood. By contrast, muscle glycogen does not serve as a reservoir for blood glucose. Instead, muscle glycogen is broken down when that tissue requires energy, releasing glucose, which is subsequently oxidized to provide energy for muscle work.

1.2.4.3 Fat or Triacylglycerol. Whereas the body's capacity to store energy in the form of glycogen is limited, its capacity for fat storage is almost limitless. After a meal, excess dietary carbohydrates are metabolized to fatty acids in the liver. Whereas some of these endogenously synthesized fatty acids, as well as some of the fatty acids obtained through the digestion of dietary fat, are used directly as fuel by peripheral tissues, most of these fatty acids are stored in adipocytes in the form of triacylglycerols. When additional metabolic fuel is required during periods of fasting or exercise, the triacylglycerol stores in adipose are mobilized and the fatty acids are made available to tissues such as muscle and liver.

1.2.5 Excretion of Potentially Harmful Substances

1.2.5.1 Urea Cycle. This metabolic pathway takes place in the liver and synthesizes urea from the ammonia (ammonium ions) derived from the catabolism of amino acids and pyrimidines. Urea synthesis is one of the body's major mechanisms for detoxifying and excreting ammonia.

1.2.5.2 Bile Acid Synthesis. Metabolism of cholesterol to bile acids in the liver serves two purposes: (1) it provides the intestine with bile salts, whose emulsifying properties facilitate fat digestion and absorption, and (2) it is a mechanism for disposing of excess cholesterol. Humans cannot break open any of the four rings of cholesterol, nor can they oxidize cholesterol to carbon dioxide and water. Thus, biliary excretion of cholesterol—both as cholesterol per se and as bile salts—is the only mechanism the body has for disposing of significant quantities of cholesterol.

1.2.5.3 Heme Catabolism. When heme-containing proteins (e.g., hemoglobin, myoglobin) and enzymes (e.g., catalase) are turned over, the heme moiety is oxidized to bilirubin, which after conjugation with glucuronic acid is excreted via the hepatobiliary system.

1.2.6 Generation of Regulatory Substances

Metabolic pathways generate molecules that play key regulatory roles. As indicated above, citric acid (produced in the TCA cycle) plays a major role in coordinating the activities of the pathways of glycolysis and gluconeogenesis. Another example of a regulatory molecule is 2,3-bisphosphoglyceric acid, which is produced in a side reaction off the glycolytic pathway and modulates the affinity of hemoglobin for oxygen.

1.3 GENERAL PRINCIPLES COMMON TO METABOLIC PATHWAYS

1.3.1 ATP Provides Energy for Synthesis

Anabolic or synthetic pathways require input of energy in the form of the high-energy bonds of ATP, which is generated directly during some catabolic reactions (such as glycolysis) as well as during mitochondrial oxidative phosphorylation.

1.3.2 Many Metabolic Reactions Involve Oxidation or Reduction

During catalysis, oxidative reactions transfer reducing equivalents (hydrogen atoms) to cofactors such as NAD^+, $NADP^+$ (nicotinamide-adenine dinucleotide phosphate) or FAD. Reduced NADH and $FADH_2$ can then be used to generate ATP through oxidative phosphorylation in mitochondria. NADPH is the main source of reducing equivalents for anabolic, energy-requiring pathways such as fatty acid and cholesterol synthesis.

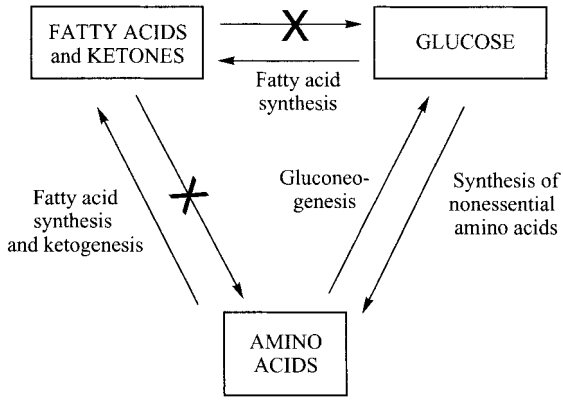

FIGURE 1-3 Possible interconversions of the three major metabolic fuels in humans. Note that glucose and amino acids cannot be synthesized from (even-carbon) fatty acids.

1.3.3 Only Certain Metabolic Reactions Occur in Human Metabolism

It is important to appreciate that although humans possess the machinery to interconvert many dietary components, not all interconversions are possible. Thus, humans can convert glucose into long-chain fatty acids, but they cannot convert even-carbon-numbered long-chain fatty acids into glucose (Fig. 1-3).

1.3.4 Some Organic Molecules Are Nutritionally Essential to Human Health

Certain key cellular components cannot be synthesized in the body and must therefore be provided preformed in the diet and are therefore designated as *essential*. These molecules include two polyunsaturated fatty acids (linoleic and α-linolenic) and the carbon skeletons of some of the amino acids. They also include the vitamins (such as thiamine and niacin), most of which serve as components of enzymatic cofactors. By contrast, other important compounds, such as glucose and palmitic acid, are not essential in the diet. Glucose, whose blood levels are crucial to homeostasis, can be synthesized from glycerol, lactate, pyruvate, and the carbon skeletons of glucogenic amino acids when dietary glucose is not available.

1.3.5 Some Metabolic Pathways Are Irreversible or Contain Irreversible Steps

One example of an irreversible pathway is glycolysis, the multistep catabolic pathway that oxidizes glucose to pyruvate or lactate. Gluconeogenesis is essentially the reverse of glycolysis and is the process by which pyruvate (or a number of other molecules such as lactate and the carbon skeleton of the amino acid alanine) can be used to synthesized glucose. Although glycolysis and gluconeogenesis share many enzymes,

specific gluconeogenic enzymes are required to bypass the steps in glycolysis that are irreversible under physiological conditions.

1.3.6 Metabolic Pathways Are Interconnected

The initial step in glycolysis is the phosphorylation of glucose to form glucose 6-phosphate. Glucose 6-phosphate is also utilized in two other key metabolic pathways: glycogen synthesis and the pentose phosphate pathway (a.k.a. the hexose monophosphate shunt), which generates ribose 5-phosphate and NADPH.

1.3.7 Metabolic Pathways Are Not Necessarily Linear

Both the tricarboxylic acid (TCA) cycle and the urea cycle are circular pathways. In each case the pathway is initiated by addition of a small molecule to a key metabolic intermediate (oxaloacetate in the TCA cycle and ornithine in the urea cycle). At the end of one cycle, the key intermediate is regenerated and available to participate in another turn of the cycle. Although the TCA and urea cycles can be depicted as simple circular pathways, metabolites can enter into—or be removed from—the pathways at intermediate steps. For example, the amino acid glutamate can be used to generate α-ketoglutarate, a key intermediate in the TCA cycle.

1.3.8 Metabolic Pathways Are Localized to Specific Compartments Within the Cell

Many metabolic pathways occur within the mitochondria, including β-oxidation of fatty acids, the TCA cycle, and oxidative phosphorylation (Fig. 1-4). Other pathways are cytosolic, including glycolysis, the pentose phosphate pathway, and fatty acid synthesis. Still others, including the urea cycle and heme synthesis, utilize both mitochondrial and cytosolic enzymes at different points in the pathways.

1.3.9 A Different Repertoire of Pathways Occurs in Different Organs

All cells are capable of oxidizing glucose to pyruvate via glycolysis to generate ATP. However, since red blood cells lack mitochondria, they cannot further oxidize the resulting pyruvate to CO_2 and water via pyruvate dehydrogenase and the TCA cycle. Instead, the pyruvate is converted to lactate and released from the red blood cells.

Most cells and organs can also utilize fatty acids as fuels. Although neural cells do contain mitochondria, they do not oxidize fatty acids. The brain is therefore dependent on a constant supply of glucose to provide energy. The gluconeogenesis pathway that provides glucose for the brain occurs in the liver and to a lesser extent in the renal cortex.

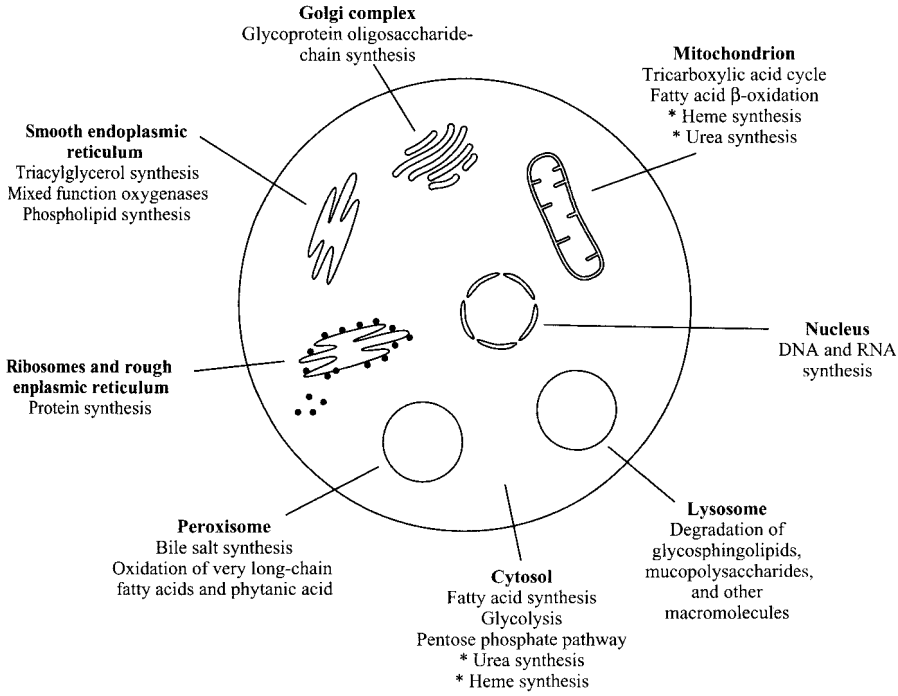

FIGURE 1-4 A liver cell, showing where various metabolic pathways occur. An asterisk indicates a pathway, portions of which occur in more than one intracellular compartment.

1.3.10 Different Metabolic Processes Occur in the Fed State Than During Fasting

After a meal, metabolic pathways are utilized to process the digested foods and store metabolites for future utilization. Postprandially, glucose is plentiful and utilized both for energy generation and to replenish glycogen stores (primarily in muscle and liver). Excess glucose is metabolized to fatty acids in liver and fat cells and the resulting triacylglycerols are stored in adipocytes.

By contrast, when a person is fasting there is a need to generate energy from endogenous fuels. Consequently, the metabolic pathways involved in fuel metabolism are regulated in such a way as to promote the oxidation of stored fuels, including the fatty acids stored in adipose tissue in the form of triacylglycerols and, to a lesser extent, glycogen stored in liver and muscle. In fact, during a fast, most of the body's energy needs are satisfied by the oxidation of fatty acids.

1.3.11 Metabolic Pathways Are Regulated

All this specialization of organs and coordination of metabolism in the fed or fasted states is a highly regulated process with several levels of regulation. One level of

regulation is gene transcription and translation, which determines which enzymes are actually present within a cell. A second level of control is substrate-level regulation, whereby concentrations of key metabolites activate or inhibit enzymatic reactions. A metabolite that acts to regulate several pathways is citrate, which both inhibits glycolysis and activates the first step in the pathway of fatty acid synthesis.

Hormones represent yet another level of control. Hormones act to coordinate processes between the organs of complex, multicellular organisms. For example, insulin, the main hormonal signal of the fed state, regulates both enzyme activity (at the level of enzyme dephosphorylation) and gene transcription.

1.4 WHAT IS THE BEST WAY TO COMPREHEND AND RETAIN A WORKING KNOWLEDGE OF INTERMEDIARY METABOLISM?

Before learning about the various enzyme-catalyzed reactions and intermediates that comprise a particular metabolic pathway, one should appreciate the major functions which that pathway serves in the body and how the pathway relates to other pathways. Particularly in the context of medical biochemistry, it is also important to understand how the pathway is regulated and how it affects (or is affected by) disease processes. As you go through this book you will find that each chapter is organized so as to answer the following questions:

1. Why does the pathway exist? That is, what are its functions?
2. Where does the pathway take place (i.e., what organ, tissue, cell, subcellular compartment, or organelle)?
3. When does the pathway operate, and when is it down-regulated: during the fasted state or the fed state; during rest or extreme physical activity; during a particular stage of development (e.g., the embryo, the neonate, old age)?
4. What are the actual steps of the pathway, and what cofactors does it require?
5. How is the pathway regulated?
6. What can go wrong? Problems can include hormonal dysregulation (e.g., diabetes mellitus), inborn errors of metabolism (e.g., adrenoleukodystrophy), and nutritional deficiencies (e.g., protein–calorie malnutrition, iron-deficiency anemia). Normal metabolic homeostasis is also profoundly altered by toxins and during infections.

CHAPTER 2

ENZYMES

2.1 THE NATURE OF ENZYMES

Enzymes are catalysts that greatly increase the rate of chemical reactions and thus make possible the numerous and diverse metabolic processes that occur in the human body. Catalysts increase the rate of a reaction without affecting its equilibrium. Enzymes can increase the rate of physiological reactions by as much as 10^{10}-fold. They accomplish this feat by decreasing the amount of energy required for activation of the initial reactants (substrates), thereby increasing the percentage of substrate molecules that have sufficient energy to react (Fig. 2-1).

With the exception of a few ribonucleic acid (RNA) molecules (ribozymes) that catalyze reactions involving nucleic acids, enzymes are proteins. Every enzyme has an active site that is composed of specific amino acid side chains which are brought into close proximity when the enzyme is folded into its active conformation. During the course of the reaction that it catalyzes, the enzyme's active site stabilizes the transition state, which is an intermediate conformation between substrates and products. The interaction between active site and substrate(s) is thus responsible for the catalytic efficiency of the enzyme as well as its substrate specificity. After the reaction occurs, the products are released from the enzyme and the active site is available to bind additional substrate molecules.

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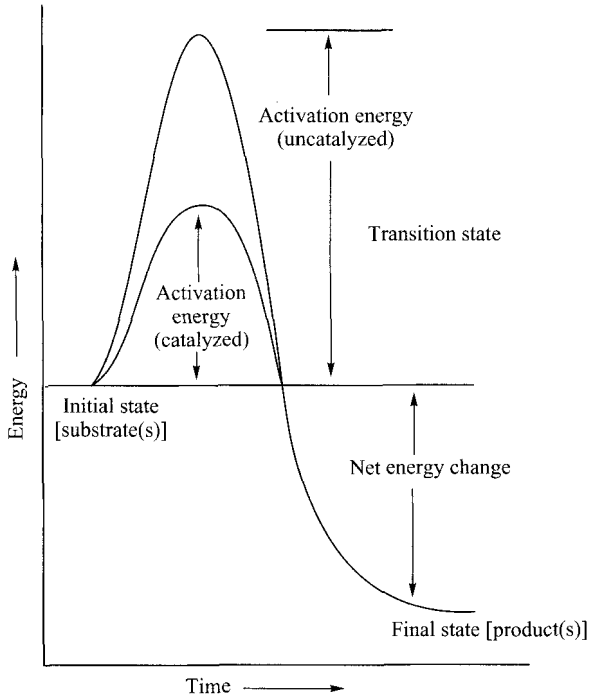


FIGURE 2-1 Activation energy of a chemical reaction.

2.2 TYPES OF ENZYMES

There are more than 2500 different enzymes in the human body. It is useful to group them into six major classes based on the type of reaction they catalyze.

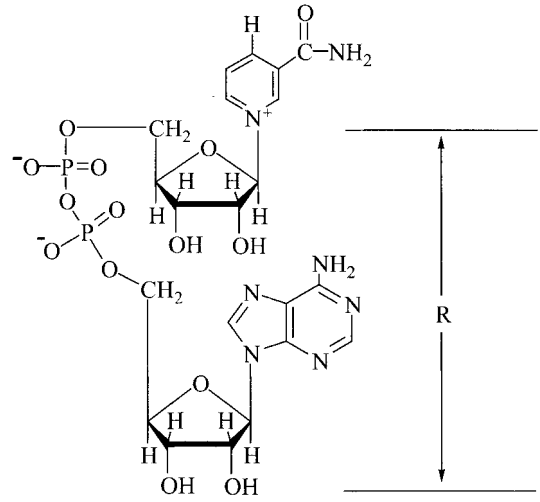
2.2.1 Oxidoreductases

Oxidative reactions remove electrons, usually one or two electrons per molecule of substrate, while reductive reactions accomplish the converse. The substrate in an oxidation–reduction reaction may be a metal, as in the case of the one-electron oxidation of the ferrous ion of hemoglobin to the ferric ion of methemoglobin, or an organic compound as illustrated by the two-electron, reversible oxidation of lactate to pyruvate.

Oxidoreductases transfer electrons from one compound to another, thus changing the oxidation state of both substrates. Some oxidoreductases, such as lactate dehydrogenase, catalyze the removal of two hydrogen atoms (two electrons plus two hydrogen ions) to an acceptor molecule such as nicotinamide-adenine dinucleotide (NAD^+) as illustrated by the lactate dehydrogenase reaction (Fig. 2-2):

$$\text{lactate} + NAD^+ \rightleftharpoons \text{pyruvate} + NADH + H^+$$

A



Oxidized nicotinamide adenine dinucleotide (NAD^+)

B

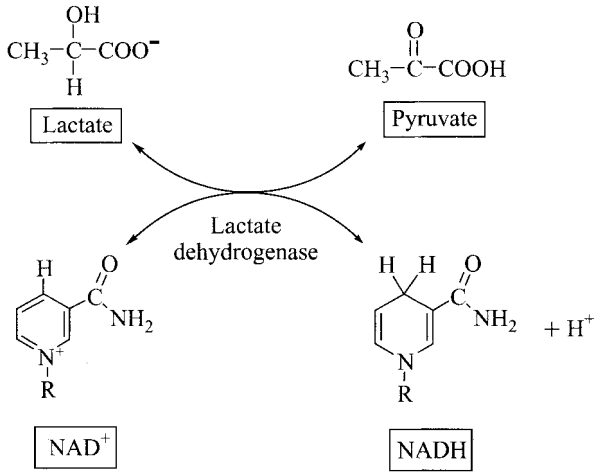


FIGURE 2-2 Lactate dehydrogenase is an oxidoreductase that uses the cofactor NAD^+ as a hydrogen acceptor: (A) structure of NAD^+; (B) lactate dehydrogenase reaction.

A second cofactor that serves as an acceptor of hydrogen atoms is flavin-adenine dinucleotide (FAD, Fig. 2-3):

$$\text{succinate} + \text{FAD} \rightarrow \text{fumarate} + \text{FADH}_2$$

In general, most oxidation–reduction (redox) reactions that oxidize oxygen-bearing carbons utilize NAD^+ (or the related cofactor $NADP^+$), whereas reductions or oxidations of carbon atoms that do not have oxygen attached utilize flavin mononucleotides (FMN or FAD).

Other oxidoreductases, such as 5-lipoxygenase (Fig. 2-4A), are dioxygenases, which catalyze the addition of both atoms of molecular oxygen into the substrate:

$$\text{arachidonic acid} + \text{O}_2 \rightarrow \text{5-hydroperoxyeicosatetraenoic acid}$$

Still other oxidoreductases are monooxygenases or mixed-function oxidases, which catalyze even more complex reactions. For example, phenylalanine hydroxylase (Fig. 2-4B) catalyzes the reaction

$$\text{phenylalanine} + \text{O}_2 + \text{BH}_4 \rightarrow \text{tyrosine} + \text{BH}_2 + \text{H}_2\text{O}$$

In this reaction, two organic substrates are oxidized: One atom of molecular oxygen is used to oxidize phenylalanine; the other combines with the two hydrogen atoms removed from tetrahydrobiopterin (BH_4), generating dihydrobiopterin (BH_2) and water.

2.2.2 Transferases

Transferases catalyze reactions in which a functional group is transferred from one compound to another. Transaminases, such as aspartate aminotransferase (Fig. 2-5A), catalyze the reversible transfer of an amino group from an amino acid to an α-ketoacid, thus generating a new amino acid and a new α-ketoacid:

$$\text{aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

Similarly, kinases transfer phosphate groups from adenosine triphosphate (ATP) to acceptor molecules such as glucose in the reaction catalyzed by hexokinase or glucokinase (Fig. 2-5B):

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{adenosine diphosphate (ADP)}$$

Unlike the aminotransferase reactions, which are reversible, most reactions catalyzed by kinases are irreversible under physiological conditions.

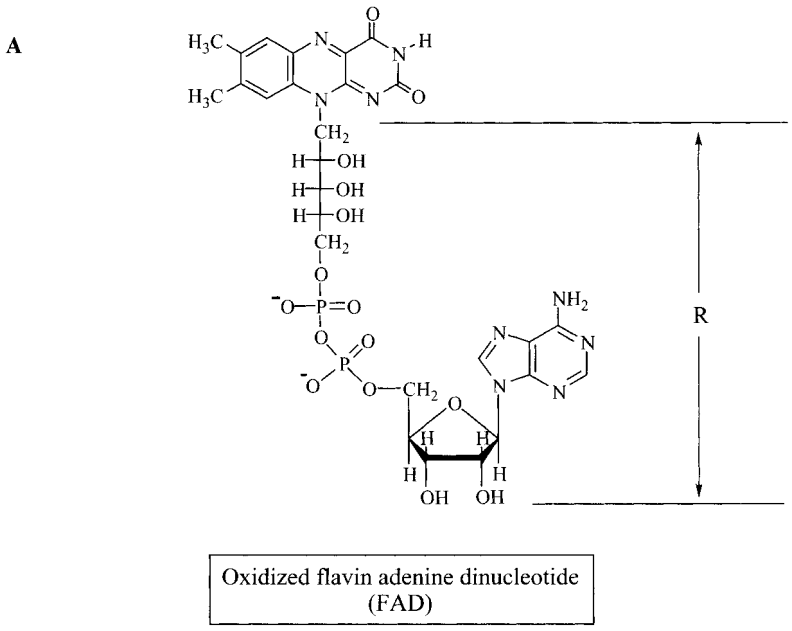


FIGURE 2-3 Succinate dehydrogenase is an oxidoreductase that uses the cofactor FAD as a hydrogen acceptor: (A) structure of FAD; (B) succinate dehydrogenase reaction.

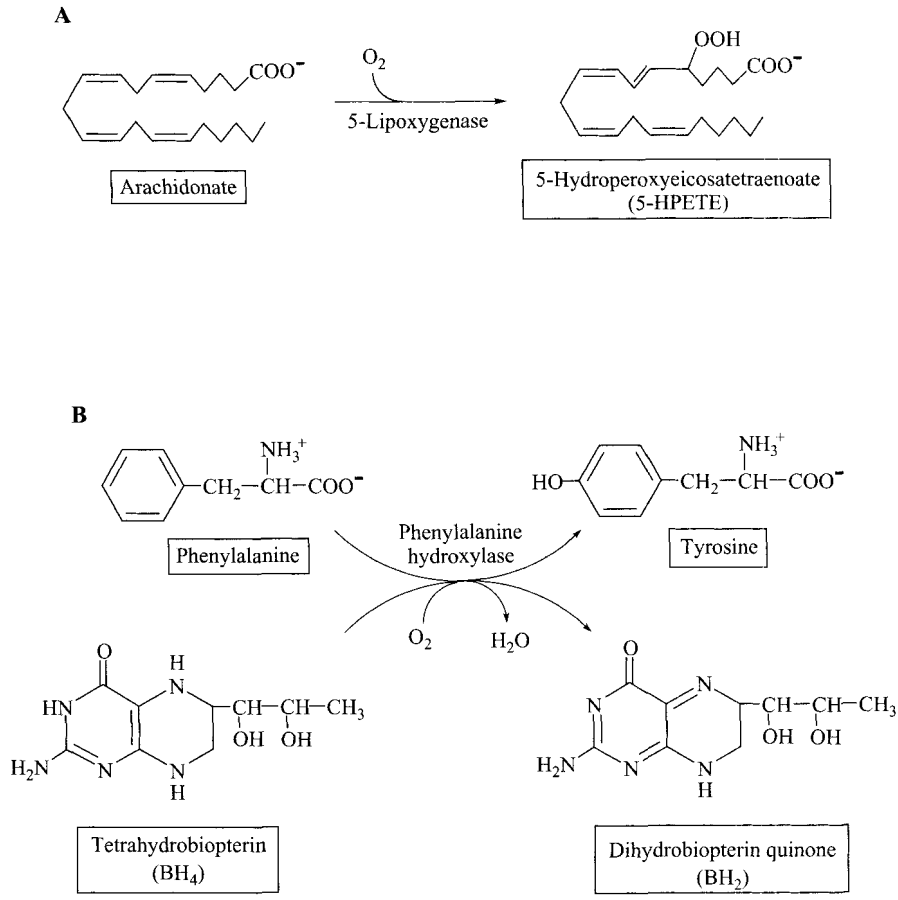


FIGURE 2-4 Oxidoreductase reactions utilizing molecular oxygen: (A) the reaction catalyzed by 5-lipoxygenase; (B) the reaction catalyzed by the sequential actions of phenylalanine hydroxylase and a subsequent dehydratase that removes water.

2.2.3 Hydrolases

Hydrolases cleave carbon–oxygen, carbon–nitrogen, or carbon–sulfur bonds by adding water across the bond. One example of a hydrolase is the digestive enzyme maltase, which hydrolyzes the glycosidic bond in the disaccharide maltose (Fig. 2-6):

$$\text{maltose} + H_2O \rightarrow 2 \text{ glucose}$$

2.2.4 Lyases

Lyases cleave carbon–oxygen, carbon–nitrogen, or carbon–sulfur bonds but do so without addition of water and without oxidizing or reducing the substrates. A good

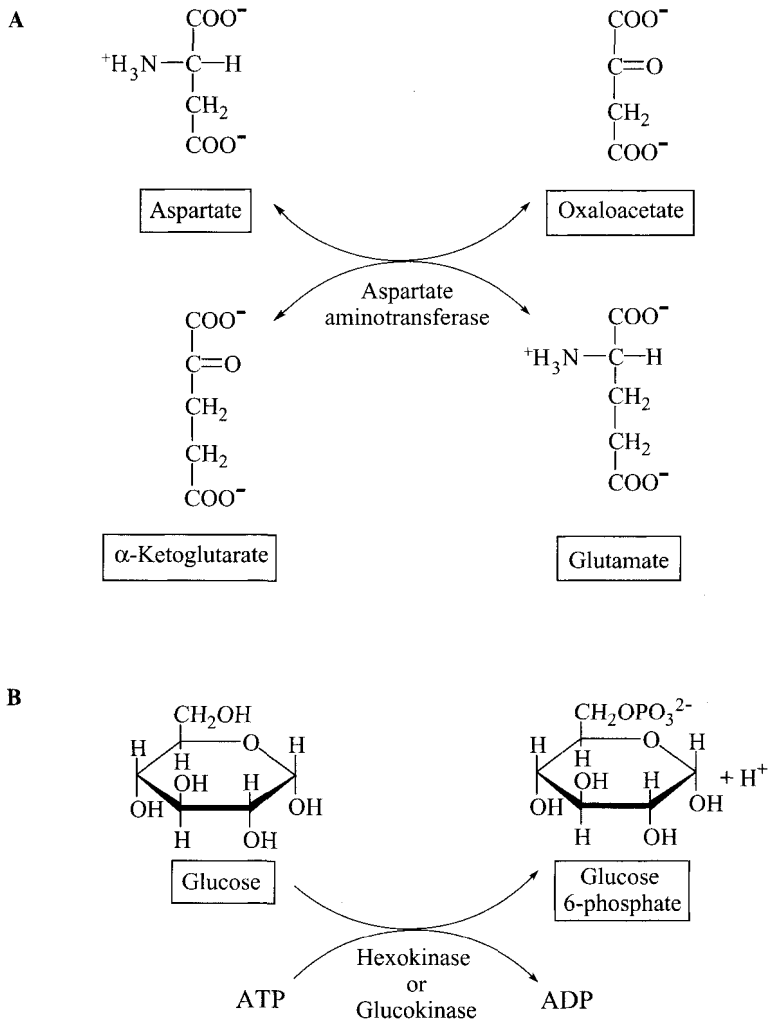


FIGURE 2-5 Transferase reactions: (A) aspartate aminotransferase uses pyridoxal phosphate (PLP) as a cofactor; (B) hexokinase and glucokinase do not utilize cofactors.

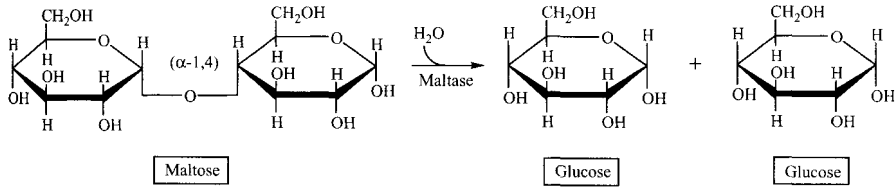


FIGURE 2-6 Maltase is a hydrolase.

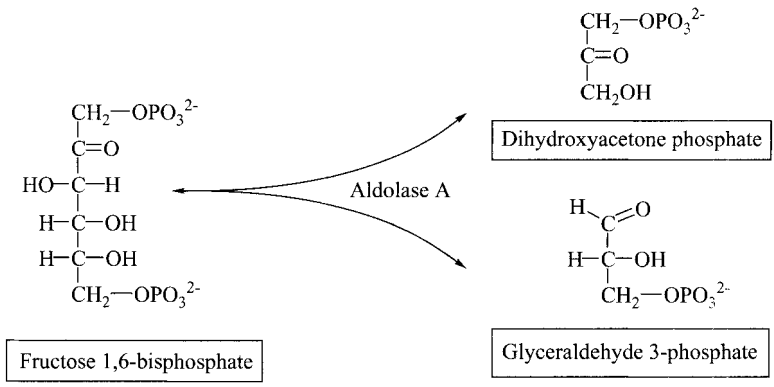


FIGURE 2-7 Aldolase A is a lyase.

example of a lyase is aldolase A (Fig. 2-7), which as an enzyme of the glycolytic pathway, catalyzes the reversible cleavage of the six-carbon sugar fructose 1,6-bisphosphate into two three-carbon sugar phosphates:

$$\text{fructose 1,6-bisphosphate} \rightleftharpoons \text{glyceraldehyde 3-phosphate} + \text{dihydroxyacetone phosphate}$$

Note that in the reverse reaction, aldolase A functions as a synthase, forming a new C–C bond.

2.2.5 Isomerases

Isomerases catalyze intramolecular rearrangements of functional groups that reversibly interconvert optical or geometric isomers. One example is glucose 6-phosphate isomerase (Fig. 2-8A), which converts glucose 6-phosphate, an aldosugar phosphate, to the isomeric keto-sugar phosphate, fructose 6-phosphate:

$$\text{glucose 6-phosphate} \rightleftharpoons \text{fructose 6-phosphate}$$

When an isomerase catalyzes an intramolecular rearrangement involving movement of a functional group, it is called a *mutase*. For example, as part of the two metabolic pathways that synthesize and break down glycogen, phosphoglucomutase (Fig. 2-8B) catalyzes the reversible transfer of a phosphate group between the hydroxyl group on C1 (of the hemiacetyl ring form of glucose) and the C6 hydroxyl group of glucose:

$$\text{glucose 6-phosphate} \rightleftharpoons \text{glucose 1-phosphate}$$

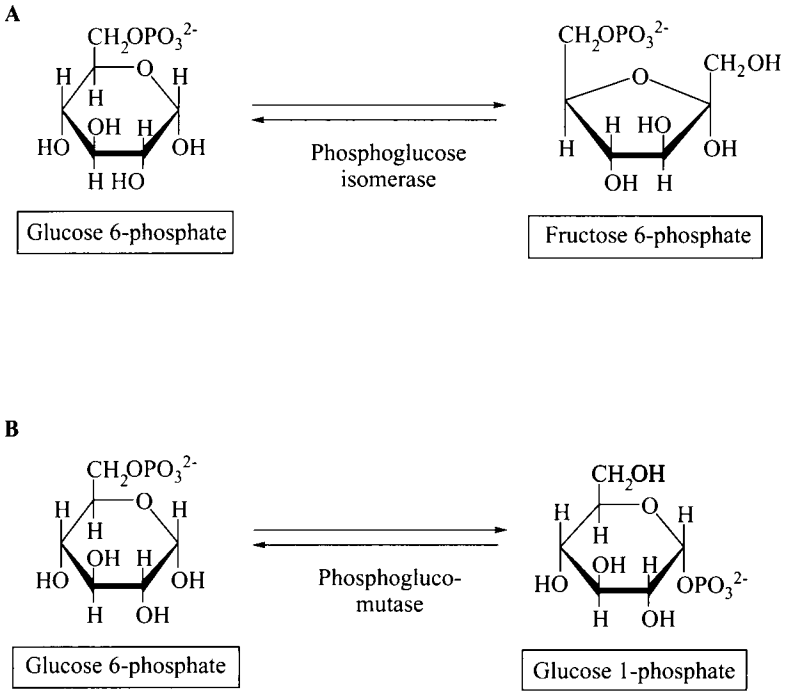


FIGURE 2-8 Reactions catalyzed by isomerases. (A) Phosphoglucose isomerase; (B) phosphoglucomutase.

2.2.6 Ligases

Ligases catalyze biosynthetic reactions that form a covalent bond between two substrates. Ligases differ from lyases such as aldolase A (discussed above) in that they utilize the energy obtained from cleavage of a high-energy bond to drive the reaction. The molecule with the high-energy bond is usually ATP, which is concurrently converted to ADP with the release of inorganic phosphate. An example of a ligase is pyruvate carboxylase, which forms a new C–C bond by adding CO_2 from bicarbonate to pyruvate, the three-carbon end product of aerobic glycolysis (Fig. 2-9):

$$\text{pyruvate} + HCO_3^- + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + P_i$$

Some ligases that catalyze synthetic reactions in which two substrates are joined and a nucleotide triphosphate (e.g., ATP) is hydrolyzed are designated by the term *synthetase*. In contrast, the term *synthase* is used to describe enzymes that catalyze reactions in which two substrates come together to form a product, but a nucleotide triphosphate is not involved in the reaction. An example of a synthase is citrate synthase, where the energy to drive the reaction is provided by the thioester of

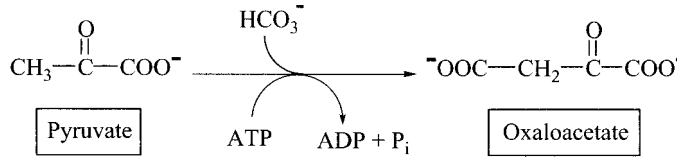


FIGURE 2-9 Pyruvate carboxylase, a biotin-containing enzyme, catalyzes a ligase reaction.

acetyl-CoA:

$$\text{oxaloacetate} + \text{acetyl-CoA} \rightarrow \text{citrate} + \text{CoASH}$$

2.2.7 Nonenzymatic Reactions

Not all physiologically or pathophysiologically relevant reactions that take place in the body are catalyzed by enzymes. For example, the covalent attachment of glucose to hemoglobin to form glycated hemoglobin (HbA1c) occurs spontaneously and does not involve an enzyme. The fact that the extent of this glycation reaction in blood is determined solely by the plasma glucose concentration is the basis for the usefulness of the HbA1c measurement as a way to monitor glucose control. The high reactivity of glucose (as well as galactose and other monosaccharides) with proteins is attributable to the intrinsic affinity of aldehyde groups for the amino groups of proteins, resulting in protein adducts that can act as neoantigens. Similarly, the covalent attachment of acetaldehyde, an intermediate in ethanol metabolism, to a wide range of proteins may account for some of the pathology associated with excessive consumption of ethanol.

Another example of an important nonenzymatic reaction in humans is the autooxidation of oxyhemoglobin to methemoglobin, which generates the superoxide anion:

$$\text{hemoglobin } (Fe^{2+}) + O_2 \rightarrow \text{methemoglobin } (Fe^{3+}) + O_2^{\bullet-}$$

Methemoglobin does not bind oxygen and is a potent oxidizing agent that can damage the red cell membrane.

2.3 SMALL MOLECULES AND METAL IONS CAN CONTRIBUTE TO ENZYME-BASED CATALYSIS

2.3.1 Cofactors

Enzymatic catalysis often involves utilization of an additional small organic molecule called a *cofactor*. Certain cofactors, such as biotin and thiamine pyrophosphate, function only when they are attached covalently to their respective enzymes. In such cases the enzyme–coenzyme complex is called a *holoenzyme*, whereas the term

apoenzyme refers to the protein component alone. In other cases, the cofactor acts more like a second substrate. A good example of this is NAD^+, which is converted to $NADH + H^+$ when it receives two hydrogen atoms (or two electrons plus protons) during the course of the redox reaction catalyzed by lactate dehydrogenase. The NADH molecule subsequently transfers the hydrogen atoms to another acceptor (e.g., FAD in the mitochondrial electron transport chain) and is thus available to participate in the catalytic dehydrogenation of another molecule of lactate. These NAD^+-utilizing enzymes are usually designated as dehydrogenases.

Most cofactors usually participate in the catalysis of many different reactions, often using a similar reaction mechanism. The cofactor does this by binding to the various enzymes, each of which has a particular active site whose structure and binding properties determine its unique substrate specificity. Thus, lactate dehydrogenase catalyzes the reaction

$$\text{lactate} + NAD^+ \rightleftharpoons \text{pyruvate} + NADH + H^+$$

whereas alcohol dehydrogenase catalyzes the reaction

$$\text{ethanol} + NAD^+ \rightleftharpoons \text{acetaldehyde} + NADH + H^+$$

2.3.2 Vitamins Are Components of Many Enzymatic Cofactors or Coenzymes

Vitamins are small organic molecules that are not synthesized in the body and are therefore essential dietary nutrients. Many of the vitamins are cofactors or components of cofactors. Because they play a catalytic role, they are required in the diet in only small amounts and are referred to as *micronutrients*. The vitamins that are cofactors or cofactor precursors include all the water-soluble B vitamins, vitamin C, and the fat-soluble vitamin K (Table 2-1).

2.3.2.1 Thiamine (Vitamin B1). Thiamine is utilized to synthesize thiamine pyrophosphate, which contributes to the transfer of active aldehyde intermediates during several reactions of carbohydrate metabolism. These include pyruvate dehydrogenase, the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase and transketolase, an enzyme that is a component of the pentose phosphate pathway.

2.3.2.2 Riboflavin (Vitamin B_2). Riboflavin is a component of FAD (flavin-adenine dinucleotide, Fig. 2-3A) and FMN (flavin mononucleotide), which participate in numerous oxidation–reduction (redox) reactions and the process of ATP generation in mitochondria. FAD-linked dehydrogenases convert succinate to fumarate in the TCA cycle and fatty acyl-CoA to β-hydroxy fatty acyl-CoA during β-oxidation of fatty acids.

TABLE 2-1 Cofactor Roles of Vitamins

Vitamin	Coenzyme	Typical Reaction Type
Thiamine (B_1)	Thiamine pyrophosphate (TPP)	Oxidative decarboxylation of α-ketoacids
Riboflavin (B_2)	Flavin-adenine dinucleotide (FAD) Flavin-adenine mononucleotide (FMN)	Oxidation–reduction
Niacin (B_3) (nicotinate)	Nicotinamide-adenine dinucleotide (NAD^+) Nicotinamide-adenine dinucleotide phosphate (NAD^+)	Oxidation–reduction
Pantothenate (B_5)	Coenzyme A (CoASH) Acyl carrier protein (ACP)	Acyl transfer
Pyridoxine (B_6) Pyridoxal (B_6) Pyridoxamine (B_6)	Pyridoxal phosphate	Transamination and deamination of amino acids
Biotin (B_7)	*N*-Carboxybiotinyl lysine	Carboxylation
Folic acid	Tetrahydrofolate (TH_4)	One-carbon transfer
Cobalamin (B_{12})	Methylcobalamin Adenosyl cobalamin	Methylation of homocysteine to methionine Conversion of methylmalony-CoA to succinyl-CoA
Ascorbic acid (C)	Ascorbic acid	Hydroxylations in the synthesis of collagen, norepinephrine, and carnitine
Phylloquinone (K) Menaquinone (K)	Vitamin K hydroquinone (KH_2)	γ-Carboxylation of glutamate residues

2.3.2.3 Niacin (Vitamin B_3). Niacin is a component of NAD^+ (nicotinamide-adenine dinucleotide) (Fig. 2-2A), and $NADP^+$ (nicotinamide-adenine dinucleotide phosphate), which participate in many redox reactions, such as those catalyzed by lactate dehydrogenase and fatty acyl-CoA dehydrogenase. $NADP^+$ differs from NAD^+ in that it has a phosphate group on C6 of the ribose moiety to which the adenosine moiety is attached. NADH, the reduced form of NAD^+, also donates electrons to the mitochondrial electron transport chain, which is a series of oxidation–reduction reactions that ultimately generate ATP. $NADP^+$ is a substrate or cofactor in the glucose 6-phosphate dehydrogenase reaction of the pentose phosphate pathway, and NADPH provides reducing equivalents for the synthesis of fatty acids and cholesterol.

2.3.2.4 Pyridoxine, Pyridoxal, and Pyridoxamine. These are forms of vitamin B_6 and precursors of pyridoxal phosphate (PLP). PLP is a cofactor for many enzymes that catalyze reactions involving amino acids, such as the various

aminotransferases, amino acid decarboxylases, and the ligase enzyme δ-amino-levulinic acid (ALA) synthetase, which catalyzes the regulated step of heme synthesis.

2.3.2.5 Biotin. Biotin is active when it is attached covalently to enzymes. It binds CO_2 and transfers this one-carbon unit to organic acceptors (e.g., acetyl-CoA, pyruvate) as part of the catalytic mechanism of enzymes such as acetyl-CoA carboxylase and pyruvate carboxylase.

2.3.2.6 Folate. Folate is the precursor of tetrahydrofolate (THF), which is the cofactor involved in the transfer of one-carbon groups other than CO_2. THF plays a central role in the synthesis of purines, which are the building blocks for both deoxyribonucleic acid (DNA) and RNA.

2.3.2.7 Cobalamin (Vitamin B_{12}). Cobalamin is the cofactor that participates in the transfer of a methyl group in the regeneration of methionine from homocysteine. Cobalamin is also the precursor of deoxyadenosylcobalamin, which is the cofactor for methylmalonyl-CoA mutase, an enzyme involved in the metabolism of propionic acid.

2.3.2.8 Pantothenic Acid. Pantothenic acid is a component of coenzyme A (CoASH) and acyl carrier protein (ACP). The sulfhydryl group of CoASH forms thioester bonds with the carboxyl groups of acetate, long-chain fatty acids, and other organic acids. CoASH serves as a carrier for the activated forms of organic acids during many reactions, including those involved in the TCA cycle, fatty acid oxidation, the catabolism of the carbon skeletons of branched-chain amino acids, and the conjugation of bile salts with glycine or taurine. Acyl carrier protein is the carrier of acyl groups during the de novo synthesis of fatty acids.

2.3.2.9 Ascorbic Acid (Vitamin C). Ascorbic acid is a cofactor in hydroxylation reactions, most prominently the hydroxylation of proline residues of collagen (Fig. 2-10) and the synthesis of norepinephrine from dopamine. Ascorbate is oxidized to dehydroascorbate during the course of these hydroxylation reactions and is regenerated by dehydroascorbate reductase, using reduced glutathione (GSH) as the source of reducing equivalents and generating oxidized glutathione (GSSG):

$$\text{dehydroascorboate} + 2\text{GSH} \rightarrow \text{ascorbic acid} + \text{GSSG}$$

2.3.2.10 Vitamin K. The two major dietary molecules with vitamin K activity are menaquinone, synthesized by bacteria, and phylloquinone, a product of green plants. Vitamin K is the cofactor for enzymes that γ-carboxylate specific glutamate residues of calcium-binding proteins (Fig. 2-11), such as prothrombin and other proteins of the blood-clotting cascade, and osteocalcin, a major bone protein. Vitamin K undergoes oxidation during γ-carboxylation reactions and is subsequently regenerated in

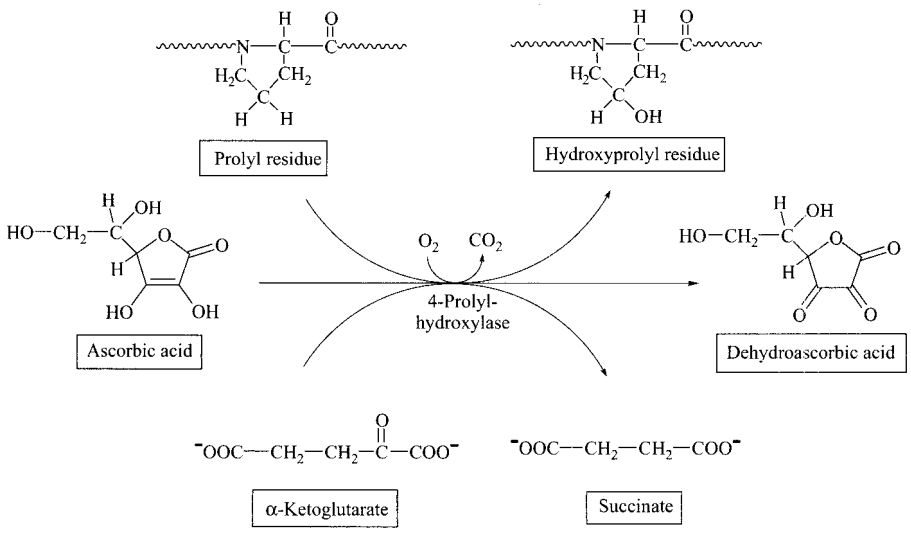


FIGURE 2-10 Role of ascorbic acid in the hydroxylation of a prolyl residue in collagen.

two reduction reactions catalyzed by vitamin K epoxide reductase and vitamin K reductase, respectively.

2.3.2.11 Not All Cofactors Are Derived from Vitamins. It is worth emphasizing that not all cofactors are synthesized from a vitamin. For example, since tetrahydrobiopterin (BH_4, Fig. 2-4B), the cofactor for phenylalanine hydroxylase and other enzymes that hydroxylate aromatic amino acids, is synthesized in the body from guanosine triphosphate (GTP), it is not a vitamin. Similarly, lipoic acid, which is one of several cofactors for the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes, is not a vitamin. It should also be noted that not all vitamins are precursors of cofactors. Indeed, vitamin K is the only one of the four fat-soluble vitamins that plays a direct catalytic role in an enzyme-catalyzed reaction in the body. Two other fat-soluble vitamins, retinol (vitamin A) and cholecalciferol (vitamin D), are actually precursors of hormones that regulate transcription of DNA, and thus gene expression. Retinol is also the precursor of 11-*cis*-retinal, which is an important constituent of rhodopsin, the visual pigment of the eye. α-Tocopherol (vitamin E), the fourth fat-soluble vitamin, is an antioxidant.

2.3.3 Many Enzymes Utilize Metal Ions as Part of Their Catalytic Mechanisms

Many enzymes utilize inorganic ions to bind the substrate and polarize critical functional groups. Examples of metal ions and the enzymes they function with include:

Zn^{2+}: alcohol dehydrogenase, carboxypeptidase
Mg^{2+}: ATP-dependent reactions such as hexokinase

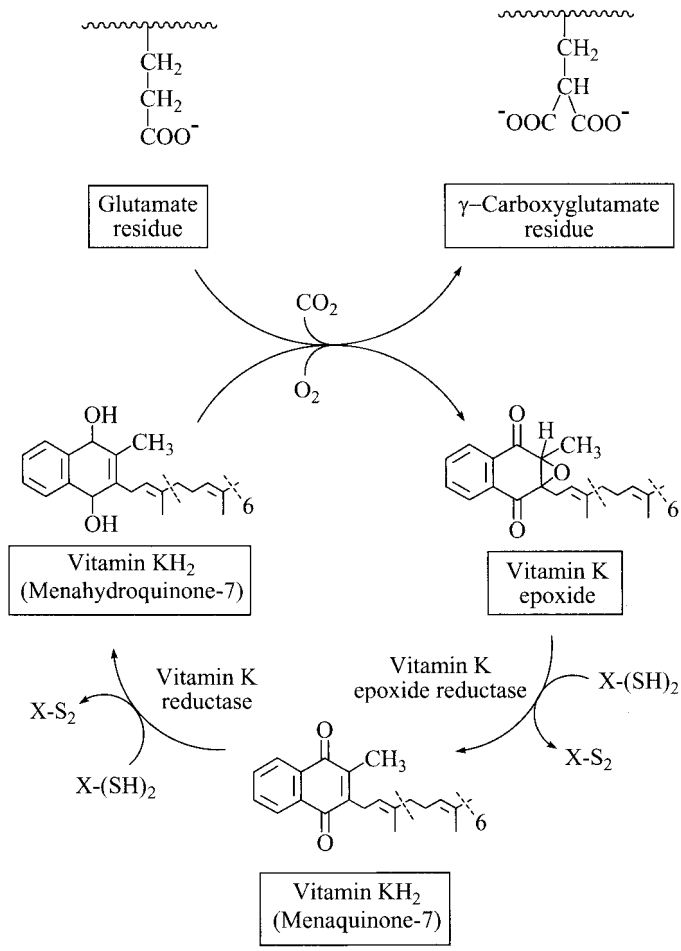


FIGURE 2-11 Role of vitamin K in the γ-carboxylation of glutamyl residues of proteins and the regeneration of reduced vitamin K. The figure shows menaquinone-7, which contains six additional isoprene units (portion between dashed lines); other menaquinones contain 6 to 13 isoprene units. Phylloquinone, obtained from plants, contains the same 2-methyl-1, 4-naphthoquinone ring, with a saturated rather than an unsaturated hydrocarbon tail. X designates the polypeptide cofactor thioredoxin, which is converted from the reduced state [X–$(SH)_2$] to the oxidized state ($X-S_2$) by both vitamin K reductase and vitamin K epoxide reductase.

Fe^{3+} and **Cu^{2+}**: components of the cytochrome oxidase complex, which catalyzes the last step in the electron transport chain in which the protons and electrons are transferred to molecular oxygen

Se^{2+}: glutathione peroxidase, which is involved in the cellular defense against free radicals

2.4 HOW DO ENZYMES WORK?

Biological catalysts increase the rate of a chemical reaction, permitting reactions to occur that would otherwise be so slow as to be incompatible with life. Mammalian enzymes have evolved to catalyze reactions under physiological conditions, that is, at 37°C and usually at a pH near neutrality. They commonly accelerate reactions by factors of 10^6 to 10^{10} and are usually highly specific for their substrates.

The active site of an enzyme is the pocket in the protein where the substrate or substrates are bound. Substrates are bound to enzymes in what is referred to an *enzyme–substrate* (ES) *complex* by multiple weak (usually noncovalent) interactions, particularly ionic and hydrogen bonds. Binding of substrates to the enzyme's active site stabilizes the reaction intermediate or transition state, thereby decreasing the amount of activation energy required for the reaction to occur (Fig. 2-1). Theoretically, all chemical reactions are reversible to some extent. Enzymes catalyze both the forward and reverse reactions.

2.4.1 What Determines the Direction in Which Reversible Reactions Proceed?

An example of a reversible reaction is the one catalyzed by lactate dehydrogenase:

$$\text{lactate} + \text{NAD}^+ \rightleftharpoons \text{pyruvate} + \text{NADH} + \text{H}^+$$

Whether one starts with the substrates (shown on the left) or the products (on the right), the lactate dehydrogenase reaction, like all reactions, will eventually reach an equilibrium or steady-state condition. At equilibrium, the relative proportion of reactants on the left and products on the right will be determined by the change in free energy of the reaction ($\Delta G^{\circ\prime}$); in other words, the reaction will proceed in the direction that releases energy ($\Delta G^{\circ\prime} < 0$) rather than one that requires a net input of energy.

For reversible reactions, the major factor that determines the rates of reactions in the forward and reverse directions is the relative concentration of substrates and products. For reactions like the one catalyzed by lactate dehydrogenase, the direction of the reaction is determined primarily by the NADH/NAD^+ ratio. Thus, when exercising muscle produces more NADH than can be utilized by the mitochondrial oxidative phosphorylation system, the buildup of NADH drives lactate dehydrogenase to produce lactate from pyruvate. Conversely, when hepatocytes are actively utilizing NADH for ATP production via oxidative phosphorylation, the NADH level falls and the concentration of NAD^+ increases, thereby causing lactate dehydrogenase to generate pyruvate from lactate.

2.4.2 Irreversible Reactions

There are many reactions that are essentially irreversible under physiological conditions. These irreversible reactions are *exergonic*, meaning that they give off

significant energy. Biochemists consider a reaction to be irreversible when the free-energy change ($\Delta G^{\circ\prime}$) is -4 kcal/mol or more negative. An example of a physiologically irreversible reaction is that catalyzed by glucose 6-phosphatase:

$$\text{glucose 6-phosphate} + H_2O \rightarrow \text{glucose} + P_i$$

The reverse reaction, that is, the formation of glucose 6-phosphate, would require the input of significant energy. Neither glucokinase nor hexokinase, the two enzymes that catalyze the synthesis of glucose 6-phosphate from free glucose, can directly reverse the reaction catalyzed by glucose 6-phosphatase. Instead, both of these enzymes utilize the energy associated with one of the high-energy bonds of ATP to phosphorylate glucose:

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{ADP}$$

Acetyl-CoA carboxylase is another enzyme that utilizes the high-energy γ-phosphate bond of ATP to drive a reaction that would be irreversible without the participation of ATP:

$$\text{acetyl-CoA} + CO_2 + \text{ATP} + H_2O \rightarrow \text{malonyl-CoA} + \text{ADP} + P_i$$

In this case, the terminal (γ) phosphate of ATP (Fig. 1-2) is not incorporated into the product of the reaction. Instead, two reactions (hydrolysis of ATP and carboxylation of acetyl-CoA) are coupled, with the favorable (energy-yielding or *exergonic*) hydrolysis of ATP being used to drive the unfavorable (energy-requiring or *endergonic*) carboxylation of acetyl-CoA.

2.4.3 Isozymes Are Different Proteins That Catalyze the Same Reaction

As described above, glucokinase and hexokinase both catalyze the synthesis of glucose 6-phosphate from glucose and ATP. However, the two enzymes differ with regard to both their catalytic properties and their protein structures, and are therefore called *isozymes* or *isoenzymes*. Hexokinase, the isozyme present in almost every cell of the body, has a high affinity for glucose and is therefore active even at relatively low concentrations of glucose (Fig. 2-12). By contrast, glucokinase, which is found primarily in liver, is relatively inactive at low concentrations of glucose. Glucokinase has a higher maximal activity than hexokinase and is able to respond to increased blood glucose concentrations by rapidly synthesizing glucose 6-phosphate. Biochemists quantify these differences by indicating that glucokinase has both a higher V_{max} (maximal reaction velocity) and a higher K_m (the substrate concentration required to support half-maximal activity) than hexokinase. As shown in Figure 2-12, the K_m of hexokinase for glucose is 0.01 mM, while the lower affinity of glucokinase for glucose is reflected by its higher K_m of 5 to 10 mM. This difference in K_m values between the two isozymes permits the liver to remove glucose rapidly from the blood

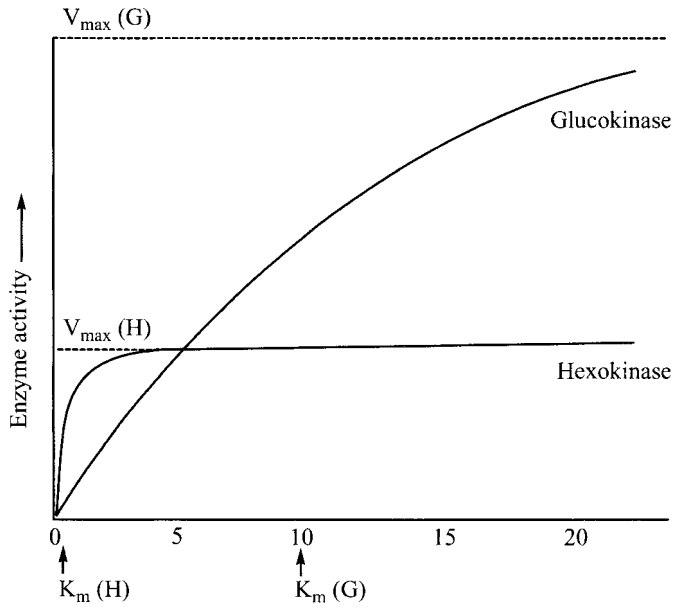


FIGURE 2-12 Glucokinase (G) and hexokinase (H) are isozymes with different kinetic properties.

when the glucose concentration is high, while leaving glucose available for glucose-dependent tissues (e.g., red blood cells, brain) when the body's glucose reserves are low.

2.5 HOW IS ENZYME ACTIVITY REGULATED?

Regulation of the functioning of the many enzymes in the body is central to coordinating the multiple pathways of metabolism and maintaining homeostasis. One key mechanism for regulating the level of activity of a particular enzyme in a cell is regulation of gene expression, since if an enzyme is not synthesized in the appropriate cell or at a particular time, the reaction it catalyzes will not occur.

The activities of existing enzymes are themselves also regulated, both by intracellular availability of metabolites and by covalent modifications (e.g., phosphorylation). In addition, many pharmaceutical agents act by inhibiting the activity of one or more enzymes. For example, patients who have elevated plasma cholesterol levels may be prescribed one of the statins, a class of drugs that inhibit HMG-CoA reductase, which catalyzes the rate-limiting step in cholesterol synthesis. The major mechanisms for regulation of enzymatic activity are described below.

2.5.1 Competitive Enzyme Inhibition

Competitive inhibition occurs when a molecule that is not a substrate for the enzyme in question, but which is structurally similar to the substrate, competes with the substrate and blocks its binding to the active site of the enzyme. Occupation of the active site by the inhibitor decreases the activity of the enzyme, particularly when the concentration of the substrate is low relative to that of the inhibitor.

Figure 2-13A depicts a Lineweaver–Burke double-reciprocal plot which illustrates how enzyme kinetics can be altered by the presence of a competitive inhibitor. For many enzymes, plotting $1/V$ (initial velocity) vs. $1/[S]$ (where $[S]$ is the substrate

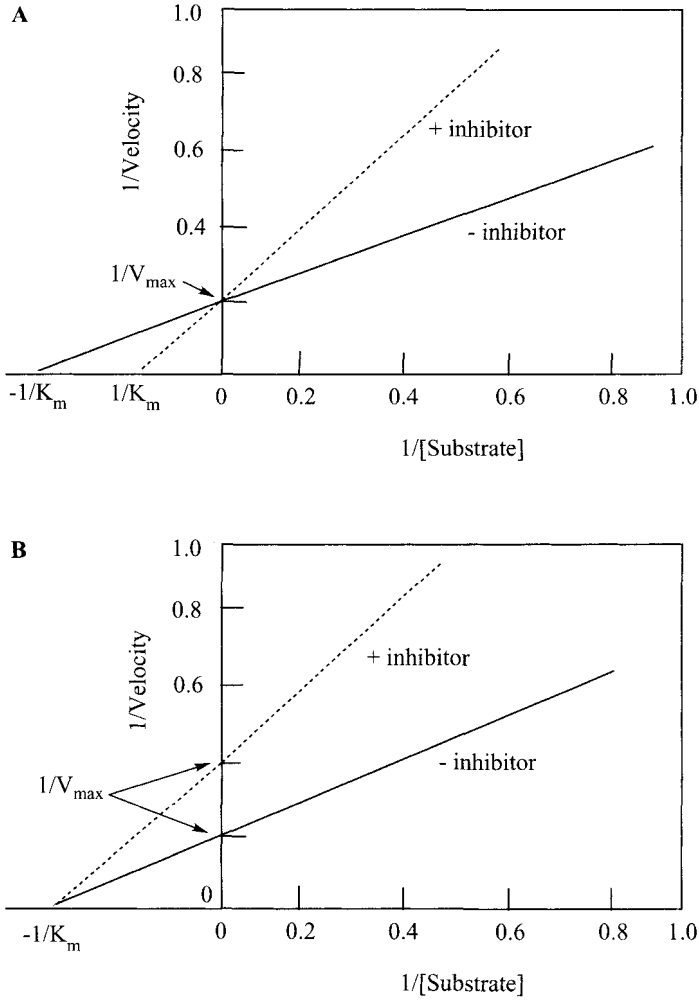


FIGURE 2-13 Enzyme inhibition illustrated with double-reciprocal Lineweaver–Burke plots: (A) competitive inhibition; (B) noncompetitive inhibition.

concentration) results in a linear graph. In this format, the X intercept of the line is $-1/K_m$ and the Y intercept is $1/V_{max}$. As shown in the figure, the competitive inhibitor increases the K_m but does not affect the V_{max} of the reaction.

Many pharmaceutical agents are competitive inhibitors of specific enzymes. For example, dicumarol inhibits catalysis involving vitamin K. Since many enzymes in the blood-clotting cascade are activated by γ-carboxylation, dicumarol acts as an anticoagulant that reduces the risk of thrombus formation.

In some cases, two different molecules may both be substrates for the same enzyme, with each acting as a competitive inhibitor of the metabolism of the other. One such example is alcohol dehydrogenase, which catalyzes the oxidation of both ethanol and methanol:

$$\text{ethanol} + \text{NAD}^+ \rightarrow \text{acetaldehyde} + \text{NADH} + \text{H}^+$$

$$\text{methanol} + \text{NAD}^+ \rightarrow \text{formaldehyde} + \text{NADH} + \text{H}^+$$

Although methanol itself is intoxicating, it is the metabolites of methanol (formaldehyde and formic acid) that are responsible for the blindness and death that result from methanol poisoning. One treatment for acute methanol poisoning involves intravenous administration of ethanol (plus glucose). Ethanol acts as a competitive inhibitor of the conversion of methanol to formaldehyde, thereby preventing accumulation of toxic metabolites until the methanol can be cleared by the kidneys. Glucose is administered to correct the hypoglycemia caused by ethanol.

2.5.2 Noncompetitive Enzyme Inhibition

A noncompetitive inhibitor binds to its target enzyme and cannot be displaced by excess substrate. Thus, this type of inhibitor diminishes the fraction of the enzyme pool that is catalytically competent. As illustrated in Figure 2-13B, noncompetitive inhibitors reduce the V_{max} of the reaction but do not change the enzyme's K_m.

Aspirin's action as a noncompetitive inhibitor is the major reason that it is a drug of choice for long-term therapy to decrease the risk of cardiovascular crises. Aspirin is a member of a class of drugs called *nonsteroidal anti-inflammatory agents* (NSAIDs) that inhibit the cyclooxygenase isozymes, thereby decreasing thromboxane production and platelet aggregation. Aspirin is an irreversible inhibitor since the molecule covalently acetylates a serine residue at the active site of the enzyme, inactivating cyclooxygenase permanently. By contrast, the inhibitory actions of other NSAIDs, such as ibuprofen, are attributable to reversible, noncovalent interactions between drug and enzyme. The reaction of aspirin with cyclooxygenase is particularly effective in platelets because platelets are incapable of synthesizing new enzyme protein.

Some of the deleterious effects of heavy metals, such as mercury and lead, result from their actions as noncompetitive enzyme inhibitors. For example, mercury inhibits glyceraldehyde 3-phosphate dehydrogenase, an enzyme in the glycolytic pathway, while lead inhibits heme synthesis.

2.5.3 Allosteric Regulation

Many enzymes contain regulatory sites that are physically separated from the active site. Binding of small molecules to one or more regulatory sites alters the three-dimensional structure of the enzyme, which increases or decreases its catalytic activity. *Allosteric regulation*, as this phenomenon is called, provides a mechanism by which enzymatic activities can be modulated by compounds that have little or no structural similarity to the substrate(s) but which instead, reflect the overall metabolic state or needs of the cell. Allosteric enzymes usually exhibit sigmoidal (S-shaped) kinetic curves (Fig. 2-14) rather than simple hyperbolic curves (Fig. 2-12). Activators of allosteric enzymes shift the V vs. S curve to the left, whereas allosteric inhibitors shift the curve to the right.

2.5.3.1 End-Product Inhibition. There are many instances in which the final endproduct of a multienzyme metabolic pathway is an allosteric inhibitor of an enzyme that catalyzes an early and irreversible step of the pathway. This form of allosteric regulation prevents accumulation of additional end product and of metabolic intermediates once a cell has sufficient supplies of that metabolic end product. Examples of this are seen in the pathways that generate heme, long-chain fatty acids, and cholesterol, where the end products inhibit δ-aminolevulinic acid synthase, acetyl-CoA carboxylase, and HMG-CoA reductase, respectively.

2.5.3.2 Regulation by Molecules That Signal the Availability of Precursors. Allosteric regulation provides a mechanism by which flux through a particular

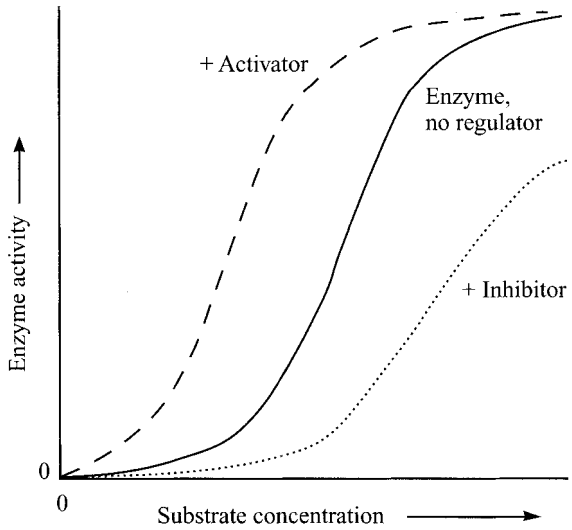


FIGURE 2-14 Sigmoidal kinetics of an allosteric enzyme and the effects of an activator and an inhibitor on the enzyme kinetics.

pathway can be rendered responsive to the overall nutritional state and needs of the cell. One such important small regulatory metabolite is citrate, an intermediate in the tricarboxylic acid cycle. Citrate allosterically stimulates liver cells to synthesize both fatty acids and glucose (gluconeogenesis) while inhibiting the breakdown of glucose by glycolysis.

2.5.3.3 Regulation by the Energy Charge of the Cell. Allosteric mechanisms also serve to regulate many metabolic pathways in response to a high ATP/ADP ratio, which is indicative of a plentiful supply of energy, or conversely, to high concentrations of ADP and adenosine 5′-monophosphate (AMP), which occur when ATP supplies have been depleted. An enzyme whose activity is regulated by the energy charge of the cell is the muscle isozyme of glycogen phosphorylase, which releases glucose (as glucose 1-phosphate) from glycogen stores. AMP is an allosteric activator of glycogen phosphorylase, and ATP is an allosteric inhibitor of the enzyme.

2.5.4 Regulation of Enzyme Activity by Covalent Modification

Many enzymes are activated or inhibited by covalent modification of their polypeptide structure. The modifications can be reversible or irreversible.

2.5.4.1 Phosphorylation/Dephosphorylation. The most common covalent modification utilized in regulating human metabolism is the reversible phosphorylation of enzyme proteins. In most cases, phosphorylation is the result of a hormone-stimulated signal-transduction cascade, thus providing a mechanism by which intracellular enzymatic activity can be modulated in response to intercellular signaling.

For example, glucagon and epinephrine both stimulate the activity of a serine–threonine protein kinase called *protein kinase A* (PKA). Briefly, this particular signaling pathway involves binding of glucagon or epinephrine to its respective transmembrane receptors, activation of a GTP-binding or G-protein, and activation of adenylyl cyclase, an enzyme that synthesizes cyclic AMP (cAMP) from ATP (Fig. 2-15):

$$\text{ATP} \rightarrow \text{cAMP} + \text{PP}_\text{i}$$

cAMP is an allosteric activator of PKA. Once activated, PKA uses ATP to phosphorylate specific serine and threonine residues on critical enzymes that regulate the flux of intermediates through key metabolic pathways. As discussed in more detail in Chapter 8, PKA-catalyzed phosphorylation activates enzymes involved with glycogen breakdown (glycogen phosphorylase). PKA-catalyzed phosphorylation also activates enzymes involved with mobilization of triacylglycerol stores and gluconeogenesis. Concurrently and conversely, PKA-catalyzed protein phosphorylation inhibits enzymes involved in glycogen and fatty acid metabolism. The simultaneous phosphorylation of multiple enzymes provides a coordinated response to the body's need to mobilize endogenous fuels during fasting or in response to stress.

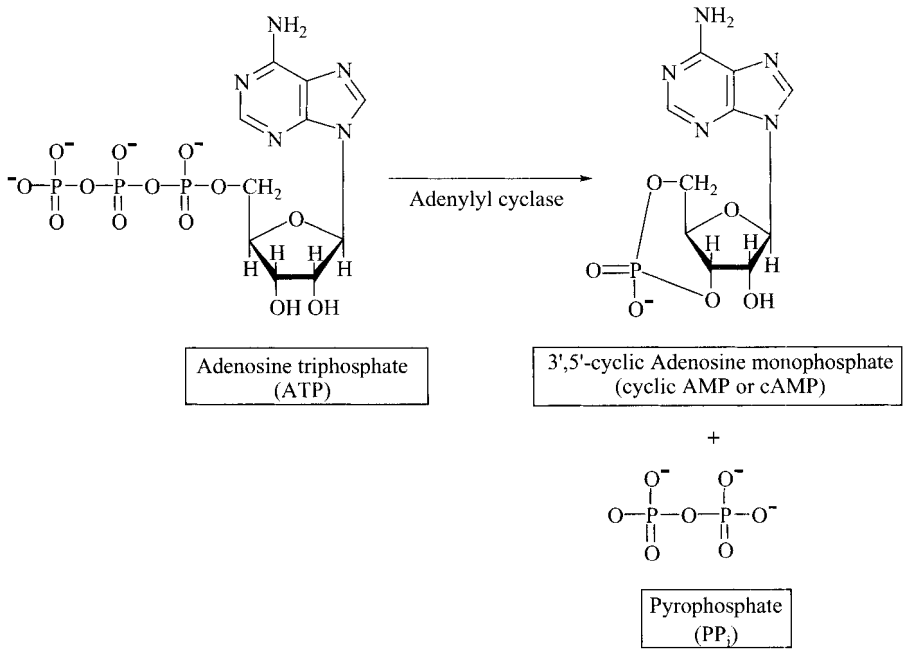


FIGURE 2-15 Synthesis of cyclic AMP by adenylyl cyclase.

Protein phosphorylation catalyzed by PKA can be reversed. Among the many effects of stimulation of cells by insulin is the activation of a signal-transduction cascade that results ultimately in the activation of protein phosphatase-1. Protein phosphatase-1 hydrolyzes the phosphate moieties from phosphoserine and phosphothreonine residues of many enzymes, thereby reversing the activation or inactivation that occurred when those enzymes were phosphorylated. Accordingly, insulin reverses the metabolic effects of glucagon and epinephrine, and switches the direction of key metabolic processes to meet the body's needs in the fed state when there is active synthesis of triacylglycerol in adipocytes and hepatocytes as well as synthesis and storage of glycogen in muscle and liver.

2.5.4.2 Hydrolytic Cleavage of Inhibitory Peptides. A number of enzymes, particularly digestive enzymes synthesized in the pancreas and the liver-synthesized proteases of the blood-clotting cascade, are secreted from their sites of synthesis in an inactive or zymogen form. Activation requires proteolytic hydrolysis of the proenzyme and release of a polypeptide, which then permits the remaining polypeptide fragment to alter its three-dimensional structure to one in which its active site and associated substrate binding pocket are configured correctly for catalysis. Thus, the digestive enzyme trypsin is secreted from the pancreas in the form of an inactive precursor, trypsinogen. Once in the lumen of the small intestine, a brush-border protease called enteropeptidase hydrolyzes one peptide bond within the trypsinogen

molecule, thereby releasing the inhibitory peptide and generating active trypsin. Secretion of trypsin in its zymogen form limits its activity to the digestive tract, thus protecting the pancreas and pancreatic duct from proteolytic damage.

2.5.5 Induction of Enzyme Synthesis

Hormonal regulation of enzymatic activity can also occur through stimulation or inhibition of transcription of genes that encode key metabolic enzymes. Hydrocortisone, a glucocorticoid hormone synthesized by the adrenal cortex, acts by entering the cell and binding to certain proteins in the cytosol that serve as glucocorticoid receptors. The hydrocortisone–glucocorticoid receptor complex then translocates to the nucleus, where it binds to specific hormone-response elements in DNA. The actions of hydrocortisone include induction of the synthesis of enzymes involved with gluconeogenesis, mobilization of adipose triacylglycerol, and degradation of muscle proteins. Hydrocortisone thus plays a major role in mediating long-term adaptations in the activities of metabolic pathways in response to starvation, sepsis, and stress.

2.6 DISEASE STATES ASSOCIATED WITH ABNORMAL ENZYME FUNCTIONING

2.6.1 Vitamin Deficiencies

Since vitamins are crucial components of many enzyme cofactors, an inadequate concentration of one or more of these essential dietary substances can result in impaired enzymatic activity. Vitamin deficiencies result from inadequate dietary intake or from impaired absorption or recycling of a vitamin. Impaired absorption of vitamins K and B_{12} is discussed in Chapters 3 and 22, respectively.

Unlike many of the other vitamin-based cofactors that attach to their respective enzymes through noncovalent bonds, biotin is covalently attached to lysyl residues of the enzymes with which it functions. Biotin deficiency can result from inadequate activity of the enzyme biotinidase, which normally hydrolyzes the biotinyl–lysyl bond and releases free biotin, thus permitting recycling of biotin when biotin-containing enzymes are degraded. A deficiency of biotin can also be induced by consumption of raw eggs, which contain avidin, a protein that binds biotin very tightly, thereby preventing absorption of biotin from the gut. Biotin deficiency reduces the activities of all four biotin-dependent enzymes: pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and β-methylcrotonyl-CoA carboxylase.

Dietary deficiencies of particular vitamins usually result from restricted diets. In each case, there is impaired activity of all of the enzymes that utilize the particular vitamin-derived cofactor, and ultimately development of a specific vitamin-deficiency disease. Thus, deficiency of folic acid results in megaloblastic anemia and is associated with congenital neural tube defects, whereas the peripheral neuropathy and cardiac manifestations of beriberi are caused by a dietary deficiency of thiamine.

2.6.1.1 Scurvy. Scurvy is the result of a dietary deficiency of vitamin C (ascorbic acid), which is usually obtained from fresh fruits and vegetables, especially citrus fruits (e.g., oranges, grapefruit, limes), cabbage, mangoes, and tomatoes. Scurvy has been recognized since ancient times, and many indigenous cultures are known to have had remedies utilizing local plant sources, including teas brewed from pine needles. Scurvy was particularly rampant among European sailors on long ocean voyages.

Ascorbic acid is a cofactor in various hydroxylation reactions including hydroxylation of specific proline and lysine residues of procollagen (Fig. 2-10) and the hydroxylation of dopamine to form norepinephrine. Scurvy is primarily a disease of defective collagen synthesis, and is characterized by bleeding gums, hemorrhages, and impaired wound heeling. Ascorbic acid also plays an important non-cofactor role as an antioxidant; it regenerates the reduced forms of other antioxidants, such as vitamin E and glutathione, as well as inactivating potentially harmful reactive oxygen species and nitrogen radicals.

2.6.1.2 Pellagra. A deficiency of niacin results in pellagra, which is characterized by dermatitis (especially in areas of the skin exposed to sunlight), diarrhea, dementia, and ultimately–if untreated–death. Since niacin is the vitamin component of NAD^+ and $NADP^+$, which are cofactors in numerous oxidation–reduction reactions, it is involved with essentially all of the major metabolic pathways including glycolysis, β-oxidation of fatty acids, the TCA cycle, electron transport and oxidative phosphorylation, and the synthesis of fatty acids and cholesterol.

Pellagra was endemic in the American Southeast between the two world wars, primarily among poor mill workers, who consumed a limited diet consisting primarily of corn (maize) and lard. The niacin in maize has relatively low bioavailability unless the maize is treated with alkali. Europeans adopted corn as a crop but not the native tradition of grinding corn with lime (calcium oxide or calcium carbonate).

Niacin is unique among the B vitamins in that the dietary requirement for this vitamin can be partially satisfied by endogenous synthesis of niacin from the essential amino acid tryptophan; unfortunately, maize also happens to be a relatively poor source of tryptophan. Prevention of pellagra was accomplished through public health measures, particularly the fortification of cereal products (e.g., bread, biscuits, pasta) with niacin. Ironically, these measures were delayed for many years because public acceptance of pellagra as a disease of malnutrition was hampered by the eugenics movement, which stereotyped the victims of pellagra in the U.S. South as inherently inferior human beings.

2.6.2 Inborn Errors of Metabolism

Inborn errors of metabolism are genetic disorders resulting from partial loss of function or from null mutations (complete absence of activity) of genes coding for particular enzymes. Examples of inborn errors of metabolism include phenylketonuria (PKU, caused by a lack of phenylalanine hydroxylase), medium-chain acyl-CoA hydrogenase deficiency, and glucose 6-phosphate dehydrogenase deficiency. Other examples of inborn errors are the lysosomal storage diseases, which result from the

loss of function of acid hydrolases required for lysosomal digestion of glycosaminoglycans, glycolipids, sphingomyelin, and glycogen.

2.6.3 Vitamin-Dependency Diseases

Inborn errors of metabolism often result from the synthesis of mutated enzymes, which have a decreased affinity (increased K_m) for their coenzyme or prosthetic group. In such cases the patient can often be treated successfully with exceptionally high intakes—or megadoses—of the vitamin precursor. For example, cystathionine synthase is a pyridoxal phosphate–dependent enzyme that synthesizes cystathionine from homocysteine and serine. Some forms of cystathionine β-synthase deficiency are responsive to treatment with pyridoxine (vitamin B_6). Similarly, some persons who are deficient in pyruvate dehydrogenase improve with thiamine therapy.

2.6.4 α_1-Antitrypsin Deficiency

α_1-Antitrypsin is a plasma glycoprotein that inhibits the activity of elastase and has inhibitory activity against a number of other serine proteases. Synthesized in and secreted by the liver, α_1-antitrypsin's major physiological function is to inhibit elastase released by neutrophils in the lung. In the absence of this bloodborne protease inhibitor, there is destruction of elastin in pulmonary alveoli, resulting in chronic obstructive pulmonary disease or emphysema. The disease occurs much earlier and is more severe in people who smoke, and some people with α_1-antitrypsin deficiency also develop cirrhosis of the liver.

2.6.5 Pancreatitis

Pancreatitis is an inflammation of the pancreas which can result from a number of conditions, including gallstones, chronic alcoholism, and the blockage of the pancreatic duct that can occur in cystic fibrosis. Damage to pancreatic cells results in premature activation of digestive proteases within the pancreas and resulting autodigestion of the pancreas. Elevated serum levels of pancreatic enzymes, particularly pancreatic lipase and amylase, are a laboratory-based diagnostic criterion of acute pancreatitis.

2.6.6 Enzymes as Markers of Disease

Many tissues produce enzymes that are relatively cell-specific. Because these enzymes are released into the circulation as a result of tissue damage, assays of the levels of certain enzymes in blood can provide useful diagnostic information. Probably the most widely requested plasma enzyme assays are those for alanine aminotransferase (ALT) and aspartate aminotransferase (AST), both of which are present in high concentration in hepatocytes. When these cells are injured, for example by viral hepatitis or acetaminophen overdose, ALT and AST are released into the blood. As indicated above, elevated serum levels of pancreatic lipase and

amylase are indicative of acute pancreatic disease. The level of the MB isozyme of creatine kinase (CPK), a marker for myocardial infarction, often rises rapidly in the plasma of a person who has experienced a heart attack. An increased level of the MM-isozyme of CPK is usually indicative of injury to skeletal muscle. Other examples of tissue-specific enzyme markers of cellular injury include bone-specific alkaline phosphatase (b-ALKP), which serves as a marker of bone turnover in patients with osteoporosis or Paget's disease, and acid phosphatase, which is elevated in plasma of patients with metastatic cancer of the prostate.

CHAPTER 3

DIGESTION AND ABSORPTION

3.1 FUNCTIONS OF DIGESTION AND ABSORPTION

The main function of digestion is to hydrolyze large dietary components such as starch, proteins, and fats into their absorbable component parts (e.g., glucose, amino acids, fatty acids). Relatively small dietary substances such as the disaccharides lactose and sucrose are also hydrolyzed into their component simple sugars. In addition, digestion serves to release some vitamins, such as biotin and vitamin B_{12}, from their protein-bound forms.

The well-orchestrated functioning of many organs of the gastrointestinal system, including the stomach, liver, gallbladder, pancreas, small intestine, and colon, is required for efficient digestion and absorption of the essential nutrients in foods. The stomach produces hydrochloric acid, which denatures proteins, rendering them more susceptible to proteolysis both by pepsin, produced by the stomach, and by pancreas-derived proteases. The liver produces and secretes bile salts, which are required for the digestion and absorption of triacylglycerols, which are comprised of long-chain fatty acids esterified to glycerol. In addition to secreting numerous digestive enzymes that act in the small intestine, the pancreas secretes large amounts of sodium bicarbonate, which neutralize stomach acid, thus providing the nearly neutral pH required for the activity of pancreatic enzymes in the lumen of the small intestine.

The enterocytes that line the lumen of the small intestine not only provide the surface to which disaccharidases and peptidases are attached, but are also the site

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where most of the small-molecular-mass products of digestion are absorbed. The ileum is an integral element of the enterohepatic circulation that accounts for the recycling of bile salts and the absorption of essential nutrients such as vitamin B_{12}. In addition, the small intestine is the body's largest endocrine organ, as it produces a variety of hormones that regulate digestion and energy balance. The colon is a major site of absorption of water and sodium and chloride ions. The colon is also the site of absorption of some of the metabolic by-products of colonic bacteria, particularly lactate, short-chain fatty acids such as propionate and butyrate, and ammonia, which is generated by hydrolysis of urea by bacterial urease.

3.2 DIGESTION AND ABSORPTION OF CARBOHYDRATES

3.2.1 Dietary Carbohydrates

The major dietary carbohydrates are starch, sucrose, and lactose. Starch, the polymeric form of glucose stored in plants, is a mixture of two macromolecular structures: amylose and amylopectin (Fig. 3-1). Amylose is a straight-chain polymer in which the glucose units are attached to one another through α-1,4 linkages. Amylopectin is a branched structure with branches formed by α-1,6 glycosidic linkages to the α-1,4 chains. Animal foods contain small quantities of glycogen, a glucose polymer that is similar to amylopectin but is more highly branched. Cellulose, the structural glucose polymer of plants, contains β-1,4 glycosidic bonds which are not hydrolyzed by human digestive enzymes. Cellulose is thus a dietary fiber rather than a bioavailable source of carbohydrate for the body.

Sucrose and lactose are disaccharides; that is, they are composed of two sugar units in glycosidic linkage (Fig. 3-2). Sucrose (table sugar), commonly extracted from sugarcane or sugar beets, consists of glucose (Glc) and fructose (Fru) and has the structure α-Glc(1 → 2)β-Fru. Lactose is the sugar found in milk and is comprised of β-galactose linked to C4 of glucose [β-Gal(1 → 4)Glc]. Fructose and glucose are also present as monosaccharides in honey and many fruits.

Most common monosaccharides and disaccharides are reducing sugars since they have a free aldehyde or ketone group. In an alkaline solution, a reducing sugar will reduce cupric ion (Cu^{2+}) to cuprous ion (Cu^{+}). By contrast, sucrose is not a reducing sugar.

3.2.2 Digestion of Starch

Salivary and pancreatic amylases are both endoglycosidases that randomly hydrolyze internal α-1,4 glycosidic bonds of amylose and amylopectin to form smaller polysaccharides called *dextrins*. Hydrolysis of the glucose polymers is initiated by salivary amylase (ptyalin), which hydrolyzes as much as 40% of dietary starch before the enzyme is inactivated by the low pH in the stomach. Pancreatic α-amylase continues the starch digestion process in the small intestine, producing maltose [α-Glc

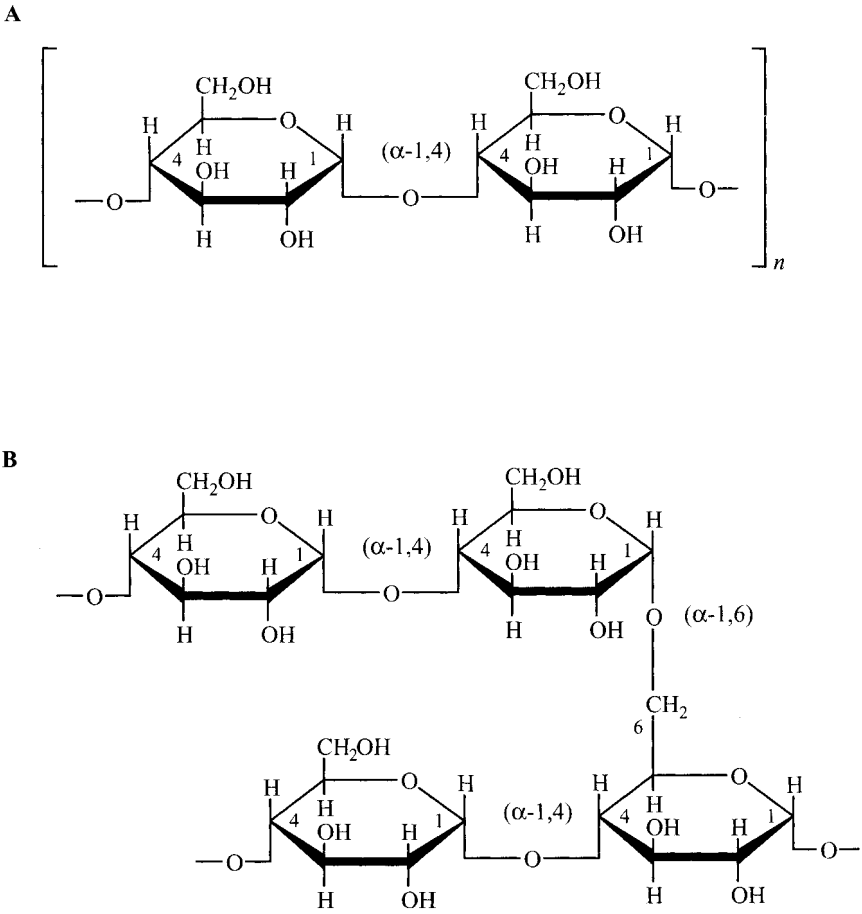


FIGURE 3-1 Structure of dietary starch: (A) the straight-chain structure of amylose (*n* indicates the number of repeating units, which may be in the thousands); (B) an α-1,6 glycosidic branch point in amylopectin.

(1 → 4)Glc], isomaltose [α-Glc(1 → 6)Glc], and limit dextrins, which are a mixture of oligosaccharides comprised of three to eight glucose units, including occasional α-1,6 branches.

3.2.3 Digestion of Oligosaccharides

The dietary disaccharides, sucrose and lactose, and the maltose, isomaltose, and oligosaccharides produced by partial digestion of dietary starch are hydrolyzed by enzymes that are localized on the surface of the brush border of the intestinal mucosa.

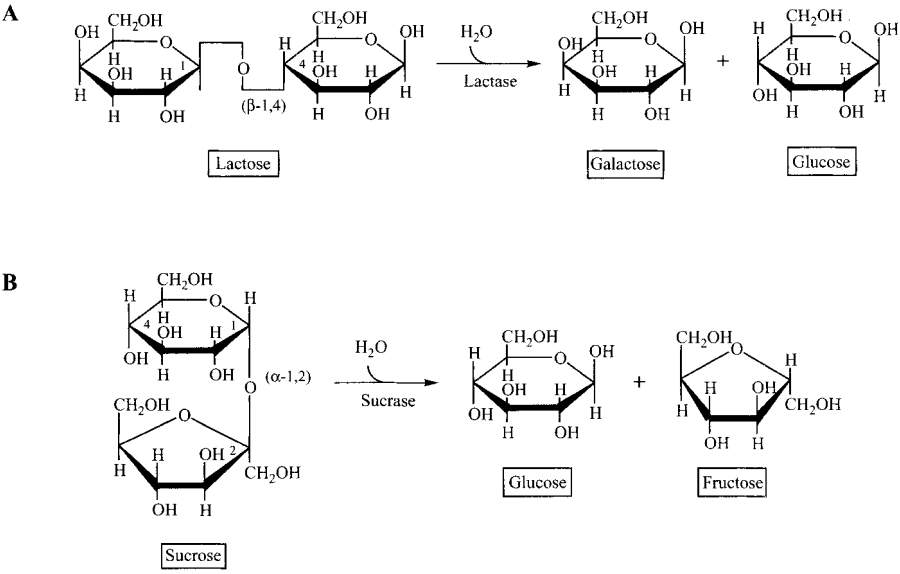


FIGURE 3-2 The disaccharides lactose (A) and sucrose (B) and their hydrolysis to simple sugars.

3.2.3.1 Maltase. Maltase is an α-glucosidase that hydrolyzes both maltose (Fig. 3-3A) and maltotriose:

$$\alpha\text{-Glc}(1 \rightarrow 4)\alpha\text{-Glc}(1 \rightarrow 4)\text{Glc [maltotriose]} + H_2O \rightarrow \text{maltose} + \text{glucose}$$

$$\alpha\text{-Glc}(1 \rightarrow 4)\text{Glc [maltose]} + H_2O \rightarrow 2\ \text{glucose}$$

3.2.3.2 Isomaltase. Isomaltase is an α-glycosidase that hydrolyzes the α-1,6 glycosidic bond of isomaltose and limit dextrans (Fig. 3-3B):

$$\alpha\text{-Glc}(1 \rightarrow 6)\text{Glc [isomaltose]} + H_2O \rightarrow 2\ \text{glucose}$$

3.2.3.3 Lactase. Lactase is a β-galactosidase that hydrolyzes lactose to glucose and galactose (Fig. 3-2A):

$$\beta\text{-Gal}(1 \rightarrow 4)\text{Glc [lactose]} + H_2O \rightarrow \text{glucose} + \text{galactose}$$

3.2.3.4 Sucrase. Sucrase is a disaccharidase that hydrolyzes sucrose (Fig. 3-2B):

$$\alpha\text{-Glc}(1 \rightarrow 2)\beta\text{-Fru[sucrose]} + H_2O \rightarrow \text{glucose} + \text{fructose}$$

It should be noted that the two polypeptides that have sucrase and isomaltase activity, respectively, are initially synthesized as a single polypeptide chain.

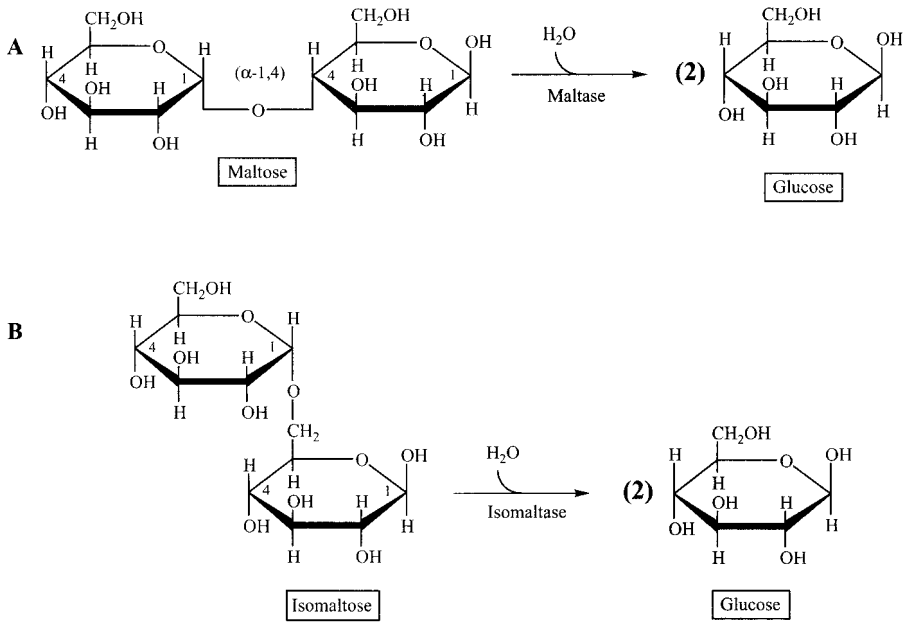


FIGURE 3-3 Hydrolysis of maltose (A) and isomaltose (B) generates glucose.

3.2.3.5 α-Dextrinase. This exoglycosidase hydrolyzes glucose α-1,4-glucose linkages starting at the nonreducing end of the oligosaccharide chain. Although α-dextrinase has greater activity for oligosaccharides with relatively longer chains, it also hydrolyzes maltose and maltotriose.

3.2.4 Absorption of Sugars

3.2.4.1 Glucose and Galactose. Glucose is absorbed into the cells of the intestinal mucosa in cotransport with Na^+ by GLUT1, the sodium glucose–dependent symporter. This process is driven by the active transport of Na^+ out of the cell through the basolateral membrane, which also serves to maintain a low concentration of intracellular Na^+. Galactose binds to the glucose-binding site of GLUT1 and is transported into the mucosa by the same cotransporter. There is also a facilitative transport mechanism for glucose absorption.

3.2.4.2 Fructose. Fructose is absorbed by facilitative diffusion, a process by which transport proteins facilitate the passage of a polar molecule across the plasma membrane. Fructose transport is driven by the concentration gradient of fructose across the membrane. All of the common dietary monosaccharides leave the enterocyte through the basolateral membrane by means of facilitated diffusion.

3.3 DIGESTION AND ABSORPTION OF DIETARY LIPIDS

3.3.1 Dietary Lipids

The major dietary lipids are triacylglycerols containing three long-chain fatty acids (usually C_{16}–C_{20}) esterified to glycerol (Fig. 3-4). Animal products also contain both free cholesterol and cholesteryl esters. Other dietary lipids include phospholipids, vitamins A, D, E, and K, and the carotenoids.

Since lipids are hydrophobic and poorly soluble in water, they have a strong tendency to aggregate into large lipid droplets. Efficient digestion of these droplets requires emulsification, the process by which large lipid droplets are dispersed into smaller ones, thus providing greater surface area for access by hydrolytic enzymes to their substrates. The process of emulsification involves both the physical effects of peristaltic churning of the food and the chemical dispersion of the droplets by the detergent action of bile salts.

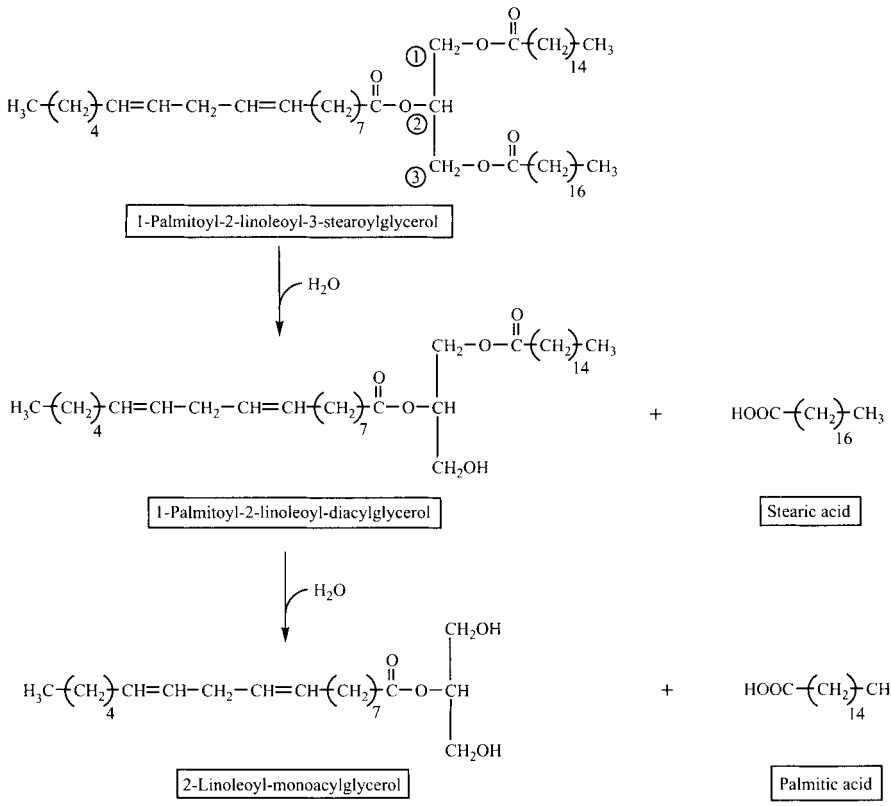


FIGURE 3-4 Structure of a typical dietary triacylglycerol and its hydrolysis in the intestine to a 2-monoacylglycerol plus two free fatty acids.

3.3.2 Bile Acid and Bile Salts

Effective digestion and absorption of dietary lipids requires both digestive enzymes and conjugated *bile acids* (a.k.a. *bile salts*). Bile acids are oxygenated derivatives of cholesterol that have several hydroxyl groups on the sterol rings and a shortened hydrocarbon tail ending in a carboxyl group (Fig. 3-5). Bile acids are weak acids with a pK_a value of about 6. The term *bile salts* refers to conjugated bile acids which contain either glycine or taurine linked via an amide bond to the carboxyl group of a bile acid (Fig. 3-5C). Conjugation decreases the pK_a of the bile salts; glycocholic acid has a pK_a of about 4, whereas the pK_a of taurocholic acid is about 2. The stronger hydrophilic domains of the bile salts renders them more amphipathic than bile acids and thus more effective emulsifiers.

3.3.2.1 Bile Salts Emulsify Dietary Lipids. The physical properties of the bile salts enable them to emulsify lipid droplets, thereby enhancing lipid digestion. Bile salts containing three hydroxyl groups (e.g., cholic acid) are better emulsifiers than those that have only two hydroxyl groups (e.g., deoxycholic acid).

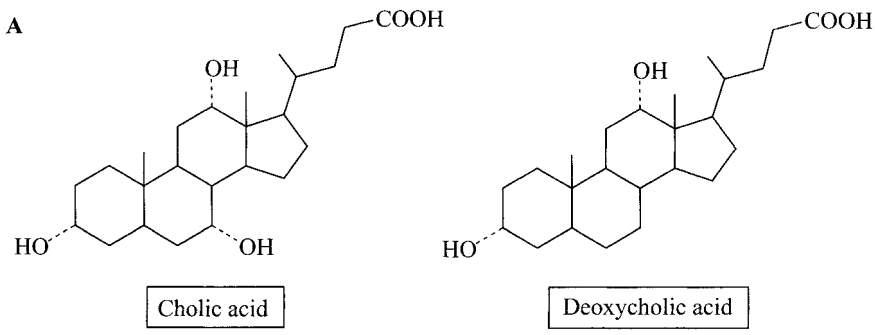
3.3.2.2 Bile Salts Stabilize Mixed Micelles. A second role that bile salts play in the process of digestion and absorption of dietary lipids is the solubilization of the relatively hydrophobic products of lipid hydrolysis in the form of small aggregates called *mixed micelles*. As shown in Figure 3-6, the stereochemistry of the hydroxyl groups of the bile salts gives the planar ring structure a hydrophobic face and a hydrophilic face. Mixed micelles have structures similar to small disks cut out of a membrane bilayer, with the bile salts stabilizing the cut edges. Stabilization of mixed micelles by bile salts is required for the products of lipid hydrolysis to diffuse through the unstirred water layer near the surface of the intestine to the plasma membrane of the enterocyte brush border where they are absorbed.

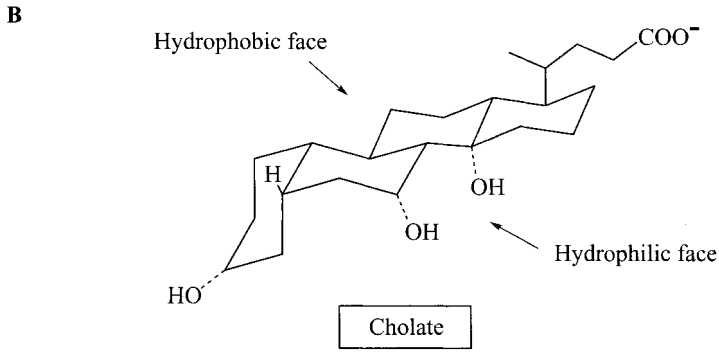
3.3.3 Hydrolysis of Dietary Lipids

One major difference between the digestion of carbohydrates and lipids is that hydrolysis of lipids is only partial. Whereas carbohydrates are absorbed only as monosaccharides, lipids are absorbed as a mixture of monoacylglycerols, diacylglycerols, and lysophospholipids, as well as free fatty acids. These products are then reassembled within the enterocytes for transport within the body.

3.3.3.1 Triacylglycerols. The enzymes that hydrolyze triacylglycerols (triglycerides) are called *lipases*. The digestive lipases catalyze the partial hydrolysis of dietary fats containing long-chain fatty acids to a mixture consisting primarily of free fatty acids and 2-monoacylglycerols. Several lipases contribute to triacylglycerol digestion, the major one being pancreatic lipase:

$$\text{triacylglycerol} + 2H_2O \rightarrow \text{2-monoacylglycerol} + 2 \text{ free fatty acids}$$





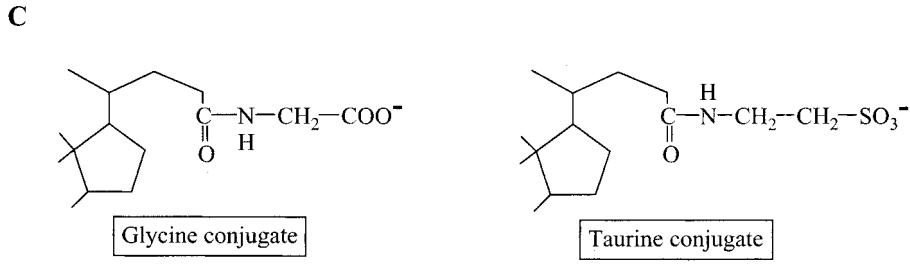


FIGURE 3-5 Structures of bile acids and bile salts: (A) the primary bile acids, cholic acid and deoxycholic acid; (B) the hydroxyl groups of bile salts such as taurocholic acid generate a polar face on an otherwise nonpolar steroid ring; (C) bile salts are formed by conjugation of bile acids with glycine or taurine.

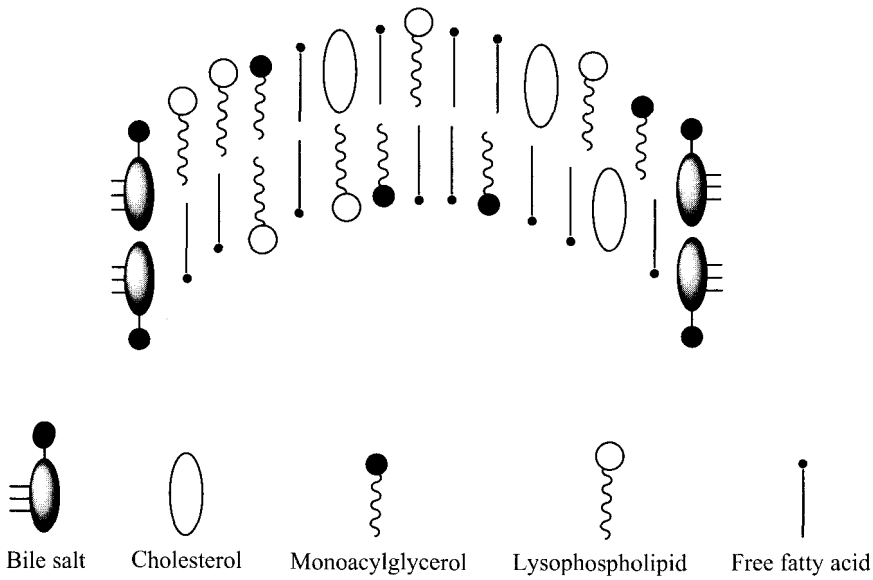


FIGURE 3-6 Cross-section of a discoidal mixed micelle.

Catalysis by pancreatic lipases requires the presence of a second pancreatic product called *colipase*. This 10-kDa nonenzyme protein reduces the surface tension at the lipid–aqueous interface, facilitating the interaction between the lipase and the lipid droplet.

The body also produces lingual and gastric lipases. Although gastric lipase is primarily active against substrates containing short-chain (C4–C6) and medium-chain (C8–C12) fatty acids, and is thus particularly important in the infant, it may also account for 10 to 30% of the hydrolysis of triacylglycerols, comprised of long-chain (C16–C20) fatty acids. The contribution of lingual lipase to fat digestion is normally quite low. Interestingly, however, lingual lipase is not inactivated by the acid pH of the stomach and, in the absence of pancreatic secretion of bicarbonate, remains active in the small intestine as well. Thus, particularly in the absence of pancreatic lipase activity, lingual lipase can contribute significantly to the digestion of dietary triacylglycerols. A neutral pH optimum, bile salt–stimulated lipase, present in human milk but not in cow's milk, contributes substantially to triacylglycerol hydrolysis in the intestine of breast-fed infants.

3.3.3.2 Hydrolysis of Phospholipids. Pancreatic phospholipase A_2 is secreted as a zymogen (inactive proenzyme), which is activated by trypsin-catalyzed hydrolysis. Phospholipase A_2 catalyzes the partial hydrolysis of both dietary phospholipids and the phosphatidylcholine secreted by the liver and contained in the bile. Pancreatic phospholipase A_2 is specific for fatty acids in the 2-position of phospholipids but has a broad specificity with respect to both the phospholipid polar head groups and the

chain length of the target fatty acid:

$$\text{phosphatidylcholine} + H_2O \rightarrow \text{2-lysophosphatidylcholine} + \text{free fatty acid}$$

3.3.3.3 Hydrolysis of Cholesteryl Esters. Dietary cholesterol is a mixture of free cholesterol and cholesterol esterified with long-chain fatty acids. Pancreatic juice also contains a cholesterol esterase or cholesteryl ester hydrolase which catalyzes the following reaction:

$$\text{cholesteryl ester} + H_2O \rightarrow \text{cholesterol} + \text{free fatty acid}$$

3.3.4 Absorption of Dietary Lipids and Chylomicron Formation

The products of lipid digestion include a mixture of partially hydrolyzed lipids (primarily monoacylglycerols and lysophospholipids), free fatty acids, cholesterol, fat-soluble vitamins, and other lipophilic molecules (e.g., carotenoids). All the products of lipid digestion ultimately become solubilized by bile salts to form small mixed micelles that diffuse from the lumen of the intestine toward the apical surface of the epithelium of the duodenum and jejunum, where the dietary lipids are absorbed. Whereas absorbed glucose can readily be transported as such to the liver and other tissues in the bloodstream, this process is not suitable for free fatty acids, because of both their limited solubility and their detergent properties, which could disrupt cell membranes and inhibit enzymes. Although free fatty acids released from adipocytes are transported in plasma bound to serum albumin, the higher concentrations of free fatty acids present after a meal would overwhelm this transport system. Instead, the absorbed fatty acids are reesterified into less polar products for transport in the form of large lipoprotein aggregates called *chylomicrons* (Fig. 3-7).

The hydrophobic core of the chylomicrons consists primarily of triacylglycerol molecules. It also contains cholesteryl esters and other absorbed lipophilic molecules, such as fat-soluble vitamins. The chylomicron particle is surrounded by a surface layer of phospholipids, free cholesterol, and proteins, primarily apoprotein B48 (apo B48) and apo A1. After assembly, the chylomicrons are secreted from the enterocytes into the lymphatic circulation, from whence they eventually enter the blood via the thoracic duct. The subsequent hydrolysis of the triacylglycerols of circulating chylomicrons is discussed in Chapter 12.

3.3.4.1 Resynthesis of Triacylglycerol. In enterocytes, synthesis of triacylglycerol occurs through the sequential action of monoacylglycerol acyltransferase and diacylglycerol acyltransferase, for a net reaction

$$\text{2-monoacylglycerol} + 2 \text{ fatty acyl-CoA} \rightarrow \text{triacylglycerol} + 2\text{CoASH}$$

This pathway is distinct from the triacylglycerol synthesis pathway in other cells, such as hepatocytes and adipocytes, which utilizes glycerol 3-phosphate as the acceptor

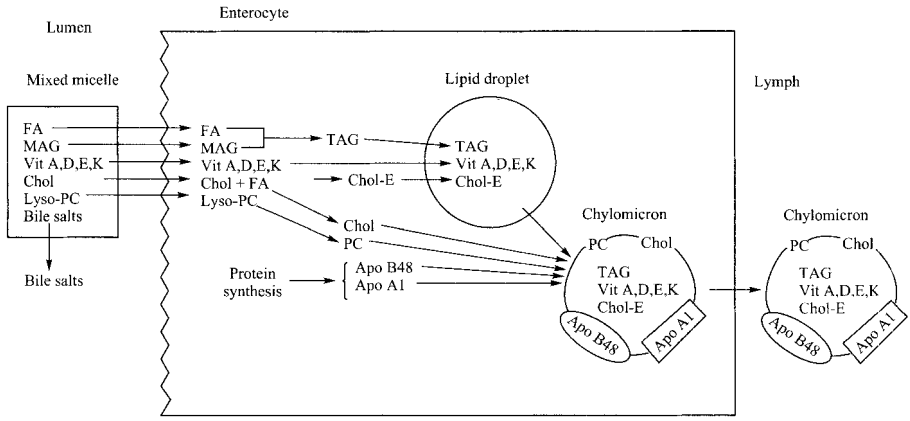


FIGURE 3-7 Absorption of dietary lipids into the enterocyte leads to chylomicron formation. apo A1, apoprotein A1; apo B48; apoprotein B48; Chol, cholesterol; Chol-E, cholesteryl ester; FA, free fatty acid; lyso-PC, 2-lysophosphatidylcholine; MAG, 2-monoacylglycerol; PC, phosphatidylcholine; TAG, triacylglycerol.

of acyl groups. The conversion of free fatty acids to fatty acyl-CoA in enterocytes utilizes the ubiquitous fatty acyl-CoA synthetase reaction

$$\text{fatty acid} + \text{ATP} + \text{CoASH} \rightarrow \text{acyl-CoA} + \text{AMP} + \text{PP}_i$$

3.3.4.2 Reesterification of Other Absorbed Lipids. As described above, the surface of chylomicrons contains cholesterol and phosphatidylcholine as well as apo B48. Secretion of cholesterol and phosphatidylcholine in bile and reabsorption of cholesterol and lysophosphatidylcholine in the small intestine provides enterocytes with a supply of the components involved in chylomicron assembly. Lysophosphatidylcholine acyltransferase catalyzes the reassembly of the phospholipid:

$$\text{2-lysophosphatidylcholine} + \text{fatty acyl-CoA} \rightarrow \text{phosphatidylcholine} + \text{CoASH}$$

Any free (nonesterified) cholesterol in excess of that which can be accommodated on the surface of the chylomicron particle is esterified by acyl-CoA:cholesterol acyltransferase (ACAT) and transported in the core of the chylomicron:

$$\text{cholesterol} + \text{fatty acyl-CoA} \rightarrow \text{cholesteryl ester} + \text{CoASH}$$

3.3.4.3 Absorption of Bile Acids. Bile salts are not absorbed together with the products of hydrolysis of dietary triglycerides, phospholipids, and cholesteryl esters, and they are not incorporated into chylomicrons. Instead, bile salts remain in the intestinal lumen until they reach the distal ileum (Fig. 3-8), where most are absorbed by an active transport mechanism that utilizes a Na^+-bile salt cotransport

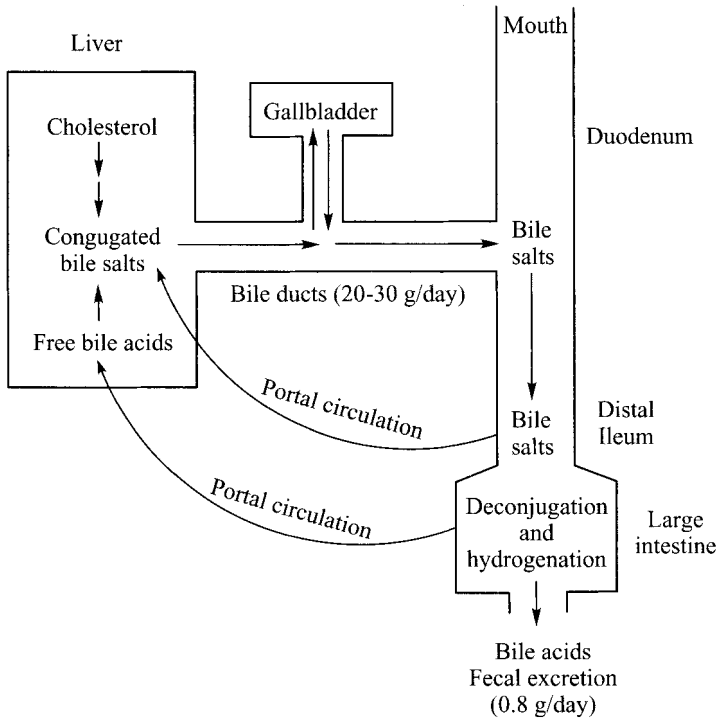


FIGURE 3-8 Enterohepatic circulation of bile salts.

system. The bile salts are transported through the portal vein to the liver, where they are extracted from the circulation by hepatocytes and then secreted back into bile. Specific transporters on both ileal and hepatic cells are required for this process. This enterohepatic circulation results in the secretion and reabsorption of the same pool of bile salts some 4 to 10 times a day, thus enabling the bile salts to be efficient promoters of fat digestion and absorption.

Those bile salts that are not reabsorbed in the ileum pass to the large intestine, where they are deconjugated through the hydrolytic removal of glycine or taurine. Bacterial metabolism also produces secondary bile acids, which have one less hydroxyl group than that of their respective primary bile acids. Some of these secondary bile acids are reabsorbed from the large intestine and returned to the liver, where they are reconjugated and reutilized. The remainder of the bile salts, approximately 0.8 g/day, is excreted in the feces.

3.3.4.4 Digestion and Absorption of Triacylglycerols Containing Medium-Chain Fatty Acids. The digestion and absorption of triacylglycerols containing short- and medium-chain fatty acids differs in several ways from that of the more common triacylglycerols that contain long-chain fatty acids. First, additional lipases are available for the hydrolysis of the shorter-chain fatty acids. Gastric lipase

preferentially hydrolyzes triacylglycerols in breast milk and some tropical oils (e.g., coconut) that contain large amounts of medium-chain fatty acids. Nonhydrolyzed triacylglycerols containing medium-chain (C6–C12) fatty acids are also absorbed intact into cells of the intestinal mucosa, where they are hydrolyzed by a mucosal lipase. Collectively, gastric- and mucosal-catalyzed lipolysis permits utilization of medium-chain triglycerides as a dietary lipid in persons who produce insufficient amounts of pancreatic lipase (e.g., patients with cystic fibrosis). Medium-chain triacylglycerols, such as those in coconut oil and human milk, can also be digested and absorbed in the absence of bile salts, although the presence of bile salts does enhance their absorption.

Since short- and medium-chain fatty acids are more soluble than C16 and C18 fatty acids, they can be transported as free fatty acids in the portal blood and are not reesterified and incorporated into chylomicrons. Thus, people with a reduced capacity for hydrolyzing triacylglycerols in circulating chylomicrons are sometimes prescribed diets in which triacylglycerols that contain medium-chain fatty acids are used in place of common dietary fats.

3.4 DIGESTION AND ABSORPTION OF PROTEINS

3.4.1 Substrates for Protein Digestion

The proteases of the digestive tract hydrolyze both exogenous or dietary proteins and endogenous proteins. Endogenous proteins include the proteases themselves as well as the proteins derived from the lining of the gastrointestinal tract. In fact, the amino acids absorbed by an average person are derived almost equally from endogenous protein (70 g/day) and dietary protein (60 to 90 g/day).

3.4.2 Enzymes That Contribute to Protein Digestion

3.4.2.1 Proteases. Proteases hydrolyze internal peptide bonds of polypeptides, producing smaller peptides and polypeptides (Fig. 3-9). The proteases involved in digestion are relatively specific for the amino acid side chain, designated R′ in Figure 3-9.

Pepsin, which is secreted by the stomach and active at acidic pH, is a relatively nonspecific protease that recognizes the R group of many different amino acids, including those that are dicarboxylic (Asp, Glu), aromatic (Phe, Tyr), or contain large, bulky side groups (Leu, Met). Pepsin can digest as much as 10 to 20% of the protein in a meal. Hydrolysis of dietary collagen by pepsin also facilitates the subsequent access of pancreatic proteases to proteins in ingested meats.

The pancreas secretes several proteases, each with its own particular substrate specificity. Trypsin cleaves peptide bonds on the C-terminal side of the basic amino acids Arg and Lys, whereas chymotrypsin cleaves peptide bonds on the C-terminal side of Leu, Met, Asn, and the aromatic amino acids Phe and Tyr. Elastase cleaves on the C-terminal side of amino acids that have a small side chain, such as Ala, Gly, and Ser.

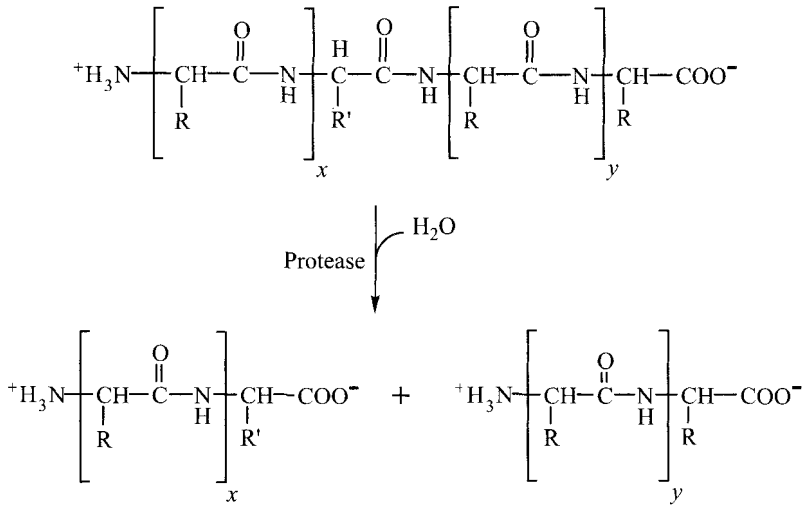


FIGURE 3-9 Hydrolysis of dietary proteins. R′ denotes the side chain of an amino acid for which the particular protease is selective.

Activation of Proteases. Pepsin is secreted in its zymogen form, pepsinogen, and is then converted to the active protease by HCl. Once activated, pepsin can hydrolyze other molecules of pepsinogen to generate additional molecules of pepsin.

Pancreatic trypsinogen is activated by the hydrolytic action of enteropeptidase, a protease that is synthesized by the brush-border cells of the small intestine. Once activated, trypsin can activate additional molecules of trypsinogen as well as other pancreatic enzymes:

$$\text{trypsinogen} \rightarrow \text{trypsin}$$
$$\text{chymotrypsinogen} \rightarrow \text{chymotrypsin}$$
$$\text{proelastase} \rightarrow \text{elastase}$$

In all cases, activation of the proenzyme involves hydrolysis of one or more peptide bonds, which results in the release of a segment of the polypeptide chain and permits the enzyme to assume a three-dimensional conformation that has a correctly configured active site.

Pancreatic Trypsin Inhibitor. The pancreas also secretes a small (6-kDa) protein called *pancreatic trypsin inhibitor* that binds very tightly to the active site of trypsin. Pancreatic trypsin inhibitor blocks the activity of any trypsin that may have resulted from premature conversion of trypsinogen to trypsin. This inhibitor thus acts to prevent a few active trypsin molecules from activating the full range of pancreatic digestive enzymes, which would otherwise damage the pancreas or pancreatic ducts.

3.4.2.2 Carboxypeptidases. Pancreatic juice also contains carboxypeptidases A and B, which are zinc-dependent exopeptidases that cleave peptide bonds and release amino acids one at a time from the C-terminal end of peptides. Both enzymes are secreted as zymogens and activated by trypsin. Carboxypeptidase A is specific for amino acids with hydrophobic side chains (e.g., valine, phenylalanine), whereas carboxypeptidase B is specific for basic amino acids (e.g., lysine, arginine).

3.4.2.3 Aminopeptidases. Cells of the intestinal mucosa produce a number of intra- and extracellular aminopeptidases which release amino acids one at a time from the N-terminal end of peptide chains.

3.4.3 Absorption of Components of Dietary Protein

Enterocytes of the small intestine absorb both amino acids and oligopeptides, particularly dipeptides and tripeptides. Indeed, oligopeptides may account for as much as two-thirds of the absorbed amino acids. There are numerous transport systems on the apical surface of the enterocyte for amino acids and peptides. Many but not all of these transport systems require cotransport of sodium. Once inside the enterocytes, the peptides are hydrolyzed to free amino acids by intracellular aminopeptidases. Free amino acids are then transported across the basolateral membrane and enter the blood.

3.5 DIGESTION AND ABSORPTION OF MICRONUTRIENTS

The body also absorbs a variety of vitamins and minerals that are termed *micronutrients* because they are required in relatively small quantities. Many of these micronutrients require specific mechanisms for absorption. In some cases, digestive processes are also required to release a bound cofactor such as vitamin B_{12} from the protein to which it is bound. The bioavailability and absorption of folate and vitamin B_{12} are discussed in Chapter 22, and the regulation of iron absorption is discussed in Chapter 23. Processes of digestion and absorption of other selected micronutrients are outlined briefly below.

3.5.1 Fat-Soluble Vitamins

Vitamins A (retinol), D (cholecalciferol), E (α-tocopherol), and K (phylloquinone and menaquinone) are lipids with limited solubility in water. In the gastrointestinal tract they are solubilized by bile salts, incorporated into mixed micelles along with the products of lipid digestion, and internalized by the intestinal mucosa. Once inside the enterocytes, the fat-soluble vitamins are incorporated into chylomicrons for transport through the lymph into the blood and eventually to the liver. Thus, conditions that impair the digestion and absorption of dietary lipids, particularly the absence of bile salts, will also compromise the absorption of fat-soluble vitamins to an extent that could lead to deficiencies of these vitamins.

β-Carotene and related retinoids are also lipids and require bile salts and mixed micelle formation for absorption. Once inside the enterocyte, β-carotene is cleaved

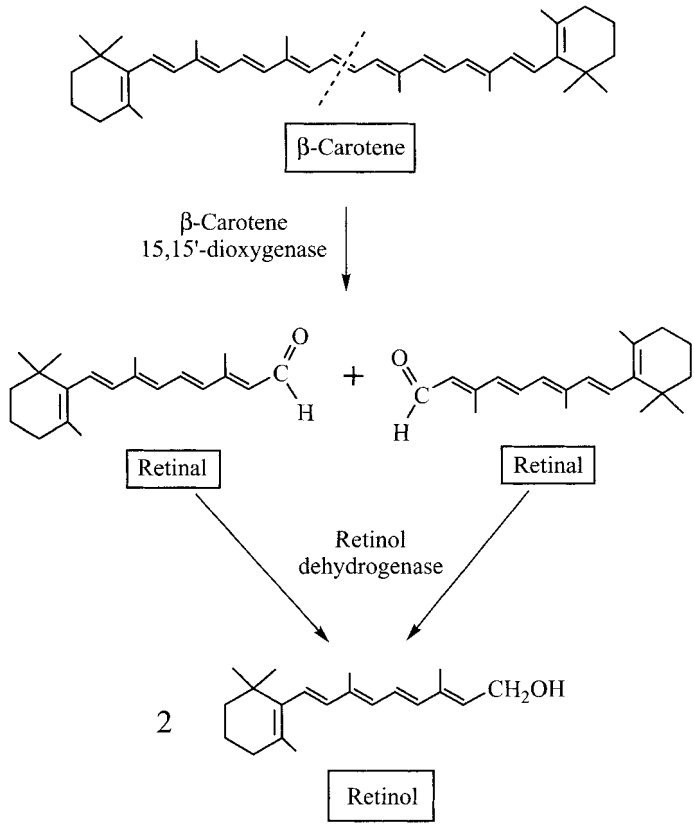


FIGURE 3-10 Hydrolysis of dietary β-carotene and the reduction of the resultant retinal to retinol.

by 15,15′-carotene dioxygenase to two molecules of *all-trans*-retinal, which are then reduced by NADPH-dependent retinol dehydrogenase to *all-trans*-retinol and incorporated into chylomicrons for transport throughout the body (Fig. 3-10).

3.5.2 Absorption of Zinc and Copper Ions

Levels of Zn^{2+} and Cu^{2+} in the body are regulated primarily by the extent of their absorption from the gut. Digestion of proteins is required to release both of these divalent cations from protein-bound dietary sources.

Once inside the enterocytes, Zn^{2+} is initially bound to cysteine-rich intestinal proteins (CRIPs), which serve as intracellular binding proteins for divalent cations. Increased plasma concentrations of zinc lead to increased synthesis of thionein, a low-molecular weight, cysteine-rich protein that binds zinc and other divalent cations. The resulting Zn^{2+}–thionein complex (metallothionein) sequesters Zn^{2+} within the enterocyte and limits its transport across the basolateral membrane into the plasma.

At the end of their lifespan, enterocytes are sloughed, returning the Zn^{2+} to the lumen of the intestine, where it is eventually excreted in the feces. This process serves to prevent absorption of excess zinc by the body.

Thionein also binds absorbed Cu^{2+} ions and prevents excess absorption of copper. Since zinc ions induce synthesis of thionein, excess dietary or pharmaceutical intakes of zinc increase the sequestration of copper ions within the enterocytes, which can lead to copper deficiency.

3.6 REGULATION OF DIGESTION

The gastrointestinal (GI) tract is a major endocrine organ. The overall function of the hormones secreted by the gut is to optimize digestion and absorption of nutrients from the gut by regulating GI motility and secretory processes. Following is a brief description of the role that some of these hormones play in digestion and absorption.

3.6.1 Gut Hormones

3.6.1.1 Gastrin. Gastrin regulates HCl secretion by the stomach and has a growth-promoting effect on the gastric mucosa. Histamine and acetylcholine also promote HCl secretion by ligand receptor–dependent mechanisms.

3.6.1.2 Cholecystokinin (CCK). Cholecystokinin stimulates secretion of pancreatic enzymes as well as contraction of the gallbladder, which enhances bile flow. It is secreted by endocrine cells located mainly in the duodenum.

3.6.1.3 Secretin. Secretin is a small polypeptide secreted by endocrine cells in the small intestine in response to a low pH (<5). It stimulates secretion of pancreatic juice containing digestive enzymes and sodium bicarbonate, which neutralizes gastric acid.

3.7 ABNORMAL FUNCTIONING OF DIGESTION AND ABSORPTION

3.7.1 Lactase Deficiency

Lack of the enzyme lactase leads to lactose intolerance (i.e., development of diarrhea and gaseous abdominal distension following ingestion of lactose or milk sugar). Congenital lactase deficiency is a rare condition characterized by a total lack of lactase activity. More commonly, the inability of adults to tolerate lactose occurs due to lactase nonpersistence (a.k.a. lactose intolerance), in which a person is born producing sufficient lactase to digest milk sugar, but within the first decade of life gradually loses the ability to produce the enzyme. Lactose nonpersistence is actually the normal condition in humans and other mammals. Multiple occurrences of genetic mutations in the promoter region of the lactase gene enabled some members of

cattle-raising populations in north-central Europe and sub-Saharan Africa to consume milk as well as meat. The mutations conveyed a powerful survival advantage and were thus subject to positive genetic selection. Loss of lactase expression may also occur secondary to disorders that damage the normal structure and function of the intestinal mucosa, such as acute diarrheal disease (e.g., enteritis), gastrointestinal parasites (e.g., giardiasis), enteropathies (e.g., celiac disease), and chronic inflammatory bowel disease (e.g., Crohn's disease).

3.7.2 Celiac Disease

Celiac disease, also called *celiac sprue*, *nontropical sprue*, or *gluten-sensitive enteropathy*, is an autoimmune enteropathy characterized by intestinal inflammation and malabsorption following ingestion of gliadin, a component of a family of wheat proteins called *glutens*. In celiac disease there is villous atrophy ("flattening"), crypt hyperplasia, and accumulation of lymphocytes in the connective tissue immediately under the intestinal epithelium. Patients with celiac disease produce antibodies not only to gliadin but also to other proteins present in connective tissue surrounding smooth muscle cells in the intestinal wall. Loss of the intestinal villus and the enzymes associated with it deprives the gastrointestinal tract of important digestive enzymes (e.g., lactase). Impaired functioning of enterocytes also results in malabsorption of the products of digestion, especially amino acids, fatty acids, and fat-soluble vitamins, as well as minerals (e.g., copper, calcium). The disease can be treated with a gluten-free diet.

3.7.3 Gallstones

Gallstones are solids that form when crystals of cholesterol or bile pigments precipitate out of the liquid stored in the gallbladder. Stones that remain in the gallbladder and do not cause blockage are said to be *silent*. However, when the stones lodge in the ducts that carry bile from the liver to the small intestine, they can lead to inflammation of the ducts, the gallbladder, the pancreas, or the liver. Gallstone attacks usually occur after high-fat meals but may also occur in the middle of the night. Acute inflammation can be extremely painful and chronic obstruction can lead to life-threatening pancreatic or liver disease.

Approximately 80% of gallstones are composed of cholesterol, which forms large yellow-green crystals or multiple tiny sandlike particles. They are most likely to form when bile contains too much cholesterol or not enough bile salts, or when the gallbladder does not empty as rapidly as it should. The other 20% of gallstone cases result from formation of solid precipitates in which the major component is bilirubin, the breakdown product of hemoglobin, which gives the stool its dark color.

The most common treatment for both types of gallstones involves surgical removal of the gallbladder. Digestion of a fatty meal proceeds relatively normally even in the absence of a gallbladder, since bile flows out of the liver directly into the small intestine. Oral treatment with bile salts can dissolve small cholesterol stones, thereby obviating the need for surgery. Since bile salt therapy requires months of treatment

and is often followed by reoccurrence of stones, it is reserved for persons for whom surgery is not an appropriate option.

3.7.4 Steatorrhea

Impaired functioning of any of the components of lipid digestion and absorption can result in steatorrhea or excretion of fat in foul-smelling, bulky stools, and poor absorption of fat-soluble vitamins.

3.7.4.1 Impaired Hydrolysis of Triacylglycerol. Many conditions impair hydrolysis of dietary triacylglycerols. One of the most common of these conditions is chronic pancreatitis, which can result in decreased secretion of pancreatic lipase. Decreased intestinal activity of pancreatic lipase is also observed in patients with gastrinomas or other conditions that result in excess production of gastric HCl. Steatorrhea is also a common side effect of the diet drug Xenical (orlistat), which inhibits pancreatic lipase activity.

3.7.4.2 Insufficient Secretion of Bile. When blockage of the bile duct reduces secretion of bile into the small intestine, the stools appear gray rather than reddish brown, reflecting the lack of excreted bile pigments. Lack of bile salts results in steatorrhea with excretion of free fatty acids rather than triacylglycerol in the stool. This occurs because bile salts are required primarily for absorption of dietary fat and because significant hydrolysis of triacylglycerol by pancreatic lipase is possible even in the absence of bile salts. Bile salt insufficiency also results in impaired absorption of other dietary lipids, including the fat-soluble vitamins.

3.7.4.3 Impaired Absorption by the Intestinal Mucosa. As indicated above, celiac disease or glutin-sensitive enteropathy results in malabsorption of dietary fat as well as other nutrients. Malabsorption of lipids may also occur as a result of mucosal inflammation, cystic fibrosis, bacterial overgrowth syndrome, and surgical resection of the small intestine.

3.7.5 Hypergastrinemia

Excessive secretion of gastrin (hypergastrinemia) is the cause of Zollinger–Ellison syndrome. The hallmark of this syndrome is gastric and duodenal ulceration due to excessive and unregulated secretion of gastric acid. Hypergastrinemia can also be caused by gastrin-secreting tumors that develop in the pancreas or duodenum or by infection with *Helicobacter pylori*, which induces mucosal inflammation.

3.7.6 Parenteral Feeding

There are many clinical conditions in which patients benefit from nutritional support which is parenteral, in that it completely bypasses the digestive system. They include various severe malabsorptive syndromes, such as necrotizing colitis in infants, severe

short bowel syndrome, and mechanical obstruction not immediately remediable by surgery. Since nutrition is provided intravenously rather than via the digestive tract, it is necessary to provide these patients with glucose rather than starch, and with amino acids rather than protein. By contrast, parenteral nutrition may include lipid emulsions (e.g., Intralipid, Liposyn) containing triacylglycerols stabilized by a surface layer of phospholipids. The lipid particles in these lipid emulsions are similar in size to chylomicrons, and like chylomicrons, are hydrolyzed in the blood, releasing free fatty acids and glycerol. Parenteral nutrition solutions must also supply essential minerals and vitamins.

CHAPTER 4

GLYCOLYSIS

4.1 FUNCTIONS OF GLYCOLYSIS

Glycolysis is a metabolic pathway that cleaves glucose into two molecules of pyruvate or lactate. During glycolysis, some of the energy in the glucose molecule is converted into ATP. Although glycolysis to pyruvate is an oxidative process, it is not dependent on molecular oxygen. By contrast, the subsequent fate of pyruvate depends on the presence of both mitochondria and sufficient oxygen. In the presence of oxygen, the product of glycolysis is pyruvate, which is further oxidized by the pyruvate dehydrogenase enzyme complex and the dehydrogenases of the tricarboxylic acid (TCA) cycle. In the absence of sufficient oxygen, or in red blood cells, where mitochondria are absent, lactate is the final product of glycolysis and there is no net oxidation. Glycolysis is not only the major pathway for oxidizing glucose, but also the main pathway for metabolizing other dietary sugars, such as galactose and fructose.

Once glucose has been trapped inside a cell in the form of glucose 6-phosphate, there may be as many as three metabolic options available for the glucose moiety (Fig. 4-1). In a hepatocyte, for example, glucose can be oxidized via glycolysis for the primary purpose of ATP production, stored as glycogen, or oxidized in the pentose phosphate pathway to generate NADPH and ribose for nucleic acid synthesis. Red blood cells, on the other hand, cannot synthesize glycogen; they can, however, metabolize glucose through the pentose phosphate pathway or through glycolysis.

Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew

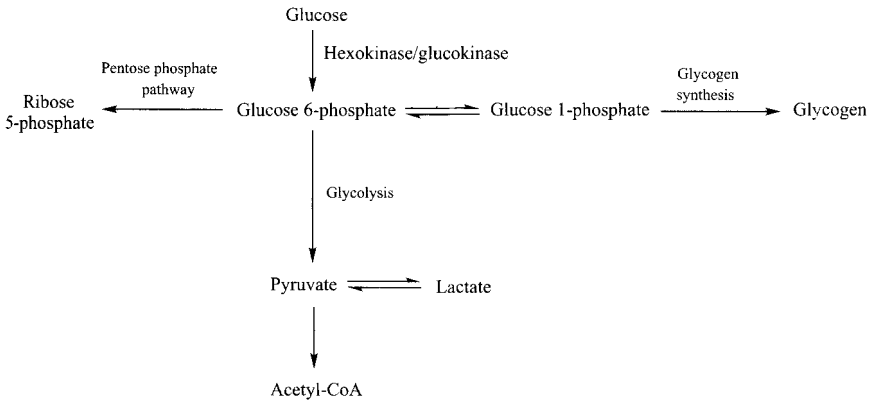


FIGURE 4-1 Three possible metabolic fates of glucose: glycolysis, the pentose phosphate pathway, and glycogen synthesis.

4.1.1 Glycolysis Provides Energy

The main function of glycolysis is energy (ATP) production. The conversion of one molecule of glucose to pyruvate or lactate is associated directly with the net production of two ATP molecules. Since the maximum number of ATP molecules that can be realized from the complete oxidation of one molecule of glucose to CO_2 and water is 30 to 32, the ATP yield from glycolysis is clearly relatively small.

Generation of energy via glycolysis is oxygen independent. In the presence of oxygen in tissues such as muscle and liver that contain mitochondria, for example, the end product of glycolysis is pyruvate. In contrast, in red blood cells lacking mitochondria or in mitochondria-containing tissues that are insufficiently oxygenated, lactate is the end product of glycolysis.

4.1.2 Glycolysis Provides Substrate for Further Oxidation

Pyruvate, the end product of aerobic glycolysis, can be further oxidized in mitochondria. The pathway (described in Chapter 5) involves the pyruvate dehydrogenase–catalyzed oxidation of pyruvate to acetyl-CoA and CO_2, followed by subsequent oxidation of acetyl-CoA to CO_2 and water in the TCA cycle.

4.1.3 Intermediates and Products of Glycolysis Can Provide Substrates for Other Pathways

Tissues such as adipose and liver that have a high capacity for triacylglycerol synthesis contain glycerol 3-phosphate dehydrogenase, which converts dihydroxyacetone

phosphate (DHAP) into glycerol 3-phosphate, which is a critical substrate in the pathways of triacylglycerol and glycerophospholipid (i.e., phospholipid) synthesis:

$$\text{dihydroxyacetonephosphate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{glycerol 3-phosphate} + \text{NAD}^+$$

Red blood cells use 1,3-bisphosphoglycerate, another intermediate in glycolysis, to generate 2,3-bisphosphoglycerate, which is an allosteric regulator of the interaction of oxygen with hemoglobin. The interconversion of these two bisphosphoglycerates is catalyzed by bisphosphoglycerate mutase:

$$1,3\text{-bisphophoglycerate} \rightleftharpoons 2,3\text{-bisphosphoglycerate}$$

Pyruvate, the end product of aerobic glycolysis, can acquire an amino group by transamination, thus producing the amino acid alanine. In addition, acetyl-CoA, produced by the mitochondrial oxidation of pyruvate, is a substrate for the synthesis of both fatty acids and cholesterol.

4.2 LOCALIZATION OF GLYCOLYSIS

Glucose is the universal fuel in humans in the sense that literally every type of cell in the body possesses the glycolytic pathway in its cytosol and can therefore metabolize glucose at least to the level of pyruvate or lactate. Although most cells can also utilize fatty acids as an energy source, some cells, such as erythrocytes and those in both the lens and cornea of the eye, contain few or no mitochondria and rely on glycolysis for essentially all of their ATP production. Even though brain cells do contain mitochondria, the impermeability of the blood–brain barrier to most long-chain fatty acids prevents fatty acids from being an important fuel source for the brain. Thus, although the brain is a highly aerobic organ that derives much of its ATP from the oxidation of acetyl-CoA in the tricarboxylic acid cycle, under all circumstances the brain metabolizes large quantities of glucose via glycolysis to generate pyruvate, which can then be oxidized to acetyl-CoA.

4.3 PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONDITIONS IN WHICH GLYCOLYSIS IS ESPECIALLY ACTIVE

4.3.1 Fed State

Glycolysis is especially active in the fed state when the body is actively digesting, absorbing, and processing nutrients. Insulin, which is secreted by the β-cells of the pancreas in response to elevated postprandial blood glucose levels, stimulates glucose metabolism in muscle, liver, and fat cells, but not in the brain.

4.3.2 Exercising Muscle

Although muscle cells at rest derive most of their energy from the oxidation of fatty acids, exercising muscle oxidizes glucose as well as fatty acids. As the intensity of exercise increases, progressively more of the energy in muscle will be derived from the oxidation of glucose.

There are two sources of glucose for muscle: localized glycogen stores within the muscle and glucose extracted from the blood. In vigorously exercising muscle, the demand for oxygen can become so great as to outstrip the oxygen supply. Under these conditions, the muscle relies extensively on glycolysis to satisfy its ATP needs, and lactate production increases.

4.3.3 Cancer Cells

Cancer cells consume glucose at a much higher rate and produce much more lactic acid than their normal counterparts, even under aerobic conditions. This phenomenon is known as the *Warburg effect*. A large fraction of the increased ATP produced by glycolysis in cancer cells is used for fatty acid, protein, and DNA synthesis, all three of which are increased in cancer cells. The Warburg effect also provides tumors with the large amounts of lactate and pyruvate that are precursors to the acetyl-CoA substrate that fatty acid synthesis requires.

4.4 PATHWAY OF GLYCOLYSIS AND RELATED REACTIONS

4.4.1 Uptake of Glucose into Cells Is Facilitated by Tissue-Specific Glucose Transporters

A family of glucose transporters (designated GLUT1 through GLUT5) facilitates movement of glucose across the plasma membrane. Glucose enters cardiac and skeletal muscle cells as well as adipocytes via the insulin-stimulated glucose-4 transporter designated GLUT4. In the absence of a strong insulin signal (i.e., a low insulin/glucagon ratio), GLUT4 is bound to intracellular vesicles. Following insulin stimulation, GLUT4-containing vesicles in muscle and adipocytes translocate to and fuse with the plasma membrane, thus providing the mechanism by which insulin stimulates uptake of glucose from the blood (Fig. 4-2). Glucose transport via GLUT4 is a major regulatory step in glucose metabolism in muscle and adipocytes.

GLUT2, which is constitutively present on the plasma membrane of liver cells, has a lower affinity for glucose then does GLUT4, but is expressed in such abundance that intracellular glucose equilibrates essentially instantaneously with glucose in the blood. As a result, the hepatic flux of glucose through glycolysis is proportional to the glucose concentration in the circulation.

Red blood cells, brain, and kidney contain GLUT1 and GLUT3 transporters, which, like GLUT2, are insulin-independent. Unlike GLUT2, GLUT1 and GLUT3 have a high affinity for glucose, thus promoting glucose uptake in the fasted state.

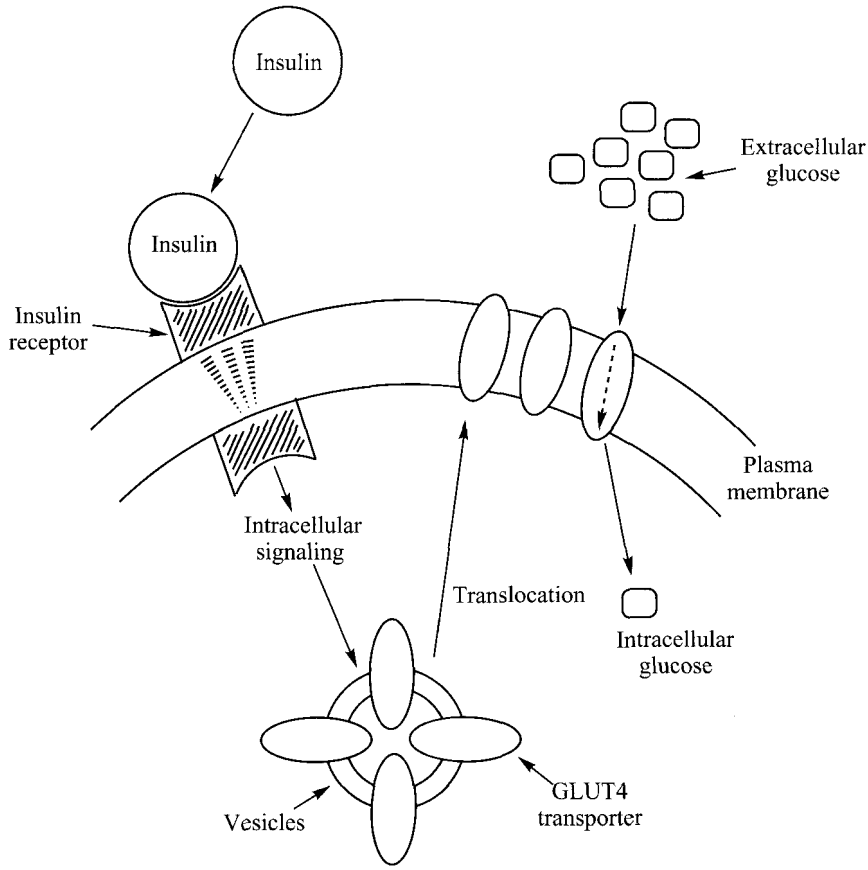


FIGURE 4-2 Insulin promotes the translocation of GLUT4 transporters from intracellular vesicles to the plasma membrane of adipocytes and muscle cells.

GLUT5 is expressed at high levels in the small intestine, where it functions primarily as a transporter of fructose rather than of glucose.

4.4.2 Trapping Glucose Intracellularly

Once glucose enters a cell it must be trapped; otherwise, it will diffuse back into the blood. All cells, including hepatocytes, contain hexokinase, which phosphorylates glucose, thereby trapping it in the cytosol (Fig. 4-3):

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{ADP}$$

Phosphorylation of glucose also serves to activate the sugar for metabolism. As more and more glucose is trapped in hepatocytes, however, the concentration

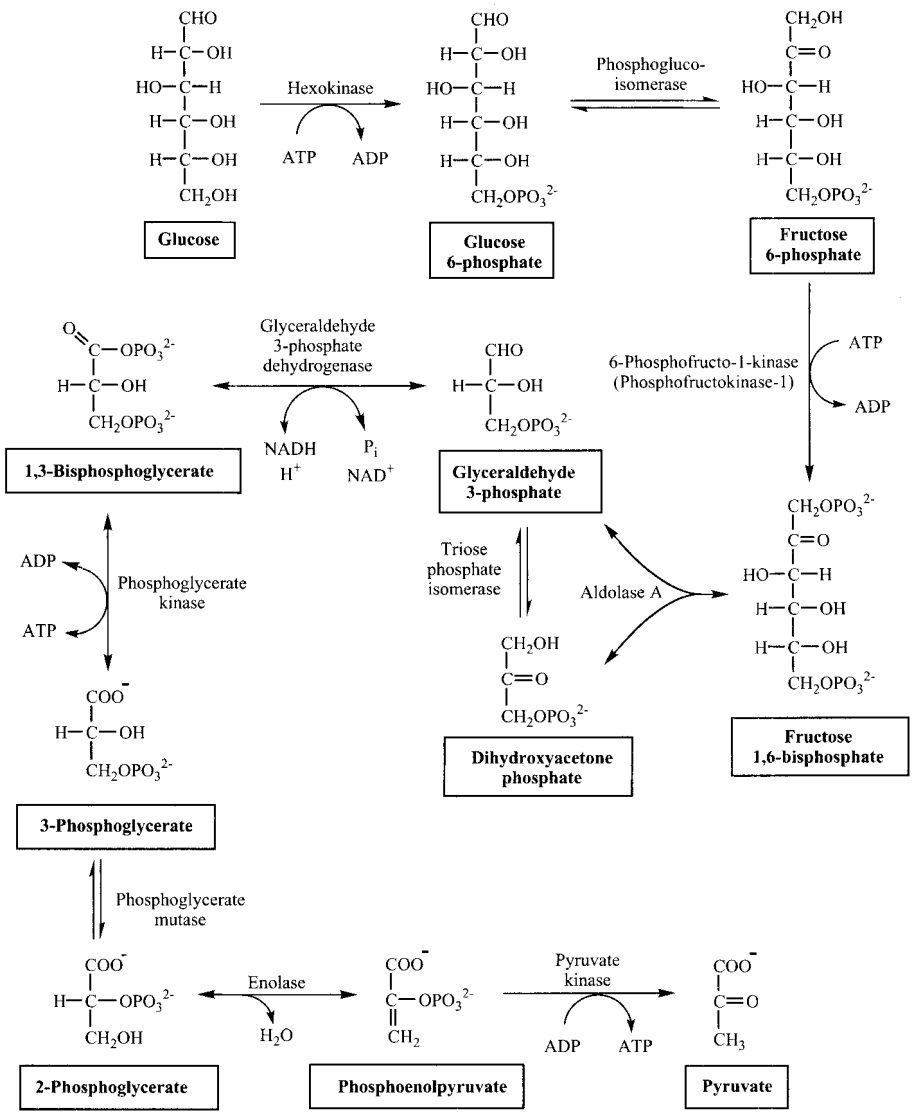


FIGURE 4-3 Glycolytic pathway. Although the structures of the sugars are illustrated in their open-chain form, in solution they exist in their respective ring forms.

of glucose 6-phosphate increases to the point where it inhibits hexokinase. This phenomenon is an example of product inhibition. If hepatocytes had no other glucose-trapping enzyme than hexokinase, the liver would soon cease extracting glucose from the blood and the body would experience a period of prolonged hyperglycemia. The problem is solved by the induction of the enzyme glucokinase by insulin.

As described in Chapter 2, glucokinase and hexokinase constitute an isoenzyme pair. Unlike hexokinase, glucokinase is not inhibited by glucose 6-phosphate. The function of glucokinase is to trap glucose when the blood glucose concentration rises after a meal.

The reaction catalyzed by both hexokinase and glucokinase is physiologically irreversible. Therefore, hepatocytes cannot run the hexokinase reaction in reverse to release the glucose moiety of glucose 6-phosphate generated when glycogen is broken down (glycogenolysis) or during gluconeogenesis. A separate enzyme called glucose 6-phosphatase dephosphorylates glucose 6-phosphate, thereby releasing free glucose that can leave the cell and enter the blood:

$$\text{glucose 6-phosphate} + H_2O \rightarrow \text{glucose} + P_i$$

4.4.3 The Individual Steps of Glycolysis

The first step following phosphorylation and trapping of glucose is the isomerization of glucose 6-phosphate to fructose 6-phosphate (Fig. 4-3). This reversible reaction is catalyzed by glucose 6-phosphate isomerase:

$$\text{glucose 6-phosphate} \rightleftharpoons \text{fructose 6-phosphate}$$

The next step in the pathway, which is catalyzed by phosphofructokinase-1 (PFK-1), is irreversible and commits fructose 6-phosphate to glycolysis. PFK-1 also represents the major regulated step in the glycolytic pathway:

$$\text{fructose 6-phosphate} + \text{ATP} \rightarrow \text{fructose 1,6-bisphosphate} + \text{ADP}$$

Next, aldolase A cleaves fructose-1,6-bisphosphate into two three-carbon fragments (called *triose phosphates*), glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP):

$$\text{fructose-1,6-bisphosphate} \rightleftharpoons \text{glyceraldehyde 3-phosphate} + \text{dihydroxyacetone phosphate}$$

Since aldolase-type reactions are reversible, aldolase A can also participate in the pathway that is essentially the reverse of glycolysis: namely, *gluconeogenesis*. The liver contains a second aldolase, designated aldolase B, which participates in the metabolism of fructose. Aldolase A and aldolase B are not isozymes.

Instead of utilizing two separate pathways for converting each of the trioses from the aldolase A reaction into pyruvate, nature has evolved a more economical strategy that involves the conversion of one of the trioses, dihydroxyacetone phosphate, into the other, glyceraldehyde 3-phosphate. The enzyme that accomplishes this freely

reversible interconversion of the two triose phosphates is triosephosphate isomerase:

$$\text{dihydroxyacetone phosphate} \rightleftharpoons \text{glyceraldehyde 3-phosphate}$$

The next step is the only oxidation–reduction reaction of glycolysis: namely, the NAD^+-dependent oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. This reversible reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase:

$$\text{glyceraldehyde 3-phosphate} + NAD^+ + P_i \rightleftharpoons \text{1,3-bisphosphoglycerate} + NADH + H^+$$

The glyceraldehyde 3-phosphate dehydrogenase reaction couples an oxidation–reduction reaction with a reaction that incorporates inorganic phosphate (P_i) into an organic compound. The overall reaction generates a high-energy phosphate ester linkage whose bond energy is greater than that of the terminal (γ) phosphate of ATP. The energy in the phosphoanhydride bond in 1,3-bisphosphoglycerate can then be transferred to ADP in a reversible reaction catalyzed by phosphoglycerate kinase:

$$\text{1,3-bisphosphoglycerate} + ADP \rightleftharpoons \text{3-phosphoglycerate} + ATP$$

Next, phosphoglyceromutase catalyzes the transfer of the phosphate group from the 3-position to the 2-position of phosphoglycerate:

$$\text{3-phosphoglycerate} \rightleftharpoons \text{2-phosphoglycerate}$$

Enolase then dehydrates 2-phosphoglycerate to generate phosphoenolpyruvate (PEP):

$$\text{2-phosphoglycerate} \rightleftharpoons \text{phosphoenolpyruvate} + H_2O$$

The phosphoenol configuration of atoms in phosphoenolpyruvate causes the carbon–oxygen–phosphorus linkage to be high energy. In fact, the free-energy change associated with the hydrolysis of the phosphate group of PEP is twofold more negative than that associated with the hydrolysis of the terminal (γ) phosphate group of ATP.

The next step in glycolysis involves the transfer of the phosphate group of PEP to ADP in a reaction catalyzed by pyruvate kinase:

$$\text{phosphoenolpyruvate} + ADP \rightarrow \text{pyruvate} + ATP$$

The pyruvate kinase reaction is irreversible and therefore cannot play a role in the pathway of gluconeogenesis. As we will see in Chapter 9, surmounting the thermodynamic barrier constituted by the direct conversion of pyruvate to phosphoenolpyruvate requires two enzymatic steps, both of which utilize the energy of nucleotide triphosphates.

4.4.4 Energy Yield from Glycolysis

The direct energy yield of glycolysis starting from free glucose and ending with either pyruvate or lactate is 2 ATP. ATP is generated in two reactions in the pathway, the glycerate kinase and pyruvate kinase reactions. Since one molecule of glucose gives rise to two molecules of glyceraldehyde 3-phosphate in glycolysis, a total of four molecules of ATP are produced; however, two molecules of ATP are consumed in the conversion of free glucose into fructose 1,6-bisphosphate.

4.4.5 Glycolysis Requires NAD^+ and Inorganic Phosphate

No matter how available glucose is, glycolysis will not function if the concentrations of two other critical substrates, NAD^+ and inorganic phosphate (orthophosphate or P_i), are suboptimal. Since inorganic phosphate is a substrate in the glyceraldehyde 3-phosphate dehydrogenase reaction, glycolysis will cease to function if sufficient inorganic phosphate is not available in the cytosol. In certain metabolic diseases, such as hereditary fructose intolerance, where cellular phosphate accumulates in a sugar phosphate form, the concentration of inorganic phosphate becomes too low to support the glyceraldehyde 3-phosphate dehydrogenase reaction, thereby reducing the rate of glycolysis.

Similarly, if cytosolic NADH is not reoxidized to NAD^+, glycolysis will not be possible. There are two ways that a cell can regenerate NAD^+: One depends on the mitochondrial electron transport system, the other on lactate dehydrogenase.

In the case of well-oxygenated tissues that contain mitochondria, the reducing equivalents in NADH are ultimately transported into mitochondria. However, since the mitochondrial inner membrane is impermeable to NADH, there is a need for alternate mechanisms for transporting the two electrons from NADH from the cytosol into the mitochondrial matrix. The two systems that transport glycolysis-derived electrons from the cytosolic NADPH into the mitochondria, the glycerol 3-phosphate shuttle and the malate–aspartate shuttle, are discussed in Chapter 6.

Alternatively, NADH can be reoxidized to NAD^+ by lactate dehydrogenase catalyzes the following reversible reaction (Fig. 4-4):

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{lactate} + \text{NAD}^+$$

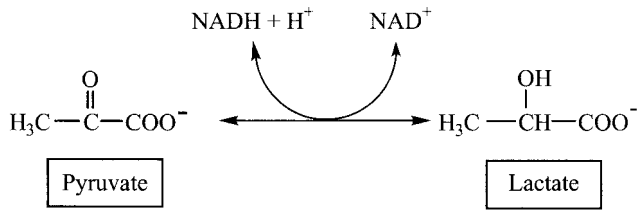


FIGURE 4-4 Lactate dehydrogenase reaction.

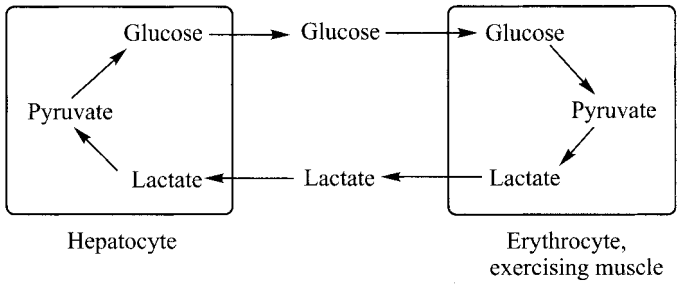


FIGURE 4-5 Pyruvate/lactate (Cori) cycle.

When glycolysis produces lactic acid, the process is referred to as *anaerobic glycolysis*. Lactate is produced both by red blood cells, which lack mitochondria, and by vigorously exercising muscle when the oxygen demand outstrips the oxygen supply. In both cases, lactate is transported in the blood to the liver, where it can provide substrate for gluconeogenesis. The newly synthesized glucose molecules can then be secreted by the liver into the blood, where they can be taken up and oxidized by red cells and muscle. This process is known as the *Cori cycle* (Fig. 4-5).

4.4.6 Metabolism of Fructose

Fructose and glucose are the monosaccharide components of sucrose, which is hydrolyzed by the enzyme sucrase that is localized on the outer surface of the brush border of the small intestine. Fructose is also present in honey and many fruits. Seminal vesicles secrete fructose into seminal fluid, where it functions as the major fuel for sperm cells.

Following its absorption into the blood, dietary fructose is extracted by the liver, where it is metabolized by glycolysis. Under normal circumstances, fructose is phosphorylated and trapped inside hepatocytes by fructokinase (Fig. 4-6):

$$\text{fructose} + \text{ATP} \rightarrow \text{fructose 1-phosphate} + \text{ADP}$$

Fructose can also be phosphorylated by hexokinase, but since the K_m of hexokinase for fructose is very high, hexokinase acts on fructose only when the fructose concentration is elevated abnormally. In contrast to fructokinase, which produces fructose 1-phosphate, hexokinase acting on fructose produces fructose 6-phosphate.

Fructose 1-phosphate is split by aldolase B into a triose (glyceraldehyde) and a triose phosphate (dihydroxyacetone phosphate):

$$\text{fructose 1-phosphate} \rightleftharpoons \text{glyceraldehyde} + \text{dihydroxyacetone phosphate}$$

Unlike aldolase A, which is present in all cells of the body, aldolase B is found only in liver, kidney, and the small intestine.

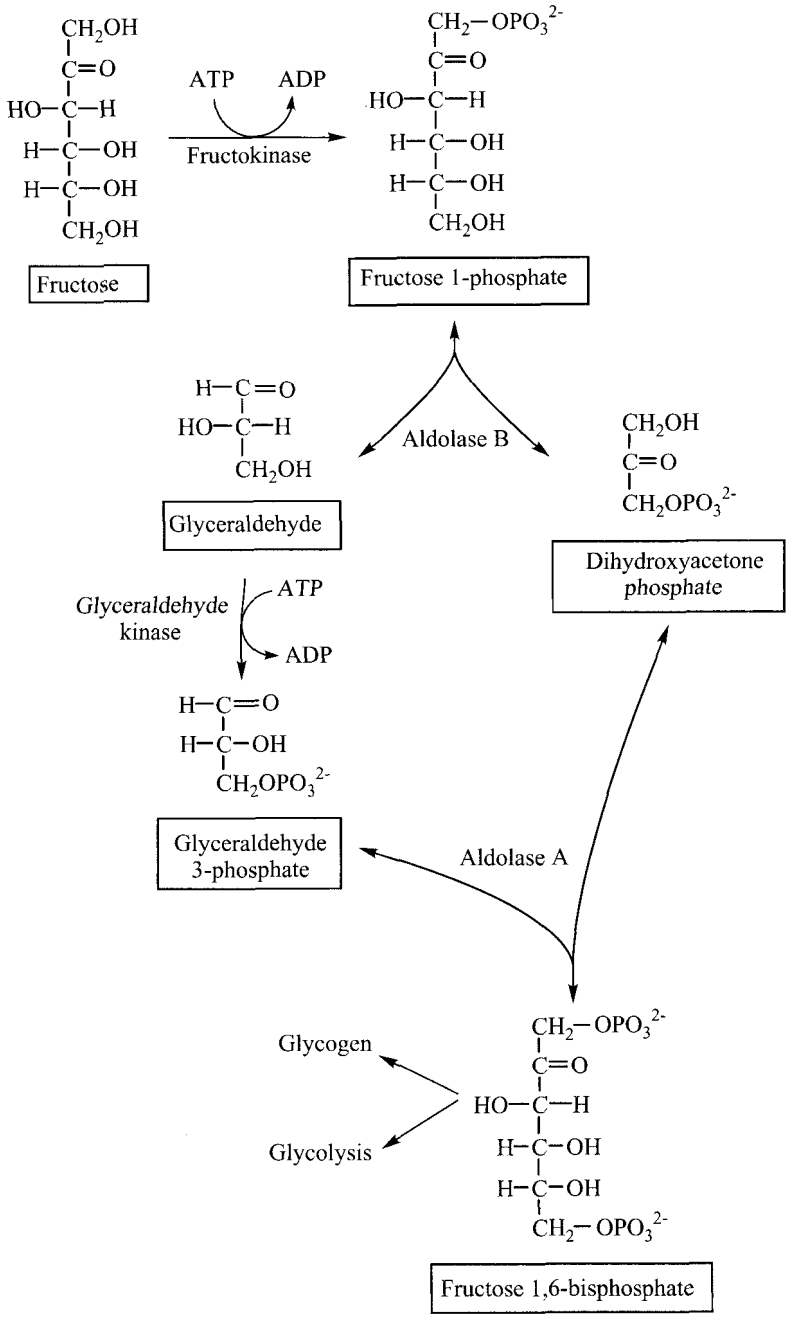


FIGURE 4-6 Metabolism of fructose to fructose 1,6-bisphosphate.

Glyceraldehyde arising from the aldolase B reaction is phosphorylated and trapped inside the hepatocyte by the action of glyceraldehyde kinase:

$$\text{glyceraldehyde} + \text{ATP} \rightarrow \text{glyceraldehyde 3-phosphate} + \text{ADP}$$

At this point, fructose has been converted into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, two triose phosphates that are the intermediates in glycolysis.

Alternatively, in hepatocytes, glyceraldehyde may be reduced by alcohol dehydrogenase:

$$\text{glyceraldehyde} + \text{NADH} + \text{H}^+ \rightarrow \text{glycerol} + \text{NAD}^+$$

The glycerol can then be phosphorylated by glycerol kinase to generate glycerol 3-phosphate, which is then available for the synthesis of triacylglycerol and glycerophospholipids.

4.4.7 The Metabolism of Galactose

Lactose (galactosyl-β-1,4-glucose) in the milk of mammals (including humans) is the major dietary source of galactose. As described in Chapter 3, lactose is hydrolyzed in the intestine by lactase. Galactose produced by hydrolysis of dietary lactose is mostly in the form of the β-isomer. β-Galactose is first converted to α-galactose by galactose mutarotase and then phosphorylated and trapped in hepatocytes by a galactose-specific kinase called *galactokinase* (Fig. 4-7):

$$\text{galactose} + \text{ATP} \rightarrow \text{galactose 1-phosphate} + \text{ADP}$$

Unlike hexokinase and glucokinase, both of which phosphorylate the C6 hydroxyl group of glucose, galactokinase and fructokinase phosphorylate the C1 hydroxyl group of their respective sugar substrates.

Isomerization of galactose 1-phosphate to glucose 1-phosphate requires two reactions. First, galactose must be incorporated into a uridine-based sugar nucleotide in a reaction catalyzed by uridyltransferase (formal name, UDP-glucose:galactose 1-phosphate uridyltransferase):

$$\text{(1) galactose 1-phosphate} + \text{UDP-1-glucose} \rightleftharpoons \text{UDP-1-galactose} + \text{glucose 1-phosphate}$$

The second step in the generation of glucose 1-phosphate is the epimerization of the galactose moiety. UDP-glucose 4-epimerase inverts the hydroxyl group on C4 of the galactose residue in UDP-1-galactose, thereby converting UDP-1-galactose into UDP-1-glucose (Fig. 4-7). NAD^+ is a cofactor in this epimerase reaction:

$$\text{(2) UDP-1-galactose} \rightleftharpoons \text{UDP-1-glucose}$$

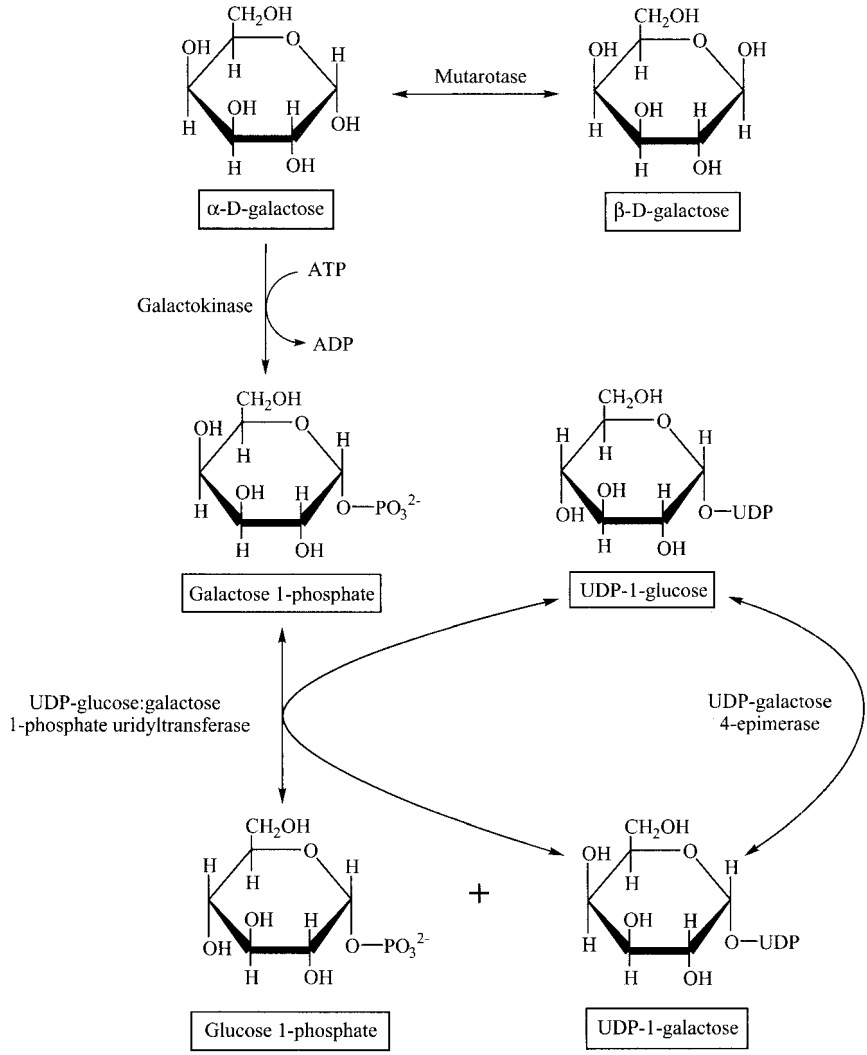


FIGURE 4-7 Metabolism of galactose to glucose 1-phosphate.

The net effect of reactions (1) and (2) is

$$\text{galactose 1-phosphate} \rightleftharpoons \text{glucose 1-phosphate}$$

Thus, UDP-1-glucose functions essentially as a catalyst in the pathway of galactose metabolism. It is consumed in the uridyltransferase reaction and regenerated in the UDP-galactose 4-epimerase reaction.

Before glucose 1-phosphate can enter glycolysis, it must first be converted into glucose 6-phosphate in the reversible reaction catalyzed by phosphoglucomutase:

$$\text{glucose 1-phosphate} \rightleftharpoons \text{glucose 6-phosphate}$$

UDP-1-glucose is also an intermediate in the pathways that synthesize glycogen and many different glycoconjugates. Indeed, it is for these reasons that lactose is an ideal dietary sugar for infants, who relative to adults, are more dependent on hepatic glycogen stores. Since UDP-1-glucose is in the direct pathway for the synthesis of glycogen, dietary galactose is converted directly into hepatic glycogen. In contrast, fructose derived from dietary sucrose must first be metabolized to triose phosphates, which are glycolytic intermediates. In the fed state, when dietary glucose stimulates insulin secretion, hepatic gluconeogenesis is suppressed and glycolytic intermediates are oxidized to pyruvate as opposed to being used to synthesize glycogen.

Humans can also synthesize UDP-1-galactose from glucose when the diet does not contain lactose or any other source of galactose. Since both the uridyltransferase and epimerase reactions are reversible, the pathway shown in Figure 4-7 can run in either direction. Synthesis of UDP-1-galactose is particularly important in the lactating mammary gland, which uses galactosyl transferase to synthesize lactose:

$$\text{UDP-1-galactose} + \text{glucose} \rightarrow \text{lactose} + \text{UDP}$$

4.5 REGULATION OF GLYCOLYSIS

The main factor that regulates glycolysis is the energy charge of the cell. When the intracellular ATP/ADP ratio is high, glycolysis is inhibited; conversely, when the ATP concentration is low and the concentrations of ADP and AMP are high, the flux of glucose through glycolysis is increased. Similarly, when the ATP/ADP ratio is high, the TCA-cycle enzyme isocitrate dehydrogenase is inhibited, resulting ultimately in an increase in the concentration of citrate in the cytosol of the cell (see Chapter 9).

Three enzymes are involved in regulating glycolysis (Fig. 4-8): phosphofructokinase-1, hexokinase, and pyruvate kinase, each of which catalyzes an irreversible reaction. In addition, translocation of GLUT4 transporters from intracellular vesicles to the plasma membrane regulates glucose metabolism in muscle and adipocytes.

4.5.1 Phosphofructokinase-1

Phosphofructokinase-1 (PFK-1) is the major regulatory enzyme in glycolysis. Allosteric regulation of PFK-1 permits the enzyme to respond to the energy needs of the cell and to hormonal signaling by insulin and glucagon. PFK-1 is inhibited by ATP and citrate, and activated by AMP. Citrate is an intermediate in the mitochondrial TCA cycle. When the energy charge of the cell is high, ATP inhibits mitochondrial

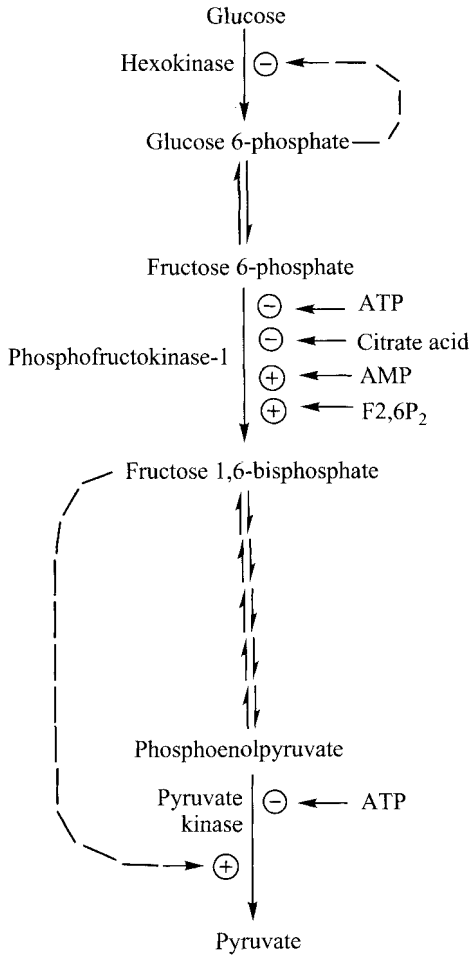


FIGURE 4-8 Regulation of hepatic glycolysis by metabolites.

isocitrate dehydrogenase, the key regulatory enzyme of the TCA cycle, resulting in a backup of the TCA cycle and accumulation of citrate.

Fructose 2,6-bisphosphate ($F2,6P_2$) is an allosteric activator of PFK-1. The intracellular concentration of $F2,6P_2$ is regulated by, and correlated directly with, the insulin/glucagon ratio. The two enzymes that determine the $F2,6P_2$ level directly are phosphofructokinase-2 (PFK-2) and fructose 2,6-bisphosphatase (FBPase-2). The activities of these two enzymes are regulated such that when PFK-2 is active, FBP-2 is inactive, and vice versa (Fig. 4-9). PFK-2 and FBPase-2 are encoded by one gene and the two catalytic activities are contained in a single polypeptide chain, hence the term *double-headed* enzyme. The activities of this double-headed enzyme system are regulated differently in different tissues.

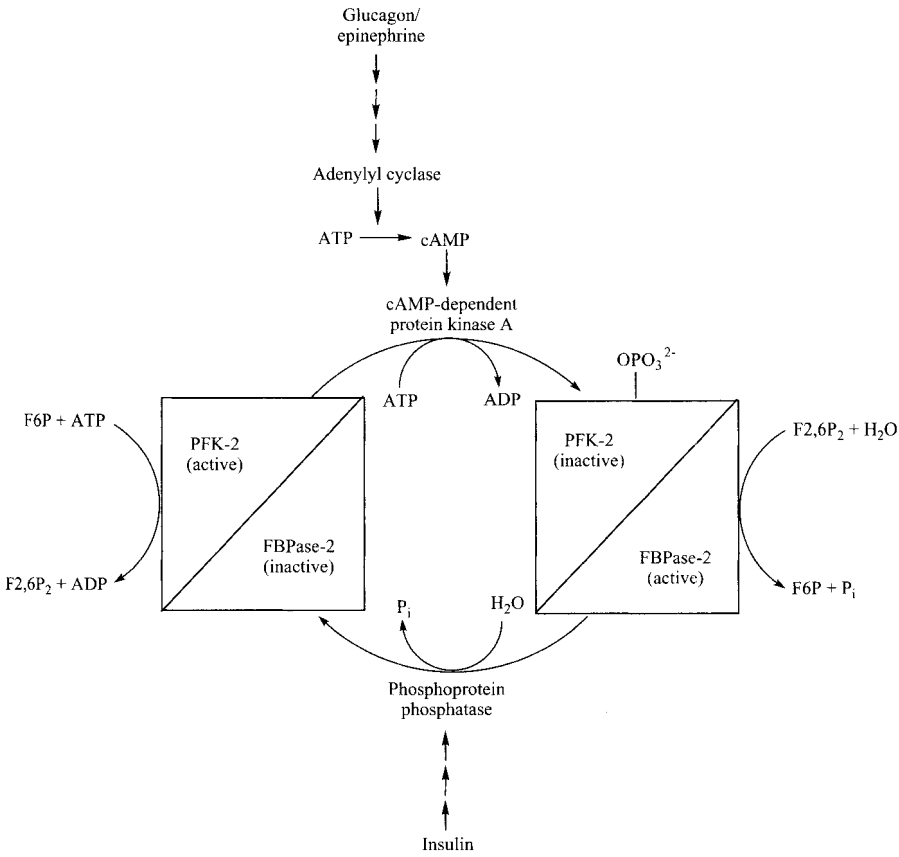


FIGURE 4-9 Hormonal regulation of the synthesis and catabolism of fructose 2,6-bisphosphate ($F2,6P_2$) in liver. PFK-2, phosphofructokinase-2; FBPase-2, fructose 2,6-bisphosphatase.

The hepatic double-headed enzyme is regulated by protein phosphorylation. As shown in Figure 4-9, when the hepatic PFK-2/FBPase-2 protein is phosphorylated by cAMP-activated protein kinase, PFK-2 is inactive and FBPase-2 is active, causing the $F2,6P_2$ level in the cell to decline. Glucagon, which activates adenylyl cyclase and thus raises the cAMP level of the cell, therefore has the effect of decreasing the F2,6P2 level, decreasing phosphofructokinase-1 activity, and ultimately slowing the flux of glucose through glycolysis. In contrast, binding of insulin to its receptor results in the activation of a phosphoprotein phosphatase that dephosphorylates the doubled-headed enzyme, thereby causing the $F2,6P_2$ concentration to rise and increasing the flux of glucose through glycolysis.

In contrast to the case in liver, the PFK-2/FBPase-2 protein in skeletal muscle has an alanine residue in place of the serine residue that is the substrate for

cAMP-activated protein kinase A. As a result, the protein constitutively synthesizes $F2,6P_2$ and glycolysis is not inhibited by epinephrine-induced intracellular signaling.

A third isozyme of PFK-2/FBP-2, present in heart muscle, has multiple phosphorylation sites including one phosphorylated by AMP-activated kinase (AMPK). Energy depletion in heart muscle results in a high AMP/ATP ratio which promotes activation of AMPK. Activated AMPK in turn phosphorylates PFK-2, which increases the intracellular concentration of F-2,6-P2, thereby stimulating glycolysis and energy production.

4.5.2 Hexokinase

As discussed above, hexokinase is regulated by direct feedback inhibition by one of the products of the reaction it catalyzes: glucose 6-phosphate.

4.5.3 Pyruvate Kinase

This enzyme, which catalyzes the last step of glycolysis, is inhibited by ATP and activated by fructose-1,6-bisphosphate (Fig. 4-8). Fructose-1,6-bisphosphate activation of pyruvate kinase is an example of feed forward activation. The activity of the liver isozyme of pyruvate kinase is also regulated by phosphorylation and dephosphorylation. Glucagon stimulates cAMP synthesis in hepatocytes, causing cAMP-activated protein kinase A in turn to phosphorylate pyruvate kinase. The phosphorylated form of protein kinase is inactive.

4.6 DISEASES INVOLVING GLYCOLYTIC ENZYMES

4.6.1 Genetic Deficiencies Directly Affecting Glycolysis

An absolute deficiency of either of the two ATP-producing enzymes of glycolysis would be lethal since virtually every cell in the body depends on glycolysis for energy production. A partial lack of pyruvate kinase or phosphoglycerate kinase activity in red blood cells depletes them of ATP, thereby compromising the cells' ability to export electrolytes and maintain proper osmotic balance between the cytosol and extracellular compartment. The end result is swelling and premature destruction of red cells, which manifests as hemolytic anemia.

4.6.2 Mercury Poisoning

Many enzymes in the body contain critical sulfhydryl groups that can react with mercury ions. The sulfhydryl group of glyceraldehyde 3-phosphate dehydrogenase that is essential for catalysis has an unusually high affinity for mercury. Binding of mercury to the enzyme's active-site thiol group inactivates this critical dehydrogenase and impedes glycolysis. Mercury can enter the body through consumption of ocean fish such as swordfish and tuna, water sources that are contaminated by industrial

waste, or by consumption of grains that had been treated with a mercury-containing rodenticide.

4.6.3 Arsenic Poisoning

In the body, arsenate is reduced to arsenite, which is the more toxic form of the metal. One deleterious effect of arsenite results from its ability to substitute for inorganic phosphate in the glyceraldehyde 3-phosphate dehydrogenase reaction. This results in the synthesis of an unstable 1-arseno-3-phosphoglycerate molecule, which hydrolyzes rapidly and spontaneously. The net effect of arsenic on glycolysis is to bypass the substrate-level phosphorylation reaction catalyzed by phosphoglycerate kinase, reducing the net energy yield from glycolysis to zero.

4.6.4 Essential Fructosuria

A genetic deficiency of fructokinase is a benign condition. A person who is fructokinase deficient will experience only a transient fructosemia (fructose in the blood) and fructosuria (fructose in the urine) following consumption of sucrose, high-fructose corn syrup, or invert sugar (an equimolar mixture of glucose and fructose). Unlike glucose, fructose is readily excreted by the kidney. In addition, hexokinase has broad substrate specificity and is capable of phosphorylating fructose as well as glucose, albeit at a much lower rate:

$$\text{fructose} + \text{ATP} \rightarrow \text{fructose 6-phosphate} + \text{ADP}$$

The fructose 6-phosphate generated by this reaction is then metabolized by the glycolytic pathway.

4.6.5 Hereditary Fructose Intolerance

In contrast to essential fructosuria, hereditary fructose intolerance is a life-threatening metabolic disorder. It is caused by a genetically based deficiency of aldolase B. Lack of aldolase B causes any ingested fructose to accumulate inside hepatocytes as fructose 1-phosphate, which has the effect of tying up intracellular phosphate in the form of a sugar phosphate. This, in turn, depletes the cell off inorganic phosphate, which is required for glycolysis in the cytosol and oxidative phosphorylation in mitochondria. Thus, a lack of aldolase B activity prevents the liver cell from making sufficient ATP from either glycolysis or mitochondrial respiration to maintain energy-requiring cellular functions.

4.6.6 Galactosemia

A genetic deficiency of any one of the enzymes of the pathway of galactose metabolism—galactokinase, uridyltransferase and galactose epimerase—will impede

galactose metabolism and will result in galactosemia if the affected infant ingests lactose. However, if a deficiency of one of these enzymes is identified in the first few days of life through a genetic-disease screening program, the potentially devastating effects of the mutation can be prevented by eliminating milk and other lactose-containing foods from the diet.

The classic and most severe form of galactosemia is caused by a deficiency of galactose 1-phosphate uridyltransferase. The resulting accumulation of galactose 1-phosphate in tissues leads to multiple-organ-system pathology, including neurological damage, cataracts, coma, and eventually death. By contrast, deficiency of galactokinase causes cataract formation but is otherwise less severe than classic galactosemia. The enzyme aldose reductase plays a role in the cataract formation associated with galactosemia by reducing galactose to galactitol and trapping this polyol inside cells of the lens. High intracellular concentrations of galactitol draw water into the tissue, thereby promoting osmotic damage to the lens.

Deficiencies of UDP-1-galactose 4-epimerase prevent both utilization of exogenous galactose and endogenous galactose synthesis. For this reason, patients are usually placed on a galactose-restricted rather than a galactose-free diet. Patients may also benefit from supplementation with *N*-acetylgalactosamine, which is needed for the synthesis of glycolipids and other glycoconjugates.

4.6.7 Diabetes Mellitus

The hallmarks of diabetes mellitus are hyperglycemia and glucosuria. The transport of glucose into muscle and adipose tissue and the stimulation of glycolysis in many tissues of a person in the fed state are dependent on normal signaling by insulin. Insulin also regulates and coordinates lipid and amino acid metabolism and glycogen storage, as well as gluconeogenesis. Type I diabetes results from inadequate synthesis of insulin by the β-cells of the pancreas, while type II diabetes is characterized primarily by insulin resistance, a condition in which peripheral cells do not respond normally to insulin.

Many of the pathological consequences of diabetes are the result of the formation of covalent bonds between glucose and proteins in various tissues (e.g., proteins in the lens of the eye). Glucose is an aldose sugar; that is, it contains an aldehyde group. Aldehydes are notoriously reactive, especially with the amino groups of proteins. Nearly all plasma and tissue proteins have an unblocked amino terminus as well as multiple lysine side chains. Even at the normal blood glucose concentration of 100 mg/dL, glucose reacts nonenzymatically with these amino groups, forming irreversible covalent sugar–protein adducts, referred to generically as *glycated proteins*. Hemoglobin A1c (HbA1c) is one such glycated protein. About 5.5% of the hemoglobin in a healthy person is glycated. In a patient with diabetes, there is a positive correlation between the percentage of HbA1c and his or her average blood glucose level over time; measurement of HbA1c therefore provides a measure of the efficacy of treatments to control the diabetes.

CHAPTER 5

PYRUVATE DEHYDROGENASE AND THE TRICARBOXYLIC ACID CYCLE

5.1 FUNCTIONS OF PYRUVATE DEHYDROGENASE AND THE TRICARBOXYLIC ACID CYCLE

5.1.1 Functions of the Pyruvate Dehydrogenase Reaction

Collectively, the pyruvate dehydrogenase (PDH) reaction and the tricarboxylic acid (TCA) cycle account for the complete combustion of pyruvate to CO_2 and water. The main functions of pyruvate dehydrogenase are to produce acetyl-CoA, which can be oxidized completely to CO_2 and water for energy in the tricarboxylic acid cycle, and to generate NADH, which can be oxidized by the mitochondrial electron transport system to support the production of ATP.

5.1.1.1 Pyruvate Dehydrogenase Generates Acetyl-CoA. Of the 30 to 32 ATP molecules that can be obtained by oxidizing one molecule of glucose to CO_2 and water, only 2 ATP are generated directly during glycolysis. An additional 3 to 5 ATP can be generated by mitochondrial oxidation of the two NADH molecules generated per molecule of glucose metabolized by glycolysis to two molecules of pyruvate. The remaining 25 ATP are produced when the two molecules of pyruvate enter the mitochondrion and are oxidized to acetyl-CoA and, in turn, the acetyl-CoA is oxidized to CO_2 and water.

Conceptually, PDH is the bridge between glycolysis and the TCA cycle. However, this bridge is unidirectional because while humans can oxidize pyruvate to

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acetyl-CoA (and ultimately to CO_2), they cannot carry out the opposite reaction of converting acetyl-CoA into pyruvate. It is the irreversibility of the PDH reaction that explains why the liver cannot use acetyl-CoA as a substrate in gluconeogenesis.

5.1.1.2 PDH Is an Important Site of Regulation. By regulating the flow of pyruvate into the TCA cycle, pyruvate dehydrogenase serves as an important site for regulating energy metabolism. Inhibition of PDH thus preserves glucose and gluconeogenic precursors such as alanine when other fuels, such as acetyl-CoA generated by the oxidation of fatty acids, are available for utilization.

5.1.2 Functions of the Tricarboxylic Acid Cycle

Each turn of the TCA cycle involves the entry into the pathway of one molecule of acetate from acetyl-CoA. The two-carbon acetate moiety combines with the four-carbon acid, oxaloacetate, to form citrate, which has six carbons. Subsequent reactions of the cycle result in the release of two carbon atoms as CO_2 and the regeneration of oxaloacetate. It is the regeneration and reutilization of oxaloacetate, which confers on the TCA cycle its cyclical character. Important functions of the TCA cycle are described below.

5.1.2.1 The TCA Cycle Generates Energy. The TCA cycle is the main source of energy in humans. It is responsible for the total oxidation of acetyl-CoA molecules that arise from the pyruvate dehydrogenase reaction, fatty acid β-oxidation, the oxidation of amino acids, ketone body catabolism, and the oxidation of ethanol. For each turn of the TCA cycle only a single molecule of high-energy nucleotide triphosphate (GTP) is produced. Most of the energy in acetyl-CoA that is released by the oxidative reactions of the TCA cycle is captured in the form of reduced electron carriers, specifically NADH and $FADH_2$. It is only when these two reduced electron carriers give up their electrons to the electron transport chain that their energy materializes in the form of ATP through the process of oxidative phosphorylation.

5.1.2.2 The TCA Cycle Provides Intermediates for Other Pathways. Some of the intermediates in the TCA cycle can be withdrawn and used in the synthesis of other cellular substances. For example, succinyl-CoA is a substrate in the pathway of heme synthesis. Similarly, the α-ketoglutarate generated when certain amino acids are broken down can enter the TCA cycle and be metabolized to malate which in turn can be exported from mitochondria into the cytosol. After cytosolic malate dehydrogenase oxidizes malate to oxaloacetate, the latter can be used to synthesize glucose by means of the gluconeogenesis pathway.

5.1.2.3 Enzymes of the TCA Cycle Make Acetyl-CoA Available for Fatty Acid and Cholesterol Synthesis. The two types of cells with the greatest capacity for fatty acid synthesis are the hepatocyte and the adipocyte. Almost all of the acetyl-CoA substrate utilized for fatty acid synthesis is generated inside mitochondria

by the pyruvate dehydrogenase reaction. The acetyl moieties are transported out of the mitochondrion in the form of the TCA-cycle intermediate citrate. Regeneration of acetyl-CoA from citrate in the cytosol then provides substrate for the synthesis of fatty acids and cholesterol.

5.1.2.4 The TCA Cycle Generates a Metabolite That Regulates Other Metabolic Pathways. The citrate concentration in a cell is strongly dependent on the energy charge of the cell. When the energy charge of the cell is high, ATP depresses TCA-cycle activity, and the citrate concentration increases. When the mitochondrial ATP concentration is high, citrate moves from the mitochondrial matrix into the cytosol, where it regulates glycolysis, gluconeogenesis, and fatty acid synthesis, all three of which are localized to the cytosol. Cytosolic citrate inhibits glycolysis by inhibiting phosphofructokinase-1 (PFK-1), stimulates gluconeogenesis by stimulating fructose 1,6-bisphosphatase (see Chapter 9), and promotes de novo fatty acid synthesis by activating acetyl-CoA carboxylase (see Chapter 11).

5.1.2.5 The TCA-Cycle Enzymes Participate in Pathways That Shuttle Reducing Equivalents into the Mitochondrion. Glycolysis generates NADH in the cytosol, but NADH cannot enter the mitochondrion. The shuttle systems that transport NADH-reducing equivalents from the cytosol into the mitochondrion for electron transport and oxidative phosphorylation utilize enzymes of the TCA cycle.

5.2 LOCATION OF PYRUVATE DEHYDROGENASE AND THE TCA-CYCLE ENZYMES

All mitochondria-containing cells possess PDH and the TCA-cycle enzymes. The pyruvate dehydrogenase complex is associated with the matrix-facing surface of the inner mitochondrial membrane. The TCA-cycle enzyme succinate dehydrogenase is an integral protein of the inner mitochondrial membrane; all the other enzymes of the TCA cycle are located in the mitochondrial matrix. In most cells, pyruvate arises mainly from glycolysis of glucose and is therefore generated in the cytosol. Pyruvate enters mitochondria through an organic-acid carrier that facilitates its transport across the inner mitochondrial membrane.

5.3 PHYSIOLOGICAL STATES WHEN PDH AND THE TCA CYCLE ARE ESPECIALLY ACTIVE OR INACTIVE

The TCA cycle is the main oxidative pathway for generating reducing equivalents in the form of NADH and $FADH_2$ that can be used to synthesize ATP. As such, flux through the TCA cycle increases in skeletal muscle and heart cells during aerobic exercise. By contrast, the TCA cycle in the liver is relatively inactive during fasting.

During a fast, intermediates of the TCA cycle are converted to malate, which is then transported out of the mitochondrion to provide substrate for gluconeogenesis. Under these conditions, the acetyl-CoA generated by β-oxidation of fatty acids in the liver is utilized to synthesize ketones, which are exported from hepatocytes into the blood. The ketones are metabolized to CO_2 and water by other tissues, primarily muscle.

PDH activity is increased in the fed state when many different types of cells are using mainly glucose as their fuel source, as opposed to fasted state when muscle, liver, and many other organs rely primarily on fatty acid oxidation to generate ATP. In addition, PDH activity in muscle increases with increased aerobic exercise, resulting in greater reliance on glucose as a fuel source.

5.4 PDH AND THE REACTIONS OF THE TCA CYCLE

5.4.1 Pyruvate Dehydrogenase

The PDH complex catalyzes a series of reactions (Fig. 5-1), the net result of which is

$$\text{pyruvate} + NAD^+ + \text{CoASH} \rightarrow \text{acetyl-CoA} + NADH + H^+ + CO_2$$

The reaction is complex in two respects. First, the pyruvate dehydrogenase enzyme is an extremely large, multisubunit complex comprised of multiple copies of

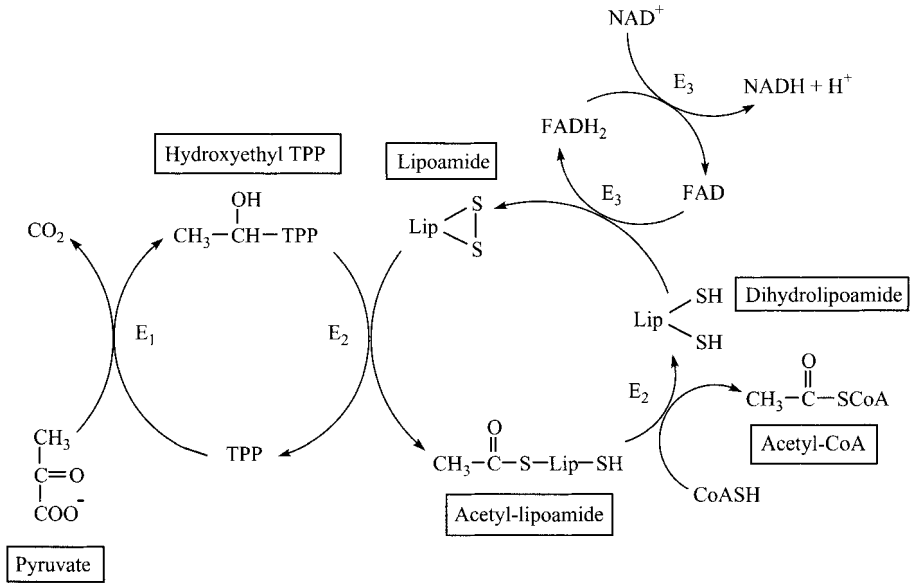

FIGURE 5-1 Sequence of reactions catalyzed by the pyruvate dehydrogenase complex. TPP, thiamine pyrophosphate.

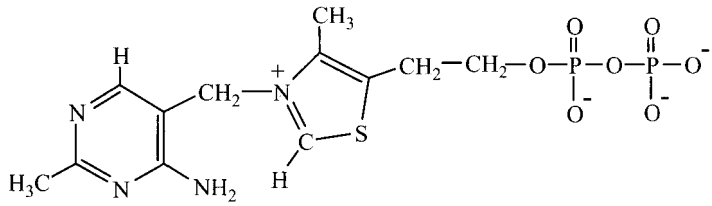

FIGURE 5-2 Structure of thiamine pyrophosphate.

each of three catalytic enzymes: pyruvate dehydrogenase, dihydrolipoamide acetyltransferase, and dihydrolipoamide dehydrogenase and two regulatory enzymes, PDH kinase and PDH phosphatase. Second, the PDH reaction requires five cofactors: coenzyme A (CoASH), NAD^+, FAD, lipoic acid, and thiamine pyrophosphate.

The pyruvate dehydrogenase subunit, designated E_1, catalyzes the oxidative decarboxylation of pyruvate and the transfer of the resultant acetyl unit to the cofactor thiamine pyrophosphate (TPP) (Fig. 5-2), which is tightly bound to E_1. The dihydrolipoamide acetyltransferase subunit, designated E_2, catalyzes the transfer of the acetyl group from the thiamine pyrophosphate of E_1 to CoASH. The prosthetic group of E_2 is lipoic acid, which is covalently attached to the ε-amino group of a lysine residue of dihydrolipoamide acetyltransferase, thereby forming lipoamide. In the process of this reaction, (oxidized) lipoamide is reduced to dihydrolipoamide (Fig. 5-3). The E_3 subunit, dihydrolipamide dehydrogenase, contains FAD as its prosthetic group and catalyzes the regeneration of lipoamide from dihydrolipoamide, generating NADH in the process.

5.4.2 The Tricarboxylic Acid Cycle

The overall process by which acetyl-CoA is oxidized to CO_2 in the TCA cycle is irreversible. The pathway itself produces just one equivalent of high-energy nucleotide triphosphate (GTP) per molecule of acetyl-CoA oxidized by the cycle (Fig. 5-4). However, for each acetyl-CoA molecule, four energy-rich reduced electron carriers (three NADH and one $FADH_2$) are generated per turn of the cycle:

$$\text{acetyl-CoA} + 3NAD^+ + P_i + GDP + FAD + 2H_2O \rightarrow$$
$$2CO_2 + \text{CoASH} + 3NADH + 3H^+ + GTP + FADH_2$$

The three NADH and one $FADH_2$ generated in one complete cycle support the production of 9 ATP by means of oxidative phosphorylation. Thus, the TCA cycle is capable of producing 10 equivalents of ATP (9 ATP and 1 GTP) from one molecule of acetyl-CoA.

The TCA cycle is initiated by the condensation of the carbon chain of acetyl-CoA with oxaloacetic acid in an irreversible reaction catalyzed by citrate synthase:

$$\text{acetyl-CoA} + \text{oxaloacetate} \rightarrow \text{citrate} + \text{CoASH}$$

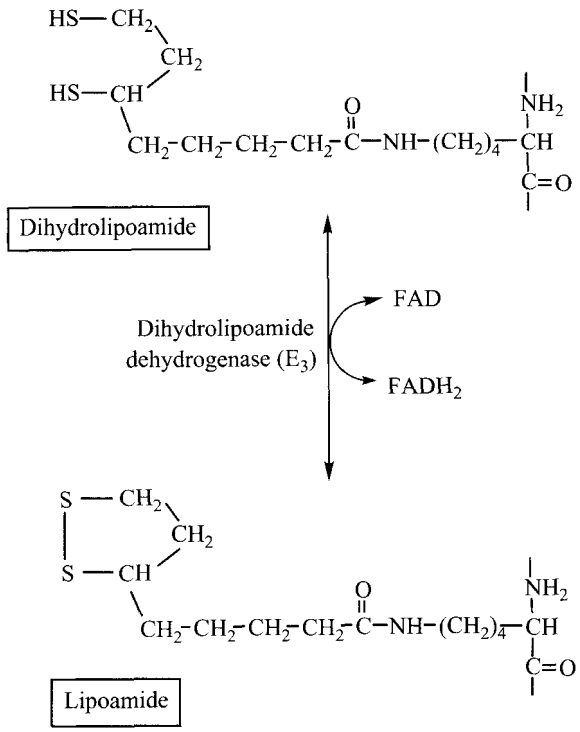

FIGURE 5-3 Reaction catalyzed by dihydrolipoamide dehydrogenase (E3) of the pyruvate dehydrogenase complex. Both oxidized lipoic acid (lipoamide) and reduced lipoic acid (dihydrolipoamide) are linked to the E2 subunit of PDH through an amide linkage.

Oxaloacetate functions in a catalytic fashion in the TCA cycle: It is the substrate that admits acetyl units into the cycle, and it is regenerated in the last step of the cycle.

The citrate formed in the first step of the TCA cycle is isomerized by aconitase to isocitrate:

$$\text{citrate} \rightleftharpoons \text{isocitrate}$$

The aconitase reaction is reversible and the equilibrium favors citrate over isocitrate by a factor of 20 to 1. Thus, when subsequent metabolism of isocitrate is inhibited, citrate levels (instead of isocitrate) increase in mitochondria. Aconitase has another function in addition to its role in the TCA cycle. The cytosolic form of the enzyme is involved in iron transport into cells and the regulation of the iron levels in the body.

The first redox reaction of the TCA cycle is catalyzed by the NAD^+-linked enzyme, isocitrate dehydrogenase:

$$\text{isocitrate} + NAD^+ \rightarrow \alpha\text{-ketoglutarate} + CO_2 + NADH + H^+$$

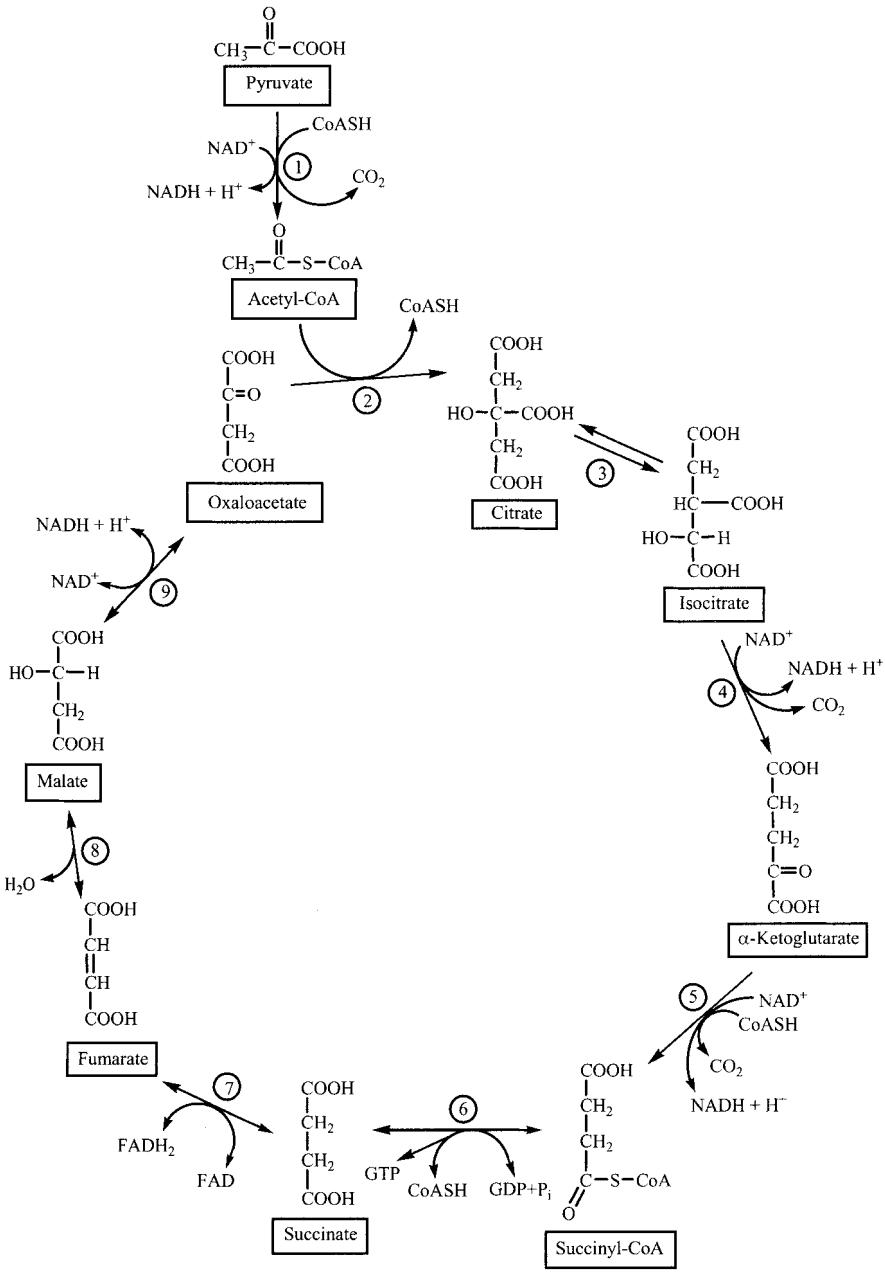

FIGURE 5-4 Reactions of the tricarboxylic acid cycle: ①, pyruvate dehydrogenase complex; ②, citrate synthase; ③, aconitase; ④, isocitrate dehydrogenase; ⑤, α-ketoglutarate dehydrogenase; ⑥, succinate thiokinase; ⑦, succinate dehydrogenase; ⑧, fumarase; ⑨, malate dehydrogenase.

When the electrons from NADH pass through the electron transport chain that is coupled to the phosphorylation of ADP, the energy that was transferred from isocitrate to NAD^+ in the isocitrate dehydrogenase reaction is ultimately used to synthesize ATP.

Next, α-ketoglutarate is oxidatively decarboxylated to produce succinyl-CoA in a reaction catalyzed by α-ketoglutarate dehydrogenase:

$$\alpha\text{-ketoglutarate} + NAD^+ + CoASH \rightarrow \text{succinyl-CoA} + CO_2 + NADH + H^+$$

The oxidative decarboxylation reaction catalyzed by α-ketoglutarate dehydrogenase is similar to the pyruvate dehydrogenase reaction, and FAD, thiamine pyrophosphate, and lipoic acid are cofactors in this reaction as well as that catalyzed by pyruvate dehydrogenase.

Succinyl-CoA contains a high-energy thioester bond which can be used to drive the synthesis of GTP. This is the only reaction in the TCA cycle that produces a high-energy nucleotide triphosphate directly, without the involvement of the machinery of oxidative phosphorylation; it is catalyzed by succinate thiokinase:

$$\text{succinyl-CoA} + GDP + P_i \rightarrow \text{succinate} + GTP + CoASH$$

The high-energy γ-phosphate bond of GTP can be transferred to ADP in a reversible reaction catalyzed by nucleotide kinase:

$$GTP + ADP \rightleftharpoons GDP + ATP$$

Proceeding through the TCA cycle, succinate is oxidized to fumarate in a reaction in which FAD is the electron acceptor:

$$\text{succinate} + FAD \rightleftharpoons \text{fumarate} + FADH_2$$

The enzyme that catalyzes this reaction, succinate dehydrogenase, is an integral membrane-bound protein localized to the inner mitochondrial membrane, thereby allowing for efficient energy transfer of the two electrons from $FADH_2$ to the co-localized electron transport chain.

Fumarate is then hydrated by fumarase:

$$\text{fumarate} + H_2O \rightleftharpoons \text{malate}$$

Fumarase exists in cytosolic and mitochondrial isoforms; however, both are encoded by the same gene. Cytosolic fumarase is active in liver, where it contributes to regenerating aspartate from the fumarate produced during the synthesis of urea.

Malate is then oxidized to oxaloacetate by malate dehydrogenase, completing the TCA cycle and bringing it full circle:

$$\text{malate} + NAD^+ \rightleftharpoons \text{oxaloacetate} + NADH + H^+$$

5.4.3 Synthesis of Catalytic Intermediates for the TCA Cycle

Since oxaloacetate functions as a catalyst in the TCA cycle, continued operation of the TCA cycle is critically dependent on maintaining the intramitochondrial concentration of oxaloacetate. Reactions that produce oxaloacetate directly or other compounds which can be metabolized to oxaloacetate are said to be anaplerotic in the sense that they can replenish oxaloacetate.

5.4.3.1 Synthesis of Oxaloacetate. The main reaction that replenishes oxaloacetate in the mitochondrion is catalyzed by the biotin-containing enzyme pyruvate carboxylase:

$$\text{pyruvate} + CO_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + P_i$$

Oxaloacetate is also synthesized by aspartate aminotransferase (AST), which transfers the amino group of aspartate to α-ketoglutarate:

$$\text{aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

5.4.3.2 Synthesis of Other TCA-Cycle Intermediates. Reactions that generate TCA-cycle intermediates such as α-ketoglutarate and succinyl-CoA are also anaplerotic, since these TCA-cycle intermediates can readily be metabolized to oxaloacetate. The major precursors of α-ketoglutarate and other TCA-cycle intermediates are the glucogenic amino acids, whose catabolism gives rise to substrates for gluconeogenesis. Another source of succinyl-CoA is propionyl-CoA, which arises from the catabolism of valine and isoleucine, the oxidation of methyl-branched fatty acids (e.g., phytanic acid) and the β-oxidation of relatively rare odd-carbon fatty acids.

5.4.4 Reactions That Deplete the TCA Cycle of Intermediates

As noted above, mitochondrial malate can be transported into the cytosol and utilized for gluconeogenesis. Intermediates in the TCA cycle can also serve as precursors in the synthesis of other cellular molecules. For example, succinyl-CoA is a substrate in the first step of heme synthesis, the δ-aminolevulinic acid synthase reaction:

$$\text{succinyl-CoA} + \text{glycine} \rightarrow \delta\text{-aminolevulinic acid} + \text{CoASH}$$

Other such examples are the synthesis of glutamate and aspartate from α-ketoglutarate and oxaloacetate, respectively:

$$\alpha\text{-ketoglutarate} + \text{alanine} \rightleftharpoons \text{glutamate} + \text{pyruvate}$$

$$\text{oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}$$

Glutamate, in turn, is the precursor of glutamine, arginine, proline, and the neurotransmitter γ-aminobutyric acid (GABA). Aspartate can be converted to asparagine.

5.5 REGULATION OF PDH AND THE TCA CYCLE

5.5.1 Pyruvate Dehydrogenase

The enzyme is regulated in two ways: by feedback inhibition involving metabolites and by phosphorylation/dephosphorylation.

5.5.1.1 Regulation of PDH by Means of Phosphorylation/Dephosphorylation. Pyruvate dehydrogenase is active in the dephosphorylated state and inactive when phosphorylated (Fig. 5-5). The enzyme is phosphorylated by a specific kinase named *pyruvate dehydrogenase kinase* (PDH kinase). PDH kinase is regulated not by cAMP but by molecules that signal changes in the energy charge of the cell. When the mitochondrial concentration of NADH, ATP, or acetyl-CoA is elevated, PDH kinase activity is stimulated, and pyruvate dehydrogenase becomes phosphorylated and inhibited. In contrast, high concentrations of pyruvate bind to PDH kinase and prevent the kinase from phosphorylating and inactivating pyruvate dehydrogenase. A PDH-specific calcium-activated phosphoprotein phosphatase (designated PDH phosphatase) removes a phosphate group from phosphorylated pyruvate dehydrogenase, thereby activating the enzyme.

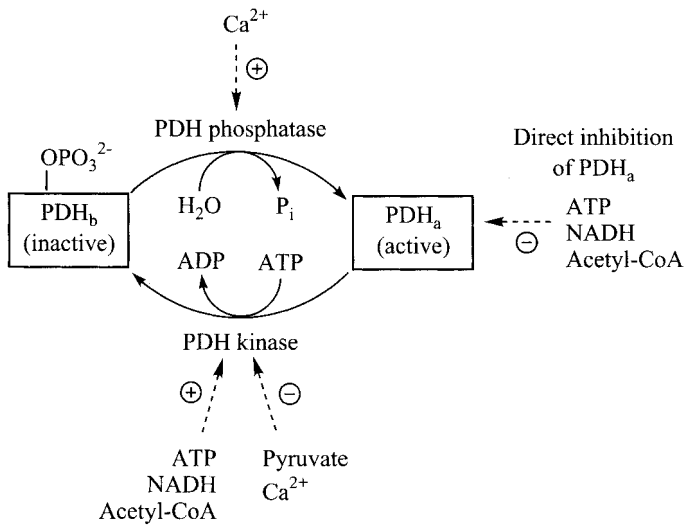

FIGURE 5-5 Regulation of pyruvate dehydrogenase (PDH).

5.5.1.2 Direct Regulation of Pyruvate Dehydrogenase by Metabolites.

The activity of the active, dephosphorylated form of pyruvate dehydrogenase is regulated directly by NADH and acetyl-CoA, which competitively inhibit the enzyme. ATP also stimulates PDH kinase, thereby inactivating PDH. PDH kinase is inhibited by a high intramitochondrial concentration of free calcium; this provides the mechanism by which epinephrine activates PDH in heart muscle.

5.5.2 Regulation of the TCA Cycle

The major locus of regulation of the TCA cycle is the isocitrate dehydrogenase reaction, and the key regulatory substance is ATP. A high-energy charge is associated with a high ATP concentration in the mitochondrion, which in turn inhibits the activity of isocitrate dehydrogenase. The TCA cycle is also inhibited by a high $NADH/NAD^+$ ratio, with NADH acting as a product inhibitor of isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase.

5.6 ABNORMAL FUNCTION OF PDH AND THE TCA CYCLE

5.6.1 Genetic Deficiency of Pyruvate Dehydrogenase

Impaired activity of PDH can result from mutations in the genes encoding any one of the three subunits of the enzyme. Most of the mutations that affect the pyruvate dehydrogenase complex adversely occur in the gene encoding the α subunit of E_1, which is located on the X chromosome; both hemizygous males and heterozygous females are affected.

Regardless of the molecular basis, a deficiency of pyruvate dehydrogenase activity is associated with severe metabolic acidosis due to the resulting high circulating levels of pyruvate and lactate, and with fatigue and hypotonia due to insufficient ATP production. Patients with mutations affecting the E_3 component of PDH also have reduced levels of activity of both α-ketoglutarate dehydrogenase and the branched-chain α-ketoacid dehydrogenase that is involved in the catabolism of leucine, isoleucine, and valine since dihydrolipoamide dehydrogenase is a common component of these enzymes as well as PDH.

Energy production is impaired in patients with PDH deficiency, especially in neural cells, which are normally dependent on a constant supply of glucose as a fuel source. Therapy in PDH-deficient persons involves a ketogenic diet (80% fat, 20% protein + carbohydrates), which creates conditions under which the brain increases its utilization of ketone bodies as an alternative fuel source. Some patients with PDH deficiency carry mutations that result in decreased affinity of their PDH for thiamine pyrophosphate cofactor; these people benefit from very high doses of thiamine. A drug called *dichloroacetate* (DCA) has also been used in some instances in an effort to stimulate residual PDH activity. DCA inhibits PDH kinase, thereby maintaining PDH in the dephosphorylated, active form and increasing the intracellular ATP level.

5.6.2 Genetic Diseases of the TCA Cycle

There are several distinct but very rare diseases that result from defective enzymes in the TCA cycle. Fumarase deficiency results in elevated plasma, urine, and tissue concentrations of fumarate, pyruvate, and lactate, muscular hypotonia, and severe neurologic impairment (encephalopathy with developmental delay, seizures, microcephaly, enlarged cerebral ventricles). Inherited deficiencies of succinate dehydrogenase and α-ketoglutarate dehydrogenase are also associated with lactic acidosis and major neurological problems.

5.6.3 Thiamine Deficiency

The classical presentation of thiamine deficiency is beriberi. A lack of dietary thiamine results in low levels of thiamine pyrophosphate (TPP) and impaired activity of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, as well as TPP-dependent transketolase, which is a component of the pentose phosphate pathway. Thiamine deficiency is especially damaging to the heart and brain, which have large energy requirements. Some of the neurological damage associated with thiamine deficiency may also be due to the impairment of direct actions of thiamine triphosphate on neural membrane proteins.

CHAPTER 6

ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

6.1 FUNCTION OF ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

The pathway of mitochondrial electron transport represents the final stage in the oxidation of carbohydrates, fats, and amino acids. This pathway transfers the reducing equivalents in NADH and $FADH_2$ to molecular oxygen. The oxidation of NADH and $FADH_2$ is accompanied by a substantial decrease in free energy, much of which is captured by the concurrent formation of ATP from ADP and P_i in a process termed *oxidative phosphorylation*. Although there is also some substrate-level phosphorylation which generates high-energy phosphate bonds during both glycolysis and the TCA cycle, oxidative phosphorylation provides most of the ATP that humans generate.

6.2 LOCALIZATION OF ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

Electron transport and oxidative phosphorylation occur within all cells except red blood cells and the cornea and lens of the eye, which lack mitochondria. The electron transport chain consists of four macromolecular complexes, three of which (complexes I, III, and IV) span the inner mitochondrial membrane, and one (complex II) which is embedded in but does not span the inner mitochondrial membrane. The

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energy released as electrons flow through the electron-transport chain is used to pump protons across the inner mitochondrial membrane to the intermembrane space, which lies between the inner and outer mitochondrial membranes, producing a proton gradient across the inner membrane.

The macromolecular complex called *ATP synthase*—the enzyme that produces ATP—also spans the inner mitochondrial membrane, forming a channel between the matrix and the cytosol. ATP synthase utilizes the electrochemical potential of the proton gradient to provide the energy for the synthesis of ATP. Thus, ATP synthesis is coupled to the collapse of the proton gradient and the return of protons through the channel of the ATP synthase complex to the matrix of the mitochondrion.

With the exception of brown fat, the rates of electron transport and oxidative phosphorylation are generally greatest in those tissues that contain large numbers of mitochondria. Mitochondria-rich tissues include the brain and central nervous tissue, red muscle fibers (relative to white muscle fibers), and the renal cortex (relative to the renal medulla). Brown fat is also rich in mitochondria (relative to white fat) and has a high rate of electron transport. Unlike other tissues, however, electron transport in brown fat is not tightly coupled to oxidative phosphorylation and functions primarily to generate heat rather than ATP.

6.3 PHYSIOLOGICAL CONDITIONS IN WHICH ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION ARE ESPECIALLY ACTIVE

Electron transport and oxidative phosphorylation are most active when there is an increased need for ATP. The utilization of oxygen is thus dramatically increased in exercising muscle relative to the relaxed state. Transport of electrons and oxidative phosphorylation are also increased in hypermetabolic states such as sepsis and trauma.

6.4 REACTIONS OF ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

6.4.1 Electron-Transport Chain

The electron-transport chain consists of four macromolecular respiratory complexes, each of which contains multiple prosthetic groups that serve as electron carriers. The electron carriers of the various respiratory complexes include iron–sulfur (Fe–S) proteins, heme-containing cytochromes, and flavin mononucleotides (FMN). Electrons are transferred between the respiratory complexes by two smaller electron carriers, ubiquinone (also called *coenzyme Q*) and the heme protein cytochrome *c* (Fig. 6-1).

6.4.1.1 Entry of Electrons from NADH into the Electron-Transport Chain. The pathway that the electrons of NADH take after they enter the electron-transport chain is shown in Figure 6-1. The two electrons from NADH are transferred

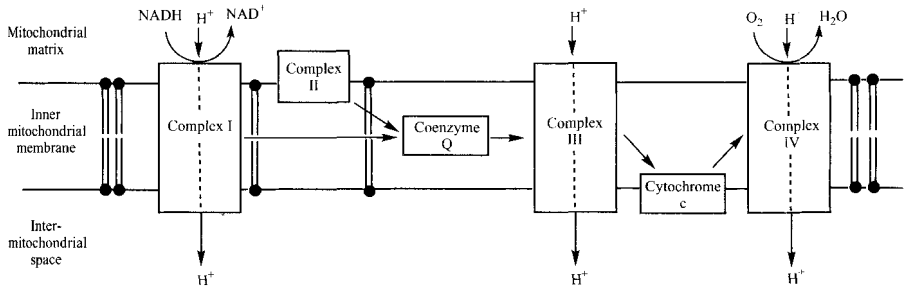

FIGURE 6-1 Components of the electron-transport chain, illustrating the pathway of electron flow from NADH to O_2. Shown are the sites where hydrogen ions are pumped out from the mitochondrial matrix into the intermitochondrial space.

to *respiratory complex I*, with the concomitant oxidation of NADH to NAD^+; after passing through the FMN and Fe–S components of the complex, these electrons are transferred to ubiquinone (CoQ):

$$NADH + H^+ + CoQ \rightarrow NAD^+ + CoQH_2$$

Reduced coenzyme Q ($CoQH_2$) in turn transfers the electrons to *respiratory complex III*, and in the process is oxidized back to the quinone state. The electrons then pass through the multiple prosthetic groups of respiratory complex III and into cytochrome *c*:

$$CoQH_2 + \text{cytochrome } c_{ox} \rightarrow CoQ + \text{cytochrome } c_{red}$$

Next, the reduced form of cytochrome *c* transfers electrons to *respiratory complex IV*, also called *cytochrome c oxidase*. Respiratory complex IV, which contains two hemeproteins (cytochrome *a* and cytochrome a_3), utilizes the electrons to reduce molecular oxygen to water:

$$\text{cytochrome } c_{red} + O_2 \rightarrow \text{cytochrome } c_{ox} + H_2O$$

The net effect of the flow of electrons from NADH through the various respiratory complexes is the oxidation of NADH by molecular oxygen:

$$NADH + H^+ + O_2 \rightarrow NAD^+ + H_2O$$

Movement of electrons through respiratory complexes I, III, and IV is accompanied by the pumping of protons from the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space. The accumulation of protons on the cytosolic side of the membrane establishes a proton gradient across the membrane

and results in a cytosolic pH which is approximately one pH unit lower than the pH of the mitochondrial matrix. This pH gradient represents potential energy or the potential to accomplish chemical work (e.g., ATP synthesis).

6.4.1.2 Entry of Electrons from $FADH_2$ into the Electron-Transport Chain. There are several ways that electrons enter the electron-transport chain from $FADH_2$ (Fig. 6-2). The major source of $FADH_2$ is that which is generated in mitochondria during β-oxidation of fatty acids. $FADH_2$ is also generated within mitochondria by the TCA cycle enzyme succinate dehydrogenase:

$$\text{succinate} + \text{FAD} \rightarrow \text{fumarate} + FADH_2$$

The succinate dehydrogenase complex of the respiratory chain contains both $FADH_2$ and Fe–S prosthetic groups and is designated *respiratory complex II*. However, the succinate dehydrogenase complex differs from respiratory complexes I, III, and IV in two important ways. First, although the succinate dehydrogenase complex is embedded in the inner mitochondrial membrane, it does not span the membrane. Second, the movement of electrons through the succinate dehydrogenase complex is not accompanied by the pumping of protons from the mitochondrial matrix across the inner mitochondrial membrane into the cytosol.

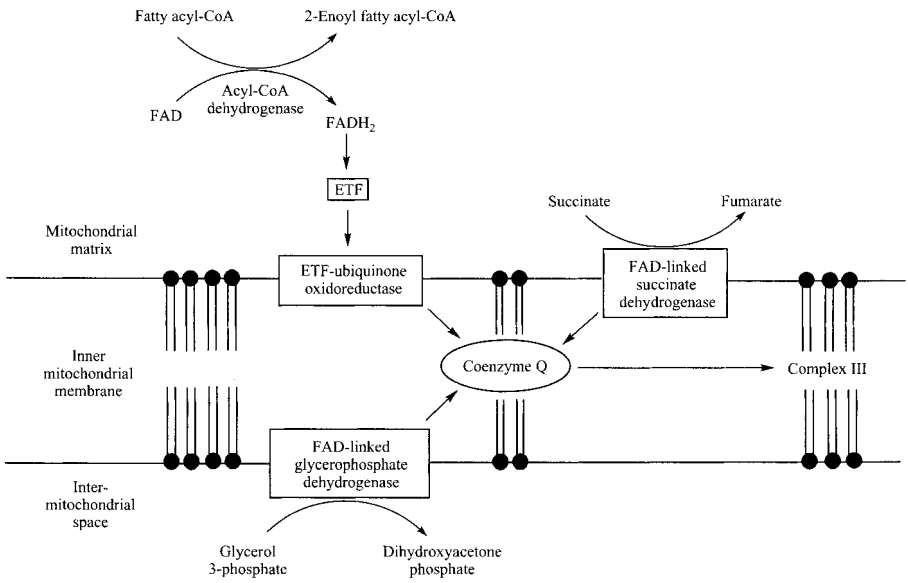

FIGURE 6-2 Entry of electrons from $FADH_2$ into the electron-transport chain at the level of coenzyme Q. ETF, electron-transfer flavoprotein.

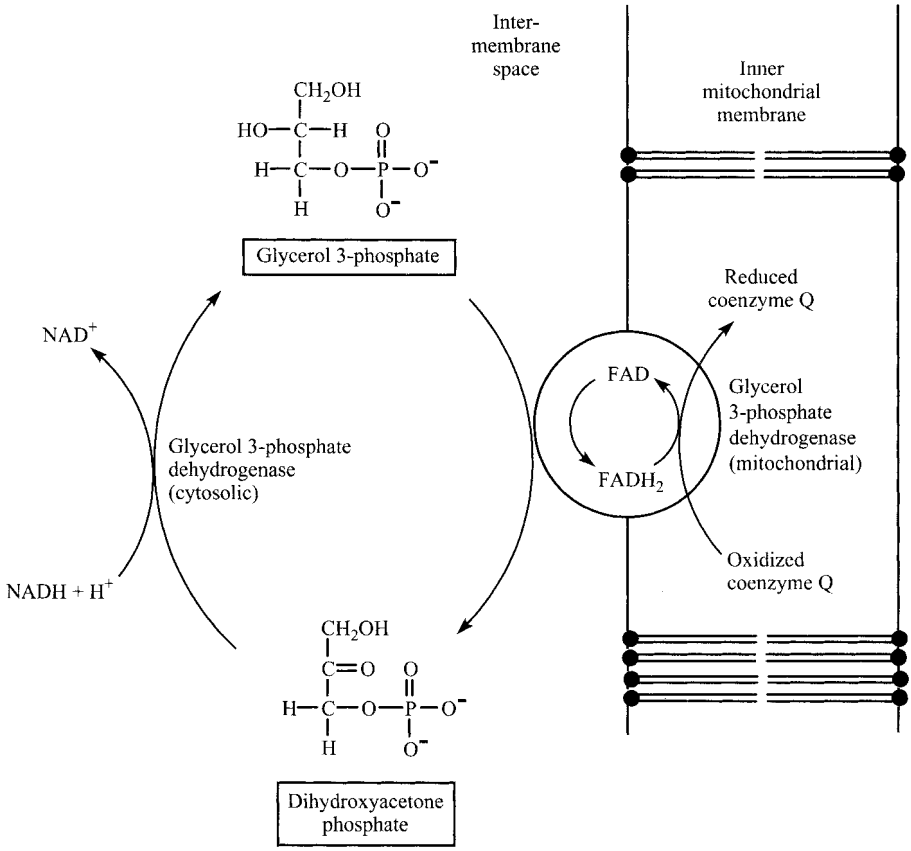

FIGURE 6-3 Entry of electrons from glycerol 3-phosphate dehydrogenase into the electron-transport chain.

$FADH_2$ is also generated in the cytosol by the action of glycerol 3-phosphate dehydrogenase (Fig. 6-3):

$$\text{glycerol 3-phosphate} + \text{FAD} \rightarrow \text{dihydroxyacetone phosphate} + FADH_2$$

The $FADH_2$ is then oxidized by the mitochondrial isozyme of glycerol 3-phosphate dehydrogenase:

$$\text{dihydroxyacetone phosphate} + FADH_2 \rightarrow \text{glycerol 3-phosphate} + \text{FAD}$$

This enzyme, also known as *flavoprotein dehydrogenase*, is embedded in the inner mitochondrial membrane, with its active site facing the cytosol, and transfers electrons through $FADH_2$ directly to coenzyme Q in the electron-transport chain.

As shown in Figure 6-2, electrons from $FADH_2$ generated by acyl-CoA dehydrogenases in the β-oxidation pathway, the succinate dehydrogenase complex, and mitochondrial glycerophosphate dehydrogenase are transferred to coenzyme Q:

$$FADH_2 + CoQ \rightarrow FAD + CoQH_2$$

Electrons are then transferred from $CoQH_2$ to respiratory complexes III and IV and, as described above, are ultimately used to reduce molecular oxygen:

$$FADH_2 + \tfrac{1}{2}O_2 \rightarrow FAD + H_2O$$

6.4.2 Synthesis of ATP

ATP synthase (a.k.a. *respiratory complex V*) catalyzes the synthesis of ATP in mitochondria (Fig. 6-4):

$$ADP + P_i \rightarrow ATP$$

ATP synthase, which was originally called mitochondrial ATPase because it also catalyzes the reverse reaction (i.e., the hydrolysis of ATP) is a large multiprotein complex comprised of two major subunits (F_0 and F_1). The F_0 *subunit* spans the inner mitochondrial membrane and contains a proton channel. Movement of protons through

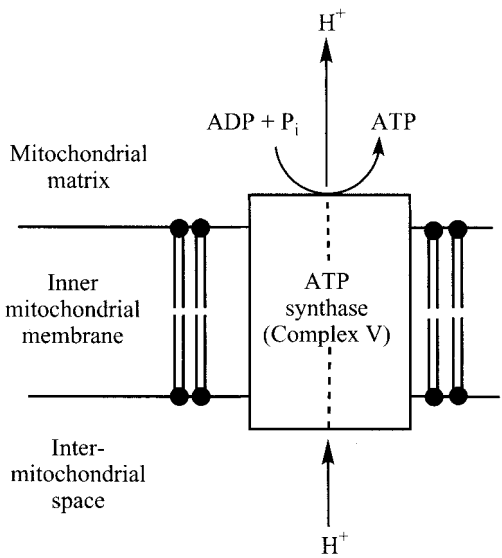

FIGURE 6-4 Generation of ATP by respiratory complex V (ATP synthase) is accompanied by the pumping of electrons back into the mitochondrial matrix.

the channel back into the mitochondrial matrix occurs down the proton gradient and provides the energy for the F_1 *subunit*, which catalyzes the synthesis of ATP.

6.4.2.1 How Much ATP Can Be Generated from the Oxidation of Mitochondrial NADH? The actual value of the P/O ratio, the ratio of the number of ATP molecules synthesized to the number of oxygen atoms consumed, has long been a subject of considerable controversy. Older studies suggested that the value was 3.0 for electrons entering the electron-transport chain from NADH. This round number was aesthetically pleasing since it suggested synthesis of one ATP from the protons pumped out of the mitochondrion at each of respiratory complexes I, III, and IV. However, more recent estimates indicate that for each NADH molecule that donates its electrons to the respiratory chain, four protons are pumped out of the mitochondrion by respiratory complex I, four protons by respiratory complex III, and two protons by respiratory complex IV. Since approximately four protons are needed to provide the energy required to synthesize one ATP, the actual P/O ratio is closer to 2.5 than to 3.0.

6.4.2.2 Why do Electrons from $FADH_2$ Generate Less ATP Than Those from NADH? As discussed above, electrons from $FADH_2$, including those arising from the succinate dehydrogenase complex, enter the electron-transport chain at coenzyme Q, bypassing respiratory complex I, which is the initial site at which protons are pumped out of the mitochondrial matrix. As a result, the passage of electrons from $FADH_2$ to molecular oxygen results in a lower net transfer to protons from the mitochondrial matrix to the cytosol, and a P/O ratio of 1.5 rather than 2.5.

6.4.3 Transport of Reducing Equivalents into the Mitochondrion

Much of the oxidative machinery of the cell is located within mitochondria, including the TCA cycle and enzymes that catalyze the β-oxidation of fatty acids and the oxidation of the carbon skeletons of most amino acids. Since the NADH and $FADH_2$ generated by these oxidative pathways are produced within the mitochondrion, the reducing equivalents in these cofactors can readily be transferred into the electron-transport chain. By contrast, the glycolytic pathway that oxidizes glucose generates NADH in the cytosol. Since the inner mitochondrial membrane is impermeable to NADH, cells need a mechanism for transporting the reducing equivalents in NADH from the cytosol into the mitochondrial matrix. Two such mechanisms exist: the malate–aspartate shuttle and the glycerol 3-phosphate shuttle.

6.4.3.1 Malate–Aspartate Shuttle. The malate–aspartate shuttle involves the reduction of a cytosolic substrate by NADH and transport of the reduced product from the cytosol into the mitochondrion, where it is reoxidized in an NAD^+-dependent reaction, thus consuming cytosolic NADH and generating mitochondrial NADH (Fig. 6-5). NADH is utilized by cytosolic malate dehydrogenase to reduce oxaloacetate to malate:

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{malate} + \text{NAD}^+$$

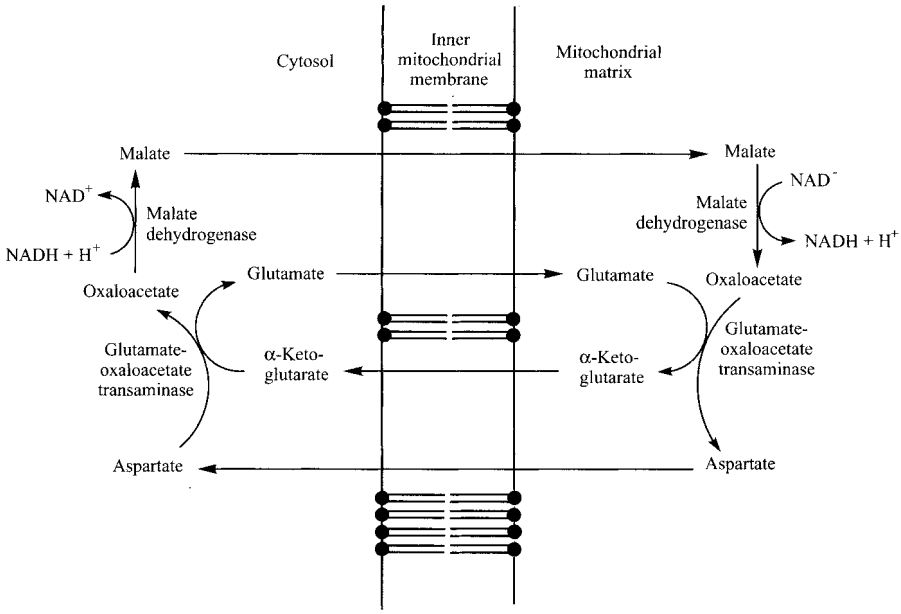

FIGURE 6-5 The malate–aspartate shuttle.

Malate is then transported from the cytosol into the mitochondrion, where it is oxidized to oxaloacetate by mitochondrial malate dehydrogenase isozyme, which also catalyzes the last step of the TCA cycle.

The actual pathway is complicated by the specific nature of the transporters that facilitate the movement of organic compounds in and out of the mitochondrion. Thus, the oxaloacetate produced by mitochondrial malate dehydrogenase is not transported directly out of the mitochondrion. Instead, the oxaloacetate molecule in the mitochondrial matrix first acquires an amino group from glutamate (through a process known as *transamination*):

$$\text{oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}$$

Aspartate is then transported out of the mitochondrion into the cytosol, where the transamination reaction occurs in the reverse direction, regenerating oxaloacetate. This process utilizes two mitochondrial transporters, one of which exchanges cytosolic malate for mitochondrial α-ketoglutarate, and a second, known as aralar 1/citrin, which exchanges mitochondrial aspartate for cytosolic glutamate.

The net effect of the malate–aspartate pathway is the transport of the reducing equivalents of cytosolic NADH into the mitochondrion:

$$\text{NADH}_{\text{cytosolic}} + \text{NAD}^+_{\text{mitochondrial}} \rightarrow \text{NAD}^+_{\text{cytolsolic}} + \text{NADH}_{\text{mitochondrial}}$$

6.4.3.2 Glycerol 3-Phosphate Shuttle. In the second electron-shuttle mechanism, the NADH generated during glycolysis is used by cytosolic glycerophosphate dehydrogenase to reduce dihydroxyacetone phosphate to glycerol 3-phosphate (Fig. 6-3). Glycerol 3-phosphate is then oxidized back to dihydroxyacetone phosphate by mitochondrial FAD-linked glycerol 3-phosphate dehydrogenase. Since the electrons from $FADH_2$ are transferred directly to ubiquinone, thus bypassing respiratory complex I, the glycerol 3-phosphate shuttle yields only 1.5 ATP per molecule of cytosolic NADH rather than the 2.5 ATP per NADH produced by means of the malate–aspartate shuttle.

6.4.3.3 Why Are There Two Different Shuttles for Transferring Reducing Equivalents from the Cytosol into the Mitochondrion? The two shuttle mechanisms are adapted to meet the needs of different metabolic conditions. Although the malate–aspartate shuttle is clearly the more efficient of the two shuttles in terms of the net yield of ATP, the pathway is readily reversible and transfers reducing equivalents into the mitochondrion only when the $NADH/NAD^+$ ratio is higher in the cytosol than in the mitochondrion. By contrast, the glycerol 3-phosphate shuttle is essentially irreversible and transfers reducing equivalents into the mitochondrion even when the $NADH/NAD^+$ ratio in the cytosol is lower than it is in the mitochondrion.

6.4.4 Export of ATP from Mitochondria

Transport of ATP out of the mitochondrion is accomplished by an adenine nucleotide translocase which moves one ADP into the mitochondrion for each ATP exported into the cytosol. Since the net charge of ATP is -4, whereas ADP has a charge of -3, the exchange is accompanied by the import of one proton per ATP into the mitochondrion and is driven by the pH gradient across the inner mitochondrial membrane.

Export of ATP from the mitochondrion in exchange for ADP also results in a net export of phosphate. Sustained ATP export thus requires a phosphate transporter, which imports cytosolic phosphate (P_i) in exchange for a hydroxyl ion. The exchange of P_i for a hydroxyl ion also results in a net import of one proton into the mitochondrion. Thus, the protons that are pumped out of the mitochondrion during electron transport provide some of the energy needed to drive the two transporters involved in ATP export from the mitochondrion.

6.4.5 Exchange of High-Energy Phosphate Bonds Between Nucleotides

Oxidative phosphorylation provides ATP for muscle work, membrane transporters, and a variety of biosynthetic reactions, such as protein synthesis. Other nucleotide triphosphates also play major roles in metabolism. For example, UTP is used to activate sugars for synthesis of glycogen and glycoconjugates, CTP in the synthesis of phospholipids, and GTP for both protein synthesis and the activation of the G proteins involved in signal transduction. Nucleoside triphosphates and deoxyribonucleoside triphosphates are also the substrates for the synthesis of DNA and RNA, respectively.