SECTION II

Bioenergetics & the Metabolism of Carbohydrates & Lipids

Bioenergetics: The Role of ATP

10

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BIOMEDICAL IMPORTANCE

Bioenergetics, or biochemical thermodynamics, is the study of the energy changes accompanying biochemical reactions. Biologic systems are essentially **isothermic** and use chemical energy to power living processes. How an animal obtains suitable fuel from its food to provide this energy is basic to the understanding of normal nutrition and metabolism. Death from **starvation** occurs when available energy reserves are depleted, and certain forms of malnutrition are associated with energy imbalance **(marasmus).** Thyroid hormones control the rate of energy release (metabolic rate), and disease results when they malfunction. Excess storage of surplus energy causes **obesity,** one of the most common diseases of Western society.

FREE ENERGY IS THE USEFUL ENERGY IN A SYSTEM

Gibbs change in free energy (ΔG) is that portion of the total energy change in a system that is available for doing work—ie, the useful energy, also known as the chemical potential.

Biologic Systems Conform to the General Laws of Thermodynamics

The first law of thermodynamics states that **the total energy of a system, including its surroundings, remains constant.** It implies that within the total system, energy is neither lost nor gained during any change. However, energy may be transferred from one part of the system to another or may be transformed into another form of energy. In living systems, chemical energy may be transformed into heat or into electrical, radiant, or mechanical energy.

The second law of thermodynamics states that **the total entropy of a system must increase if a process is to occur spontaneously. Entropy** is the extent of disorder or randomness of the system and becomes maximum as equilibrium is approached. Under conditions of constant temperature and pressure, the relationship between the free energy change (ΔG) of a reacting system and the change in entropy (ΔS) is expressed by the following equation, which combines the two laws of thermodynamics:

$$\Delta G = \Delta H - T\Delta S$$

where ΔH is the change in **enthalpy** (heat) and T is the absolute temperature.

In biochemical reactions, because ΔH is approximately equal to ΔE, the total change in internal energy of the reaction, the above relationship may be expressed in the following way:

$$\Delta G = \Delta E - T\Delta S$$

If ΔG is negative, the reaction proceeds spontaneously with loss of free energy; ie, it is **exergonic.** If, in addition, ΔG is of great magnitude, the reaction goes virtually to completion and is essentially irreversible. On the other hand, if ΔG is positive, the reaction proceeds only if free energy can be gained; ie, it is **endergonic.** If, in addition, the magnitude of ΔG is great, the

system is stable, with little or no tendency for a reaction to occur. If ΔG is zero, the system is at equilibrium and no net change takes place.

When the reactants are present in concentrations of 1.0 mol/L, ΔG^0 is the standard free energy change. For biochemical reactions, a standard state is defined as having a pH of 7.0. The standard free energy change at this standard state is denoted by $\Delta G^{0'}$.

The standard free energy change can be calculated from the equilibrium constant K_{eq}.

$$\Delta G^{0'} = -RT \ln K'_{eq}$$

where R is the gas constant and T is the absolute temperature (Chapter 8). It is important to note that the actual ΔG may be larger or smaller than $\Delta G^{0'}$ depending on the concentrations of the various reactants, including the solvent, various ions, and proteins.

In a biochemical system, an enzyme only speeds up the attainment of equilibrium; it never alters the final concentrations of the reactants at equilibrium.

ENDERGONIC PROCESSES PROCEED BY COUPLING TO EXERGONIC PROCESSES

The vital processes—eg, synthetic reactions, muscular contraction, nerve impulse conduction, and active transport—obtain energy by chemical linkage, or **coupling,** to oxidative reactions. In its simplest form, this type of coupling may be represented as shown in Figure 10–1. The conversion of metabolite A to metabolite B occurs with release of free energy. It is coupled to another reaction, in which free energy is required to convert metabolite C to metabolite D. The terms **exergonic** and **endergonic** rather than the normal chemical terms "exothermic" and "endothermic" are used to indicate that a process is accompanied by loss or gain, respectively, of free energy in any form, not necessarily as heat. In practice, an endergonic process cannot exist independently but must be a component of a coupled exergonic-endergonic system where the overall net change is exergonic. The exergonic reactions are termed **catabolism** (generally, the breakdown or oxidation of fuel molecules), whereas the synthetic reactions that build up substances are termed **anabolism.** The combined catabolic and anabolic processes constitute **metabolism.**

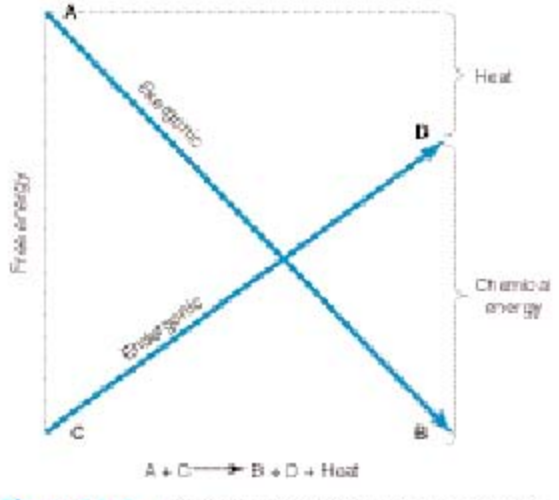


Figure 10–1. Coupling of an exergonic to an endergonic reaction.

If the reaction shown in Figure 10–1 is to go from left to right, then the overall process must be accompanied by loss of free energy as heat. One possible mechanism of coupling could be envisaged if a common obligatory intermediate (I) took part in both reactions, ie,

$$A + C \rightarrow I \rightarrow B + D$$

Some exergonic and endergonic reactions in biologic systems are coupled in this way. This type of system has a built-in mechanism for biologic control of the rate of oxidative processes since the common obligatory intermediate allows the rate of utilization of the product of the synthetic path (D) to determine by mass action the rate at which A is oxidized. Indeed, these relationships supply a basis for the concept of **respiratory control,** the process that prevents an organism from burning out of control. An extension of the coupling concept is provided by dehydrogenation reactions, which are coupled to hydrogenations by an intermediate carrier (Figure 10–2).

An alternative method of coupling an exergonic to an endergonic process is to synthesize a compound of high-energy potential in the exergonic reaction and to incorporate this new compound into the endergonic reaction, thus effecting a transference of free energy from the exergonic to the endergonic pathway (Figure 10–3). The biologic advantage of this mechanism is that the compound of high potential energy, ~Ⓔ, unlike I

$$\Delta G = \Delta H - T\Delta S$$

Figure 10–2. Coupling of dehydrogenation and hydrogenation reactions by an intermediate carrier.

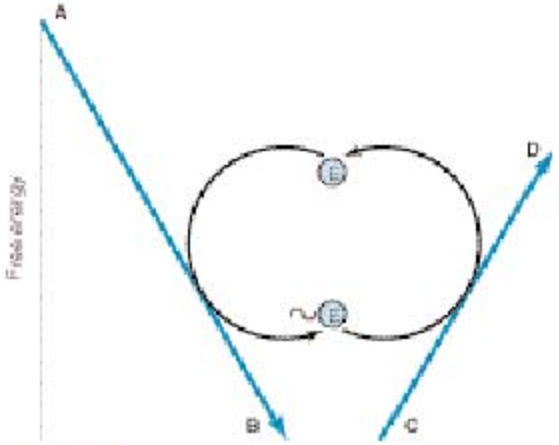


Figure 10–3. Transfer of free energy from an exergonic to an endergonic reaction via a high-energy intermediate compound (~Ⓔ).

in the previous system, need not be structurally related to A, B, C, or D, allowing Ⓔ to serve as a transducer of energy from a wide range of exergonic reactions to an equally wide range of endergonic reactions or processes, such as biosyntheses, muscular contraction, nervous excitation, and active transport. In the living cell, the principal high-energy intermediate or carrier compound (designated ~Ⓔ in Figure 10–3) is **adenosine triphosphate (ATP).**

HIGH-ENERGY PHOSPHATES PLAY A CENTRAL ROLE IN ENERGY CAPTURE AND TRANSFER

In order to maintain living processes, all organisms must obtain supplies of free energy from their environment. **Autotrophic** organisms utilize simple exergonic processes; eg, the energy of sunlight (green plants), the reaction $Fe^{2+} \rightarrow Fe^{3+}$ (some bacteria). On the other hand, **heterotrophic** organisms obtain free energy by coupling their metabolism to the breakdown of complex organic molecules in their environment. In all these organisms, ATP plays a central role in the transference of free energy from the exergonic to the endergonic processes (Figure 10–3). ATP is a nucleoside triphosphate containing adenine, ribose, and three phosphate groups. In its reactions in the cell, it functions as the Mg^{2+} complex (Figure 10–4).

The importance of phosphates in intermediary metabolism became evident with the discovery of the role of ATP, adenosine diphosphate (ADP), and inorganic phosphate (P_i) in glycolysis (Chapter 17).

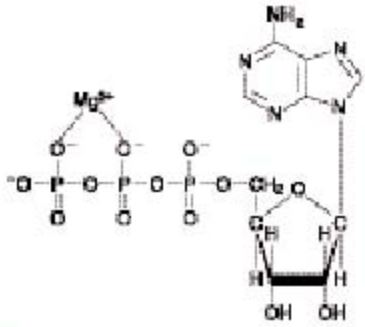


Figure 10–4. Adenosine triphosphate (ATP) shown as the magnesium complex. ADP forms a similar complex with Mg^{2+}.

The Intermediate Value for the Free Energy of Hydrolysis of ATP Has Important Bioenergetic Significance

The standard free energy of hydrolysis of a number of biochemically important phosphates is shown in Table 10–1. An estimate of the comparative tendency of each of the phosphate groups to transfer to a suitable acceptor may be obtained from the $\Delta G^{0'}$ of hydrolysis at 37 °C. The value for the hydrolysis of the terminal

Table 10–1. Standard free energy of hydrolysis of some organophosphates of biochemical importance.[1,2]

Compound	$\Delta G^{0'}$	
	kJ/mol	kcal/mol
Phosphoenolpyruvate	–61.9	–14.8
Carbamoyl phosphate	–51.4	–12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	–49.3	–11.8
Creatine phosphate	–43.1	–10.3
ATP → ADP + P_i	–30.5	–7.3
ADP → AMP + P_i	–27.6	–6.6
Pyrophosphate	–27.6	–6.6
Glucose 1-phosphate	–20.9	–5.0
Fructose 6-phosphate	–15.9	–3.8
AMP	–14.2	–3.4
Glucose 6-phosphate	–13.8	–3.3
Glycerol 3-phosphate	–9.2	–2.2

[1] P_i, inorganic orthophosphate.
[2] Values for ATP and most others taken from Krebs and Kornberg (1957). They differ between investigators depending on the precise conditions under which the measurements are made.

phosphate of ATP divides the list into two groups. **Low-energy phosphates,** exemplified by the ester phosphates found in the intermediates of glycolysis, have $\Delta G^{0'}$ values smaller than that of ATP, while in **high-energy phosphates** the value is higher than that of ATP. The components of this latter group, including ATP, are usually anhydrides (eg, the 1-phosphate of 1,3-bisphosphoglycerate), enolphosphates (eg, phosphoenolpyruvate), and phosphoguanidines (eg, creatine phosphate, arginine phosphate). The intermediate position of ATP allows it to play an important role in energy transfer. The high free energy change on hydrolysis of ATP is due to relief of charge repulsion of adjacent negatively charged oxygen atoms and to stabilization of the reaction products, especially phosphate, as resonance hybrids. Other "high-energy compounds" are thiol esters involving coenzyme A (eg, acetyl-CoA), acyl carrier protein, amino acid esters involved in protein synthesis, *S*-adenosylmethionine (active methionine), UDPGlc (uridine diphosphate glucose), and PRPP (5-phosphoribosyl-1-pyrophosphate).

High-Energy Phosphates Are Designated by ~ Ⓟ

The symbol ~Ⓟ indicates that the group attached to the bond, on transfer to an appropriate acceptor, results in transfer of the larger quantity of free energy. For this reason, the term **group transfer potential** is preferred by some to "high-energy bond." Thus, ATP contains two high-energy phosphate groups and ADP contains one, whereas the phosphate in AMP (adenosine monophosphate) is of the low-energy type, since it is a normal ester link (Figure 10–5).

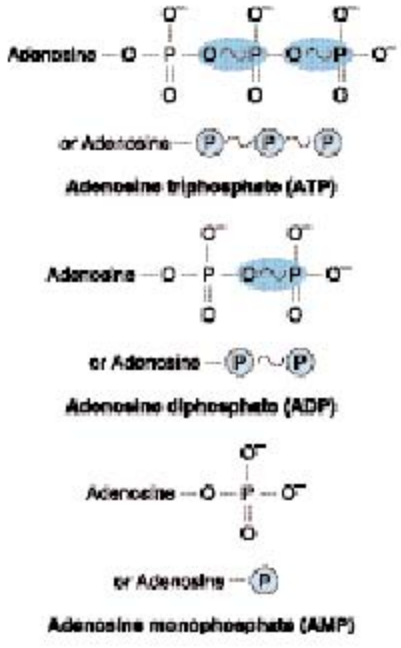


Figure 10–5. Structure of ATP, ADP, and AMP showing the position and the number of high-energy phosphates (~Ⓟ).

HIGH-ENERGY PHOSPHATES ACT AS THE "ENERGY CURRENCY" OF THE CELL

ATP is able to act as a donor of high-energy phosphate to form those compounds below it in Table 10–1. Likewise, with the necessary enzymes, ADP can accept high-energy phosphate to form ATP from those compounds above ATP in the table. In effect, an **ATP/ADP cycle** connects those processes that generate ~Ⓟ to those processes that utilize ~Ⓟ (Figure 10–6), continuously consuming and regenerating ATP. This occurs at a very rapid rate, since the total ATP/ADP pool is extremely small and sufficient to maintain an active tissue for only a few seconds.

There are three major sources of ~Ⓟ taking part in **energy conservation** or **energy capture:**

(1) **Oxidative phosphorylation:** The greatest quantitative source of ~Ⓟ in aerobic organisms. Free energy

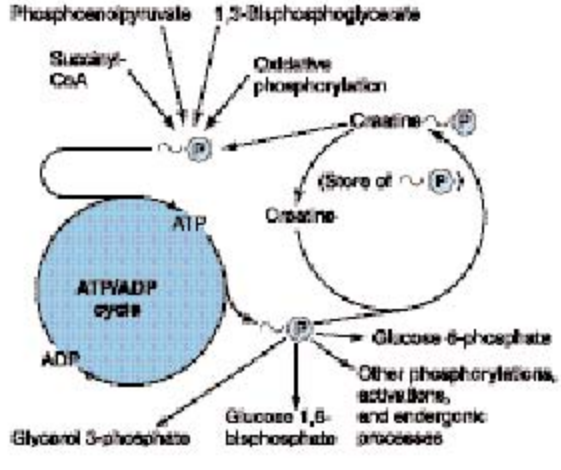


Figure 10–6. Role of ATP/ADP cycle in transfer of high-energy phosphate.

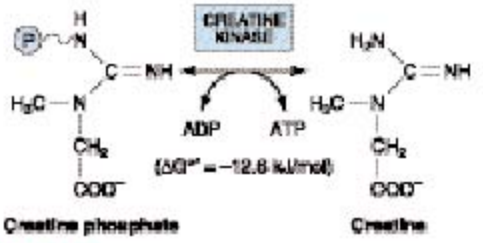


Figure 10–7. Transfer of high-energy phosphate between ATP and creatine.

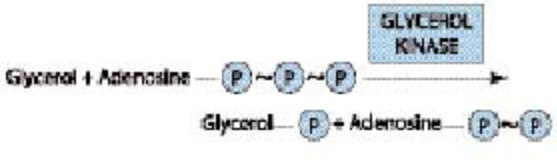
comes from respiratory chain oxidation using molecular O_2 within mitochondria (Chapter 11).

(2) **Glycolysis:** A net formation of two −Ⓟ results from the formation of lactate from one molecule of glucose, generated in two reactions catalyzed by phosphoglycerate kinase and pyruvate kinase, respectively (Figure 17–2).

(3) **The citric acid cycle:** One −Ⓟ is generated directly in the cycle at the succinyl thiokinase step (Figure 16–3).

Phosphagens act as storage forms of high-energy phosphate and include creatine phosphate, occurring in vertebrate skeletal muscle, heart, spermatozoa, and brain; and arginine phosphate, occurring in invertebrate muscle. When ATP is rapidly being utilized as a source of energy for muscular contraction, phosphagens permit its concentrations to be maintained, but when the ATP/ADP ratio is high, their concentration can increase to act as a store of high-energy phosphate (Figure 10–7).

When ATP acts as a phosphate donor to form those compounds of lower free energy of hydrolysis (Table 10–1), the phosphate group is invariably converted to one of low energy, eg,



ATP Allows the Coupling of Thermodynamically Unfavorable Reactions to Favorable Ones

The phosphorylation of glucose to glucose 6-phosphate, the first reaction of glycolysis (Figure 17–2), is highly endergonic and cannot proceed under physiologic conditions.

$$\text{(1) Glucose} + P_i \rightarrow \text{Glucose 6-phosphate} + H_2O$$
$$(\Delta G^{0'} = +13.8 \text{ kJ/mol})$$

To take place, the reaction must be coupled with another—more exergonic—reaction such as the hydrolysis of the terminal phosphate of ATP.

$$\text{(2) ATP} \rightarrow \text{ADP} + P_i \; (\Delta G^{0'} = -30.5 \text{ kJ/mol})$$

When (1) and (2) are coupled in a reaction catalyzed by hexokinase, phosphorylation of glucose readily proceeds in a highly exergonic reaction that under physiologic conditions is irreversible. Many "activation" reactions follow this pattern.

Adenylyl Kinase (Myokinase) Interconverts Adenine Nucleotides

This enzyme is present in most cells. It catalyzes the following reaction:

This allows:

(1) High-energy phosphate in ADP to be used in the synthesis of ATP.

(2) AMP, formed as a consequence of several activating reactions involving ATP, to be recovered by rephosphorylation to ADP.

(3) AMP to increase in concentration when ATP becomes depleted and act as a metabolic (allosteric) signal to increase the rate of catabolic reactions, which in turn lead to the generation of more ATP (Chapter 19).

When ATP Forms AMP, Inorganic Pyrophosphate (PP_i) Is Produced

This occurs, for example, in the activation of long-chain fatty acids (Chapter 22):

This reaction is accompanied by loss of free energy as heat, which ensures that the activation reaction will go to the right; and is further aided by the hydrolytic splitting of PP_i, catalyzed by **inorganic pyrophosphatase,** a reaction that itself has a large $\Delta G^{0'}$ of −27.6 kJ/

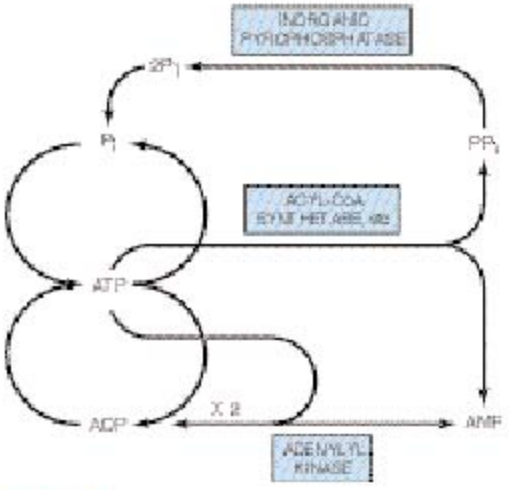


Figure 10–8. Phosphate cycles and interchange of adenine nucleotides.

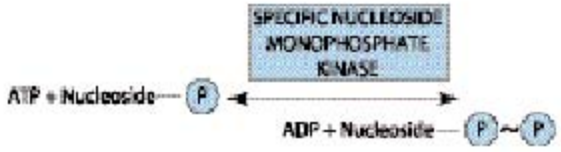
mol. Note that activations via the pyrophosphate pathway result in the loss of two ~Ⓟ rather than one ~Ⓟ as occurs when ADP and P_i are formed.

A combination of the above reactions makes it possible for phosphate to be recycled and the adenine nucleotides to interchange (Figure 10–8).

Other Nucleoside Triphosphates Participate in the Transfer of High-Energy Phosphate

By means of the enzyme **nucleoside diphosphate kinase,** UTP, GTP, and CTP can be synthesized from their diphosphates, eg,

All of these triphosphates take part in phosphorylations in the cell. Similarly, specific nucleoside monophosphate kinases catalyze the formation of nucleoside diphosphates from the corresponding monophosphates.



Thus, adenylyl kinase is a specialized monophosphate kinase.

SUMMARY

- Biologic systems use chemical energy to power the living processes.
- Exergonic reactions take place spontaneously with loss of free energy (ΔG is negative). Endergonic reactions require the gain of free energy (ΔG is positive) and only occur when coupled to exergonic reactions.
- ATP acts as the "energy currency" of the cell, transferring free energy derived from substances of higher energy potential to those of lower energy potential.

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Biologic Oxidation

11

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BIOMEDICAL IMPORTANCE

Chemically, **oxidation** is defined as the removal of electrons and **reduction** as the gain of electrons. Thus, oxidation is always accompanied by reduction of an electron acceptor. This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying understanding of the nature of biologic oxidation. Note that many biologic oxidations can take place without the participation of molecular oxygen, eg, dehydrogenations. The life of higher animals is absolutely dependent upon a supply of oxygen for **respiration,** the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water. In addition, molecular oxygen is incorporated into a variety of substrates by enzymes designated as **oxygenases;** many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized by enzymes of this class, known as the **cytochrome P450 system.** Administration of oxygen can be lifesaving in the treatment of patients with respiratory or circulatory failure.

FREE ENERGY CHANGES CAN BE EXPRESSED IN TERMS OF REDOX POTENTIAL

In reactions involving oxidation and reduction, the free energy change is proportionate to the tendency of reactants to donate or accept electrons. Thus, in addition to expressing free energy change in terms of $\Delta G^{0'}$ (Chapter 10), it is possible, in an analogous manner, to express it numerically as an **oxidation-reduction** or **redox potential** (E'_0). The redox potential of a system (E_0) is usually compared with the potential of the hydrogen electrode (0.0 volts at pH 0.0). However, for biologic systems, the redox potential (E'_0)is normally expressed at pH 7.0, at which pH the electrode potential of the hydrogen electrode is −0.42 volts. The redox potentials of some redox systems of special interest in mammalian biochemistry are shown in Table 11–1. The relative positions of redox systems in the table allows prediction of the direction of flow of electrons from one redox couple to another.

Enzymes involved in oxidation and reduction are called **oxidoreductases** and are classified into four groups: **oxidases, dehydrogenases, hydroperoxidases,** and **oxygenases.**

OXIDASES USE OXYGEN AS A HYDROGEN ACCEPTOR

Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor.* They form water or hydrogen peroxide as a reaction product (Figure 11–1).

Some Oxidases Contain Copper

Cytochrome oxidase is a hemoprotein widely distributed in many tissues, having the typical heme prosthetic group present in myoglobin, hemoglobin, and other cytochromes (Chapter 6). It is the terminal component of the chain of respiratory carriers found in mitochondria and transfers electrons resulting from the oxidation of substrate molecules by dehydrogenases to their final acceptor, oxygen. The enzyme is poisoned by carbon monoxide, cyanide, and hydrogen sulfide. It has also been termed cytochrome a_3. It is now known that cytochromes a and a_3 are combined in a single protein, and the complex is known as **cytochrome aa_3.** It contains two molecules of heme, each having one Fe atom that oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction. Furthermore, two atoms of Cu are present, each associated with a heme unit.

Other Oxidases Are Flavoproteins

Flavoprotein enzymes contain **flavin mononucleotide (FMN)** or **flavin adenine dinucleotide (FAD)** as prosthetic groups. FMN and FAD are formed in the body from the vitamin **riboflavin** (Chapter 45). FMN and FAD are usually tightly—but not covalently—bound to their respective apoenzyme proteins. Metalloflavoproteins contain one or more metals as essential cofactors.

Examples of flavoprotein enzymes include **L-amino acid oxidase,** an FMN-linked enzyme found in kidney with general specificity for the oxidative deamination of

* The term "oxidase" is sometimes used collectively to denote all enzymes that catalyze reactions involving molecular oxygen.

Table 11–1. Some redox potentials of special interest in mammalian oxidation systems.

System	E'_0 Volts
H^+/H_2	−0.42
NAD^+/NADH	−0.32
Lipoate; ox/red	−0.29
Acetoacetate/3-hydroxybutyrate	−0.27
Pyruvate/lactate	−0.19
Oxaloacetate/malate	−0.17
Fumarate/succinate	+0.03
Cytochrome *b*; Fe^{3+}/Fe^{2+}	+0.08
Ubiquinone; ox/red	+0.10
Cytochrome c_1; Fe^{3+}/Fe^{2+}	+0.22
Cytochrome *a*; Fe^{3+}/Fe^{2+}	+0.29
Oxygen/water	+0.82

the naturally occurring L-amino acids; **xanthine oxidase,** which contains molybdenum and plays an important role in the conversion of purine bases to uric acid (Chapter 34), and is of particular significance in uricotelic animals (Chapter 29); and **aldehyde dehydrogenase,** an FAD-linked enzyme present in mammalian livers, which contains molybdenum and nonheme iron and acts upon aldehydes and N-heterocyclic substrates. The mechanisms of oxidation and reduction of these enzymes are complex. Evidence suggests a two-step reaction as shown in Figure 11–2.

DEHYDROGENASES CANNOT USE OXYGEN AS A HYDROGEN ACCEPTOR

There are a large number of enzymes in this class. They perform two main functions:

(1) Transfer of hydrogen from one substrate to another in a coupled oxidation-reduction reaction (Figure 11–3). These dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD^+. Since the reactions are reversible, these properties enable reducing equivalents to be freely transferred within the cell. This type of reaction, which enables one substrate to be oxidized at the expense of another, is particularly useful in enabling oxidative processes to occur in the absence of oxygen, such as during the anaerobic phase of glycolysis (Figure 17–2).

(2) As components in the **respiratory chain** of electron transport from substrate to oxygen (Figure 12–3).

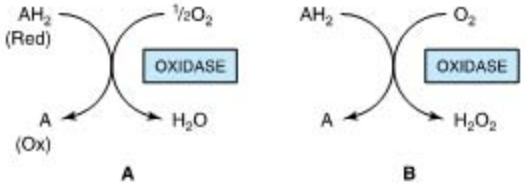

Figure 11–1. Oxidation of a metabolite catalyzed by an oxidase **(A)** forming H_2O, **(B)** forming H_2O_2.

Many Dehydrogenases Depend on Nicotinamide Coenzymes

These dehydrogenases use **nicotinamide adenine dinucleotide (NAD$^+$)** or **nicotinamide adenine dinucleotide phosphate (NADP$^+$)**—or both—and are formed in the body from the vitamin **niacin** (Chapter 45). The coenzymes are reduced by the specific substrate of the dehydrogenase and reoxidized by a suitable electron acceptor (Figure 11–4).They may freely and reversibly dissociate from their respective apoenzymes.

Generally, NAD-linked dehydrogenases catalyze oxidoreduction reactions in the oxidative pathways of metabolism, particularly in glycolysis, in the citric acid cycle, and in the respiratory chain of mitochondria. NADP-linked dehydrogenases are found characteristically in reductive syntheses, as in the extramitochondrial pathway of fatty acid synthesis and steroid synthesis—and also in the pentose phosphate pathway.

Other Dehydrogenases Depend on Riboflavin

The flavin groups associated with these dehydrogenases are similar to FMN and FAD occurring in oxidases. They are generally more tightly bound to their apoenzymes than are the nicotinamide coenzymes. Most of the riboflavin-linked dehydrogenases are concerned with electron transport in (or to) the respiratory chain (Chapter 12). **NADH dehydrogenase** acts as a carrier of electrons between NADH and the components of higher redox potential (Figure 12–3). Other dehydrogenases such as **succinate dehydrogenase, acyl-CoA dehydrogenase,** and **mitochondrial glycerol-3-phosphate dehydrogenase** transfer reducing equivalents directly from the substrate to the respiratory chain (Figure 12–4). Another role of the flavin-dependent dehydrogenases is in the dehydrogenation (by **dihydrolipoyl dehydrogenase**) of reduced lipoate, an intermediate in the oxidative decarboxylation of pyruvate and α-ketoglutarate (Figures 12–4 and 17–5). The **electron-transferring flavoprotein** is an intermediary carrier of electrons between acyl-CoA dehydrogenase and the respiratory chain (Figure 12–4).

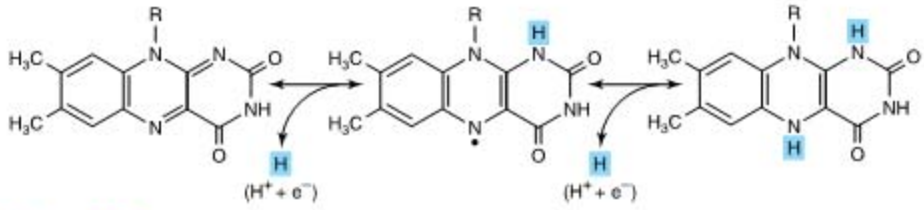

Figure 11–2. Oxidoreduction of isoalloxazine ring in flavin nucleotides via a semiquinone (free radical) intermediate (center).

Cytochromes May Also Be Regarded as Dehydrogenases

The cytochromes are iron-containing hemoproteins in which the iron atom oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction. Except for cytochrome oxidase (previously described), they are classified as dehydrogenases. In the respiratory chain, they are involved as carriers of electrons from flavoproteins on the one hand to cytochrome oxidase on the other (Figure 12–4). Several identifiable cytochromes occur in the respiratory chain, ie, cytochromes *b*, c_1, *c*, *a*, and a_3 (cytochrome oxidase). Cytochromes are also found in other locations, eg, the endoplasmic reticulum (cytochromes P450 and b_5), and in plant cells, bacteria, and yeasts.

HYDROPEROXIDASES USE HYDROGEN PEROXIDE OR AN ORGANIC PEROXIDE AS SUBSTRATE

Two type of enzymes found both in animals and plants fall into this category: **peroxidases** and **catalase.**

Hydroperoxidases protect the body against harmful peroxides. Accumulation of peroxides can lead to generation of free radicals, which in turn can disrupt membranes and perhaps cause cancer and atherosclerosis. (See Chapters 14 and 45.)

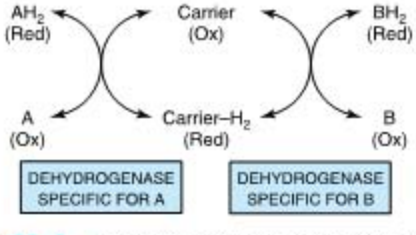

Figure 11–3. Oxidation of a metabolite catalyzed by coupled dehydrogenases.

Peroxidases Reduce Peroxides Using Various Electron Acceptors

Peroxidases are found in milk and in leukocytes, platelets, and other tissues involved in eicosanoid metabolism (Chapter 23). The prosthetic group is protoheme. In the reaction catalyzed by peroxidase, hydrogen peroxide is reduced at the expense of several substances that will act as electron acceptors, such as ascorbate, quinones, and cytochrome *c*. The reaction catalyzed by peroxidase is complex, but the overall reaction is as follows:

$$H_2O_2 + AH_2 \xrightarrow{\text{PEROXIDASE}} 2H_2O + A$$

In erythrocytes and other tissues, the enzyme **glutathione peroxidase,** containing **selenium** as a prosthetic group, catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione, protecting membrane lipids and hemoglobin against oxidation by peroxides (Chapter 20).

Catalase Uses Hydrogen Peroxide as Electron Donor & Electron Acceptor

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as a substrate electron donor and another molecule of H_2O_2 as an oxidant or electron acceptor.

$$2H_2O_2 \xrightarrow{\text{CATALASE}} 2H_2O + O_2$$

Under most conditions in vivo, the peroxidase activity of catalase seems to be favored. Catalase is found in blood, bone marrow, mucous membranes, kidney, and liver. Its function is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases.

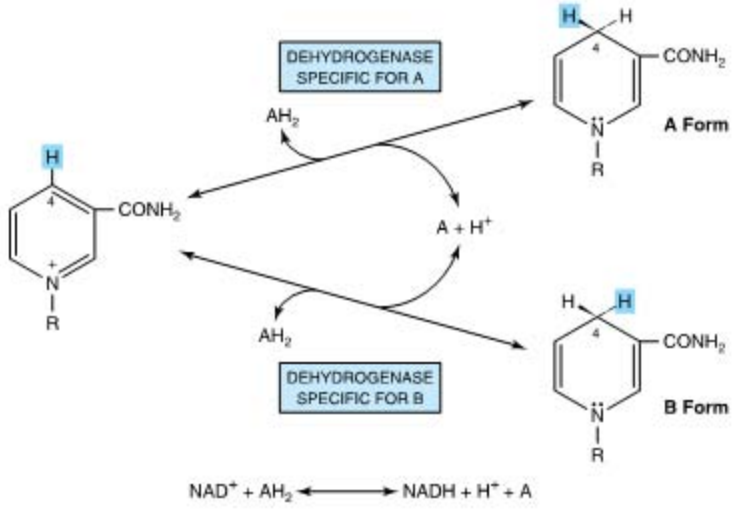

Figure 11–4. Mechanism of oxidation and reduction of nicotinamide coenzymes. There is stereospecificity about position 4 of nicotinamide when it is reduced by a substrate AH_2. One of the hydrogen atoms is removed from the substrate as a hydrogen nucleus with two electrons (hydride ion, H^-) and is transferred to the 4 position, where it may be attached in either the A or the B position according to the specificity determined by the particular dehydrogenase catalyzing the reaction. The remaining hydrogen of the hydrogen pair removed from the substrate remains free as a hydrogen ion.

Peroxisomes are found in many tissues, including liver. They are rich in oxidases and in catalase, Thus, the enzymes that produce H_2O_2 are grouped with the enzyme that destroys it. However, mitochondrial and microsomal electron transport systems as well as xanthine oxidase must be considered as additional sources of H_2O_2.

OXYGENASES CATALYZE THE DIRECT TRANSFER & INCORPORATION OF OXYGEN INTO A SUBSTRATE MOLECULE

Oxygenases are concerned with the synthesis or degradation of many different types of metabolites. They catalyze the incorporation of oxygen into a substrate molecule in two steps: (1) oxygen is bound to the enzyme at the active site, and (2) the bound oxygen is reduced or transferred to the substrate. Oxygenases may be divided into two subgroups, as follows.

Dioxygenases Incorporate Both Atoms of Molecular Oxygen Into the Substrate

The basic reaction is shown below:

$$A + O_2 \rightarrow AO_2$$

Examples include the liver enzymes, **homogentisate dioxygenase** (oxidase) and **3-hydroxyanthranilate dioxygenase** (oxidase), that contain iron; and **L-tryptophan dioxygenase** (tryptophan pyrrolase) (Chapter 30), that utilizes heme.

Monooxygenases (Mixed-Function Oxidases, Hydroxylases) Incorporate Only One Atom of Molecular Oxygen Into the Substrate

The other oxygen atom is reduced to water, an additional electron donor or cosubstrate (Z) being necessary for this purpose.

$$A—H + O_2 + ZH_2 \rightarrow A—OH + H_2O + Z$$

Cytochromes P450 Are Monooxygenases Important for the Detoxification of Many Drugs & for the Hydroxylation of Steroids

Cytochromes P450 are an important superfamily of heme-containing monooxgenases, and more than 1000 such enzymes are known. Both NADH and NADPH donate reducing equivalents for the reduction of these cytochromes (Figure 11–5), which in turn are oxidized by substrates in a series of enzymatic reactions collectively known as the **hydroxylase cycle** (Figure 11–6). In liver microsomes, cytochromes P450 are found together with **cytochrome b_5** and have an important role in detoxification. Benzpyrene, aminopyrine, aniline, morphine, and benzphetamine are hydroxylated, increasing their solubility and aiding their excretion. Many drugs such as phenobarbital have the ability to induce the formation of microsomal enzymes and of cytochromes P450.

Mitochondrial cytochrome P450 systems are found in steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta and are concerned with the biosyn-

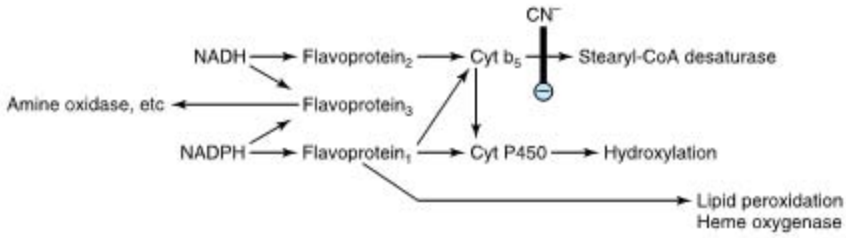

Figure 11–5. Electron transport chain in microsomes. Cyanide (CN^-) inhibits the indicated step.

thesis of steroid hormones from cholesterol (hydroxylation at C_{22} and C_{20} in side-chain cleavage and at the 11β and 18 positions). In addition, renal systems catalyzing 1α- and 24-hydroxylations of 25-hydroxycholecalciferol in vitamin D metabolism—and cholesterol 7α-hydroxylase and sterol 27-hydroxylase involved in bile acid biosynthesis in the liver (Chapter 26)—are P450 enzymes.

SUPEROXIDE DISMUTASE PROTECTS AEROBIC ORGANISMS AGAINST OXYGEN TOXICITY

Transfer of a single electron to O_2 generates the potentially damaging **superoxide anion free radical** ($O_2^{\cdot-}$), the destructive effects of which are amplified by its giving rise to free radical chain reactions (Chapter 14). The ease with which superoxide can be formed from oxygen in tissues and the occurrence of **superoxide dismutase,** the enzyme responsible for its removal in all aerobic organisms (although not in obligate anaerobes) indicate that the potential toxicity of oxygen is due to its conversion to superoxide.

Superoxide is formed when reduced flavins—present, for example, in xanthine oxidase—are reoxidized univalently by molecular oxygen.

$$\text{Enz–Flavin–H}_2 + O_2 \rightarrow \text{Enz–Flavin–H} + O_2^{\cdot-} + H^+$$

Superoxide can reduce oxidized cytochrome *c*

$$O_2^{\cdot-} + \text{Cyt } c\ (Fe^{3+}) \rightarrow O_2 + \text{Cyt } c\ (Fe^{2+})$$

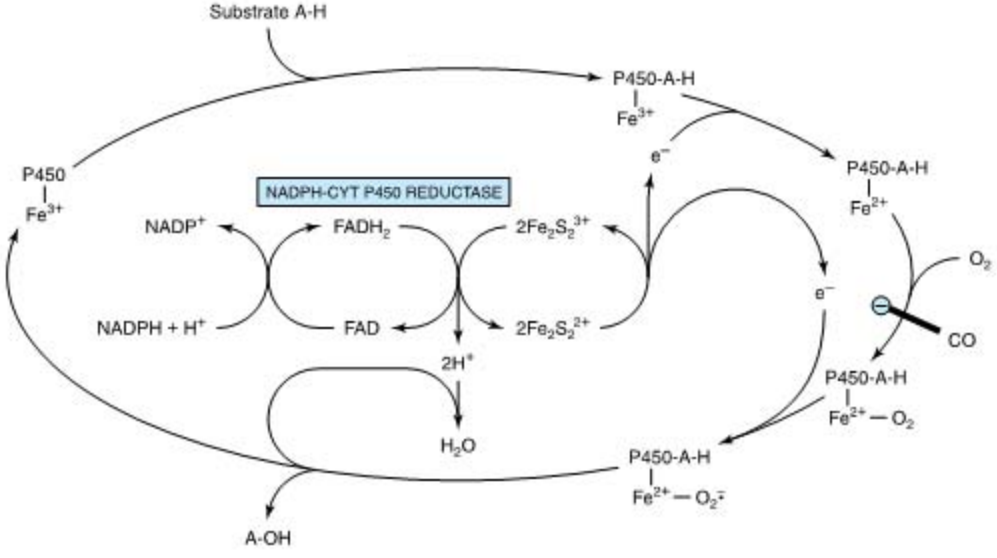

Figure 11–6. Cytochrome P450 hydroxylase cycle in microsomes. The system shown is typical of steroid hydroxylases of the adrenal cortex. Liver microsomal cytochrome P450 hydroxylase does not require the iron-sulfur protein Fe_2S_2. Carbon monoxide (CO) inhibits the indicated step.

or be removed by superoxide dismutase.

In this reaction, superoxide acts as both oxidant and reductant. Thus, superoxide dismutase protects aerobic organisms against the potential deleterious effects of superoxide. The enzyme occurs in all major aerobic tissues in the mitochondria and the cytosol. Although exposure of animals to an atmosphere of 100% oxygen causes an adaptive increase in superoxide dismutase, particularly in the lungs, prolonged exposure leads to lung damage and death. Antioxidants, eg, α-tocopherol (vitamin E), act as scavengers of free radicals and reduce the toxicity of oxygen (Chapter 45).

SUMMARY

- In biologic systems, as in chemical systems, oxidation (loss of electrons) is always accompanied by reduction of an electron acceptor.
- Oxidoreductases have a variety of functions in metabolism; oxidases and dehydrogenases play major roles in respiration; hydroperoxidases protect the body against damage by free radicals; and oxygenases mediate the hydroxylation of drugs and steroids.
- Tissues are protected from oxygen toxicity caused by the superoxide free radical by the specific enzyme superoxide dismutase.

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The Respiratory Chain & Oxidative Phosphorylation

12

Peter A. Mayes, PhD, DSc, & Kathleen M. Botham, PhD, DSc

BIOMEDICAL IMPORTANCE

Aerobic organisms are able to capture a far greater proportion of the available free energy of respiratory substrates than anaerobic organisms. Most of this takes place inside mitochondria, which have been termed the "powerhouses" of the cell. Respiration is coupled to the generation of the high-energy intermediate, ATP, by **oxidative phosphorylation,** and the **chemiosmotic theory** offers insight into how this is accomplished. A number of drugs (eg, **amobarbital**) and poisons (eg, **cyanide, carbon monoxide**) inhibit oxidative phosphorylation, usually with fatal consequences. Several inherited defects of mitochondria involving components of the respiratory chain and oxidative phosphorylation have been reported. Patients present with **myopathy** and **encephalopathy** and often have **lactic acidosis.**

SPECIFIC ENZYMES ACT AS MARKERS OF COMPARTMENTS SEPARATED BY THE MITOCHONDRIAL MEMBRANES

Mitochondria have an **outer membrane** that is permeable to most metabolites, an **inner membrane** that is selectively permeable, and a **matrix** within (Figure 12–1). The outer membrane is characterized by the presence of various enzymes, including acyl-CoA synthetase and glycerolphosphate acyltransferase. Adenylyl kinase and creatine kinase are found in the intermembrane space. The phospholipid cardiolipin is concentrated in the inner membrane together with the enzymes of the respiratory chain.

THE RESPIRATORY CHAIN COLLECTS & OXIDIZES REDUCING EQUIVALENTS

Most of the energy liberated during the oxidation of carbohydrate, fatty acids, and amino acids is made available within mitochondria as reducing equivalents (—H or electrons) (Figure 12–2). Mitochondria contain the **respiratory chain,** which collects and transports reducing equivalents directing them to their final reaction with oxygen to form water, the machinery for trapping the liberated free energy as high-energy phosphate, and the enzymes of β-oxidation and of the citric acid cycle (Chapters 22 and 16) that produce most of the reducing equivalents.

Components of the Respiratory Chain Are Arranged in Order of Increasing Redox Potential

Hydrogen and electrons flow through the respiratory chain (Figure 12–3) through a redox span of 1.1 V from $NAD^+/NADH$ to $O_2/2H_2O$ (Table 11–1). The respiratory chain consists of a number of redox carriers that proceed from the NAD-linked dehydrogenase systems, through flavoproteins and cytochromes, to molecular oxygen. Not all substrates are linked to the respiratory chain through NAD-specific dehydrogenases; some, because their redox potentials are more positive (eg, fumarate/succinate; Table 11–1), are linked directly to flavoprotein dehydrogenases, which in turn are linked to the cytochromes of the respiratory chain (Figure 12–4).

Ubiquinone or Q (coenzyme Q) (Figure 12–5) links the flavoproteins to cytochrome *b*, the member of the cytochrome chain of lowest redox potential. Q exists in the oxidized quinone or reduced quinol form under aerobic or anaerobic conditions, respectively. The structure of Q is very similar to that of vitamin K and vitamin E (Chapter 45) and of plastoquinone, found in chloroplasts. Q acts as a mobile component of the respiratory chain that collects reducing equivalents from the more fixed flavoprotein complexes and passes them on to the cytochromes.

An additional component is the **iron-sulfur protein** (**FeS;** nonheme iron) (Figure 12–6). It is associated with the flavoproteins (metalloflavoproteins) and with cytochrome *b*. The sulfur and iron are thought to take part in the oxidoreduction mechanism between flavin and Q, which involves only a single e^- change, the iron atom undergoing oxidoreduction between Fe^{2+} and Fe^{3+}.

Pyruvate and α-ketoglutarate dehydrogenase have complex systems involving lipoate and FAD prior to the passage of electrons to NAD, while electron trans-

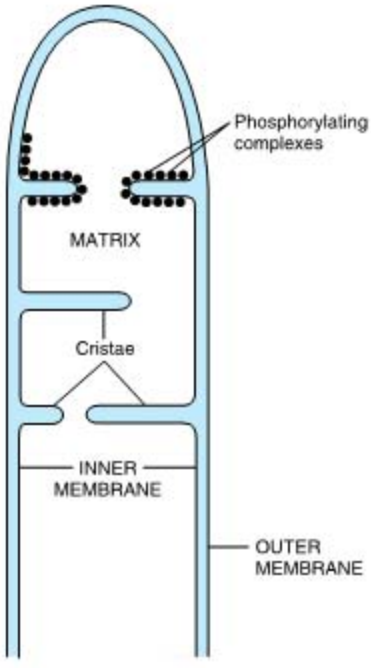

Figure 12–1. Structure of the mitochondrial membranes. Note that the inner membrane contains many folds, or cristae.

fers from other dehydrogenases, eg, L(+)-3-hydroxyacyl-CoA dehydrogenase, couple directly with NAD.

The reduced NADH of the respiratory chain is in turn oxidized by a metalloflavoprotein enzyme—**NADH dehydrogenase.** This enzyme contains FeS and FMN, is tightly bound to the respiratory chain, and passes reducing equivalents on to Q.

Electrons flow from Q through the series of cytochromes in order of increasing redox potential to molecular oxygen (Figure 12–4). The terminal cytochrome aa_3 (cytochrome oxidase), responsible for the final combination of reducing equivalents with molecular oxygen, has a very high affinity for oxygen, allowing the respiratory chain to function at maximum rate until the tissue has become depleted of O_2. Since this is an irreversible reaction (the only one in the chain), it gives direction to the movement of reducing equivalents and to the production of ATP, to which it is coupled.

Functionally and structurally, the components of the respiratory chain are present in the inner mitochondrial membrane as four **protein-lipid respiratory chain complexes** that span the membrane. Cytochrome *c* is the only soluble cytochrome and, together with Q, seems to be a more mobile component of the respiratory chain connecting the fixed complexes (Figures 12–7 and 12–8).

THE RESPIRATORY CHAIN PROVIDES MOST OF THE ENERGY CAPTURED DURING CATABOLISM

ADP captures, in the form of high-energy phosphate, a significant proportion of the free energy released by catabolic processes. The resulting ATP has been called the energy "currency" of the cell because it passes on this free energy to drive those processes requiring energy (Figure 10–6).

There is a net direct capture of two high-energy phosphate groups in the glycolytic reactions (Table 17–1), equivalent to approximately 103.2 kJ/mol of glucose. (In vivo, ΔG for the synthesis of ATP from ADP has been calculated as approximately 51.6 kJ/mol. (It is greater than $\Delta G^{0'}$ for the hydrolysis of ATP as given in Table 10–1, which is obtained under standard

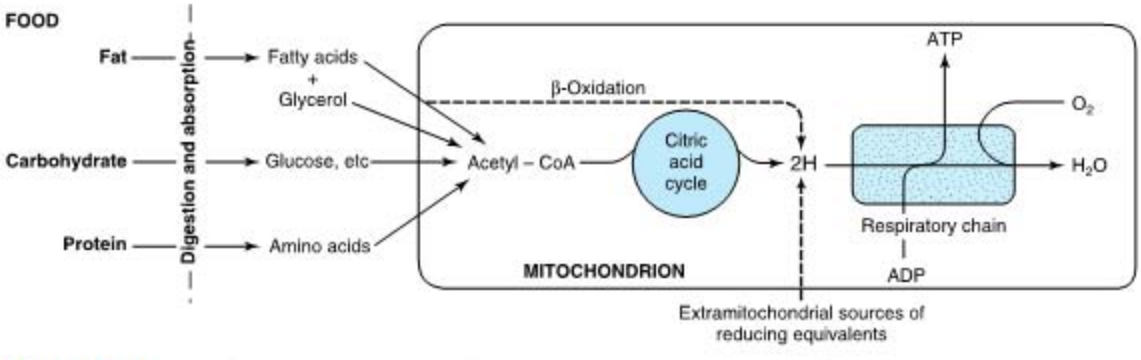

Figure 12–2. Role of the respiratory chain of mitochondria in the conversion of food energy to ATP. Oxidation of the major foodstuffs leads to the generation of reducing equivalents (2H) that are collected by the respiratory chain for oxidation and coupled generation of ATP.

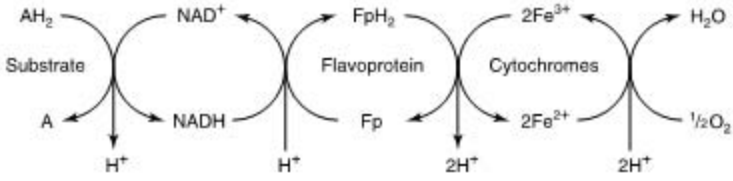

Figure 12–3. Transport of reducing equivalents through the respiratory chain.

concentrations of 1.0 mol/L.) Since 1 mol of glucose yields approximately 2870 kJ on complete combustion, the energy captured by phosphorylation in glycolysis is small. Two more high-energy phosphates per mole of glucose are captured in the citric acid cycle during the conversion of succinyl CoA to succinate. All of these phosphorylations occur at the **substrate level.** When substrates are oxidized via an NAD-linked dehydrogenase and the respiratory chain, approximately 3 mol of inorganic phosphate are incorporated into 3 mol of ADP to form 3 mol of ATP per half mol of O_2 consumed; ie, the P:O ratio = 3 (Figure 12–7). On the other hand, when a substrate is oxidized via a flavoprotein-linked dehydrogenase, only 2 mol of ATP are formed; ie, P:O = 2. These reactions are known as **oxidative phosphorylation at the respiratory chain level.** Such dehydrogenations plus phosphorylations at the substrate level can now account for 68% of the free energy resulting from the combustion of glucose, captured in the form of high-energy phosphate. It is evident that the respiratory chain is responsible for a large proportion of total ATP formation.

Respiratory Control Ensures a Constant Supply of ATP

The rate of respiration of mitochondria can be controlled by the availability of ADP. This is because oxidation and phosphorylation are tightly coupled; ie, oxidation cannot proceed via the respiratory chain without concomitant phosphorylation of ADP. Table 12–1 shows the five conditions controlling the rate of respiration in mitochondria. Most cells in the resting state are in state 4, and respiration is controlled by the availability of ADP. When work is performed, ATP is converted to ADP, allowing more respiration to occur, which in turn replenishes the store of ATP. Under certain conditions, the concentration of inorganic phosphate can also affect the rate of functioning of the respiratory chain. As respiration increases (as in exercise),

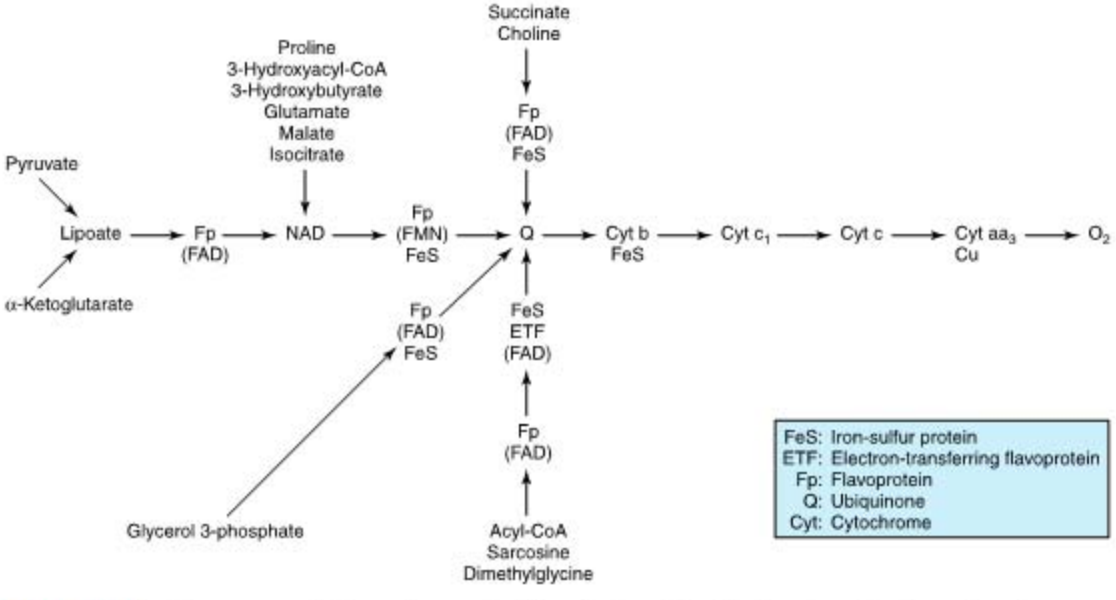

Figure 12–4. Components of the respiratory chain in mitochondria, showing the collecting points for reducing equivalents from important substrates. FeS occurs in the sequences on the O_2 side of Fp or Cyt *b*.

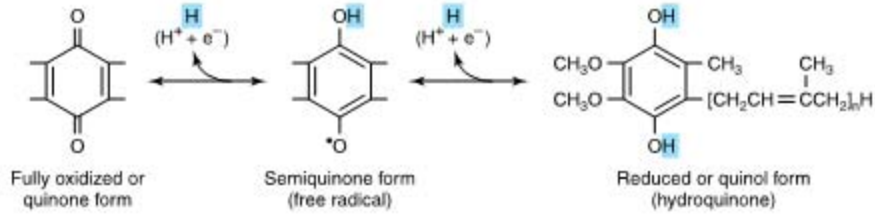

Figure 12–5. Structure of ubiquinone (Q). n = Number of isoprenoid units, which is 10 in higher animals, ie, Q_{10}.

the cell approaches state 3 or state 5 when either the capacity of the respiratory chain becomes saturated or the Po_2 decreases below the K_m for cytochrome a_3. There is also the possibility that the ADP/ATP transporter (Figure 12–9), which facilitates entry of cytosolic ADP into and ATP out of the mitochondrion, becomes rate-limiting.

Thus, the manner in which biologic oxidative processes allow the free energy resulting from the oxidation of foodstuffs to become available and to be captured is stepwise, efficient (approximately 68%), and controlled—rather than explosive, inefficient, and uncontrolled, as in many nonbiologic processes. The remaining free energy that is not captured as high-energy phosphate is liberated as **heat.** This need not be considered "wasted," since it ensures that the respiratory system as a whole is sufficiently exergonic to be removed from equilibrium, allowing continuous unidirectional flow and constant provision of ATP. It also contributes to maintenance of body temperature.

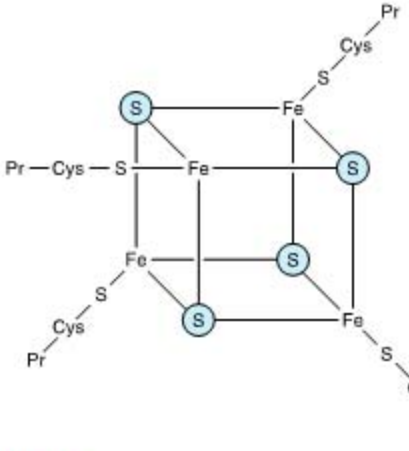

Figure 12–6. Iron-sulfur-protein complex (Fe_4S_4). Ⓢ, acid-labile sulfur; Pr, apoprotein; Cys, cysteine residue. Some iron-sulfur proteins contain two iron atoms and two sulfur atoms (Fe_2S_2).

MANY POISONS INHIBIT THE RESPIRATORY CHAIN

Much information about the respiratory chain has been obtained by the use of inhibitors, and, conversely, this has provided knowledge about the mechanism of action of several poisons (Figure 12–7). They may be classified as inhibitors of the respiratory chain, inhibitors of oxidative phosphorylation, and uncouplers of oxidative phosphorylation.

Barbiturates such as amobarbital inhibit NAD-linked dehydrogenases by blocking the transfer from FeS to Q. At sufficient dosage, they are fatal in vivo. **Antimycin A** and **dimercaprol** inhibit the respiratory chain between cytochrome *b* and cytochrome *c*. The classic poisons **H_2S**, **carbon monoxide,** and **cyanide** inhibit cytochrome oxidase and can therefore totally arrest respiration. **Malonate** is a competitive inhibitor of succinate dehydrogenase.

Atractyloside inhibits oxidative phosphorylation by inhibiting the transporter of ADP into and ATP out of the mitochondrion (Figure 12–10).

The action of **uncouplers** is to dissociate oxidation in the respiratory chain from phosphorylation. These compounds are toxic in vivo, causing respiration to become uncontrolled, since the rate is no longer limited by the concentration of ADP or P_i. The uncoupler that has been used most frequently is **2,4-dinitrophenol,** but other compounds act in a similar manner. The antibiotic **oligomycin** completely blocks oxidation and phosphorylation by acting on a step in phosphorylation (Figures 12–7 and 12–8).

THE CHEMIOSMOTIC THEORY EXPLAINS THE MECHANISM OF OXIDATIVE PHOSPHORYLATION

Mitchell's chemiosmotic theory postulates that the energy from oxidation of components in the respiratory chain is coupled to the translocation of hydrogen ions (protons, H^+) from the inside to the outside of the inner mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric dis-

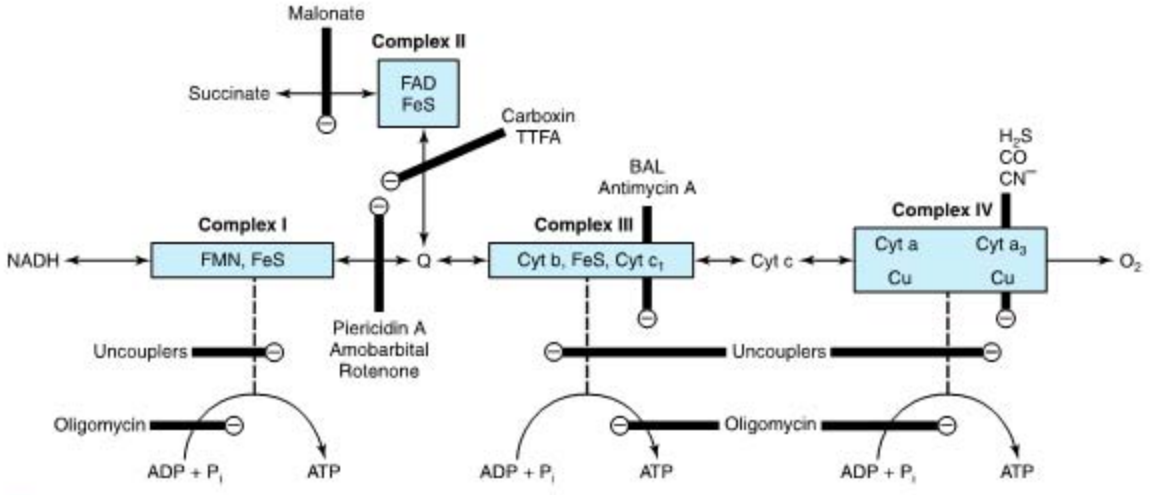

Figure 12–7. Proposed sites of inhibition (⊖) of the respiratory chain by specific drugs, chemicals, and antibiotics. The sites that appear to support phosphorylation are indicated. BAL, dimercaprol. TTFA, an Fe-chelating agent. Complex I, NADH:ubiquinone oxidoreductase; complex II, succinate:ubiquinone oxidoreductase; complex III, ubiquinol:ferricytochrome c oxidoreductase; complex IV, ferrocytochrome c:oxygen oxidoreductase. Other abbreviations as in Figure 12–4.

tribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP (Figure 12–8).

The Respiratory Chain Is a Proton Pump

Each of the respiratory chain complexes I, III, and IV (Figures 12–7 and 12–8) acts as a **proton pump.** The inner membrane is impermeable to ions in general but particularly to protons, which accumulate outside the membrane, creating an **electrochemical potential difference** across the membrane ($\Delta\mu_{H^+}$).This consists of a chemical potential (difference in pH) and an electrical potential.

A Membrane-Located ATP Synthase Functions as a Rotary Motor to Form ATP

The electrochemical potential difference is used to drive a membrane-located **ATP synthase** which in the presence of P_i + ADP forms ATP (Figure 12–8). Scattered over the surface of the inner membrane are the phosphorylating complexes, ATP synthase, responsible for the production of ATP (Figure 12–1). These consist of several protein subunits, collectively known as F_1, which project into the matrix and which contain the phosphorylation mechanism (Figure 12–8). These subunits are attached to a membrane protein complex known as F_0, which also consists of several protein subunits. F_0 spans the membrane and forms the proton channel. The flow of protons through F_0 causes it to rotate, driving the production of ATP in the F_1 complex (Figure 12–9). Estimates suggest that for each NADH oxidized, complex I translocates four protons and complexes III and IV translocate 6 between them. As four protons are taken into the mitochondrion for each ATP exported, the P:O ratio would not necessarily be a complete integer, ie, 3, but possibly 2.5. However, for simplicity, a value of 3 for the oxidation of NADH + H^+ and 2 for the oxidation of $FADH_2$ will continue to be used throughout this text.

Experimental Findings Support the Chemiosmotic Theory

(1) Addition of protons (acid) to the external medium of intact mitochondria leads to the generation of ATP.

(2) Oxidative phosphorylation does not occur in soluble systems where there is no possibility of a vectorial ATP synthase. A closed membrane must be present in order to achieve oxidative phosphorylation (Figure 12–8).

(3) The respiratory chain contains components organized in a sided manner (transverse asymmetry) as required by the chemiosmotic theory.

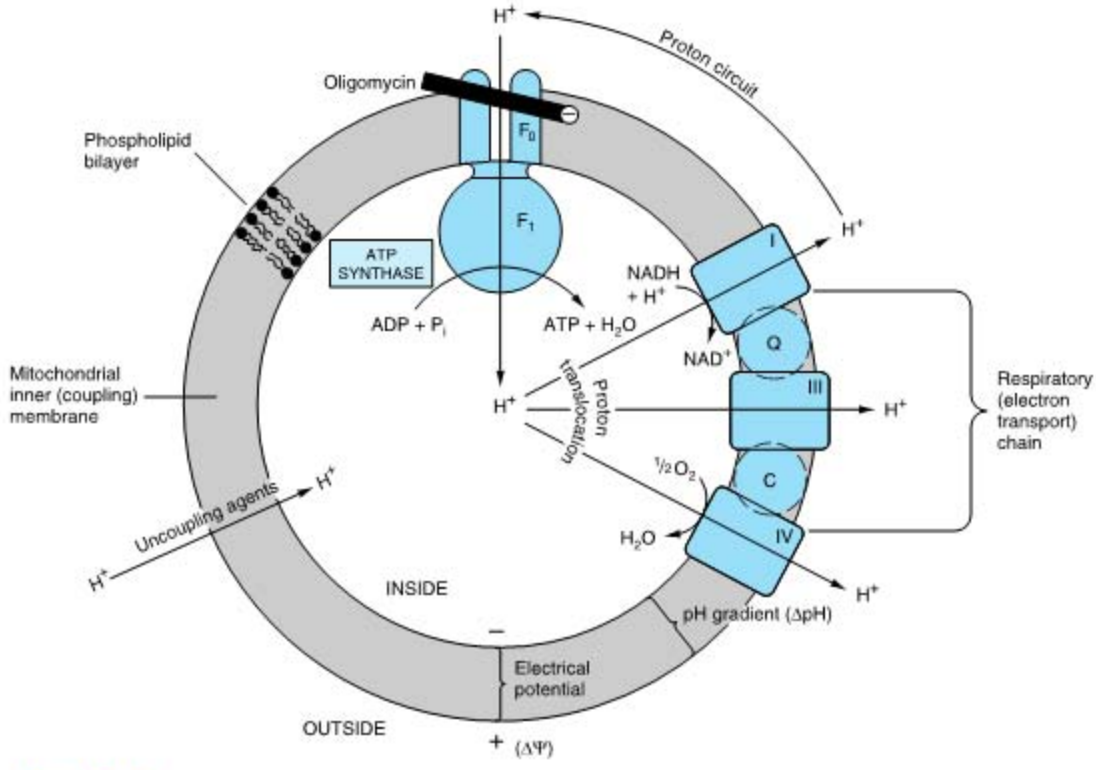

Figure 12–8. Principles of the chemiosmotic theory of oxidative phosphorylation. The main proton circuit is created by the coupling of oxidation in the respiratory chain to proton translocation from the inside to the outside of the membrane, driven by the respiratory chain complexes I, III, and IV, each of which acts as a *proton pump*. Q, ubiquinone; C, cytochrome c; F_1, F_0, protein subunits which utilize energy from the proton gradient to promote phosphorylation. Uncoupling agents such as dinitrophenol allow leakage of H^+ across the membrane, thus collapsing the electrochemical proton gradient. Oligomycin specifically blocks conduction of H^+ through F_0.

The Chemiosmotic Theory Can Account for Respiratory Control and the Action of Uncouplers

The electrochemical potential difference across the membrane, once established as a result of proton translocation, inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back-translocation of protons across the membrane through the vectorial ATP synthase. This in turn depends on availability of ADP and P_i.

Uncouplers (eg, dinitrophenol) are amphipathic (Chapter 14) and increase the permeability of the lipoid inner mitochondrial membrane to protons (Figure 12–8), thus reducing the electrochemical potential and short-circuiting the ATP synthase. In this way, oxidation can proceed without phosphorylation.

Table 12–1. States of respiratory control.

	Conditions Limiting the Rate of Respiration
State 1	Availability of ADP and substrate
State 2	Availability of substrate only
State 3	The capacity of the respiratory chain itself, when all substrates and components are present in saturating amounts
State 4	Availability of ADP only
State 5	Availability of oxygen only

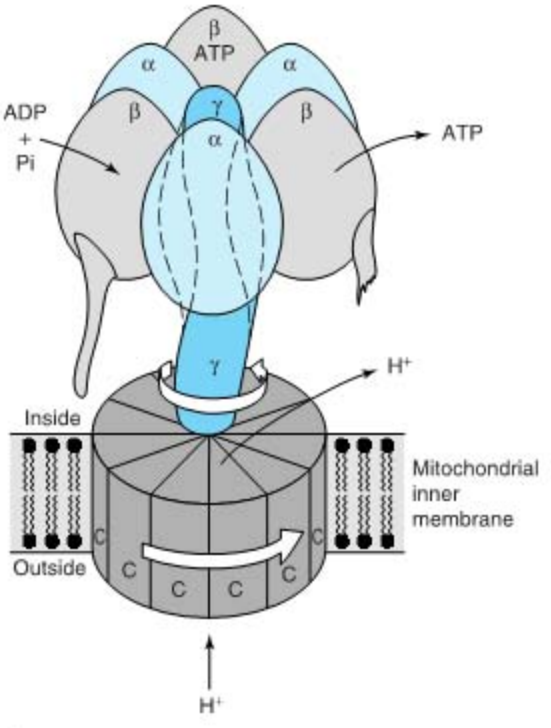

Figure 12–9. Mechanism of ATP production by ATP synthase. The enzyme complex consists of an F_0 subcomplex which is a disk of "C" protein subunits. Attached is a γ-subunit in the form of a "bent axle." Protons passing through the disk of "C" units cause it and the attached γ-subunit to rotate. The γ-subunit fits inside the F_1 subcomplex of three α- and three β-subunits, which are fixed to the membrane and do not rotate. ADP and P_i are taken up sequentially by the β-subunits to form ATP, which is expelled as the rotating γ-subunit squeezes each β-subunit in turn. Thus, three ATP molecules are generated per revolution. For clarity, not all the subunits that have been identified are shown—eg, the "axle" also contains an ε-subunit.

THE RELATIVE IMPERMEABILITY OF THE INNER MITOCHONDRIAL MEMBRANE NECESSITATES EXCHANGE TRANSPORTERS

Exchange diffusion systems are present in the membrane for exchange of anions against OH^- ions and cations against H^+ ions. Such systems are necessary for uptake and output of ionized metabolites while preserving electrical and osmotic equilibrium. The inner bilipoid mitochondrial membrane is freely permeable to uncharged small molecules, such as oxygen, water, CO_2, and NH_3, and to monocarboxylic acids, such as 3-hydroxybutyric, acetoacetic, and acetic. Long-chain fatty acids are transported into mitochondria via the carnitine system (Figure 22–1), and there is also a special carrier for pyruvate involving a symport that utilizes the H^+ gradient from outside to inside the mitochondrion (Figure 12–10). However, dicarboxylate and tri-

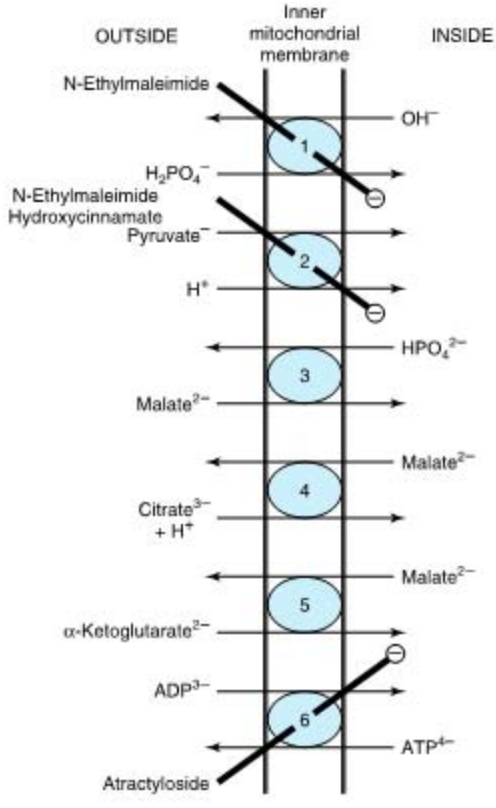

Figure 12–10. Transporter systems in the inner mitochondrial membrane. ①, phosphate transporter; ②, pyruvate symport; ③, dicarboxylate transporter; ④, tricarboxylate transporter; ⑤, α-ketoglutarate transporter; ⑥, adenine nucleotide transporter. *N*-Ethylmaleimide, hydroxycinnamate, and atractyloside inhibit (⊖) the indicated systems. Also present (but not shown) are transporter systems for glutamate/aspartate (Figure 12–13), glutamine, ornithine, neutral amino acids, and carnitine (Figure 22–1).

carboxylate anions and amino acids require specific transporter or carrier systems to facilitate their passage across the membrane. Monocarboxylic acids penetrate more readily in their undissociated and more lipid-soluble form.

The transport of di- and tricarboxylate anions is closely linked to that of inorganic phosphate, which penetrates readily as the $H_2PO_4^-$ ion in exchange for OH^-. The net uptake of malate by the dicarboxylate transporter requires inorganic phosphate for exchange in the opposite direction. The net uptake of citrate, isocitrate, or *cis*-aconitate by the tricarboxylate transporter requires malate in exchange. α-Ketoglutarate transport also requires an exchange with malate. The adenine nucleotide transporter allows the exchange of ATP and ADP but not AMP. It is vital in allowing ATP exit from mitochondria to the sites of extramitochondrial utilization and in allowing the return of ADP for ATP production within the mitochondrion (Figure 12–11). Na^+ can be exchanged for H^+, driven by the proton gradient. It is believed that active uptake of Ca^{2+} by mitochondria occurs with a net charge transfer of 1 (Ca^+ uniport), possibly through a Ca^{2+}/H^+ antiport. Calcium release from mitochondria is facilitated by exchange with Na^+.

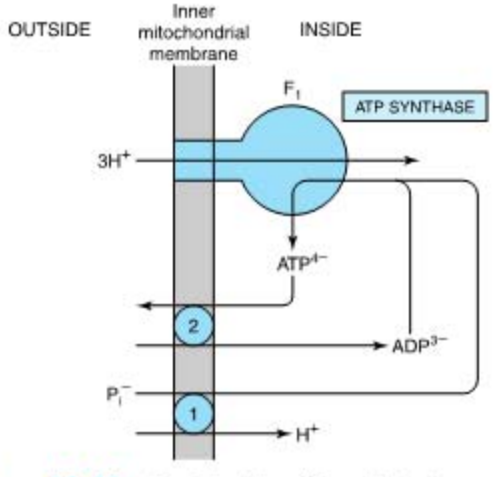

Figure 12–11. Combination of phosphate transporter (①) with the adenine nucleotide transporter (②) in ATP synthesis. The H^+/P_i symport shown is equivalent to the P_i/OH^- antiport shown in Figure 12–10. Four protons are taken into the mitochondrion for each ATP exported. However, one less proton would be taken in when ATP is used inside the mitochondrion.

Ionophores Permit Specific Cations to Penetrate Membranes

Ionophores are lipophilic molecules that complex specific cations and facilitate their transport through biologic membranes, eg, **valinomycin** (K^+). The classic uncouplers such as dinitrophenol are, in fact, proton ionophores.

A Proton-Translocating Transhydrogenase Is a Source of Intramitochondrial NADPH

Energy-linked transhydrogenase, a protein in the inner mitochondrial membrane, couples the passage of protons down the electrochemical gradient from outside to inside the mitochondrion with the transfer of H from intramitochondrial NADH to NADPH for intramitochondrial enzymes such as glutamate dehydrogenase and hydroxylases involved in steroid synthesis.

Oxidation of Extramitochondrial NADH Is Mediated by Substrate Shuttles

NADH cannot penetrate the mitochondrial membrane, but it is produced continuously in the cytosol by 3-phosphoglyceraldehyde dehydrogenase, an enzyme in the glycolysis sequence (Figure 17–2). However, under aerobic conditions, extramitochondrial NADH does not accumulate and is presumed to be oxidized by the respiratory chain in mitochondria. The transfer of reducing equivalents through the mitochondrial membrane requires substrate pairs, linked by suitable dehydrogenases on each side of the mitochondrial membrane. The mechanism of transfer using the **glycerophosphate shuttle** is shown in Figure 12–12). Since the mitochondrial enzyme is linked to the respiratory chain via a flavoprotein rather than NAD, only 2 mol rather than 3 mol of ATP are formed per atom of oxygen consumed. Although this shuttle is present in some tissues (eg, brain, white muscle), in others (eg, heart muscle) it is deficient. It is therefore believed that the **malate shuttle** system (Figure 12–13) is of more universal utility. The complexity of this system is due to the impermeability of the mitochondrial membrane to oxaloacetate, which must react with glutamate and transaminate to aspartate and α-ketoglutarate before transport through the mitochondrial membrane and reconstitution to oxaloacetate in the cytosol.

Ion Transport in Mitochondria Is Energy-Linked

Mitochondria maintain or accumulate cations such as K^+, Na^+, Ca^{2+}, and Mg^{2+}, and P_i. It is assumed that a primary proton pump drives cation exchange.

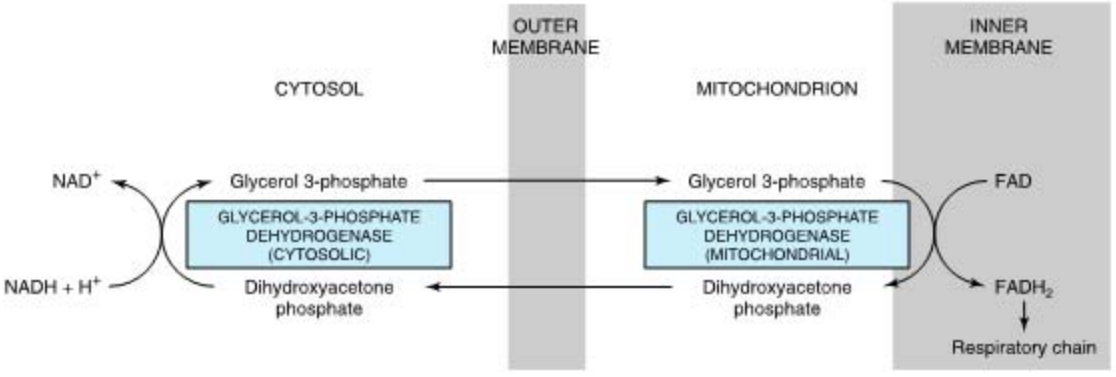

Figure 12–12. Glycerophosphate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion.

The Creatine Phosphate Shuttle Facilitates Transport of High-Energy Phosphate From Mitochondria

This shuttle (Figure 12–14) augments the functions of creatine phosphate as an energy buffer by acting as a dynamic system for transfer of high-energy phosphate from mitochondria in active tissues such as heart and skeletal muscle. An isoenzyme of creatine kinase (CK_m) is found in the mitochondrial intermembrane space, catalyzing the transfer of high-energy phosphate to creatine from ATP emerging from the adenine nucleotide transporter. In turn, the creatine phosphate is transported into the cytosol via protein pores in the outer mitochondrial membrane, becoming available for generation of extramitochondrial ATP.

CLINICAL ASPECTS

The condition known as **fatal infantile mitochondrial myopathy and renal dysfunction** involves severe diminution or absence of most oxidoreductases of the respiratory chain. **MELAS** (mitochondrial encephalopathy, lactic acidosis, and stroke) is an inherited condition due to NADH:ubiquinone oxidoreductase (complex I) or cytochrome oxidase deficiency. It is caused by a muta-

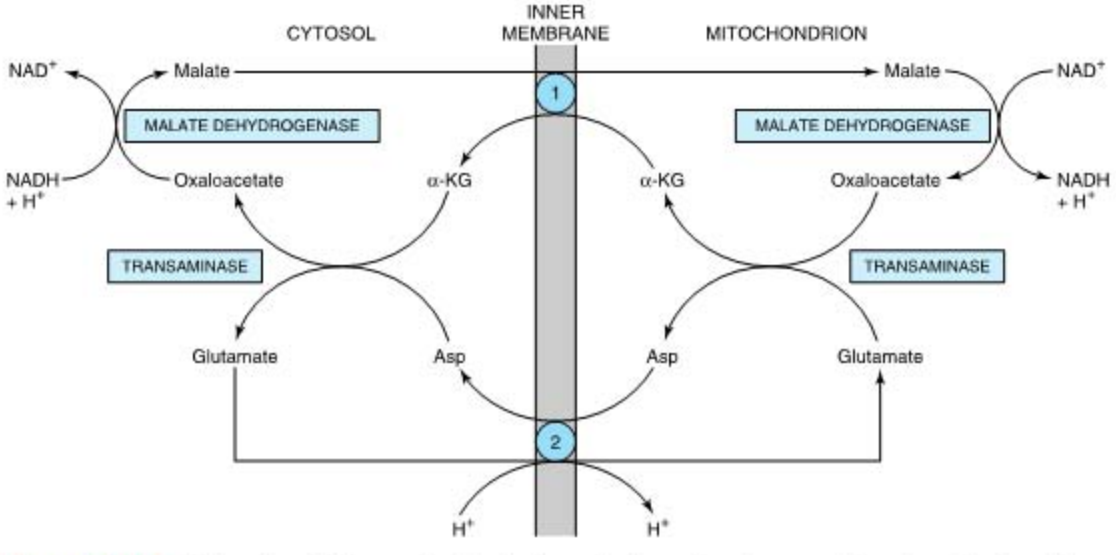

Figure 12–13. Malate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion. ① Ketoglutarate transporter; ②, glutamate/aspartate transporter (note the proton symport with glutamate).

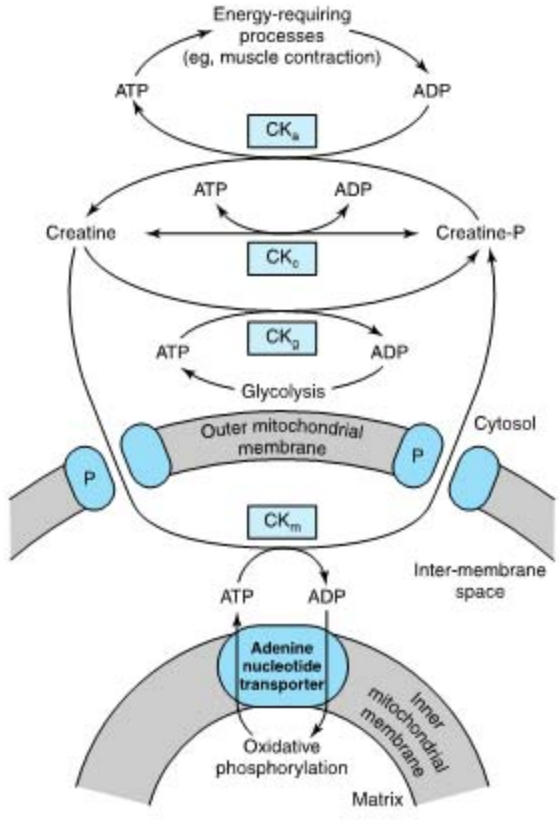

Figure 12–14. The creatine phosphate shuttle of heart and skeletal muscle. The shuttle allows rapid transport of high-energy phosphate from the mitochondrial matrix into the cytosol. CK_a, creatine kinase concerned with large requirements for ATP, eg, muscular contraction; CK_c, creatine kinase for maintaining equilibrium between creatine and creatine phosphate and ATP/ADP; CK_g, creatine kinase coupling glycolysis to creatine phosphate synthesis; CK_m, mitochondrial creatine kinase mediating creatine phosphate production from ATP formed in oxidative phosphorylation; P, pore protein in outer mitochondrial membrane.

tion in mitochondrial DNA and may be involved in Alzheimer's disease and diabetes mellitus. A number of drugs and poisons act by inhibition of oxidative phosphorylation (see above).

SUMMARY

- Virtually all energy released from the oxidation of carbohydrate, fat, and protein is made available in mitochondria as reducing equivalents (—H or e^-). These are funneled into the respiratory chain, where they are passed down a redox gradient of carriers to their final reaction with oxygen to form water.
- The redox carriers are grouped into respiratory chain complexes in the inner mitochondrial membrane. These use the energy released in the redox gradient to pump protons to the outside of the membrane, creating an electrochemical potential across the membrane.
- Spanning the membrane are ATP synthase complexes that use the potential energy of the proton gradient to synthesize ATP from ADP and P_i. In this way, oxidation is closely coupled to phosphorylation to meet the energy needs of the cell.
- Because the inner mitochondrial membrane is impermeable to protons and other ions, special exchange transporters span the membrane to allow passage of ions such as OH^-, P_i^-, ATP^{4-}, ADP^{3-}, and metabolites, without discharging the electrochemical gradient across the membrane.
- Many well-known poisons such as cyanide arrest respiration by inhibition of the respiratory chain.

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Carbohydrates of Physiologic Significance

13

Peter A. Mayes, PhD, DSc, & David A. Bender, PhD

BIOMEDICAL IMPORTANCE

Carbohydrates are widely distributed in plants and animals; they have important structural and metabolic roles. In plants, glucose is synthesized from carbon dioxide and water by photosynthesis and stored as starch or used to synthesize cellulose of the plant framework. Animals can synthesize carbohydrate from lipid glycerol and amino acids, but most animal carbohydrate is derived ultimately from plants. **Glucose** is the most important carbohydrate; most dietary carbohydrate is absorbed into the bloodstream as glucose, and other sugars are converted into glucose in the liver. Glucose is the major metabolic fuel of mammals (except ruminants) and a universal fuel of the fetus. It is the precursor for synthesis of all the other carbohydrates in the body, including **glycogen** for storage; **ribose** and **deoxyribose** in nucleic acids; and **galactose** in lactose of milk, in glycolipids, and in combination with protein in glycoproteins and proteoglycans. Diseases associated with carbohydrate metabolism include **diabetes mellitus, galactosemia, glycogen storage diseases,** and **lactose intolerance.**

CARBOHYDRATES ARE ALDEHYDE OR KETONE DERIVATIVES OF POLYHYDRIC ALCOHOLS

(1) **Monosaccharides** are those carbohydrates that cannot be hydrolyzed into simpler carbohydrates: They may be classified as **trioses, tetroses, pentoses, hexoses,** or **heptoses,** depending upon the number of carbon atoms; and as **aldoses** or **ketoses** depending upon whether they have an aldehyde or ketone group. Examples are listed in Table 13–1.

(2) **Disaccharides** are condensation products of two monosaccharide units. Examples are maltose and sucrose.

(3) **Oligosaccharides** are condensation products of two to ten monosaccharides; maltotriose* is an example.

*Note that this is not a true triose but a trisaccharide containing three α-glucose residues.

(4) **Polysaccharides** are condensation products of more than ten monosaccharide units; examples are the starches and dextrins, which may be linear or branched polymers. Polysaccharides are sometimes classified as hexosans or pentosans, depending upon the identity of the constituent monosaccharides.

BIOMEDICALLY, GLUCOSE IS THE MOST IMPORTANT MONOSACCHARIDE

The Structure of Glucose Can Be Represented in Three Ways

The straight-chain structural formula (aldohexose; Figure 13–1A) can account for some of the properties of glucose, but a cyclic structure is favored on thermodynamic grounds and accounts for the remainder of its chemical properties. For most purposes, the structural formula is represented as a simple ring in perspective as proposed by Haworth (Figure 13–1B). In this representation, the molecule is viewed from the side and above the plane of the ring. By convention, the bonds nearest to the viewer are bold and thickened. The six-membered ring containing one oxygen atom is in the form of a chair (Figure 13–1C).

Sugars Exhibit Various Forms of Isomerism

Glucose, with four asymmetric carbon atoms, can form 16 isomers. The more important types of isomerism found with glucose are as follows.

(1) **D and L isomerism:** The designation of a sugar isomer as the D form or of its mirror image as the L form

Table 13–1. Classification of important sugars.

	Aldoses	Ketoses
Trioses ($C_3H_6O_3$)	Glycerose	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose

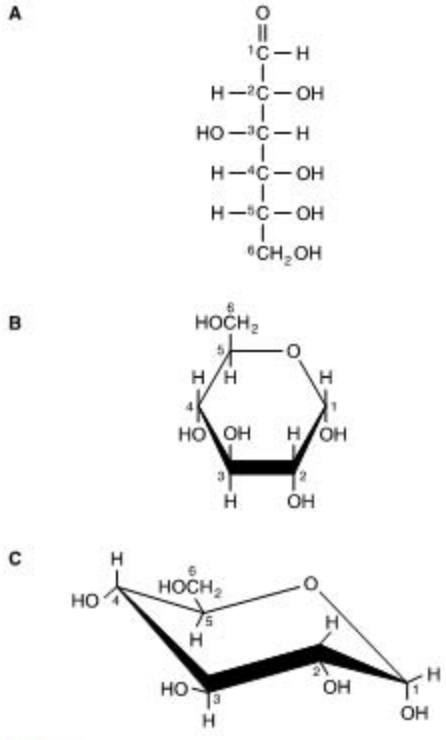


Figure 13–1. D-Glucose. A: straight chain form. B: α-D-glucose; Haworth projection. C: α-D-glucose; chair form.

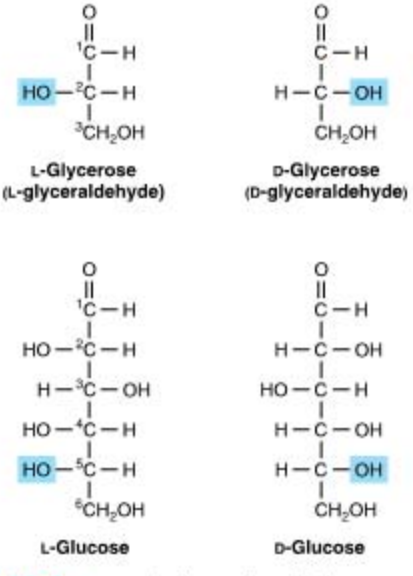


Figure 13–2. D- and L-isomerism of glycerose and glucose.

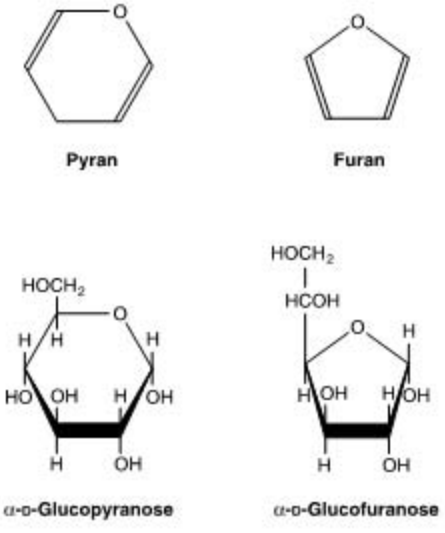


Figure 13–3. Pyranose and furanose forms of glucose.

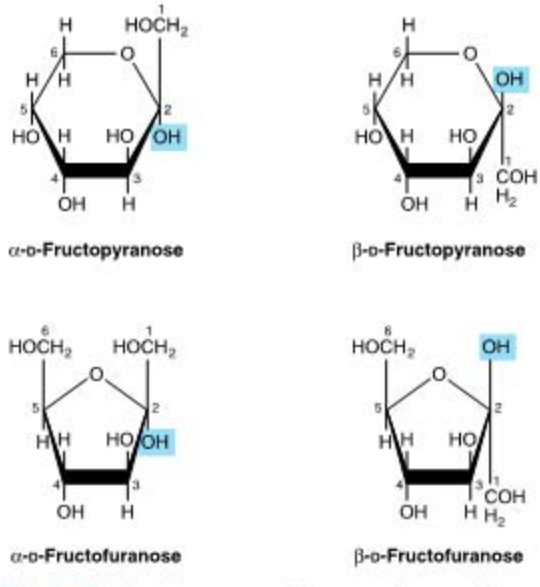


Figure 13–4. Pyranose and furanose forms of fructose.

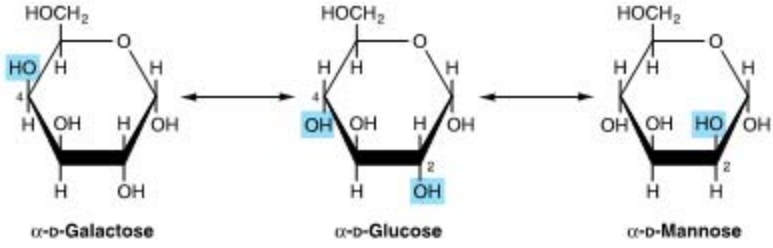


Figure 13–5. Epimerization of glucose.

is determined by its spatial relationship to the parent compound of the carbohydrates, the three-carbon sugar glycerose (glyceraldehyde). The L and D forms of this sugar, and of glucose, are shown in Figure 13–2. The orientation of the —H and —OH groups around the carbon atom adjacent to the terminal primary alcohol carbon (carbon 5 in glucose) determines whether the sugar belongs to the D or L series. When the —OH group on this carbon is on the right (as seen in Figure 13–2), the sugar is the D-isomer; when it is on the left, it is the L-isomer. Most of the monosaccharides occurring in mammals are D sugars, and the enzymes responsible for their metabolism are specific for this configuration. In solution, glucose is dextrorotatory—hence the alternative name **dextrose,** often used in clinical practice.

The presence of asymmetric carbon atoms also confers **optical activity** on the compound. When a beam of plane-polarized light is passed through a solution of an **optical isomer,** it will be rotated either to the right, dextrorotatory (+); or to the left, levorotatory (−). The direction of rotation is independent of the stereochemistry of the sugar, so it may be designated D(−), D(+), L(−), or L(+). For example, the naturally occurring form of fructose is the D(−) isomer.

(2) **Pyranose and furanose ring structures:** The stable ring structures of monosaccharides are similar to the ring structures of either pyran (a six-membered ring) or furan (a five-membered ring) (Figures 13–3 and 13–4). For glucose in solution, more than 99% is in the pyranose form.

(3) **Alpha and beta anomers:** The ring structure of an aldose is a hemiacetal, since it is formed by combination of an aldehyde and an alcohol group. Similarly, the ring structure of a ketose is a hemiketal. Crystalline glucose is α-D-glucopyranose. The cyclic structure is retained in solution, but isomerism occurs about position 1, the carbonyl or **anomeric carbon atom,** to give a mixture of α-glucopyranose (38%) and β-glucopyranose (62%). Less than 0.3% is represented by α and β anomers of glucofuranose.

(4) **Epimers:** Isomers differing as a result of variations in configuration of the —OH and —H on carbon atoms 2, 3, and 4 of glucose are known as epimers. Biologically, the most important epimers of glucose are mannose and galactose, formed by epimerization at carbons 2 and 4, respectively (Figure 13–5).

(5) **Aldose-ketose isomerism:** Fructose has the same molecular formula as glucose but differs in its structural formula, since there is a potential keto group in position 2, the anomeric carbon of fructose (Figures 13–4 and 13–7), whereas there is a potential aldehyde group in position 1, the anomeric carbon of glucose (Figures 13–2 and 13–6).

Many Monosaccharides Are Physiologically Important

Derivatives of trioses, tetroses, and pentoses and of a seven-carbon sugar (sedoheptulose) are formed as metabolic intermediates in glycolysis and the pentose phosphate pathway. Pentoses are important in nucleotides,

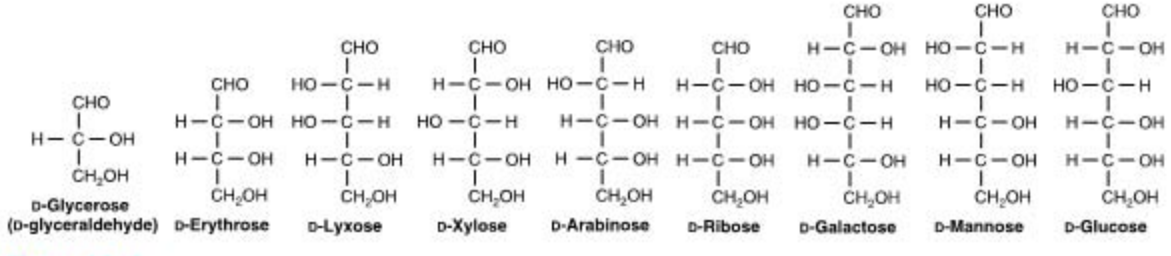


Figure 13–6. Examples of aldoses of physiologic significance.

Table 13–2. Pentoses of physiologic importance.

Sugar	Where Found	Biochemical Importance	Clinical Significance
D-Ribose	Nucleic acids.	Structural elements of nucleic acids and coenzymes, eg, ATP, NAD, NADP, flavoproteins. Ribose phosphates are intermediates in pentose phosphate pathway.	
D-Ribulose	Formed in metabolic processes.	Ribulose phosphate is an intermediate in pentose phosphate pathway.	
D-Arabinose	Gum arabic. Plum and cherry gums.	Constituent of glycoproteins.	
D-Xylose	Wood gums, proteoglycans, glycosaminoglycans.	Constituent of glycoproteins.	
D-Lyxose	Heart muscle.	A constituent of a lyxoflavin isolated from human heart muscle.	
L-Xylulose	Intermediate in uronic acid pathway.		Found in urine in essential pentosuria.

nucleic acids, and several coenzymes (Table 13–2). Glucose, galactose, fructose, and mannose are physiologically the most important hexoses (Table 13–3). The biochemically important aldoses are shown in Figure 13–6, and important ketoses in Figure 13–7.

In addition, carboxylic acid derivatives of glucose are important, including D-glucuronate (for glucuronide formation and in glycosaminoglycans) and its metabolic derivative, L-iduronate (in glycosaminoglycans) (Figure 13–8) and L-gulonate (an intermediate in the uronic acid pathway; see Figure 20–4).

Sugars Form Glycosides With Other Compounds & With Each Other

Glycosides are formed by condensation between the hydroxyl group of the anomeric carbon of a monosaccharide, or monosaccharide residue, and a second compound that may—or may not (in the case of an **aglycone**)—be another monosaccharide. If the second group is a hydroxyl, the O-glycosidic bond is an **acetal** link because it results from a reaction between a hemiacetal group (formed from an aldehyde and an —OH group) and an-

Table 13–3. Hexoses of physiologic importance.

Sugar	Source	Importance	Clinical Significance
D-Glucose	Fruit juices. Hydrolysis of starch, cane sugar, maltose, and lactose.	The "sugar" of the body. The sugar carried by the blood, and the principal one used by the tissues.	Present in the urine (glycosuria) in diabetes mellitus owing to raised blood glucose (hyperglycemia).
D-Fructose	Fruit juices. Honey. Hydrolysis of cane sugar and of inulin (from the Jerusalem artichoke).	Can be changed to glucose in the liver and so used in the body.	Hereditary fructose intolerance leads to fructose accumulation and hypoglycemia.
D-Galactose	Hydrolysis of lactose.	Can be changed to glucose in the liver and metabolized. Synthesized in the mammary gland to make the lactose of milk. A constituent of glycolipids and glycoproteins.	Failure to metabolize leads to galactosemia and cataract.
D-Mannose	Hydrolysis of plant mannans and gums.	A constituent of many glycoproteins.	

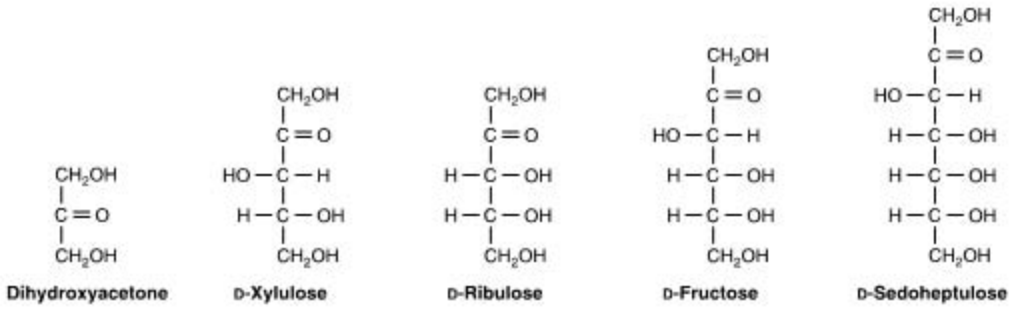


Figure 13–7. Examples of ketoses of physiologic significance.

other —OH group. If the hemiacetal portion is glucose, the resulting compound is a **glucoside;** if galactose, a **galactoside;** and so on. If the second group is an amine, an N-glycosidic bond is formed, eg, between adenine and ribose in nucleotides such as ATP (Figure 10–4).

Glycosides are widely distributed in nature; the aglycone may be methanol, glycerol, a sterol, a phenol, or a base such as adenine. The glycosides that are important in medicine because of their action on the heart **(cardiac glycosides)** all contain steroids as the aglycone. These include derivatives of digitalis and strophanthus such as **ouabain,** an inhibitor of the Na^+-K^+ ATPase of cell membranes. Other glycosides include antibiotics such as **streptomycin.**

Deoxy Sugars Lack an Oxygen Atom

Deoxy sugars are those in which a hydroxyl group has been replaced by hydrogen. An example is **deoxyribose** (Figure 13–9) in DNA. The deoxy sugar L-fucose (Figure 13–15) occurs in glycoproteins; 2-deoxyglucose is used experimentally as an inhibitor of glucose metabolism.

Amino Sugars (Hexosamines) Are Components of Glycoproteins, Gangliosides, & Glycosaminoglycans

The amino sugars include D-glucosamine, a constituent of hyaluronic acid (Figure 13–10), D-galactosamine (chondrosamine), a constituent of chondroitin; and D-mannosamine. Several **antibiotics** (eg, erythromycin) contain amino sugars believed to be important for their antibiotic activity.

MALTOSE, SUCROSE, & LACTOSE ARE IMPORTANT DISACCHARIDES

The physiologically important disaccharides are maltose, sucrose, and lactose (Table 13–4; Figure 13–11). Hydrolysis of sucrose yields a mixture of glucose and

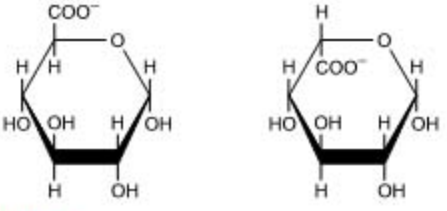


Figure 13–8. α-D-Glucuronate (left) and β-L-iduronate (right).

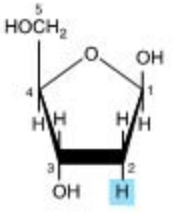


Figure 13–9. 2-Deoxy-D-ribofuranose (β form).

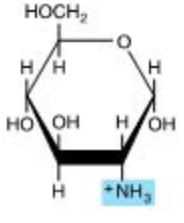


Figure 13–10. Glucosamine (2-amino-D-glucopyranose) (α form). Galactosamine is 2-amino-D-galactopyranose. Both glucosamine and galactosamine occur as N-acetyl derivatives in more complex carbohydrates, eg, glycoproteins.

Table 13–4. Disaccharides.

Sugar	Source	Clinical Significance
Maltose	Digestion by amylase or hydrolysis of starch. Germinating cereals and malt.	
Lactose	Milk. May occur in urine during pregnancy.	In lactase deficiency, malabsorption leads to diarrhea and flatulence.
Sucrose	Cane and beet sugar. Sorghum. Pineapple. Carrot roots.	In sucrase deficiency, malabsorption leads to diarrhea and flatulence.
Trehalose[1]	Fungi and yeasts. The major sugar of insect hemolymph.	

[1]*O*-α-D-Glucopyranosyl-(1 → 1)-α-D-glucopyranoside.

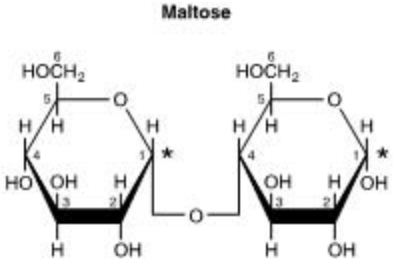
fructose which is called "invert sugar" because the strongly levorotatory fructose changes (inverts) the previous dextrorotatory action of sucrose.

POLYSACCHARIDES SERVE STORAGE & STRUCTURAL FUNCTIONS

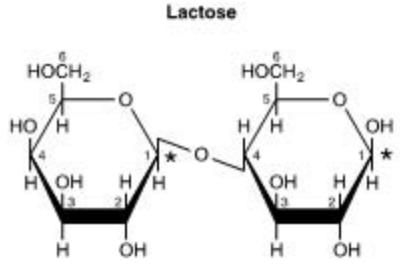
Polysaccharides include the following physiologically important carbohydrates.

Starch is a homopolymer of glucose forming an α-glucosidic chain, called a **glucosan** or **glucan.** It is the most abundant dietary carbohydrate in cereals, potatoes, legumes, and other vegetables. The two main constituents are **amylose** (15–20%), which has a nonbranching helical structure (Figure 13–12); and **amylopectin** (80–85%), which consists of branched chains composed of 24–30 glucose residues united by 1 → 4 linkages in the chains and by 1 → 6 linkages at the branch points.

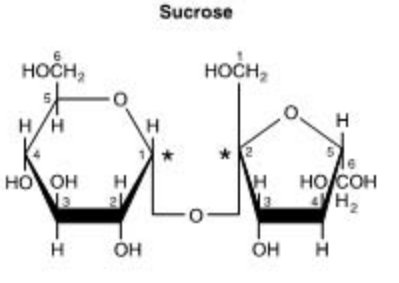
Glycogen (Figure 13–13) is the storage polysaccharide in animals. It is a more highly branched structure than amylopectin, with chains of 12–14 α-D-glucopyranose residues (in α[1 → 4]-glucosidic linkage), with branching by means of α(1 → 6)-glucosidic bonds.



O-α-D-Glucopyranosyl-(1 → 4)-α-D-glucopyranose



O-β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranose



O-α-D-Glucopyranosyl-(1 → 2)-β-D-fructofuranoside

Figure 13–11. Structures of important disaccharides. The α and β refer to the configuration at the anomeric carbon atom (asterisk). When the anomeric carbon of the second residue takes part in the formation of the glycosidic bond, as in sucrose, the residue becomes a glycoside known as a furanoside or pyranoside. As the disaccharide no longer has an anomeric carbon with a free potential aldehyde or ketone group, it no longer exhibits reducing properties. The configuration of the β-fructofuranose residue in sucrose results from turning the β-fructofuranose molecule depicted in Figure 13–4 through 180 degrees and inverting it.

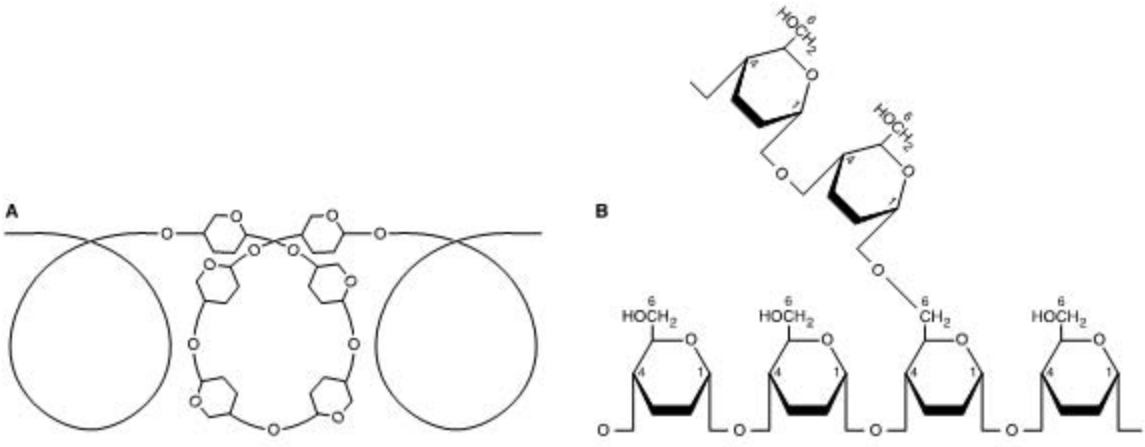


Figure 13–12. Structure of starch. **A:** Amylose, showing helical coil structure. **B:** Amylopectin, showing 1 → 6 branch point.

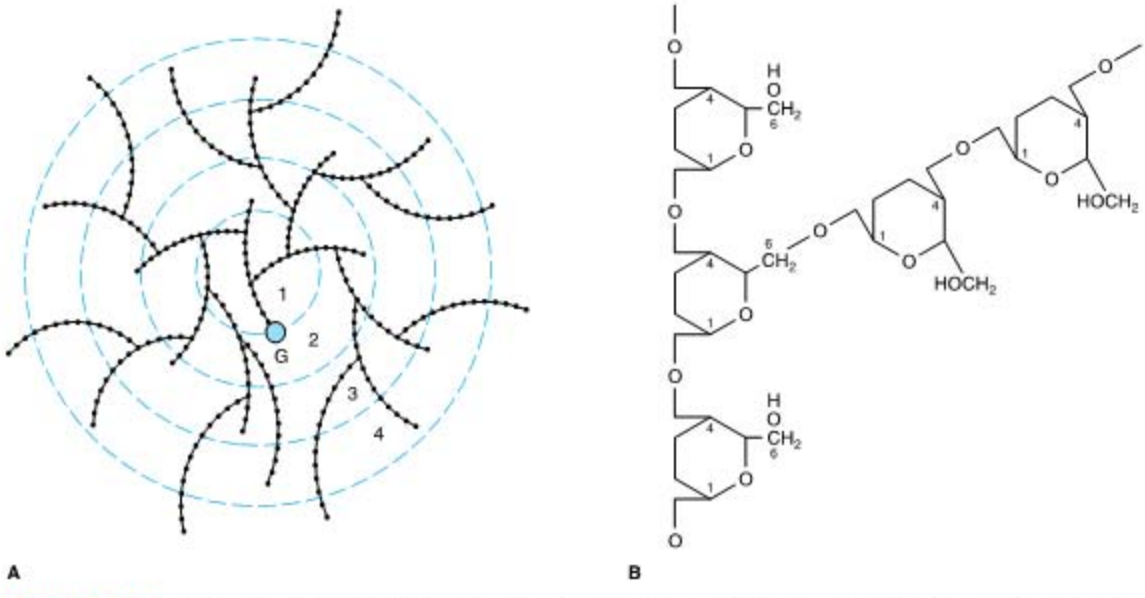


Figure 13–13. The glycogen molecule. **A:** General structure. **B:** Enlargement of structure at a branch point. The molecule is a sphere approximately 21 nm in diameter that can be visualized in electron micrographs. It has a molecular mass of 10^7 Da and consists of polysaccharide chains each containing about 13 glucose residues. The chains are either branched or unbranched and are arranged in 12 concentric layers (only four are shown in the figure). The branched chains (each has two branches) are found in the inner layers and the unbranched chains in the outer layer. (G, glycogenin, the primer molecule for glycogen synthesis.)

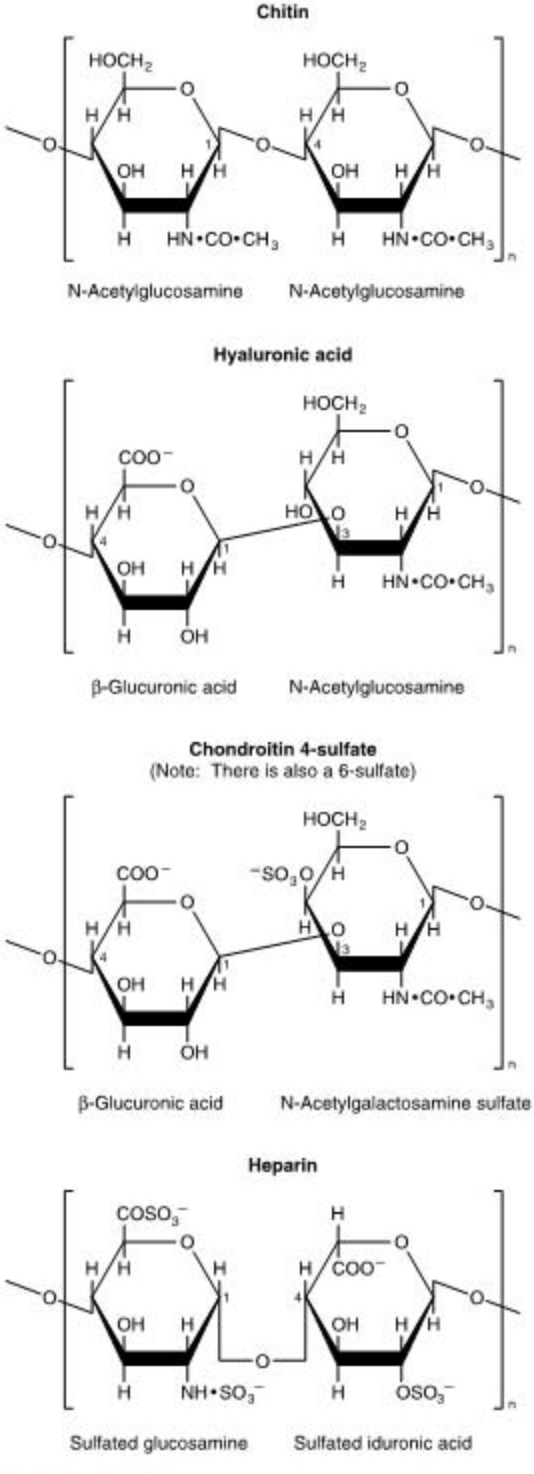


Figure 13–14. Structure of some complex polysaccharides and glycosaminoglycans.

Inulin is a polysaccharide of fructose (and hence a fructosan) found in tubers and roots of dahlias, artichokes, and dandelions. It is readily soluble in water and is used to determine the glomerular filtration rate. **Dextrins** are intermediates in the hydrolysis of starch. **Cellulose** is the chief constituent of the framework of plants. It is insoluble and consists of β-D-glucopyranose units linked by β(1 → 4) bonds to form long, straight chains strengthened by cross-linked hydrogen bonds. Cellulose cannot be digested by mammals because of the absence of an enzyme that hydrolyzes the β linkage. It is an important source of "bulk" in the diet. Microorganisms in the gut of ruminants and other herbivores can hydrolyze the β linkage and ferment the products to short-chain fatty acids as a major energy source. There is limited bacterial metabolism of cellulose in the human colon. **Chitin** is a structural polysaccharide in the exoskeleton of crustaceans and insects and also in mushrooms. It consists of *N*-acetyl-D-glucosamine units joined by β (1 → 4)-glycosidic linkages (Figure 13–14).

Glycosaminoglycans (mucopolysaccharides) are complex carbohydrates characterized by their content of **amino sugars** and **uronic acids.** When these chains are attached to a protein molecule, the result is a **proteoglycan.** Proteoglycans provide the ground or packing substance of connective tissues. Their property of holding large quantities of water and occupying space, thus cushioning or lubricating other structures, is due to the large number of —OH groups and negative charges on the molecules, which, by repulsion, keep the carbohydrate chains apart. Examples are **hyaluronic acid, chondroitin sulfate,** and **heparin** (Figure 13–14).

Glycoproteins (mucoproteins) occur in many different situations in fluids and tissues, including the cell membranes (Chapters 41 and 47). They are proteins

Table 13–5. Carbohydrates found in glycoproteins.

Hexoses	Mannose (Man) Galactose (Gal)
Acetyl hexosamines	*N*-Acetylglucosamine (GlcNAc) *N*-Acetylgalactosamine (GalNAc)
Pentoses	Arabinose (Ara) Xylose (Xyl)
Methyl pentose	L-Fucose (Fuc; see Figure 13–15)
Sialic acids	N-Acyl derivatives of neuraminic acid, eg, *N*-acetylneuraminic acid (NeuAc; see Figure 13–16), the predominant sialic acid.

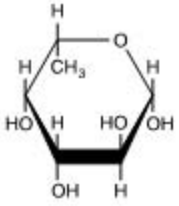


Figure 13–15. β-L-Fucose (6-deoxy-β-L-galactose).

containing branched or unbranched oligosaccharide chains (see Table 13–5). The **sialic acids** are N- or O-acyl derivatives of neuraminic acid (Figure 13–16). **Neuraminic acid** is a nine-carbon sugar derived from mannosamine (an epimer of glucosamine) and pyruvate. Sialic acids are constituents of both **glycoproteins** and **gangliosides** (Chapters 14 and 47).

CARBOHYDRATES OCCUR IN CELL MEMBRANES & IN LIPOPROTEINS

In addition to the lipid of cell membranes (see Chapters 14 and 41), approximately 5% is carbohydrate in glycoproteins and glycolipids. Carbohydrates are also present in apo B of lipoproteins. Their presence on the outer surface of the plasma membrane (the **glycocalyx**) has been shown with the use of plant **lectins,** protein agglutinins that bind with specific glycosyl residues. For example, **concanavalin A** binds α-glucosyl and α-mannosyl residues. **Glycophorin** is a major integral membrane glycoprotein of human erythrocytes and spans the lipid membrane, having free polypeptide portions outside both the external and internal (cytoplasmic) surfaces. Carbohydrate chains are only attached to the amino terminal portion outside the external surface (Chapter 41).

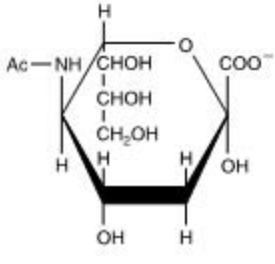


Figure 13–16. Structure of *N*-acetylneuraminic acid, a sialic acid (Ac = CH_3—CO—).

SUMMARY

- Carbohydrates are major constituents of animal food and animal tissues. They are characterized by the type and number of monosaccharide residues in their molecules.
- Glucose is the most important carbohydrate in mammalian biochemistry because nearly all carbohydrate in food is converted to glucose for metabolism.
- Sugars have large numbers of stereoisomers because they contain several asymmetric carbon atoms.
- The monosaccharides include glucose, the "blood sugar"; and ribose, an important constituent of nucleotides and nucleic acids.
- The disaccharides include maltose (glucosyl glucose), an intermediate in the digestion of starch; sucrose (glucosyl fructose), important as a dietary constituent containing fructose; and lactose (galactosyl glucose), in milk.
- Starch and glycogen are storage polymers of glucose in plants and animals, respectively. Starch is the major source of energy in the diet.
- Complex carbohydrates contain other sugar derivatives such as amino sugars, uronic acids, and sialic acids. They include proteoglycans and glycosaminoglycans, associated with structural elements of the tissues; and glycoproteins, proteins containing attached oligosaccharide chains. They are found in many situations including the cell membrane.

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Lipids of Physiologic Significance

14

Peter A. Mayes, PhD, DSc, & Kathleen M. Botham, PhD, DSc

BIOMEDICAL IMPORTANCE

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. They have the common property of being (1) relatively **insoluble in water** and (2) **soluble in nonpolar solvents** such as ether and chloroform. They are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods. Fat is stored in **adipose tissue,** where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. Nonpolar lipids act as **electrical insulators,** allowing rapid propagation of depolarization waves along **myelinated nerves.** Combinations of lipid and protein (lipoproteins) are important cellular constituents, occurring both in the cell **membrane** and in the mitochondria, and serving also as the means of **transporting lipids** in the blood. Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas, eg, **obesity, diabetes mellitus, atherosclerosis,** and the role of various **polyunsaturated fatty acids** in nutrition and health.

LIPIDS ARE CLASSIFIED AS SIMPLE OR COMPLEX

1. **Simple lipids:** Esters of fatty acids with various alcohols.
   a. **Fats:** Esters of fatty acids with glycerol. **Oils** are fats in the liquid state.
   b. **Waxes:** Esters of fatty acids with higher molecular weight monohydric alcohols.
2. **Complex lipids:** Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.
   a. **Phospholipids:** Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents, eg, in **glycerophospholipids** the alcohol is glycerol and in **sphingophospholipids** the alcohol is sphingosine.
   b. **Glycolipids (glycosphingolipids):** Lipids containing a fatty acid, sphingosine, and carbohydrate.
   c. **Other complex lipids:** Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.
3. **Precursor and derived lipids:** These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies (Chapter 22), hydrocarbons, lipid-soluble vitamins, and hormones.

Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed **neutral lipids.**

FATTY ACIDS ARE ALIPHATIC CARBOXYLIC ACIDS

Fatty acids occur mainly as esters in natural fats and oils but do occur in the unesterified form as **free fatty acids,** a transport form found in the plasma. Fatty acids that occur in natural fats are usually straight-chain derivatives containing an even number of carbon atoms. The chain may be **saturated** (containing no double bonds) or **unsaturated** (containing one or more double bonds).

Fatty Acids Are Named After Corresponding Hydrocarbons

The most frequently used systematic nomenclature names the fatty acid after the hydrocarbon with the same number and arrangement of carbon atoms, with **-oic** being substituted for the final **-e** (Genevan system). Thus, saturated acids end in **-anoic,** eg, octanoic acid, and unsaturated acids with double bonds end in **-enoic,** eg, octadecenoic acid (oleic acid).

Carbon atoms are numbered from the carboxyl carbon (carbon No. 1). The carbon atoms adjacent to the carboxyl carbon (Nos. 2, 3, and 4) are also known as the α, β, and γ carbons, respectively, and the terminal methyl carbon is known as the ω or n-carbon.

Various conventions use Δ for indicating the number and position of the double bonds (Figure 14–1); eg, Δ^9 indicates a double bond between carbons 9 and 10 of the fatty acid; ω9 indicates a double bond on the ninth carbon counting from the ω- carbon. In animals, additional double bonds are introduced only between the existing double bond (eg, ω9, ω6, or ω3) and the

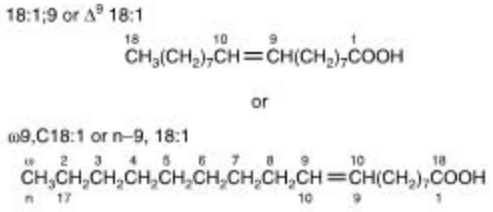

Figure 14–1. Oleic acid. n – 9 (n minus 9) is equivalent to ω9.

carboxyl carbon, leading to three series of fatty acids known as the ω9, ω6, and ω3 families, respectively.

Saturated Fatty Acids Contain No Double Bonds

Saturated fatty acids may be envisaged as based on acetic acid (CH_3—COOH) as the first member of the series in which —CH_2— is progressively added between the terminal CH_3— and —COOH groups. Examples are shown in Table 14–1. Other higher members of the series are known to occur, particularly in waxes. A few branched-chain fatty acids have also been isolated from both plant and animal sources.

Table 14–1. Saturated fatty acids.

Common Name	Number of C Atoms	
Acetic	2	Major end product of carbohydrate fermentation by rumen organisms[1]
Propionic	3	An end product of carbohydrate fermentation by rumen organisms[1]
Butyric	4	In certain fats in small amounts (especially butter). An end product of carbohydrate fermentation by rumen organisms[1]
Valeric	5	
Caproic	6	
Lauric	12	Spermaceti, cinnamon, palm kernel, coconut oils, laurels, butter
Myristic	14	Nutmeg, palm kernel, coconut oils, myrtles, butter
Palmitic	16	Common in all animal and plant fats
Stearic	18	

[1]Also formed in the cecum of herbivores and to a lesser extent in the colon of humans.

Unsaturated Fatty Acids Contain One or More Double Bonds (Table 14–2)

Fatty acids may be further subdivided as follows:

(1) **Monounsaturated** (monoethenoid, monoenoic) acids, containing one double bond.

(2) **Polyunsaturated** (polyethenoid, polyenoic) acids, containing two or more double bonds.

(3) **Eicosanoids:** These compounds, derived from eicosa- (20-carbon) polyenoic fatty acids, comprise the **prostanoids, leukotrienes (LTs),** and **lipoxins (LXs).** Prostanoids include **prostaglandins (PGs), prostacyclins (PGIs),** and **thromboxanes (TXs).**

Prostaglandins exist in virtually every mammalian tissue, acting as local hormones; they have important physiologic and pharmacologic activities. They are synthesized in vivo by cyclization of the center of the carbon chain of 20-carbon (eicosanoic) polyunsaturated fatty acids (eg, arachidonic acid) to form a cyclopentane ring (Figure 14–2). A related series of compounds, the **thromboxanes,** have the cyclopentane ring interrupted with an oxygen atom (oxane ring) (Figure 14–3). Three different eicosanoic fatty acids give rise to three groups of eicosanoids characterized by the number of double bonds in the side chains, eg, PG_1, PG_2, PG_3. Different substituent groups attached to the rings give rise to series of prostaglandins and thromboxanes, labeled A, B, etc—eg, the "E" type of prostaglandin (as in PGE_2) has a keto group in position 9, whereas the "F" type has a hydroxyl group in this position. The **leukotrienes** and **lipoxins** are a third group of eicosanoid derivatives formed via the lipoxygenase pathway (Figure 14–4). They are characterized by the presence of three or four conjugated double bonds, respectively. Leukotrienes cause bronchoconstriction as well as being potent proinflammatory agents and play a part in **asthma.**

Most Naturally Occurring Unsaturated Fatty Acids Have *cis* Double Bonds

The carbon chains of saturated fatty acids form a zigzag pattern when extended, as at low temperatures. At higher temperatures, some bonds rotate, causing chain shortening, which explains why biomembranes become thinner with increases in temperature. A type of **geometric isomerism** occurs in unsaturated fatty acids, depending on the orientation of atoms or groups around the axes of double bonds, which do not allow rotation. If the acyl chains are on the same side of the bond, it is *cis*-, as in oleic acid; if on opposite sides, it is *trans*-, as in elaidic acid, the *trans* isomer of oleic acid (Fig-

Table 14–2. Unsaturated fatty acids of physiologic and nutritional significance.

Number of C Atoms and Number and Position of Double Bonds	Family	Common Name	Systematic Name	Occurrence
Monoenoic acids (one double bond)				
16:1;9	ω7	Palmitoleic	*cis*-9-Hexadecenoic	In nearly all fats.
18:1;9	ω9	Oleic	*cis*-9-Octadecenoic	Possibly the most common fatty acid in natural fats.
18:1;9	ω9	Elaidic	*trans*-9-Octadecenoic	Hydrogenated and ruminant fats.
Dienoic acids (two double bonds)				
18:2;9,12	ω6	Linoleic	all-*cis*-9,12-Octadecadienoic	Corn, peanut, cottonseed, soybean, and many plant oils.
Trienoic acids (three double bonds)				
18:3;6,9,12	ω6	γ-Linolenic	all-*cis*-6,9,12-Octadecatrienoic	Some plants, eg, oil of evening primrose, borage oil; minor fatty acid in animals.
18:3;9,12,15	ω3	α-Linolenic	all-*cis*-9,12,15-Octadecatrienoic	Frequently found with linoleic acid but particularly in linseed oil.
Tetraenoic acids (four double bonds)				
20:4;5,8,11,14	ω6	Arachidonic	all-*cis*-5,8,11,14-Eicosatetraenoic	Found in animal fats and in peanut oil; important component of phospholipids in animals.
Pentaenoic acids (five double bonds)				
20:5;5,8,11,14,17	ω3	Timnodonic	all-*cis*-5,8,11,14,17-Eicosapentaenoic	Important component of fish oils, eg, cod liver, mackerel, menhaden, salmon oils.
Hexaenoic acids (six double bonds)				
22:6;4,7,10,13,16,19	ω3	Cervonic	all-*cis*-4,7,10,13,16,19-Docosahexaenoic	Fish oils, phospholipids in brain.

ure 14–5). Naturally occurring unsaturated long-chain fatty acids are nearly all of the *cis* configuration, the molecules being "bent" 120 degrees at the double bond. Thus, oleic acid has an L shape, whereas elaidic acid remains "straight." Increase in the number of *cis* double bonds in a fatty acid leads to a variety of possible spatial configurations of the molecule—eg, arachidonic acid, with four *cis* double bonds, has "kinks" or a U shape. This has profound significance on molecular packing in membranes and on the positions occupied by fatty acids in more complex molecules such as phospholipids. *Trans* double bonds alter these spatial relationships. *Trans* fatty acids are present in certain foods, arising as a by-product of the saturation of fatty acids during hydrogenation, or "hardening," of natural oils in the manufacture of margarine. An additional small

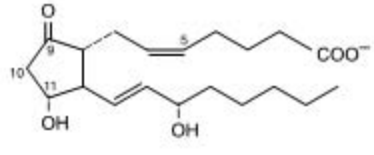

Figure 14–2. Prostaglandin E_2 (PGE_2).

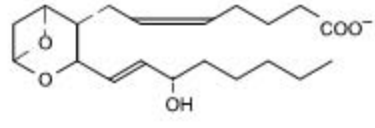

Figure 14–3. Thromboxane A_2 (TXA_2).

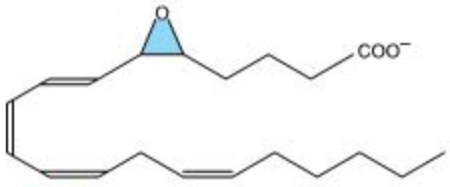

Figure 14–4. Leukotriene A_4 (LTA_4).

contribution comes from the ingestion of ruminant fat that contains *trans* fatty acids arising from the action of microorganisms in the rumen.

Physical and Physiologic Properties of Fatty Acids Reflect Chain Length and Degree of Unsaturation

The melting points of even-numbered-carbon fatty acids increase with chain length and decrease according to unsaturation. A triacylglycerol containing three saturated fatty acids of 12 carbons or more is solid at body temperature, whereas if the fatty acid residues are 18:2, it is liquid to below 0 °C. In practice, natural acylglycerols contain a mixture of fatty acids tailored to suit their functional roles. The membrane lipids, which must be fluid at all environmental temperatures, are more unsaturated than storage lipids. Lipids in tissues that are subject to cooling, eg, in hibernators or in the extremities of animals, are more unsaturated.

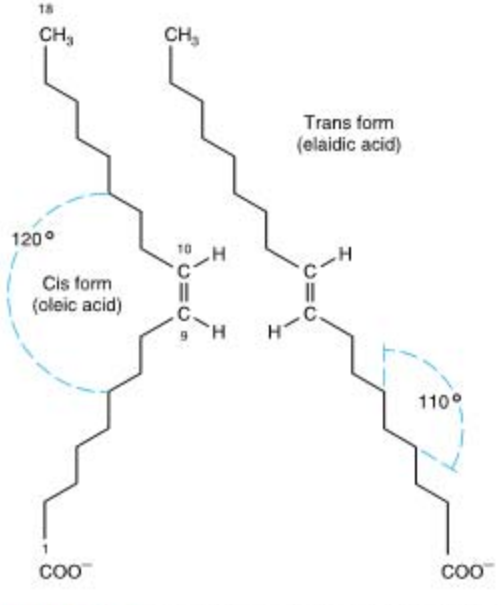

Figure 14–5. Geometric isomerism of Δ^9, 18:1 fatty acids (oleic and elaidic acids).

TRIACYLGLYCEROLS (TRIGLYCERIDES)* ARE THE MAIN STORAGE FORMS OF FATTY ACIDS

The triacylglycerols (Figure 14–6) are esters of the trihydric alcohol glycerol and fatty acids. Mono- and diacylglycerols wherein one or two fatty acids are esterified with glycerol are also found in the tissues. These are of particular significance in the synthesis and hydrolysis of triacylglycerols.

Carbons 1 & 3 of Glycerol Are Not Identical

To number the carbon atoms of glycerol unambiguously, the *-sn-* (stereochemical numbering) system is used. It is important to realize that carbons 1 and 3 of glycerol are not identical when viewed in three dimensions (shown as a projection formula in Figure 14–7). Enzymes readily distinguish between them and are nearly always specific for one or the other carbon; eg, glycerol is always phosphorylated on *sn*-3 by glycerol kinase to give glycerol 3-phosphate and not glycerol 1-phosphate.

PHOSPHOLIPIDS ARE THE MAIN LIPID CONSTITUENTS OF MEMBRANES

Phospholipids may be regarded as derivatives of **phosphatidic acid** (Figure 14–8), in which the phosphate is esterified with the —OH of a suitable alcohol. Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols as well as phosphoglycerols but is not found in any great quantity in tissues.

Phosphatidylcholines (Lecithins) Occur in Cell Membranes

Phosphoacylglycerols containing choline (Figure 14–8) are the most abundant phospholipids of the cell mem-

* According to the standardized terminology of the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB), the monoglycerides, diglycerides, and triglycerides should be designated monoacylglycerols, diacylglycerols, and triacylglycerols, respectively. However, the older terminology is still widely used, particularly in clinical medicine.

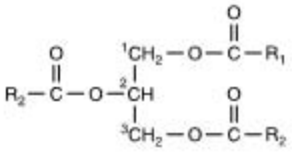

Figure 14–6. Triacylglycerol.

brane and represent a large proportion of the body's store of choline. Choline is important in nervous transmission, as acetylcholine, and as a store of labile methyl groups. **Dipalmitoyl lecithin** is a very effective surface-active agent and a major constituent of the **surfactant** preventing adherence, due to surface tension, of the inner surfaces of the lungs. Its absence from the lungs of premature infants causes **respiratory distress syndrome.** Most phospholipids have a saturated acyl radical in the *sn*-1 position but an unsaturated radical in the *sn*-2 position of glycerol.

Phosphatidylethanolamine (cephalin) and **phosphatidylserine** (found in most tissues) differ from phosphatidylcholine only in that ethanolamine or serine, respectively, replaces choline (Figure 14–8).

Phosphatidylinositol Is a Precursor of Second Messengers

The inositol is present in **phosphatidylinositol** as the stereoisomer, myoinositol (Figure 14–8). **Phosphatidylinositol 4,5-bisphosphate** is an important constituent of cell membrane phospholipids; upon stimulation by a suitable hormone agonist, it is cleaved into **diacylglycerol** and **inositol trisphosphate,** both of which act as internal signals or second messengers.

Cardiolipin Is a Major Lipid of Mitochondrial Membranes

Phosphatidic acid is a precursor of **phosphatidylglycerol** which, in turn, gives rise to **cardiolipin** (Figure 14–8).

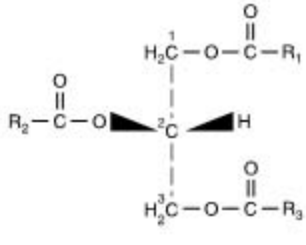

Figure 14–7. Triacyl-*sn*-glycerol.

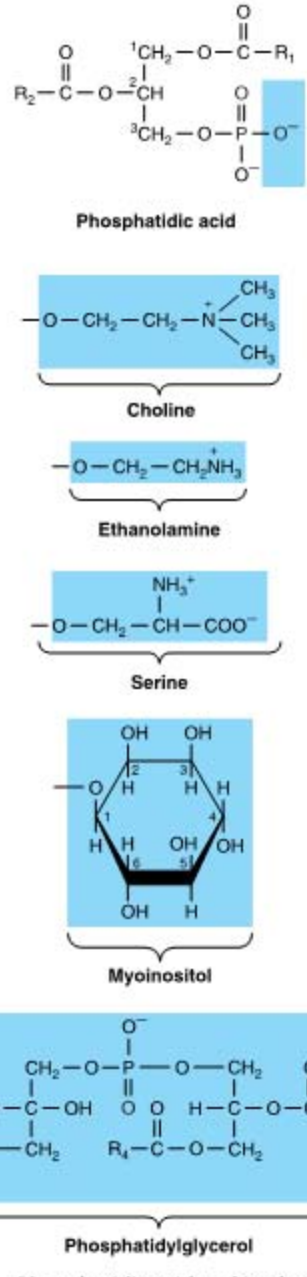

Figure 14–8. Phosphatidic acid and its derivatives. The O^- shown shaded in phosphatidic acid is substituted by the substituents shown to form in **(A)** 3-phosphatidylcholine, **(B)** 3-phosphatidylethanolamine, **(C)** 3-phosphatidylserine, **(D)** 3-phosphatidylinositol, and **(E)** cardiolipin (diphosphatidylglycerol).

Lysophospholipids Are Intermediates in the Metabolism of Phosphoglycerols

These are phosphoacylglycerols containing only one acyl radical, eg, **lysophosphatidylcholine (lysolecithin),** important in the metabolism and interconversion of phospholipids (Figure 14–9).It is also found in oxidized lipoproteins and has been implicated in some of their effects in promoting **atherosclerosis**.

Plasmalogens Occur in Brain & Muscle

These compounds constitute as much as 10% of the phospholipids of brain and muscle. Structurally, the plasmalogens resemble phosphatidylethanolamine but possess an ether link on the *sn*-1 carbon instead of the ester link found in acylglycerols. Typically, the alkyl radical is an unsaturated alcohol (Figure 14–10). In some instances, choline, serine, or inositol may be substituted for ethanolamine.

Sphingomyelins Are Found in the Nervous System

Sphingomyelins are found in large quantities in brain and nerve tissue. On hydrolysis, the sphingomyelins yield a fatty acid, phosphoric acid, choline, and a complex amino alcohol, **sphingosine** (Figure 14–11). No glycerol is present. The combination of sphingosine plus fatty acid is known as **ceramide,** a structure also found in the glycosphingolipids (see below).

GLYCOLIPIDS (GLYCOSPHINGOLIPIDS) ARE IMPORTANT IN NERVE TISSUES & IN THE CELL MEMBRANE

Glycolipids are widely distributed in every tissue of the body, particularly in nervous tissue such as brain. They occur particularly in the outer leaflet of the plasma membrane, where they contribute to **cell surface carbohydrates.**

The major glycolipids found in animal tissues are glycosphingolipids. They contain ceramide and one or more sugars. **Galactosylceramide** is a major glycosphingolipid of brain and other nervous tissue, found in relatively low amounts elsewhere. It contains a number of characteristic C_{24} fatty acids, eg, cerebronic acid. Galactosylceramide (Figure 14–12) can be converted to sulfogalactosylceramide **(sulfatide),** present in high amounts in myelin. Glucosylceramide is the predominant simple glycosphingolipid of extraneural tissues, also occurring in the brain in small amounts. **Gangliosides** are complex glycosphingolipids derived from glucosylceramide that contain in addition one or more molecules of a **sialic acid.** Neuraminic acid (NeuAc; see Chapter 13) is the principal sialic acid found in human tissues. Gangliosides are also present in nervous tissues in high concentration. They appear to have receptor and other functions. The simplest ganglioside found in tissues is G_{M3}, which contains ceramide, one molecule of glucose, one molecule of galactose, and one molecule of NeuAc. In the shorthand nomenclature used, G represents ganglioside; M is a monosialo-containing species; and the subscript 3 is a number assigned on the basis of chromatographic migration. G_{M1} (Figure 14–13), a more complex ganglioside derived from G_{M3}, is of considerable biologic interest, as it is known to be the receptor in human intestine for cholera toxin. Other gangliosides can contain anywhere from one to five molecules of sialic acid, giving rise to di-, trisialogangliosides, etc.

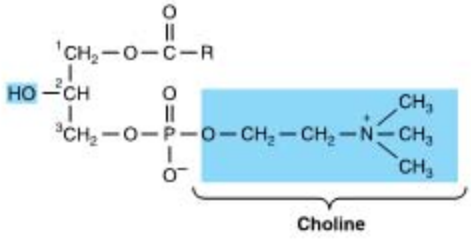

Figure 14–9. Lysophosphatidylcholine (lysolecithin).

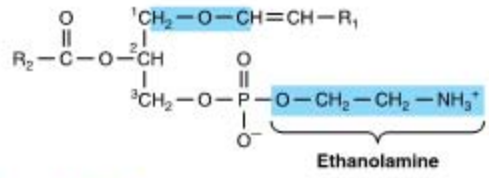

Figure 14–10. Plasmalogen.

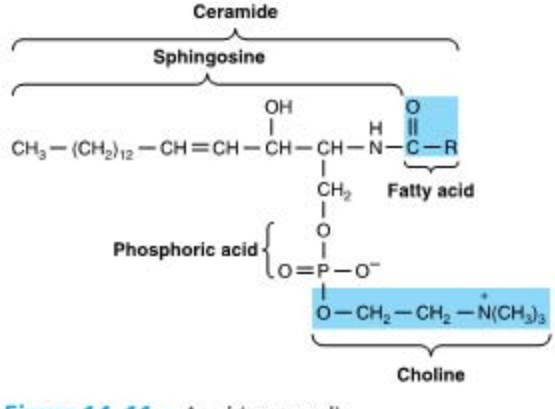

Figure 14–11. A sphingomyelin.

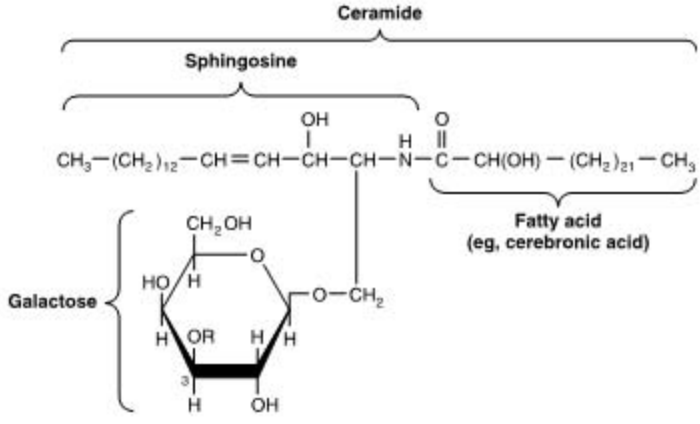

Figure 14–12. Structure of galactosylceramide (galactocerebroside, R = H), and sulfogalactosylceramide (a sulfatide, R = SO_4^{2-}).

STEROIDS PLAY MANY PHYSIOLOGICALLY IMPORTANT ROLES

Cholesterol is probably the best known steroid because of its association with **atherosclerosis.** However, biochemically it is also of significance because it is the precursor of a large number of equally important steroids that include the bile acids, adrenocortical hormones, sex hormones, D vitamins, cardiac glycosides, sitosterols of the plant kingdom, and some alkaloids.

All of the steroids have a similar cyclic nucleus resembling phenanthrene (rings A, B, and C) to which a cyclopentane ring (D) is attached. The carbon positions on the steroid nucleus are numbered as shown in Figure 14–14. It is important to realize that in structural formulas of steroids, a simple hexagonal ring denotes a completely saturated six-carbon ring with all valences satisfied by hydrogen bonds unless shown otherwise; ie, it is not a benzene ring. All double bonds are shown as such. Methyl side chains are shown as single bonds unattached at the farther (methyl) end. These occur typically at positions 10 and 13 (constituting C atoms 19 and 18). A side chain at position 17 is usual (as in cholesterol). If the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a **sterol,** and the name terminates in -ol.

Because of Asymmetry in the Steroid Molecule, Many Stereoisomers Are Possible

Each of the six-carbon rings of the steroid nucleus is capable of existing in the three-dimensional conformation either of a "chair" or a "boat" (Figure 14–15). In naturally occurring steroids, virtually all the rings are in the "chair" form, which is the more stable conformation. With respect to each other, the rings can be either *cis* or *trans* (Figure 14–16). The junction between the A and B rings can be *cis* or *trans* in naturally occurring steroids. That between B and C is *trans,* as is usually the C/D junction. Bonds attaching substituent groups above the plane of the rings (β bonds) are shown with bold solid lines, whereas those bonds attaching groups below (α bonds) are indicated with broken lines. The A ring of a 5α steroid is always *trans* to the B ring, whereas it is *cis* in a 5β steroid. The methyl groups attached to C_{10} and C_{13} are invariably in the β configuration.

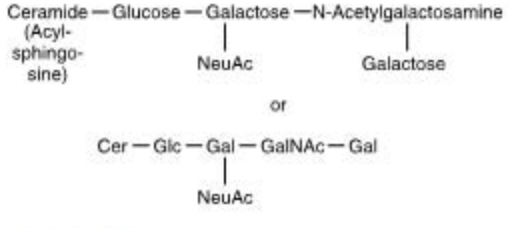

Figure 14–13. G_{M1} ganglioside, a monosialoganglioside, the receptor in human intestine for cholera toxin.

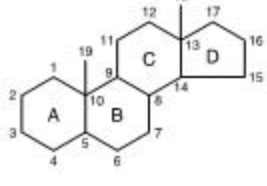

Figure 14–14. The steroid nucleus.

Figure 14–15. Conformations of stereoisomers of the steroid nucleus.

Cholesterol Is a Significant Constituent of Many Tissues

Cholesterol (Figure 14–17) is widely distributed in all cells of the body but particularly in nervous tissue. It is a major constituent of the plasma membrane and of plasma lipoproteins. It is often found as **cholesteryl ester,** where the hydroxyl group on position 3 is esterified with a long-chain fatty acid. It occurs in animals but not in plants.

Ergosterol Is a Precursor of Vitamin D

Ergosterol occurs in plants and yeast and is important as a precursor of vitamin D (Figure 14–18). When irradiated with ultraviolet light, it acquires antirachitic properties consequent to the opening of ring B.

Polyprenoids Share the Same Parent Compound as Cholesterol

Although not steroids, these compounds are related because they are synthesized, like cholesterol (Figure 26–2), from five-carbon isoprene units (Figure 14–19). They include **ubiquinone** (Chapter 12), a member of the respiratory chain in mitochondria, and the long-chain alcohol **dolichol** (Figure 14–20), which takes part in glycoprotein synthesis by transferring carbohydrate residues to asparagine residues of the polypeptide (Chapter 47). Plant-derived isoprenoid compounds include rubber, camphor, the fat-soluble vitamins A, D, E, and K, and β-carotene (provitamin A).

LIPID PEROXIDATION IS A SOURCE OF FREE RADICALS

Peroxidation **(auto-oxidation)** of lipids exposed to oxygen is responsible not only for deterioration of foods **(rancidity)** but also for damage to tissues in vivo, where it may be a cause of cancer, inflammatory diseases, atherosclerosis, and aging. The deleterious effects are considered to be caused by free radicals ($ROO^{\bullet}$, $RO^{\bullet}$, $OH^{\bullet}$) produced during peroxide formation from fatty acids containing methylene-interrupted double bonds, ie, those found in the naturally occurring polyunsaturated fatty acids (Figure 14–21). Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. The whole process can be depicted as follows:

(1) Initiation:

$$ROOH + Metal^{(n)+} \rightarrow ROO^{\bullet} + Metal^{(n-1)+} + H^{+}$$

$$X^{\bullet} + RH \rightarrow R^{\bullet} + XH$$

(2) Propagation:

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}, \text{ etc}$$

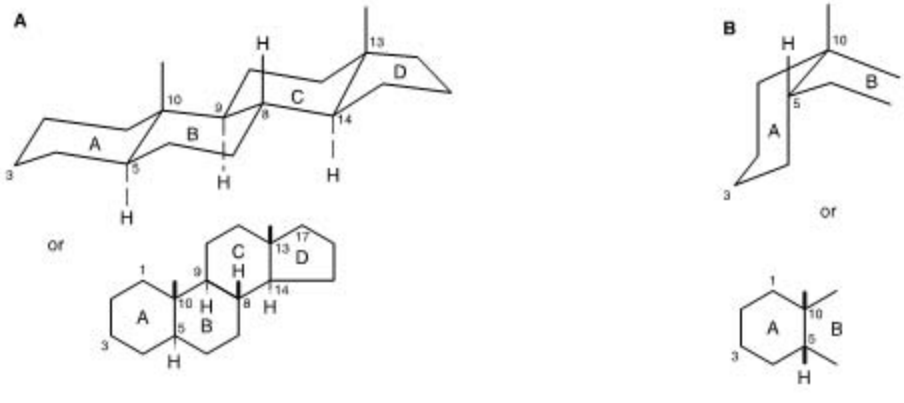

Figure 14–16. Generalized steroid nucleus, showing **(A)** an all-*trans* configuration between adjacent rings and **(B)** a *cis* configuration between rings A and B.

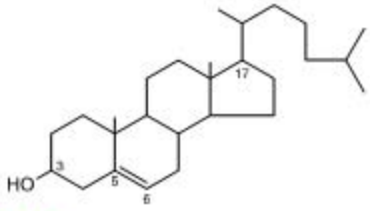

Figure 14–17. Cholesterol, 3-hydroxy-5,6-cholestene.

(3) Termination:

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$$

$$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$$

$$R^{\bullet} + R^{\bullet} \rightarrow RR$$

Since the molecular precursor for the initiation process is generally the hydroperoxide product ROOH, lipid peroxidation is a chain reaction with potentially devastating effects. To control and reduce lipid peroxidation, both humans in their activities and nature invoke the use of **antioxidants.** Propyl gallate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are antioxidants used as food additives. Naturally occurring antioxidants include vitamin E (tocopherol), which is lipid-soluble, and urate and vitamin C, which are water-soluble. Beta-carotene is an antioxidant at low P_{O_2}. Antioxidants fall into two classes: (1) preventive antioxidants, which reduce the rate of chain initiation; and (2) chain-breaking antioxidants, which interfere with chain propagation. Preventive antioxidants include catalase and other peroxidases that react with ROOH and chelators of metal ions such as EDTA (ethylenediaminetetraacetate) and DTPA (diethylenetriaminepentaacetate). In vivo, the principal chain-breaking antioxidants are superoxide dismutase, which acts in the aqueous phase to trap superoxide free radicals ($O_2^{\bullet}$); perhaps urate; and vitamin E, which acts in the lipid phase to trap $ROO^{\bullet}$ radicals (Figure 45–6).

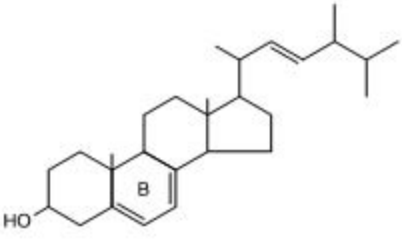

Figure 14–18. Ergosterol.

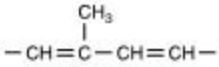

Figure 14–19. Isoprene unit.

Peroxidation is also catalyzed in vivo by heme compounds and by **lipoxygenases** found in platelets and leukocytes. Other products of auto-oxidation or enzymic oxidation of physiologic significance include **oxysterols** (formed from cholesterol) and **isoprostanes** (prostanoids).

AMPHIPATHIC LIPIDS SELF-ORIENT AT OIL:WATER INTERFACES

They Form Membranes, Micelles, Liposomes, & Emulsions

In general, lipids are insoluble in water since they contain a predominance of nonpolar (hydrocarbon) groups. However, fatty acids, phospholipids, sphingolipids, bile salts, and, to a lesser extent, cholesterol contain polar groups. Therefore, part of the molecule is **hydrophobic,** or water-insoluble; and part is **hydrophilic,** or water-soluble. Such molecules are described as **amphipathic** (Figure 14–22). They become oriented at oil:water interfaces with the polar group in the water phase and the nonpolar group in the oil phase. A bilayer of such amphipathic lipids has been regarded as a basic structure in biologic membranes (Chapter 41). When a critical concentration of these lipids is present in an aqueous medium, they form **micelles.** Aggregations of bile salts into micelles and liposomes and the formation of mixed micelles with the products of fat digestion are important in facilitating absorption of lipids from the intestine. Liposomes may be formed by sonicating an amphipathic lipid in an aqueous medium. They consist of spheres of lipid bilayers that enclose part of the aqueous medium. They are of potential clinical use—particularly when combined with tissue-specific antibodies—as carriers of drugs in the circulation, targeted to specific organs, eg, in cancer therapy. In addition, they are being used for gene transfer into vascular cells and as carriers for topical and transdermal

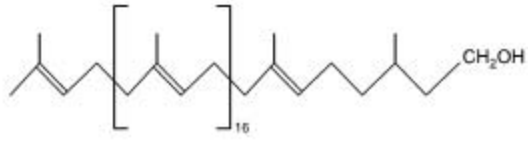

Figure 14–20. Dolichol—a C_{95} alcohol.

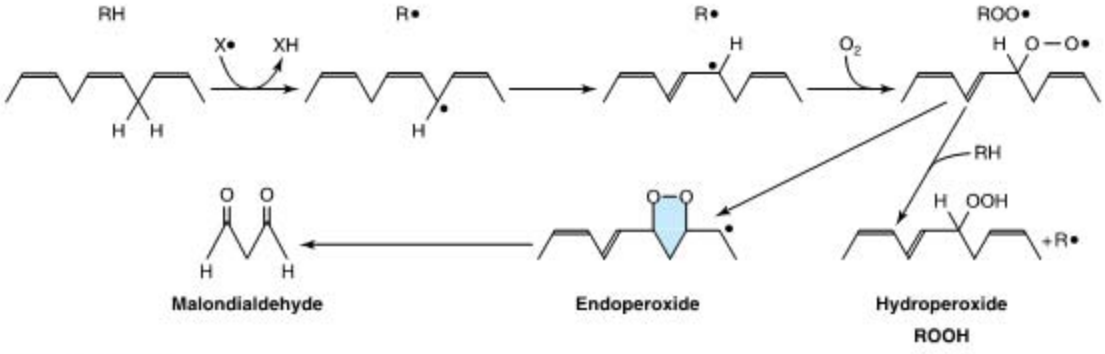

Figure 14–21. Lipid peroxidation. The reaction is initiated by an existing free radical (X•), by light, or by metal ions. Malondialdehyde is only formed by fatty acids with three or more double bonds and is used as a measure of lipid peroxidation together with ethane from the terminal two carbons of ω3 fatty acids and pentane from the terminal five carbons of ω6 fatty acids.

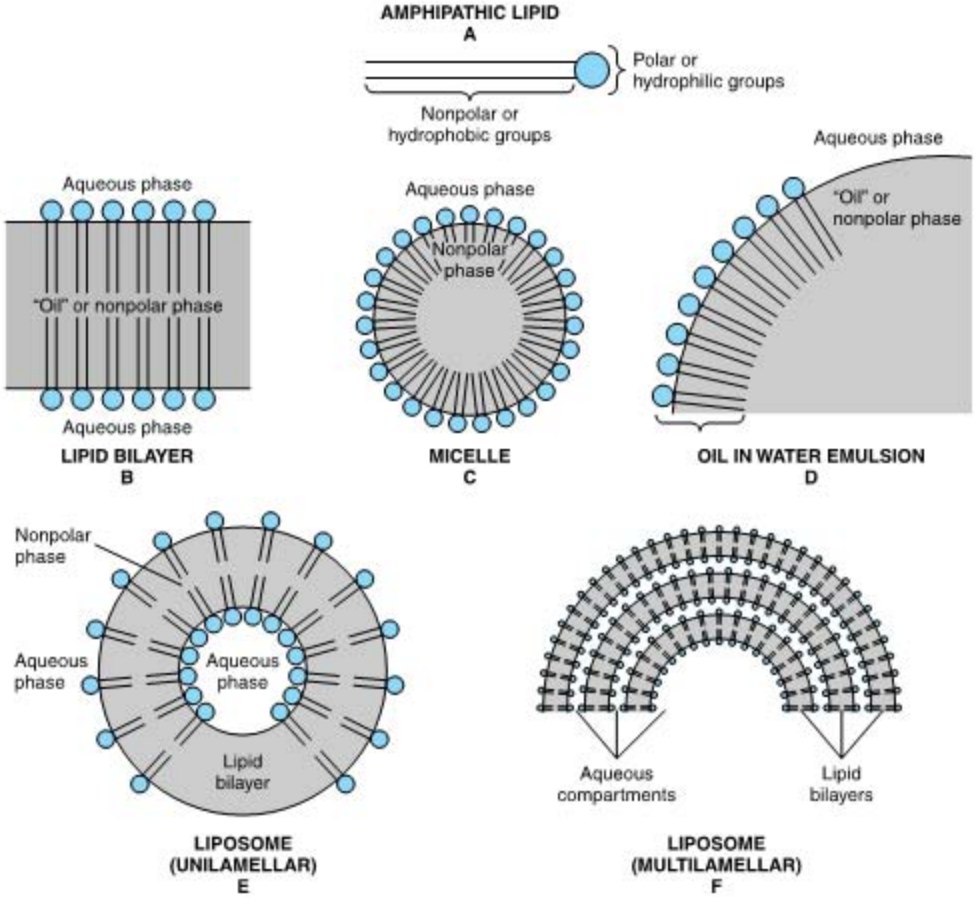

Figure 14–22. Formation of lipid membranes, micelles, emulsions, and liposomes from amphipathic lipids, eg, phospholipids.

delivery of drugs and cosmetics. **Emulsions** are much larger particles, formed usually by nonpolar lipids in an aqueous medium. These are stabilized by emulsifying agents such as amphipathic lipids (eg, lecithin), which form a surface layer separating the main bulk of the nonpolar material from the aqueous phase (Figure 14–22).

SUMMARY

- Lipids have the common property of being relatively insoluble in water (hydrophobic) but soluble in nonpolar solvents. Amphipathic lipids also contain one or more polar groups, making them suitable as constituents of membranes at lipid:water interfaces.
- The lipids of major physiologic significance are fatty acids and their esters, together with cholesterol and other steroids.
- Long-chain fatty acids may be saturated, monounsaturated, or polyunsaturated, according to the number of double bonds present. Their fluidity decreases with chain length and increases according to degree of unsaturation.
- Eicosanoids are formed from 20-carbon polyunsaturated fatty acids and make up an important group of physiologically and pharmacologically active compounds known as prostaglandins, thromboxanes, leukotrienes, and lipoxins.
- The esters of glycerol are quantitatively the most significant lipids, represented by triacylglycerol ("fat"), a major constituent of lipoproteins and the storage form of lipid in adipose tissue. Phosphoacylglycerols are amphipathic lipids and have important roles—as major constituents of membranes and the outer layer of lipoproteins, as surfactant in the lung, as precursors of second messengers, and as constituents of nervous tissue.
- Glycolipids are also important constituents of nervous tissue such as brain and the outer leaflet of the cell membrane, where they contribute to the carbohydrates on the cell surface.
- Cholesterol, an amphipathic lipid, is an important component of membranes. It is the parent molecule from which all other steroids in the body, including major hormones such as the adrenocortical and sex hormones, D vitamins, and bile acids, are synthesized.
- Peroxidation of lipids containing polyunsaturated fatty acids leads to generation of free radicals that may damage tissues and cause disease.

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Overview of Metabolism

15

Peter A. Mayes, PhD, DSc, & David A. Bender, PhD

BIOMEDICAL IMPORTANCE

The fate of dietary components after digestion and absorption constitutes metabolism—the metabolic pathways taken by individual molecules, their interrelationships, and the mechanisms that regulate the flow of metabolites through the pathways. Metabolic pathways fall into three categories: (1) **Anabolic pathways** are those involved in the synthesis of compounds. Protein synthesis is such a pathway, as is the synthesis of fuel reserves of triacylglycerol and glycogen. Anabolic pathways are endergonic. (2) **Catabolic pathways** are involved in the breakdown of larger molecules, commonly involving oxidative reactions; they are exergonic, producing reducing equivalents and, mainly via the respiratory chain, ATP. (3) **Amphibolic pathways** occur at the "crossroads" of metabolism, acting as links between the anabolic and catabolic pathways, eg, the citric acid cycle.

A knowledge of normal metabolism is essential for an understanding of abnormalities underlying disease. Normal metabolism includes adaptation to periods of starvation, exercise, pregnancy, and lactation. Abnormal metabolism may result from nutritional deficiency, enzyme deficiency, abnormal secretion of hormones, or the actions of drugs and toxins. An important example of a metabolic disease is **diabetes mellitus.**

PATHWAYS THAT PROCESS THE MAJOR PRODUCTS OF DIGESTION

The nature of the diet sets the basic pattern of metabolism. There is a need to process the products of digestion of dietary carbohydrate, lipid, and protein. These are mainly glucose, fatty acids and glycerol, and amino acids, respectively. In ruminants (and to a lesser extent in other herbivores), dietary cellulose is fermented by symbiotic microorganisms to short-chain fatty acids (acetic, propionic, butyric), and metabolism in these animals is adapted to use these fatty acids as major substrates. All the products of digestion are metabolized to a **common product, acetyl-CoA,** which is then oxidized by the **citric acid cycle** (Figure 15–1).

Carbohydrate Metabolism Is Centered on the Provision & Fate of Glucose (Figure 15–2)

Glucose is metabolized to pyruvate by the pathway of **glycolysis,** which can occur anaerobically (in the absence of oxygen), when the end product is lactate. Aerobic tissues metabolize pyruvate to **acetyl-CoA,** which can enter the **citric acid cycle** for complete oxidation to CO_2 and H_2O, linked to the formation of ATP in the process of **oxidative phosphorylation** (Figure 16–2). Glucose is the major fuel of most tissues.

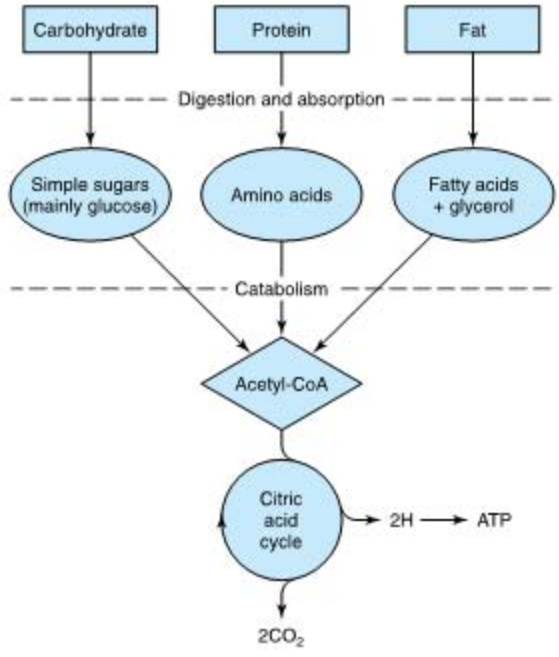

Figure 15–1. Outline of the pathways for the catabolism of dietary carbohydrate, protein, and fat. All the pathways lead to the production of acetyl-CoA, which is oxidized in the citric acid cycle, ultimately yielding ATP in the process of oxidative phosphorylation.

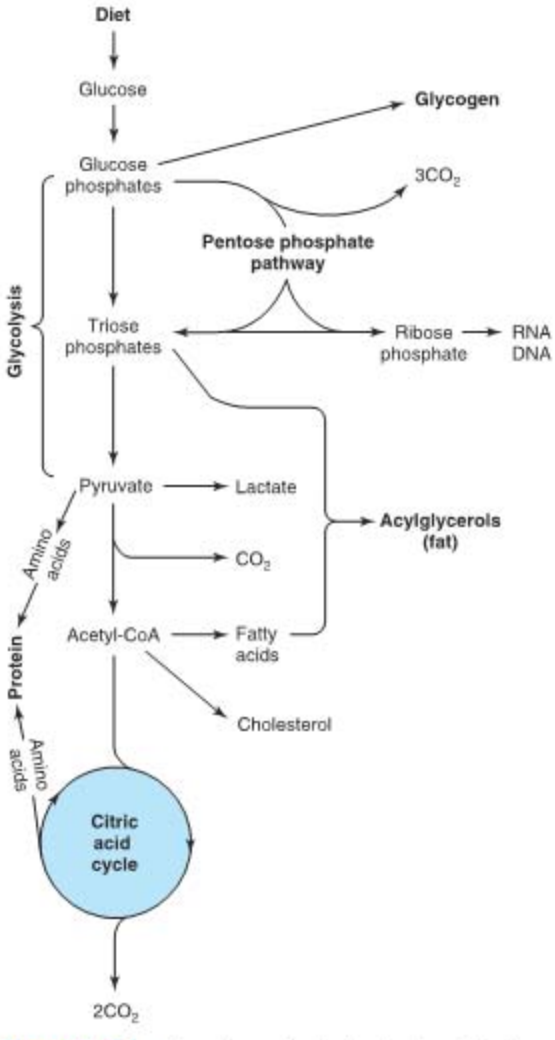

Figure 15–2. Overview of carbohydrate metabolism showing the major pathways and end products. Gluconeogenesis is not shown.

Glucose and its metabolites also take part in other processes. Examples: (1) Conversion to the storage polymer **glycogen** in skeletal muscle and liver. (2) The **pentose phosphate pathway,** an alternative to part of the pathway of glycolysis, is a source of reducing equivalents (NADPH) for biosynthesis and the source of **ribose** for nucleotide and nucleic acid synthesis. (3) Triose phosphate gives rise to the **glycerol moiety** of triacylglycerols. (4) Pyruvate and intermediates of the citric acid cycle provide the carbon skeletons for the synthesis of **amino acids;** and acetyl-CoA, the precursor of **fatty acids** and **cholesterol** (and hence of all steroids synthesized in the body). **Gluconeogenesis** is the process of forming glucose from noncarbohydrate precursors, eg, lactate, amino acids, and glycerol.

Lipid Metabolism Is Concerned Mainly With Fatty Acids & Cholesterol (Figure 15–3)

The source of long-chain fatty acids is either dietary lipid or de novo synthesis from acetyl-CoA derived from carbohydrate. Fatty acids may be oxidized to **acetyl-CoA** (β-oxidation) or esterified with glycerol, forming **triacylglycerol** (fat) as the body's main fuel reserve.

Acetyl-CoA formed by β-oxidation may undergo several fates:

(1) As with acetyl-CoA arising from glycolysis, it is **oxidized** to $CO_2 + H_2O$ via the **citric acid cycle.**

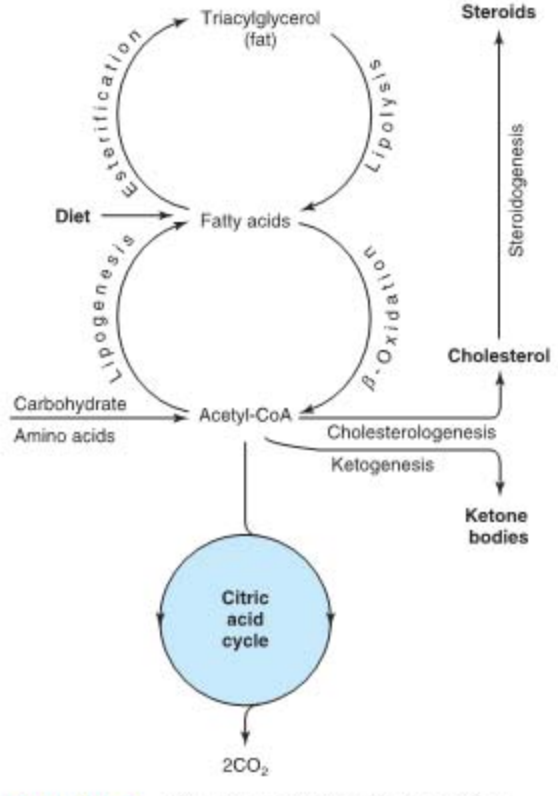

Figure 15–3. Overview of fatty acid metabolism showing the major pathways and end products. Ketone bodies comprise the substances acetoacetate, 3-hydroxybutyrate, and acetone.

(2) It is the precursor for synthesis of **cholesterol** and other **steroids.**

(3) In the liver, it forms **ketone bodies** (acetone, acetoacetate, and 3-hydroxybutyrate) that are important fuels in prolonged starvation.

Much of Amino Acid Metabolism Involves Transamination (Figure 15–4)

The amino acids are required for protein synthesis. Some must be supplied in the diet (the **essential amino acids**) since they cannot be synthesized in the body. The remainder are **nonessential amino acids** that are supplied in the diet but can be formed from metabolic intermediates by **transamination,** using the amino nitrogen from other amino acids. After **deamination,** amino nitrogen is excreted as **urea,** and the carbon skeletons that remain after transamination (1) are oxidized to CO_2 via the citric acid cycle, (2) form glucose (gluconeogenesis), or (3) form ketone bodies.

Several amino acids are also the precursors of other compounds, eg, purines, pyrimidines, hormones such as epinephrine and thyroxine, and neurotransmitters.

METABOLIC PATHWAYS MAY BE STUDIED AT DIFFERENT LEVELS OF ORGANIZATION

In addition to studies in the whole organism, the location and integration of metabolic pathways is revealed by studies at several levels of organization. At the **tissue and organ level,** the nature of the substrates entering and metabolites leaving tissues and organs is defined. At the **subcellular level,** each cell organelle (eg, the mitochondrion) or compartment (eg, the cytosol) has specific roles that form part of a subcellular pattern of metabolic pathways.

At the Tissue and Organ Level, the Blood Circulation Integrates Metabolism

Amino acids resulting from the digestion of dietary protein and glucose resulting from the digestion of carbohydrate are absorbed and directed to the liver via the **hepatic portal vein.** The liver has the role of regulating the blood concentration of most water-soluble metabolites (Figure 15–5). In the case of glucose, this is achieved by taking up glucose in excess of immediate requirements and converting it to glycogen (**glycogene-**

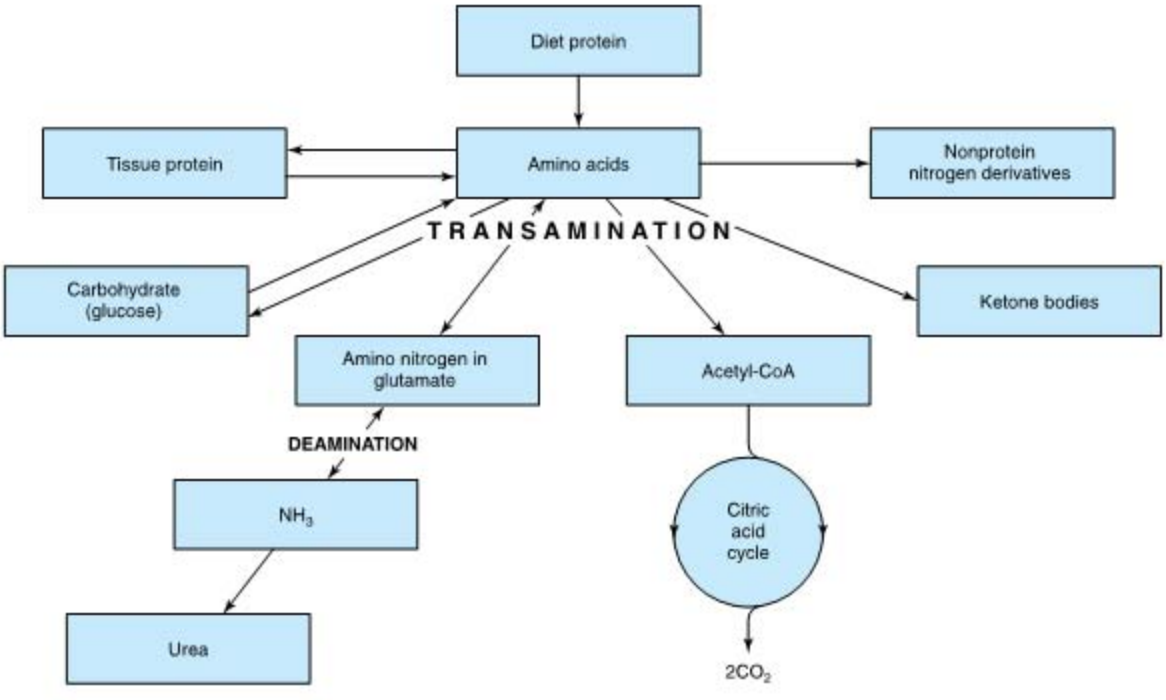

Figure 15–4. Overview of amino acid metabolism showing the major pathways and end products.

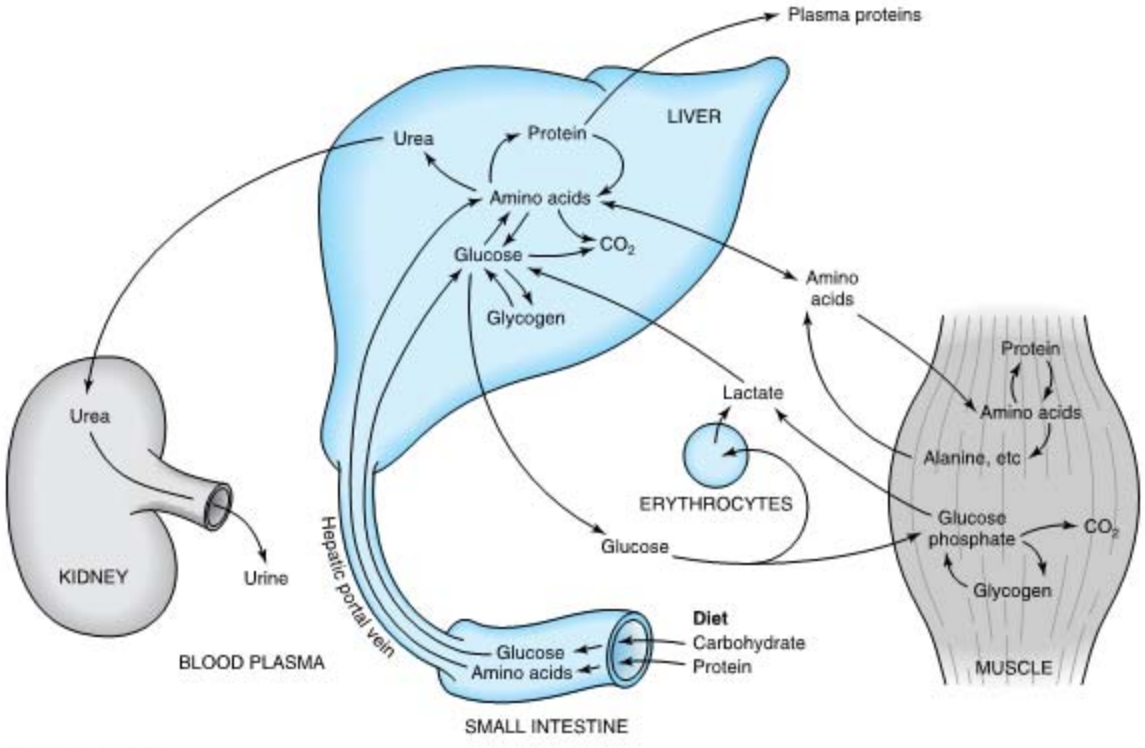

Figure 15–5. Transport and fate of major carbohydrate and amino acid substrates and metabolites. Note that there is little free glucose in muscle, since it is rapidly phosphorylated upon entry.

sis) or to fat **(lipogenesis).** Between meals, the liver acts to maintain the blood glucose concentration from glycogen **(glycogenolysis)** and, together with the kidney, by converting noncarbohydrate metabolites such as lactate, glycerol, and amino acids to glucose **(gluconeogenesis).** Maintenance of an adequate concentration of blood glucose is vital for those tissues in which it is the major fuel (the brain) or the only fuel (the erythrocytes). The liver also **synthesizes the major plasma proteins** (eg, albumin) and **deaminates amino acids** that are in excess of requirements, forming urea, which is transported to the kidney and excreted.

Skeletal muscle utilizes glucose as a fuel, forming both lactate and CO_2. It stores glycogen as a fuel for its use in muscular contraction and synthesizes muscle protein from plasma amino acids. Muscle accounts for approximately 50% of body mass and consequently represents a considerable store of protein that can be drawn upon to supply amino acids for gluconeogenesis in starvation.

Lipids in the diet (Figure 15–6) are mainly triacylglycerol and are hydrolyzed to monoacylglycerols and fatty acids in the gut, then reesterified in the intestinal mucosa. Here they are packaged with protein and secreted into the lymphatic system and thence into the blood stream as **chylomicrons,** the largest of the plasma **lipoproteins.** Chylomicrons also contain other lipid-soluble nutrients, eg, vitamins. Unlike glucose and amino acids, chylomicron triacylglycerol is not taken up directly by the liver. It is first metabolized by tissues that have **lipoprotein lipase,** which hydrolyzes the triacylglycerol, releasing fatty acids that are incorporated into tissue lipids or oxidized as fuel. The other major source of long-chain fatty acid is synthesis **(lipogenesis)** from carbohydrate, mainly in adipose tissue and the liver.

Adipose tissue triacylglycerol is the main fuel reserve of the body. On hydrolysis **(lipolysis)** free fatty acids are released into the circulation. These are taken up by most tissues (but not brain or erythrocytes) and esterified to acylglycerols or oxidized as a fuel. In the liver, triacylglycerol arising from lipogenesis, free fatty acids, and chylomicron remnants (see Figures 25–3 and 25–4) is secreted into the circulation as **very low density lipoprotein** (VLDL). This triacylglycerol undergoes a fate similar to that of chylomicrons. Partial oxidation of fatty acids in the liver leads to **ketone body** production (keto-

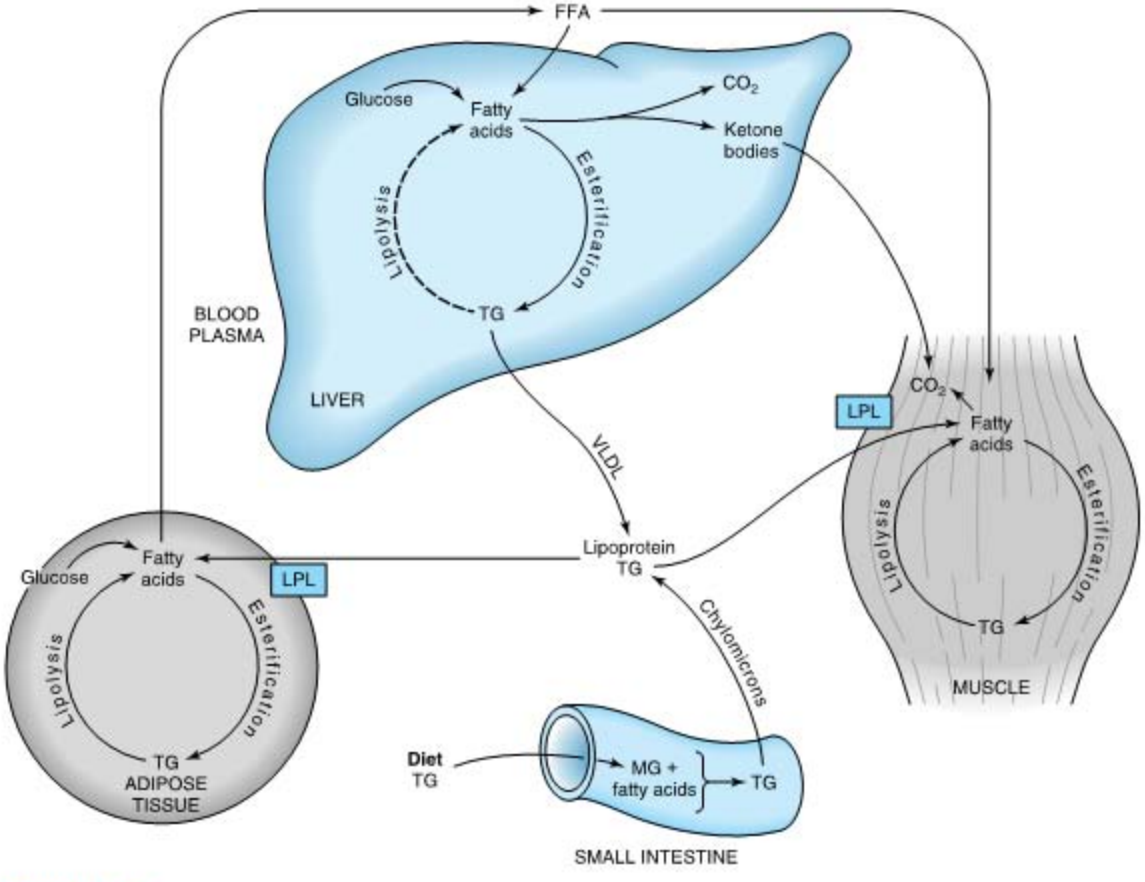

Figure 15–6. Transport and fate of major lipid substrates and metabolites. (FFA, free fatty acids; LPL, lipoprotein lipase; MG, monoacylglycerol; TG, triacylglycerol; VLDL, very low density lipoprotein.)

genesis). Ketone bodies are transported to extrahepatic tissues, where they act as a fuel source in starvation.

At the Subcellular Level, Glycolysis Occurs in the Cytosol & the Citric Acid Cycle in the Mitochondria

Compartmentation of pathways in separate subcellular compartments or organelles permits integration and regulation of metabolism. Not all pathways are of equal importance in all cells. Figure 15–7 depicts the subcellular compartmentation of metabolic pathways in a hepatic parenchymal cell.

The central role of the **mitochondrion** is immediately apparent, since it acts as the focus of carbohydrate, lipid, and amino acid metabolism. It contains the enzymes of the citric acid cycle, β-oxidation of fatty acids, and ketogenesis, as well as the respiratory chain and ATP synthase.

Glycolysis, the pentose phosphate pathway, and fatty acid synthesis are all found in the cytosol. In gluconeogenesis, substrates such as lactate and pyruvate, which are formed in the cytosol, enter the mitochondrion to yield **oxaloacetate** before formation of glucose.

The membranes of the **endoplasmic reticulum** contain the enzyme system for **acylglycerol synthesis,** and the **ribosomes** are responsible for **protein synthesis.**

THE FLUX OF METABOLITES IN METABOLIC PATHWAYS MUST BE REGULATED IN A CONCERTED MANNER

Regulation of the overall flux through a pathway is important to ensure an appropriate supply, when required, of the products of that pathway. Regulation is achieved by control of one or more key reactions in the pathway, catalyzed by **"regulatory enzymes."** The physicochemical factors that control the rate of an

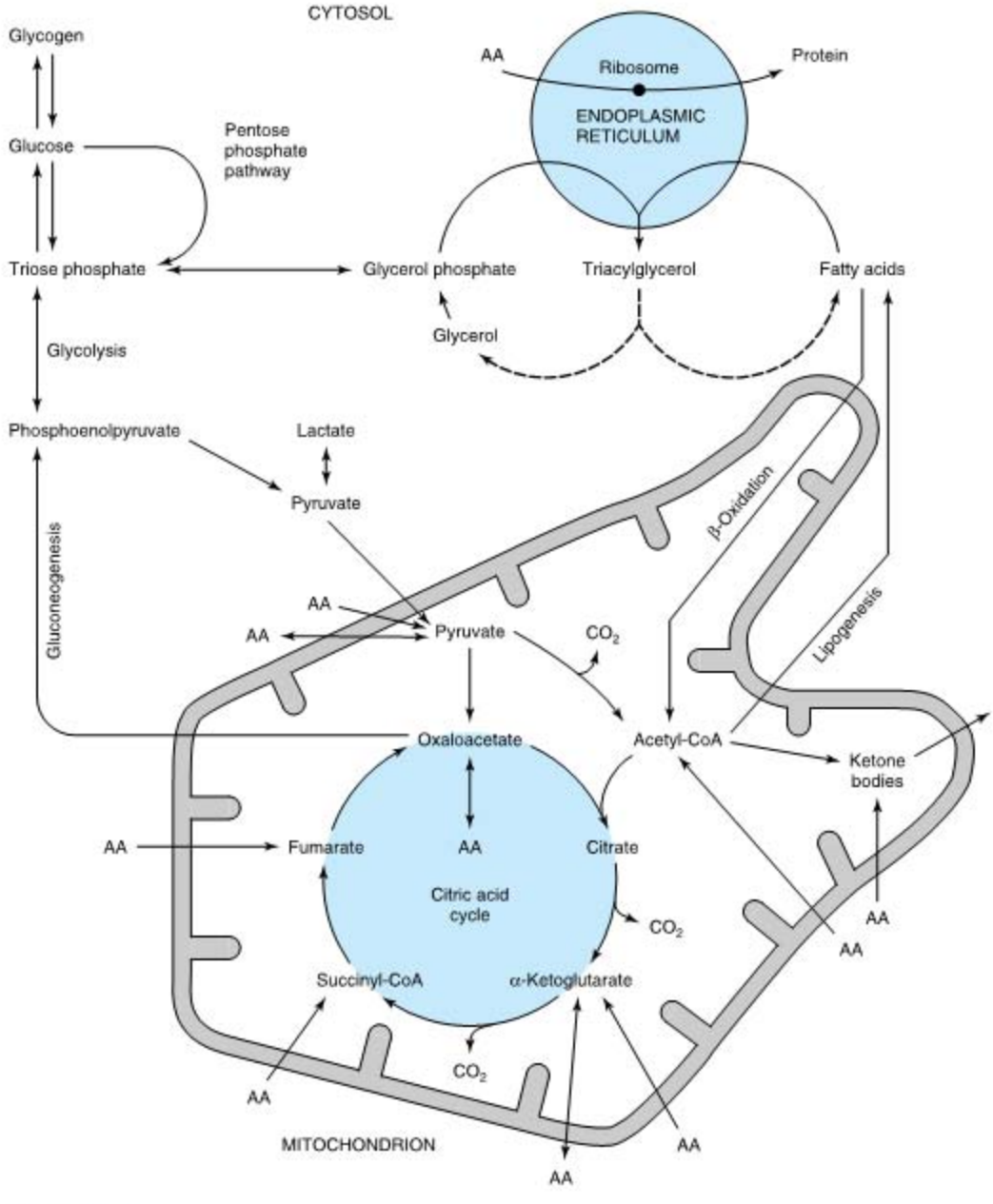

Figure 15–7. Intracellular location and overview of major metabolic pathways in a liver parenchymal cell. (AA →, metabolism of one or more essential amino acids; AA ↔, metabolism of one or more nonessential amino acids.)

enzyme-catalyzed reaction, eg, substrate concentration, are of primary importance in the control of the overall rate of a metabolic pathway (Chapter 9).

"Nonequilibrium" Reactions Are Potential Control Points

In a reaction at equilibrium, the forward and reverse reactions occur at equal rates, and there is therefore no net flux in either direction:

$$A \leftrightarrow B \leftrightarrow C \leftrightarrow D$$

In vivo, under "steady-state" conditions, there is a net flux from left to right because there is a continuous supply of A and removal of D. In practice, there are invariably one or more **nonequilibrium reactions** in a metabolic pathway, where the reactants are present in concentrations that are far from equilibrium. In attempting to reach equilibrium, large losses of free energy occur as heat, making this type of reaction essentially irreversible, eg,

$$A \leftrightarrow B \xrightarrow{\text{Heat}} C \leftrightarrow D$$

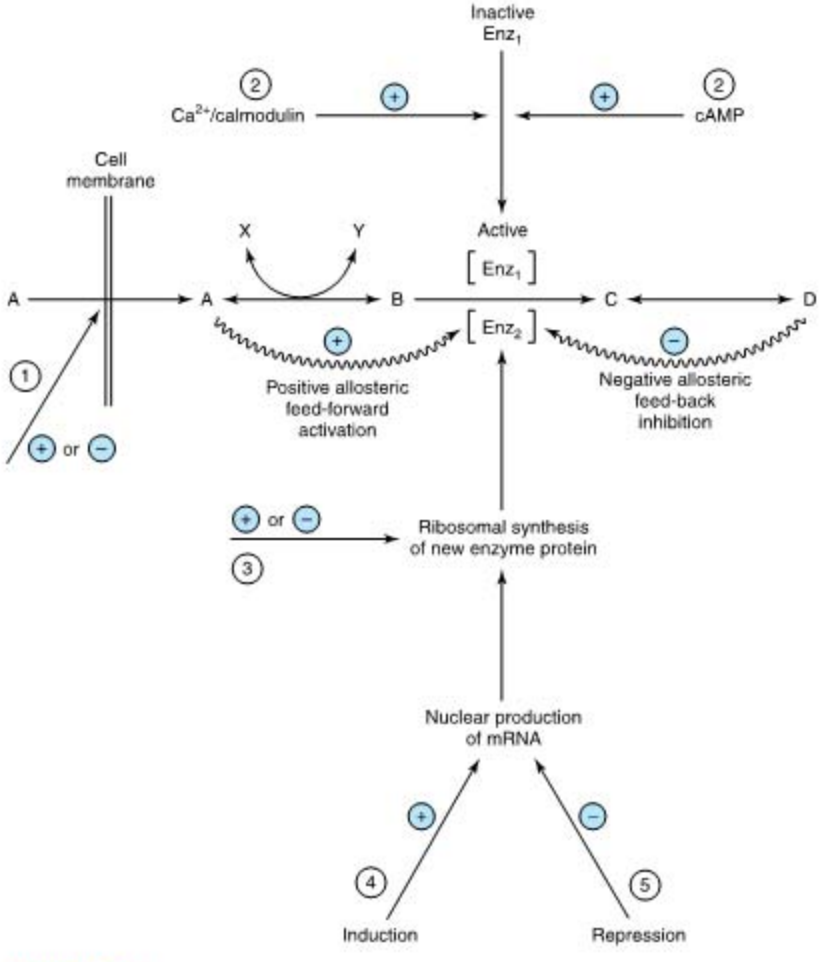

Figure 15–8. Mechanisms of control of an enzyme-catalyzed reaction. Circled numbers indicate possible sites of action of hormones. ①, Alteration of membrane permeability; ②, conversion of an inactive to an active enzyme, usually involving phosphorylation/dephosphorylation reactions; ③, alteration of the rate of translation of mRNA at the ribosomal level; ④, induction of new mRNA formation; and ⑤, repression of mRNA formation. ① and ② are rapid, whereas ③–⑤ are slower ways of regulating enzyme activity.

Such a pathway has both flow and direction. The enzymes catalyzing nonequilibrium reactions are usually present in low concentrations and are subject to a variety of regulatory mechanisms. However, many of the reactions in metabolic pathways cannot be classified as equilibrium or nonequilibrium but fall somewhere between the two extremes.

The Flux-Generating Reaction Is the First Reaction in a Pathway That Is Saturated With Substrate

It may be identified as a nonequilibrium reaction in which the K_m of the enzyme is considerably lower than the normal substrate concentration. The first reaction in glycolysis, catalyzed by **hexokinase** (Figure 17–2), is such a flux-generating step because its K_m for glucose of 0.05 mmol/L is well below the normal blood glucose concentration of 5 mmol/L.

ALLOSTERIC & HORMONAL MECHANISMS ARE IMPORTANT IN THE METABOLIC CONTROL OF ENZYME-CATALYZED REACTIONS

A hypothetical metabolic pathway is shown in Figure 15–8, in which reactions A ↔ B and C ↔ D are equilibrium reactions and B → C is a nonequilibrium reaction. The flux through such a pathway can be regulated by the availability of substrate A. This depends on its supply from the blood, which in turn depends on either food intake or key reactions that maintain and release substrates from tissue reserves to the blood, eg, the glycogen phosphorylase in liver (Figure 18–1) and hormone-sensitive lipase in adipose tissue (Figure 25–7). The flux also depends on the transport of substrate A across the cell membrane. Flux is also determined by the removal of the end product D and the availability of cosubstrate or cofactors represented by X and Y. Enzymes catalyzing nonequilibrium reactions are often allosteric proteins subject to the rapid actions of "feedback" or "feed-forward" control by **allosteric modifiers** in immediate response to the needs of the cell (Chapter 9). Frequently, the product of a biosynthetic pathway will inhibit the enzyme catalyzing the first reaction in the pathway. Other control mechanisms depend on the action of **hormones** responding to the needs of the body as a whole; they may act rapidly, by altering the activity of existing enzyme molecules, or slowly, by altering the rate of enzyme synthesis.

SUMMARY

- The products of digestion provide the tissues with the building blocks for the biosynthesis of complex molecules and also with the fuel to power the living processes.
- Nearly all products of digestion of carbohydrate, fat, and protein are metabolized to a common metabolite, acetyl-CoA, before final oxidation to CO_2 in the citric acid cycle.
- Acetyl-CoA is also used as the precursor for biosynthesis of long-chain fatty acids; steroids, including cholesterol; and ketone bodies.
- Glucose provides carbon skeletons for the glycerol moiety of fat and of several nonessential amino acids.
- Water-soluble products of digestion are transported directly to the liver via the hepatic portal vein. The liver regulates the blood concentrations of glucose and amino acids.
- Pathways are compartmentalized within the cell. Glycolysis, glycogenesis, glycogenolysis, the pentose phosphate pathway, and lipogenesis occur in the cytosol. The mitochondrion contains the enzymes of the citric acid cycle, β-oxidation of fatty acids, and of oxidative phosphorylation. The endoplasmic reticulum also contains the enzymes for many other processes, including protein synthesis, glycerolipid formation, and drug metabolism.
- Metabolic pathways are regulated by rapid mechanisms affecting the activity of existing enzymes, eg, allosteric and covalent modification (often in response to hormone action); and slow mechanisms affecting the synthesis of enzymes.

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The Citric Acid Cycle: The Catabolism of Acetyl-CoA

16

Peter A. Mayes, PhD, DSc, & David A. Bender, PhD

BIOMEDICAL IMPORTANCE

The citric acid cycle (Krebs cycle, tricarboxylic acid cycle) is a series of reactions in mitochondria that oxidize acetyl residues (as acetyl-CoA) and reduce coenzymes that upon reoxidation are linked to the formation of ATP.

The citric acid cycle is the final common pathway for the aerobic oxidation of carbohydrate, lipid, and protein because glucose, fatty acids, and most amino acids are metabolized to acetyl-CoA or intermediates of the cycle. It also has a central role in gluconeogenesis, lipogenesis, and interconversion of amino acids. Many of these processes occur in most tissues, but the liver is the only tissue in which all occur to a significant extent. The repercussions are therefore profound when, for example, large numbers of hepatic cells are damaged as in acute **hepatitis** or replaced by connective tissue (as in **cirrhosis**). Very few, if any, genetic abnormalities of citric acid cycle enzymes have been reported; such abnormalities would be incompatible with life or normal development.

THE CITRIC ACID CYCLE PROVIDES SUBSTRATE FOR THE RESPIRATORY CHAIN

The cycle starts with reaction between the acetyl moiety of acetyl-CoA and the four-carbon dicarboxylic acid oxaloacetate, forming a six-carbon tricarboxylic acid, citrate. In the subsequent reactions, two molecules of CO_2 are released and oxaloacetate is regenerated (Figure 16–1). Only a small quantity of oxaloacetate is needed for the oxidation of a large quantity of acetyl-CoA; oxaloacetate may be considered to play a **catalytic role.**

The citric acid cycle is an integral part of the process by which much of the free energy liberated during the oxidation of fuels is made available. During oxidation of acetyl-CoA, coenzymes are reduced and subsequently reoxidized in the respiratory chain, linked to the formation of ATP (oxidative phosphorylation; see Figure 16–2 and also Chapter 12). This process is **aerobic,** requiring oxygen as the final oxidant of the reduced coenzymes. The enzymes of the citric acid cycle are located in the **mitochondrial matrix,** either free or attached to the inner mitochondrial membrane, where the enzymes of the respiratory chain are also found.

REACTIONS OF THE CITRIC ACID CYCLE LIBERATE REDUCING EQUIVALENTS & CO_2 (Figure 16–3)*

The initial reaction between acetyl-CoA and oxaloacetate to form citrate is catalyzed by **citrate synthase** which forms a carbon-carbon bond between the methyl carbon of acetyl-CoA and the carbonyl carbon of oxaloacetate. The thioester bond of the resultant citryl-CoA is hydrolyzed, releasing citrate and CoASH—an exergonic reaction.

Citrate is isomerized to isocitrate by the enzyme **aconitase** (aconitate hydratase); the reaction occurs in two steps: dehydration to *cis*-aconitate, some of which remains bound to the enzyme; and rehydration to isocitrate. Although citrate is a symmetric molecule, aconitase reacts with citrate asymmetrically, so that the two carbon atoms that are lost in subsequent reactions of the cycle are not those that were added from acetyl-CoA. This asymmetric behavior is due to **channeling**—transfer of the product of citrate synthase directly onto the active site of aconitase without entering free solution. This provides integration of citric acid cycle activity and the provision of citrate in the cytosol as a source of acetyl-CoA for fatty acid synthesis. The poison **fluoroacetate** is toxic because fluoroacetyl-CoA condenses with oxaloacetate to form fluorocitrate, which inhibits aconitase, causing citrate to accumulate.

Isocitrate undergoes dehydrogenation catalyzed by **isocitrate dehydrogenase** to form, initially, oxalosuccinate, which remains enzyme-bound and undergoes decarboxylation to α-ketoglutarate. The decarboxylation

*From Circular No. 200 of the Committee of Editors of Biochemical Journals Recommendations (1975): "According to standard biochemical convention, the ending *ate* in, eg, palmitate, denotes any mixture of free acid and the ionized form(s) (according to pH) in which the cations are not specified." The same convention is adopted in this text for all carboxylic acids.

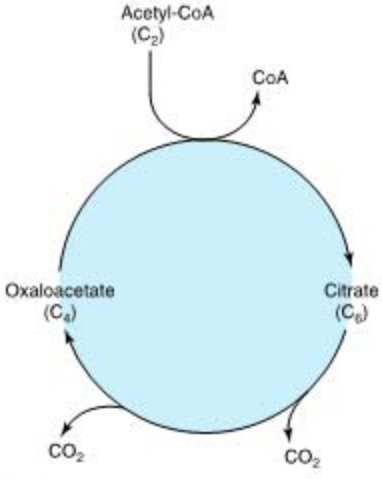

Figure 16–1. Citric acid cycle, illustrating the catalytic role of oxaloacetate.

requires Mg^{2+} or Mn^{2+} ions. There are three isoenzymes of isocitrate dehydrogenase. One, which uses NAD^+, is found only in mitochondria. The other two use $NADP^+$ and are found in mitochondria and the cytosol. Respiratory chain-linked oxidation of isocitrate proceeds almost completely through the NAD^+-dependent enzyme.

α-Ketoglutarate undergoes **oxidative decarboxylation** in a reaction catalyzed by a multi-enzyme complex similar to that involved in the oxidative decarboxylation of pyruvate (Figure 17–5). The **α-ketoglutarate dehydrogenase complex** requires the same cofactors as the pyruvate dehydrogenase complex—thiamin diphosphate, lipoate, NAD^+, FAD, and CoA—and results in the formation of succinyl-CoA. The equilibrium of this reaction is so much in favor of succinyl-CoA formation that it must be considered physiologically unidirectional. As in the case of pyruvate oxidation (Chapter 17), arsenite inhibits the reaction, causing the substrate, **α-ketoglutarate,** to accumulate.

Succinyl-CoA is converted to succinate by the enzyme **succinate thiokinase (succinyl-CoA synthetase).** This is the only example in the citric acid cycle of substrate-level phosphorylation. Tissues in which gluconeogenesis occurs (the liver and kidney) contain two isoenzymes of succinate thiokinase, one specific for GDP and the other for ADP. The GTP formed is used for the decarboxylation of oxaloacetate to phosphoenolpyruvate in gluconeogenesis and provides a regulatory link between citric acid cycle activity and the withdrawal of oxaloacetate for gluconeogenesis. Nongluconeogenic tissues have only the isoenzyme that uses ADP.

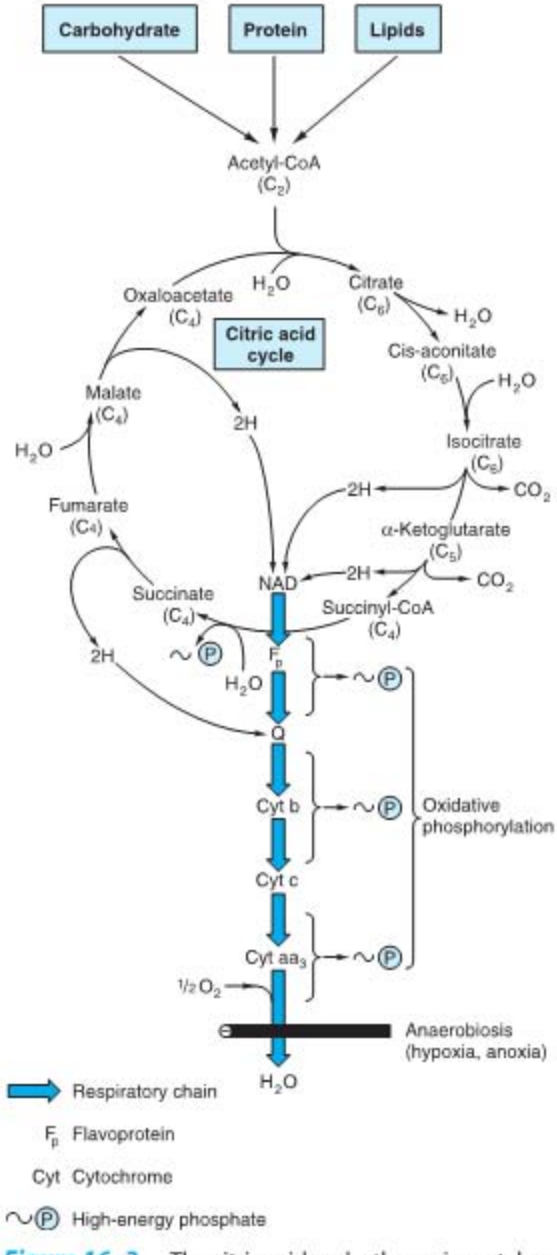

Figure 16–2. The citric acid cycle: the major catabolic pathway for acetyl-CoA in aerobic organisms. Acetyl-CoA, the product of carbohydrate, protein, and lipid catabolism, is taken into the cycle, together with H_2O, and oxidized to CO_2 with the release of reducing equivalents (2H). Subsequent oxidation of 2H in the respiratory chain leads to coupled phosphorylation of ADP to ATP. For one turn of the cycle, 11~Ⓟ are generated via oxidative phosphorylation and one ~Ⓟ arises at substrate level from the conversion of succinyl-CoA to succinate.

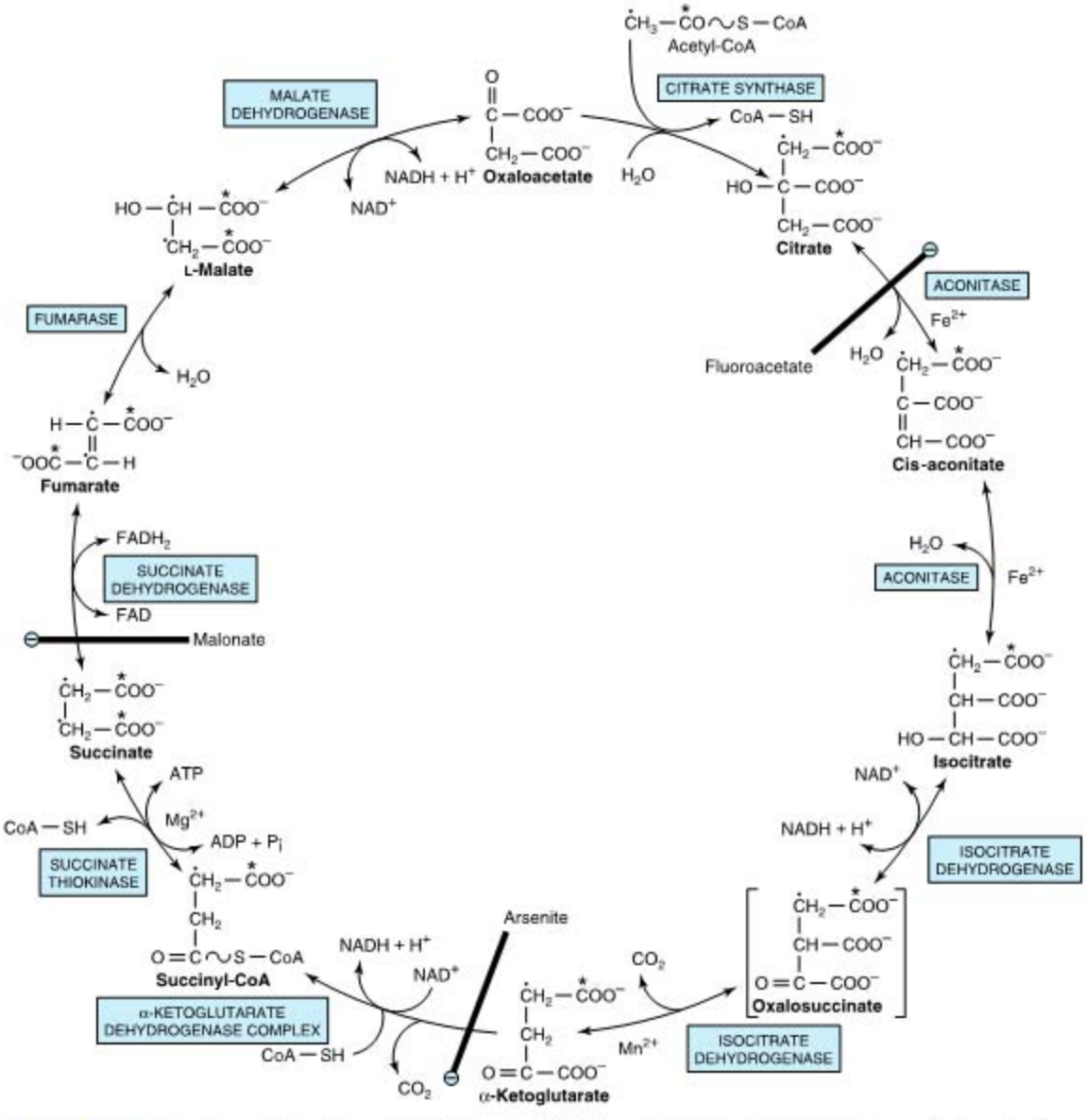

Figure 16–3. Reactions of the citric acid (Krebs) cycle. Oxidation of NADH and $FADH_2$ in the respiratory chain leads to the generation of ATP via oxidative phosphorylation. In order to follow the passage of acetyl-CoA through the cycle, the two carbon atoms of the acetyl radical are shown labeled on the carboxyl carbon (designated by asterisk) and on the methyl carbon (using the designation ˙). Although two carbon atoms are lost as CO_2 in one revolution of the cycle, these atoms are not derived from the acetyl-CoA that has immediately entered the cycle but from that portion of the citrate molecule that was derived from oxaloacetate. However, on completion of a single turn of the cycle, the oxaloacetate that is regenerated is now labeled, which leads to labeled CO_2 being evolved during the second turn of the cycle. Because succinate is a symmetric compound and because succinate dehydrogenase does not differentiate between its two carboxyl groups, "randomization" of label occurs at this step such that all four carbon atoms of oxaloacetate appear to be labeled after one turn of the cycle. During gluconeogenesis, some of the label in oxaloacetate is incorporated into glucose and glycogen (Figure 19–1). For a discussion of the stereochemical aspects of the citric acid cycle, see Greville (1968). The sites of inhibition (⊖) by fluoroacetate, malonate, and arsenite are indicated.

When ketone bodies are being metabolized in extrahepatic tissues there is an alternative reaction catalyzed by **succinyl-CoA–acetoacetate-CoA transferase (thiophorase)**—involving transfer of CoA from succinyl-CoA to acetoacetate, forming acetoacetyl-CoA (Chapter 22).

The onward metabolism of succinate, leading to the regeneration of oxaloacetate, is the same sequence of chemical reactions as occurs in the β-oxidation of fatty acids: dehydrogenation to form a carbon-carbon double bond, addition of water to form a hydroxyl group, and a further dehydrogenation to yield the oxo- group of oxaloacetate.

The first dehydrogenation reaction, forming fumarate, is catalyzed by **succinate dehydrogenase,** which is bound to the inner surface of the inner mitochondrial membrane. The enzyme contains FAD and iron-sulfur (Fe:S) protein and directly reduces ubiquinone in the respiratory chain. **Fumarase (fumarate hydratase)** catalyzes the addition of water across the double bond of fumarate, yielding malate. Malate is converted to oxaloacetate by **malate dehydrogenase,** a reaction requiring NAD^+. Although the equilibrium of this reaction strongly favors malate, the net flux is toward the direction of oxaloacetate because of the continual removal of oxaloacetate (either to form citrate, as a substrate for gluconeogenesis, or to undergo transamination to aspartate) and also because of the continual reoxidation of NADH.

TWELVE ATP ARE FORMED PER TURN OF THE CITRIC ACID CYCLE

As a result of oxidations catalyzed by the dehydrogenases of the citric acid cycle, three molecules of NADH and one of $FADH_2$ are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle. These reducing equivalents are transferred to the respiratory chain (Figure 16–2), where reoxidation of each NADH results in formation of 3 ATP and reoxidation of $FADH_2$ in formation of 2 ATP. In addition, 1 ATP (or GTP) is formed by substrate-level phosphorylation catalyzed by succinate thiokinase.

VITAMINS PLAY KEY ROLES IN THE CITRIC ACID CYCLE

Four of the B vitamins are essential in the citric acid cycle and therefore in energy-yielding metabolism: (1) **riboflavin,** in the form of flavin adenine dinucleotide (FAD), a cofactor in the α-ketoglutarate dehydrogenase complex and in succinate dehydrogenase; (2) **niacin,** in the form of nicotinamide adenine dinucleotide (NAD), the coenzyme for three dehydrogenases in the cycle—isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase; (3) **thiamin (vitamin B_1),** as thiamin diphosphate, the coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction; and (4) **pantothenic acid,** as part of coenzyme A, the cofactor attached to "active" carboxylic acid residues such as acetyl-CoA and succinyl-CoA.

THE CITRIC ACID CYCLE PLAYS A PIVOTAL ROLE IN METABOLISM

The citric acid cycle is not only a pathway for oxidation of two-carbon units—it is also a major pathway for interconversion of metabolites arising from **transamination** and **deamination** of amino acids. It also provides the substrates for **amino acid synthesis** by transamination, as well as for **gluconeogenesis** and **fatty acid synthesis**. Because it functions in both oxidative and synthetic processes, it is **amphibolic** (Figure 16–4).

The Citric Acid Cycle Takes Part in Gluconeogenesis, Transamination, & Deamination

All the intermediates of the cycle are potentially glucogenic, since they can give rise to oxaloacetate and thus net production of glucose (in the liver and kidney, the organs that carry out gluconeogenesis; see Chapter 19). The key enzyme that catalyzes net transfer out of the cycle into gluconeogenesis is **phosphoenolpyruvate carboxykinase,** which decarboxylates oxaloacetate to phosphoenolpyruvate, with GTP acting as the donor phosphate (Figure 16–4).

Net transfer into the cycle occurs as a result of several different reactions. Among the most important of such **anaplerotic reactions** is the formation of oxaloacetate by the carboxylation of pyruvate, catalyzed by **pyruvate carboxylase.** This reaction is important in maintaining an adequate concentration of oxaloacetate for the condensation reaction with acetyl-CoA. If acetyl-CoA accumulates, it acts both as an allosteric activator of pyruvate carboxylase and as an inhibitor of pyruvate dehydrogenase, thereby ensuring a supply of oxaloacetate. Lactate, an important substrate for gluconeogenesis, enters the cycle via oxidation to pyruvate and then carboxylation to oxaloacetate.

Aminotransferase (transaminase) reactions form pyruvate from alanine, oxaloacetate from aspartate, and α-ketoglutarate from glutamate. Because these reactions are reversible, the cycle also serves as a source of carbon skeletons for the synthesis of these amino acids. Other amino acids contribute to gluconeogenesis because their carbon skeletons give rise to citric acid cycle

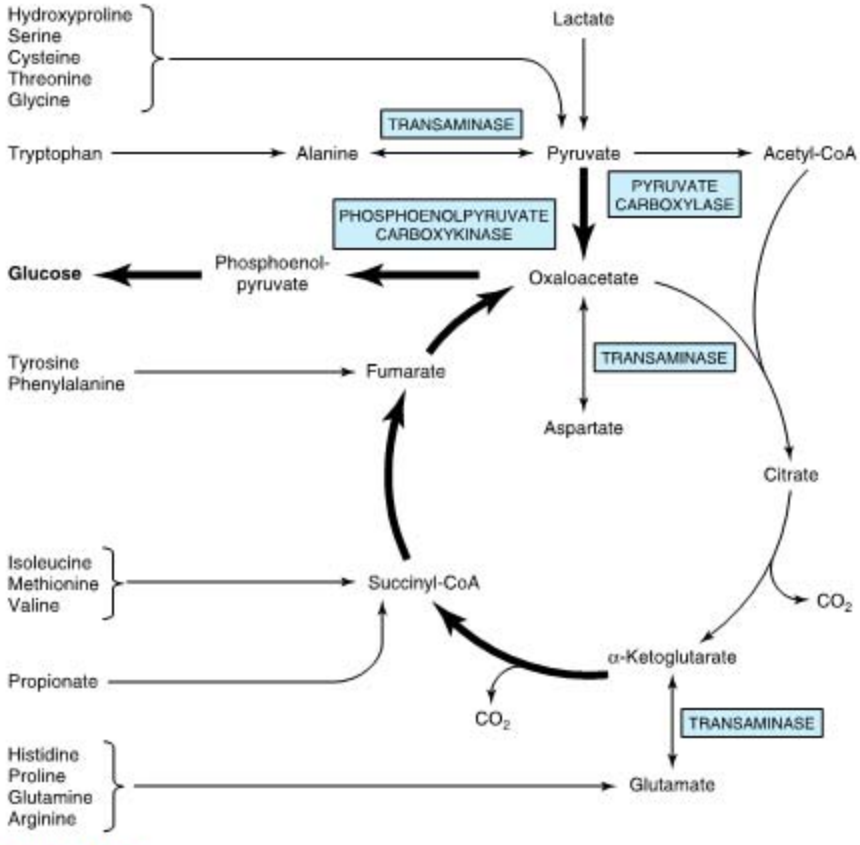

Figure 16–4. Involvement of the citric acid cycle in transamination and gluconeogenesis. The bold arrows indicate the main pathway of gluconeogenesis.

intermediates. Alanine, cysteine, glycine, hydroxyproline, serine, threonine, and tryptophan yield pyruvate; arginine, histidine, glutamine, and proline yield α-ketoglutarate; isoleucine, methionine, and valine yield succinyl-CoA; and tyrosine and phenylalanine yield fumarate (Figure 16–4).

In ruminants, whose main metabolic fuel is short-chain fatty acids formed by bacterial fermentation, the conversion of propionate, the major glucogenic product of rumen fermentation, to succinyl-CoA via the methylmalonyl-CoA pathway (Figure 19–2) is especially important.

The Citric Acid Cycle Takes Part in Fatty Acid Synthesis (Figure 16–5)

Acetyl-CoA, formed from pyruvate by the action of pyruvate dehydrogenase, is the major building block for long-chain fatty acid synthesis in nonruminants. (In ruminants, acetyl-CoA is derived directly from acetate.) Pyruvate dehydrogenase is a mitochondrial enzyme, and fatty acid synthesis is a cytosolic pathway, but the mitochondrial membrane is impermeable to acetyl-CoA. Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol and cleaved in a reaction catalyzed by **ATP-citrate lyase.**

Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors

In most tissues, where the primary role of the citric acid cycle is in energy-yielding metabolism, **respiratory control** via the respiratory chain and oxidative phosphorylation regulates citric acid cycle activity (Chapter 14). Thus, activity is immediately dependent on the supply of NAD^+, which in turn, because of the tight coupling between oxidation and phosphorylation, is dependent on the availability of ADP and hence, ulti-

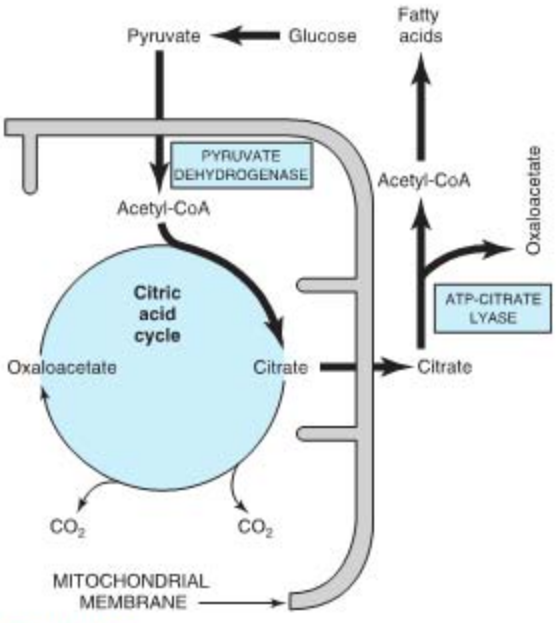

Figure 16–5. Participation of the citric acid cycle in fatty acid synthesis from glucose. See also Figure 21–5.

mately, on the rate of utilization of ATP in chemical and physical work. In addition, individual enzymes of the cycle are regulated. The most likely sites for regulation are the nonequilibrium reactions catalyzed by pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The dehydrogenases are activated by Ca^{2+}, which increases in concentration during muscular contraction and secretion, when there is increased energy demand. In a tissue such as brain, which is largely dependent on carbohydrate to supply acetyl-CoA, control of the citric acid cycle may occur at pyruvate dehydrogenase. Several enzymes are responsive to the energy status, as shown by the [ATP]/[ADP] and [NADH]/[NAD^+] ratios. Thus, there is allosteric inhibition of citrate synthase by ATP and long-chain fatty acyl-CoA. Allosteric activation of mitochondrial NAD-dependent isocitrate dehydrogenase by ADP is counteracted by ATP and NADH. The α-ketoglutarate dehydrogenase complex is regulated in the same way as is pyruvate dehydrogenase (Figure 17–6). Succinate dehydrogenase is inhibited by oxaloacetate, and the availability of oxaloacetate, as controlled by malate dehydrogenase, depends on the [NADH]/[NAD^+] ratio. Since the K_m for oxaloacetate of citrate synthase is of the same order of magnitude as the intramitochondrial concentration, it is likely that the concentration of oxaloacetate controls the rate of citrate formation. Which of these mechanisms are important in vivo has still to be resolved.

SUMMARY

- The citric acid cycle is the final pathway for the oxidation of carbohydrate, lipid, and protein whose common end-metabolite, acetyl-CoA, reacts with oxaloacetate to form citrate. By a series of dehydrogenations and decarboxylations, citrate is degraded, releasing reduced coenzymes and $2CO_2$ and regenerating oxaloacetate.
- The reduced coenzymes are oxidized by the respiratory chain linked to formation of ATP. Thus, the cycle is the major route for the generation of ATP and is located in the matrix of mitochondria adjacent to the enzymes of the respiratory chain and oxidative phosphorylation.
- The citric acid cycle is amphibolic, since in addition to oxidation it is important in the provision of carbon skeletons for gluconeogenesis, fatty acid synthesis, and interconversion of amino acids.

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Glycolysis & the Oxidation of Pyruvate

17

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BIOMEDICAL IMPORTANCE

Most tissues have at least some requirement for glucose. In brain, the requirement is substantial. Glycolysis, the major pathway for glucose metabolism, occurs in the cytosol of all cells. It is unique in that it can function either aerobically or anaerobically. Erythrocytes, which lack mitochondria, are completely reliant on glucose as their metabolic fuel and metabolize it by anaerobic glycolysis. However, to oxidize glucose beyond pyruvate (the end product of glycolysis) requires both oxygen and mitochondrial enzyme systems such as the pyruvate dehydrogenase complex, the citric acid cycle, and the respiratory chain.

Glycolysis is both the principal route for glucose metabolism and the main pathway for the metabolism of fructose, galactose, and other carbohydrates derived from the diet. The ability of glycolysis to provide ATP in the absence of oxygen is especially important because it allows skeletal muscle to perform at very high levels when oxygen supply is insufficient and because it allows tissues to survive anoxic episodes. However, heart muscle, which is adapted for aerobic performance, has relatively low glycolytic activity and poor survival under conditions of **ischemia.** Diseases in which enzymes of glycolysis (eg, pyruvate kinase) are deficient are mainly seen as **hemolytic anemias** or, if the defect affects skeletal muscle (eg, phosphofructokinase), as **fatigue.** In fast-growing cancer cells, glycolysis proceeds at a higher rate than is required by the citric acid cycle, forming large amounts of pyruvate, which is reduced to lactate and exported. This produces a relatively acidic local environment in the tumor which may have implications for cancer therapy. The lactate is used for gluconeogenesis in the liver, an energy-expensive process responsible for much of the **hypermetabolism** seen in **cancer cachexia. Lactic acidosis** results from several causes, including impaired activity of pyruvate dehydrogenase.

GLYCOLYSIS CAN FUNCTION UNDER ANAEROBIC CONDITIONS

When a muscle contracts in an anaerobic medium, ie, one from which oxygen is excluded, **glycogen disappears** and **lactate appears** as the principal end product. When oxygen is admitted, aerobic recovery takes place and lactate disappears. However, if contraction occurs under aerobic conditions, lactate does not accumulate and pyruvate is the major end product of glycolysis. Pyruvate is oxidized further to CO_2 and water (Figure 17–1). When oxygen is in short supply, mitochondrial reoxidation of NADH formed from NAD^+ during glycolysis is impaired, and NADH is reoxidized by reducing pyruvate to lactate, so permitting glycolysis to proceed (Figure 17–1). While glycolysis can occur under anaerobic conditions, this has a price, for it limits the amount of ATP formed per mole of glucose oxidized, so that much more glucose must be metabolized under anaerobic than under aerobic conditions.

THE REACTIONS OF GLYCOLYSIS CONSTITUTE THE MAIN PATHWAY OF GLUCOSE UTILIZATION

The overall equation for glycolysis from glucose to lactate is as follows:

$$\text{Glucose} + 2\text{ADP} + 2P_i \rightarrow 2\text{L(+)} - \text{Lactate} + 2\text{ATP} + 2H_2O$$

All of the enzymes of glycolysis (Figure 17–2) are found in the cytosol. Glucose enters glycolysis by phosphorylation to glucose 6-phosphate, catalyzed by **hexokinase,** using ATP as the phosphate donor. Under physiologic conditions, the phosphorylation of glucose to glucose 6-phosphate can be regarded as irreversible. Hexokinase is inhibited allosterically by its product, glucose 6-phosphate. In tissues other than the liver and pancreatic B islet cells, the availability of glucose for

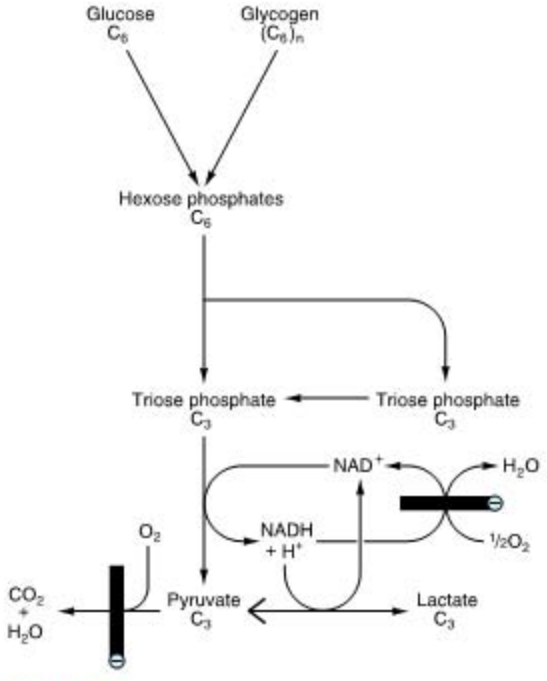

Figure 17–1. Summary of glycolysis. ⊖, blocked by anaerobic conditions or by absence of mitochondria containing key respiratory enzymes, eg, as in erythrocytes.

glycolysis (or glycogen synthesis in muscle and lipogenesis in adipose tissue) is controlled by transport into the cell, which in turn is regulated by **insulin.** Hexokinase has a high affinity (low K_m) for its substrate, glucose, and in the liver and pancreatic B islet cells is saturated under all normal conditions and so acts at a constant rate to provide glucose 6-phosphate to meet the cell's need. Liver and pancreatic B islet cells also contain an isoenzyme of hexokinase, **glucokinase,** which has a K_m very much higher than the normal intracellular concentration of glucose. The function of glucokinase in the liver is to remove glucose from the blood following a meal, providing glucose 6-phosphate in excess of requirements for glycolysis, which will be used for glycogen synthesis and lipogenesis. In the pancreas, the glucose 6-phosphate formed by glucokinase signals increased glucose availability and leads to the secretion of insulin.

Glucose 6-phosphate is an important compound at the junction of several metabolic pathways (glycolysis, gluconeogenesis, the pentose phosphate pathway, glycogenesis, and glycogenolysis). In glycolysis, it is converted to fructose 6-phosphate by **phosphohexose-isomerase,** which involves an aldose-ketose isomerization. This reaction is followed by another phosphorylation with ATP catalyzed by the enzyme **phosphofructokinase (phosphofructokinase-1),** forming fructose 1,6-bisphosphate. The phosphofructokinase reaction may be considered to be functionally irreversible under physiologic conditions; it is both inducible and subject to allosteric regulation and has a major role in regulating the rate of glycolysis. Fructose 1,6-bisphosphate is cleaved by **aldolase** (fructose 1,6-bisphosphate aldolase) into two triose phosphates, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are interconverted by the enzyme **phosphotriose isomerase.**

Glycolysis continues with the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. The enzyme catalyzing this oxidation, **glyceraldehyde 3-phosphate dehydrogenase,** is NAD-dependent. Structurally, it consists of four identical polypeptides (monomers) forming a tetramer. —SH groups are present on each polypeptide, derived from cysteine residues within the polypeptide chain. One of the —SH groups at the active site of the enzyme (Figure 17–3) combines with the substrate forming a thiohemiacetal that is oxidized to a thiol ester; the hydrogens removed in this oxidation are transferred to NAD^+. The thiol ester then undergoes phosphorolysis; inorganic phosphate (P_i) is added, forming 1,3-bisphosphoglycerate, and the —SH group is reconstituted.

In the next reaction, catalyzed by **phosphoglycerate kinase,** phosphate is transferred from 1,3-bisphosphoglycerate onto ADP, forming ATP (substrate-level phosphorylation) and 3-phosphoglycerate. Since two molecules of triose phosphate are formed per molecule of glucose, two molecules of ATP are generated at this stage per molecule of glucose undergoing glycolysis. The toxicity of arsenic is due to competition of arsenate with inorganic phosphate (P_i) in the above reactions to give 1-arseno-3-phosphoglycerate, which hydrolyzes spontaneously to give 3-phosphoglycerate plus heat, without generating ATP. 3-Phosphoglycerate is isomerized to 2-phosphoglycerate by **phosphoglycerate mutase.** It is likely that 2,3-bisphosphoglycerate (diphosphoglycerate; DPG) is an intermediate in this reaction.

The subsequent step is catalyzed by **enolase** and involves a dehydration, forming phosphoenolpyruvate. Enolase is inhibited by **fluoride.** To prevent glycolysis in the estimation of glucose, blood is collected in tubes containing fluoride. The enzyme is also dependent on the presence of either Mg^{2+} or Mn^{2+}. The phosphate of phosphoenolpyruvate is transferred to ADP by **pyruvate kinase** to generate, at this stage, two molecules of ATP per molecule of glucose oxidized. The product of the enzyme-catalyzed reaction, enolpyruvate, undergoes spontaneous (nonenzymic) isomerization to pyruvate and so is not available to

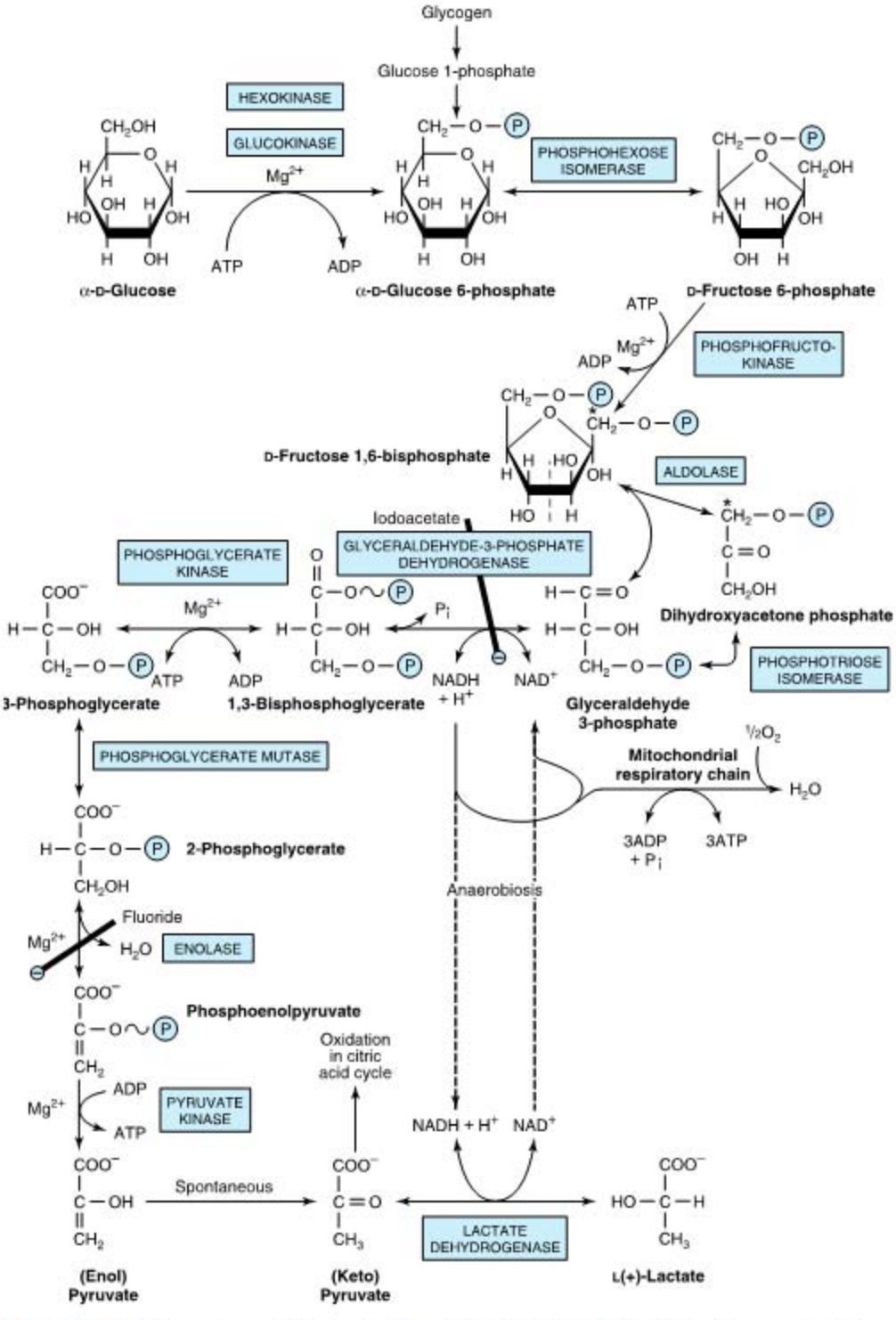

Figure 17–2. The pathway of glycolysis. (Ⓟ, —PO_3^{2-}; P_i, $HOPO_3^{2-}$; ⊖, inhibition.) At asterisk: Carbon atoms 1–3 of fructose bisphosphate form dihydroxyacetone phosphate, whereas carbons 4–6 form glyceraldehyde 3-phosphate. The term "bis-," as in bisphosphate, indicates that the phosphate groups are separated, whereas diphosphate, as in adenosine diphosphate, indicates that they are joined.

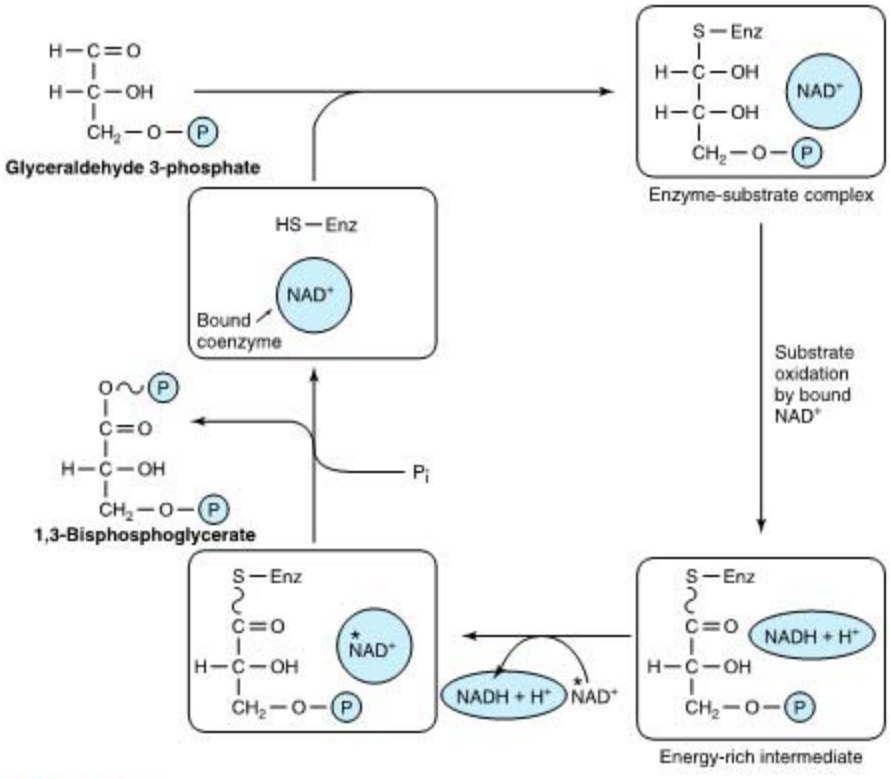

Figure 17–3. Mechanism of oxidation of glyceraldehyde 3-phosphate. (Enz, glyceraldehyde-3-phosphate dehydrogenase.) The enzyme is inhibited by the —SH poison iodoacetate, which is thus able to inhibit glycolysis. The NADH produced on the enzyme is not as firmly bound to the enzyme as is NAD^+. Consequently, NADH is easily displaced by another molecule of NAD^+.

undergo the reverse reaction. The pyruvate kinase reaction is thus also irreversible under physiologic conditions.

The redox state of the tissue now determines which of two pathways is followed. Under **anaerobic** conditions, the reoxidation of NADH through the respiratory chain to oxygen is prevented. Pyruvate is reduced by the NADH to lactate, the reaction being catalyzed by **lactate dehydrogenase.** Several tissue-specific isoenzymes of this enzyme have been described and have clinical significance (Chapter 7). The reoxidation of NADH via lactate formation allows glycolysis to proceed in the absence of oxygen by regenerating sufficient NAD^+ for another cycle of the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase. Under **aerobic conditions,** pyruvate is taken up into mitochondria and after conversion to acetyl-CoA is oxidized to CO_2 by the citric acid cycle. The reducing equivalents from the NADH + H^+ formed in glycolysis are taken up into mitochondria for oxidation via one of the two shuttles described in Chapter 12.

Tissues That Function Under Hypoxic Circumstances Tend to Produce Lactate (Figure 17–2)

This is true of skeletal muscle, particularly the white fibers, where the rate of work output—and therefore the need for ATP formation—may exceed the rate at which oxygen can be taken up and utilized. Glycolysis in erythrocytes, even under aerobic conditions, always terminates in lactate, because the subsequent reactions of pyruvate are mitochondrial, and erythrocytes lack mitochondria. Other tissues that normally derive much of their energy from glycolysis and produce lactate include brain, gastrointestinal tract, renal medulla, retina, and skin. The liver, kidneys, and heart usually take up

lactate and oxidize it but will produce it under hypoxic conditions.

Glycolysis Is Regulated at Three Steps Involving Nonequilibrium Reactions

Although most of the reactions of glycolysis are reversible, three are markedly exergonic and must therefore be considered physiologically irreversible. These reactions, catalyzed by **hexokinase** (and glucokinase), **phosphofructokinase,** and **pyruvate kinase,** are the major sites of regulation of glycolysis. Cells that are capable of reversing the glycolytic pathway **(gluconeogenesis)** have different enzymes that catalyze reactions which effectively reverse these irreversible reactions. The importance of these steps in the regulation of glycolysis and gluconeogenesis is discussed in Chapter 19.

In Erythrocytes, the First Site in Glycolysis for ATP Generation May Be Bypassed

In the erythrocytes of many mammals, the reaction catalyzed by **phosphoglycerate kinase** may be bypassed by a process that effectively dissipates as heat the free energy associated with the high-energy phosphate of 1,3-bisphosphoglycerate (Figure 17–4). **Bisphosphoglycerate mutase** catalyzes the conversion of 1,3-bisphosphoglycerate to 2,3-bisphosphoglycerate, which is converted to 3-phosphoglycerate by **2,3-bisphosphoglycerate phosphatase** (and possibly also phosphoglycerate mutase). This alternative pathway involves no net yield of ATP from glycolysis. However, it does serve to provide 2,3-bisphosphoglycerate, which binds to hemoglobin, decreasing its affinity for oxygen and so making oxygen more readily available to tissues (see Chapter 6).

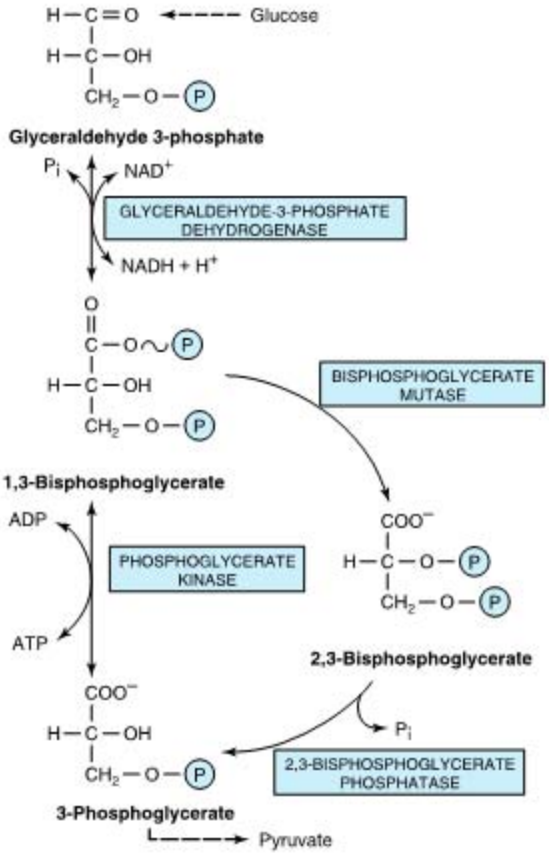

Figure 17–4. 2,3-Bisphosphoglycerate pathway in erythrocytes.

THE OXIDATION OF PYRUVATE TO ACETYL-CoA IS THE IRREVERSIBLE ROUTE FROM GLYCOLYSIS TO THE CITRIC ACID CYCLE

Pyruvate, formed in the cytosol, is transported into the mitochondrion by a proton symporter (Figure 12–10). Inside the mitochondrion, pyruvate is oxidatively decarboxylated to acetyl-CoA by a multienzyme complex that is associated with the inner mitochondrial membrane. This **pyruvate dehydrogenase complex** is analogous to the α-ketoglutarate dehydrogenase complex of the citric acid cycle (Figure 16–3). Pyruvate is decarboxylated by the **pyruvate dehydrogenase** component of the enzyme complex to a hydroxyethyl derivative of the thiazole ring of enzyme-bound **thiamin diphosphate,** which in turn reacts with oxidized lipoamide, the prosthetic group of **dihydrolipoyl transacetylase,** to form acetyl lipoamide (Figure 17–5). Thiamin is vitamin B_1 (Chapter 45), and in thiamin deficiency glucose metabolism is impaired and there is significant (and potentially life-threatening) lactic and pyruvic acidosis. Acetyl lipoamide reacts with coenzyme A to form acetyl-CoA and reduced lipoamide. The cycle of reaction is completed when the reduced lipoamide is reoxidized by a flavoprotein, **dihydrolipoyl dehydrogenase,** containing FAD. Finally, the reduced flavoprotein is oxidized by NAD^+, which in turn transfers reducing equivalents to the respiratory chain.

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{Acetyl-CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2$$

The pyruvate dehydrogenase complex consists of a number of polypeptide chains of each of the three component enzymes, all organized in a regular spatial configuration. Movement of the individual enzymes appears to be restricted, and the metabolic intermediates do not dissociate freely but remain bound to the enzymes. Such a complex of enzymes, in which the sub-

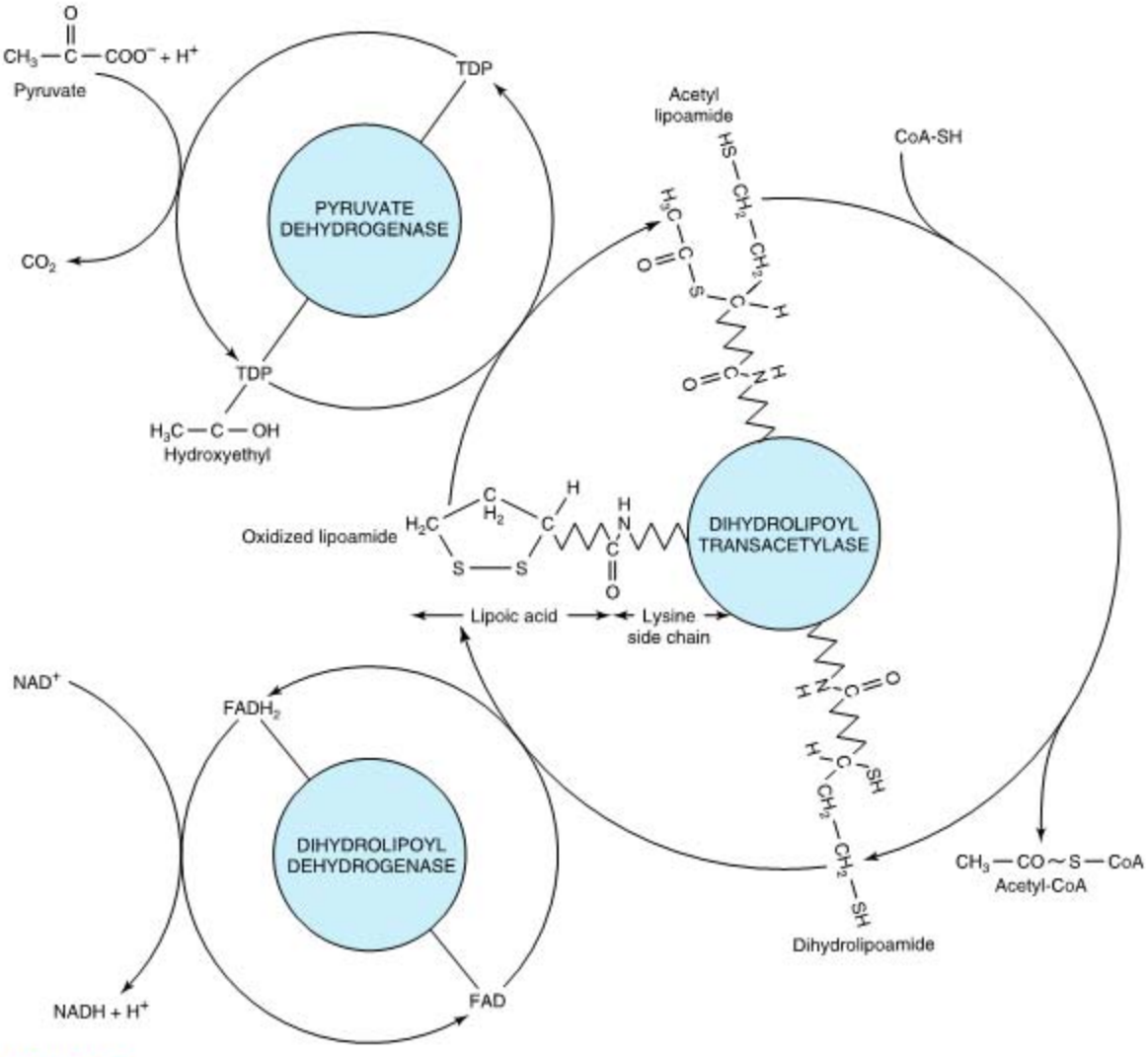

Figure 17–5. Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex. Lipoic acid is joined by an amide link to a lysine residue of the transacetylase component of the enzyme complex. It forms a long flexible arm, allowing the lipoic acid prosthetic group to rotate sequentially between the active sites of each of the enzymes of the complex. (NAD^+, nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide; TDP, thiamin diphosphate.)

strates are handed on from one enzyme to the next, increases the reaction rate and eliminates side reactions, increasing overall efficiency.

Pyruvate Dehydrogenase Is Regulated by End-Product Inhibition & Covalent Modification

Pyruvate dehydrogenase is inhibited by its products, acetyl-CoA and NADH (Figure 17–6). It is also regulated by phosphorylation by a kinase of three serine residues on the pyruvate dehydrogenase component of the multienzyme complex, resulting in decreased activity, and by dephosphorylation by a phosphatase that causes an increase in activity. The kinase is activated by increases in the [ATP]/[ADP], [acetyl-CoA]/[CoA], and [NADH]/[NAD^+] ratios. Thus, pyruvate dehydrogenase—and therefore glycolysis—is inhibited not only by a high-energy potential but also when fatty acids are being oxidized. Thus, in starvation, when free fatty acid

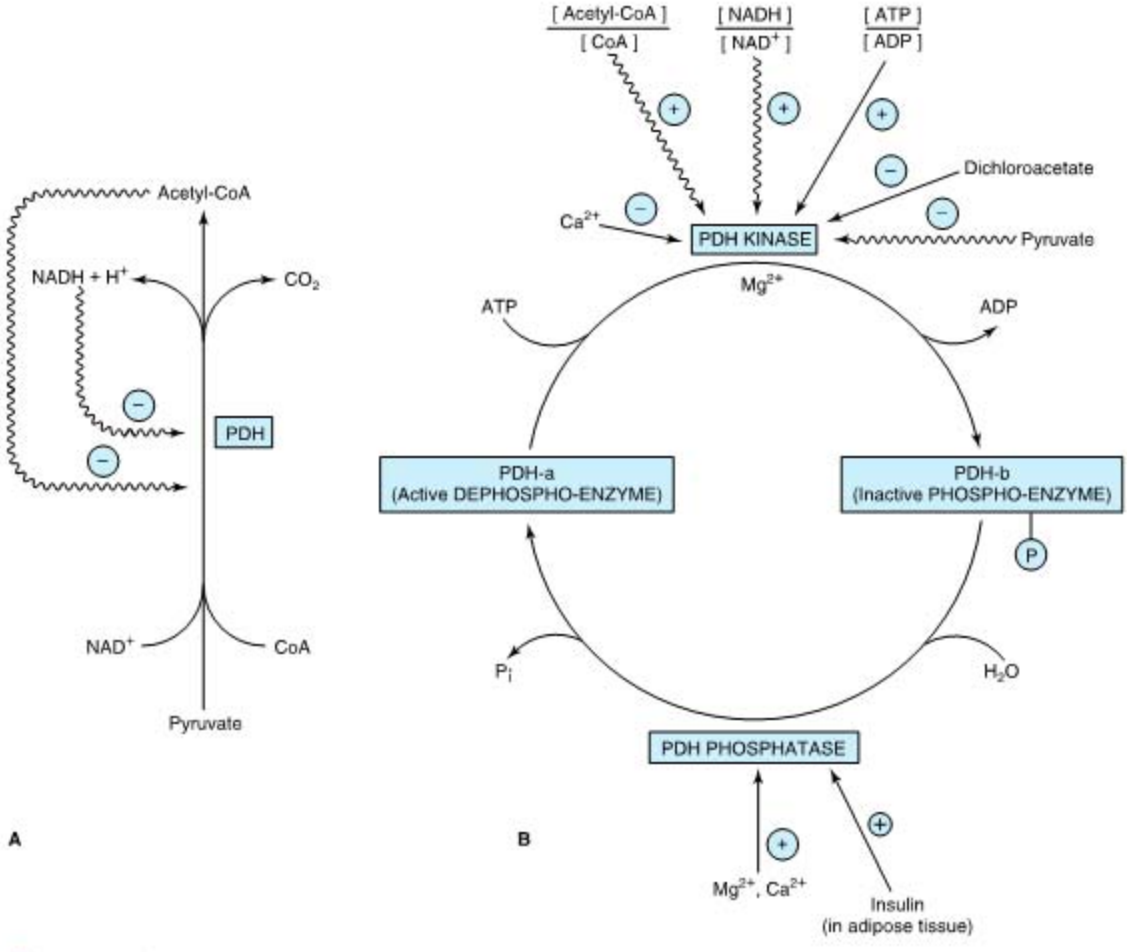

Figure 17–6. Regulation of pyruvate dehydrogenase (PDH). Arrows with wavy shafts indicate allosteric effects. **A:** Regulation by end-product inhibition. **B:** Regulation by interconversion of active and inactive forms.

concentrations increase, there is a decrease in the proportion of the enzyme in the active form, leading to a sparing of carbohydrate. In adipose tissue, where glucose provides acetyl CoA for lipogenesis, the enzyme is activated in response to insulin.

Oxidation of Glucose Yields Up to 38 Mol of ATP Under Aerobic Conditions But Only 2 Mol When O_2 Is Absent

When 1 mol of glucose is combusted in a calorimeter to CO_2 and water, approximately 2870 kJ are liberated as heat. When oxidation occurs in the tissues, approximately 38 mol of ATP are generated per molecule of glucose oxidized to CO_2 and water. In vivo, ΔG for the ATP synthase reaction has been calculated as approximately 51.6 kJ. It follows that the total energy captured in ATP per mole of glucose oxidized is 1961 kJ, or approximately 68% of the energy of combustion. Most of the ATP is formed by oxidative phosphorylation resulting from the reoxidation of reduced coenzymes by the respiratory chain. The remainder is formed by substrate-level phosphorylation (Table 17–1).

CLINICAL ASPECTS

Inhibition of Pyruvate Metabolism Leads to Lactic Acidosis

Arsenite and mercuric ions react with the —SH groups of lipoic acid and inhibit pyruvate dehydrogenase, as

Table 17–1. Generation of high-energy phosphate in the catabolism of glucose.

Pathway	Reaction Catalyzed by	Method of ~Ⓟ Production	Number of ~Ⓟ Formed per Mole of Glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	6*
	Phosphoglycerate kinase	Phosphorylation at substrate level	2
	Pyruvate kinase	Phosphorylation at substrate level	2
			10
	Allow for consumption of ATP by reactions catalyzed by hexokinase and phosphofructokinase		−2
			Net 8
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	α-Ketoglutarate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Succinate thiokinase	Phosphorylation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 $FADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
			Net 30
Total per mole of glucose under aerobic conditions			38
Total per mole of glucose under anaerobic conditions			2

*It is assumed that NADH formed in glycolysis is transported into mitochondria via the malate shuttle (see Figure 12–13). If the glycerophosphate shuttle is used, only 2 ~Ⓟ would be formed per mole of NADH, the total net production being 26 instead of 38. The calculation ignores the small loss of ATP due to a transport of H^+ into the mitochondrion with pyruvate and a similar transport of H^+ in the operation of the malate shuttle, totaling about 1 mol of ATP. Note that there is a substantial benefit under anaerobic conditions if glycogen is the starting point, since the net production of high-energy phosphate in glycolysis is increased from 2 to 3, as ATP is no longer required by the hexokinase reaction.

does a **dietary deficiency of thiamin,** allowing pyruvate to accumulate. Nutritionally deprived alcoholics are thiamin-deficient and may develop potentially fatal pyruvic and lactic acidosis. Patients with **inherited pyruvate dehydrogenase deficiency,** which can be due to defects in one or more of the components of the enzyme complex, also present with lactic acidosis, particularly after a glucose load. Because of its dependence on glucose as a fuel, brain is a prominent tissue where these metabolic defects manifest themselves in neurologic disturbances.

Inherited aldolase A deficiency and pyruvate kinase deficiency in erythrocytes cause **hemolytic anemia.** The exercise capacity of patients with **muscle phosphofructokinase deficiency** is low, particularly on high-carbohydrate diets. By providing an alternative lipid fuel, eg, during starvation, when blood free fatty acids and ketone bodies are increased, work capacity is improved.

SUMMARY

- Glycolysis is the cytosolic pathway of all mammalian cells for the metabolism of glucose (or glycogen) to pyruvate and lactate.
- It can function anaerobically by regenerating oxidized NAD^+ (required in the glyceraldehyde-3-phosphate dehydrogenase reaction) by reducing pyruvate to lactate.
- Lactate is the end product of glycolysis under anaerobic conditions (eg, in exercising muscle) or when the metabolic machinery is absent for the further oxidation of pyruvate (eg, in erythrocytes).
- Glycolysis is regulated by three enzymes catalyzing nonequilibrium reactions: hexokinase, phosphofructokinase, and pyruvate kinase.
- In erythrocytes, the first site in glycolysis for generation of ATP may be bypassed, leading to the formation of 2,3-bisphosphoglycerate, which is important in decreasing the affinity of hemoglobin for O_2.
- Pyruvate is oxidized to acetyl-CoA by a multienzyme complex, pyruvate dehydrogenase, that is dependent on the vitamin cofactor thiamin diphosphate.
- Conditions that involve an inability to metabolize pyruvate frequently lead to lactic acidosis.

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Metabolism of Glycogen

18

Peter A. Mayes, PhD, DSc, & David A. Bender, PhD

BIOMEDICAL IMPORTANCE

Glycogen is the major storage carbohydrate in animals, corresponding to starch in plants; it is a branched polymer of α-D-glucose. It occurs mainly in liver (up to 6%) and muscle, where it rarely exceeds 1%. However, because of its greater mass, muscle contains about three to four times as much glycogen as does liver (Table 18–1).

Muscle glycogen is a readily available source of glucose for glycolysis within the muscle itself. Liver glycogen functions to store and export glucose to maintain **blood glucose** between meals. After 12–18 hours of fasting, the liver glycogen is almost totally depleted. **Glycogen storage diseases** are a group of inherited disorders characterized by deficient mobilization of glycogen or deposition of abnormal forms of glycogen, leading to muscular weakness or even death.

GLYCOGENESIS OCCURS MAINLY IN MUSCLE & LIVER

The Pathway of Glycogen Biosynthesis Involves a Special Nucleotide of Glucose (Figure 18–1)

As in glycolysis, glucose is phosphorylated to glucose 6-phosphate, catalyzed by **hexokinase** in muscle and **glucokinase** in liver. Glucose 6-phosphate is isomerized to glucose 1-phosphate by **phosphoglucomutase.** The enzyme itself is phosphorylated, and the phosphogroup takes part in a reversible reaction in which glucose 1,6-bisphosphate is an intermediate. Next, glucose 1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide **uridine diphosphate glucose (UDPGlc)*** and pyrophosphate (Figure 18–2), catalyzed by **UDPGlc pyrophosphorylase. Pyrophosphatase** catalyzes hydrolysis of pyrophosphate to 2 mol of inorganic phosphate, shifting the equilibrium of the main reaction by removing one of its products.

Glycogen synthase catalyzes the formation of a glycoside bond between C_1 of the activated glucose of UDPGlc and C_4 of a terminal glucose residue of glycogen, liberating uridine diphosphate (UDP). A preexisting glycogen molecule, or "glycogen primer," must be present to initiate this reaction. The glycogen primer may in turn be formed on a primer known as **glycogenin,** which is a 37-kDa protein that is glycosylated on a specific tyrosine residue by UDPGlc. Further glucose residues are attached in the 1→4 position to make a short chain that is a substrate for glycogen synthase. In skeletal muscle, glycogenin remains attached in the center of the glycogen molecule (Figure 13–15), whereas in liver the number of glycogen molecules is greater than the number of glycogenin molecules.

Branching Involves Detachment of Existing Glycogen Chains

The addition of a glucose residue to a preexisting glycogen chain, or "primer," occurs at the nonreducing, outer end of the molecule so that the "branches" of the glycogen "tree" become elongated as successive 1→4 linkages are formed (Figure 18–3). When the chain has been lengthened to at least 11 glucose residues, **branching enzyme** transfers a part of the 1→4 chain (at least six glucose residues) to a neighboring chain to form a 1→6 linkage, establishing a **branch point.** The branches grow by further additions of 1→4-glucosyl units and further branching.

GLYCOGENOLYSIS IS NOT THE REVERSE OF GLYCOGENESIS BUT IS A SEPARATE PATHWAY (Figure 18–1)

Glycogen phosphorylase catalyzes the rate-limiting step in glycogenolysis by promoting the phosphorylytic cleavage by inorganic phosphate (phosphorylysis; cf hy-

* Other nucleoside diphosphate sugar compounds are known, eg, UDPGal. In addition, the same sugar may be linked to different nucleotides. For example, glucose may be linked to uridine (as shown above) as well as to guanosine, thymidine, adenosine, or cytidine nucleotides.

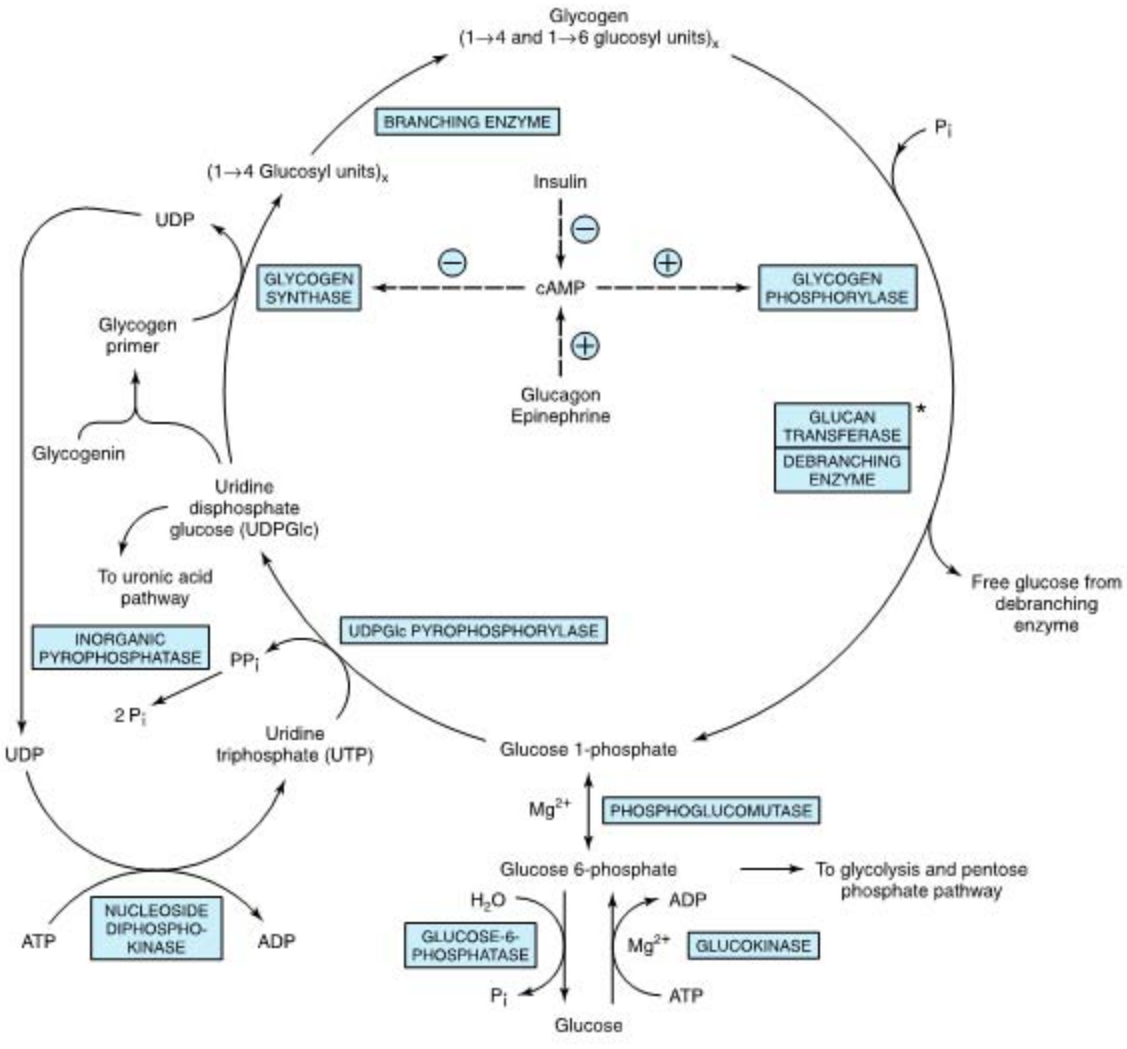

Figure 18–1. Pathway of glycogenesis and of glycogenolysis in the liver. Two high-energy phosphates are used in the incorporation of 1 mol of glucose into glycogen. ⊕, stimulation; ⊖, inhibition. Insulin decreases the level of cAMP only after it has been raised by glucagon or epinephrine—ie, it antagonizes their action. Glucagon is active in heart muscle but not in skeletal muscle. At asterisk: Glucan transferase and debranching enzyme appear to be two separate activities of the same enzyme.

Table 18–1. Storage of carbohydrate in postabsorptive normal adult humans (70 kg).

Liver glycogen	4.0%	=	72 g[1]
Muscle glycogen	0.7%	=	245 g[2]
Extracellular glucose	0.1%	=	10 g[3]
			327 g

[1]Liver weight 1800 g.
[2]Muscle mass 35 kg.
[3]Total volume 10 L.

drolysis) of the 1→4 linkages of glycogen to yield glucose 1-phosphate. The terminal glucosyl residues from the outermost chains of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a 1→6 branch (Figure 18–4). Another enzyme **(α-[1→4]→α-[1→4] glucan transferase)** transfers a trisaccharide unit from one branch to the other, exposing the 1→6 branch point. **Hydrolysis** of the 1→6 linkages requires the **debranching enzyme.** Further phosphorylase action can

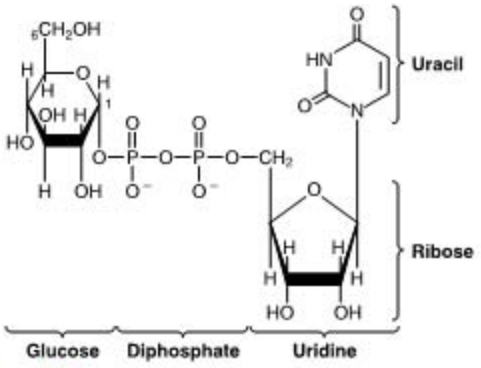

Figure 18–2. Uridine diphosphate glucose (UDPGlc).

then proceed. The combined action of phosphorylase and these other enzymes leads to the complete breakdown of glycogen. The reaction catalyzed by phosphoglucomutase is reversible, so that glucose 6-phosphate can be formed from glucose 1-phosphate. In **liver** (and **kidney**), but not in muscle, there is a specific enzyme, **glucose-6-phosphatase,** that hydrolyzes glucose 6-phosphate, yielding glucose that is exported, leading to an increase in the blood glucose concentration.

CYCLIC AMP INTEGRATES THE REGULATION OF GLYCOGENOLYSIS & GLYCOGENESIS

The principal enzymes controlling glycogen metabolism—glycogen phosphorylase and glycogen synthase—are regulated by allosteric mechanisms and covalent modifications due to reversible phosphorylation and dephosphorylation of enzyme protein in response to hormone action (Chapter 9).

Cyclic AMP (cAMP) (Figure 18–5) is formed from ATP by **adenylyl cyclase** at the inner surface of cell membranes and acts as an intracellular **second messenger** in response to hormones such as **epinephrine, norepinephrine,** and **glucagon.** cAMP is hydrolyzed by **phosphodiesterase,** so terminating hormone action. In liver, insulin increases the activity of phosphodiesterase.

Phosphorylase Differs Between Liver & Muscle

In liver, one of the serine hydroxyl groups of active **phosphorylase a** is phosphorylated. It is inactivated by hydrolytic removal of the phosphate by **protein phosphatase-1** to form **phosphorylase b.** Reactivation requires rephosphorylation catalyzed by **phosphorylase kinase.**

Muscle phosphorylase is distinct from that of liver. It is a dimer, each monomer containing 1 mol of pyridoxal phosphate (vitamin B_6). It is present in two forms: **phosphorylase a,** which is phosphorylated and active in either the presence or absence of 5′-AMP (its allosteric modifier); and **phosphorylase b,** which is dephosphorylated and active only in the presence of 5′-AMP. This occurs during exercise when the level of 5′-AMP rises, providing, by this mechanism, fuel for the muscle. Phosphorylase a is the normal physiologically active form of the enzyme.

cAMP Activates Muscle Phosphorylase

Phosphorylase in muscle is activated in response to epinephrine (Figure 18–6) acting via cAMP. Increasing the concentration of cAMP activates **cAMP-dependent**

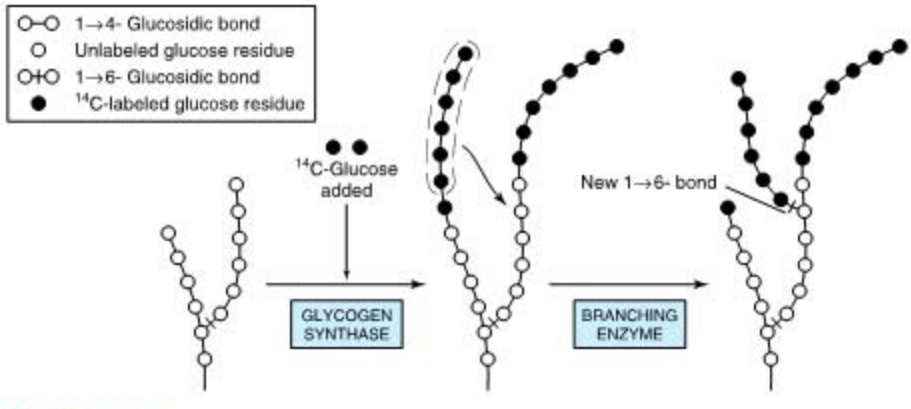

Figure 18–3. The biosynthesis of glycogen. The mechanism of branching as revealed by adding ^{14}C-labeled glucose to the diet in the living animal and examining the liver glycogen at further intervals.

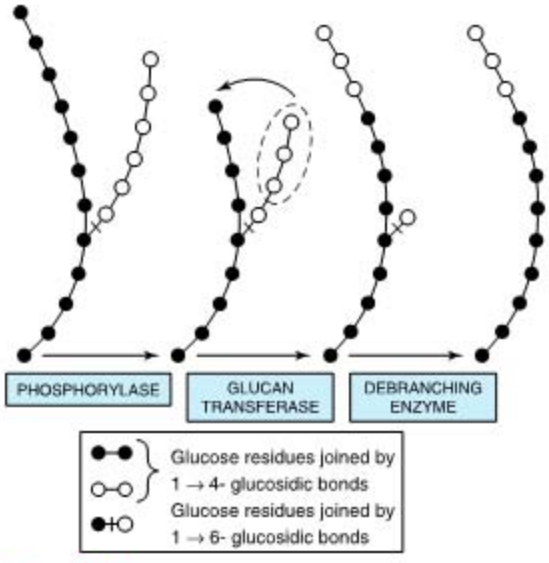

Figure 18–4. Steps in glycogenolysis.

protein kinase, which catalyzes the phosphorylation by ATP of inactive **phosphorylase kinase b** to active **phosphorylase kinase a,** which in turn, by means of a further phosphorylation, activates phosphorylase b to phosphorylase a.

Ca^{2+} Synchronizes the Activation of Phosphorylase With Muscle Contraction

Glycogenolysis increases in muscle several hundred-fold immediately after the onset of contraction. This involves the rapid activation of phosphorylase by activation of phosphorylase kinase by Ca^{2+}, the same signal as that which initiates contraction in response to nerve stimulation. Muscle phosphorylase kinase has four types of subunits—α, β, γ, and δ—in a structure represented as $(\alpha\beta\gamma\delta)_4$. The α and β subunits contain serine residues that are phosphorylated by cAMP-dependent protein kinase. The δ subunit binds four Ca^{2+} and is identical to the Ca^{2+}-binding protein **calmodulin** (Chapter 43). The binding of Ca^{2+} activates the catalytic site of the γ subunit while the molecule remains in the dephosphorylated b configuration. However, the phosphorylated a form is only fully activated in the presence of Ca^{2+}. A second molecule of calmodulin, or TpC (the structurally similar Ca^{2+}-binding protein in muscle), can interact with phosphorylase kinase, causing further activation. Thus, activation of muscle contraction and glycogenolysis are carried out by the same Ca^{2+}-binding protein, ensuring their synchronization.

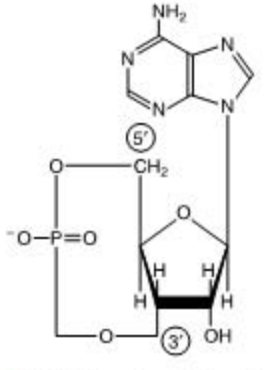

Figure 18–5. 3′,5′-Adenylic acid (cyclic AMP; cAMP).

Glycogenolysis in Liver Can Be cAMP-Independent

In addition to the action of **glucagon** in causing formation of cAMP and activation of phosphorylase in liver, **α_1-adrenergic** receptors mediate stimulation of glycogenolysis by epinephrine and norepinephrine. This involves a **cAMP-independent** mobilization of Ca^{2+} from mitochondria into the cytosol, followed by the stimulation of a **Ca^{2+}**/calmodulin-sensitive phosphorylase kinase. cAMP-independent glycogenolysis is also caused by vasopressin, oxytocin, and angiotensin II acting through calcium or the phosphatidylinositol bisphosphate pathway (Figure 43–7).

Protein Phosphatase-1 Inactivates Phosphorylase

Both phosphorylase a and phosphorylase kinase a are dephosphorylated and inactivated by **protein phosphatase-1.** Protein phosphatase-1 is inhibited by a protein, **inhibitor-1,** which is active only after it has been phosphorylated by cAMP-dependent protein kinase. Thus, cAMP controls both the activation and inactivation of phosphorylase (Figure 18–6). **Insulin** reinforces this effect by inhibiting the activation of phosphorylase b. It does this indirectly by increasing uptake of glucose, leading to increased formation of glucose 6-phosphate, which is an inhibitor of phosphorylase kinase.

Glycogen Synthase & Phosphorylase Activity Are Reciprocally Regulated (Figure 18–7)

Like phosphorylase, glycogen synthase exists in either a phosphorylated or nonphosphorylated state. However, unlike phosphorylase, the active form is dephosphorylated **(glycogen synthase a)** and may be inactivated to

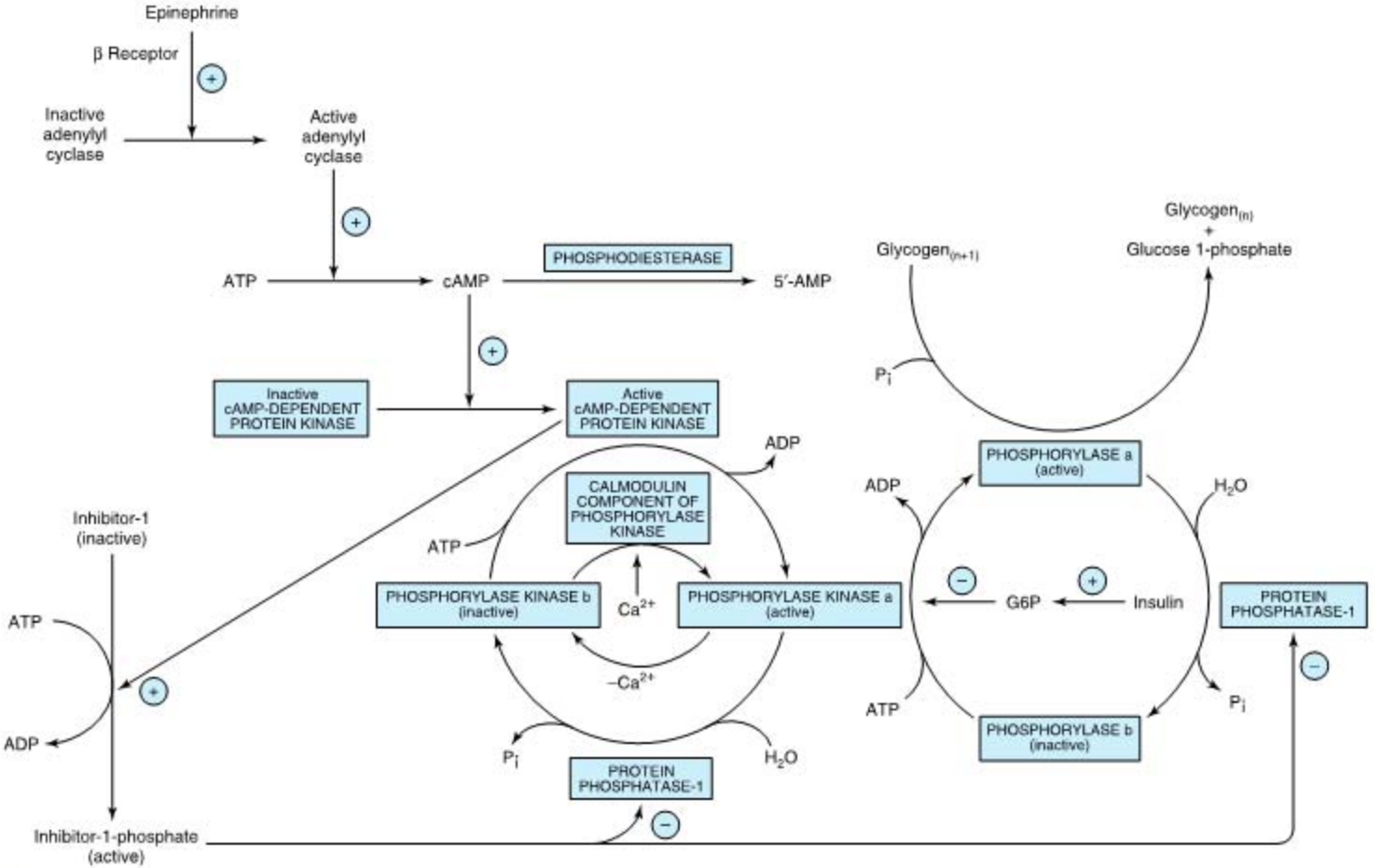

Figure 18–6. Control of phosphorylase in muscle. The sequence of reactions arranged as a cascade allows amplification of the hormonal signal at each step. (n = number of glucose residues; G6P, glucose 6-phosphate.)

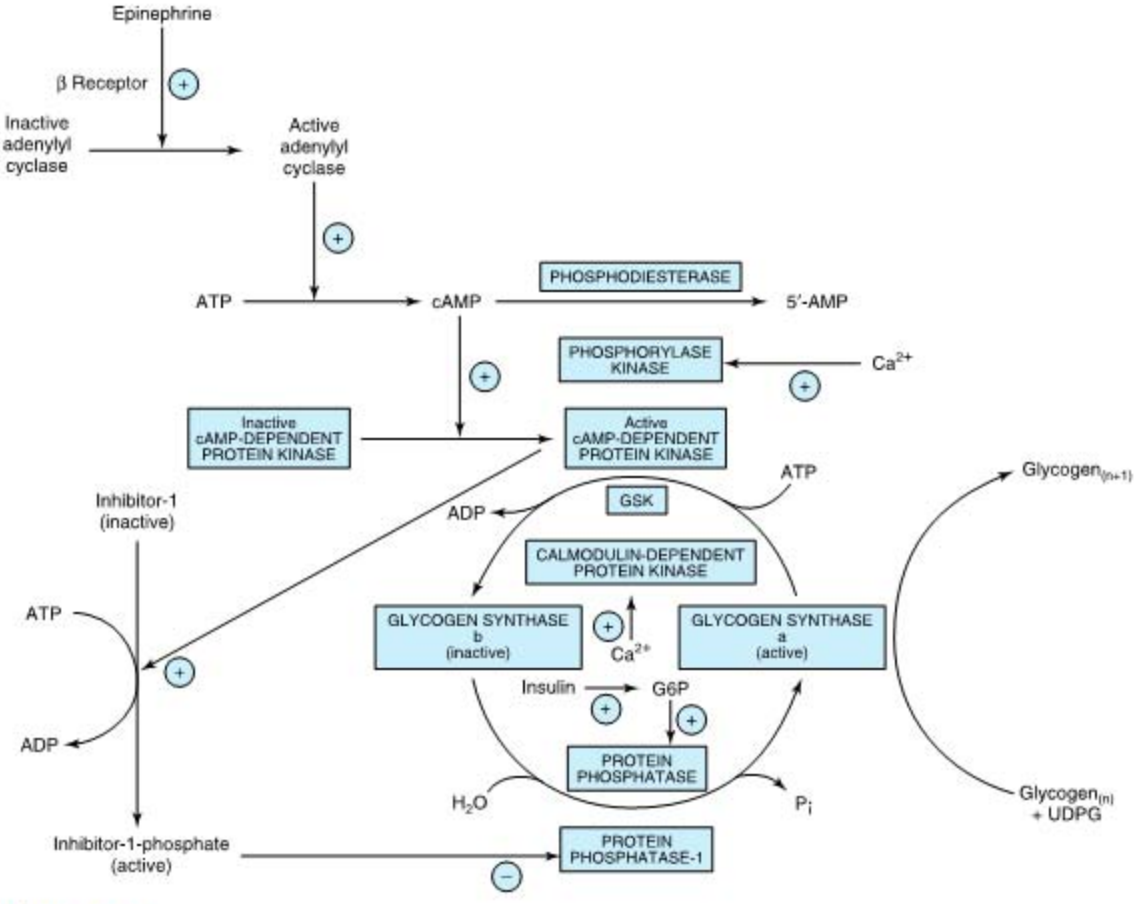

Figure 18–7. Control of glycogen synthase in muscle (n = number of glucose residues). The sequence of reactions arranged in a cascade causes amplification at each step, allowing only nanomole quantities of hormone to cause major changes in glycogen concentration. (GSK, glycogen synthase kinase-3, -4, and -5; G6P, glucose 6-phosphate.)

glycogen synthase b by phosphorylation on serine residues by no fewer than six different protein kinases. Two of the protein kinases are Ca^{2+}/calmodulin-dependent (one of these is phosphorylase kinase). Another kinase is cAMP-dependent protein kinase, which allows cAMP-mediated hormonal action to inhibit glycogen synthesis synchronously with the activation of glycogenolysis. Insulin also promotes glycogenesis in muscle at the same time as inhibiting glycogenolysis by raising glucose 6-phosphate concentrations, which stimulates the dephosphorylation and activation of glycogen synthase. Dephosphorylation of glycogen synthase b is carried out by protein phosphatase-1, which is under the control of cAMP-dependent protein kinase.

REGULATION OF GLYCOGEN METABOLISM IS EFFECTED BY A BALANCE IN ACTIVITIES BETWEEN GLYCOGEN SYNTHASE & PHOSPHORYLASE (Figure 18–8)

Not only is phosphorylase activated by a rise in concentration of cAMP (via phosphorylase kinase), but glycogen synthase is at the same time converted to the inactive form; both effects are mediated via **cAMP-dependent protein kinase.** Thus, inhibition of glycogenolysis enhances net glycogenesis, and inhibition of glycogenesis enhances net glycogenolysis. Furthermore,

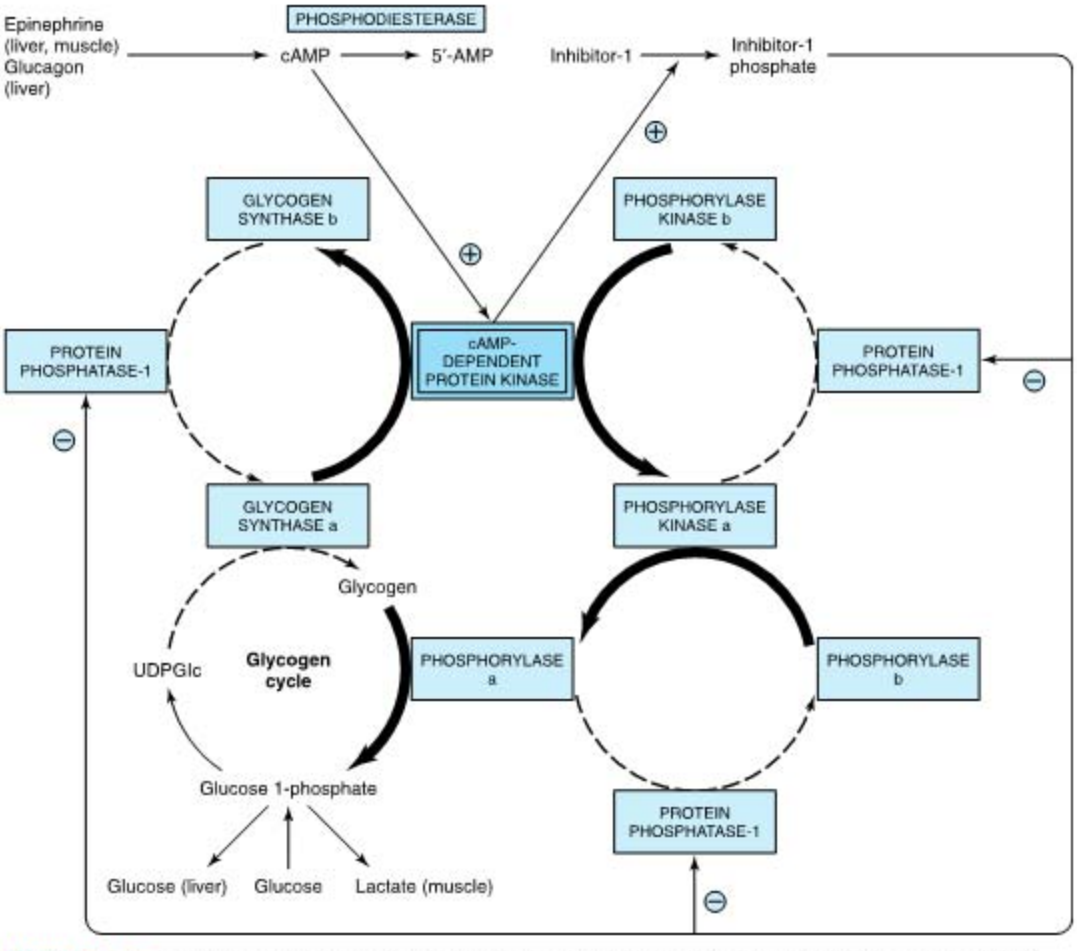

Figure 18–8. Coordinated control of glycogenolysis and glycogenesis by cAMP-dependent protein kinase. The reactions that lead to glycogenolysis as a result of an increase in cAMP concentrations are shown with bold arrows, and those that are inhibited by activation of protein phosphatase-1 are shown as broken arrows. The reverse occurs when cAMP concentrations decrease as a result of phosphodiesterase activity, leading to glycogenesis.

the dephosphorylation of phosphorylase a, phosphorylase kinase a, and glycogen synthase b is catalyzed by a single enzyme of wide specificity—**protein phosphatase-1.** In turn, protein phosphatase-1 is inhibited by cAMP-dependent protein kinase via inhibitor-1. Thus, glycogenolysis can be terminated and glycogenesis can be stimulated synchronously, or vice versa, because both processes are keyed to the activity of cAMP-dependent protein kinase. Both phosphorylase kinase and glycogen synthase may be reversibly phosphorylated in more than one site by separate kinases and phosphatases. These secondary phosphorylations modify the sensitivity of the primary sites to phosphorylation and dephosphorylation **(multisite phosphorylation).** What is more, they allow insulin, via glucose 6-phosphate elevation, to have effects that act reciprocally to those of cAMP (Figures 18–6 and 18–7).

CLINICAL ASPECTS

Glycogen Storage Diseases Are Inherited

"Glycogen storage disease" is a generic term to describe a group of inherited disorders characterized by deposition of an abnormal type or quantity of glycogen in the tissues. The principal glycogenoses are summarized in Table 18–2. Deficiencies of **adenylyl kinase** and **cAMP-dependent protein kinase** have also been re-

Table 18–2. Glycogen storage diseases.

Glycogenosis	Name	Cause of Disorder	Characteristics
Type I	Von Gierke's disease	Deficiency of glucose-6-phosphatase	Liver cells and renal tubule cells loaded with glycogen. Hypoglycemia, lactic-acidemia, ketosis, hyperlipemia.
Type II	Pompe's disease	Deficiency of lysosomal α-1→4- and 1→6-glucosidase (acid maltase)	Fatal, accumulation of glycogen in lysosomes, heart failure.
Type III	Limit dextrinosis, Forbes' or Cori's disease	Absence of debranching enzyme	Accumulation of a characteristic branched polysaccharide.
Type IV	Amylopectinosis, Andersen's disease	Absence of branching enzyme	Accumulation of a polysaccharide having few branch points. Death due to cardiac or liver failure in first year of life.
Type V	Myophosphorylase deficiency, McArdle's syndrome	Absence of muscle phosphorylase	Diminished exercise tolerance; muscles have abnormally high glycogen content (2.5–4.1%). Little or no lactate in blood after exercise.
Type VI	Hers' disease	Deficiency of liver phosphorylase	High glycogen content in liver, tendency toward hypoglycemia.
Type VII	Tarui's disease	Deficiency of phosphofructokinase in muscle and erythrocytes	As for type V but also possibility of hemolytic anemia.
Type VIII		Deficiency of liver phosphorylase kinase	As for type VI.

ported. Some of the conditions described have benefited from liver transplantation.

SUMMARY

- Glycogen represents the principal storage form of carbohydrate in the mammalian body, mainly in the liver and muscle.
- In the liver, its major function is to provide glucose for extrahepatic tissues. In muscle, it serves mainly as a ready source of metabolic fuel for use in muscle.
- Glycogen is synthesized from glucose by the pathway of glycogenesis. It is broken down by a separate pathway known as glycogenolysis. Glycogenolysis leads to glucose formation in liver and lactate formation in muscle owing to the respective presence or absence of glucose-6-phosphatase.
- Cyclic AMP integrates the regulation of glycogenolysis and glycogenesis by promoting the simultaneous activation of phosphorylase and inhibition of glycogen synthase. Insulin acts reciprocally by inhibiting glycogenolysis and stimulating glycogenesis.
- Inherited deficiencies in specific enzymes of glycogen metabolism in both liver and muscle are the causes of glycogen storage diseases.

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Gluconeogenesis & Control of the Blood Glucose

19

Peter A. Mayes, PhD, DSc, & David A. Bender, PhD

BIOMEDICAL IMPORTANCE

Gluconeogenesis is the term used to include all pathways responsible for converting noncarbohydrate precursors to glucose or glycogen. The major substrates are the glucogenic amino acids and lactate, glycerol, and propionate. Liver and kidney are the major gluconeogenic tissues. Gluconeogenesis meets the needs of the body for glucose when carbohydrate is not available in sufficient amounts from the diet or from glycogen reserves. A supply of glucose is necessary especially for the nervous system and erythrocytes. Failure of gluconeogenesis is usually fatal. **Hypoglycemia** causes brain dysfunction, which can lead to coma and death. Glucose is also important in maintaining the level of intermediates of the citric acid cycle even when fatty acids are the main source of acetyl-CoA in the tissues. In addition, gluconeogenesis clears lactate produced by muscle and erythrocytes and glycerol produced by adipose tissue. Propionate, the principal glucogenic fatty acid produced in the digestion of carbohydrates by ruminants, is a major substrate for gluconeogenesis in these species.

GLUCONEOGENESIS INVOLVES GLYCOLYSIS, THE CITRIC ACID CYCLE, & SOME SPECIAL REACTIONS (Figure 19–1)

Thermodynamic Barriers Prevent a Simple Reversal of Glycolysis

Three nonequilibrium reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase prevent simple reversal of glycolysis for glucose synthesis (Chapter 17). They are circumvented as follows:

A. Pyruvate & Phosphoenolpyruvate

Mitochondrial **pyruvate carboxylase** catalyzes the carboxylation of pyruvate to oxaloacetate, an ATP-requiring reaction in which the vitamin biotin is the coenzyme. Biotin binds CO_2 from bicarbonate as carboxybiotin prior to the addition of the CO_2 to pyruvate (Figure 45–17). A second enzyme, **phosphoenolpyruvate carboxykinase,** catalyzes the decarboxylation and phosphorylation of oxaloacetate to phosphoenolpyruvate using GTP (or ITP) as the phosphate donor. Thus, reversal of the reaction catalyzed by pyruvate kinase in glycolysis involves two endergonic reactions.

In pigeon, chicken, and rabbit liver, phosphoenolpyruvate carboxykinase is a mitochondrial enzyme, and phosphoenolpyruvate is transported into the cytosol for gluconeogenesis. In the rat and the mouse, the enzyme is cytosolic. Oxaloacetate does not cross the mitochondrial inner membrane; it is converted to malate, which is transported into the cytosol, and converted back to oxaloacetate by cytosolic malate dehydrogenase. In humans, the guinea pig, and the cow, the enzyme is equally distributed between mitochondria and cytosol.

The main source of GTP for phosphoenolpyruvate carboxykinase inside the mitochondrion is the reaction of succinyl-CoA synthetase (Chapter 16). This provides a link and limit between citric acid cycle activity and the extent of withdrawal of oxaloacetate for gluconeogenesis.

B. Fructose 1,6-Bisphosphate & Fructose 6-Phosphate

The conversion of fructose 1,6-bisphosphate to fructose 6-phosphate, to achieve a reversal of glycolysis, is catalyzed by **fructose-1,6-bisphosphatase.** Its presence determines whether or not a tissue is capable of synthesizing glycogen not only from pyruvate but also from triosephosphates. It is present in liver, kidney, and skeletal muscle but is probably absent from heart and smooth muscle.

C. Glucose 6-Phosphate & Glucose

The conversion of glucose 6-phosphate to glucose is catalyzed by **glucose-6-phosphatase.** It is present in liver and kidney but absent from muscle and adipose tissue, which, therefore, cannot export glucose into the bloodstream.

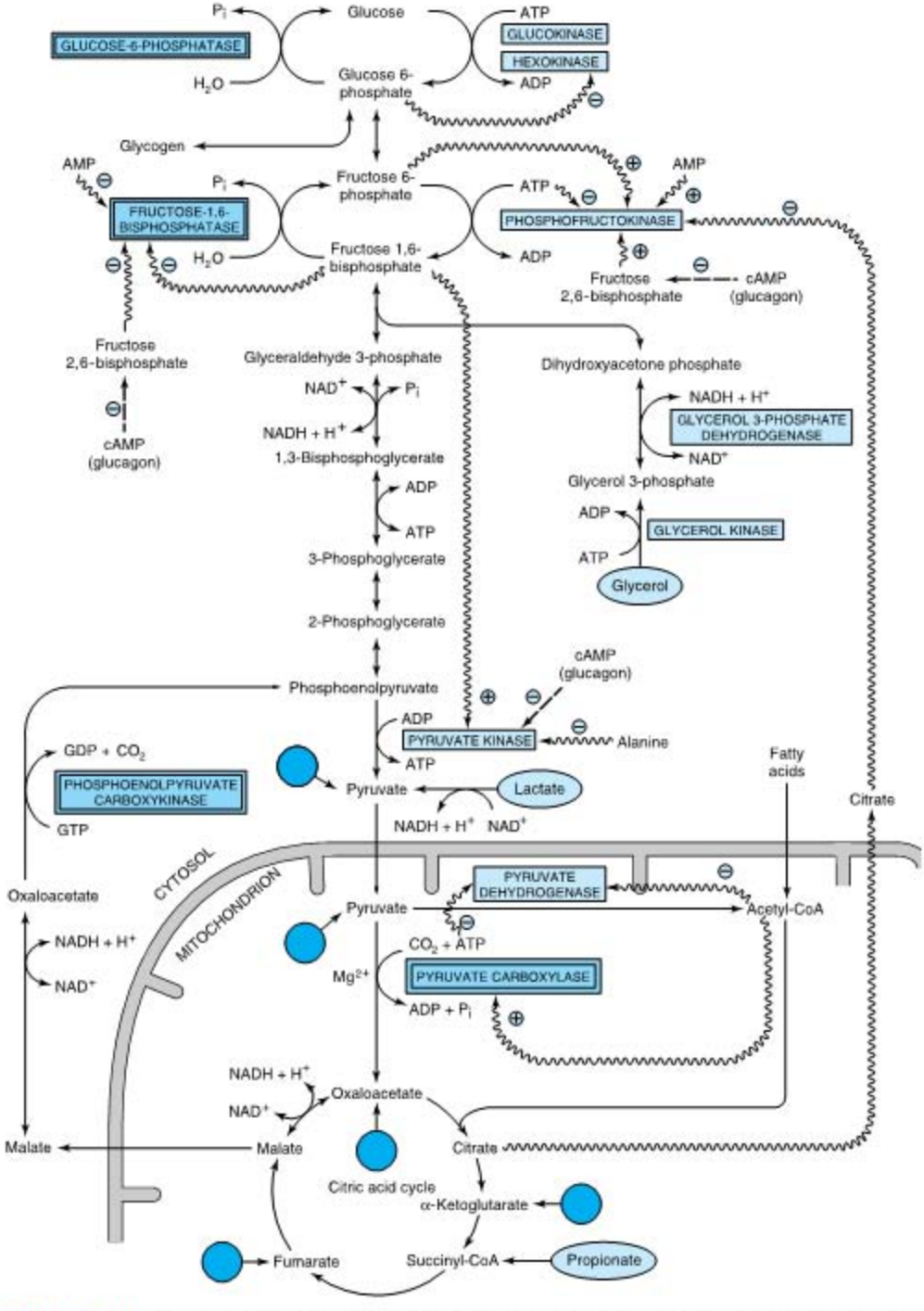

Figure 19–1. Major pathways and regulation of gluconeogenesis and glycolysis in the liver. Entry points of glucogenic amino acids after transamination are indicated by arrows extended from circles. (See also Figure 16–4.) The key gluconeogenic enzymes are enclosed in double-bordered boxes. The ATP required for gluconeogenesis is supplied by the oxidation of long-chain fatty acids. Propionate is of quantitative importance only in ruminants. Arrows with wavy shafts signify allosteric effects; dash-shafted arrows, covalent modification by reversible phosphorylation. High concentrations of alanine act as a "gluconeogenic signal" by inhibiting glycolysis at the pyruvate kinase step.

D. Glucose 1-Phosphate & Glycogen

The breakdown of glycogen to glucose 1-phosphate is catalyzed by phosphorylase. Glycogen synthesis involves a different pathway via uridine diphosphate glucose and **glycogen synthase** (Figure 18–1).

The relationships between gluconeogenesis and the glycolytic pathway are shown in Figure 19–1. After transamination or deamination, glucogenic amino acids yield either pyruvate or intermediates of the citric acid cycle. Therefore, the reactions described above can account for the conversion of both glucogenic amino acids and lactate to glucose or glycogen. Propionate is a major source of glucose in ruminants and enters gluconeogenesis via the citric acid cycle. Propionate is esterified with CoA, then propionyl-CoA, is carboxylated to D-methylmalonyl-CoA, catalyzed by **propionyl-CoA carboxylase,** a biotin-dependent enzyme (Figure 19–2). **Methylmalonyl-CoA racemase** catalyzes the conversion of D-methylmalonyl-CoA to L-methylmalonyl-CoA, which then undergoes isomerization to succinyl-CoA catalyzed by **methylmalonyl-CoA isomerase.** This enzyme requires vitamin B_{12} as a coenzyme, and deficiency of this vitamin results in the excretion of methylmalonate **(methylmalonic aciduria).**

C_{15} and C_{17} fatty acids are found particularly in the lipids of ruminants. Dietary odd-carbon fatty acids upon oxidation yield propionate (Chapter 22), which is a substrate for gluconeogenesis in human liver.

Glycerol is released from adipose tissue as a result of lipolysis, and only tissues such as liver and kidney that possess **glycerol kinase,** which catalyzes the conversion of glycerol to glycerol 3-phosphate, can utilize it. Glycerol 3-phosphate may be oxidized to dihydroxyacetone phosphate by NAD^+ catalyzed by **glycerol-3-phosphate dehydrogenase.**

SINCE GLYCOLYSIS & GLUCONEOGENESIS SHARE THE SAME PATHWAY BUT IN OPPOSITE DIRECTIONS, THEY MUST BE REGULATED RECIPROCALLY

Changes in the availability of substrates are responsible for most changes in metabolism either directly or indirectly acting via changes in hormone secretion. Three mechanisms are responsible for regulating the activity of enzymes in carbohydrate metabolism: (1) changes in the rate of enzyme synthesis, (2) covalent modification by reversible phosphorylation, and (3) allosteric effects.

Induction & Repression of Key Enzyme Synthesis Requires Several Hours

The changes in enzyme activity in the liver that occur under various metabolic conditions are listed in Table 19–1. The enzymes involved catalyze nonequilibrium (physiologically irreversible) reactions. The effects are generally reinforced because the activity of the enzymes catalyzing the changes in the opposite direction varies reciprocally (Figure 19–1). The enzymes involved in the utilization of glucose (ie, those of glycolysis and lipogenesis) all become more active when there is a superfluity of glucose, and under these conditions the enzymes responsible for gluconeogenesis all have low activity. The secretion of insulin, in response to increased blood glucose, enhances the synthesis of the key

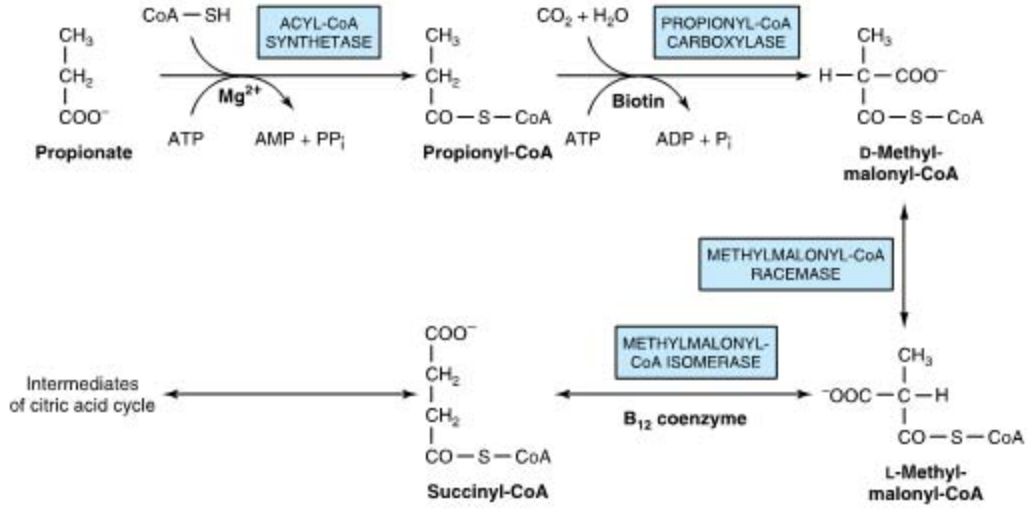

Figure 19–2. Metabolism of propionate.

Table 19–1. Regulatory and adaptive enzymes of the rat (mainly liver).

	Activity In					
	Carbohydrate Feeding	Starvation and Diabetes	Inducer	Repressor	Activator	Inhibitor
Enzymes of glycogenesis, glycolysis, and pyruvate oxidation						
Glycogen synthase system	↑	↓	Insulin		Insulin Glucose 6-phosphate[1]	Glucagon (cAMP) phosphorylase, glycogen
Hexokinase						Glucose 6-phosphate[1]
Glucokinase	↑	↓	Insulin	Glucagon (cAMP)		
Phosphofructokinase-1	↑	↓	Insulin	Glucagon (cAMP)	AMP, fructose 6-phosphate, P_i, fructose 2,6-bisphosphate[1]	Citrate (fatty acids, ketone bodies),[1] ATP,[1] glucagon (cAMP)
Pyruvate kinase	↑	↓	Insulin, fructose	Glucagon (cAMP)	Fructose 1,6-bisphosphate[1], insulin	ATP, alanine, glucagon (cAMP), epinephrine
Pyruvate dehydrogenase	↑	↓			CoA, NAD^+, insulin,[2] ADP, pyruvate	Acetyl-CoA, NADH, ATP (fatty acids, ketone bodies)
Enzymes of gluconeogenesis						
Pyruvate carboxylase	↓	↑	Glucocorticoids, glucagon, epinephrine (cAMP)	Insulin	Acetyl-CoA[1]	ADP[1]
Phosphoenolpyruvate carboxykinase	↓	↑	Glucocorticoids, glucagon, epinephrine (cAMP)	Insulin	Glucagon?	
Fructose-1,6-bisphosphatase	↓	↑	Glucocorticoids, glucagon, epinephrine (cAMP)	Insulin	Glucagon (cAMP)	Fructose 1,6- bisphosphate, AMP, fructose 2,6-bisphosphate[1]
Glucose-6-phosphatase	↓	↑	Glucocorticoids, glucagon, epinephrine (cAMP)	Insulin		
Enzymes of the pentose phosphate pathway and lipogenesis						
Glucose-6-phosphate dehydrogenase	↑	↓	Insulin			
6-Phosphogluconate dehydrogenase	↑	↓	Insulin			
"Malic enzyme"	↑	↓	Insulin			
ATP-citrate lyase	↑	↓	Insulin			
Acetyl-CoA carboxylase	↑	↓	Insulin?		Citrate,[1] insulin	Long-chain acyl-CoA, cAMP, glucagon
Fatty acid synthase	↑	↓	Insulin?			

[1]Allosteric.
[2]In adipose tissue but not in liver.

enzymes in glycolysis. Likewise, it antagonizes the effect of the glucocorticoids and glucagon-stimulated cAMP, which induce synthesis of the key enzymes responsible for gluconeogenesis.

Both dehydrogenases of the pentose phosphate pathway can be classified as adaptive enzymes, since they increase in activity in the well-fed animal and when insulin is given to a diabetic animal. Activity is low in diabetes or starvation. "Malic enzyme" and ATP-citrate lyase behave similarly, indicating that these two enzymes are involved in lipogenesis rather than gluconeogenesis (Chapter 21).

Covalent Modification by Reversible Phosphorylation Is Rapid

Glucagon, and to a lesser extent **epinephrine,** hormones that are responsive to decreases in blood glucose, inhibit glycolysis and stimulate gluconeogenesis in the liver by increasing the concentration of cAMP. This in turn activates cAMP-dependent protein kinase, leading to the phosphorylation and inactivation of **pyruvate kinase.** They also affect the concentration of fructose 2,6-bisphosphate and therefore glycolysis and gluconeogenesis, as explained below.

Allosteric Modification Is Instantaneous

In gluconeogenesis, pyruvate carboxylase, which catalyzes the synthesis of oxaloacetate from pyruvate, requires acetyl-CoA as an **allosteric activator.** The presence of acetyl-CoA results in a change in the tertiary structure of the protein, lowering the K_m value for bicarbonate. This means that as acetyl-CoA is formed from pyruvate, it automatically ensures the provision of oxaloacetate and, therefore, its further oxidation in the citric acid cycle. The activation of pyruvate carboxylase and the reciprocal inhibition of pyruvate dehydrogenase by acetyl-CoA derived from the oxidation of fatty acids explains the action of fatty acid oxidation in sparing the oxidation of pyruvate and in stimulating gluconeogenesis. The reciprocal relationship between these two enzymes in both liver and kidney alters the metabolic fate of pyruvate as the tissue changes from carbohydrate oxidation, via glycolysis, to gluconeogenesis during transition from a fed to a starved state (Figure 19–1). A major role of fatty acid oxidation in promoting gluconeogenesis is to supply the requirement for ATP. **Phosphofructokinase (phosphofructokinase-1)** occupies a key position in regulating glycolysis and is also subject to feedback control. It is inhibited by citrate and by ATP and is activated by 5′-AMP. 5′-AMP acts as an indicator of the energy status of the cell. The presence of **adenylyl kinase** in liver and many other tissues allows rapid equilibration of the reaction:

$$\text{ATP} + \text{AMP} \leftrightarrow 2\text{ADP}$$

Thus, when ATP is used in energy-requiring processes resulting in formation of ADP, [AMP] increases. As [ATP] may be 50 times [AMP] at equilibrium, a small fractional decrease in [ATP] will cause a severalfold increase in [AMP]. Thus, a large change in [AMP] acts as a metabolic amplifier of a small change in [ATP]. This mechanism allows the activity of phosphofructokinase-1 to be highly sensitive to even small changes in energy status of the cell and to control the quantity of carbohydrate undergoing glycolysis prior to its entry into the citric acid cycle. The increase in [AMP] can also explain why glycolysis is increased during hypoxia when [ATP] decreases. Simultaneously, AMP activates phosphorylase, increasing glycogenolysis. The inhibition of phosphofructokinase-1 by citrate and ATP is another explanation of the sparing action of fatty acid oxidation on glucose oxidation and also of the **Pasteur effect,** whereby aerobic oxidation (via the citric acid cycle) inhibits the anaerobic degradation of glucose. A consequence of the inhibition of phosphofructokinase-1 is an accumulation of glucose 6-phosphate that, in turn, inhibits further uptake of glucose in extrahepatic tissues by allosteric inhibition of hexokinase.

Fructose 2,6-Bisphosphate Plays a Unique Role in the Regulation of Glycolysis & Gluconeogenesis in Liver

The most potent positive allosteric effector of phosphofructokinase-1 and inhibitor of fructose-1,6-bisphosphatase in liver is **fructose 2,6-bisphosphate.** It relieves inhibition of phosphofructokinase-1 by ATP and increases affinity for fructose 6-phosphate. It inhibits fructose-1,6-bisphosphatase by increasing the K_m for fructose 1,6-bisphosphate. Its concentration is under both substrate (allosteric) and hormonal control (covalent modification) (Figure 19–3).

Fructose 2,6-bisphosphate is formed by phosphorylation of fructose 6-phosphate by **phosphofructokinase-2.** The same enzyme protein is also responsible for its breakdown, since it has **fructose-2,6-bisphosphatase** activity. This **bifunctional enzyme** is under the allosteric control of fructose 6-phosphate, which stimulates the kinase and inhibits the phosphatase. Hence, when glucose is abundant, the concentration of fructose 2,6-bisphosphate increases, stimulating glycolysis by activating phosphofructokinase-1 and inhibiting

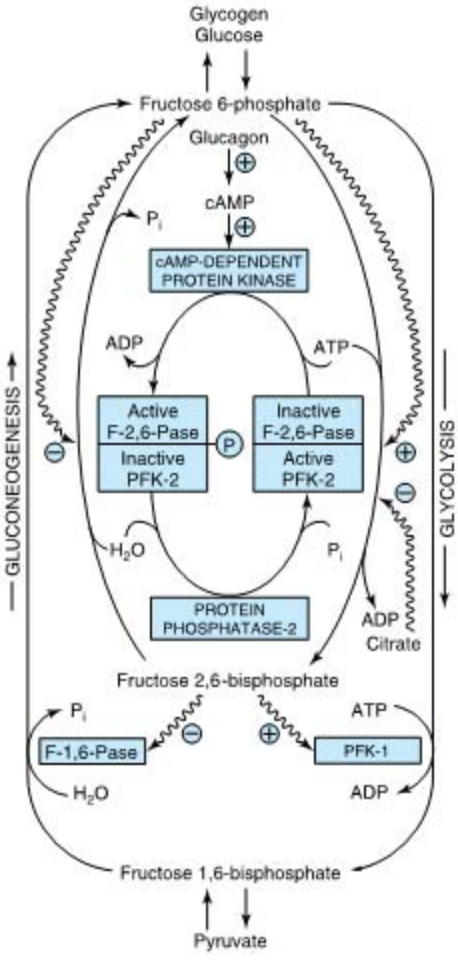

Figure 19–3. Control of glycolysis and gluconeogenesis in the liver by fructose 2,6-bisphosphate and the bifunctional enzyme PFK-2/F-2,6-Pase (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase). (PFK-1, phosphofructokinase-1 [6-phosphofructo-1-kinase]; F-1,6-Pase, fructose-1,6-bisphosphatase. Arrows with wavy shafts indicate allosteric effects.)

fructose-1,6-bisphosphatase. When glucose is short, glucagon stimulates the production of cAMP, activating cAMP-dependent protein kinase, which in turn inactivates phosphofructokinase-2 and activates fructose 2,6-bisphosphatase by phosphorylation. Therefore, gluconeogenesis is stimulated by a decrease in the concentration of fructose 2,6-bisphosphate, which deactivates phosphofructokinase-1 and deinhibits fructose-1,6-bisphosphatase. This mechanism also ensures that glucagon stimulation of glycogenolysis in liver results in glucose release rather than glycolysis.

Substrate (Futile) Cycles Allow Fine Tuning

It will be apparent that the control points in glycolysis and glycogen metabolism involve a cycle of phosphorylation and dephosphorylation catalyzed by: glucokinase and glucose-6-phosphatase; phosphofructokinase-1 and fructose-1,6-bisphosphatase; pyruvate kinase, pyruvate carboxylase, and phosphoenolypyruvate carboxykinase; and glycogen synthase and phosphorylase. If these were allowed to cycle unchecked, they would amount to futile cycles whose net result was hydrolysis of ATP. This does not occur extensively due to the various control mechanisms, which ensure that one reaction is inhibited as the other is stimulated. However, there is a physiologic advantage in allowing some cycling. The rate of net glycolysis may increase several thousand-fold in response to stimulation, and this is more readily achieved by both increasing the activity of phosphofructokinase and decreasing that of fructose bisphosphatase if both are active, than by switching one enzyme "on" and the other "off" completely. This "fine tuning" of metabolic control occurs at the expense of some loss of ATP.

THE CONCENTRATION OF BLOOD GLUCOSE IS REGULATED WITHIN NARROW LIMITS

In the postabsorptive state, the concentration of blood glucose in most mammals is maintained between 4.5 and 5.5 mmol/L. After the ingestion of a carbohydrate meal, it may rise to 6.5–7.2 mmol/L, and in starvation, it may fall to 3.3–3.9 mmol/L. A sudden decrease in blood glucose will cause convulsions, as in insulin overdose, owing to the immediate dependence of the brain on a supply of glucose. However, much lower concentrations can be tolerated, provided progressive adaptation is allowed. The blood glucose level in birds is considerably higher (14.0 mmol/L) and in ruminants considerably lower (approximately 2.2 mmol/L in sheep and 3.3 mmol/L in cattle). These lower normal levels appear to be associated with the fact that ruminants ferment virtually all dietary carbohydrate to lower (volatile) fatty acids, and these largely replace glucose as the main metabolic fuel of the tissues in the fed condition.

BLOOD GLUCOSE IS DERIVED FROM THE DIET, GLUCONEOGENESIS, & GLYCOGENOLYSIS

The digestible dietary carbohydrates yield glucose, galactose, and fructose that are transported via the **hepatic portal vein** to the liver where galactose and fructose are readily converted to glucose (Chapter 20).

Glucose is formed from two groups of compounds that undergo gluconeogenesis (Figures 16–4 and 19–1): (1) those which involve a direct net conversion to glucose without significant recycling, such as some **amino acids** and **propionate;** and (2) those which are the products of the metabolism of glucose in tissues. Thus, **lactate,** formed by glycolysis in skeletal muscle and erythrocytes, is transported to the liver and kidney where it re-forms glucose, which again becomes available via the circulation for oxidation in the tissues. This process is known as the **Cori cycle,** or **lactic acid cycle** (Figure 19–4). Triacylglycerol glycerol in adipose tissue is derived from blood glucose. This triacylglycerol is continuously undergoing hydrolysis to form free **glycerol,** which cannot be utilized by adipose tissue and is converted back to glucose by gluconeogenic mechanisms in the liver and kidney (Figure 19–1).

Of the amino acids transported from muscle to the liver during starvation, alanine predominates. The **glucose-alanine cycle** (Figure 19–4) transports glucose from liver to muscle with formation of pyruvate, followed by transamination to alanine, then transports alanine to the liver, followed by gluconeogenesis back to glucose. A net transfer of amino nitrogen from muscle to liver and of free energy from liver to muscle is effected. The energy required for the hepatic synthesis of glucose from pyruvate is derived from the oxidation of fatty acids.

Glucose is also formed from liver glycogen by glycogenolysis (Chapter 18).

Metabolic & Hormonal Mechanisms Regulate the Concentration of the Blood Glucose

The maintenance of stable levels of glucose in the blood is one of the most finely regulated of all homeostatic mechanisms, involving the liver, extrahepatic tissues, and several hormones. Liver cells are freely permeable to glucose (via the GLUT 2 transporter), whereas cells of extrahepatic tissues (apart from pancreatic B islets) are relatively impermeable, and their glucose transporters are regulated by insulin. As a result, uptake from the bloodstream is the rate-limiting step in the utilization of glucose in extrahepatic tissues. The role of various glucose transporter proteins found in cell membranes, each having 12 transmembrane domains, is shown in Table 19–2.

Glucokinase Is Important in Regulating Blood Glucose After a Meal

Hexokinase has a low K_m for glucose and in the liver is saturated and acting at a constant rate under all normal conditions. Glucokinase has a considerably higher K_m (lower affinity) for glucose, so that its activity increases over the physiologic range of glucose concentrations (Figure 19–5). It promotes hepatic uptake of large amounts of glucose at the high concentrations found in the hepatic portal vein after a carbohydrate meal. It is absent from the liver of ruminants, which have little

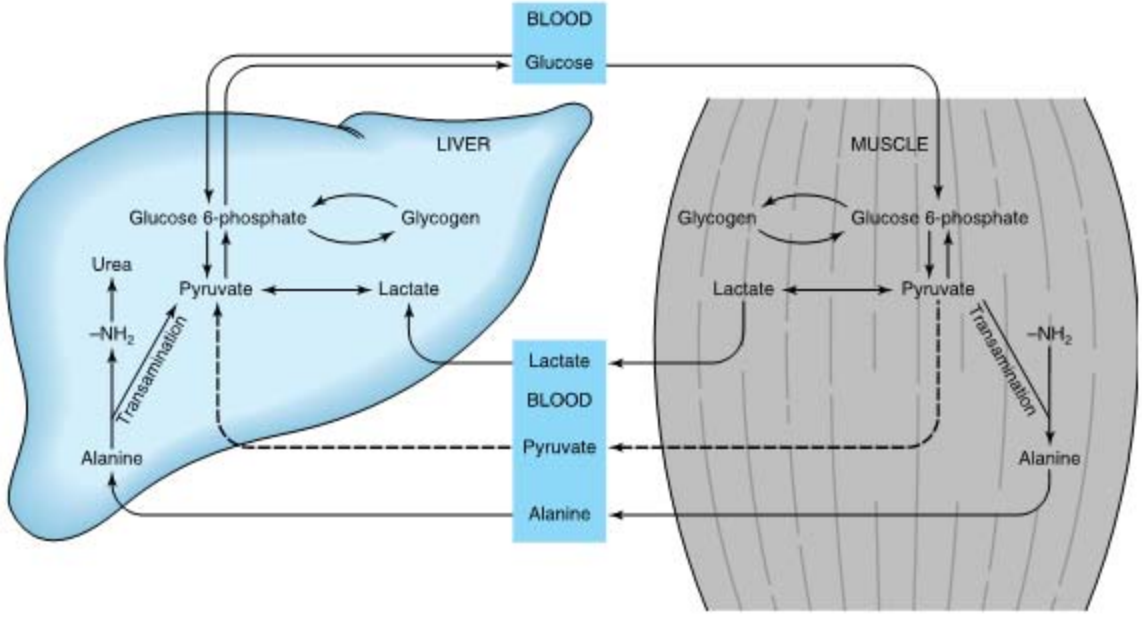

Figure 19–4. The lactic acid (Cori) cycle and glucose-alanine cycle.

Table 19–2. Glucose transporters.

	Tissue Location	Functions
Facilitative bidirectional transporters		
GLUT 1	Brain, kidney, colon, placenta, erythrocyte	Uptake of glucose
GLUT 2	Liver, pancreatic B cell, small intestine, kidney	Rapid uptake and release of glucose
GLUT 3	Brain, kidney, placenta	Uptake of glucose
GLUT 4	Heart and skeletal muscle, adipose tissue	Insulin-stimulated uptake of glucose
GLUT 5	Small intestine	Absorption of glucose
Sodium-dependent unidirectional transporter		
SGLT 1	Small intestine and kidney	Active uptake of glucose from lumen of intestine and reabsorption of glucose in proximal tubule of kidney against a concentration gradient

glucose entering the portal circulation from the intestines.

At normal systemic-blood glucose concentrations (4.5–5.5 mmol/L), the liver is a net producer of glucose. However, as the glucose level rises, the output of glucose ceases, and there is a net uptake.

Insulin Plays a Central Role in Regulating Blood Glucose

In addition to the direct effects of hyperglycemia in enhancing the uptake of glucose into the liver, the hormone insulin plays a central role in regulating blood glucose. It is produced by the B cells of the islets of Langerhans in the pancreas in response to hyperglycemia. The B islet cells are freely permeable to glucose via the GLUT 2 transporter, and the glucose is phosphorylated by glucokinase. Therefore, increasing blood glucose increases metabolic flux through glycolysis, the citric acid cycle, and the generation of ATP. Increase in [ATP] inhibits ATP-sensitive K^+ channels, causing depolarization of the B cell membrane, which increases Ca^{2+} influx via voltage-sensitive Ca^{2+} channels, stimulating exocytosis of insulin. Thus, the concentration of insulin in the blood parallels that of the blood glucose. Other substances causing release of insulin from the pancreas include amino acids, free fatty acids, ketone bodies, glucagon, secretin, and the sulfonylurea drugs tolbutamide and glyburide. These drugs are used to stimulate insulin secretion in type 2 diabetes mellitus (NIDDM, non-insulin-dependent diabetes mellitus); they act by inhibiting the ATP-sensitive K^+ channels. Epinephrine and norepinephrine block the release of insulin. Insulin lowers blood glucose immediately by enhancing glucose transport into adipose tissue and muscle by recruitment of glucose transporters (GLUT 4) from the interior of the cell to the plasma membrane. Although it does not affect glucose uptake into the liver directly, insulin does enhance long-term uptake as a result of its actions on the enzymes controlling glycolysis, glycogenesis, and gluconeogenesis (Chapter 18).

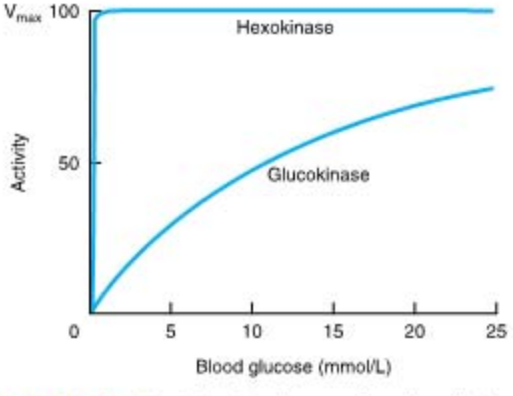

Figure 19–5. Variation in glucose phosphorylating activity of hexokinase and glucokinase with increase of blood glucose concentration. The K_m for glucose of hexokinase is 0.05 mmol/L and of glucokinase is 10 mmol/L.

Glucagon Opposes the Actions of Insulin

Glucagon is the hormone produced by the A cells of the pancreatic islets. Its secretion is stimulated by hypoglycemia. In the liver, it stimulates glycogenolysis by activating phosphorylase. Unlike epinephrine, glucagon does not have an effect on muscle phosphorylase. Glucagon also enhances gluconeogenesis from amino acids and lactate. In all these actions, glucagon acts via generation of cAMP (Table 19–1). Both hepatic glycogenolysis and gluconeogenesis contribute to the

hyperglycemic effect of glucagon, whose actions oppose those of insulin. Most of the endogenous glucagon (and insulin) is cleared from the circulation by the liver.

Other Hormones Affect Blood Glucose

The **anterior pituitary gland** secretes hormones that tend to elevate the blood glucose and therefore antagonize the action of insulin. These are growth hormone, ACTH (corticotropin), and possibly other "diabetogenic" hormones. Growth hormone secretion is stimulated by hypoglycemia; it decreases glucose uptake in muscle. Some of this effect may not be direct, since it stimulates mobilization of free fatty acids from adipose tissue, which themselves inhibit glucose utilization. The **glucocorticoids** (11-oxysteroids) are secreted by the adrenal cortex and increase gluconeogenesis. This is a result of enhanced hepatic uptake of amino acids and increased activity of aminotransferases and key enzymes of gluconeogenesis. In addition, glucocorticoids inhibit the utilization of glucose in extrahepatic tissues. In all these actions, glucocorticoids act in a manner antagonistic to insulin.

Epinephrine is secreted by the adrenal medulla as a result of stressful stimuli (fear, excitement, hemorrhage, hypoxia, hypoglycemia, etc) and leads to glycogenolysis in liver and muscle owing to stimulation of phosphorylase via generation of cAMP. In muscle, glycogenolysis results in increased glycolysis, whereas in liver glucose is the main product leading to increase in blood glucose.

FURTHER CLINICAL ASPECTS

Glucosuria Occurs When the Renal Threshold for Glucose Is Exceeded

When the blood glucose rises to relatively high levels, the kidney also exerts a regulatory effect. Glucose is continuously filtered by the glomeruli but is normally completely reabsorbed in the renal tubules by active transport. The capacity of the tubular system to reabsorb glucose is limited to a rate of about 350 mg/min, and in hyperglycemia (as occurs in poorly controlled diabetes mellitus) the glomerular filtrate may contain more glucose than can be reabsorbed, resulting in **glucosuria.** Glucosuria occurs when the venous blood glucose concentration exceeds 9.5–10.0 mmol/L; this is termed the **renal threshold** for glucose.

Hypoglycemia May Occur During Pregnancy & in the Neonate

During pregnancy, fetal glucose consumption increases and there is a risk of maternal and possibly fetal hypoglycemia, particularly if there are long intervals between meals or at night. Furthermore, premature and low-birth-weight babies are more susceptible to hypoglycemia, since they have little adipose tissue to generate alternative fuels such as free fatty acids or ketone bodies during the transition from fetal dependency to the free-living state. The enzymes of gluconeogenesis may not be completely functional at this time, and the process is dependent on a supply of free fatty acids for energy. Glycerol, which would normally be released from adipose tissue, is less available for gluconeogenesis.

The Body's Ability to Utilize Glucose May Be Ascertained by Measuring Its Glucose Tolerance

Glucose tolerance is the ability to regulate the blood glucose concentration after the administration of a test dose of glucose (normally 1 g/kg body weight) (Figure 19–6). **Diabetes mellitus** (type 1, or insulin-dependent diabetes mellitus; IDDM) is characterized by decreased glucose tolerance due to decreased secretion of insulin in response to the glucose challenge. Glucose tolerance is also impaired in type 2 diabetes mellitus (NIDDM), which is often associated with obesity and raised levels of plasma free fatty acids and in conditions where the liver is damaged; in some infections; and in response to some drugs. Poor glucose tolerance can also be expected

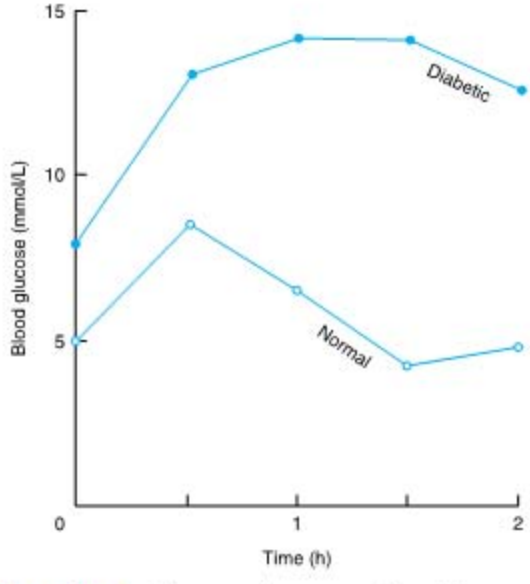

Figure 19–6. Glucose tolerance test. Blood glucose curves of a normal and a diabetic individual after oral administration of 50 g of glucose. Note the initial raised concentration in the diabetic. A criterion of normality is the return of the curve to the initial value within 2 hours.

due to hyperactivity of the pituitary or adrenal cortex because of the antagonism of the hormones secreted by these glands to the action of insulin.

Administration of insulin (as in the treatment of diabetes mellitus type 1) lowers the blood glucose and increases its utilization and storage in the liver and muscle as glycogen. An excess of insulin may cause **hypoglycemia,** resulting in convulsions and even in death unless glucose is administered promptly. Increased tolerance to glucose is observed in pituitary or adrenocortical insufficiency—attributable to a decrease in the antagonism to insulin by the hormones normally secreted by these glands.

SUMMARY

- Gluconeogenesis is the process of converting noncarbohydrates to glucose or glycogen. It is of particular importance when carbohydrate is not available from the diet. Significant substrates are amino acids, lactate, glycerol, and propionate.
- The pathway of gluconeogenesis in the liver and kidney utilizes those reactions in glycolysis which are reversible plus four additional reactions that circumvent the irreversible nonequilibrium reactions.
- Since glycolysis and gluconeogenesis share the same pathway but operate in opposite directions, their activities are regulated reciprocally.
- The liver regulates the blood glucose after a meal because it contains the high-K_m glucokinase that promotes increased hepatic utilization of glucose.
- Insulin is secreted as a direct response to hyperglycemia; it stimulates the liver to store glucose as glycogen and facilitates uptake of glucose into extrahepatic tissues.
- Glucagon is secreted as a response to hypoglycemia and activates both glycogenolysis and gluconeogenesis in the liver, causing release of glucose into the blood.

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The Pentose Phosphate Pathway & Other Pathways of Hexose Metabolism

20

Peter A. Mayes, PhD, DSc, & David A. Bender, PhD

BIOMEDICAL IMPORTANCE

The pentose phosphate pathway is an alternative route for the metabolism of glucose. It does not generate ATP but has two major functions: (1) The formation of **NADPH** for synthesis of fatty acids and steroids and (2) the synthesis of **ribose** for nucleotide and nucleic acid formation. Glucose, fructose, and galactose are the main hexoses absorbed from the gastrointestinal tract, derived principally from dietary starch, sucrose, and lactose, respectively. Fructose and galactose are converted to glucose, mainly in the liver.

Genetic deficiency of glucose 6-phosphate dehydrogenase, the first enzyme of the pentose phosphate pathway, is a major cause of hemolysis of red blood cells, resulting in **hemolytic anemia** and affecting approximately 100 million people worldwide. Glucuronic acid is synthesized from glucose via the **uronic acid pathway,** of major significance for the excretion of metabolites and foreign chemicals (xenobiotics) as **glucuronides.** A deficiency in the pathway leads to **essential pentosuria.** The lack of one enzyme of the pathway (gulonolactone oxidase) in primates and some other animals explains why **ascorbic acid** (vitamin C) is a dietary requirement for humans but not most other mammals. Deficiencies in the enzymes of fructose and galactose metabolism lead to **essential fructosuria** and the **galactosemias.**

THE PENTOSE PHOSPHATE PATHWAY GENERATES NADPH & RIBOSE PHOSPHATE (Figure 20–1)

The pentose phosphate pathway (hexose monophosphate shunt) is a more complex pathway than glycolysis. Three molecules of glucose 6-phosphate give rise to three molecules of CO_2 and three five-carbon sugars. These are rearranged to regenerate two molecules of glucose 6-phosphate and one molecule of the glycolytic intermediate, glyceraldehyde 3-phosphate. Since two molecules of glyceraldehyde 3-phosphate can regenerate glucose 6-phosphate, the pathway can account for the complete oxidation of glucose.

REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY OCCUR IN THE CYTOSOL

The enzymes of the pentose phosphate pathway, as of glycolysis, are cytosolic. As in glycolysis, oxidation is achieved by dehydrogenation; but $NADP^+$ and not NAD^+ is the hydrogen acceptor. The sequence of reactions of the pathway may be divided into two phases: an **oxidative nonreversible phase** and a **nonoxidative reversible phase.** In the first phase, glucose 6-phosphate undergoes dehydrogenation and decarboxylation to yield a pentose, ribulose 5-phosphate. In the second phase, ribulose 5-phosphate is converted back to glucose 6-phosphate by a series of reactions involving mainly two enzymes: **transketolase** and **transaldolase** (Figure 20–1).

The Oxidative Phase Generates NADPH (Figures 20–1 and 20–2)

Dehydrogenation of glucose 6-phosphate to 6-phosphogluconate occurs via the formation of 6-phosphogluconolactone, catalyzed by **glucose-6-phosphate dehydrogenase,** an NADP-dependent enzyme. The hydrolysis of 6-phosphogluconolactone is accomplished by the enzyme **gluconolactone hydrolase.** A second oxidative step is catalyzed by **6-phosphogluconate dehydrogenase,** which also requires $NADP^+$ as hydrogen acceptor and involves decarboxylation followed by formation of the ketopentose, ribulose 5-phosphate.

The Nonoxidative Phase Generates Ribose Precursors

Ribulose 5-phosphate is the substrate for two enzymes. **Ribulose 5-phosphate 3-epimerase** alters the configuration about carbon 3, forming another ketopentose, xylulose 5-phosphate. **Ribose 5-phosphate ketoisomerase** converts ribulose 5-phosphate to the corresponding aldopentose, ribose 5-phosphate, which is the precursor of the ribose required for nucleotide and nucleic acid synthesis. **Transketolase** transfers the two-carbon

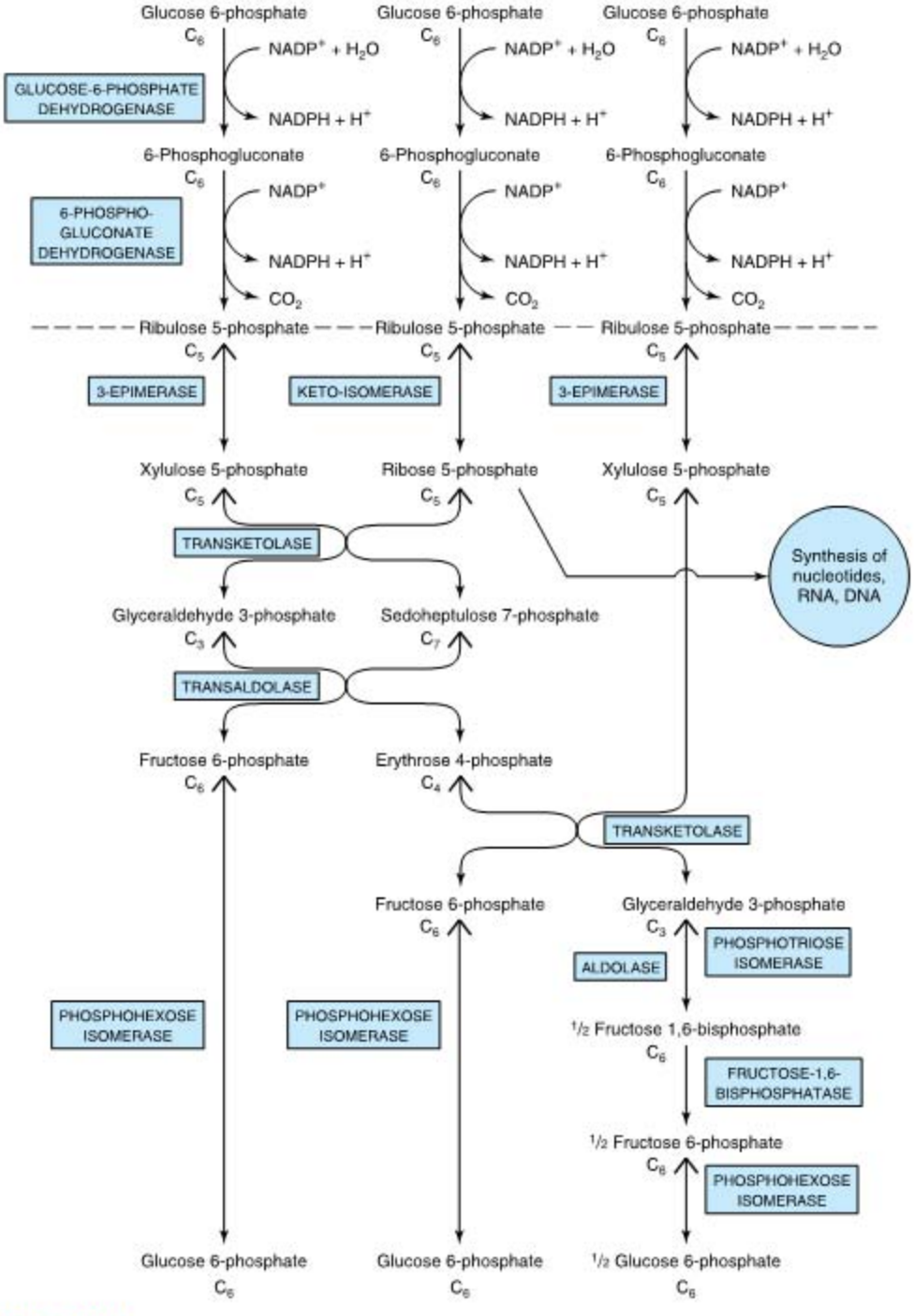

Figure 20–1. Flow chart of pentose phosphate pathway and its connections with the pathway of glycolysis. The full pathway, as indicated, consists of three interconnected cycles in which glucose 6-phosphate is both substrate and end product. The reactions above the broken line are nonreversible, whereas all reactions under that line are freely reversible apart from that catalyzed by fructose-1,6-bisphosphatase.

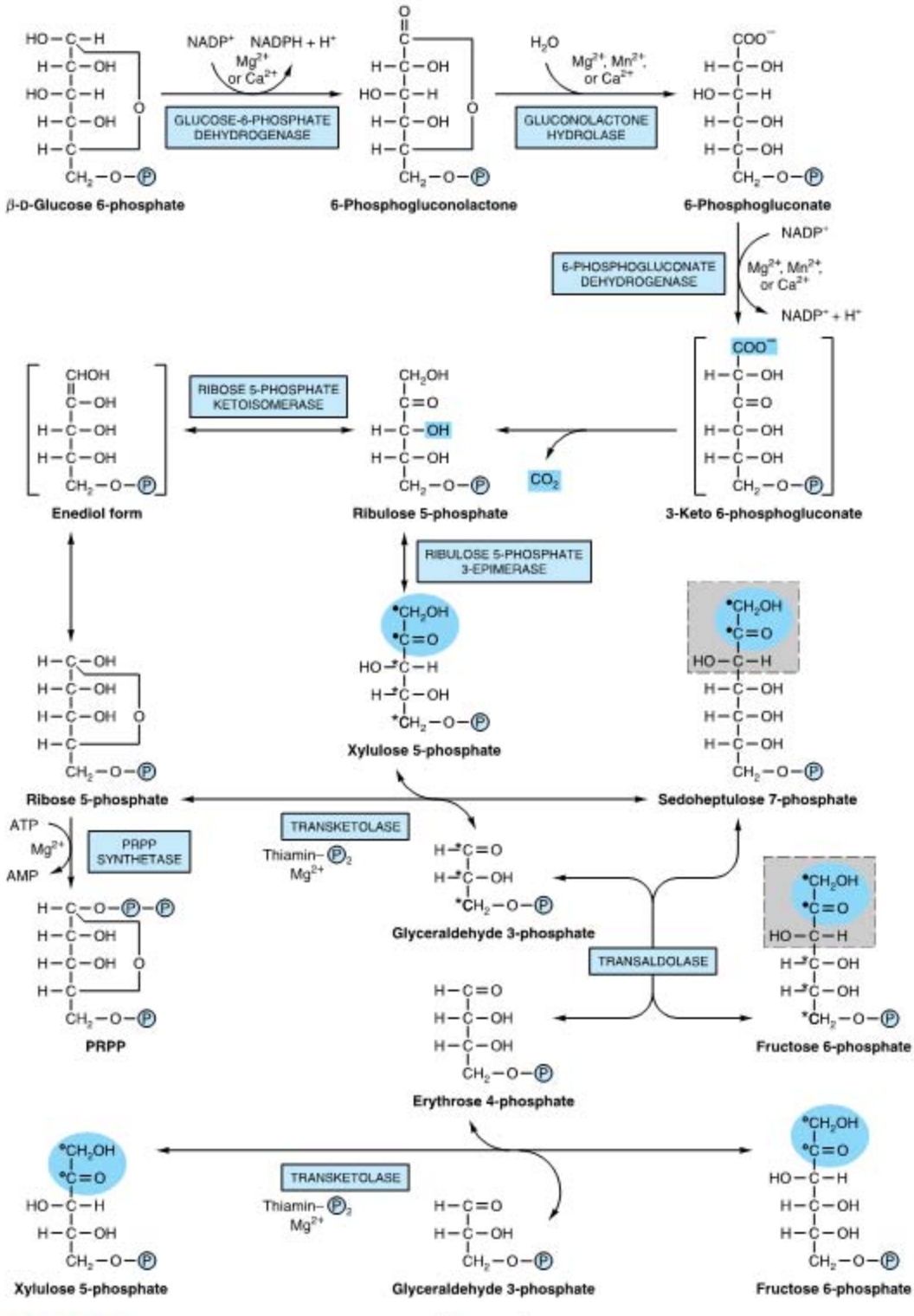

Figure 20–2. The pentose phosphate pathway. (Ⓟ, —PO_3^{2-}; PRPP, 5-phosphoribosyl 1-pyrophosphate.)

unit comprising carbons 1 and 2 of a ketose onto the aldehyde carbon of an aldose sugar. It therefore effects the conversion of a ketose sugar into an aldose with two carbons less and simultaneously converts an aldose sugar into a ketose with two carbons more. The reaction requires Mg^{2+} and **thiamin diphosphate** (vitamin B_1) as coenzyme. Thus, transketolase catalyzes the transfer of the two-carbon unit from xylulose 5-phosphate to ribose 5-phosphate, producing the seven-carbon ketose sedoheptulose 7-phosphate and the aldose glyceraldehyde 3-phosphate. **Transaldolase** allows the transfer of a three-carbon dihydroxyacetone moiety (carbons 1–3) from the ketose sedoheptulose 7-phosphate onto the aldose glyceraldehyde 3-phosphate to form the ketose fructose 6-phosphate and the four-carbon aldose erythrose 4-phosphate. In a further reaction catalyzed by **transketolase,** xylulose 5-phosphate donates a two-carbon unit to erythrose 4-phosphate to form fructose 6-phosphate and glyceraldehyde 3-phosphate.

In order to oxidize glucose completely to CO_2 via the pentose phosphate pathway, there must be enzymes present in the tissue to convert glyceraldehyde 3-phosphate to glucose 6-phosphate. This involves reversal of glycolysis and the gluconeogenic enzyme **fructose 1,6-bisphosphatase.** In tissues that lack this enzyme, glyceraldehyde 3-phosphate follows the normal pathway of glycolysis to pyruvate.

The Two Major Pathways for the Catabolism of Glucose Have Little in Common

Although glucose 6-phosphate is common to both pathways, the pentose phosphate pathway is markedly different from glycolysis. Oxidation utilizes NADP rather than NAD, and CO_2, which is not produced in glycolysis, is a characteristic product. No ATP is generated in the pentose phosphate pathway, whereas ATP is a major product of glycolysis.

Reducing Equivalents Are Generated in Those Tissues Specializing in Reductive Syntheses

The pentose phosphate pathway is active in liver, adipose tissue, adrenal cortex, thyroid, erythrocytes, testis, and lactating mammary gland. Its activity is low in nonlactating mammary gland and skeletal muscle. Those tissues in which the pathway is active use NADPH in reductive syntheses, eg, of fatty acids, steroids, amino acids via glutamate dehydrogenase, and reduced glutathione. The synthesis of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase may also be induced by insulin during conditions associated with the "fed state" (Table 19–1), when lipogenesis increases.

Ribose Can Be Synthesized in Virtually All Tissues

Little or no ribose circulates in the bloodstream, so tissues must synthesize the ribose required for nucleotide and nucleic acid synthesis (Chapter 34). The source of ribose 5-phosphate is the pentose phosphate pathway (Figure 20–2). Muscle has only low activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Nevertheless, like most other tissues, it is capable of synthesizing ribose 5-phosphate by reversal of the nonoxidative phase of the pentose phosphate pathway utilizing fructose 6-phosphate. It is not necessary to have a completely functioning pentose phosphate pathway for a tissue to synthesize ribose phosphates.

THE PENTOSE PHOSPHATE PATHWAY & GLUTATHIONE PEROXIDASE PROTECT ERYTHROCYTES AGAINST HEMOLYSIS

In erythrocytes, the pentose phosphate pathway provides NADPH for the reduction of oxidized glutathione catalyzed by **glutathione reductase,** a flavoprotein containing FAD. Reduced glutathione removes H_2O_2 in a reaction catalyzed by **glutathione peroxidase,** an enzyme that contains the **selenium** analogue of cysteine (selenocysteine) at the active site (Figure 20–3). This reaction is important, since accumulation of H_2O_2 may decrease the life span of the erythrocyte by causing oxidative damage to the cell membrane, leading to hemolysis.

GLUCURONATE, A PRECURSOR OF PROTEOGLYCANS & CONJUGATED GLUCURONIDES, IS A PRODUCT OF THE URONIC ACID PATHWAY

In liver, the **uronic acid pathway** catalyzes the conversion of glucose to glucuronic acid, ascorbic acid, and pentoses (Figure 20–4). It is also an alternative oxidative pathway for glucose, but—like the pentose phosphate pathway—it does not lead to the generation of ATP. Glucose 6-phosphate is isomerized to glucose 1-phosphate, which then reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDPGlc) in a reaction catalyzed by **UDPGlc pyrophosphorylase,** as occurs in glycogen synthesis (Chapter 18). UDPGlc is oxidized at carbon 6 by NAD-dependent **UDPGlc dehydrogenase** in a two-step reaction to yield UDP-glucuronate. UDP-glucuronate is the "active" form of glucuronate for reactions involving incorporation of glucuronic acid into proteoglycans or for reactions in which substrates such as steroid hormones, bilirubin, and a number of drugs are conjugated with glucuronate for excretion in urine or bile (Figure 32–14).

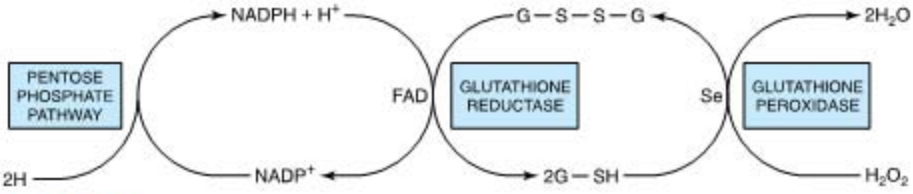

Figure 20–3. Role of the pentose phosphate pathway in the glutathione peroxidase reaction of erythrocytes. (G-S-S-G, oxidized glutathione; G-SH, reduced glutathione; Se, selenium cofactor.)

Glucuronate is reduced to L-gulonate in an NADPH-dependent reaction; L-gulonate is the direct precursor of **ascorbate** in those animals capable of synthesizing this vitamin. In humans and other primates as well as guinea pigs, ascorbic acid cannot be synthesized because of the absence of **L-gulonolactone oxidase.** L-Gulonate is metabolized ultimately to D-xylulose 5-phosphate, a constituent of the pentose phosphate pathway.

INGESTION OF LARGE QUANTITIES OF FRUCTOSE HAS PROFOUND METABOLIC CONSEQUENCES

Diets high in sucrose or in high-fructose syrups used in manufactured foods and beverages lead to large amounts of fructose (and glucose) entering the hepatic portal vein. Fructose undergoes more rapid glycolysis in the liver than does glucose because it bypasses the regulatory step catalyzed by phosphofructokinase (Figure 20–5). This allows fructose to flood the pathways in the liver, leading to enhanced fatty acid synthesis, increased esterification of fatty acids, and increased VLDL secretion, which may raise serum triacylglycerols and ultimately raise LDL cholesterol concentrations (Figure 25–6). A specific kinase, **fructokinase,** in liver (and kidney and intestine) catalyzes the phosphorylation of fructose to fructose 1-phosphate. This enzyme does not act on glucose, and, unlike glucokinase, its activity is not affected by fasting or by insulin, which may explain why fructose is cleared from the blood of diabetic patients at a normal rate. Fructose 1-phosphate is cleaved to D-glyceraldehyde and dihydroxyacetone phosphate by **aldolase B,** an enzyme found in the liver, which also functions in glycolysis by cleaving fructose 1,6-bisphosphate. D-Glyceraldehyde enters glycolysis via phosphorylation to glyceraldehyde 3-phosphate, catalyzed by **triokinase.** The two triose phosphates, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, may be degraded by glycolysis or may be substrates for aldolase and hence gluconeogenesis, which is the fate of much of the fructose metabolized in the liver.

In extrahepatic tissues, hexokinase catalyzes the phosphorylation of most hexose sugars, including fructose. However, glucose inhibits the phosphorylation of fructose since it is a better substrate for hexokinase. Nevertheless, some fructose can be metabolized in adipose tissue and muscle. Fructose, a potential fuel, is found in seminal plasma and in the fetal circulation of ungulates and whales. **Aldose reductase** is found in the placenta of the ewe and is responsible for the secretion of sorbitol into the fetal blood. The presence of **sorbitol dehydrogenase** in the liver, including the fetal liver, is responsible for the conversion of sorbitol into fructose. This pathway is also responsible for the occurrence of fructose in seminal fluid.

GALACTOSE IS NEEDED FOR THE SYNTHESIS OF LACTOSE, GLYCOLIPIDS, PROTEOGLYCANS, & GLYCOPROTEINS

Galactose is derived from intestinal hydrolysis of the disaccharide **lactose,** the sugar of milk. It is readily converted in the liver to glucose. **Galactokinase** catalyzes the phosphorylation of galactose, using ATP as phosphate donor (Figure 20–6A). Galactose 1-phosphate reacts with uridine diphosphate glucose (UDPGlc) to form uridine diphosphate galactose (UDPGal) and glucose 1-phosphate, in a reaction catalyzed by **galactose 1-phosphate uridyl transferase.** The conversion of UDPGal to UDPGlc is catalyzed by **UDPGal 4-epimerase.** Epimerization involves an oxidation and reduction at carbon 4 with NAD^+ as coenzyme. Finally, glucose is liberated from UDPGlc after conversion to glucose 1-phosphate, probably via incorporation into glycogen followed by phosphorolysis (Chapter 18).

Since the epimerase reaction is freely reversible, glucose can be converted to galactose, so that galactose is not a dietary essential. Galactose is required in the body not only in the formation of lactose but also as a constituent of glycolipids (cerebrosides), proteoglycans, and glycoproteins. In the synthesis of lactose in the mammary gland, UDPGal condenses with glucose to yield lactose, catalyzed by **lactose synthase** (Figure 20–6B).

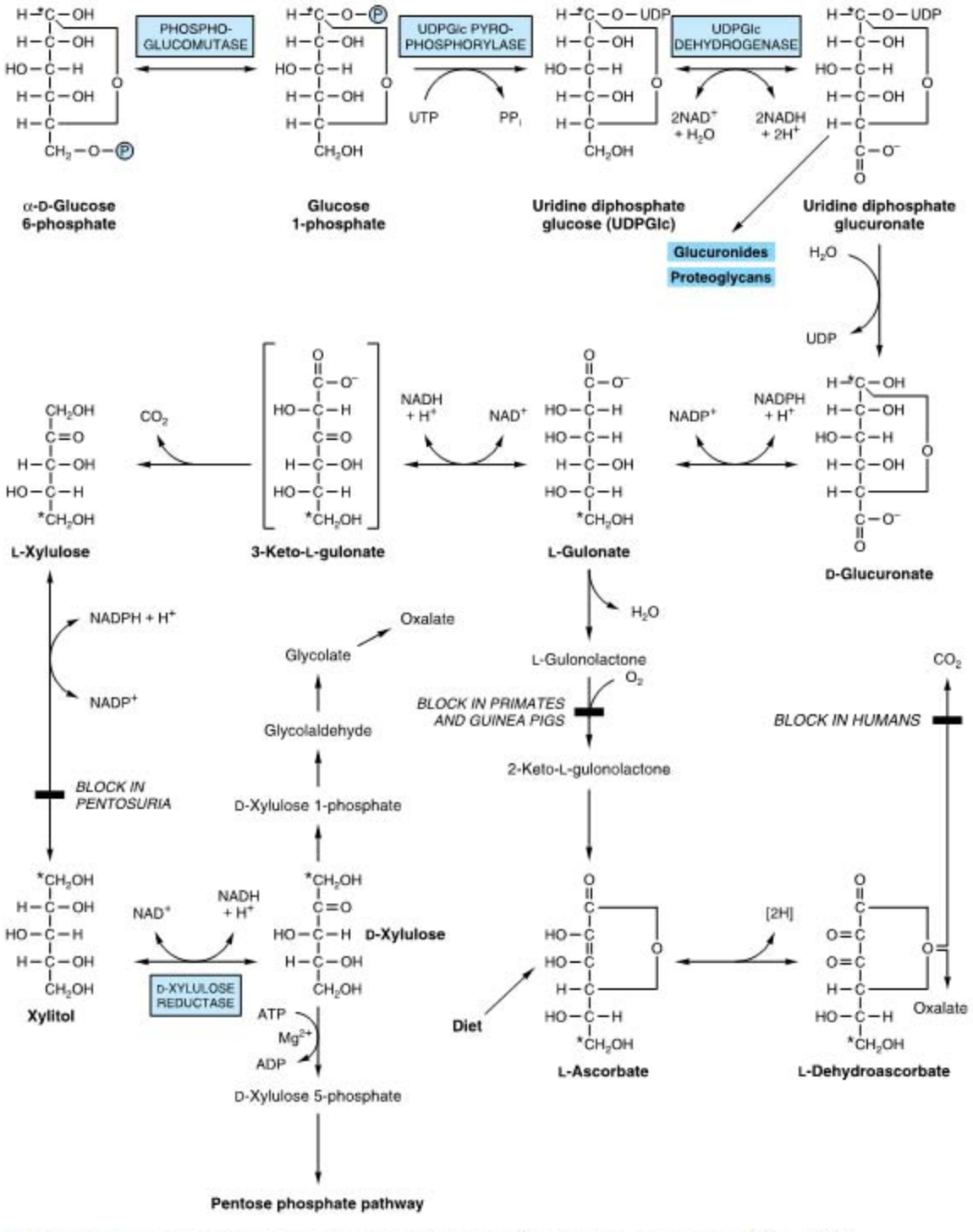

Figure 20–4. Uronic acid pathway. (Asterisk indicates the fate of carbon 1 of glucose; Ⓟ, —PO_3^{2-}.)

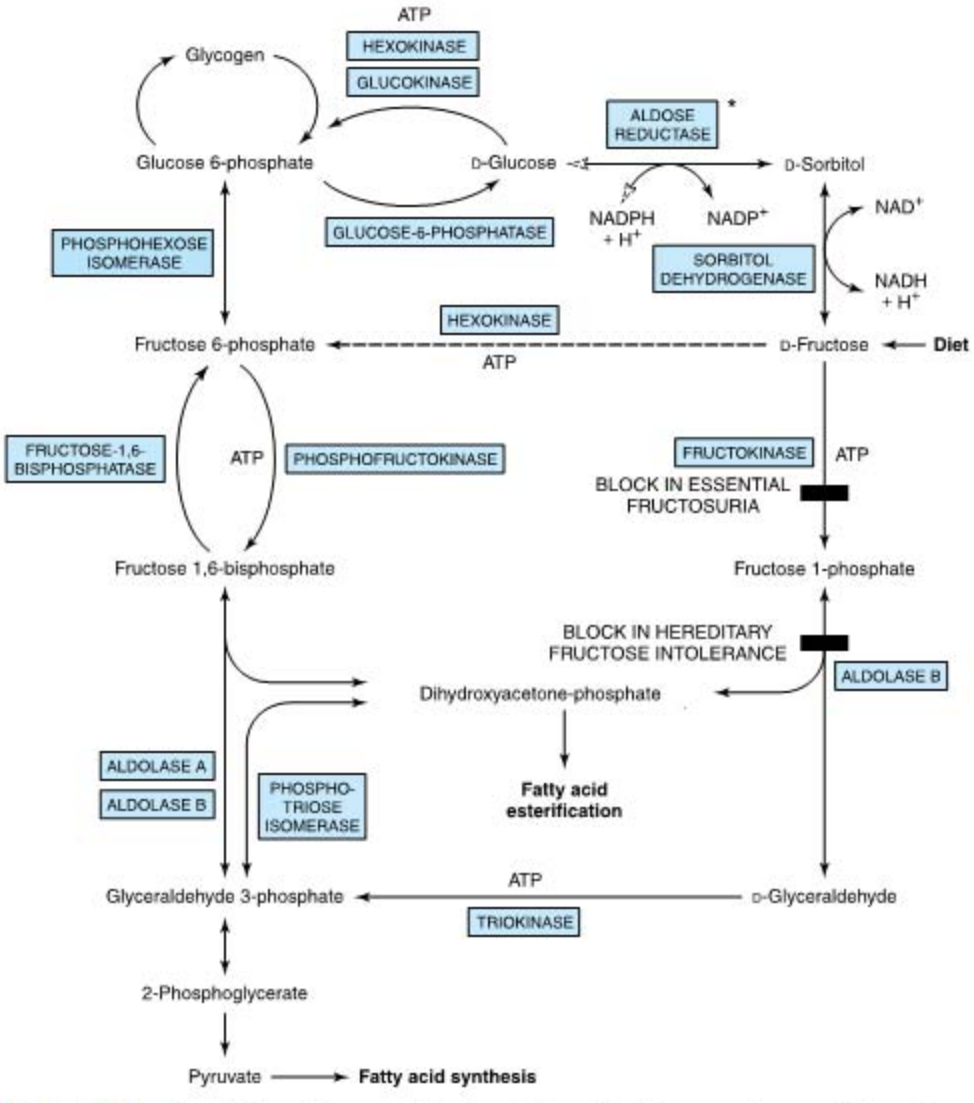

Figure 20–5. Metabolism of fructose. Aldolase A is found in all tissues, whereas aldolase B is the predominant form in liver. (*, not found in liver.)

Glucose Is the Precursor of All Amino Sugars (Hexosamines)

Amino sugars are important components of **glycoproteins** (Chapter 47), of certain **glycosphingolipids** (eg, gangliosides) (Chapter 14), and of **glycosaminoglycans** (Chapter 48). The major amino sugars are **glucosamine, galactosamine,** and **mannosamine** and the nine-carbon compound **sialic acid.** The principal sialic acid found in human tissues is ***N*-acetylneuraminic acid** (NeuAc). A summary of the metabolic interrelationships among the amino sugars is shown in Figure 20–7.

CLINICAL ASPECTS

Impairment of the Pentose Phosphate Pathway Leads to Erythrocyte Hemolysis

Genetic deficiency of glucose-6-phosphate dehydrogenase, with consequent impairment of the generation of NADPH, is common in populations of Mediterranean and Afro-Caribbean origin. The defect is manifested as red cell hemolysis **(hemolytic anemia)** when susceptible individuals are subjected to oxidants, such as the antimalarial primaquine, aspirin, or sulfonamides or when

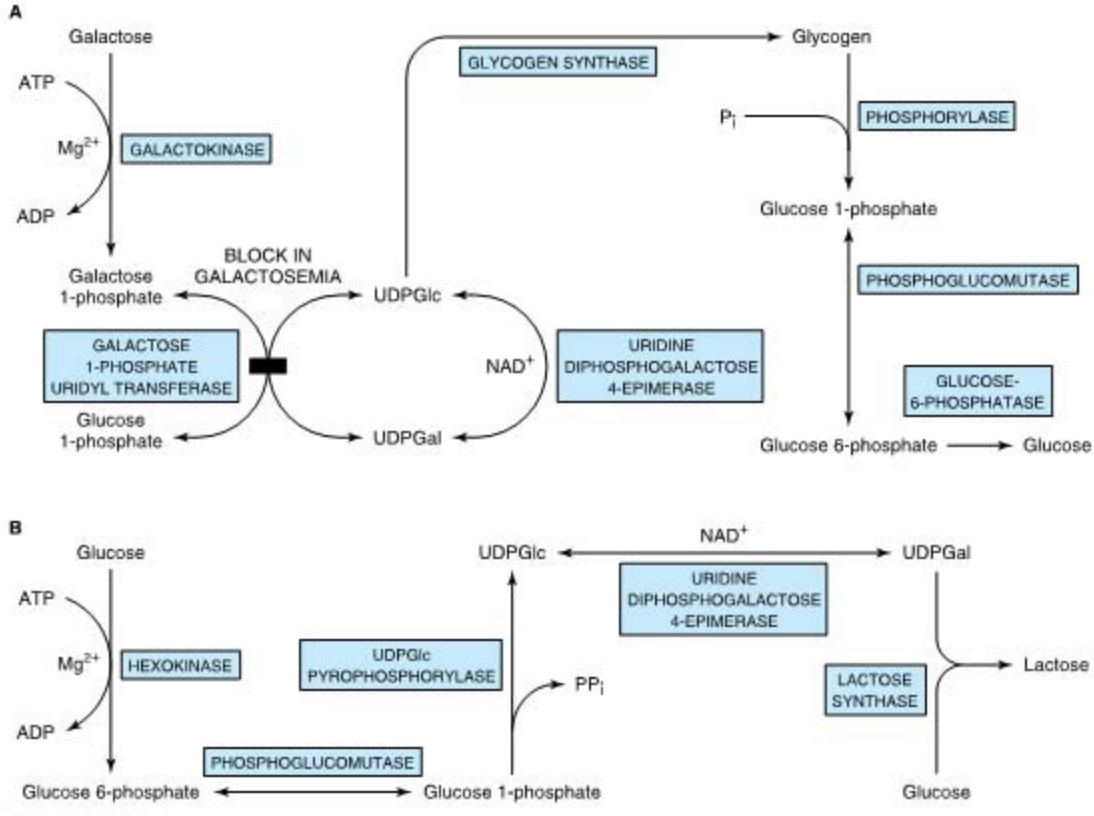

Figure 20–6. Pathway of conversion of **(A)** galactose to glucose in the liver and **(B)** glucose to lactose in the lactating mammary gland.

they have eaten fava beans (*Vicia fava*—hence the term **favism**). Glutathione peroxidase is dependent upon a supply of NADPH, which in erythrocytes can be formed only via the pentose phosphate pathway. It reduces organic peroxides and H_2O_2 as part of the body's defense against lipid peroxidation (Figure 14–21). Measurement of erythrocyte **transketolase** and its activation by thiamin diphosphate is used to assess thiamin nutritional status (Chapter 45).

Disruption of the Uronic Acid Pathway Is Caused by Enzyme Defects & Some Drugs

In the rare hereditary disease **essential pentosuria,** considerable quantities of **L-xylulose** appear in the urine because of absence of the enzyme necessary to reduce L-xylulose to xylitol. Parenteral administration of xylitol may lead to **oxalosis,** involving calcium oxalate deposition in brain and kidneys (Figure 20–4). Various drugs markedly increase the rate at which glucose enters the uronic acid pathway. For example, administration of barbital or of chlorobutanol to rats results in a significant increase in the conversion of glucose to glucuronate, L-gulonate, and ascorbate.

Loading of the Liver With Fructose May Potentiate Hyperlipidemia & Hyperuricemia

In the liver, fructose increases triacylglycerol synthesis and VLDL secretion, leading to hypertriacylglycerolemia—and increased LDL cholesterol—which can be regarded as potentially atherogenic (Chapter 26). In addition, acute loading of the liver with fructose, as can occur with intravenous infusion or following very high fructose intakes, causes sequestration of inorganic phosphate in fructose 1-phosphate and diminished ATP synthesis. As a result there is less inhibition of de novo purine synthesis by ATP and uric acid formation is in-

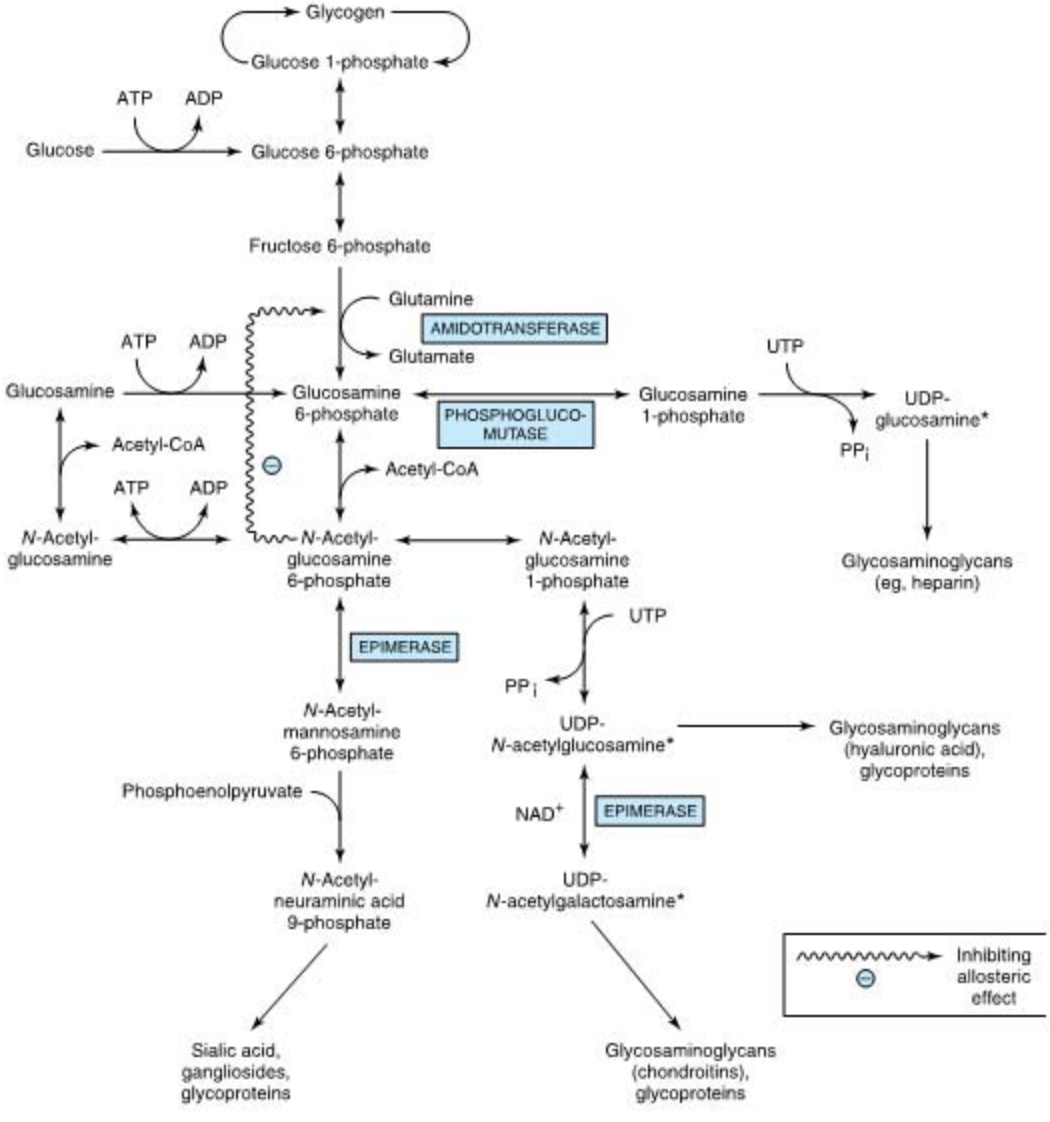

Figure 20–7. Summary of the interrelationships in metabolism of amino sugars. (At asterisk: Analogous to UDPGlc.) Other purine or pyrimidine nucleotides may be similarly linked to sugars or amino sugars. Examples are thymidine diphosphate (TDP)-glucosamine and TDP-*N*-acetylglucosamine.

creased, causing hyperuricemia, which is a cause of gout (Chapter 34).

Defects in Fructose Metabolism Cause Disease (Figure 20–5)

Lack of hepatic fructokinase causes **essential fructosuria,** and absence of hepatic aldolase B, which cleaves fructose 1-phosphate, leads to **hereditary fructose intolerance.** Diets low in fructose, sorbitol, and sucrose are beneficial for both conditions. One consequence of hereditary fructose intolerance and of another condition due to **fructose-1,6-bisphosphatase deficiency** is fructose-induced **hypoglycemia** despite the presence of high glycogen reserves. The accumulation of fructose 1-phosphate and fructose 1,6-bisphosphate allosterically

inhibits the activity of liver phosphorylase. The sequestration of inorganic phosphate also leads to depletion of ATP and hyperuricemia.

Fructose & Sorbitol in the Lens Are Associated With Diabetic Cataract

Both fructose and sorbitol are found in the lens of the eye in increased concentrations in diabetes mellitus and may be involved in the pathogenesis of **diabetic cataract.** The **sorbitol (polyol) pathway** (not found in liver) is responsible for fructose formation from glucose (Figure 20–5) and increases in activity as the glucose concentration rises in diabetes in those tissues that are not insulin-sensitive, ie, the lens, peripheral nerves, and renal glomeruli. Glucose is reduced to sorbitol by **aldose reductase,** followed by oxidation of sorbitol to fructose in the presence of NAD^+ and sorbitol dehydrogenase (polyol dehydrogenase). Sorbitol does not diffuse through cell membranes easily and accumulates, causing osmotic damage. Simultaneously, myoinositol levels fall. Sorbitol accumulation, myoinositol depletion, and diabetic cataract can be prevented by aldose reductase inhibitors in diabetic rats, and promising results have been obtained in clinical trials.

When sorbitol is administered intravenously, it is converted to fructose rather than to glucose. It is poorly absorbed in the small intestine, and much is fermented by colonic bacteria to short-chain fatty acids, CO_2, and H_2, leading to abdominal pain and diarrhea **(sorbitol intolerance).**

Enzyme Deficiencies in the Galactose Pathway Cause Galactosemia

Inability to metabolize galactose occurs in the **galactosemias,** which may be caused by inherited defects in galactokinase, uridyl transferase, or 4-epimerase (Figure 20–6A), though a deficiency in **uridyl transferase** is the best known cause. The galactose concentration in the blood and in the eye is reduced by aldose reductase to galactitol, which accumulates, causing cataract. In uridyl transferase deficiency, galactose 1-phosphate accumulates and depletes the liver of inorganic phosphate. Ultimately, liver failure and mental deterioration result. As the epimerase is present in adequate amounts, the galactosemic individual can still form UDPGal from glucose, and normal growth and development can occur regardless of the galactose-free diets used to control the symptoms of the disease.

SUMMARY

- The pentose phosphate pathway, present in the cytosol, can account for the complete oxidation of glucose, producing NADPH and CO_2 but not ATP.
- The pathway has an oxidative phase, which is irreversible and generates NADPH; and a nonoxidative phase, which is reversible and provides ribose precursors for nucleotide synthesis. The complete pathway is present only in those tissues having a requirement for NADPH for reductive syntheses, eg, lipogenesis or steroidogenesis, whereas the nonoxidative phase is present in all cells requiring ribose.
- In erythrocytes, the pathway has a major function in preventing hemolysis by providing NADPH to maintain glutathione in the reduced state as the substrate for glutathione peroxidase.
- The uronic acid pathway is the source of glucuronic acid for conjugation of many endogenous and exogenous substances before excretion as glucuronides in urine and bile.
- Fructose bypasses the main regulatory step in glycolysis, catalyzed by phosphofructokinase, and stimulates fatty acid synthesis and hepatic triacylglycerol secretion.
- Galactose is synthesized from glucose in the lactating mammary gland and in other tissues where it is required for the synthesis of glycolipids, proteoglycans, and glycoproteins.

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Biosynthesis of Fatty Acids

21

Peter A. Mayes, PhD, DSc, & Kathleen M. Botham, PhD, DSc

BIOMEDICAL IMPORTANCE

Fatty acids are synthesized by an **extramitochondrial system**, which is responsible for the complete synthesis of palmitate from acetyl-CoA in the cytosol. In the rat, the pathway is well represented in adipose tissue and liver, whereas in humans adipose tissue may not be an important site, and liver has only low activity. In birds, lipogenesis is confined to the liver, where it is particularly important in providing lipids for egg formation. In most mammals, glucose is the primary substrate for lipogenesis, but in ruminants it is acetate, the main fuel molecule produced by the diet. Critical diseases of the pathway have not been reported in humans. However, inhibition of lipogenesis occurs in type 1 (insulin-dependent) **diabetes mellitus,** and variations in its activity may affect the nature and extent of **obesity**.

THE MAIN PATHWAY FOR DE NOVO SYNTHESIS OF FATTY ACIDS (LIPOGENESIS) OCCURS IN THE CYTOSOL

This system is present in many tissues, including liver, kidney, brain, lung, mammary gland, and adipose tissue. Its cofactor requirements include NADPH, ATP, Mn^{2+}, biotin, and HCO_3^- (as a source of CO_2). **Acetyl-CoA** is the immediate substrate, and **free palmitate** is the end product.

Production of Malonyl-CoA Is the Initial & Controlling Step in Fatty Acid Synthesis

Bicarbonate as a source of CO_2 is required in the initial reaction for the carboxylation of acetyl-CoA to **malonyl-CoA** in the presence of ATP and **acetyl-CoA carboxylase.** Acetyl-CoA carboxylase has a requirement for the vitamin **biotin** (Figure 21–1). The enzyme is a **multienzyme protein** containing a variable number of identical subunits, each containing biotin, biotin carboxylase, biotin carboxyl carrier protein, and transcarboxylase, as well as a regulatory allosteric site. The reaction takes place in two steps: (1) carboxylation of biotin involving ATP and (2) transfer of the carboxyl to acetyl-CoA to form malonyl-CoA.

The Fatty Acid Synthase Complex Is a Polypeptide Containing Seven Enzyme Activities

In bacteria and plants, the individual enzymes of the **fatty acid synthase** system are separate, and the acyl radicals are found in combination with a protein called the **acyl carrier protein (ACP).** However, in yeast, mammals, and birds, the synthase system is a multienzyme polypeptide complex that incorporates ACP, which takes over the role of CoA. It contains the vitamin **pantothenic acid** in the form of 4′-phosphopantetheine (Figure 45–18). The use of one multienzyme functional unit has the advantages of achieving the effect of compartmentalization of the process within the cell without the erection of permeability barriers, and synthesis of all enzymes in the complex is coordinated since it is encoded by a single gene.

In mammals, the fatty acid synthase complex is a dimer comprising two identical monomers, each containing all seven enzyme activities of fatty acid synthase on one polypeptide chain (Figure 21–2). Initially, a priming molecule of acetyl-CoA combines with a cysteine —SH group catalyzed by **acetyl transacylase** (Figure 21–3, reaction 1a). Malonyl-CoA combines with the adjacent —SH on the 4′-phosphopantetheine of ACP of the other monomer, catalyzed by **malonyl transacylase** (reaction 1b), to form **acetyl (acyl)-malonyl enzyme.** The acetyl group attacks the methylene group of the malonyl residue, catalyzed by **3-ketoacyl synthase,** and liberates CO_2, forming 3-ketoacyl enzyme (acetoacetyl enzyme) (reaction 2), freeing the cysteine —SH group. Decarboxylation allows the reaction to go to completion, pulling the whole sequence of reactions in the forward direction. The 3-ketoacyl group is reduced, dehydrated, and reduced again (reactions 3, 4, 5) to form the corresponding saturated acyl-S-enzyme. A new malonyl-CoA molecule combines with the —SH of 4′-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine —SH group. The sequence of reactions is repeated six more times until a saturated 16-carbon acyl radical (palmityl) has been assembled. It is liberated from the enzyme complex by the activity of a seventh enzyme in the complex, **thioesterase** (deacylase). The free palmitate must be activated to acyl-CoA before it can proceed via any other

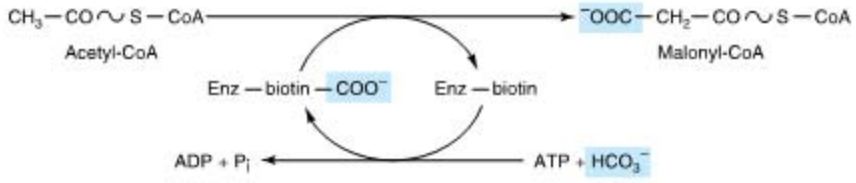

Figure 21–1. Biosynthesis of malonyl-CoA. (Enz, acetyl-CoA carboxylase.)

metabolic pathway. Its usual fate is esterification into acylglycerols, chain elongation or desaturation, or esterification to cholesteryl ester. In mammary gland, there is a separate thioesterase specific for acyl residues of C_8, C_{10}, or C_{12}, which are subsequently found in milk lipids.

The equation for the overall synthesis of palmitate from acetyl-CoA and malonyl-CoA is:

$$CH_2CO \cdot S \cdot CoA + 7HOOC \cdot CH_2CO \cdot S \cdot CoA + 14NADPH + 14H^+ \rightarrow CH_3(CH_2)_{14}COOH + 7CO_2 + 6H_2O + 8CoA \cdot SH + 14NADP^+$$

The acetyl-CoA used as a primer forms carbon atoms 15 and 16 of palmitate. The addition of all the subsequent C_2 units is via malonyl-CoA. Propionyl-CoA acts as primer for the synthesis of long-chain fatty

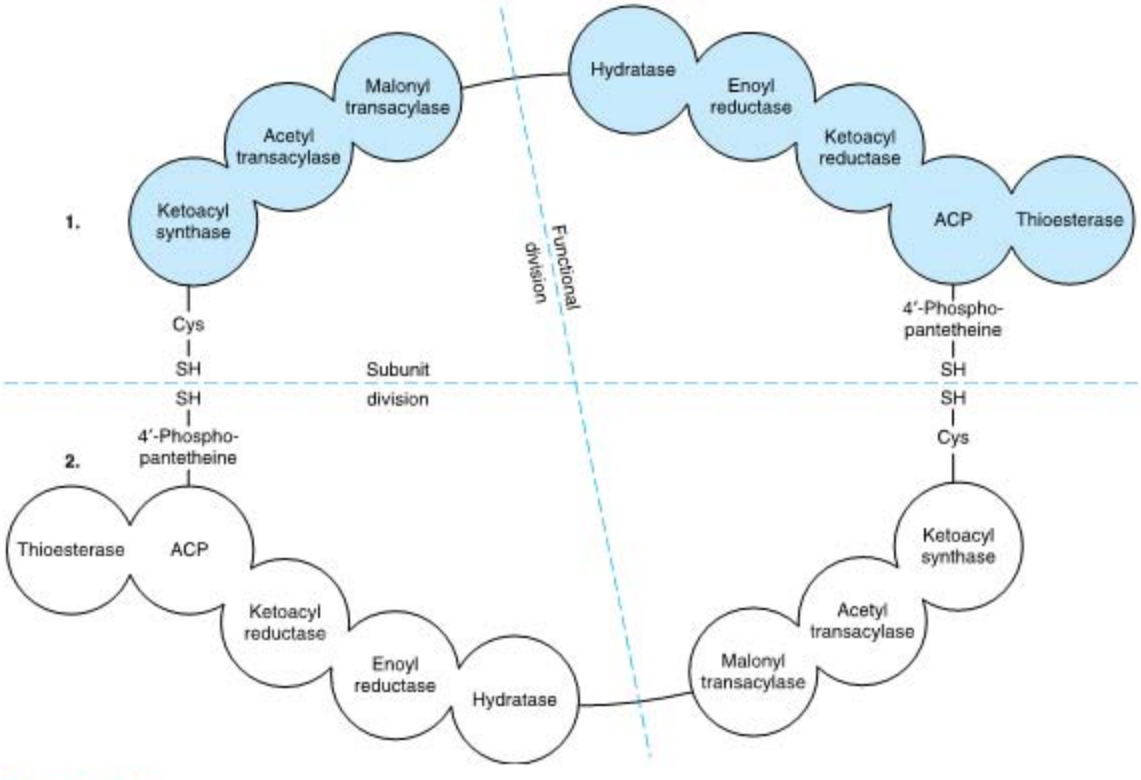

Figure 21–2. Fatty acid synthase multienzyme complex. The complex is a dimer of two identical polypeptide monomers, 1 and 2, each consisting of seven enzyme activities and the acyl carrier protein (ACP). (Cys—SH, cysteine thiol.) The —SH of the 4′-phosphopantetheine of one monomer is in close proximity to the —SH of the cysteine residue of the ketoacyl synthase of the other monomer, suggesting a "head-to-tail" arrangement of the two monomers. Though each monomer contains all the partial activities of the reaction sequence, the actual functional unit consists of one-half of one monomer interacting with the complementary half of the other. Thus, two acyl chains are produced simultaneously. The sequence of the enzymes in each monomer is based on Wakil.

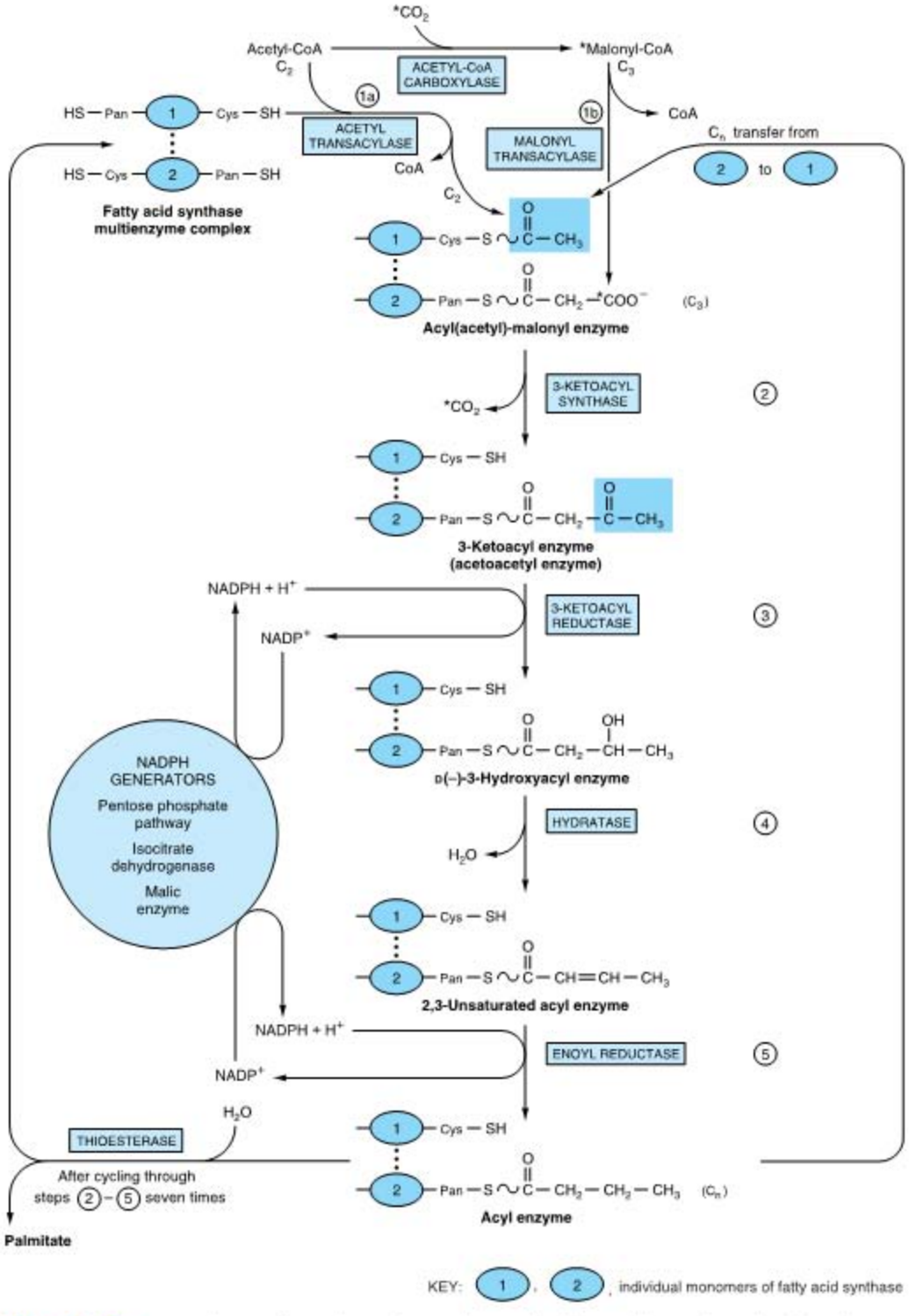

Figure 21–3. Biosynthesis of long-chain fatty acids. Details of how addition of a malonyl residue causes the acyl chain to grow by two carbon atoms. (Cys, cysteine residue; Pan, 4′-phosphopantetheine.) The blocks shown in dark blue contain initially a C_2 unit derived from acetyl-CoA (as illustrated) and subsequently the C_n unit formed in reaction 5.

acids having an odd number of carbon atoms, found particularly in ruminant fat and milk.

The Main Source of NADPH for Lipogenesis Is the Pentose Phosphate Pathway

NADPH is involved as donor of reducing equivalents in both the reduction of the 3-ketoacyl and of the 2,3-unsaturated acyl derivatives (Figure 21–3, reactions 3 and 5). The oxidative reactions of the pentose phosphate pathway (see Chapter 20) are the chief source of the hydrogen required for the reductive synthesis of fatty acids. Significantly, tissues specializing in active lipogenesis—ie, liver, adipose tissue, and the lactating mammary gland—also possess an active pentose phosphate pathway. Moreover, both metabolic pathways are found in the cytosol of the cell, so there are no membranes or permeability barriers against the transfer of NADPH. Other sources of NADPH include the reaction that converts malate to pyruvate catalyzed by the **"malic enzyme"** (NADP malate dehydrogenase) (Figure 21–4) and the extramitochondrial **isocitrate dehydrogenase** reaction (probably not a substantial source, except in ruminants).

Acetyl-CoA Is the Principal Building Block of Fatty Acids

Acetyl-CoA is formed from glucose via the oxidation of pyruvate within the mitochondria. However, it does not diffuse readily into the extramitochondrial cytosol,

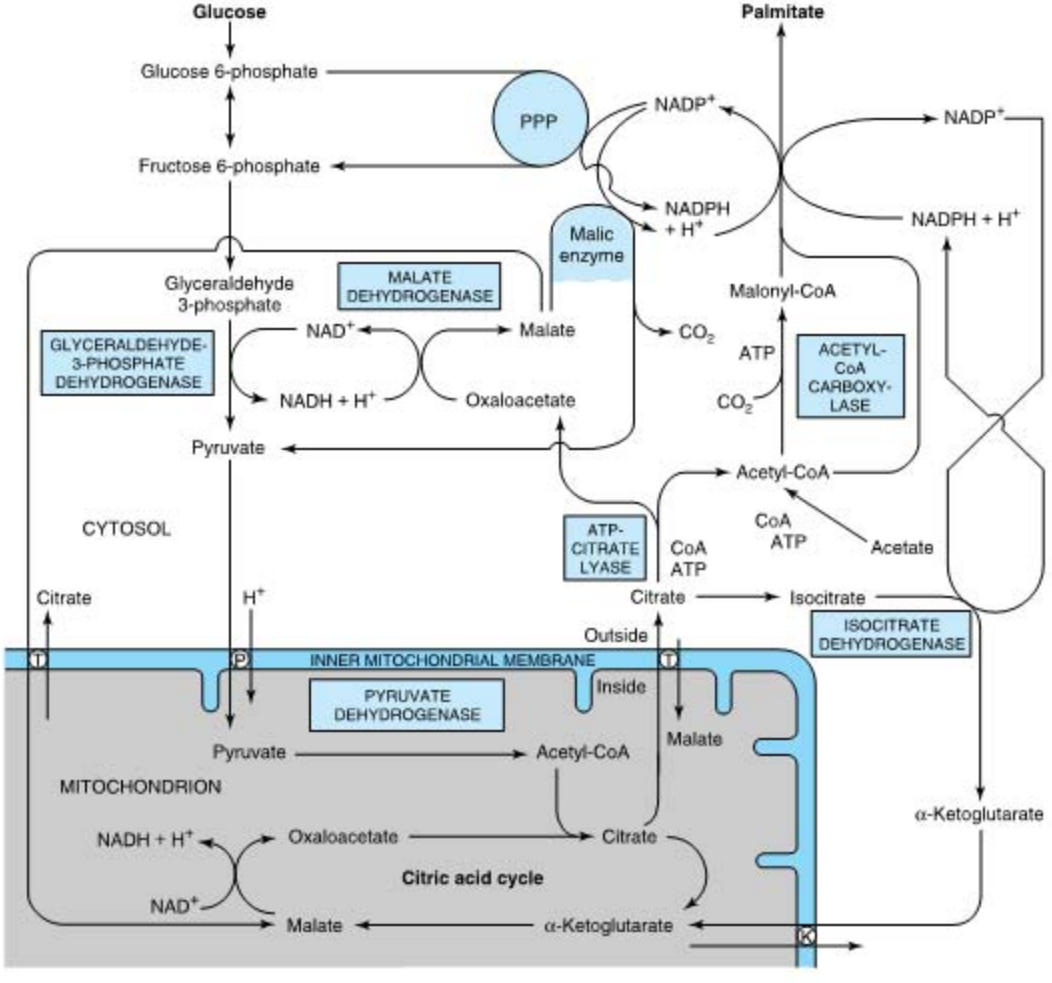

Figure 21–4. The provision of acetyl-CoA and NADPH for lipogenesis. (PPP, pentose phosphate pathway; T, tricarboxylate transporter; K, α-ketoglutarate transporter; P, pyruvate transporter.)

the principal site of fatty acid synthesis. Citrate, formed after condensation of acetyl-CoA with oxaloacetate in the citric acid cycle within mitochondria, is translocated into the extramitochondrial compartment via the tricarboxylate transporter, where in the presence of CoA and ATP it undergoes cleavage to acetyl-CoA and oxaloacetate catalyzed by **ATP-citrate lyase,** which increases in activity in the well-fed state. The acetyl-CoA is then available for malonyl-CoA formation and synthesis to palmitate (Figure 21–4). The resulting oxaloacetate can form malate via NADH-linked malate dehydrogenase, followed by the generation of NADPH via the malic enzyme. The NADPH becomes available for lipogenesis, and the pyruvate can be used to regenerate acetyl-CoA after transport into the mitochondrion. This pathway is a means of transferring reducing equivalents from extramitochondrial NADH to NADP. Alternatively, malate itself can be transported into the mitochondrion, where it is able to re-form oxaloacetate. Note that the citrate (tricarboxylate) transporter in the mitochondrial membrane requires malate to exchange with citrate (see Figure 12-10). There is little ATP-citrate lyase or malic enzyme in ruminants, probably because in these species acetate (derived from the rumen and activated to acetyl CoA extramitochondrially) is the main source of acetyl-CoA.

Elongation of Fatty Acid Chains Occurs in the Endoplasmic Reticulum

This pathway (the "microsomal system") elongates saturated and unsaturated fatty acyl-CoAs (from C_{10} upward) by two carbons, using malonyl-CoA as acetyl donor and NADPH as reductant, and is catalyzed by the microsomal **fatty acid elongase** system of enzymes (Figure 21–5). Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide C_{22} and C_{24} fatty acids for sphingolipids.

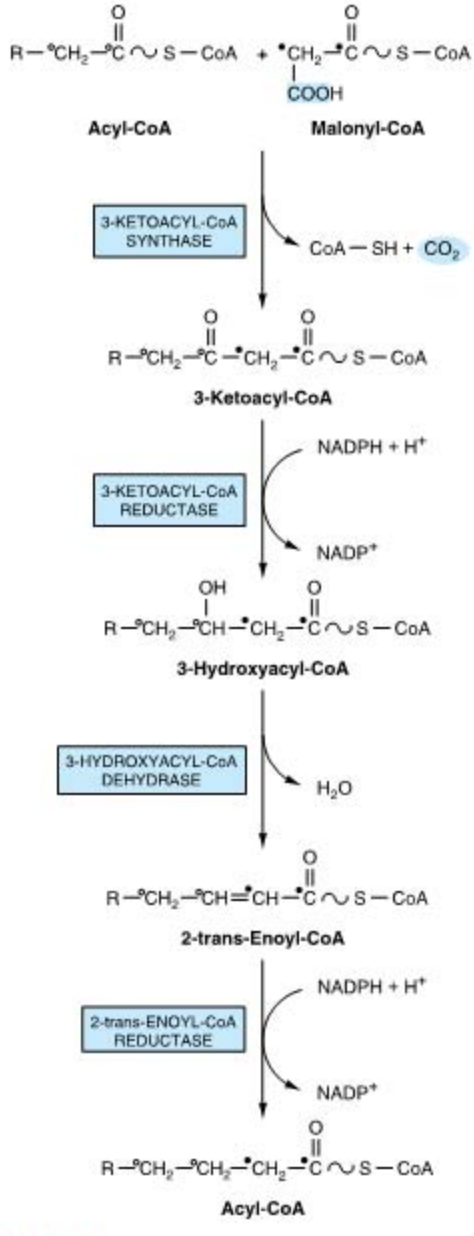

Figure 21–5. Microsomal elongase system for fatty acid chain elongation. NADH is also used by the reductases, but NADPH is preferred.

THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, etc, and to provide energy for use between meals in animals, including humans, that take their food at spaced intervals. Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle. The nutritional state of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is depressed under conditions of restricted caloric intake, on a fat diet, or when there is a deficiency of insulin, as in diabetes mellitus. These latter conditions are associated with increased concentrations of plasma free fatty acids, and an inverse relationship has been demonstrated between hepatic lipogenesis and the concentration of serum-free fatty acids. Lipogenesis is increased when su-

crose is fed instead of glucose because fructose bypasses the phosphofructokinase control point in glycolysis and floods the lipogenic pathway (Figure 20–5).

SHORT- & LONG-TERM MECHANISMS REGULATE LIPOGENESIS

Long-chain fatty acid synthesis is controlled in the short term by allosteric and covalent modification of enzymes and in the long term by changes in gene expression governing rates of synthesis of enzymes.

Acetyl-CoA Carboxylase Is the Most Important Enzyme in the Regulation of Lipogenesis

Acetyl-CoA carboxylase is an allosteric enzyme and is activated by **citrate,** which increases in concentration in the well-fed state and is an indicator of a plentiful supply of acetyl-CoA. Citrate converts the enzyme from an inactive dimer to an active polymeric form, having a molecular mass of several million. Inactivation is promoted by phosphorylation of the enzyme and by long-chain acyl-CoA molecules, an example of negative feedback inhibition by a product of a reaction. Thus, if acyl-CoA accumulates because it is not esterified quickly enough or because of increased lipolysis or an influx of free fatty acids into the tissue, it will automatically reduce the synthesis of new fatty acid. Acyl-CoA may also inhibit the mitochondrial **tricarboxylate transporter,** thus preventing activation of the enzyme by egress of citrate from the mitochondria into the cytosol.

Acetyl-CoA carboxylase is also regulated by hormones such as **glucagon, epinephrine,** and **insulin** via changes in its phosphorylation state (details in Figure 21–6).

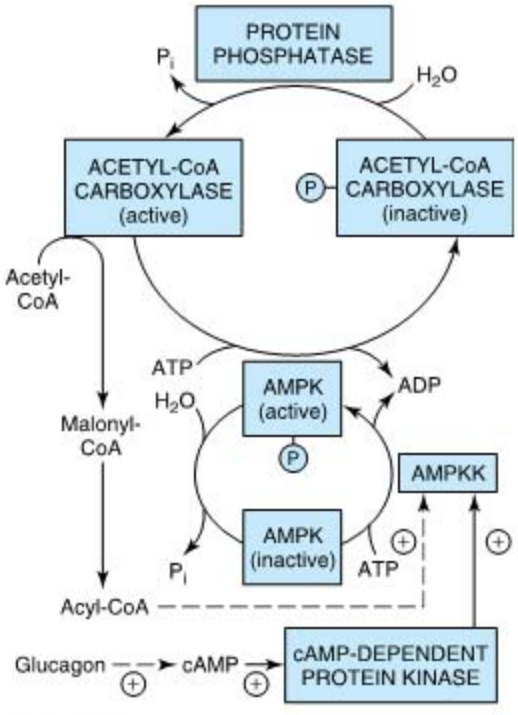

Figure 21–6. Regulation of acetyl-CoA carboxylase by phosphorylation/dephosphorylation. The enzyme is inactivated by phosphorylation by AMP-activated protein kinase (AMPK), which in turn is phosphorylated and activated by AMP-activated protein kinase kinase (AMPKK). Glucagon (and epinephrine), after increasing cAMP, activate this latter enzyme via cAMP-dependent protein kinase. The kinase kinase enzyme is also believed to be activated by acyl-CoA. Insulin activates acetyl-CoA carboxylase, probably through an "activator" protein and an insulin-stimulated protein kinase.

Pyruvate Dehydrogenase Is Also Regulated by Acyl-CoA

Acyl-CoA causes an inhibition of pyruvate dehydrogenase by inhibiting the ATP-ADP exchange transporter of the inner mitochondrial membrane, which leads to increased intramitochondrial [ATP]/[ADP] ratios and therefore to conversion of active to inactive pyruvate dehydrogenase (see Figure 17–6), thus regulating the availability of acetyl-CoA for lipogenesis. Furthermore, oxidation of acyl-CoA due to increased levels of free fatty acids may increase the ratios of [acetyl-CoA]/[CoA] and [NADH]/[NAD^+] in mitochondria, inhibiting pyruvate dehydrogenase.

Insulin Also Regulates Lipogenesis by Other Mechanisms

Insulin stimulates lipogenesis by several other mechanisms as well as by increasing acetyl-CoA carboxylase activity. It increases the transport of glucose into the cell (eg, in adipose tissue), increasing the availability of both pyruvate for fatty acid synthesis and glycerol 3-phosphate for esterification of the newly formed fatty acids, and also converts the inactive form of pyruvate dehydrogenase to the active form in adipose tissue but not in liver. Insulin also—by its ability to depress the level of intracellular cAMP—**inhibits lipolysis** in adipose tissue and thereby reduces the concentration of

plasma free fatty acids and therefore long-chain acyl-CoA, an inhibitor of lipogenesis.

The Fatty Acid Synthase Complex & Acetyl-CoA Carboxylase Are Adaptive Enzymes

These enzymes adapt to the body's physiologic needs by increasing in total amount in the fed state and by decreasing in starvation, feeding of fat, and in diabetes. **Insulin** is an important hormone causing gene expression and induction of enzyme biosynthesis, and glucagon (via cAMP) antagonizes this effect. Feeding fats containing polyunsaturated fatty acids coordinately regulates the inhibition of expression of key enzymes of glycolysis and lipogenesis. These mechanisms for longer-term regulation of lipogenesis take several days to become fully manifested and augment the direct and immediate effect of free fatty acids and hormones such as insulin and glucagon.

SUMMARY

- The synthesis of long-chain fatty acids (lipogenesis) is carried out by two enzyme systems: acetyl-CoA carboxylase and fatty acid synthase.
- The pathway converts acetyl-CoA to palmitate and requires NADPH, ATP, Mn^{2+}, biotin, pantothenic acid, and HCO_3^- as cofactors.
- Acetyl-CoA carboxylase is required to convert acetyl-CoA to malonyl-CoA. In turn, fatty acid synthase, a multienzyme complex of one polypeptide chain with seven separate enzymatic activities, catalyzes the assembly of palmitate from one acetyl-CoA and seven malonyl-CoA molecules.
- Lipogenesis is regulated at the acetyl-CoA carboxylase step by allosteric modifiers, phosphorylation/dephosphorylation, and induction and repression of enzyme synthesis. Citrate activates the enzyme, and long-chain acyl-CoA inhibits its activity. Insulin activates acetyl-CoA carboxylase whereas glucagon and epinephrine have opposite actions.

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Oxidation of Fatty Acids: Ketogenesis

22

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BIOMEDICAL IMPORTANCE

Although fatty acids are both oxidized to acetyl-CoA and synthesized from acetyl-CoA, fatty acid oxidation is not the simple reverse of fatty acid biosynthesis but an entirely different process taking place in a separate compartment of the cell. The separation of fatty acid oxidation in mitochondria from biosynthesis in the cytosol allows each process to be individually controlled and integrated with tissue requirements. Each step in fatty acid oxidation involves acyl-CoA derivatives catalyzed by separate enzymes, utilizes NAD^+ and FAD as coenzymes, and generates ATP. It is an aerobic process, requiring the presence of oxygen.

Increased fatty acid oxidation is a characteristic of starvation and of diabetes mellitus, leading to **ketone body** production by the liver **(ketosis).** Ketone bodies are acidic and when produced in excess over long periods, as in diabetes, cause **ketoacidosis,** which is ultimately fatal. Because gluconeogenesis is dependent upon fatty acid oxidation, any impairment in fatty acid oxidation leads to **hypoglycemia.** This occurs in various states of **carnitine deficiency** or deficiency of essential enzymes in fatty acid oxidation, eg, **carnitine palmitoyltransferase,** or inhibition of fatty acid oxidation by poisons, eg, **hypoglycin.**

OXIDATION OF FATTY ACIDS OCCURS IN MITOCHONDRIA

Fatty Acids Are Transported in the Blood as Free Fatty Acids (FFA)

Free fatty acids—also called unesterified (UFA) or nonesterified (NEFA) fatty acids—are fatty acids that are in the **unesterified state.** In plasma, longer-chain FFA are combined with **albumin,** and in the cell they are attached to a **fatty acid-binding protein,** so that in fact they are never really "free." Shorter-chain fatty acids are more water-soluble and exist as the un-ionized acid or as a fatty acid anion.

Fatty Acids Are Activated Before Being Catabolized

Fatty acids must first be converted to an active intermediate before they can be catabolized. This is the only step in the complete degradation of a fatty acid that requires energy from ATP. In the presence of ATP and coenzyme A, the enzyme **acyl-CoA synthetase (thiokinase)** catalyzes the conversion of a fatty acid (or free fatty acid) to an "active fatty acid" or acyl-CoA, which uses one high-energy phosphate with the formation of AMP and PP_i (Figure 22–1). The PP_i is hydrolyzed by **inorganic pyrophosphatase** with the loss of a further high-energy phosphate, ensuring that the overall reaction goes to completion. Acyl-CoA synthetases are found in the endoplasmic reticulum, peroxisomes, and inside and on the outer membrane of mitochondria.

Long-Chain Fatty Acids Penetrate the Inner Mitochondrial Membrane as Carnitine Derivatives

Carnitine (β-hydroxy-γ-trimethylammonium butyrate), $(CH_3)_3N^+—CH_2—CH(OH)—CH_2—COO^-$, is widely distributed and is particularly abundant in muscle. Long-chain acyl-CoA (or FFA) will not penetrate the inner membrane of mitochondria. However, **carnitine palmitoyltransferase-I,** present in the outer mitochondrial membrane, converts long-chain acyl-CoA to acylcarnitine, which is able to penetrate the inner membrane and gain access to the β-oxidation system of enzymes (Figure 22–1). **Carnitine-acylcarnitine translocase** acts as an inner membrane exchange transporter. Acylcarnitine is transported in, coupled with the transport out of one molecule of carnitine. The acylcarnitine then reacts with CoA, cat-

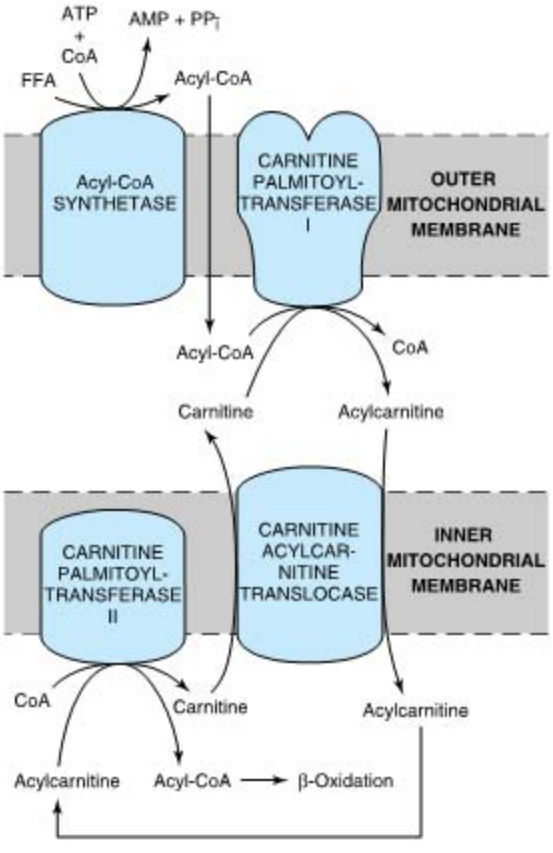

Figure 22–1. Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane. Long-chain acyl-CoA cannot pass through the inner mitochondrial membrane, but its metabolic product, acylcarnitine, can.

alyzed by **carnitine palmitoyltransferase-II,** located on the inside of the inner membrane. Acyl-CoA is reformed in the mitochondrial matrix, and carnitine is liberated.

β-OXIDATION OF FATTY ACIDS INVOLVES SUCCESSIVE CLEAVAGE WITH RELEASE OF ACETYL-CoA

In β-oxidation (Figure 22–2), two carbons at a time are cleaved from acyl-CoA molecules, starting at the carboxyl end. The chain is broken between the α(2)- and β(3)-carbon atoms—hence the name β-oxidation. The two-carbon units formed are acetyl-CoA; thus, palmitoyl-CoA forms eight acetyl-CoA molecules.

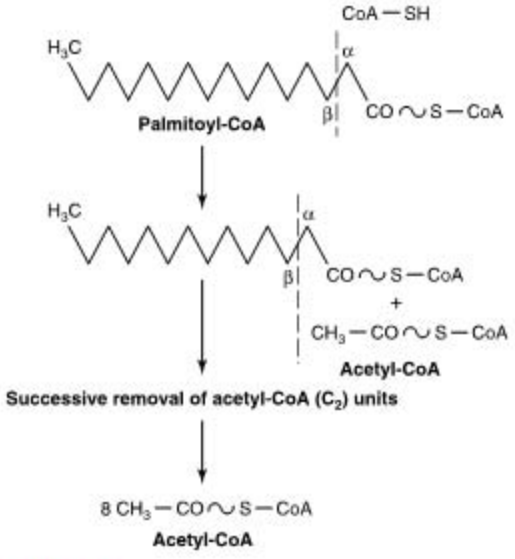

Figure 22–2. Overview of β-oxidation of fatty acids.

The Cyclic Reaction Sequence Generates $FADH_2$ & NADH

Several enzymes, known collectively as "fatty acid oxidase," are found in the mitochondrial matrix or inner membrane adjacent to the respiratory chain. These catalyze the oxidation of acyl-CoA to acetyl-CoA, the system being coupled with the phosphorylation of ADP to ATP (Figure 22–3).

The first step is the removal of two hydrogen atoms from the 2(α)- and 3(β)-carbon atoms, catalyzed by **acyl-CoA dehydrogenase** and requiring FAD. This results in the formation of Δ^2-*trans*-enoyl-CoA and $FADH_2$. The reoxidation of $FADH_2$ by the respiratory chain requires the mediation of another flavoprotein, termed **electron-transferring flavoprotein** (Chapter 11). Water is added to saturate the double bond and form 3-hydroxyacyl-CoA, catalyzed by **Δ^2-enoyl-CoA hydratase.** The 3-hydroxy derivative undergoes further dehydrogenation on the 3-carbon catalyzed by **L(+)-3-hydroxyacyl-CoA dehydrogenase** to form the corresponding 3-ketoacyl-CoA compound. In this case, NAD^+ is the coenzyme involved. Finally, 3-ketoacyl-CoA is split at the 2,3- position by **thiolase** (3-ketoacyl-CoA-thiolase), forming acetyl-CoA and a new acyl-CoA two carbons shorter than the original acyl-CoA molecule. The acyl-CoA formed in the cleavage reaction reenters the oxidative pathway at reaction 2 (Figure 22–3). In this way, a long-chain fatty acid may be degraded completely to acetyl-CoA (C_2 units). Since acetyl-CoA can be oxidized to CO_2 and water via the

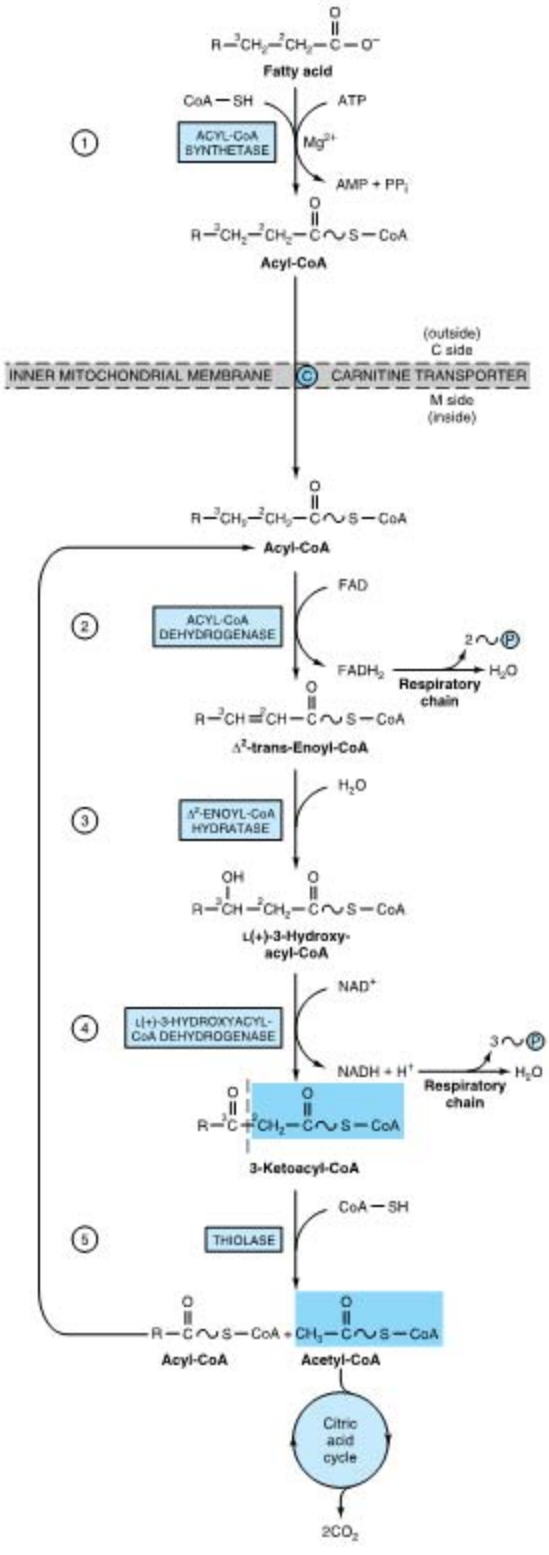

Figure 22–3. β-Oxidation of fatty acids. Long-chain acyl-CoA is cycled through reactions 2–5, acetyl-CoA being split off, each cycle, by thiolase (reaction 5). When the acyl radical is only four carbon atoms in length, two acetyl-CoA molecules are formed in reaction 5.

citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.

Oxidation of a Fatty Acid With an Odd Number of Carbon Atoms Yields Acetyl-CoA Plus a Molecule of Propionyl-CoA

Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β-oxidation, producing acetyl-CoA, until a three-carbon (propionyl-CoA) residue remains. This compound is converted to succinyl-CoA, a constituent of the citric acid cycle (Figure 19–2). Hence, **the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.**

Oxidation of Fatty Acids Produces a Large Quantity of ATP

Transport in the respiratory chain of electrons from $FADH_2$ and NADH will lead to the synthesis of five high-energy phosphates (Chapter 12) for each of the first seven acetyl-CoA molecules formed by β-oxidation of palmitate ($7 \times 5 = 35$). A total of 8 mol of acetyl-CoA is formed, and each will give rise to 12 mol of ATP on oxidation in the citric acid cycle, making $8 \times 12 = 96$ mol. Two must be subtracted for the initial activation of the fatty acid, yielding a net gain of 129 mol of ATP per mole of palmitate, or $129 \times 51.6^* = 6656$ kJ. This represents 68% of the free energy of combustion of palmitic acid.

Peroxisomes Oxidize Very Long Chain Fatty Acids

A modified form of β-oxidation is found in peroxisomes and leads to the formation of acetyl-CoA and H_2O_2 (from the flavoprotein-linked dehydrogenase step), which is broken down by catalase. Thus, this dehydrogenation in peroxisomes is not linked directly to phosphorylation and the generation of ATP. The system facilitates the oxidation of very long chain fatty acids (eg, C_{20}, C_{22}). These enzymes are induced by

* ΔG for the ATP reaction, as explained in Chapter 17.

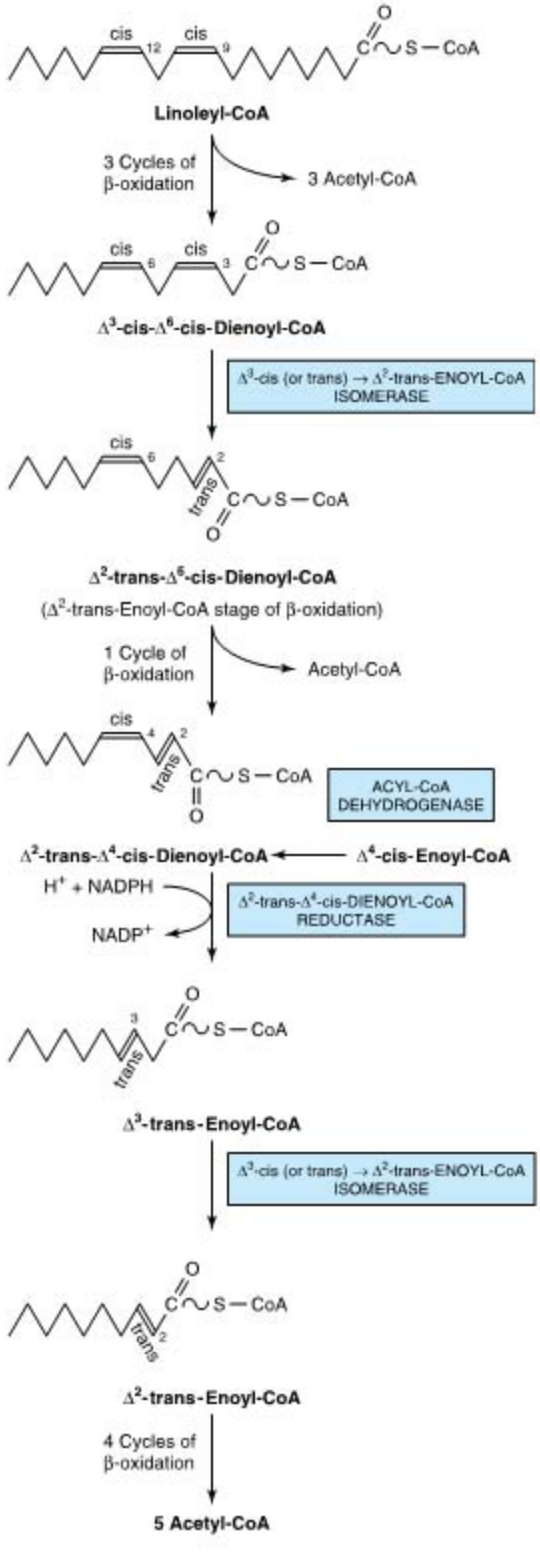

Figure 22–4. Sequence of reactions in the oxidation of unsaturated fatty acids, eg, linoleic acid. Δ^4-*cis*-fatty acids or fatty acids forming Δ^4-*cis*-enoyl-CoA enter the pathway at the position shown. NADPH for the dienoyl-CoA reductase step is supplied by intramitochondrial sources such as glutamate dehydrogenase, isocitrate dehydrogenase, and NAD(P)H transhydrogenase.

high-fat diets and in some species by hypolipidemic drugs such as clofibrate.

The enzymes in peroxisomes do not attack shorter-chain fatty acids; the β-oxidation sequence ends at octanoyl-CoA. Octanoyl and acetyl groups are both further oxidized in mitochondria. Another role of peroxisomal β-oxidation is to shorten the side chain of cholesterol in bile acid formation (Chapter 26). Peroxisomes also take part in the synthesis of ether glycerolipids (Chapter 24), cholesterol, and dolichol (Figure 26–2).

OXIDATION OF UNSATURATED FATTY ACIDS OCCURS BY A MODIFIED β-OXIDATION PATHWAY

The CoA esters of these acids are degraded by the enzymes normally responsible for β-oxidation until either a Δ^3-*cis*-acyl-CoA compound or a Δ^4-*cis*-acyl-CoA compound is formed, depending upon the position of the double bonds (Figure 22–4). The former compound is isomerized (**Δ^3*cis* → Δ^2-*trans*-enoyl-CoA isomerase**) to the corresponding Δ^2-*trans*-CoA stage of β-oxidation for subsequent hydration and oxidation. Any Δ^4-*cis*-acyl-CoA either remaining, as in the case of linoleic acid, or entering the pathway at this point after conversion by acyl-CoA dehydrogenase to Δ^2-*trans*-Δ^4-*cis*-dienoyl-CoA, is then metabolized as indicated in Figure 22–4.

KETOGENESIS OCCURS WHEN THERE IS A HIGH RATE OF FATTY ACID OXIDATION IN THE LIVER

Under metabolic conditions associated with a high rate of fatty acid oxidation, the liver produces considerable quantities of **acetoacetate** and **D(−)-3-hydroxybutyrate** (β-hydroxybutyrate). Acetoacetate continually undergoes spontaneous decarboxylation to yield **acetone.** These three substances are collectively known as the **ketone bodies** (also called acetone bodies or [incorrectly*] "ketones") (Figure 22–5). Acetoacetate and 3-hydroxybu-

* The term "ketones" should not be used because 3-hydroxybutyrate is not a ketone and there are ketones in blood that are not ketone bodies, eg, pyruvate, fructose.

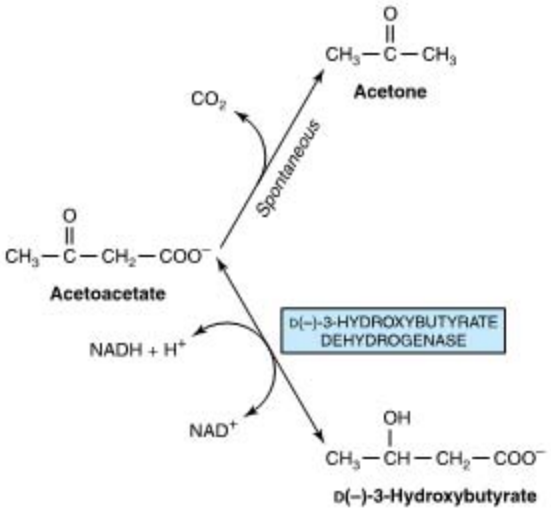

Figure 22–5. Interrelationships of the ketone bodies. D(–)-3-hydroxybutyrate dehydrogenase is a mitochondrial enzyme.

tyrate are interconverted by the mitochondrial enzyme **D(−)-3-hydroxybutyrate dehydrogenase;** the equilibrium is controlled by the mitochondrial [NAD^+]/[NADH] ratio, ie, the **redox state.** The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/L except in ruminants, where 3-hydroxybutyrate is formed continuously from butyric acid (a product of ruminal fermentation) in the rumen wall. In vivo, the liver appears to be the only organ in nonruminants to add significant quantities of ketone bodies to the blood. Extrahepatic tissues utilize them as respiratory substrates. The net flow of ketone bodies from the liver to the extrahepatic tissues results from active hepatic synthesis coupled with very low utilization. The reverse situation occurs in extrahepatic tissues (Figure 22–6).

3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) Is an Intermediate in the Pathway of Ketogenesis

Enzymes responsible for ketone body formation are associated mainly with the mitochondria. Two acetyl-CoA molecules formed in β-oxidation condense with one another to form acetoacetyl-CoA by a reversal of the **thiolase** reaction. Acetoacetyl-CoA, which is the

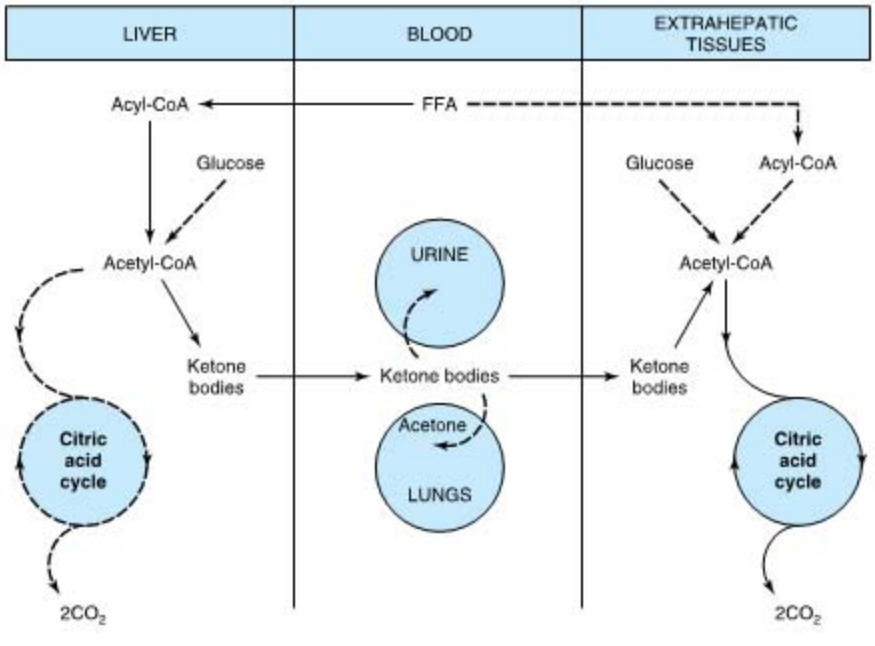

Figure 22–6. Formation, utilization, and excretion of ketone bodies. (The main pathway is indicated by the solid arrows.)

starting material for ketogenesis, also arises directly from the terminal four carbons of a fatty acid during β-oxidation (Figure 22–7). Condensation of acetoacetyl-CoA with another molecule of acetyl-CoA by **3-hydroxy-3-methylglutaryl-CoA synthase** forms HMG-CoA. **3-Hydroxy-3-methylglutaryl-CoA lyase** then causes acetyl-CoA to split off from the HMG-CoA, leaving free acetoacetate. The carbon atoms split off in the acetyl-CoA molecule are derived from the original acetoacetyl-CoA molecule. **Both enzymes must be present in mitochondria for ketogenesis to take place.** This occurs solely in liver and rumen epithelium. D(−)-3-Hydroxybutyrate is quantitatively the predominant ketone body present in the blood and urine in ketosis.

Ketone Bodies Serve as a Fuel for Extrahepatic Tissues

While an active enzymatic mechanism produces acetoacetate from acetoacetyl-CoA in the liver, acetoacetate once formed cannot be reactivated directly except in the cytosol, where it is used in a much less active pathway as a precursor in cholesterol synthesis. This accounts for the net production of ketone bodies by the liver.

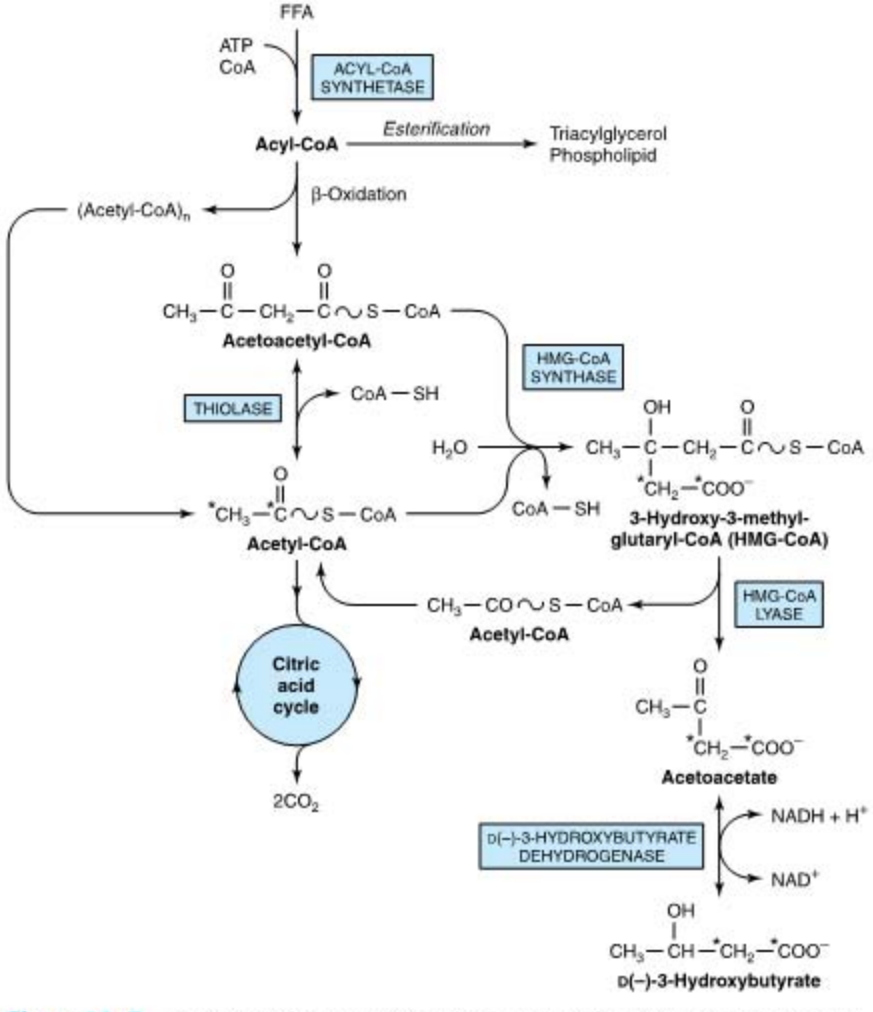

Figure 22–7. Pathways of ketogenesis in the liver. (FFA, free fatty acids; HMG, 3-hydroxy-3-methylglutaryl.)

In extrahepatic tissues, acetoacetate is activated to acetoacetyl-CoA by **succinyl-CoA-acetoacetate CoA transferase.** CoA is transferred from succinyl-CoA to form acetoacetyl-CoA (Figure 22–8). The acetoacetyl-CoA is split to acetyl-CoA by thiolase and oxidized in the citric acid cycle. If the blood level is raised, oxidation of ketone bodies increases until, at a concentration of approximately 12 mmol/L, they saturate the oxidative machinery. When this occurs, a large proportion of the oxygen consumption may be accounted for by the oxidation of ketone bodies.

In most cases, **ketonemia is due to increased production of ketone bodies** by the liver rather than to a deficiency in their utilization by extrahepatic tissues. While acetoacetate and D(−)-3-hydroxybutyrate are readily oxidized by extrahepatic tissues, acetone is difficult to oxidize in vivo and to a large extent is volatilized in the lungs.

In moderate ketonemia, the loss of ketone bodies via the urine is only a few percent of the total ketone body production and utilization. Since there are renal threshold-like effects (there is not a true threshold) that vary between species and individuals, measurement of the ketonemia, not the ketonuria, is the preferred method of assessing the severity of ketosis.

KETOGENESIS IS REGULATED AT THREE CRUCIAL STEPS

(1) Ketosis does not occur in vivo unless there is an increase in the level of circulating free fatty acids that arise from lipolysis of triacylglycerol in adipose tissue. **Free fatty acids are the precursors of ketone bodies in the liver.** The liver, both in fed and in fasting conditions, extracts about 30% of the free fatty acids passing through it, so that at high concentrations the flux passing into the liver is substantial. **Therefore, the factors regulating mobilization of free fatty acids from adipose tissue are important in controlling ketogenesis** (Figures 22–9 and 25–8).

(2) After uptake by the liver, free fatty acids are either **β-oxidized** to CO_2 or ketone bodies or **esterified** to triacylglycerol and phospholipid. There is regulation of entry of fatty acids into the oxidative pathway by **carnitine palmitoyltransferase-I** (CPT-I), and the remainder of the fatty acid uptake is esterified. CPT-I activity is

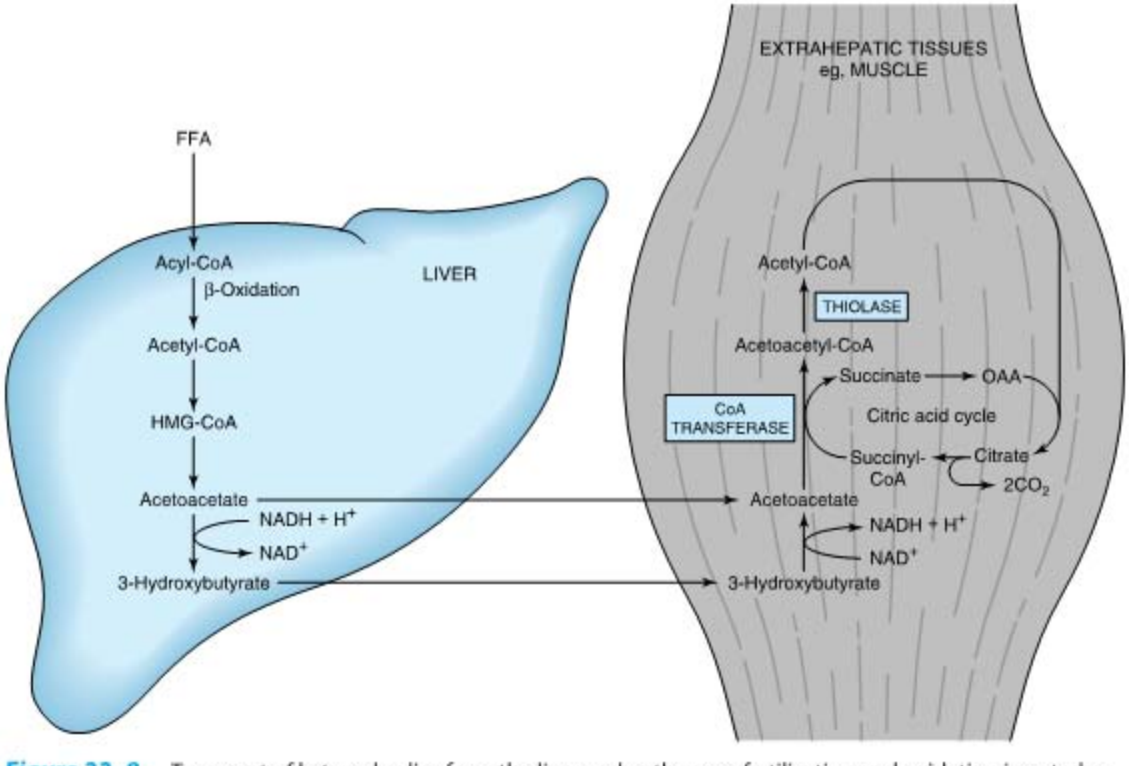

Figure 22–8. Transport of ketone bodies from the liver and pathways of utilization and oxidation in extrahepatic tissues.

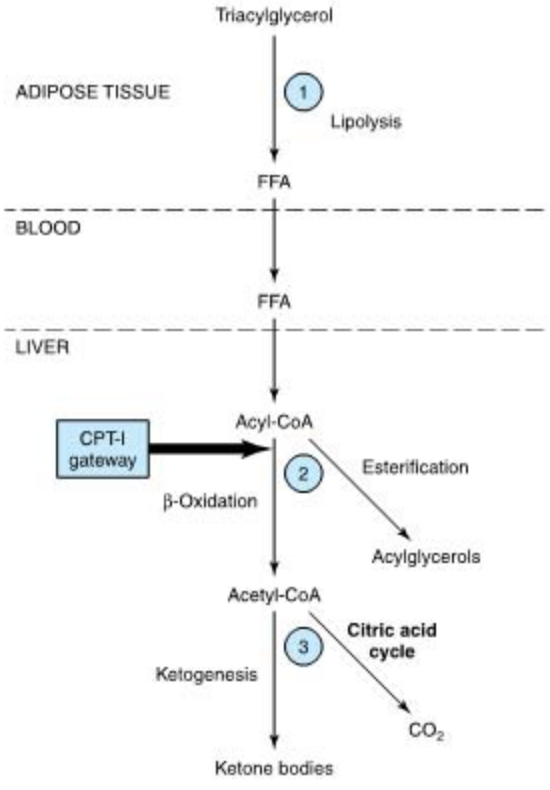

Figure 22–9. Regulation of ketogenesis. ①–③ show three crucial steps in the pathway of metabolism of free fatty acids (FFA) that determine the magnitude of ketogenesis. (CPT-I, carnitine palmitoyltransferase-I.)

low in the fed state, leading to depression of fatty acid oxidation, and high in starvation, allowing fatty acid oxidation to increase. Malonyl-CoA, the initial intermediate in fatty acid biosynthesis (Figure 21–1), formed by acetyl-CoA carboxylase in the fed state, is a potent inhibitor of CPT-I (Figure 22–10). Under these conditions, free fatty acids enter the liver cell in low concentrations and are nearly all esterified to acylglycerols and transported out of the liver in very low density lipoproteins (VLDL). However, as the concentration of free fatty acids increases with the onset of starvation, acetyl-CoA carboxylase is inhibited directly by acyl-CoA, and [malonyl-CoA] decreases, releasing the inhibition of CPT-I and allowing more acyl-CoA to be β-oxidized. These events are reinforced in starvation by decrease in the **[insulin]/[glucagon] ratio.** Thus, β-oxidation from free fatty acids is controlled by the CPT-I gateway into the mitochondria, and the balance of the free fatty acid uptake not oxidized is esterified.

(3) In turn, the acetyl-CoA formed in β-oxidation is oxidized in the citric acid cycle, or it enters the pathway of ketogenesis to form ketone bodies. As the level of serum free fatty acids is raised, proportionately more free fatty acid is converted to ketone bodies and less is oxidized via the citric acid cycle to CO_2. The partition of acetyl-CoA between the ketogenic pathway and the pathway of oxidation to CO_2 is so regulated that the total free energy captured in ATP which results from the oxidation of free fatty acids remains constant. This may be appreciated when it is realized that complete oxidation of 1 mol of palmitate involves a net production of 129 mol of ATP via β-oxidation and CO_2 production in the citric acid cycle (see above), whereas only 33 mol of ATP are produced when acetoacetate is the end product and only 21 mol when 3-hydroxybutyrate is the end product. Thus, ketogenesis may be regarded as a mechanism that allows the liver to oxidize increasing quantities of fatty acids within the constraints of a tightly coupled system of oxidative phosphorylation—without increasing its total energy expenditure.

Theoretically, a fall in concentration of oxaloacetate, particularly within the mitochondria, could impair the ability of the citric acid cycle to metabolize acetyl-CoA and divert fatty acid oxidation toward ketogenesis. Such a fall may occur because of an increase in the [NADH]/[NAD^+] ratio caused by increased β-oxidation affecting the equilibrium between oxaloacetate and malate and decreasing the concentration of oxaloacetate. However, pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate, is activated by acetyl-CoA. Consequently, when there are significant amounts of acetyl-CoA, there should be sufficient oxaloacetate to initiate the condensing reaction of the citric acid cycle.

CLINICAL ASPECTS

Impaired Oxidation of Fatty Acids Gives Rise to Diseases Often Associated With Hypoglycemia

Carnitine deficiency can occur particularly in the newborn—and especially in preterm infants—owing to inadequate biosynthesis or renal leakage. Losses can also occur in hemodialysis. This suggests a vitamin-like dietary requirement for carnitine in some individuals. Symptoms of deficiency include hypoglycemia, which is a consequence of impaired fatty acid oxidation and lipid accumulation with muscular weakness. Treatment is by oral supplementation with carnitine.

Inherited **CPT-I deficiency** affects only the liver, resulting in reduced fatty acid oxidation and ketogenesis, with hypoglycemia. **CPT-II deficiency** affects pri-

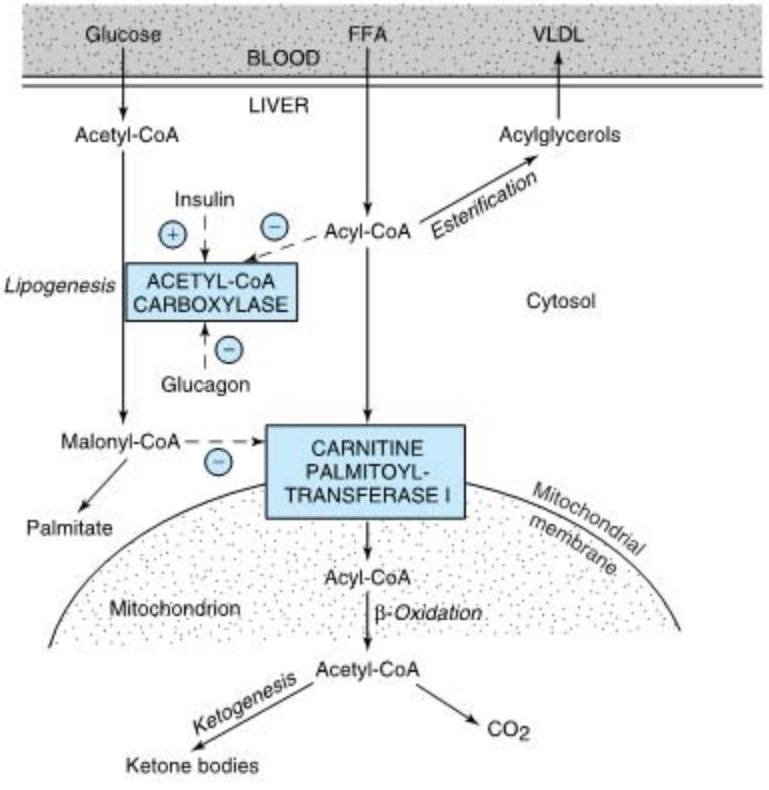

Figure 22–10. Regulation of long-chain fatty acid oxidation in the liver. (FFA, free fatty acids; VLDL, very low density lipoprotein.) Positive (⊕) and negative (⊖) regulatory effects are represented by broken arrows and substrate flow by solid arrows.

marily skeletal muscle and, when severe, the liver. The sulfonylurea drugs **(glyburide [glibenclamide]** and **tolbutamide),** used in the treatment of type 2 diabetes mellitus, reduce fatty acid oxidation and, therefore, hyperglycemia by inhibiting CPT-I.

Inherited defects in the enzymes of β-oxidation and ketogenesis also lead to nonketotic hypoglycemia, coma, and fatty liver. Defects are known in long- and short-chain 3-hydroxyacyl-CoA dehydrogenase (deficiency of the long-chain enzyme may be a cause of **acute fatty liver of pregnancy). 3-Ketoacyl-CoA thiolase** and **HMG-CoA lyase deficiency** also affect the degradation of leucine, a ketogenic amino acid (Chapter 30).

Jamaican vomiting sickness is caused by eating the unripe fruit of the akee tree, which contains a toxin, **hypoglycin,** that inactivates medium- and short-chain acyl-CoA dehydrogenase, inhibiting β-oxidation and causing hypoglycemia. **Dicarboxylic aciduria** is characterized by the excretion of C_6–C_{10} ω-dicarboxylic acids and by nonketotic hypoglycemia. It is caused by a lack of mitochondrial **medium-chain acyl-CoA dehydrogenase. Refsum's disease** is a rare neurologic disorder due to a defect that causes the accumulation of phytanic acid, which is found in plant foodstuffs and blocks β-oxidation. **Zellweger's (cerebrohepatorenal) syndrome** occurs in individuals with a rare inherited absence of peroxisomes in all tissues. They accumulate C_{26}–C_{38} polyenoic acids in brain tissue and also exhibit a generalized loss of peroxisomal functions, eg, impaired bile acid and ether lipid synthesis.

Ketoacidosis Results From Prolonged Ketosis

Higher than normal quantities of ketone bodies present in the blood or urine constitute **ketonemia** (hyperketonemia) or **ketonuria,** respectively. The overall condition is called **ketosis.** Acetoacetic and 3-hydroxybutyric acids are both moderately strong acids and are buffered when present in blood or other tissues. However, their continual excretion in quantity progressively depletes the alkali reserve, causing **ketoacidosis.** This may be fatal in uncontrolled **diabetes mellitus.**

The basic form of ketosis occurs in **starvation** and involves depletion of available carbohydrate coupled with mobilization of free fatty acids. This general pattern of metabolism is exaggerated to produce the pathologic states found in **diabetes mellitus, twin lamb disease,** and **ketosis in lactating cattle.** Nonpathologic forms of ketosis are found under conditions of high-fat

feeding and after severe exercise in the postabsorptive state.

SUMMARY

- Fatty acid oxidation in mitochondria leads to the generation of large quantities of ATP by a process called β-oxidation that cleaves acetyl-CoA units sequentially from fatty acyl chains. The acetyl-CoA is oxidized in the citric acid cycle, generating further ATP.
- The ketone bodies (acetoacetate, 3-hydroxybutyrate, and acetone) are formed in hepatic mitochondria when there is a high rate of fatty acid oxidation. The pathway of ketogenesis involves synthesis and breakdown of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by two key enzymes, HMG-CoA synthase and HMG-CoA lyase.
- Ketone bodies are important fuels in extrahepatic tissues.
- Ketogenesis is regulated at three crucial steps: (1) control of free fatty acid mobilization from adipose tissue; (2) the activity of carnitine palmitoyltransferase-I in liver, which determines the proportion of the fatty acid flux that is oxidized rather than esterified; and (3) partition of acetyl-CoA between the pathway of ketogenesis and the citric acid cycle.
- Diseases associated with impairment of fatty acid oxidation lead to hypoglycemia, fatty infiltration of organs, and hypoketonemia.
- Ketosis is mild in starvation but severe in diabetes mellitus and ruminant ketosis.

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Metabolism of Unsaturated Fatty Acids & Eicosanoids

23

Peter A. Mayes, PhD, DSc, & Kathleen M. Botham, PhD, DSc

BIOMEDICAL IMPORTANCE

Unsaturated fatty acids in phospholipids of the cell membrane are important in maintaining membrane fluidity. A high ratio of polyunsaturated fatty acids to saturated fatty acids (P:S ratio) in the diet is a major factor in lowering plasma cholesterol concentrations and is considered to be beneficial in preventing coronary heart disease. Animal tissues have limited capacity for desaturating fatty acids, and that process requires certain dietary polyunsaturated fatty acids derived from plants. These **essential fatty acids** are used to form eicosanoic (C_{20}) fatty acids, which in turn give rise to the prostaglandins and thromboxanes and to leukotrienes and lipoxins—known collectively as **eicosanoids.** The prostaglandins and thromboxanes are local hormones that are synthesized rapidly when required. Prostaglandins mediate **inflammation,** produce **pain,** and induce **sleep** as well as being involved in the regulation of **blood coagulation** and **reproduction.** Nonsteroidal anti-inflammatory drugs such as **aspirin** act by inhibiting prostaglandin synthesis. Leukotrienes have muscle contractant and chemotactic properties and are important in allergic reactions and inflammation.

SOME POLYUNSATURATED FATTY ACIDS CANNOT BE SYNTHESIZED BY MAMMALS & ARE NUTRITIONALLY ESSENTIAL

Certain long-chain unsaturated fatty acids of metabolic significance in mammals are shown in Figure 23–1. Other C_{20}, C_{22}, and C_{24} polyenoic fatty acids may be derived from oleic, linoleic, and α-linolenic acids by chain elongation. Palmitoleic and oleic acids are not essential in the diet because the tissues can introduce a double bond at the Δ^9 position of a saturated fatty acid. **Linoleic and α-linolenic acids** are the only fatty acids known to be essential for the complete nutrition of many species of animals, including humans, and are known as the **nutritionally essential fatty acids.** In most mammals, **arachidonic acid** can be formed from linoleic acid (Figure 23–4). Double bonds can be introduced at the Δ^4, Δ^5, Δ^6, and Δ^9 positions (see Chapter 14) in most animals, but never beyond the Δ^9 position. In contrast, plants are able to synthesize the nutritionally essential fatty acids by introducing double bonds at the Δ^{12} and Δ^{15} positions.

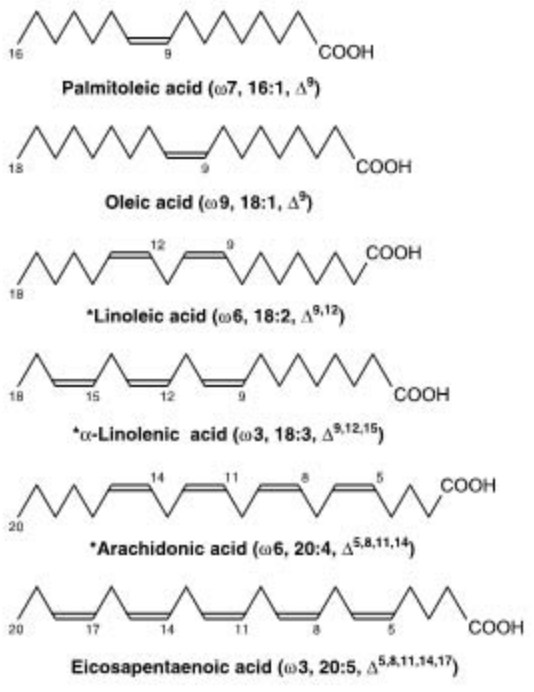

Figure 23–1. Structure of some unsaturated fatty acids. Although the carbon atoms in the molecules are conventionally numbered—ie, numbered from the carboxyl terminal—the ω numbers (eg, ω7 in palmitoleic acid) are calculated from the reverse end (the methyl terminal) of the molecules. The information in parentheses shows, for instance, that α-linolenic acid contains double bonds starting at the third carbon from the methyl terminal, has 18 carbons and 3 double bonds, and has these double bonds at the 9th, 12th, and 15th carbons from the carboxyl terminal. (Asterisks: Classified as "essential fatty acids.")

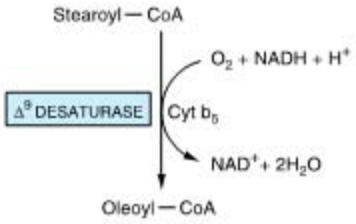

Figure 23–2. Microsomal Δ^9 desaturase.

MONOUNSATURATED FATTY ACIDS ARE SYNTHESIZED BY A Δ^9 DESATURASE SYSTEM

Several tissues including the liver are considered to be responsible for the formation of nonessential monounsaturated fatty acids from saturated fatty acids. The first double bond introduced into a saturated fatty acid is nearly always in the Δ^9 position. An enzyme system—**Δ^9 desaturase** (Figure 23–2)—in the endoplasmic reticulum will catalyze the conversion of palmitoyl-CoA or stearoyl-CoA to palmitoleoyl-CoA or oleoyl-CoA, respectively. Oxygen and either NADH or NADPH are necessary for the reaction. The enzymes appear to be similar to a monooxygenase system involving cytochrome b_5 (Chapter 11).

SYNTHESIS OF POLYUNSATURATED FATTY ACIDS INVOLVES DESATURASE & ELONGASE ENZYME SYSTEMS

Additional double bonds introduced into existing monounsaturated fatty acids are always separated from each other by a methylene group (methylene interrupted) except in bacteria. Since animals have a Δ^9 desaturase, they are able to synthesize the ω9 (oleic acid) family of unsaturated fatty acids completely by a combination of chain elongation and desaturation (Figure 23–3). However, as indicated above, linoleic (ω6) or α-linolenic (ω3) acids required for the synthesis of the other members of the ω6 or ω3 families must be supplied in the diet. Linoleate may be converted to arachidonate via **γ-linolenate** by the pathway shown in Figure 23–4. The nutritional requirement for arachidonate may thus be dispensed with if there is adequate linoleate in the diet. The desaturation and chain elongation system is greatly diminished in the starving state, in response to glucagon and epinephrine administration, and in the absence of insulin as in type 1 diabetes mellitus.

DEFICIENCY SYMPTOMS ARE PRODUCED WHEN THE ESSENTIAL FATTY ACIDS (EFA) ARE ABSENT FROM THE DIET

Rats fed a purified nonlipid diet containing vitamins A and D exhibit a reduced growth rate and reproductive deficiency which may be cured by the addition of **linoleic, α-linolenic,** and **arachidonic acids** to the diet. These fatty acids are found in high concentrations in vegetable oils (Table 14–2) and in small amounts in animal carcasses. These essential fatty acids are required for prostaglandin, thromboxane, leukotriene, and lipoxin formation (see below), and they also have various other functions which are less well defined. Essential fatty acids are found in the structural lipids of the cell, often in the 2 position of phospholipids, and are concerned with the structural integrity of the mitochondrial membrane.

Arachidonic acid is present in membranes and accounts for 5–15% of the fatty acids in phospholipids. Docosahexaenoic acid (DHA; ω3, 22:6), which is syn-

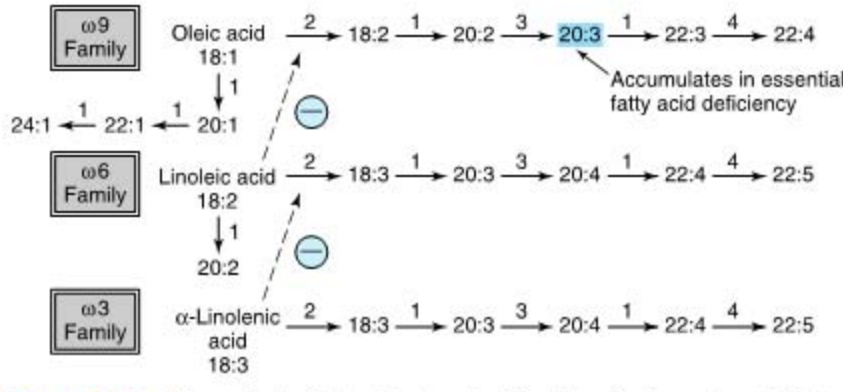

Figure 23–3. Biosynthesis of the ω9, ω6, and ω3 families of polyunsaturated fatty acids. Each step is catalyzed by the microsomal chain elongation or desaturase system: 1, elongase; 2, Δ^6 desaturase; 3, Δ^5 desaturase; 4, Δ^4 desaturase. (⊖, Inhibition.)

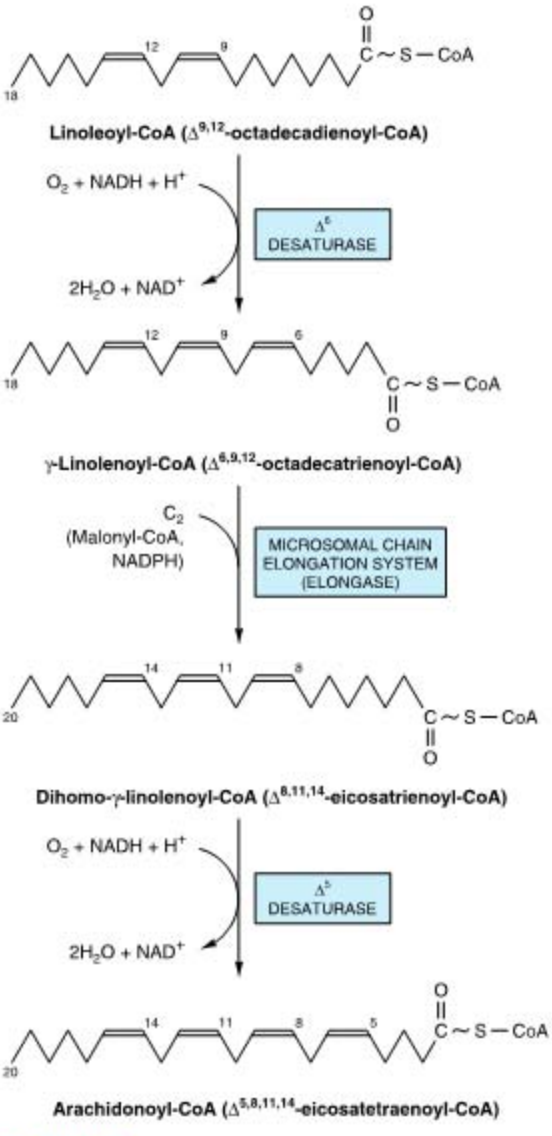

Figure 23–4. Conversion of linoleate to arachidonate. Cats cannot carry out this conversion owing to absence of Δ^6 desaturase and must obtain arachidonate in their diet.

thesized from α-linolenic acid or obtained directly from fish oils, is present in high concentrations in retina, cerebral cortex, testis, and sperm. DHA is particularly needed for development of the brain and retina and is supplied via the placenta and milk. Patients with **retinitis pigmentosa** are reported to have low blood levels of DHA. In **essential fatty acid deficiency,** nonessential polyenoic acids of the ω9 family replace the essential fatty acids in phospholipids, other complex lipids, and membranes, particularly with $\Delta^{5,8,11}$-eicosatrienoic acid (ω9 20:3) (Figure 23–3). The triene:tetraene ratio in plasma lipids can be used to diagnose the extent of essential fatty acid deficiency.

Trans Fatty Acids Are Implicated in Various Disorders

Small amounts of trans-unsaturated fatty acids are found in ruminant fat (eg, butter fat has 2–7%), where they arise from the action of microorganisms in the rumen, but the main source in the human diet is from partially hydrogenated vegetable oils (eg, margarine). Trans fatty acids compete with essential fatty acids and may exacerbate essential fatty acid deficiency. Moreover, they are structurally similar to saturated fatty acids (Chapter 14) and have comparable effects in the promotion of hypercholesterolemia and atherosclerosis (Chapter 26).

EICOSANOIDS ARE FORMED FROM C_{20} POLYUNSATURATED FATTY ACIDS

Arachidonate and some other C_{20} polyunsaturated fatty acids give rise to **eicosanoids,** physiologically and pharmacologically active compounds known as **prostaglandins (PG), thromboxanes (TX), leukotrienes (LT),** and **lipoxins (LX)** (Chapter 14). Physiologically, they are considered to act as local hormones functioning through G-protein-linked receptors to elicit their biochemical effects.

There are three groups of eicosanoids that are synthesized from C_{20} eicosanoic acids derived from the essential fatty acids **linoleate** and **α-linolenate,** or directly from dietary arachidonate and eicosapentaenoate (Figure 23–5). Arachidonate, usually derived from the 2 position of phospholipids in the plasma membrane by the action of phospholipase A_2 (Figure 24–6)—but also from the diet—is the substrate for the synthesis of the PG_2, TX_2 series **(prostanoids)** by the **cyclooxygenase pathway,** or the LT_4 and LX_4 series by the **lipoxygenase pathway,** with the two pathways competing for the arachidonate substrate (Figure 23–5).

THE CYCLOOXYGENASE PATHWAY IS RESPONSIBLE FOR PROSTANOID SYNTHESIS

Prostanoid synthesis (Figure 23–6) involves the consumption of two molecules of O_2 catalyzed by **prostaglandin H synthase (PGHS),** which consists of two enzymes, **cyclooxygenase** and **peroxidase.** PGHS is present as two isoenzymes, PGHS-1 and PGHS-2. The product, an endoperoxide (PGH), is converted to prostaglandins D, E, and F as well as to a thromboxane

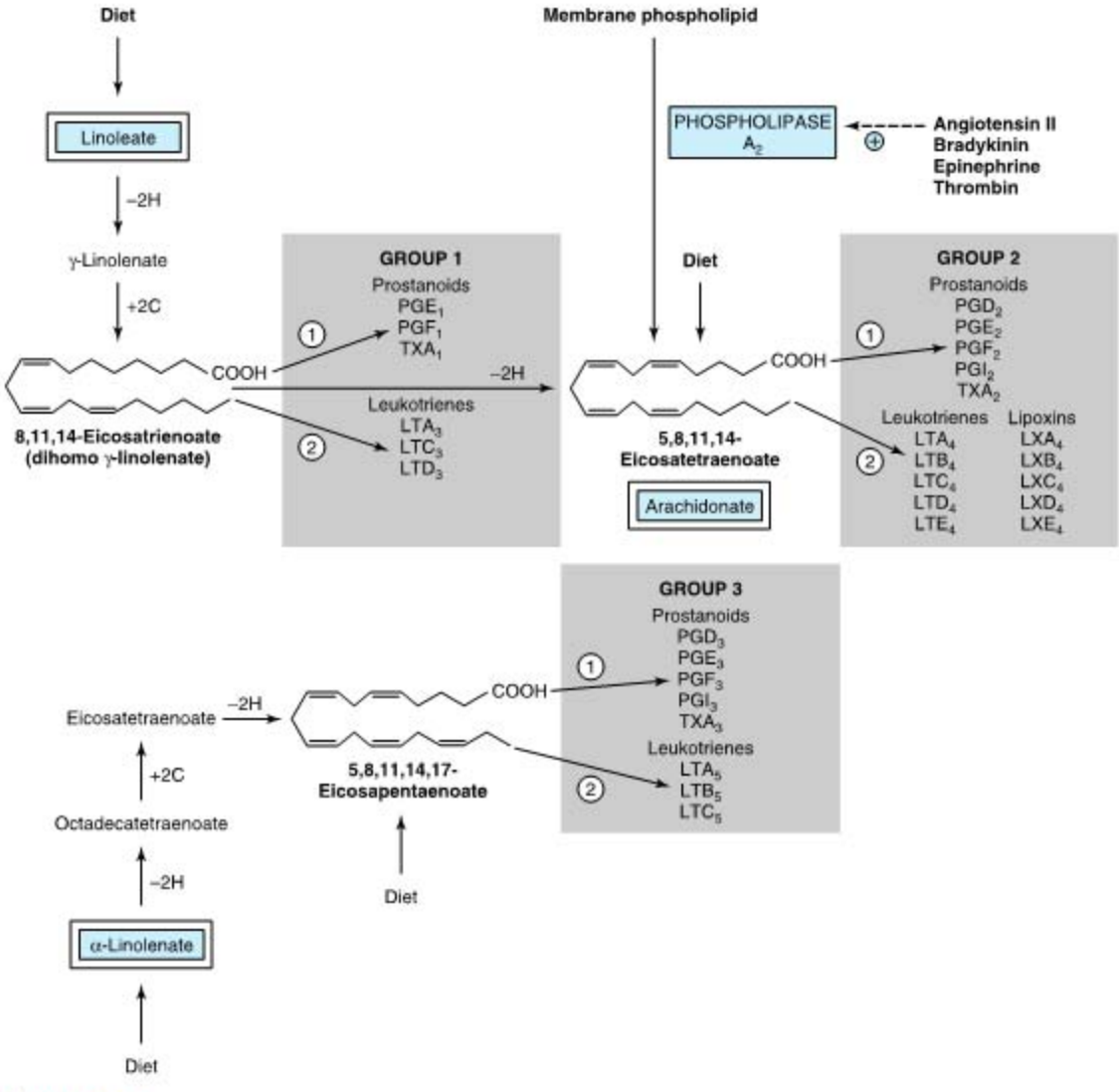

Figure 23–5. The three groups of eicosanoids and their biosynthetic origins. (PG, prostaglandin; PGI, prostacyclin; TX, thromboxane; LT, leukotriene; LX, lipoxin; ①, cyclooxygenase pathway; ②, lipoxygenase pathway.) The subscript denotes the total number of double bonds in the molecule and the series to which the compound belongs.

(TXA_2) and prostacyclin (PGI_2). Each cell type produces only one type of prostanoid. **Aspirin,** a nonsteroidal anti-inflammatory drug (NSAID), inhibits cyclooxygenase of both PGHS-1 and PGHS-2 by acetylation. Most other NSAIDs, such as indomethacin and ibuprofen, inhibit cyclooxygenases by competing with arachidonate. Transcription of PGHS-2—but not of PGHS-1—is completely inhibited by **anti-inflammatory corticosteroids.**

Essential Fatty Acids Do Not Exert All Their Physiologic Effects Via Prostaglandin Synthesis

The role of essential fatty acids in membrane formation is unrelated to prostaglandin formation. Prostaglandins do not relieve symptoms of essential fatty acid deficiency, and an essential fatty acid deficiency is not caused by inhibition of prostaglandin synthesis.

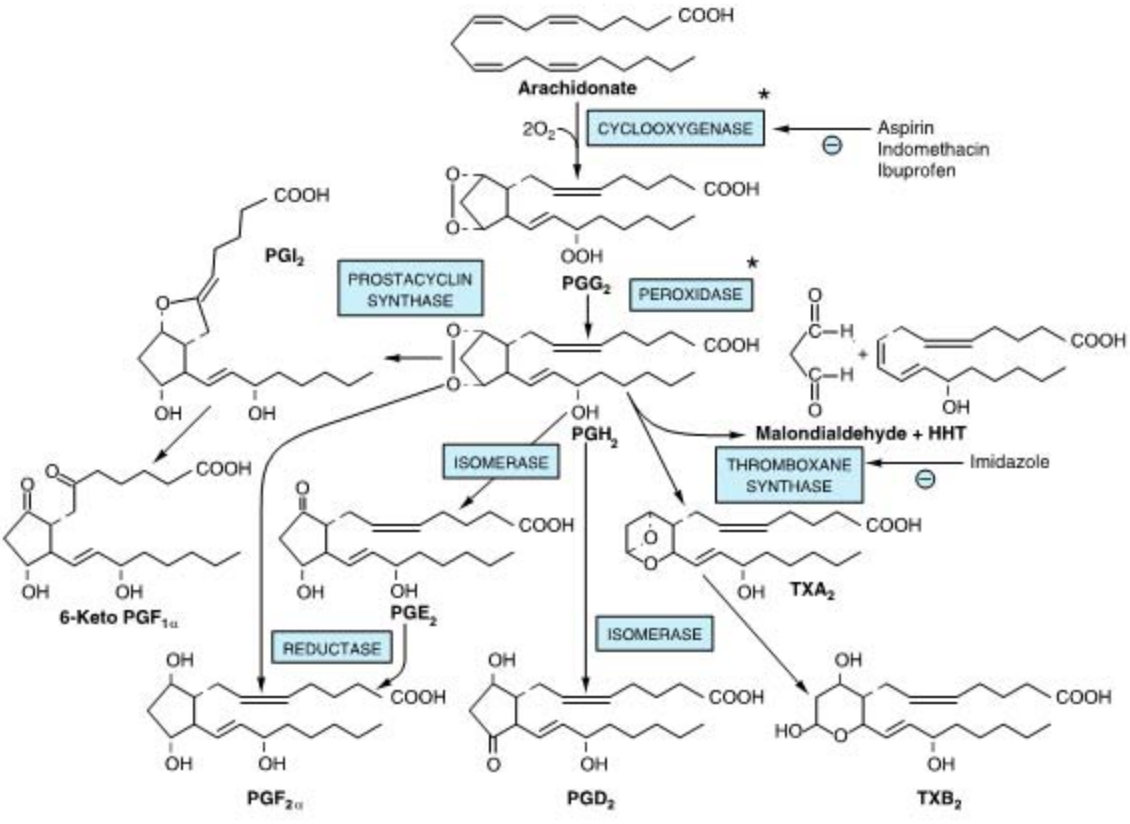

Figure 23–6. Conversion of arachidonic acid to prostaglandins and thromboxanes of series 2. (PG, prostaglandin; TX, thromboxane; PGI, prostacyclin; HHT, hydroxyheptadecatrienoate.) (Asterisk: Both of these starred activities are attributed to one enzyme: prostaglandin H synthase. Similar conversions occur in prostaglandins and thromboxanes of series 1 and 3.)

Cyclooxygenase Is a "Suicide Enzyme"

"Switching off" of prostaglandin activity is partly achieved by a remarkable property of cyclooxygenase—that of self-catalyzed destruction; ie, it is a "suicide enzyme." Furthermore, the inactivation of prostaglandins by **15-hydroxyprostaglandin dehydrogenase** is rapid. Blocking the action of this enzyme with sulfasalazine or indomethacin can prolong the half-life of prostaglandins in the body.

LEUKOTRIENES & LIPOXINS ARE FORMED BY THE LIPOXYGENASE PATHWAY

The leukotrienes are a family of conjugated trienes formed from eicosanoic acids in leukocytes, mastocytoma cells, platelets, and macrophages by the **lipoxygenase pathway** in response to both immunologic and nonimmunologic stimuli. Three different lipoxygenases (dioxygenases) insert oxygen into the 5, 12, and 15 positions of arachidonic acid, giving rise to hydroperoxides (HPETE). Only **5-lipoxygenase** forms leukotrienes (details in Figure 23–7). Lipoxins are a family of conjugated tetraenes also arising in leukocytes. They are formed by the combined action of more than one lipoxygenase (Figure 23–7).

CLINICAL ASPECTS

Symptoms of Essential Fatty Acid Deficiency in Humans Include Skin Lesions & Impairment of Lipid Transport

In adults subsisting on ordinary diets, no signs of essential fatty acid deficiencies have been reported. How-

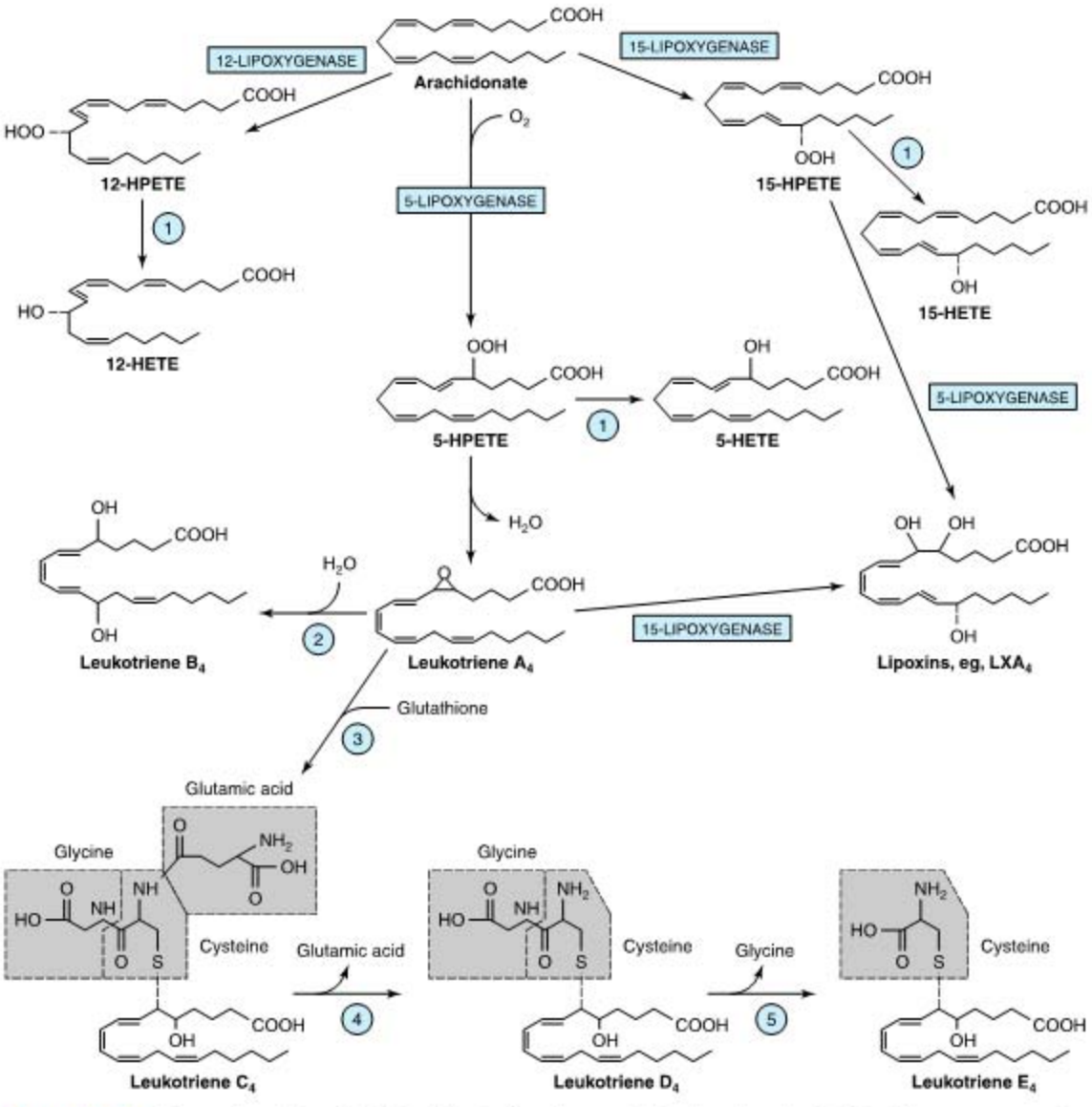

Figure 23–7. Conversion of arachidonic acid to leukotrienes and lipoxins of series 4 via the lipoxygenase pathway. Some similar conversions occur in series 3 and 5 leukotrienes. (HPETE, hydroperoxyeicosatetraenoate; HETE, hydroxyeicosatetraenoate; ①, peroxidase; ②, leukotriene A_4 epoxide hydrolase; ③, glutathione *S*-transferase; ④, γ-glutamyltranspeptidase; ⑤, cysteinyl-glycine dipeptidase.)

ever, infants receiving formula diets low in fat and patients maintained for long periods exclusively by intravenous nutrition low in essential fatty acids show deficiency symptoms that can be prevented by an essential fatty acid intake of 1–2% of the total caloric requirement.

Abnormal Metabolism of Essential Fatty Acids Occurs in Several Diseases

Abnormal metabolism of essential fatty acids, which may be connected with dietary insufficiency, has been noted in cystic fibrosis, acrodermatitis enteropathica,

hepatorenal syndrome, Sjögren-Larsson syndrome, multisystem neuronal degeneration, Crohn's disease, cirrhosis and alcoholism, and Reye's syndrome. Elevated levels of very long chain polyenoic acids have been found in the brains of patients with Zellweger's syndrome (Chapter 22). Diets with a high P:S (polyunsaturated:saturated fatty acid) ratio reduce serum cholesterol levels and are considered to be beneficial in terms of the risk of development of coronary heart disease.

Prostanoids Are Potent Biologically Active Substances

Thromboxanes are synthesized in platelets and upon release cause vasoconstriction and platelet aggregation. Their synthesis is specifically inhibited by low-dose aspirin. **Prostacyclins (PGI_2)** are produced by blood vessel walls and are potent inhibitors of platelet aggregation. Thus, thromboxanes and prostacyclins are antagonistic. PG_3 and TX_3, formed from eicosapentaenoic acid (EPA) in fish oils, inhibit the release of arachidonate from phospholipids and the formation of PG_2 and TX_2. PGI_3 is as potent an antiaggregator of platelets as PGI_2, but TXA_3 is a weaker aggregator than TXA_2, changing the balance of activity and favoring longer clotting times. As little as 1 ng/mL of plasma prostaglandins causes contraction of smooth muscle in animals. Potential therapeutic uses include prevention of conception, induction of labor at term, termination of pregnancy, prevention or alleviation of gastric ulcers, control of inflammation and of blood pressure, and relief of asthma and nasal congestion. In addition, PGD_2 is a potent sleep-promoting substance. Prostaglandins increase cAMP in platelets, thyroid, corpus luteum, fetal bone, adenohypophysis, and lung but reduce cAMP in renal tubule cells and adipose tissue (Chapter 25).

Leukotrienes & Lipoxins Are Potent Regulators of Many Disease Processes

Slow-reacting substance of anaphylaxis **(SRS-A)** is a mixture of leukotrienes C_4, D_4, and E_4. This mixture of leukotrienes is a potent constrictor of the bronchial airway musculature. These leukotrienes together with leukotriene B_4 also cause vascular permeability and attraction and activation of leukocytes and are important regulators in many diseases involving inflammatory or immediate hypersensitivity reactions, such as asthma. Leukotrienes are vasoactive, and 5-lipoxygenase has been found in arterial walls. Evidence supports a role for lipoxins in vasoactive and immunoregulatory function, eg, as counterregulatory compounds (chalones) of the immune response.

SUMMARY

- Biosynthesis of unsaturated long-chain fatty acids is achieved by desaturase and elongase enzymes, which introduce double bonds and lengthen existing acyl chains, respectively.
- Higher animals have Δ^4, Δ^5, Δ^6, and Δ^9 desaturases but cannot insert new double bonds beyond the 9 position of fatty acids. Thus, the essential fatty acids linoleic (ω6) and α-linolenic (ω3) must be obtained from the diet.
- Eicosanoids are derived from C_{20} (eicosanoic) fatty acids synthesized from the essential fatty acids and comprise important groups of physiologically and pharmacologically active compounds, including the prostaglandins, thromboxanes, leukotrienes, and lipoxins.

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Metabolism of Acylglycerols & Sphingolipids

24

Peter A. Mayes, PhD, DSc, & Kathleen M. Botham, PhD, DSc

BIOMEDICAL IMPORTANCE

Acylglycerols constitute the majority of lipids in the body. Triacylglycerols are the major lipids in fat deposits and in food, and their roles in lipid transport and storage and in various diseases such as obesity, diabetes, and hyperlipoproteinemia will be described in subsequent chapters. The amphipathic nature of phospholipids and sphingolipids makes them ideally suitable as the main lipid component of cell membranes. Phospholipids also take part in the metabolism of many other lipids. Some phospholipids have specialized functions; eg, dipalmitoyl lecithin is a major component of **lung surfactant,** which is lacking in respiratory distress syndrome of the newborn. Inositol phospholipids in the cell membrane act as precursors of **hormone second messengers,** and **platelet-activating factor** is an alkylphospholipid. Glycosphingolipids, containing sphingosine and sugar residues as well as fatty acid and found in the outer leaflet of the plasma membrane with their oligosaccharide chains facing outward, form part of the glycocalyx of the cell surface and are important (1) in cell adhesion and cell recognition; (2) as receptors for bacterial toxins (eg, the toxin that causes cholera); and (3) as ABO blood group substances. A dozen or so **glycolipid storage diseases** have been described (eg, Gaucher's disease, Tay-Sachs disease), each due to a genetic defect in the pathway for glycolipid degradation in the lysosomes.

HYDROLYSIS INITIATES CATABOLISM OF TRIACYLGLYCEROLS

Triacylglycerols must be hydrolyzed by a **lipase** to their constituent fatty acids and glycerol before further catabolism can proceed. Much of this hydrolysis (lipolysis) occurs in adipose tissue with release of free fatty acids into the plasma, where they are found combined with serum albumin. This is followed by free fatty acid uptake into tissues (including liver, heart, kidney, muscle, lung, testis, and adipose tissue, but not readily by brain), where they are oxidized or reesterified. The utilization of glycerol depends upon whether such tissues possess **glycerol kinase,** found in significant amounts in liver, kidney, intestine, brown adipose tissue, and lactating mammary gland.

TRIACYLGLYCEROLS & PHOSPHOGLYCEROLS ARE FORMED BY ACYLATION OF TRIOSE PHOSPHATES

The major pathways of triacylglycerol and phosphoglycerol biosynthesis are outlined in Figure 24–1. Important substances such as triacylglycerols, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and cardiolipin, a constituent of mitochondrial membranes, are formed from glycerol-3-phosphate. Significant branch points in the pathway occur at the phosphatidate and diacylglycerol steps. From dihydroxyacetone phosphate are derived phosphoglycerols containing an ether link (—C—O—C—), the best-known of which are plasmalogens and platelet-activating factor (PAF). Glycerol 3-phosphate and dihydroxyacetone phosphate are intermediates in glycolysis, making a very important connection between carbohydrate and lipid metabolism.

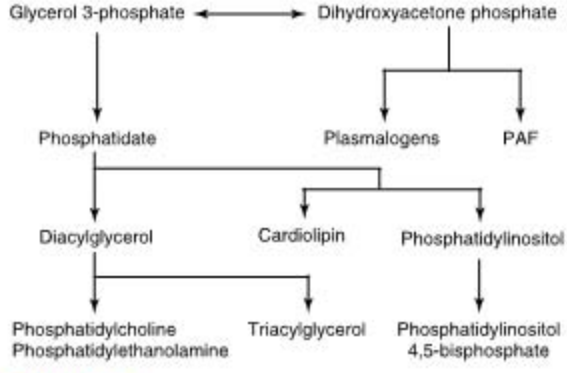

Figure 24–1. Overview of acylglycerol biosynthesis. (PAF, platelet-activating factor.)

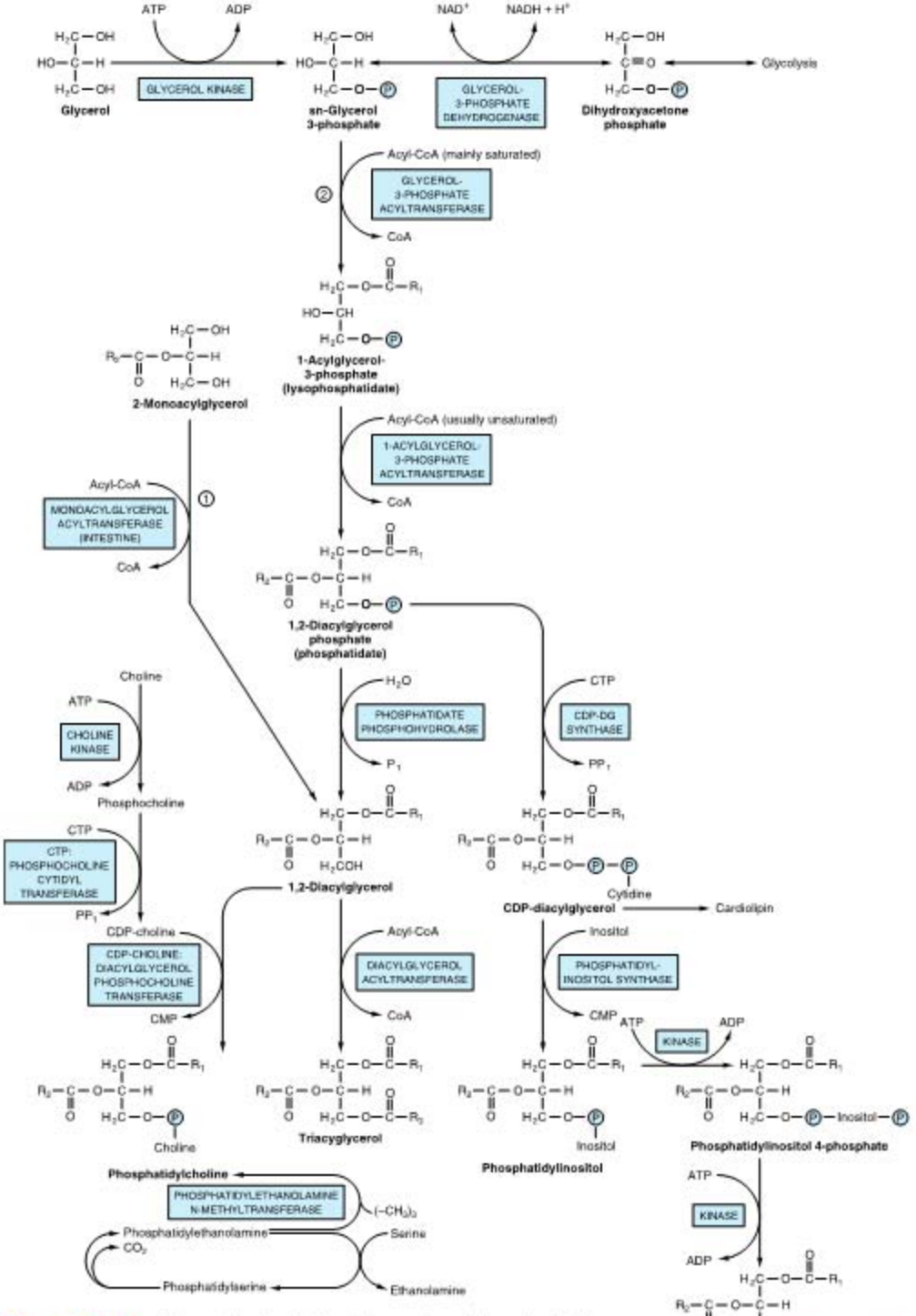

Figure 24–2. Biosynthesis of triacylglycerol and phospholipids. (①, Monoacylglycerol pathway; ②, glycerol phosphate pathway.) Phosphatidylethanolamine may be formed from ethanolamine by a pathway similar to that shown for the formation of phosphatidylcholine from choline.

Phosphatidate Is the Common Precursor in the Biosynthesis of Triacylglycerols, Many Phosphoglycerols, & Cardiolipin

Both glycerol and fatty acids must be activated by ATP before they can be incorporated into acylglycerols. Glycerol kinase catalyzes the activation of glycerol to *sn*-glycerol 3-phosphate. If the activity of this enzyme is absent or low, as in muscle or adipose tissue, most of the glycerol 3-phosphate is formed from dihydroxyacetone phosphate by **glycerol-3-phosphate dehydrogenase** (Figure 24–2).

A. Biosynthesis of Triacylglycerols

Two molecules of acyl-CoA, formed by the activation of fatty acids by **acyl-CoA synthetase** (Chapter 22), combine with glycerol 3-phosphate to form **phosphatidate** (1,2-diacylglycerol phosphate). This takes place in two stages, catalyzed by **glycerol-3-phosphate acyltransferase** and **1-acylglycerol-3-phosphate acyltransferase.** Phosphatidate is converted by **phosphatidate phosphohydrolase** and **diacylglycerol acyltransferase** to 1,2-diacylglycerol and then triacylglycerol. In intestinal mucosa, **monoacylglycerol acyltransferase** converts **monoacylglycerol** to 1,2-diacylglycerol in the **monoacylglycerol pathway.** Most of the activity of these enzymes resides in the endoplasmic reticulum of the cell, but some is found in mitochondria. Phosphatidate phosphohydrolase is found mainly in the cytosol, but the active form of the enzyme is membrane-bound.

In the biosynthesis of phosphatidylcholine and phosphatidylethanolamine (Figure 24–2), choline or ethanolamine must first be activated by phosphorylation by ATP followed by linkage to CTP. The resulting CDP-choline or CDP-ethanolamine reacts with 1,2-diacylglycerol to form either phosphatidylcholine or phosphatidylethanolamine, respectively. Phosphatidylserine is formed from phosphatidylethanolamine directly by reaction with serine (Figure 24–2). Phosphatidylserine may re-form phosphatidylethanolamine by decarboxylation. An alternative pathway in liver enables phosphatidylethanolamine to give rise directly to phosphatidylcholine by progressive methylation of the ethanolamine residue. In spite of these sources of choline, it is considered to be an essential nutrient in many mammalian species, but this has not been established in humans.

The regulation of triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine biosynthesis is driven by the availability of free fatty acids. Those that escape oxidation are preferentially converted to phospholipids, and when this requirement is satisfied they are used for triacylglycerol synthesis.

A phospholipid present in mitochondria is **cardiolipin** (diphosphatidylglycerol; Figure 14–8). It is formed from phosphatidylglycerol, which in turn is synthesized from CDP-diacylglycerol (Figure 24–2) and glycerol 3-phosphate according to the scheme shown in Figure 24–3. Cardiolipin, found in the inner membrane of mitochondria, is specifically required for the functioning of the phosphate transporter and for cytochrome oxidase activity.

B. Biosynthesis of Glycerol Ether Phospholipids

This pathway is located in peroxisomes. Dihydroxyacetone phosphate is the precursor of the glycerol moiety of glycerol ether phospholipids (Figure 24–4). This compound combines with acyl-CoA to give 1-acyldihydroxyacetone phosphate. The ether link is formed in the next reaction, producing 1-alkyldihydroxyacetone phosphate, which is then converted to 1-alkylglycerol 3-phosphate. After further acylation in the 2 position, the resulting 1-alkyl-2-acylglycerol 3-phosphate (analogous to phosphatidate in Figure 24–2) is hydrolyzed to give the free glycerol derivative. **Plasmalogens,** which comprise much of the phospholipid in mitochondria, are formed by desaturation of the analogous 3-phosphoethanolamine derivative (Figure 24–4). **Platelet-activating factor (PAF)** (1-alkyl-2-acetyl-*sn*-glycerol-3-phosphocholine) is synthesized from the corresponding 3-phosphocholine derivative. It is formed by many blood cells and other tissues and aggregates platelets at concentrations as low as 10^{-11} mol/L. It also has hypotensive and ulcerogenic properties and is involved in a variety of biologic responses, including inflammation, chemotaxis, and protein phosphorylation.

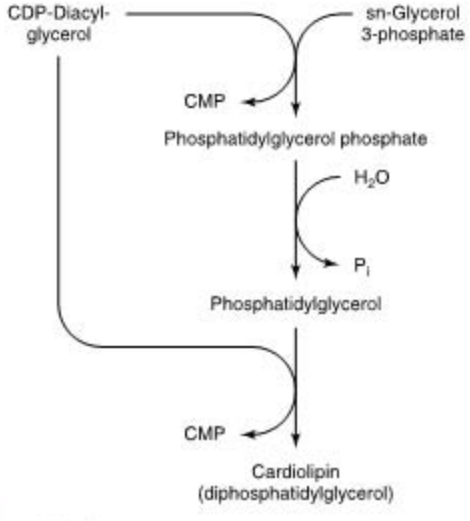

Figure 24–3. Biosynthesis of cardiolipin.

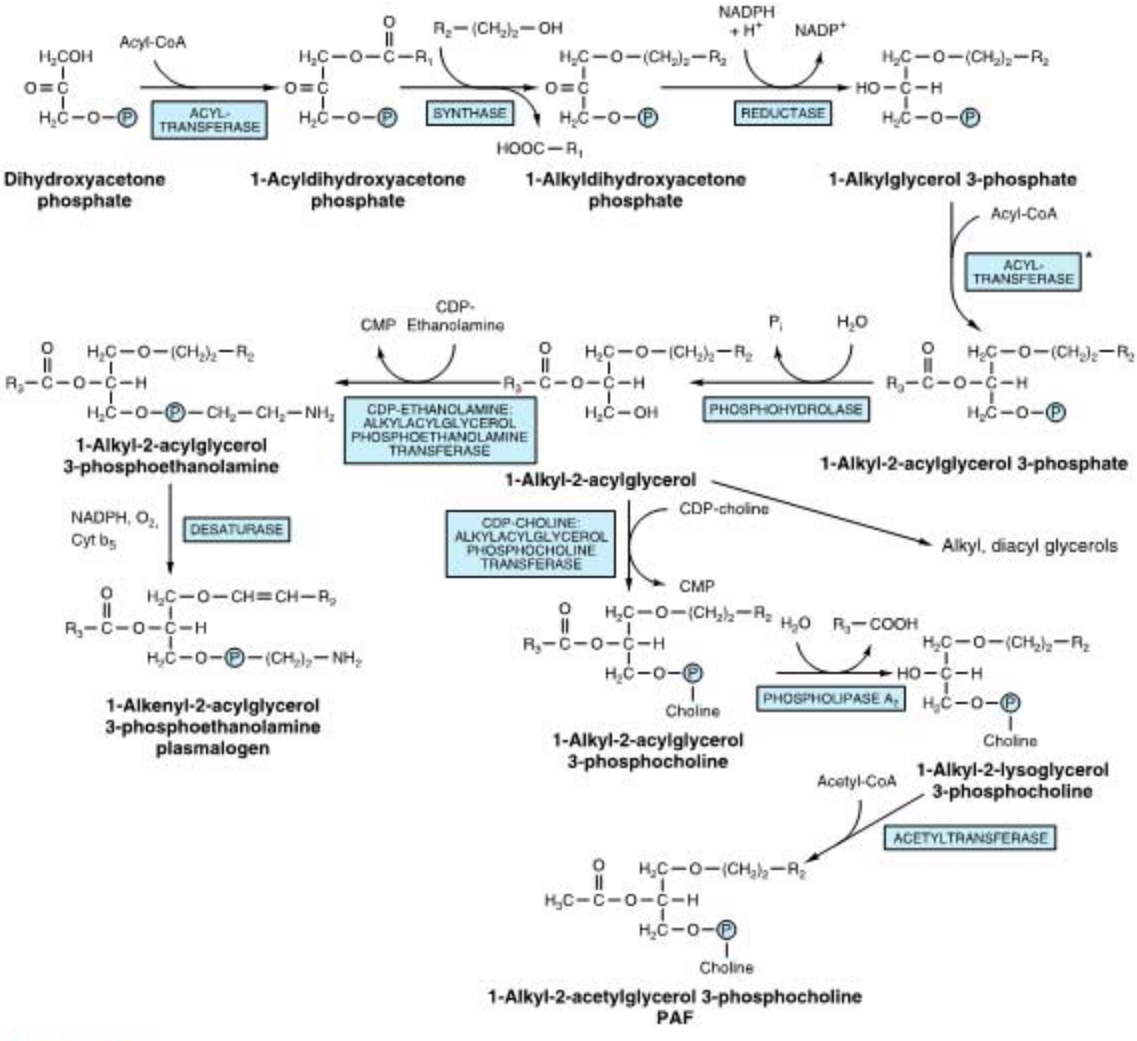

Figure 24–4. Biosynthesis of ether lipids, including plasmalogens, and platelet-activating factor (PAF). In the de novo pathway for PAF synthesis, acetyl-CoA is incorporated at stage *, avoiding the last two steps in the pathway shown here.

Phospholipases Allow Degradation & Remodeling of Phosphoglycerols

Although phospholipids are actively degraded, each portion of the molecule turns over at a different rate—eg, the turnover time of the phosphate group is different from that of the 1-acyl group. This is due to the presence of enzymes that allow partial degradation followed by resynthesis (Figure 24–5). **Phospholipase A_2** catalyzes the hydrolysis of glycerophospholipids to form a free fatty acid and lysophospholipid, which in turn may be reacylated by acyl-CoA in the presence of an acyltransferase. Alternatively, lysophospholipid (eg, lysolecithin) is attacked by **lysophospholipase**, forming the corresponding glyceryl phosphoryl base, which in turn may be split by a hydrolase liberating glycerol 3-phosphate plus base. **Phospholipases A_1, A_2, B, C, and D** attack the bonds indicated in Figure 24–6. **Phospholipase A_2** is found in pancreatic fluid and snake venom as well as in many types of cells; **phospholipase C** is one of the major toxins secreted by bacteria; and **phospholipase D** is known to be involved in mammalian signal transduction.

Lysolecithin (lysophosphatidylcholine) may be formed by an alternative route that involves **lecithin: cholesterol acyltransferase (LCAT).** This enzyme,

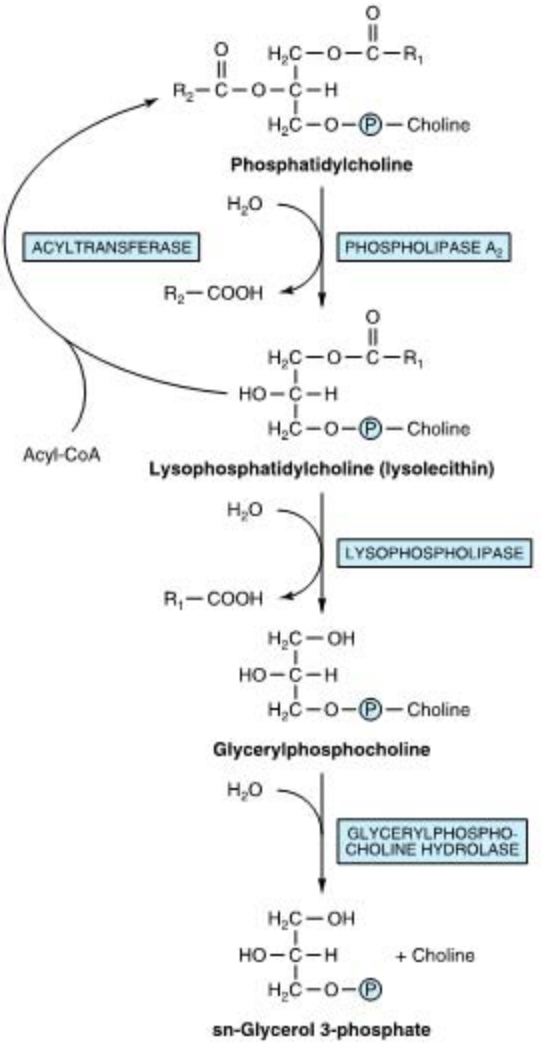

Figure 24–5. Metabolism of phosphatidylcholine (lecithin).

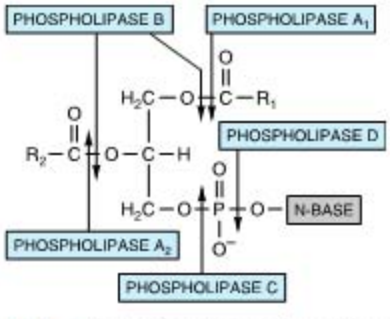

Figure 24–6. Sites of the hydrolytic activity of phospholipases on a phospholipid substrate.

found in plasma, catalyzes the transfer of a fatty acid residue from the 2 position of lecithin to cholesterol to form cholesteryl ester and lysolecithin and is considered to be responsible for much of the cholesteryl ester in plasma lipoproteins. Long-chain saturated fatty acids are found predominantly in the 1 position of phospholipids, whereas the polyunsaturated acids (eg, the precursors of prostaglandins) are incorporated more into the 2 position. The incorporation of fatty acids into lecithin occurs by complete synthesis of the phospholipid, by transacylation between cholesteryl ester and lysolecithin, and by direct acylation of lysolecithin by acyl-CoA. Thus, a continuous exchange of the fatty acids is possible, particularly with regard to introducing essential fatty acids into phospholipid molecules.

ALL SPHINGOLIPIDS ARE FORMED FROM CERAMIDE

Ceramide is synthesized in the endoplasmic reticulum from the amino acid serine according to Figure 24–7. Ceramide is an important signaling molecule (second messenger) regulating pathways including **apoptosis** (processes leading to cell death), cell senescence, and differentiation, and opposes some of the actions of diacylglycerol.

Sphingomyelins (Figure 14–11) are phospholipids and are formed when ceramide reacts with phosphatidylcholine to form sphingomyelin plus diacylglycerol (Figure 24–8A). This occurs mainly in the Golgi apparatus and to a lesser extent in the plasma membrane.

Glycosphingolipids Are a Combination of Ceramide With One or More Sugar Residues

The simplest glycosphingolipids **(cerebrosides)** are **galactosylceramide (GalCer)** and **glucosylceramide (GlcCer).** GalCer is a major lipid of myelin, whereas GlcCer is the major glycosphingolipid of extraneural tissues and a precursor of most of the more complex glycosphingolipids. Galactosylceramide (Figure 24–8B) is formed in a reaction between ceramide and UDPGal (formed by epimerization from UDPGlc—Figure 20–6). **Sulfogalactosylceramide** and other sulfolipids such as the **sulfo(galacto)-glycerolipids** and the **steroid sulfates** are formed after further reactions involving 3′-phosphoadenosine-5′-phosphosulfate (PAPS; "active sulfate"). **Gangliosides** are synthesized from ceramide by the stepwise addition of activated sugars (eg, UDPGlc and UDPGal) and a **sialic acid,** usually ***N*-acetylneuraminic acid** (Figure 24–9). A large number of gangliosides of increasing molecular weight may be formed. Most of the enzymes transferring sugars from

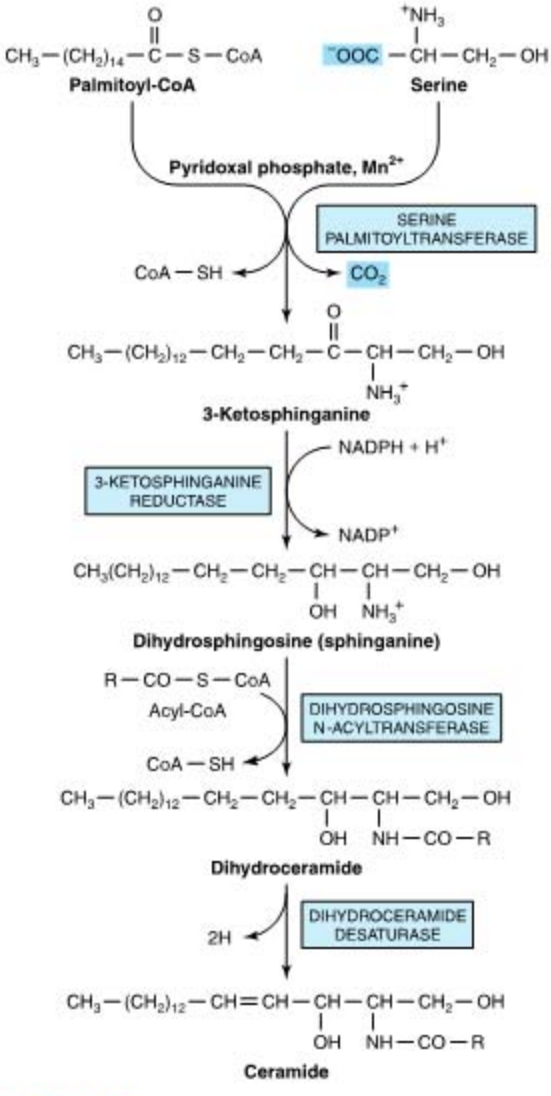

Figure 24–7. Biosynthesis of ceramide.

nucleotide sugars (glycosyl transferases) are found in the Golgi apparatus.

Glycosphingolipids are constituents of the outer leaflet of plasma membranes and are important in **cell adhesion** and **cell recognition.** Some are antigens, eg, ABO blood group substances. Certain gangliosides function as receptors for bacterial toxins (eg, for cholera toxin, which subsequently activates adenylyl cyclase).

CLINICAL ASPECTS

Deficiency of Lung Surfactant Causes Respiratory Distress Syndrome

Lung surfactant is composed mainly of lipid with some proteins and carbohydrate and prevents the alveoli from collapsing. Surfactant activity is largely attributed to **dipalmitoylphosphatidylcholine,** which is synthesized shortly before parturition in full-term infants. Deficiency of lung surfactant in the lungs of many preterm newborns gives rise to **respiratory distress syndrome.** Administration of either natural or artificial surfactant has been of therapeutic benefit.

Phospholipids & Sphingolipids Are Involved in Multiple Sclerosis and Lipidoses

Certain diseases are characterized by abnormal quantities of these lipids in the tissues, often in the nervous system. They may be classified into two groups: (1) true demyelinating diseases and (2) sphingolipidoses.

In **multiple sclerosis,** which is a demyelinating disease, there is loss of both phospholipids (particularly ethanolamine plasmalogen) and of sphingolipids from white matter. Thus, the lipid composition of white matter resembles that of gray matter. The cerebrospinal fluid shows raised phospholipid levels.

The **sphingolipidoses (lipid storage diseases)** are a group of inherited diseases that are often manifested in childhood. These diseases are part of a larger group of lysosomal disorders and exhibit several constant features: (1) Complex lipids containing ceramide accumulate in cells, particularly neurons, causing neurodegen-

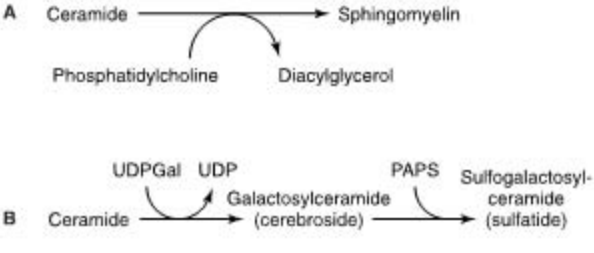

Figure 24–8. Biosynthesis of sphingomyelin (A), galactosylceramide and its sulfo derivative (B). (PAPS, "active sulfate," adenosine 3′-phosphate-5′-phosphosulfate.)

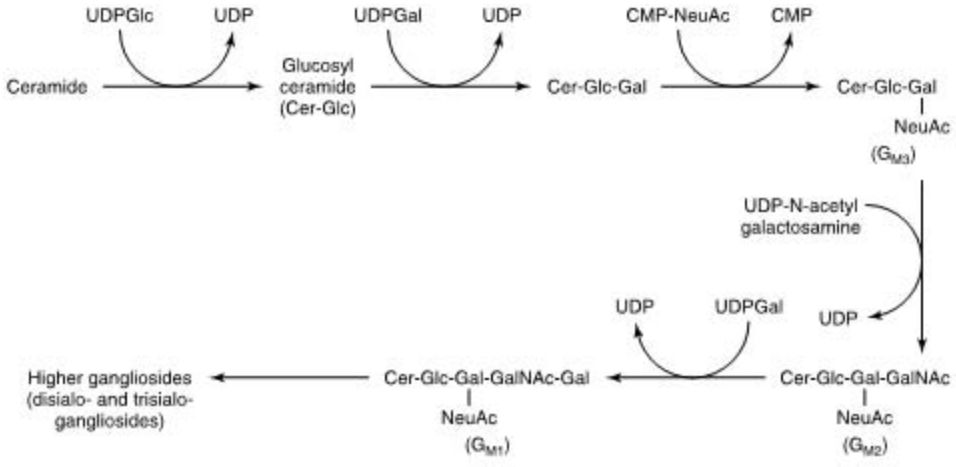

Figure 24–9. Biosynthesis of gangliosides. (NeuAc, *N*-acetylneuraminic acid.)

eration and shortening the life span. (2) The rate of **synthesis** of the stored lipid is normal. (3) The enzymatic defect is in the **lysosomal degradation pathway** of sphingolipids. (4) The extent to which the activity of the affected enzyme is decreased is similar in all tissues. There is no effective treatment for many of the diseases, though some success has been achieved with enzymes that have been chemically modified to ensure binding to receptors of target cells, eg, to macrophages in the liver in order to deliver β-glucosidase (glucocerebrosidase) in the treatment of Gaucher's disease. A recent promising approach is substrate reduction therapy to inhibit the synthesis of sphingolipids, and gene therapy for lysosomal disorders is currently under investigation. Some examples of the more important lipid storage diseases are shown in Table 24–1.

Multiple sulfatase deficiency results in accumulation of sulfogalactosylceramide, steroid sulfates, and proteoglycans owing to a combined deficiency of arylsulfatases A, B, and C and steroid sulfatase.

Table 24–1. Examples of sphingolipidoses.

Disease	Enzyme Deficiency	Lipid Accumulating[1]	Clinical Symptoms
Tay-Sachs disease	Hexosaminidase A	Cer—Glc—Gal(NeuAc)÷GalNAc G_{M2} Ganglioside	Mental retardation, blindness, muscular weakness.
Fabry's disease	α-Galactosidase	Cer—Glc—Gal÷Gal Globotriaosylceramide	Skin rash, kidney failure (full symptoms only in males; X-linked recessive).
Metachromatic leukodystrophy	Arylsulfatase A	Cer—Gal÷OSO_3 3-Sulfogalactosylceramide	Mental retardation and psychologic disturbances in adults; demyelination.
Krabbe's disease	β-Galactosidase	Cer÷Gal Galactosylceramide	Mental retardation; myelin almost absent.
Gaucher's disease	β-Glucosidase	Cer÷Glc Glucosylceramide	Enlarged liver and spleen, erosion of long bones, mental retardation in infants.
Niemann-Pick disease	Sphingomyelinase	Cer÷P—choline Sphingomyelin	Enlarged liver and spleen, mental retardation; fatal in early life.
Farber's disease	Ceramidase	Acyl÷Sphingosine Ceramide	Hoarseness, dermatitis, skeletal deformation, mental retardation; fatal in early life.

[1]NeuAc, *N*-acetylneuraminic acid; Cer, ceramide; Glc, glucose; Gal, galactose. ÷, site of deficient enzyme reaction.

SUMMARY

- Triacylglycerols are the major energy-storing lipids, whereas phosphoglycerols, sphingomyelin, and glycosphingolipids are amphipathic and have structural functions in cell membranes as well as other specialized roles.
- Triacylglycerols and some phosphoglycerols are synthesized by progressive acylation of glycerol 3-phosphate. The pathway bifurcates at phosphatidate, forming inositol phospholipids and cardiolipin on the one hand and triacylglycerol and choline and ethanolamine phospholipids on the other.
- Plasmalogens and platelet-activating factor (PAF) are ether phospholipids formed from dihydroxyacetone phosphate.
- Sphingolipids are formed from ceramide (*N*-acylsphingosine). Sphingomyelin is present in membranes of organelles involved in secretory processes (eg, Golgi apparatus). The simplest glycosphingolipids are a combination of ceramide plus a sugar residue (eg, GalCer in myelin). Gangliosides are more complex glycosphingolipids containing more sugar residues plus sialic acid. They are present in the outer layer of the plasma membrane, where they contribute to the glycocalyx and are important as antigens and cell receptors.
- Phospholipids and sphingolipids are involved in several disease processes, including respiratory distress syndrome (lack of lung surfactant), multiple sclerosis (demyelination), and sphingolipidoses (inability to break down sphingolipids in lysosomes due to inherited defects in hydrolase enzymes).

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Lipid Transport & Storage

25

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BIOMEDICAL IMPORTANCE

Fat absorbed from the diet and lipids synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. Since lipids are insoluble in water, the problem of how to transport them in the aqueous blood plasma is solved by associating nonpolar lipids (triacylglycerol and cholesteryl esters) with amphipathic lipids (phospholipids and cholesterol) and proteins to make water-miscible lipoproteins.

In a meal-eating omnivore such as the human, excess calories are ingested in the anabolic phase of the feeding cycle, followed by a period of negative caloric balance when the organism draws upon its carbohydrate and fat stores. Lipoproteins mediate this cycle by transporting lipids from the intestines as chylomicrons—and from the liver as very low density lipoproteins (VLDL)—to most tissues for oxidation and to adipose tissue for storage. Lipid is mobilized from adipose tissue as free fatty acids (FFA) attached to serum albumin. Abnormalities of lipoprotein metabolism cause various **hypo-** or **hyperlipoproteinemias.** The most common of these is **diabetes mellitus,** where insulin deficiency causes excessive mobilization of FFA and underutilization of chylomicrons and VLDL, leading to **hypertriacylglycerolemia.** Most other pathologic conditions affecting lipid transport are due primarily to inherited defects, some of which cause **hypercholesterolemia,** and premature **atherosclerosis. Obesity**—particularly abdominal obesity—is a risk factor for increased mortality, hypertension, type 2 diabetes mellitus, hyperlipidemia, hyperglycemia, and various endocrine dysfunctions.

LIPIDS ARE TRANSPORTED IN THE PLASMA AS LIPOPROTEINS

Four Major Lipid Classes Are Present in Lipoproteins

Plasma lipids consist of **triacylglycerols** (16%), **phospholipids** (30%), **cholesterol** (14%), and **cholesteryl esters** (36%) and a much smaller fraction of unesterified long-chain fatty acids (free fatty acids) (4%). This latter fraction, the **free fatty acids (FFA),** is metabolically the most active of the plasma lipids.

Four Major Groups of Plasma Lipoproteins Have Been Identified

Because fat is less dense than water, the density of a lipoprotein decreases as the proportion of lipid to protein increases (Table 25–1). In addition to FFA, four major groups of lipoproteins have been identified that are important physiologically and in clinical diagnosis. These are (1) **chylomicrons,** derived from intestinal absorption of triacylglycerol and other lipids; (2) **very low density lipoproteins** (VLDL, or pre-β-lipoproteins), derived from the liver for the export of triacylglycerol; (3) **low-density lipoproteins** (LDL, or β-lipoproteins), representing a final stage in the catabolism of VLDL; and (4) **high-density lipoproteins** (HDL, or α-lipoproteins), involved in VLDL and chylomicron metabolism and also in cholesterol transport. Triacylglycerol is the predominant lipid in chylomicrons and VLDL, whereas cholesterol and phospholipid are the predominant lipids in LDL and HDL, respectively (Table 25–1). Lipoproteins may be separated according to their electrophoretic properties into **α-, β-,** and **pre-β-lipoproteins.**

Lipoproteins Consist of a Nonpolar Core & a Single Surface Layer of Amphipathic Lipids

The **nonpolar lipid core** consists of mainly **triacylglycerol** and **cholesteryl ester** and is surrounded by a **single surface layer** of **amphipathic phospholipid** and **cholesterol** molecules (Figure 25–1). These are oriented so that their polar groups face outward to the aqueous medium, as in the cell membrane (Chapter 14). The protein moiety of a lipoprotein is known as an **apolipoprotein** or **apoprotein,** constituting nearly 70% of some HDL and as little as 1% of chylomicrons. Some apolipoproteins are integral and cannot be removed, whereas others are free to transfer to other lipoproteins.

The Distribution of Apolipoproteins Characterizes the Lipoprotein

One or more apolipoproteins (proteins or polypeptides) are present in each lipoprotein. The major apolipoproteins of HDL (α-lipoprotein) are designated A (Table

Table 25–1. Composition of the lipoproteins in plasma of humans.

Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Composition		Main Lipid Components	Apolipoproteins
				Protein (%)	Lipid (%)		
Chylomicrons	Intestine	90–1000	< 0.95	1–2	98–99	Triacylglycerol	A-I, A-II, A-IV,[1] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45–150	< 1.006	6–8	92–94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30–90	0.95–1.006	7–10	90–93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25–35	1.006–1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20–25	1.019–1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylomicrons					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[2] E
HDL_1		20–25	1.019–1.063	32	68		
HDL_2		10–20	1.063–1.125	33	67		
HDL_3		5–10	1.125–1.210	57	43		
Preβ-HDL[3]		< 5	> 1.210				A-I
Albumin/free fatty acids	Adipose tissue		> 1.281	99	1	Free fatty acids	

Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low density lipoproteins.
[1]Secreted with chylomicrons but transfers to HDL.
[2]Associated with HDL_2 and HDL_3 subfractions.
[3]Part of a minor fraction known as very high density lipoproteins (VHDL).

25–1). The main apolipoprotein of LDL (β-lipoprotein) is apolipoprotein B (B-100) and is found also in VLDL. Chylomicrons contain a truncated form of apo B (B-48) that is synthesized in the intestine, while B-100 is synthesized in the liver. Apo B-100 is one of the longest single polypeptide chains known, having 4536 amino acids and a molecular mass of 550,000 Da. Apo B-48 (48% of B-100) is formed from the same mRNA as apo B-100 after the introduction of a stop signal by an RNA editing enzyme. Apo C-I, C-II, and C-III are smaller polypeptides (molecular mass 7000–9000 Da) freely transferable between several different lipoproteins. Apo E is found in VLDL, HDL, chylomicrons, and chylomicron remnants; it accounts for 5–10% of total VLDL apolipoproteins in normal subjects.

Apolipoproteins carry out several roles: (1) they can form part of the structure of the lipoprotein, eg, apo B; (2) they are enzyme cofactors, eg, C-II for lipoprotein lipase, A-I for lecithin:cholesterol acyltransferase, or enzyme inhibitors, eg, apo A-II and apo C-III for lipoprotein lipase, apo C-I for cholesteryl ester transfer protein; and (3) they act as ligands for interaction with lipoprotein receptors in tissues, eg, apo B-100 and apo E for the LDL receptor, apo E for the LDL receptor-related protein (LRP), which has been identified as the remnant receptor, and apo A-I for the HDL receptor. The functions of apo A-IV and apo D, however, are not yet clearly defined.

FREE FATTY ACIDS ARE RAPIDLY METABOLIZED

The free fatty acids (FFA, nonesterified fatty acids, unesterified fatty acids) arise in the plasma from lipolysis of triacylglycerol in adipose tissue or as a result of the action of lipoprotein lipase during uptake of plasma triacylglycerols into tissues. They are found **in combination with albumin,** a very effective solubilizer, in concentrations varying between 0.1 and 2.0 μeq/mL of plasma. Levels are low in the fully fed condition and rise to 0.7–0.8 μeq/mL in the starved state. In uncontrolled **diabetes mellitus,** the level may rise to as much as 2 μeq/mL.

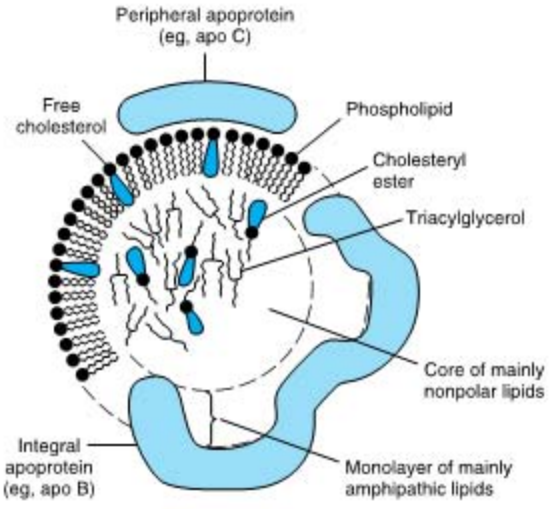


Figure 25–1. Generalized structure of a plasma lipoprotein. The similarities with the structure of the plasma membrane are to be noted. Small amounts of cholesteryl ester and triacylglycerol are to be found in the surface layer and a little free cholesterol in the core.

Free fatty acids are removed from the blood extremely rapidly and oxidized (fulfilling 25–50% of energy requirements in starvation) or esterified to form triacylglycerol in the tissues. In starvation, esterified lipids from the circulation or in the tissues are oxidized as well, particularly in heart and skeletal muscle cells, where considerable stores of lipid are to be found.

The free fatty acid uptake by tissues is related directly to the plasma free fatty acid concentration, which in turn is determined by the rate of lipolysis in adipose tissue. After dissociation of the fatty acid-albumin complex at the plasma membrane, fatty acids bind to a **membrane fatty acid transport protein** that acts as a transmembrane cotransporter with Na^+. On entering the cytosol, free fatty acids are bound by intracellular **fatty acid-binding proteins.** The role of these proteins in intracellular transport is thought to be similar to that of serum albumin in extracellular transport of long-chain fatty acids.

TRIACYLGLYCEROL IS TRANSPORTED FROM THE INTESTINES IN CHYLOMICRONS & FROM THE LIVER IN VERY LOW DENSITY LIPOPROTEINS

By definition, **chylomicrons** are found in **chyle** formed only by the lymphatic system **draining the intestine.** They are responsible for the transport of all dietary lipids into the circulation. Small quantities of VLDL are also to be found in chyle; however, most of the plasma **VLDL** are of hepatic origin. **They are the vehicles of transport of triacylglycerol from the liver to the extrahepatic tissues.**

There are striking similarities in the mechanisms of formation of chylomicrons by intestinal cells and of VLDL by hepatic parenchymal cells (Figure 25–2), perhaps because—apart from the mammary gland—the intestine and liver are the only tissues from which particulate lipid is secreted. Newly secreted or "nascent" chylomicrons and VLDL contain only a small amount of apolipoproteins C and E, and the full complement is acquired from HDL in the circulation (Figures 25–3 and 25–4). Apo B is essential for chylomicron and VLDL formation. In **abetalipoproteinemia** (a rare disease), lipoproteins containing apo B are not formed and lipid droplets accumulate in the intestine and liver.

A more detailed account of the factors controlling hepatic VLDL secretion is given below.

CHYLOMICRONS & VERY LOW DENSITY LIPOPROTEINS ARE RAPIDLY CATABOLIZED

The clearance of labeled chylomicrons from the blood is rapid, the half-time of disappearance being under 1 hour in humans. Larger particles are catabolized more quickly than smaller ones. Fatty acids originating from chylomicron triacylglycerol are delivered mainly to adipose tissue, heart, and muscle (80%), while about 20% goes to the liver. However, **the liver does not metabolize native chylomicrons or VLDL significantly;** thus, the fatty acids in the liver must be secondary to their metabolism in extrahepatic tissues.

Triacylglycerols of Chylomicrons & VLDL Are Hydrolyzed by Lipoprotein Lipase

Lipoprotein lipase is located on the walls of blood capillaries, anchored to the endothelium by negatively charged proteoglycan chains of heparan sulfate. It has been found in heart, adipose tissue, spleen, lung, renal medulla, aorta, diaphragm, and lactating mammary gland, though it is not active in adult liver. It is not normally found in blood; however, following injection of **heparin,** lipoprotein lipase is released from its heparan sulfate binding into the circulation. **Hepatic lipase** is bound to the sinusoidal surface of liver cells and is released by heparin. This enzyme, however, does not react readily with chylomicrons or VLDL but is concerned with chylomicron remnant and HDL metabolism.

Both **phospholipids** and **apo C-II** are required as cofactors for lipoprotein lipase activity, while apo A-II

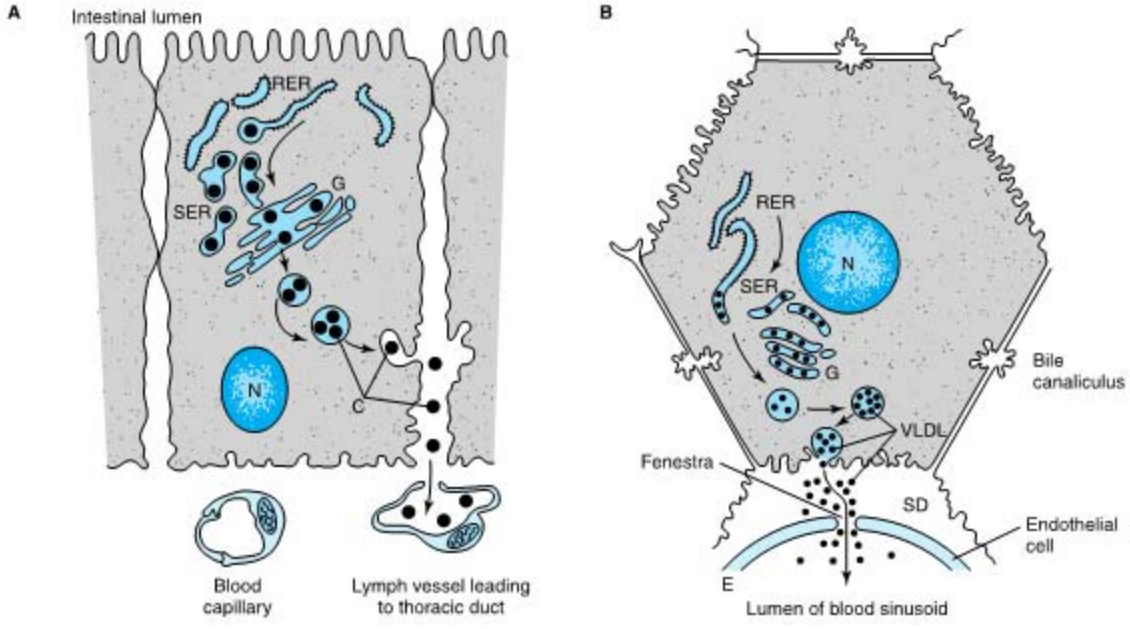


Figure 25–2. The formation and secretion of **(A)** chylomicrons by an intestinal cell and **(B)** very low density lipoproteins by a hepatic cell. (RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; G, Golgi apparatus; N, nucleus; C, chylomicrons; VLDL, very low density lipoproteins; E, endothelium; SD, space of Disse, containing blood plasma.) Apolipoprotein B, synthesized in the RER, is incorporated into lipoproteins in the SER, the main site of synthesis of triacylglycerol. After addition of carbohydrate residues in G, they are released from the cell by reverse pinocytosis. Chylomicrons pass into the lymphatic system. VLDL are secreted into the space of Disse and then into the hepatic sinusoids through fenestrae in the endothelial lining.

and apo C-III act as inhibitors. Hydrolysis takes place while the lipoproteins are attached to the enzyme on the endothelium. Triacylglycerol is hydrolyzed progressively through a diacylglycerol to a monoacylglycerol that is finally hydrolyzed to free fatty acid plus glycerol. Some of the released free fatty acids return to the circulation, attached to albumin, but the bulk is transported into the tissue (Figures 25–3 and 25–4). Heart lipoprotein lipase has a low K_m for triacylglycerol, about one-tenth of that for the enzyme in adipose tissue. This enables the delivery of fatty acids from triacylglycerol to be **redirected from adipose tissue to the heart in the starved state** when the plasma triacylglycerol decreases. A similar redirection to the mammary gland occurs during lactation, allowing uptake of lipoprotein triacylglycerol fatty acid for milk fat synthesis. The **VLDL receptor** plays an important part in the delivery of fatty acids from VLDL triacylglycerol to adipocytes by binding VLDL and bringing it into close contact with lipoprotein lipase. In adipose tissue, insulin enhances lipoprotein lipase synthesis in adipocytes and its translocation to the luminal surface of the capillary endothelium.

The Action of Lipoprotein Lipase Forms Remnant Lipoproteins

Reaction with lipoprotein lipase results in the loss of approximately 90% of the triacylglycerol of chylomicrons and in the loss of apo C (which returns to HDL) but not apo E, which is retained. The resulting **chylomicron remnant** is about half the diameter of the parent chylomicron and is relatively enriched in cholesterol and cholesteryl esters because of the loss of triacylglycerol (Figure 25–3). Similar changes occur to VLDL, with the formation of VLDL remnants or IDL (intermediate-density lipoprotein) (Figure 25–4).

The Liver Is Responsible for the Uptake of Remnant Lipoproteins

Chylomicron remnants are taken up by the liver by receptor-mediated endocytosis, and the cholesteryl esters and triacylglycerols are hydrolyzed and metabolized. Uptake is mediated by a **receptor specific for apo E** (Figure 25–3), and both the **LDL (apo B-100, E) receptor** and the **LRP (LDL receptor-related protein)**

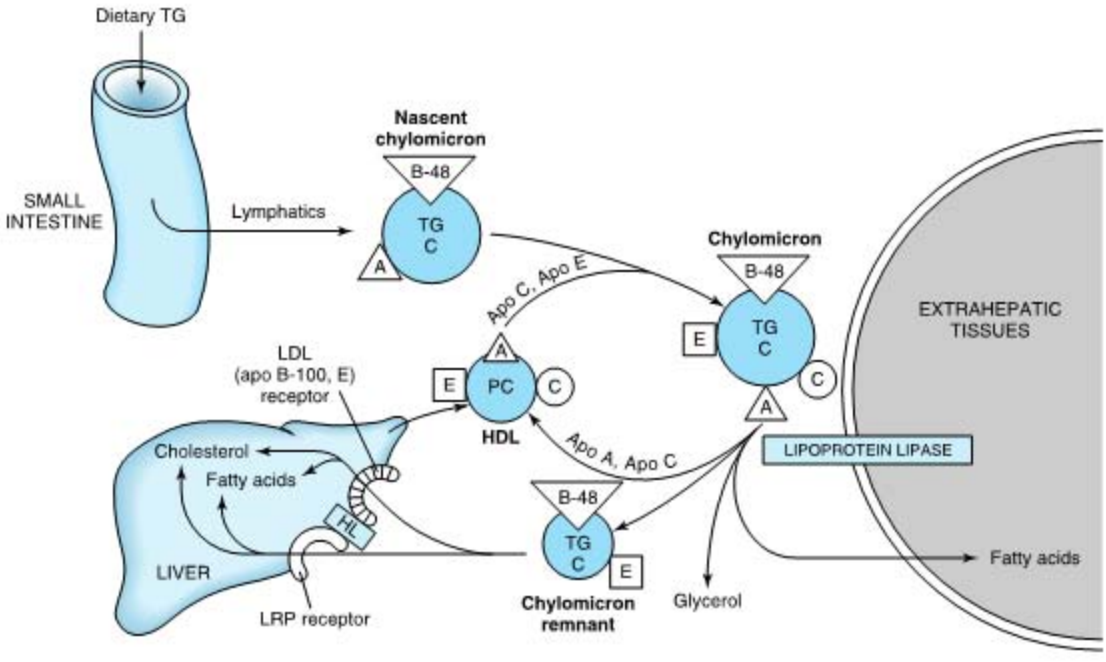


Figure 25–3. Metabolic fate of chylomicrons. (A, apolipoprotein A; B-48, apolipoprotein B-48; ℂ, apolipoprotein C; E, apolipoprotein E; HDL, high-density lipoprotein; TG, triacylglycerol; C, cholesterol and cholesteryl ester; P, phospholipid; HL, hepatic lipase; LRP, LDL receptor-related protein.) Only the predominant lipids are shown.

are believed to take part. Hepatic lipase has a dual role: (1) in acting as a ligand to the lipoprotein and (2) in hydrolyzing its triacylglycerol and phospholipid.

VLDL is the precursor of IDL, which is then converted to LDL. Only one molecule of apo B-100 is present in each of these lipoprotein particles, and this is conserved during the transformations. Thus, each LDL particle is derived from only one VLDL particle (Figure 25–4). Two possible fates await IDL. It can be taken up by the liver directly via the LDL (apo B-100, E) receptor, or it is converted to LDL. In humans, a relatively large proportion forms LDL, accounting for the increased concentrations of LDL in humans compared with many other mammals.

LDL IS METABOLIZED VIA THE LDL RECEPTOR

The liver and many extrahepatic tissues express the **LDL (B-100, E) receptor.** It is so designated because it is specific for apo B-100 but not B-48, which lacks the carboxyl terminal domain of B-100 containing the LDL receptor ligand, and it also takes up lipoproteins rich in apo E. This receptor is defective in **familial hypercholesterolemia.** Approximately 30% of LDL is degraded in extrahepatic tissues and 70% in the liver. A positive correlation exists between the incidence of **coronary atherosclerosis** and the plasma concentration of LDL cholesterol. For further discussion of the regulation of the LDL receptor, see Chapter 26.

HDL TAKES PART IN BOTH LIPOPROTEIN TRIACYLGLYCEROL & CHOLESTEROL METABOLISM

HDL is synthesized and secreted from both liver and intestine (Figure 25–5). However, apo C and apo E are synthesized in the liver and transferred from liver HDL to intestinal HDL when the latter enters the plasma. A major function of HDL is to act as a repository for the apo C and apo E required in the metabolism of chylomicrons and VLDL. Nascent HDL consists of discoid phospholipid bilayers containing apo A and free cholesterol. These lipoproteins are similar to the particles found in the plasma of patients with a deficiency of the plasma enzyme **lecithin:cholesterol acyltransferase (LCAT)** and in the plasma of patients with obstructive jaundice. LCAT—and the LCAT activator apo A-I—bind to the disk, and the surface phospholipid and free cholesterol are converted into cholesteryl esters and

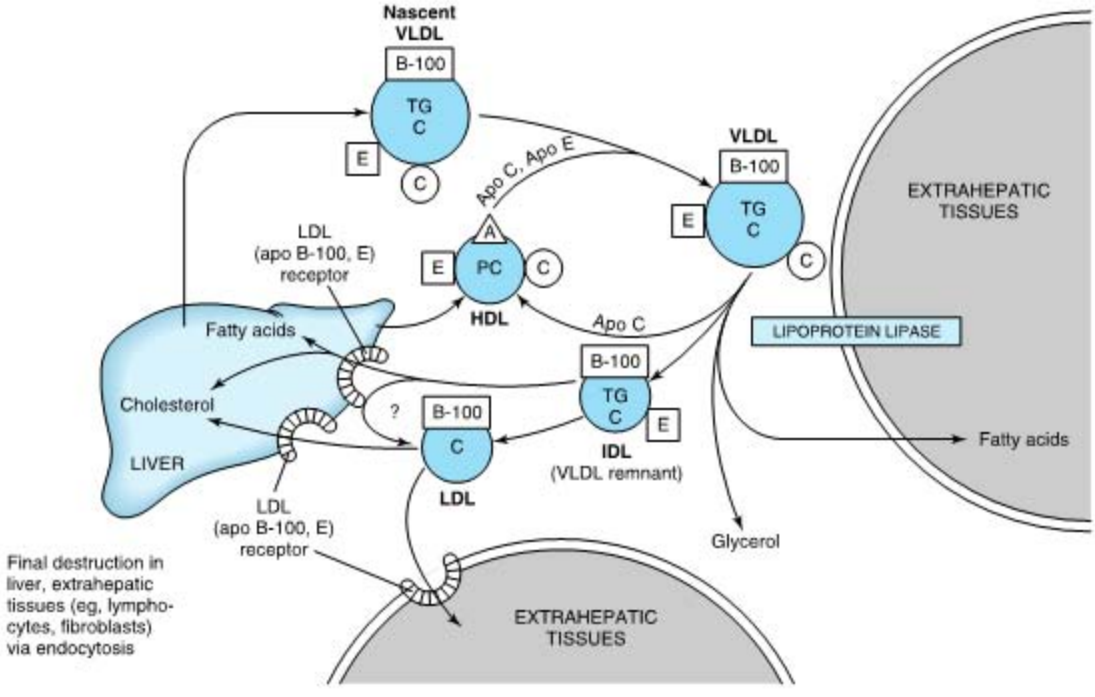


Figure 25–4. Metabolic fate of very low density lipoproteins (VLDL) and production of low-density lipoproteins (LDL). (A, apolipoprotein A; B-100, apolipoprotein B-100; ©, apolipoprotein C; E, apolipoprotein E; HDL, high-density lipoprotein; TG, triacylglycerol; IDL, intermediate-density lipoprotein; C, cholesterol and cholesteryl ester; P, phospholipid.) Only the predominant lipids are shown. It is possible that some IDL is also metabolized via the LRP.

lysolecithin (Chapter 24). The nonpolar cholesteryl esters move into the hydrophobic interior of the bilayer, whereas lysolecithin is transferred to plasma albumin. Thus, a nonpolar core is generated, forming a spherical, pseudomicellar HDL covered by a surface film of polar lipids and apolipoproteins. In this way, the LCAT system is involved in the removal of excess unesterified cholesterol from lipoproteins and tissues. The **class B scavenger receptor B1 (SR-B1)** has recently been identified as an **HDL receptor** in the liver and in steroidogenic tissues. HDL binds to the receptor via apo A-I and cholesteryl ester is selectively delivered to the cells, but the particle itself, including apo A-I, is not taken up. The transport of cholesterol from the tissues to the liver is known as **reverse cholesterol transport** and is mediated by an HDL cycle (Figure 25–5). The smaller HDL_3 accepts cholesterol from the tissues via the **ATP-binding cassette transporter-1 (ABC-1).** ABC-1 is a member of a family of transporter proteins that couple the hydrolysis of ATP to the binding of a substrate, enabling it to be transported across the membrane. After being accepted by HDL_3, the cholesterol is then esterified by LCAT, increasing the size of the particles to form the less dense HDL_2. The cycle is completed by the re-formation of HDL_3, either after selective delivery of cholesteryl ester to the liver via the SR-B1 or by hydrolysis of HDL_2 phospholipid and triacylglycerol by hepatic lipase. In addition, free apo A-I is released by these processes and forms **preβ-HDL** after associating with a minimum amount of phospholipid and cholesterol. Preβ-HDL is the most potent form of HDL in inducing cholesterol efflux from the tissues to form discoidal HDL. Surplus apo A-I is destroyed in the kidney.

HDL concentrations vary reciprocally with plasma triacylglycerol concentrations and directly with the activity of lipoprotein lipase. This may be due to surplus surface constituents, eg, phospholipid and apo A-I being released during hydrolysis of chylomicrons and VLDL and contributing toward the formation of preβ-HDL and discoidal HDL. HDL_2 concentrations are **inversely related to the incidence of coronary atherosclerosis,** possibly because they reflect the efficiency of reverse cholesterol transport. HDL_c (HDL_1) is found in

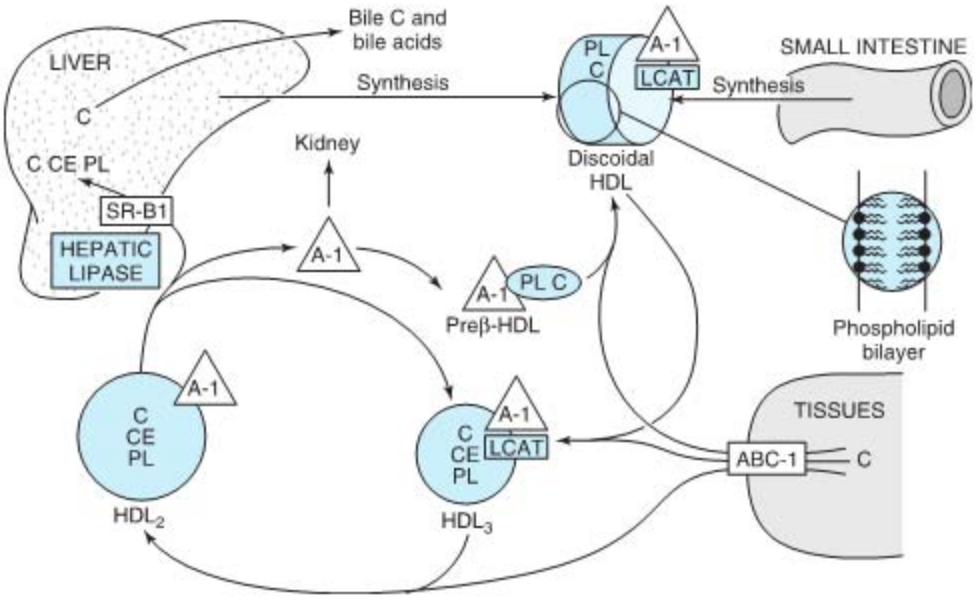


Figure 25–5. Metabolism of high-density lipoprotein (HDL) in reverse cholesterol transport. (LCAT, lecithin:cholesterol acyltransferase; C, cholesterol; CE, cholesteryl ester; PL, phospholipid; A-I, apolipoprotein A-I; SR-B1, scavenger receptor B1; ABC-1, ATP binding cassette transporter 1.) Preβ-HDL, HDL_2, HDL_3—see Table 25–1. Surplus surface constituents from the action of lipoprotein lipase on chylomicrons and VLDL are another source of preβ-HDL. Hepatic lipase activity is increased by androgens and decreased by estrogens, which may account for higher concentrations of plasma HDL_2 in women.

the blood of diet-induced hypercholesterolemic animals. It is rich in cholesterol, and its sole apolipoprotein is apo E. It appears that all plasma lipoproteins are interrelated components of one or more metabolic cycles that together are responsible for the complex process of plasma lipid transport.

THE LIVER PLAYS A CENTRAL ROLE IN LIPID TRANSPORT & METABOLISM

The liver carries out the following major functions in lipid metabolism: (1) It facilitates the digestion and absorption of lipids by the production of bile, which contains cholesterol and bile salts synthesized within the liver de novo or from uptake of lipoprotein cholesterol (Chapter 26). (2) The liver has active enzyme systems for synthesizing and oxidizing fatty acids (Chapters 21 and 22) and for synthesizing triacylglycerols and phospholipids (Chapter 24). (3) It converts fatty acids to ketone bodies (ketogenesis) (Chapter 22). (4) It plays an integral part in the synthesis and metabolism of plasma lipoproteins (this chapter).

Hepatic VLDL Secretion Is Related to Dietary & Hormonal Status

The cellular events involved in VLDL formation and secretion have been described above. Hepatic triacylglycerol synthesis provides the immediate stimulus for the formation and secretion of VLDL. The fatty acids used are derived from two possible sources: (1) synthesis within the liver from acetyl-CoA derived mainly from carbohydrate (perhaps not so important in humans) and (2) uptake of free fatty acids from the circulation. The first source is predominant in the well-fed condition, when fatty acid synthesis is high and the level of circulating free fatty acids is low. As triacylglycerol does not normally accumulate in the liver under this condition, it must be inferred that it is transported from the liver in VLDL as rapidly as it is synthesized and that the synthesis of apo B-100 is not rate-limiting. Free fatty acids from the circulation are the main source during starvation, the feeding of high-fat diets, or in diabetes mellitus, when hepatic lipogenesis is inhibited. Factors that enhance both the synthesis of triacylglycerol and the secretion of VLDL by the liver include (1)

the fed state rather than the starved state; (2) the feeding of diets high in carbohydrate (particularly if they contain sucrose or fructose), leading to high rates of lipogenesis and esterification of fatty acids; (3) high levels of circulating free fatty acids; (4) ingestion of ethanol; and (5) the presence of high concentrations of insulin and low concentrations of glucagon, which enhance fatty acid synthesis and esterification and inhibit their oxidation (Figure 25–6).

CLINICAL ASPECTS

Imbalance in the Rate of Triacylglycerol Formation & Export Causes Fatty Liver

For a variety of reasons, lipid—mainly as triacylglycerol—can accumulate in the liver (Figure 25–6). Extensive accumulation is regarded as a pathologic condition. When accumulation of lipid in the liver becomes chronic, fibrotic changes occur in the cells that progress to **cirrhosis** and impaired liver function.

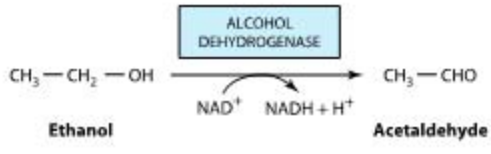
Fatty livers fall into two main categories. The first type is associated with **raised levels of plasma free fatty acids** resulting from mobilization of fat from adipose tissue or from the hydrolysis of lipoprotein triacylglycerol by lipoprotein lipase in extrahepatic tissues. The production of VLDL does not keep pace with the increasing influx and esterification of free fatty acids, allowing triacylglycerol to accumulate, causing a fatty liver. This occurs during **starvation** and the feeding of **high-fat diets.** The ability to secrete VLDL may also be impaired (eg, in starvation). In uncontrolled **diabetes mellitus, twin lamb disease,** and **ketosis in cattle,** fatty infiltration is sufficiently severe to cause visible pallor (fatty appearance) and enlargement of the liver with possible liver dysfunction.

The second type of fatty liver is usually due to a **metabolic block in the production of plasma lipoproteins,** thus allowing triacylglycerol to accumulate. Theoretically, the lesion may be due to (1) a block in apolipoprotein synthesis, (2) a block in the synthesis of the lipoprotein from lipid and apolipoprotein, (3) a failure in provision of phospholipids that are found in lipoproteins, or (4) a failure in the secretory mechanism itself.

One type of fatty liver that has been studied extensively in rats is due to a deficiency of **choline,** which has therefore been called a **lipotropic factor.** The antibiotic puromycin, ethionine (α-amino-γ-mercaptobutyric acid), carbon tetrachloride, chloroform, phosphorus, lead, and arsenic all cause fatty liver and a marked reduction in concentration of VLDL in rats. Choline will not protect the organism against these agents but appears to aid in recovery. The action of carbon tetrachloride probably involves formation of free radicals causing lipid peroxidation. Some protection against this is provided by the antioxidant action of vitamin E-supplemented diets. The action of ethionine is thought to be due to a reduction in availability of ATP due to its replacing methionine in *S*-adenosylmethionine, trapping available adenine and preventing synthesis of ATP. Orotic acid also causes fatty liver; it is believed to interfere with glycosylation of the lipoprotein, thus inhibiting release, and may also impair the recruitment of triacylglycerol to the particles. A deficiency of vitamin E enhances the hepatic necrosis of the choline deficiency type of fatty liver. Added vitamin E or a source of selenium has a protective effect by combating lipid peroxidation. In addition to protein deficiency, essential fatty acid and vitamin deficiencies (eg, linoleic acid, pyridoxine, and pantothenic acid) can cause fatty infiltration of the liver.

Ethanol Also Causes Fatty Liver

Alcoholism leads to fat accumulation in the liver, hyperlipidemia, and ultimately **cirrhosis.** The exact mechanism of action of ethanol in the long term is still uncertain. Ethanol consumption over a long period leads to the accumulation of fatty acids in the liver that are derived from endogenous synthesis rather than from increased mobilization from adipose tissue. There is no impairment of hepatic synthesis of protein after ethanol ingestion. Oxidation of ethanol by **alcohol dehydrogenase** leads to excess production of NADH.



The NADH generated competes with reducing equivalents from other substrates, including fatty acids, for the respiratory chain, inhibiting their oxidation, and decreasing activity of the citric acid cycle. The net effect of inhibiting fatty acid oxidation is to cause increased esterification of fatty acids in triacylglycerol, resulting in the fatty liver. Oxidation of ethanol leads to the formation of acetaldehyde, which is oxidized by **aldehyde dehydrogenase,** producing acetate. Other effects of ethanol may include increased lipogenesis and cholesterol synthesis from acetyl-CoA, and lipid peroxidation. The increased [NADH]/[NAD^+] ratio also causes increased [lactate]/[pyruvate], resulting in **hyperlacticacidemia,** which decreases excretion of uric acid, aggravating gout. Some metabolism of ethanol takes place via a cytochrome P450-dependent microsomal ethanol oxidizing system (MEOS) involving NADPH and O_2. This system increases in activity in **chronic alcoholism**

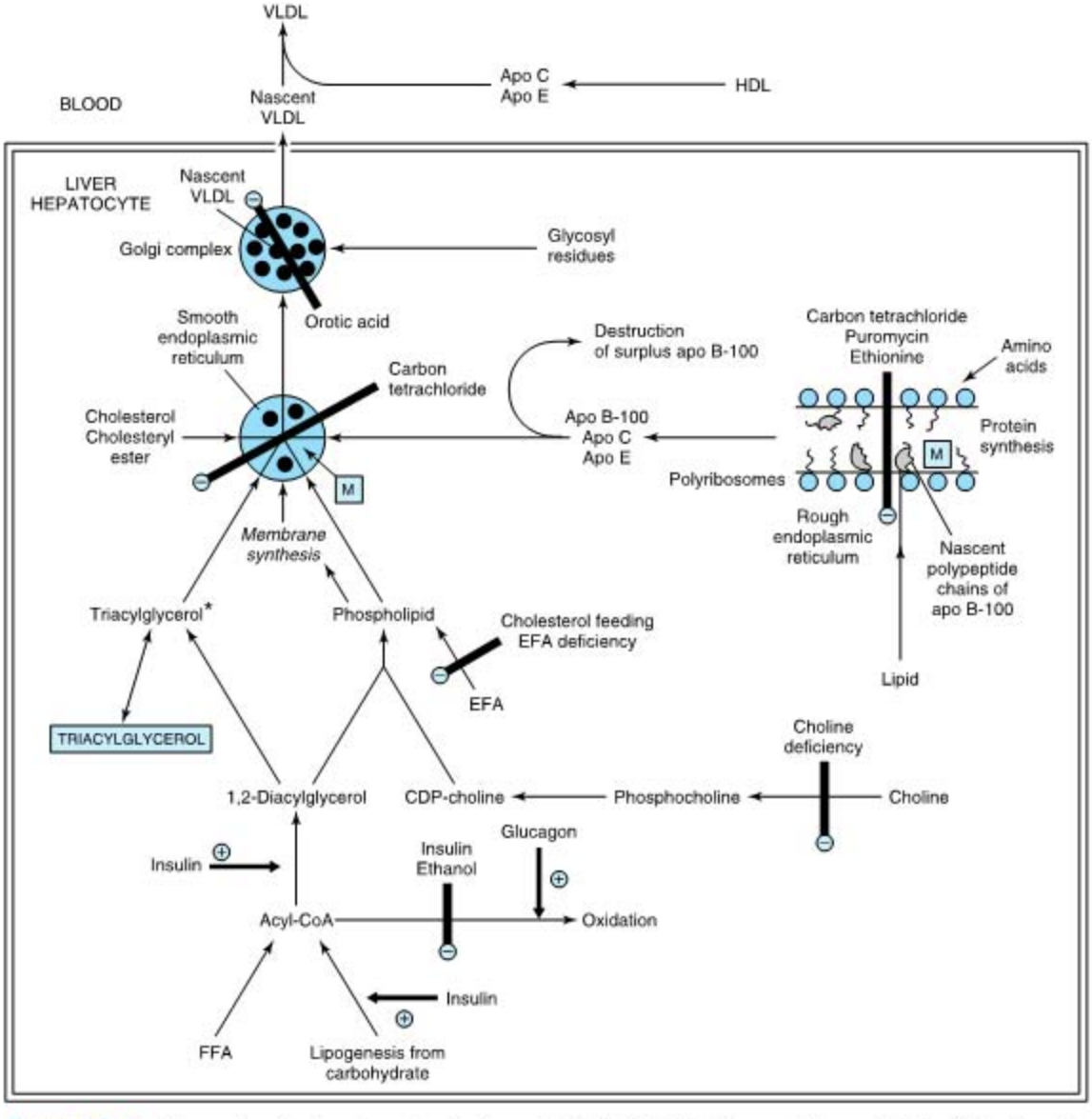
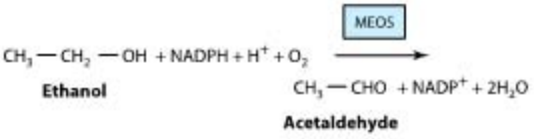


Figure 25–6. The synthesis of very low density lipoprotein (VLDL) in the liver and the possible loci of action of factors causing accumulation of triacylglycerol and a fatty liver. (EFA, essential fatty acids; FFA, free fatty acids; HDL, high-density lipoproteins; Apo, apolipoprotein; M, microsomal triacylglycerol transfer protein.) The pathways indicated form a basis for events depicted in Figure 25–2. The main triacylglycerol pool in liver is not on the direct pathway of VLDL synthesis from acyl-CoA. Thus, FFA, insulin, and glucagon have immediate effects on VLDL secretion as their effects impinge directly on the small triacylglycerol* precursor pool. In the fully fed state, apo B-100 is synthesized in excess of requirements for VLDL secretion and the surplus is destroyed in the liver. During translation of apo B-100, microsomal transfer protein-mediated lipid transport enables lipid to become associated with the nascent polypeptide chain. After release from the ribosomes, these particles fuse with more lipids from the smooth endoplasmic reticulum, producing nascent VLDL.

and may account for the increased metabolic clearance in this condition. Ethanol will also inhibit the metabolism of some drugs, eg, barbiturates, by competing for cytochrome P450-dependent enzymes.



In some Asian populations and Native Americans, alcohol consumption results in increased adverse reactions to acetaldehyde owing to a genetic defect of mitochondrial aldehyde dehydrogenase.

ADIPOSE TISSUE IS THE MAIN STORE OF TRIACYLGLYCEROL IN THE BODY

The triacylglycerol stores in adipose tissue are continually undergoing lipolysis (hydrolysis) and reesterification (Figure 25–7). These two processes are entirely different pathways involving different reactants and enzymes. This allows the processes of esterification or lipolysis to be regulated separately by many nutritional, metabolic, and hormonal factors. The resultant of these two processes determines the magnitude of the free fatty acid pool in adipose tissue, which in turn determines the level of free fatty acids circulating in the plasma. Since the latter has most profound effects upon the metabolism of other tissues, particularly liver and muscle, the factors operating in adipose tissue that regulate the outflow of free fatty acids exert an influence far beyond the tissue itself.

The Provision of Glycerol 3-Phosphate Regulates Esterification: Lipolysis Is Controlled by Hormone-Sensitive Lipase (Figure 25–7)

Triacylglycerol is synthesized from acyl-CoA and glycerol 3-phosphate (Figure 24–2). Because the enzyme **glycerol kinase** is not expressed in adipose tissue, glycerol cannot be utilized for the provision of glycerol 3-phosphate, which must be supplied by glucose via glycolysis.

Triacylglycerol undergoes hydrolysis by a **hormone-sensitive lipase** to form free fatty acids and glycerol. This lipase is distinct from lipoprotein lipase that catalyzes lipoprotein triacylglycerol hydrolysis before its uptake into extrahepatic tissues (see above). Since glycerol cannot be utilized, it diffuses into the blood, whence it is utilized by tissues such as those of the liver and kidney, which possess an active glycerol kinase.

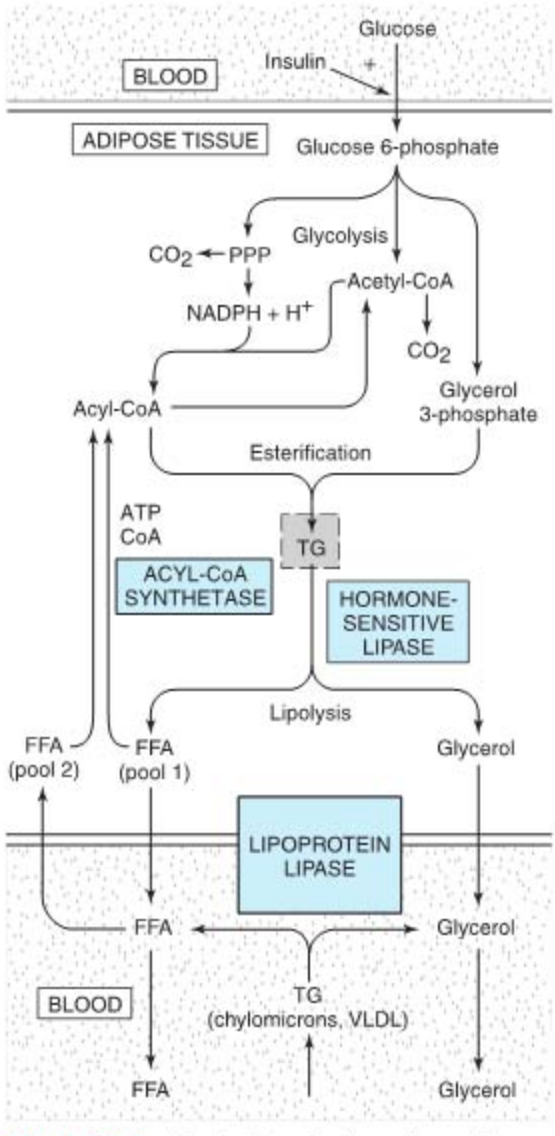


Figure 25–7. Metabolism of adipose tissue. Hormone-sensitive lipase is activated by ACTH, TSH, glucagon, epinephrine, norepinephrine, and vasopressin and inhibited by insulin, prostaglandin E_1, and nicotinic acid. Details of the formation of glycerol 3-phosphate from intermediates of glycolysis are shown in Figure 24–2. (PPP, pentose phosphate pathway; TG, triacylglycerol; FFA, free fatty acids; VLDL, very low density lipoprotein.)

The free fatty acids formed by lipolysis can be reconverted in the tissue to acyl-CoA by **acyl-CoA synthetase** and reesterified with glycerol 3-phosphate to form triacylglycerol. Thus, **there is a continuous cycle of lipolysis and reesterification within the tissue.** However, when the rate of reesterification is not sufficient to match the rate of lipolysis, free fatty acids accumulate and diffuse into the plasma, where they bind to albumin and raise the concentration of plasma free fatty acids.

Increased Glucose Metabolism Reduces the Output of Free Fatty Acids

When the utilization of glucose by adipose tissue is increased, the free fatty acid outflow decreases. However, the release of glycerol continues, demonstrating that the effect of glucose is not mediated by reducing the rate of lipolysis. The effect is due to the provision of glycerol 3-phosphate, which enhances esterification of free fatty acids. Glucose can take several pathways in adipose tissue, including oxidation to CO_2 via the citric acid cycle, oxidation in the pentose phosphate pathway, conversion to long-chain fatty acids, and formation of acylglycerol via glycerol 3-phosphate (Figure 25–7). When glucose utilization is high, a larger proportion of the uptake is oxidized to CO_2 and converted to fatty acids. However, as total glucose utilization decreases, the greater proportion of the glucose is directed to the formation of glycerol 3-phosphate for the esterification of acyl-CoA, which helps to minimize the efflux of free fatty acids.

HORMONES REGULATE FAT MOBILIZATION

Insulin Reduces the Output of Free Fatty Acids

The rate of release of free fatty acids from adipose tissue is affected by many hormones that influence either the rate of esterification or the rate of lipolysis. Insulin inhibits the release of free fatty acids from adipose tissue, which is followed by a fall in circulating plasma free fatty acids. It enhances lipogenesis and the synthesis of acylglycerol and increases the oxidation of glucose to CO_2 via the pentose phosphate pathway. All of these effects are dependent on the presence of glucose and can be explained, to a large extent, on the basis of the ability of insulin to enhance the uptake of glucose into adipose cells via the GLUT 4 transporter. Insulin also increases the activity of pyruvate dehydrogenase, acetyl-CoA carboxylase, and glycerol phosphate acyltransferase, reinforcing the effects of increased glucose uptake on the enhancement of fatty acid and acylglycerol synthesis. These three enzymes are now known to be regulated in a coordinate manner by phosphorylation-dephosphorylation mechanisms.

A principal action of insulin in adipose tissue is to inhibit the activity of **hormone-sensitive lipase,** reducing the release not only of free fatty acids but of glycerol as well. Adipose tissue is much more sensitive to insulin than are many other tissues, which points to adipose tissue as a major site of insulin action in vivo.

Several Hormones Promote Lipolysis

Other hormones accelerate the release of free fatty acids from adipose tissue and raise the plasma free fatty acid concentration by increasing the rate of lipolysis of the triacylglycerol stores (Figure 25–8). These include epinephrine, norepinephrine, glucagon, adrenocorticotropic hormone (ACTH), α- and β-melanocyte-stimulating hormones (MSH), thyroid-stimulating hormone (TSH), growth hormone (GH), and vasopressin. Many of these activate the hormone-sensitive lipase. For an optimal effect, most of these lipolytic processes require the presence of **glucocorticoids** and **thyroid hormones.** These hormones act in a **facilitatory** or **permissive** capacity with respect to other lipolytic endocrine factors.

The hormones that act rapidly in promoting lipolysis, ie, catecholamines, do so by stimulating the activity of **adenylyl cyclase,** the enzyme that converts ATP to cAMP. The mechanism is analogous to that responsible for hormonal stimulation of glycogenolysis (Chapter 18). cAMP, by stimulating **cAMP-dependent protein kinase,** activates hormone-sensitive lipase. Thus, processes which destroy or preserve cAMP influence lipolysis. cAMP is degraded to 5′-AMP by the enzyme **cyclic 3′,5′-nucleotide phosphodiesterase.** This enzyme is inhibited by methylxanthines such as **caffeine** and **theophylline. Insulin** antagonizes the effect of the lipolytic hormones. Lipolysis appears to be more sensitive to changes in concentration of insulin than are glucose utilization and esterification. The antilipolytic effects of insulin, nicotinic acid, and prostaglandin E_1 are accounted for by inhibition of the synthesis of cAMP at the adenylyl cyclase site, acting through a G_i protein. Insulin also stimulates phosphodiesterase and the lipase phosphatase that inactivates hormone-sensitive lipase. The effect of growth hormone in promoting lipolysis is dependent on synthesis of proteins involved in the formation of cAMP. Glucocorticoids promote lipolysis via synthesis of new lipase protein by a cAMP-independent pathway, which may be inhibited by insulin, and also by promoting transcription of genes involved in the cAMP signal cascade. These findings help to explain the role of the pituitary gland and the adrenal cortex in enhancing fat mobilization. The recently discovered body weight regulatory hormone, **leptin,** stimulates

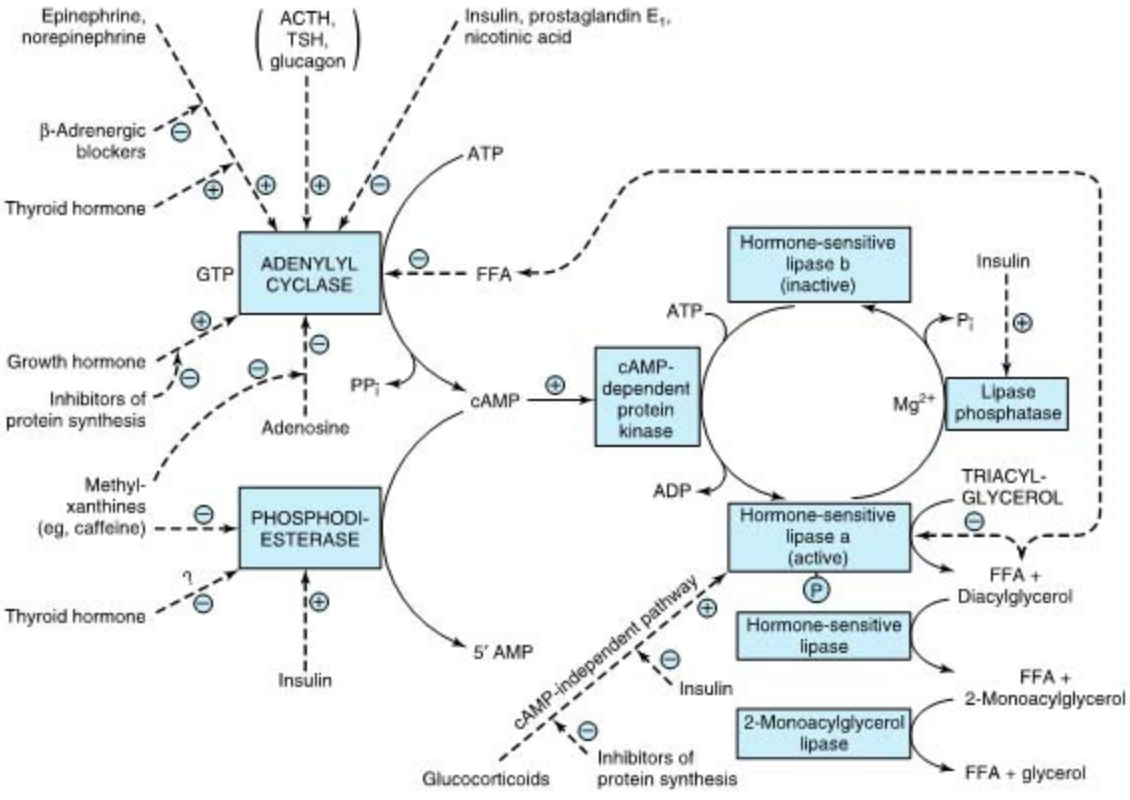


Figure 25–8. Control of adipose tissue lipolysis. (TSH, thyroid-stimulating hormone; FFA, free fatty acids.) Note the cascade sequence of reactions affording amplification at each step. The lipolytic stimulus is "switched off" by removal of the stimulating hormone; the action of lipase phosphatase; the inhibition of the lipase and adenylyl cyclase by high concentrations of FFA; the inhibition of adenylyl cyclase by adenosine; and the removal of cAMP by the action of phosphodiesterase. ACTH, TSH, and glucagon may not activate adenylyl cyclase in vivo, since the concentration of each hormone required in vitro is much higher than is found in the circulation. Positive (⊕) and negative (⊖) regulatory effects are represented by broken lines and substrate flow by solid lines.

lipolysis and inhibits lipogenesis by influencing the activity of the enzymes in the pathways for the breakdown and synthesis of fatty acids.

The sympathetic nervous system, through liberation of norepinephrine in adipose tissue, plays a central role in the mobilization of free fatty acids. Thus, the increased lipolysis caused by many of the factors described above can be reduced or abolished by denervation of adipose tissue or by ganglionic blockade.

A Variety of Mechanisms Have Evolved for Fine Control of Adipose Tissue Metabolism

Human adipose tissue may not be an important site of lipogenesis. There is no significant incorporation of glucose or pyruvate into long-chain fatty acids; ATP-citrate lyase, a key enzyme in lipogenesis, does not appear to be present, and other lipogenic enzymes—eg, glucose-6-phosphate dehydrogenase and the malic enzyme—do not undergo adaptive changes. Indeed, it has been suggested that in humans there is a "carbohydrate excess syndrome" due to a unique limitation in ability to dispose of excess carbohydrate by lipogenesis. In birds, lipogenesis is confined to the liver, where it is particularly important in providing lipids for egg formation, stimulated by estrogens. Human adipose tissue is unresponsive to most of the lipolytic hormones apart from the catecholamines.

On consideration of the profound derangement of metabolism in **diabetes mellitus** (due in large part to increased release of free fatty acids from the depots) and the fact that insulin to a large extent corrects the condi-

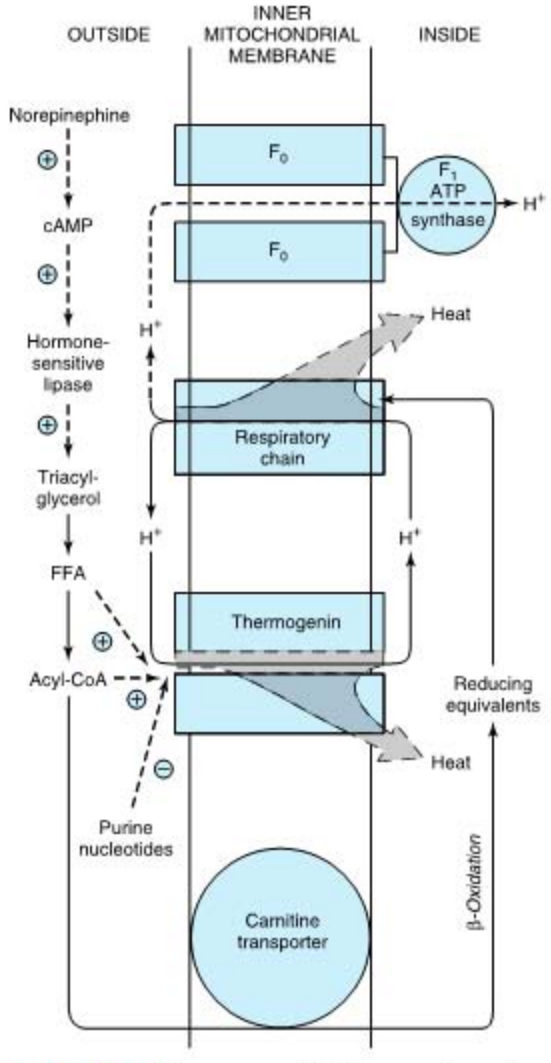


Figure 25–9. Thermogenesis in brown adipose tissue. Activity of the respiratory chain produces heat in addition to translocating protons (Chapter 12). These protons dissipate more heat when returned to the inner mitochondrial compartment via thermogenin instead of generating ATP when returning via the F_1 ATP synthase. The passage of H^+ via thermogenin is inhibited by purine nucleotides when brown adipose tissue is unstimulated. Under the influence of norepinephrine, the inhibition is removed by the production of free fatty acids (FFA) and acyl-CoA. Note the dual role of acyl-CoA in both facilitating the action of thermogenin and supplying reducing equivalents for the respiratory chain. ⊕ and ⊖ signify positive or negative regulatory effects.

tion, it must be concluded that insulin plays a prominent role in the regulation of adipose tissue metabolism.

BROWN ADIPOSE TISSUE PROMOTES THERMOGENESIS

Brown adipose tissue is involved in metabolism particularly at times when heat generation is necessary. Thus, the tissue is extremely active in some species in arousal from hibernation, in animals exposed to cold (nonshivering thermogenesis), and in heat production in the newborn animal. Though not a prominent tissue in humans, it is present in normal individuals, where it could be responsible for **"diet-induced thermogenesis."** It is noteworthy that brown adipose tissue is reduced or absent in obese persons. The tissue is characterized by a well-developed blood supply and a high content of mitochondria and cytochromes but low activity of ATP synthase. Metabolic emphasis is placed on oxidation of both glucose and fatty acids. **Norepinephrine** liberated from sympathetic nerve endings is important in increasing lipolysis in the tissue and increasing synthesis of lipoprotein lipase to enhance utilization of triacylglycerol-rich lipoproteins from the circulation. Oxidation and phosphorylation are not coupled in mitochondria of this tissue, and the phosphorylation that does occur is at the substrate level, eg, at the succinate thiokinase step and in glycolysis. Thus, **oxidation produces much heat, and little free energy is trapped in ATP.** A thermogenic uncoupling protein, **thermogenin,** acts as a proton conductance pathway dissipating the electrochemical potential across the mitochondrial membrane (Figure 25–9).

SUMMARY

- Since nonpolar lipids are insoluble in water, for transport between the tissues in the aqueous blood plasma they are combined with amphipathic lipids and proteins to make water-miscible lipoproteins.
- Four major groups of lipoproteins are recognized: Chylomicrons transport lipids resulting from digestion and absorption. Very low density lipoproteins (VLDL) transport triacylglycerol from the liver. Low-density lipoproteins (LDL) deliver cholesterol to the tissues, and high-density lipoproteins (HDL) remove cholesterol from the tissues in the process known as reverse cholesterol transport.
- Chylomicrons and VLDL are metabolized by hydrolysis of their triacylglycerol, and lipoprotein remnants are left in the circulation. These are taken up by liver, but some of the remnants (IDL) resulting from VLDL form LDL which is taken up by the liver and other tissues via the LDL receptor.

- Apolipoproteins constitute the protein moiety of lipoproteins. They act as enzyme activators (eg, apo C-II and apo A-I) or as ligands for cell receptors (eg, apo A-I, apo E, and apo B-100).
- Triacylglycerol is the main storage lipid in adipose tissue. Upon mobilization, free fatty acids and glycerol are released. Free fatty acids are an important fuel source.
- Brown adipose tissue is the site of "nonshivering thermogenesis." It is found in hibernating and newborn animals and is present in small quantity in humans. Thermogenesis results from the presence of an uncoupling protein, thermogenin, in the inner mitochondrial membrane.

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Cholesterol Synthesis, Transport, & Excretion

26

Peter A. Mayes, PhD, DSc, & Kathleen M. Botham, PhD, DSc

BIOMEDICAL IMPORTANCE

Cholesterol is present in tissues and in plasma either as free cholesterol or as a storage form, combined with a long-chain fatty acid as cholesteryl ester. In plasma, both forms are transported in lipoproteins (Chapter 25). Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. It is synthesized in many tissues from acetyl-CoA and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids, and vitamin D. As a typical product of animal metabolism, cholesterol occurs in foods of animal origin such as egg yolk, meat, liver, and brain. Plasma low-density lipoprotein (LDL) is the vehicle of uptake of cholesterol and cholesteryl ester into many tissues. Free cholesterol is removed from tissues by plasma high-density lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion to bile acids in the process known as **reverse cholesterol transport.** Cholesterol is a major constituent of **gallstones.** However, its chief role in pathologic processes is as a factor in the genesis of **atherosclerosis** of vital arteries, causing cerebrovascular, coronary, and peripheral vascular disease.

CHOLESTEROL IS DERIVED ABOUT EQUALLY FROM THE DIET & FROM BIOSYNTHESIS

A little more than half the cholesterol of the body arises by synthesis (about 700 mg/d), and the remainder is provided by the average diet. The liver and intestine account for approximately 10% each of total synthesis in humans. Virtually all tissues containing nucleated cells are capable of cholesterol synthesis, which occurs in the endoplasmic reticulum and the cytosol.

Acetyl-CoA Is the Source of All Carbon Atoms in Cholesterol

The biosynthesis of cholesterol may be divided into five steps: (1) Synthesis of mevalonate occurs from acetyl-CoA (Figure 26–1). (2) Isoprenoid units are formed from mevalonate by loss of CO_2 (Figure 26–2). (3) Six isoprenoid units condense to form squalene. (4) Squalene cyclizes to give rise to the parent steroid, lanosterol. (5) Cholesterol is formed from lanosterol (Figure 26–3).

Step 1—Biosynthesis of Mevalonate: HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) is formed by the reactions used in mitochondria to synthesize ketone bodies (Figure 22–7). However, since cholesterol synthesis is extramitochondrial, the two pathways are distinct. Initially, two molecules of acetyl-CoA condense to form acetoacetyl-CoA catalyzed by cytosolic **thiolase.** Acetoacetyl-CoA condenses with a further molecule of acetyl-CoA catalyzed by **HMG-CoA synthase** to form HMG-CoA, which is reduced to **mevalonate** by NADPH catalyzed by **HMG-CoA reductase.** This is the principal regulatory step in the pathway of cholesterol synthesis and is the site of action of the most effective class of cholesterol-lowering drugs, the HMG-CoA reductase inhibitors (statins) (Figure 26–1).

Step 2—Formation of Isoprenoid Units: Mevalonate is phosphorylated sequentially by ATP by three kinases, and after decarboxylation (Figure 26–2) the active isoprenoid unit, **isopentenyl diphosphate,** is formed.

Step 3—Six Isoprenoid Units Form Squalene: Isopentenyl diphosphate is isomerized by a shift of the double bond to form **dimethylallyl diphosphate,** then condensed with another molecule of isopentenyl diphosphate to form the ten-carbon intermediate **geranyl diphosphate** (Figure 26–2). A further condensation with isopentenyl diphosphate forms **farnesyl diphosphate.** Two molecules of farnesyl diphosphate condense at the diphosphate end to form **squalene.** Initially, inorganic pyrophosphate is eliminated, forming presqualene diphosphate, which is then reduced by NADPH with elimination of a further inorganic pyrophosphate molecule.

Step 4—Formation of Lanosterol: Squalene can fold into a structure that closely resembles the steroid nucleus (Figure 26–3). Before ring closure occurs, squalene is converted to squalene 2,3-epoxide by a mixed-

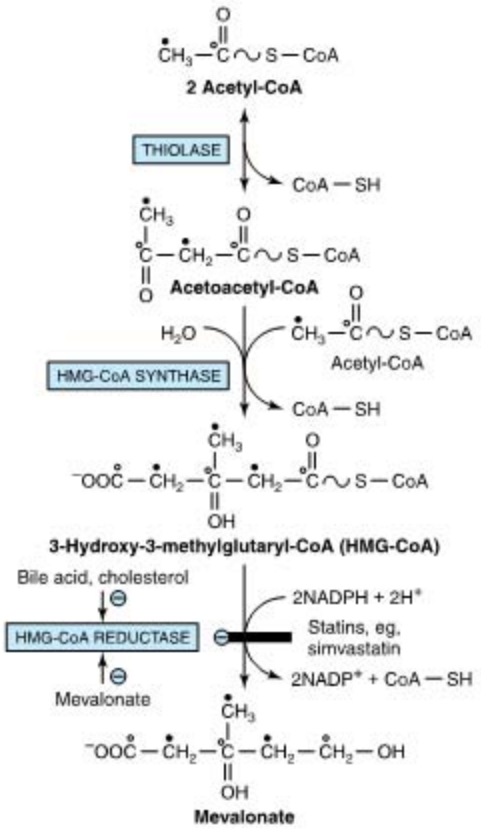

Figure 26–1. Biosynthesis of mevalonate. HMG-CoA reductase is inhibited by atorvastatin, pravastatin, and simvastatin. The open and solid circles indicate the fate of each of the carbons in the acetyl moiety of acetyl-CoA.

function oxidase in the endoplasmic reticulum, **squalene epoxidase.** The methyl group on C_{14} is transferred to C_{13} and that on C_8 to C_{14} as cyclization occurs, catalyzed by **oxidosqualene:lanosterol cyclase.**

Step 5—Formation of Cholesterol: The formation of cholesterol from **lanosterol** takes place in the membranes of the endoplasmic reticulum and involves changes in the steroid nucleus and side chain (Figure 26–3). The methyl groups on C_{14} and C_4 are removed to form 14-desmethyl lanosterol and then zymosterol. The double bond at C_8–C_9 is subsequently moved to C_5–C_6 in two steps, forming **desmosterol.** Finally, the double bond of the side chain is reduced, producing cholesterol. The exact order in which the steps described actually take place is not known with certainty.

Farnesyl Diphosphate Gives Rise to Dolichol & Ubiquinone

The polyisoprenoids **dolichol** (Figure 14–20 and Chapter 47) and **ubiquinone** (Figure 12–5) are formed from farnesyl diphosphate by the further addition of up to 16 (dolichol) or 3–7 (ubiquinone) isopentenyl diphosphate residues, respectively. Some GTP-binding proteins in the cell membrane are prenylated with farnesyl or geranylgeranyl (20 carbon) residues. Protein prenylation is believed to facilitate the anchoring of proteins into lipoid membranes and may also be involved in protein-protein interactions and membrane-associated protein trafficking.

CHOLESTEROL SYNTHESIS IS CONTROLLED BY REGULATION OF HMG-CoA REDUCTASE

Regulation of cholesterol synthesis is exerted near the beginning of the pathway, at the HMG-CoA reductase step. The reduced synthesis of cholesterol in starving animals is accompanied by a decrease in the activity of the enzyme. However, it is only hepatic synthesis that is inhibited by dietary cholesterol. HMG-CoA reductase in liver is inhibited by mevalonate, the immediate product of the pathway, and by cholesterol, the main product. Cholesterol (or a metabolite, eg, oxygenated sterol) represses transcription of the HMG-CoA reductase gene and is also believed to influence translation. A **diurnal variation** occurs in both cholesterol synthesis and reductase activity. In addition to these mechanisms regulating the rate of protein synthesis, the enzyme activity is also modulated more rapidly by posttranslational modification (Figure 26–4). Insulin or thyroid hormone increases HMG-CoA reductase activity, whereas glucagon or glucocorticoids decrease it. Activity is reversibly modified by phosphorylation-dephosphorylation mechanisms, some of which may be cAMP-dependent and therefore immediately responsive to glucagon. Attempts to lower plasma cholesterol in humans by reducing the amount of cholesterol in the diet produce variable results. Generally, a decrease of 100 mg in dietary cholesterol causes a decrease of approximately 0.13 mmol/L of serum.

MANY FACTORS INFLUENCE THE CHOLESTEROL BALANCE IN TISSUES

In tissues, cholesterol balance is regulated as follows (Figure 26–5): Cell cholesterol increase is due to uptake of cholesterol-containing lipoproteins by receptors, eg, the LDL receptor or the scavenger receptor; uptake of free cholesterol from cholesterol-rich lipoproteins to the cell

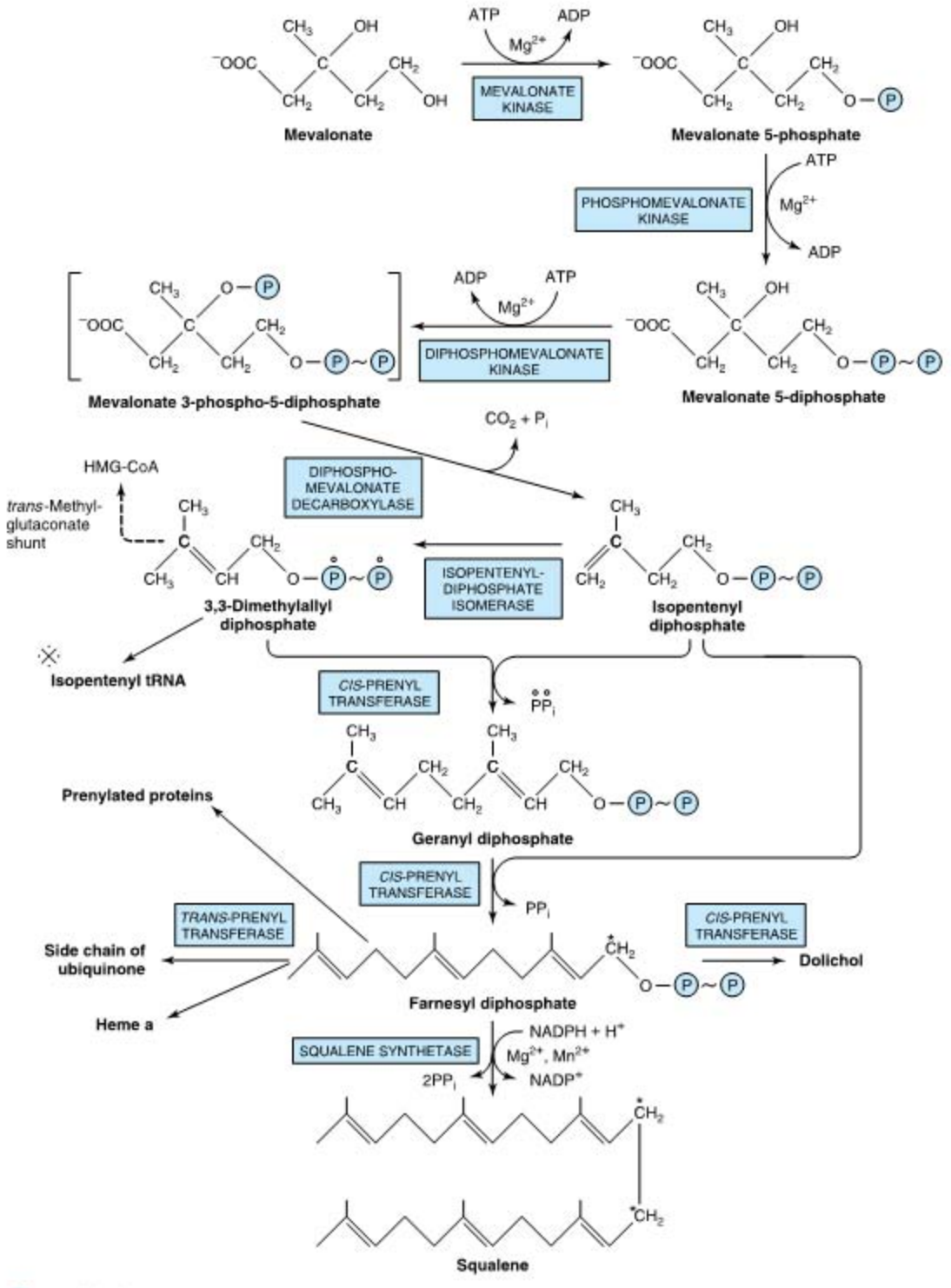

Figure 26–2. Biosynthesis of squalene, ubiquinone, dolichol, and other polyisoprene derivatives. (HMG, 3-hydroxy-3-methylglutaryl; ※, cytokinin.) A farnesyl residue is present in heme a of cytochrome oxidase. The carbon marked with asterisk becomes C_{11} or C_{12} in squalene. Squalene synthetase is a microsomal enzyme; all other enzymes indicated are soluble cytosolic proteins, and some are found in peroxisomes.

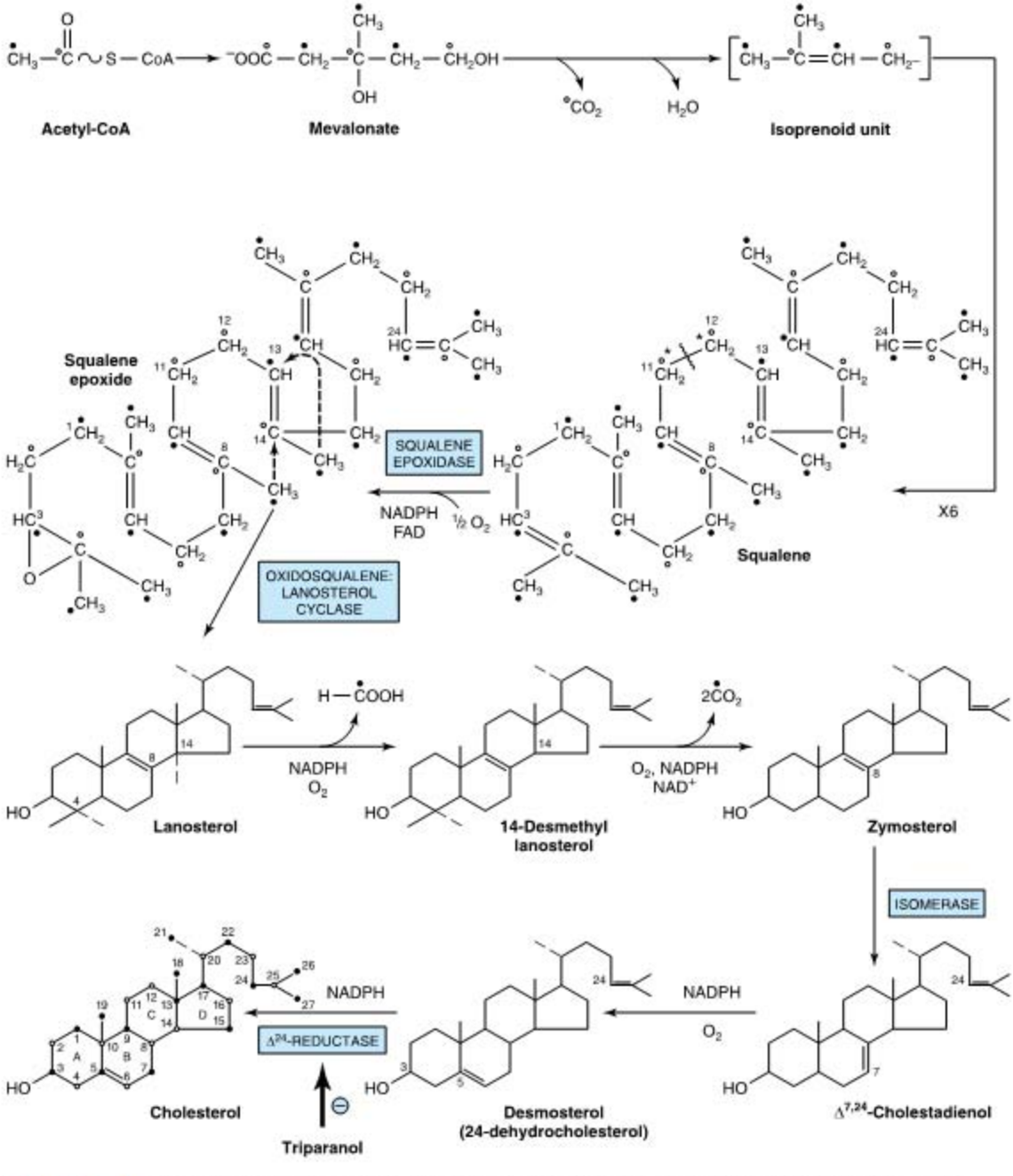

Figure 26–3. Biosynthesis of cholesterol. The numbered positions are those of the steroid nucleus and the open and solid circles indicate the fate of each of the carbons in the acetyl moiety of acetyl-CoA. Asterisks: Refer to labeling of squalene in Figure 26–2.

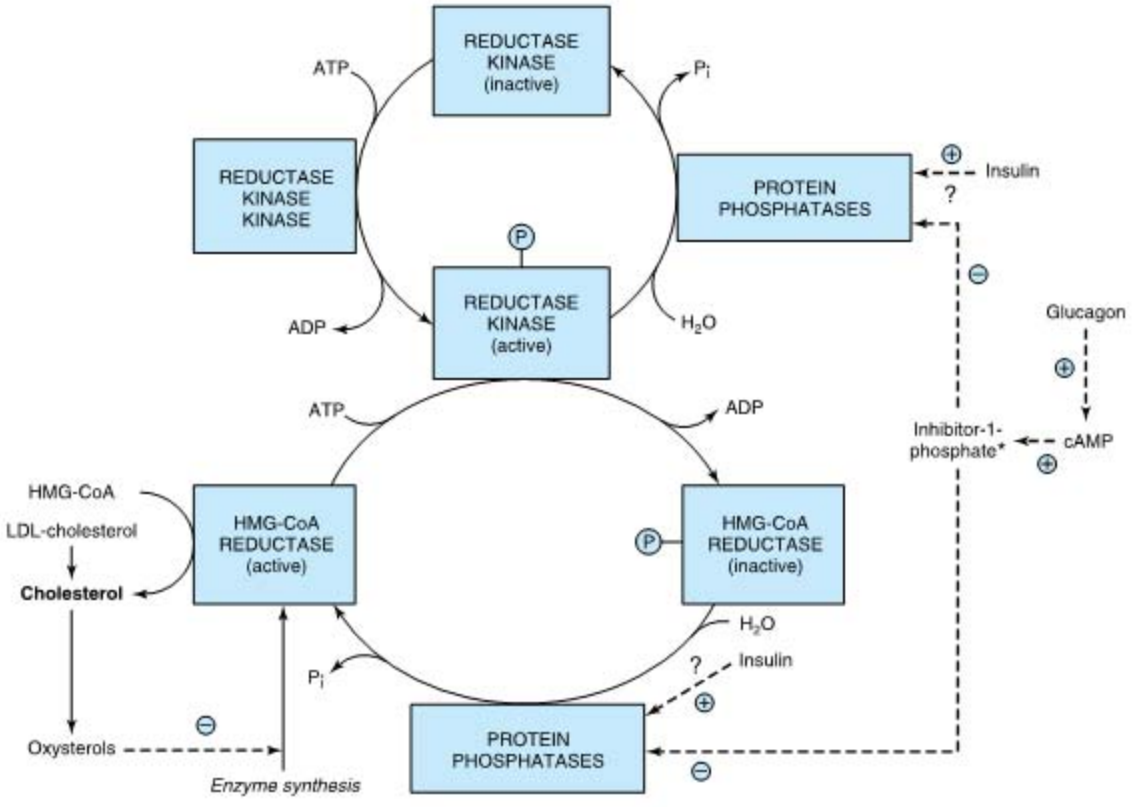

Figure 26–4. Possible mechanisms in the regulation of cholesterol synthesis by HMG-CoA reductase. Insulin has a dominant role compared with glucagon. Asterisk: See Figure 18–6.

membrane; cholesterol synthesis; and hydrolysis of cholesteryl esters by the enzyme **cholesteryl ester hydrolase.** Decrease is due to efflux of cholesterol from the membrane to HDL, promoted by **LCAT** (lecithin:cholesterol acyltransferase) (Chapter 25); esterification of cholesterol by **ACAT** (acyl-CoA:cholesterol acyltransferase); and utilization of cholesterol for synthesis of other steroids, such as hormones, or bile acids in the liver.

The LDL Receptor Is Highly Regulated

LDL (apo B-100, E) receptors occur on the cell surface in pits that are coated on the cytosolic side of the cell membrane with a protein called clathrin. The glycoprotein receptor spans the membrane, the B-100 binding region being at the exposed amino terminal end. After binding, LDL is taken up intact by endocytosis. The apoprotein and cholesteryl ester are then hydrolyzed in the lysosomes, and cholesterol is translocated into the cell. The receptors are recycled to the cell surface. This influx of cholesterol inhibits in a coordinated manner HMG-CoA synthase, HMG-CoA reductase, and, therefore, cholesterol synthesis; stimulates ACAT activity; and down-regulates synthesis of the LDL receptor. Thus, the number of LDL receptors on the cell surface is regulated by the cholesterol requirement for membranes, steroid hormones, or bile acid synthesis (Figure 26–5). The apo B-100, E receptor is a "high-affinity" LDL receptor, which may be saturated under most circumstances. Other "low-affinity" LDL receptors also appear to be present in addition to a scavenger pathway, which is not regulated.

CHOLESTEROL IS TRANSPORTED BETWEEN TISSUES IN PLASMA LIPOPROTEINS (Figure 26–6)

In Western countries, the total plasma cholesterol in humans is about 5.2 mmol/L, rising with age, though there are wide variations between individuals. The greater part is found in the esterified form. It is transported in lipoproteins of the plasma, and the highest proportion of cholesterol is found in the LDL. Dietary cholesterol equilibrates with plasma cholesterol in days

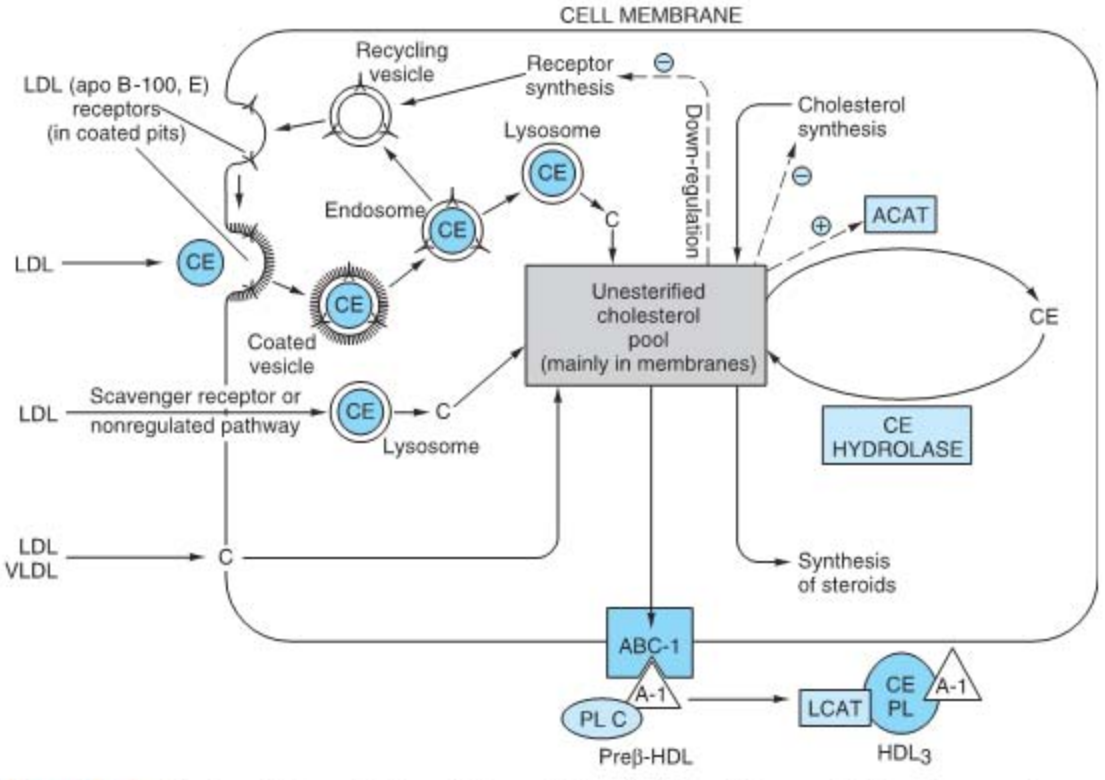

Figure 26–5. Factors affecting cholesterol balance at the cellular level. Reverse cholesterol transport may be initiated by preβ HDL binding to the ABC-1 transporter protein via apo A-I. Cholesterol is then moved out of the cell via the transporter, lipidating the HDL, and the larger particles then dissociate from the ABC-1 molecule. (C, cholesterol; CE, cholesteryl ester; PL, phospholipid; ACAT, acyl-CoA:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; A-I, apolipoprotein A-I; LDL, low-density lipoprotein; VLDL, very low density lipoprotein.) LDL and HDL are not shown to scale.

and with tissue cholesterol in weeks. Cholesteryl ester in the diet is hydrolyzed to cholesterol, which is then absorbed by the intestine together with dietary unesterified cholesterol and other lipids. With cholesterol synthesized in the intestines, it is then incorporated into chylomicrons. Of the cholesterol absorbed, 80–90% is esterified with long-chain fatty acids in the intestinal mucosa. Ninety-five percent of the chylomicron cholesterol is delivered to the liver in chylomicron remnants, and most of the cholesterol secreted by the liver in VLDL is retained during the formation of IDL and ultimately LDL, which is taken up by the LDL receptor in liver and extrahepatic tissues (Chapter 25).

Plasma LCAT Is Responsible for Virtually All Plasma Cholesteryl Ester in Humans

LCAT activity is associated with HDL containing apo A-I. As cholesterol in HDL becomes esterified, it creates a concentration gradient and draws in cholesterol from tissues and from other lipoproteins (Figures 26–5 and 26–6), thus enabling HDL to function in **reverse cholesterol transport** (Figure 25–5).

Cholesteryl Ester Transfer Protein Facilitates Transfer of Cholesteryl Ester From HDL to Other Lipoproteins

This protein is found in plasma of humans and many other species, associated with HDL. It facilitates transfer of cholesteryl ester from HDL to VLDL, IDL, and LDL in exchange for triacylglycerol, relieving product inhibition of LCAT activity in HDL. Thus, in humans, much of the cholesteryl ester formed by LCAT finds its way to the liver via VLDL remnants (IDL) or LDL (Figure 26–6). The triacylglycerol-enriched HDL_2 delivers its cholesterol to the liver in the HDL cycle (Figure 25–5).

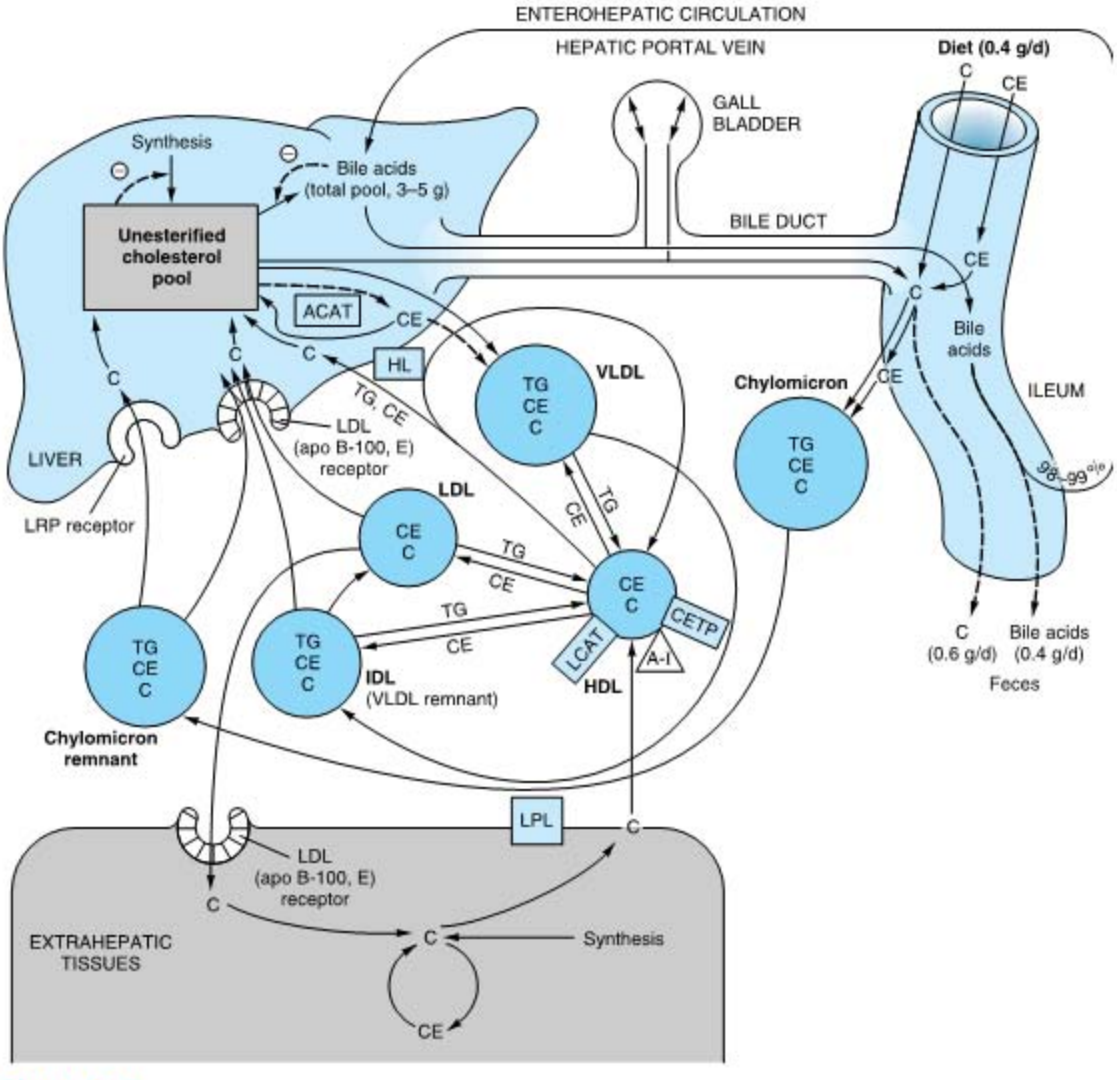

Figure 26–6. Transport of cholesterol between the tissues in humans. (C, unesterified cholesterol; CE, cholesteryl ester; TG, triacylglycerol; VLDL, very low density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ACAT, acyl-CoA:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase; A-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; LRP, LDL receptor-related protein.)

CHOLESTEROL IS EXCRETED FROM THE BODY IN THE BILE AS CHOLESTEROL OR BILE ACIDS (SALTS)

About 1 g of cholesterol is eliminated from the body per day. Approximately half is excreted in the feces after conversion to bile acids. The remainder is excreted as cholesterol. **Coprostanol** is the principal sterol in the feces; it is formed from cholesterol by the bacteria in the lower intestine.

Bile Acids Are Formed From Cholesterol

The **primary bile acids** are synthesized in the liver from cholesterol. These are **cholic acid** (found in the largest amount) and **chenodeoxycholic acid** (Figure 26–7).

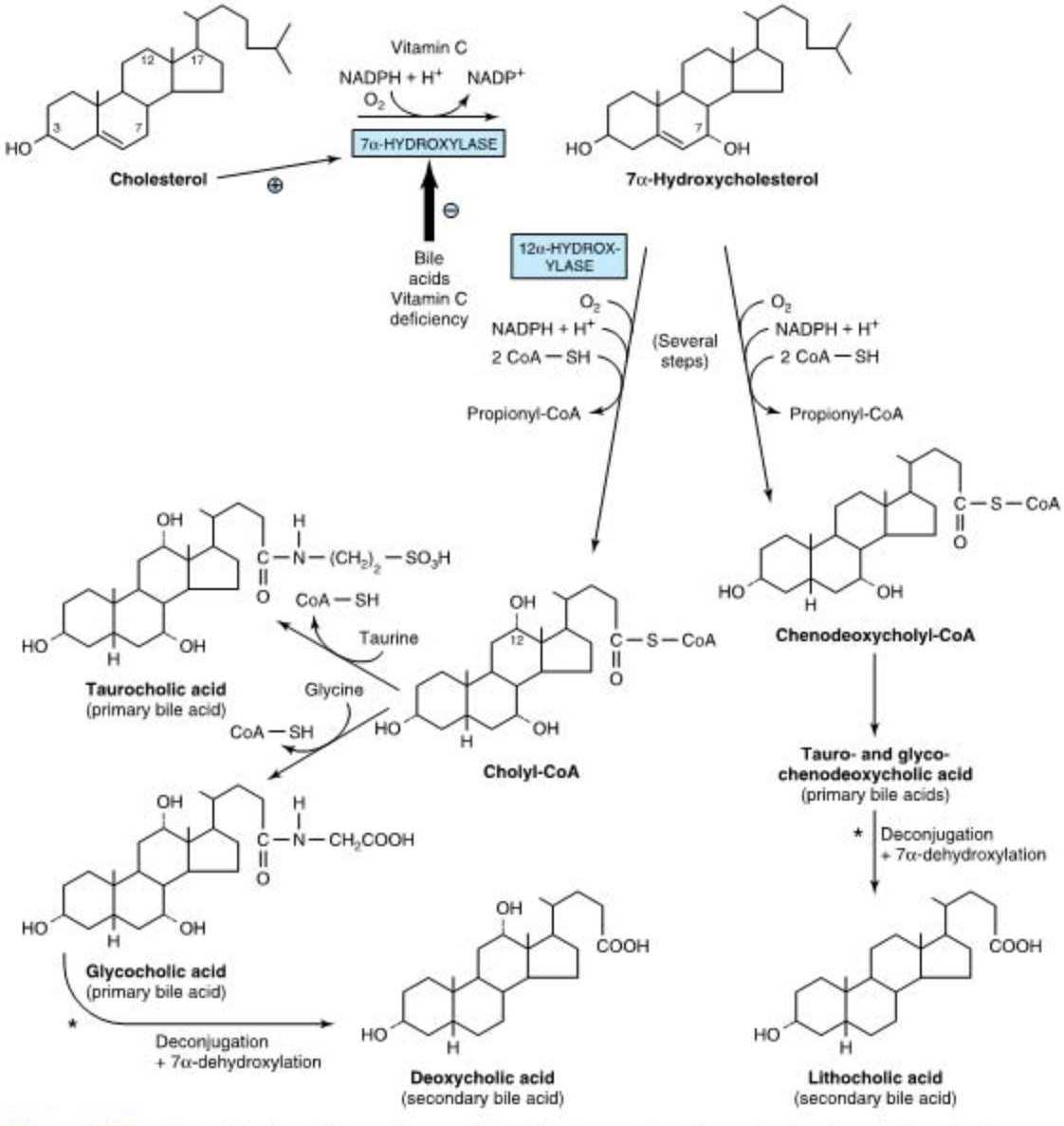

Figure 26–7. Biosynthesis and degradation of bile acids. A second pathway in mitochondria involves hydroxylation of cholesterol by sterol 27-hydroxylase. Asterisk: Catalyzed by microbial enzymes.

The 7α-hydroxylation of cholesterol is the first and principal regulatory step in the biosynthesis of bile acids catalyzed by **7α-hydroxylase,** a microsomal enzyme. A typical monooxygenase, it requires oxygen, NADPH, and cytochrome P450. Subsequent hydroxylation steps are also catalyzed by monooxygenases. The pathway of bile acid biosynthesis divides early into one subpathway leading to **cholyl-CoA,** characterized by an extra α-OH group on position 12, and another pathway leading to **chenodeoxycholyl-CoA** (Figure 26–7). A second pathway in mitochondria involving the 27-hydroxylation of cholesterol by **sterol 27-hydroxylase** as the first step is responsible for a significant proportion of the primary bile acids synthesized. The primary bile acids (Figure 26–7) enter the bile as glycine or taurine conjugates. Conjugation takes place in peroxisomes. In humans, the ratio of the glycine to the taurine conjugates is normally 3:1. In the alkaline bile, the bile acids and their conju-

gates are assumed to be in a salt form—hence the term "bile salts."

A portion of the primary bile acids in the intestine is subjected to further changes by the activity of the intestinal bacteria. These include deconjugation and 7α-dehydroxylation, which produce the **secondary bile acids,** deoxycholic acid and lithocholic acid.

Most Bile Acids Return to the Liver in the Enterohepatic Circulation

Although products of fat digestion, including cholesterol, are absorbed in the first 100 cm of small intestine, the primary and secondary bile acids are absorbed almost exclusively in the ileum, and 98–99% are returned to the liver via the portal circulation. This is known as the **enterohepatic circulation** (Figure 26–6). However, lithocholic acid, because of its insolubility, is not reabsorbed to any significant extent. Only a small fraction of the bile salts escapes absorption and is therefore eliminated in the feces. Nonetheless, this represents a major pathway for the elimination of cholesterol. Each day the small pool of bile acids (about 3–5 g) is cycled through the intestine six to ten times and an amount of bile acid equivalent to that lost in the feces is synthesized from cholesterol, so that a pool of bile acids of constant size is maintained. This is accomplished by a system of feedback controls.

Bile Acid Synthesis Is Regulated at the 7α-Hydroxylase Step

The principal rate-limiting step in the biosynthesis of bile acids is at the **cholesterol 7α-hydroxylase reaction** (Figure 26–7). The activity of the enzyme is feedback-regulated via the nuclear bile acid-binding receptor **farnesoid X receptor (FXR).** When the size of the bile acid pool in the enterohepatic circulation increases, FXR is activated and transcription of the cholesterol 7α-hydroxylase gene is suppressed. Chenodeoxycholic acid is particularly important in activating FXR. Cholesterol 7α-hydroxylase activity is also enhanced by cholesterol of endogenous and dietary origin and regulated by insulin, glucagon, glucocorticoids, and thyroid hormone.

CLINICAL ASPECTS

The Serum Cholesterol Is Correlated With the Incidence of Atherosclerosis & Coronary Heart Disease

While cholesterol is believed to be chiefly concerned in the relationship, other serum lipids such as triacylglycerols may also play a role. Atherosclerosis is characterized by the deposition of cholesterol and cholesteryl ester from the plasma lipoproteins into the artery wall. Diseases in which prolonged elevated levels of VLDL, IDL, chylomicron remnants, or LDL occur in the blood (eg, diabetes mellitus, lipid nephrosis, hypothyroidism, and other conditions of hyperlipidemia) are often accompanied by premature or more severe atherosclerosis. There is also an inverse relationship between HDL (HDL_2) concentrations and coronary heart disease, and some consider that the most predictive relationship is the **LDL:HDL cholesterol ratio.** This is consistent with the function of HDL in reverse cholesterol transport. Susceptibility to atherosclerosis varies widely among species, and humans are one of the few in which the disease can be induced by diets high in cholesterol.

Diet Can Play an Important Role in Reducing Serum Cholesterol

Hereditary factors play the greatest role in determining individual serum cholesterol concentrations; however, dietary and environmental factors also play a part, and the most beneficial of these is the substitution in the diet of **polyunsaturated and monounsaturated fatty acids** for saturated fatty acids. Plant oils such as corn oil and sunflower seed oil contain a high proportion of polyunsaturated fatty acids, while olive oil contains a high concentration of monounsaturated fatty acids. On the other hand, butterfat, beef fat, and palm oil contain a high proportion of saturated fatty acids. Sucrose and fructose have a greater effect in raising blood lipids, particularly triacylglycerols, than do other carbohydrates.

The reason for the cholesterol-lowering effect of polyunsaturated fatty acids is still not fully understood. It is clear, however, that one of the mechanisms involved is the up-regulation of LDL receptors by poly- and monounsaturated as compared with saturated fatty acids, causing an increase in the catabolic rate of LDL, the main atherogenic lipoprotein. In addition, saturated fatty acids cause the formation of smaller VLDL particles that contain relatively more cholesterol, and they are utilized by extrahepatic tissues at a slower rate than are larger particles—tendencies that may be regarded as atherogenic.

Lifestyle Affects the Serum Cholesterol Level

Additional factors considered to play a part in coronary heart disease include high blood pressure, smoking, male gender, obesity (particularly abdominal obesity), lack of exercise, and drinking soft as opposed to hard water. Factors associated with elevation of plasma FFA followed by increased output of triacylglycerol and cho-

lesterol into the circulation in VLDL include emotional stress and coffee drinking. Premenopausal women appear to be protected against many of these deleterious factors, and this is thought to be related to the beneficial effects of estrogen. There is an association between moderate alcohol consumption and a lower incidence of coronary heart disease. This may be due to elevation of HDL concentrations resulting from increased synthesis of apo A-I and changes in activity of cholesteryl ester transfer protein. It has been claimed that red wine is particularly beneficial, perhaps because of its content of antioxidants. Regular exercise lowers plasma LDL

Table 26–1. Primary disorders of plasma lipoproteins (dyslipoproteinemias).

Name	Defect	Remarks
Hypolipoproteinemias Abetalipoproteinemia	No chylomicrons, VLDL, or LDL are formed because of defect in the loading of apo B with lipid.	Rare; blood acylglycerols low; intestine and liver accumulate acylglycerols. Intestinal malabsorption. Early death avoidable by administration of large doses of fat-soluble vitamins, particularly vitamin E.
Familial alpha-lipoprotein deficiency Tangier disease Fish-eye disease Apo-A-I deficiencies	All have low or near absence of HDL.	Tendency toward hypertriacylglycerolemia as a result of absence of apo C-II, causing inactive LPL. Low LDL levels. Atherosclerosis in the elderly.
Hyperlipoproteinemias Familial lipoprotein lipase deficiency (type I)	Hypertriacylglycerolemia due to deficiency of LPL, abnormal LPL, or apo C-II deficiency causing inactive LPL.	Slow clearance of chylomicrons and VLDL. Low levels of LDL and HDL. No increased risk of coronary disease.
Familial hypercholesterolemia (type IIa)	Defective LDL receptors or mutation in ligand region of apo B-100.	Elevated LDL levels and hypercholesterolemia, resulting in atherosclerosis and coronary disease.
Familial type III hyperlipoproteinemia (broad beta disease, remnant removal disease, familial dysbetalipoproteinemia)	Deficiency in remnant clearance by the liver is due to abnormality in apo E. Patients lack isoforms E3 and E4 and have only E2, which does not react with the E receptor.[1]	Increase in chylomicron and VLDL remnants of density < 1.019 (β-VLDL). Causes hypercholesterolemia, xanthomas, and atherosclerosis.
Familial hypertriacylglycerolemia (type IV)	Overproduction of VLDL often associated with glucose intolerance and hyperinsulinemia.	Cholesterol levels rise with the VLDL concentration. LDL and HDL tend to be subnormal. This type of pattern is commonly associated with coronary heart disease, type II diabetes mellitus, obesity, alcoholism, and administration of progestational hormones.
Familial hyperalphalipoproteinemia	Increased concentrations of HDL.	A rare condition apparently beneficial to health and longevity.
Hepatic lipase deficiency	Deficiency of the enzyme leads to accumulation of large triacylglycerol-rich HDL and VLDL remnants.	Patients have xanthomas and coronary heart disease.
Familial lecithin:cholesterol acyltransferase (LCAT) deficiency	Absence of LCAT leads to block in reverse cholesterol transport. HDL remains as nascent disks incapable of taking up and esterifying cholesterol.	Plasma concentrations of cholesteryl esters and lysolecithin are low. Present is an abnormal LDL fraction, lipoprotein X, found also in patients with cholestasis. VLDL is abnormal (β-VLDL).
Familial lipoprotein(a) excess	Lp(a) consists of 1 mol of LDL attached to 1 mol of apo(a). Apo(a) shows structural homologies to plasminogen.	Premature coronary heart disease due to atherosclerosis, plus thrombosis due to inhibition of fibrinolysis.

[1]There is an association between patients possessing the apo E4 allele and the incidence of Alzheimer's disease. Apparently, apo E4 binds more avidly to β-amyloid found in neuritic plaques.

but raises HDL. Triacylglycerol concentrations are also reduced, due most likely to increased insulin sensitivity, which enhances expression of lipoprotein lipase.

When Diet Changes Fail, Hypolipidemic Drugs Will Reduce Serum Cholesterol & Triacylglycerol

Significant reductions of plasma cholesterol can be effected medically by the use of **cholestyramine resin** or surgically by the ileal exclusion operations. Both procedures block the reabsorption of bile acids, causing increased bile acid synthesis in the liver. This increases cholesterol excretion and up-regulates LDL receptors, lowering plasma cholesterol. **Sitosterol** is a hypocholesterolemic agent that acts by blocking the absorption of cholesterol from the gastrointestinal tract.

Several drugs are known to block the formation of cholesterol at various stages in the biosynthetic pathway. The **statins** inhibit HMG-CoA reductase, thus up-regulating LDL receptors. Statins currently in use include **atorvastatin, simvastatin,** and **pravastatin.** Fibrates such as **clofibrate** and **gemfibrozil** act mainly to lower plasma triacylglycerols by decreasing the secretion of triacylglycerol and cholesterol-containing VLDL by the liver. In addition, they stimulate hydrolysis of VLDL triacylglycerols by lipoprotein lipase. **Probucol** appears to increase LDL catabolism via receptor-independent pathways, but its antioxidant properties may be more important in preventing accumulation of oxidized LDL, which has enhanced atherogenic properties, in arterial walls. **Nicotinic acid** reduces the flux of FFA by inhibiting adipose tissue lipolysis, thereby inhibiting VLDL production by the liver.

Primary Disorders of the Plasma Lipoproteins (Dyslipoproteinemias) Are Inherited

Inherited defects in lipoprotein metabolism lead to the primary condition of either **hypo-** or **hyperlipoproteinemia** (Table 26–1). In addition, diseases such as diabetes mellitus, hypothyroidism, kidney disease (nephrotic syndrome), and atherosclerosis are associated with secondary abnormal lipoprotein patterns that are very similar to one or another of the primary inherited conditions. Virtually all of the primary conditions are due to a defect at a stage in lipoprotein formation, transport, or destruction (see Figures 25–4, 26–5, and 26–6). Not all of the abnormalities are harmful.

SUMMARY

- Cholesterol is the precursor of all other steroids in the body, eg, corticosteroids, sex hormones, bile acids, and vitamin D. It also plays an important structural role in membranes and in the outer layer of lipoproteins.
- Cholesterol is synthesized in the body entirely from acetyl-CoA. Three molecules of acetyl-CoA form mevalonate via the important regulatory reaction for the pathway, catalyzed by HMG-CoA reductase. Next, a five-carbon isoprenoid unit is formed, and six of these condense to form squalene. Squalene undergoes cyclization to form the parent steroid lanosterol, which, after the loss of three methyl groups, forms cholesterol.
- Cholesterol synthesis in the liver is regulated partly by cholesterol in the diet. In tissues, cholesterol balance is maintained between the factors causing gain of cholesterol (eg, synthesis, uptake via the LDL or scavenger receptors) and the factors causing loss of cholesterol (eg, steroid synthesis, cholesteryl ester formation, excretion). The activity of the LDL receptor is modulated by cellular cholesterol levels to achieve this balance. In reverse cholesterol transport, HDL (preβ-HDL, discoidal, or HDL_3) takes up cholesterol from the tissues and LCAT esterifies it and deposits it in the core of HDL, which is converted to HDL_2. The cholesteryl ester in HDL_2 is taken up by the liver, either directly or after transfer to VLDL, IDL, or LDL via the cholesteryl ester transfer protein.
- Excess cholesterol is excreted from the liver in the bile as cholesterol or bile salts. A large proportion of bile salts is absorbed into the portal circulation and returned to the liver as part of the enterohepatic circulation.
- Elevated levels of cholesterol present in VLDL, IDL, or LDL are associated with atherosclerosis, whereas high levels of HDL have a protective effect.
- Inherited defects in lipoprotein metabolism lead to a primary condition of hypo- or hyperlipoproteinemia. Conditions such as diabetes mellitus, hypothyroidism, kidney disease, and atherosclerosis exhibit secondary abnormal lipoprotein patterns that resemble certain primary conditions.

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Integration of Metabolism—The Provision of Metabolic Fuels

27

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BIOMEDICAL IMPORTANCE

An adult human weighing 70 kg requires about 10–12 MJ (2400–2900 kcal) from metabolic fuels each day. This requirement is met from carbohydrates (40–60%), lipids (mainly triacylglycerol, 30–40%), protein (10–15%), and alcohol if consumed. The mix being oxidized varies depending on whether the subject is in the fed or starving state and on the intensity of physical work. The requirement for metabolic fuels is relatively constant throughout the day, since average physical activity only increases metabolic rate by about 40–50% over the basal metabolic rate. However, most people consume their daily intake of metabolic fuels in two or three meals, so there is a need to form reserves of carbohydrate (glycogen in liver and muscle) and lipid (triacylglycerol in adipose tissue) for use between meals.

If the intake of fuels is consistently greater than energy expenditure, the surplus is stored, largely as fat, leading to the development of **obesity** and its associated health hazards. If the intake of fuels is consistently lower than energy expenditure, there will be negligible fat and carbohydrate reserves, and amino acids arising from protein turnover will be used for energy rather than replacement protein synthesis, leading to emaciation and eventually death.

After a normal meal there is an ample supply of carbohydrate, and the fuel for most tissues is glucose. In the starving state, glucose must be spared for use by the central nervous system (which is largely dependent on glucose) and the erythrocytes (which are wholly reliant on glucose). Other tissues can utilize alternative fuels such as fatty acids and ketone bodies. As glycogen reserves become depleted, so amino acids arising from protein turnover and glycerol arising from lipolysis are used for gluconeogenesis. These events are largely controlled by the hormones insulin and glucagon. In **diabetes mellitus** there is either impaired synthesis and secretion of insulin (type 1 diabetes mellitus) or impaired sensitivity of tissues to insulin action (type 2 diabetes mellitus), leading to severe metabolic derangement. In cattle the demands of heavy lactation can lead to ketosis, as can the demands of twin pregnancy in sheep.

MANY METABOLIC FUELS ARE INTERCONVERTIBLE

Carbohydrate in excess of immediate requirements as fuel or for synthesis of glycogen in muscle and liver may be used for lipogenesis (Chapter 21) and hence triacylglycerol synthesis in both adipose tissue and liver (whence it is exported in very low density lipoprotein). The importance of lipogenesis in human beings is unclear; in Western countries, dietary fat provides 35–45% of energy intake, while in less developed countries where carbohydrate may provide 60–75% of energy intake the total intake of food may be so low that there is little surplus for lipogenesis. A high intake of fat inhibits lipogenesis.

Fatty acids (and ketone bodies formed from them) cannot be used for the synthesis of glucose. The reaction of pyruvate dehydrogenase, forming acetyl-CoA, is irreversible, and for every two-carbon unit from acetyl-CoA that enters the citric acid cycle there is a loss of two carbon atoms as carbon dioxide before only one molecule of oxaloacetate is re-formed—ie, there is no net increase. This means that acetyl-CoA (and therefore any substrates that yield acetyl-CoA) can never be used for gluconeogenesis (Chapter 19). The (relatively rare) fatty acids with an odd number of carbon atoms yield propionyl-CoA as the product of the final cycle of β-oxidation (Chapter 22), and this can be a substrate for gluconeogenesis, as can the glycerol released by lipolysis of adipose tissue triacylglycerol reserves. Most of the amino acids in excess of requirements for protein synthesis (arising from the diet or from tissue protein turnover) yield pyruvate, or five- and four-carbon intermediates of the citric acid cycle. Pyruvate can be carboxylated to oxaloacetate, which is the primary substrate for gluconeogenesis, and the five- and four-carbon intermediates also result in a net increase in the formation of oxaloacetate, which is then available for gluconeogenesis. These amino acids are classified as **glucogenic.** Lysine and leucine yield only acetyl-CoA on oxidation and thus cannot be used for gluconeogenesis, while phenylalanine, tyrosine, tryptophan, and isoleucine give rise to both acetyl-CoA and to intermediates of the citric acid cycle that can be used for gluco-

neogenesis. Those amino acids that give rise to acetyl-CoA are classified as **ketogenic** because in the starving state much of the acetyl-CoA will be used for synthesis of ketone bodies in the liver.

A SUPPLY OF METABOLIC FUELS IS PROVIDED IN BOTH THE FED & STARVING STATES (Figure 27–1)

Glucose Is Always Required by the Central Nervous System & Erythrocytes

Erythrocytes lack mitochondria and hence are wholly reliant on glycolysis and the pentose phosphate pathway. The brain can metabolize ketone bodies to meet about 20% of its energy requirements; the remainder must be supplied by glucose. The metabolic changes that occur in starvation are the consequences of the need to preserve glucose and the limited reserves of glycogen in liver for use by the brain and erythrocytes and to ensure the provision of alternative fuels for other tissues. The fetus and synthesis of lactose in milk also require a significant amount of glucose.

In the Fed State, Metabolic Fuel Reserves Are Laid Down

For several hours after a meal, while the products of digestion are being absorbed, there is an abundant supply of metabolic fuels. Under these conditions, glucose is the major fuel for oxidation in most tissues; this is observed as an increase in the respiratory quotient (the ratio of carbon dioxide produced to oxygen consumed) from about 0.8 in the starved state to near 1 (Table 27–1).

Glucose uptake into muscle and adipose tissue is controlled by insulin, which is secreted by the B islet cells of the pancreas in response to an increased concentration of glucose in the portal blood. An early response to insulin in muscle and adipose tissue is the migration of glucose transporter vesicles to the cell surface, exposing active glucose transporters (GLUT 4). These insulin-sensitive tissues will only take up glucose from the blood stream to any significant extent in the presence of the hormone. As insulin secretion falls in the starved state, so the transporters are internalized again, reducing glucose uptake.

The uptake of glucose into the liver is independent of insulin, but liver has an isoenzyme of hexokinase (glucokinase) with a high K_m, so that as the concentration of glucose entering the liver increases, so does the rate of synthesis of glucose 6-phosphate. This is in excess of the liver's requirement for energy and is used mainly for synthesis of glycogen. In both liver and skeletal muscle, insulin acts to stimulate glycogen synthase and inhibit glycogen phosphorylase. Some of the glucose entering the liver may also be used for lipogenesis and synthesis of triacylglycerol. In adipose tissue, insulin stimulates glucose uptake, its conversion to fatty acids, and their esterification; and inhibits intracellular lipolysis and the release of free fatty acids.

The products of lipid digestion enter the circulation as triacylglycerol-rich chylomicrons (Chapter 25). In adipose tissue and skeletal muscle, lipoprotein lipase is activated in response to insulin; the resultant free fatty acids are largely taken up to form triacylglycerol reserves, while the glycerol remains in the blood stream and is taken up by the liver and used for glycogen synthesis or lipogenesis. Free fatty acids remaining in the blood stream are taken up by the liver and reesterified. The lipid-depleted chylomicron remnants are also cleared by the liver, and surplus liver triacylglycerol—including that from lipogenesis—is exported in very low density lipoprotein.

Under normal feeding patterns the rate of tissue protein catabolism is more or less constant throughout the day; it is only in cachexia that there is an increased rate of protein catabolism. There is net protein catabolism in the postabsorptive phase of the feeding cycle and net protein synthesis in the absorptive phase, when the rate of synthesis increases by about 20–25%. The increased rate of protein synthesis is, again, a response to insulin action. Protein synthesis is an energy-expensive process, accounting for up to almost 20% of energy expenditure in the fed state, when there is an ample supply of amino acids from the diet, but under 9% in the starved state.

Metabolic Fuel Reserves Are Mobilized in the Starving State

There is a small fall in plasma glucose upon starvation, then little change as starvation progresses (Table 27–2; Figure 27–2). Plasma free fatty acids increase with onset of starvation but then plateau. There is an initial delay in ketone body production, but as starvation progresses the plasma concentration of ketone bodies increases markedly.

In the postabsorptive state, as the concentration of glucose in the portal blood falls, so insulin secretion decreases, resulting in skeletal muscle and adipose tissue taking up less glucose. The increase in secretion of glucagon from the A cells of the pancreas inhibits glycogen synthase and activates glycogen phosphorylase in liver. The resulting glucose 6-phosphate in liver is

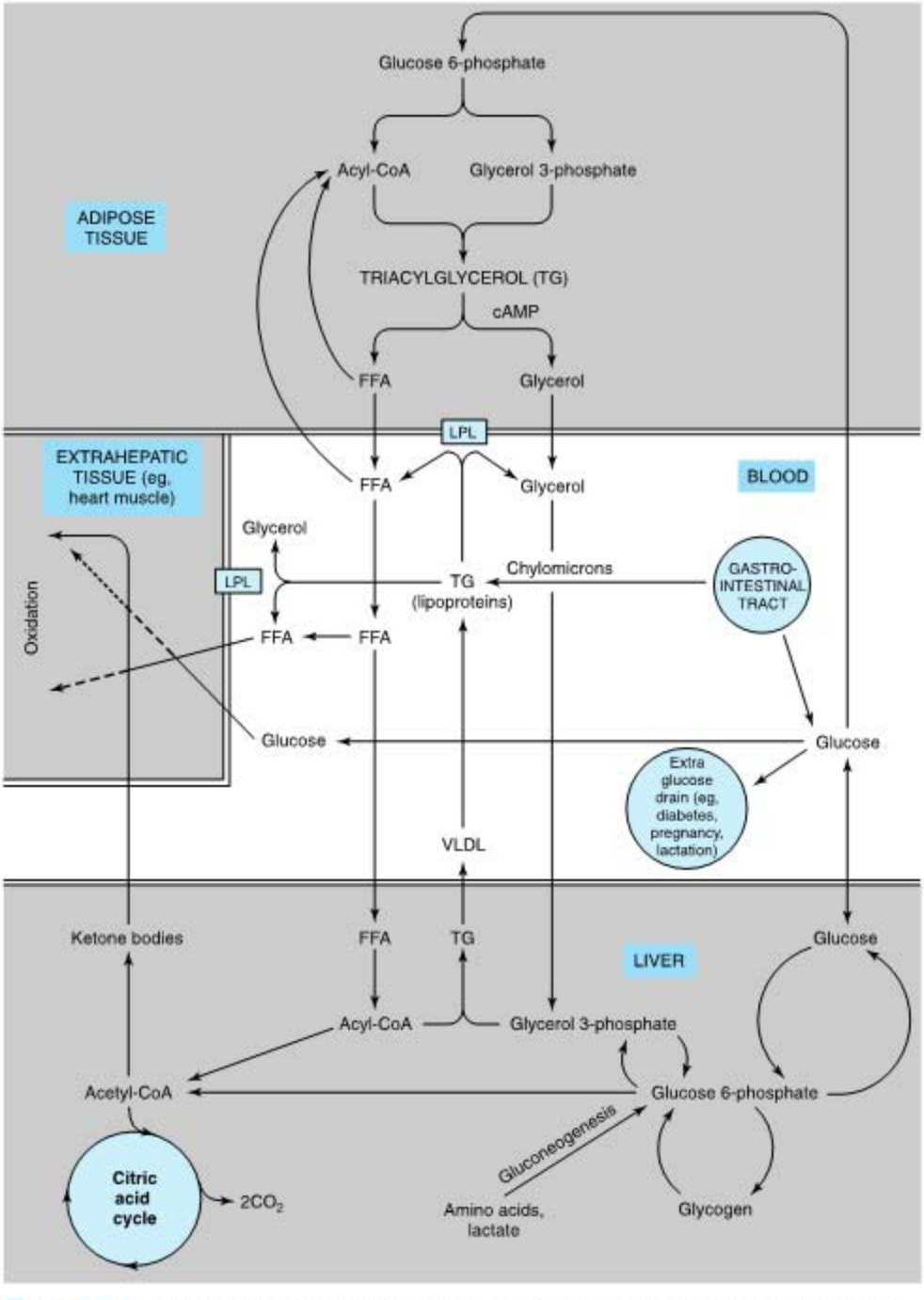

Figure 27–1. Metabolic interrelationships between adipose tissue, the liver, and extrahepatic tissues. In extrahepatic tissues such as heart, metabolic fuels are oxidized in the following order of preference: (1) ketone bodies, (2) fatty acids, (3) glucose. (LPL, lipoprotein lipase; FFA, free fatty acids; VLDL, very low density lipoproteins.)

Table 27–1. Energy yields, oxygen consumption, and carbon dioxide production in the oxidation of metabolic fuels.

	Energy Yield (kJ/g)	O_2 Consumed (L/g)	CO_2 Produced (L/g)	RQ	Oxygen (kJ/L)
Carbohydrate	16	0.829	0.829	1.00	20
Protein	17	0.966	0.782	0.81	20
Fat	37	2.016	1.427	0.71	20

hydrolyzed by glucose-6-phosphatase, and glucose is released into the blood stream for use by other tissues, particularly the brain and erythrocytes.

Muscle glycogen cannot contribute directly to plasma glucose, since muscle lacks glucose-6-phosphatase, and the primary purpose of muscle glycogen is to provide a source of glucose 6-phosphate for energy-yielding metabolism in the muscle itself. However, acetyl-CoA formed by oxidation of fatty acids in muscle inhibits pyruvate dehydrogenase and leads to citrate accumulation, which in turn inhibits phosphofructokinase and therefore glycolysis, thus sparing glucose. Any accumulated pyruvate is transaminated to alanine at the expense of amino acids arising from breakdown of protein reserves. The alanine—and much of the keto acids resulting from this transamination—are exported from muscle and taken up by the liver, where the alanine is transaminated to yield pyruvate. The resultant amino acids are largely exported back to muscle to provide amino groups for formation of more alanine, while the pyruvate is a major substrate for gluconeogenesis in the liver.

In adipose tissue, the effect of the decrease in insulin and increase in glucagon results in inhibition of lipogenesis, inactivation of lipoprotein lipase, and activation of hormone-sensitive lipase (Chapter 25). This leads to release of increased amounts of glycerol (a substrate for gluconeogenesis in the liver) and free fatty acids, which are used by skeletal muscle and liver as their preferred metabolic fuels, so sparing glucose.

Table 27–2. Plasma concentrations of metabolic fuels (mmol/L) in the fed and starving states.

	Fed	40 Hours Starvation	7 Days Starvation
Glucose	5.5	3.6	3.5
Free fatty acids	0.30	1.15	1.19
Ketone bodies	Negligible	2.9	4.5

Although muscle takes up and preferentially oxidizes free fatty acids in the starving state, it cannot meet all of its energy requirements by β-oxidation. By contrast, the liver has a greater capacity for β-oxidation than it requires to meet its own energy needs and forms more acetyl-CoA than can be oxidized. This acetyl-CoA is used to synthesize ketone bodies (Chapter 22), which are major metabolic fuels for skeletal and heart muscle and can meet some of the brain's energy needs. In prolonged starvation, glucose may represent less than 10% of whole body energy-yielding metabolism. Furthermore, as a result of protein catabolism, an increasing number of amino acids are released and utilized in the liver and kidneys for gluconeogenesis.

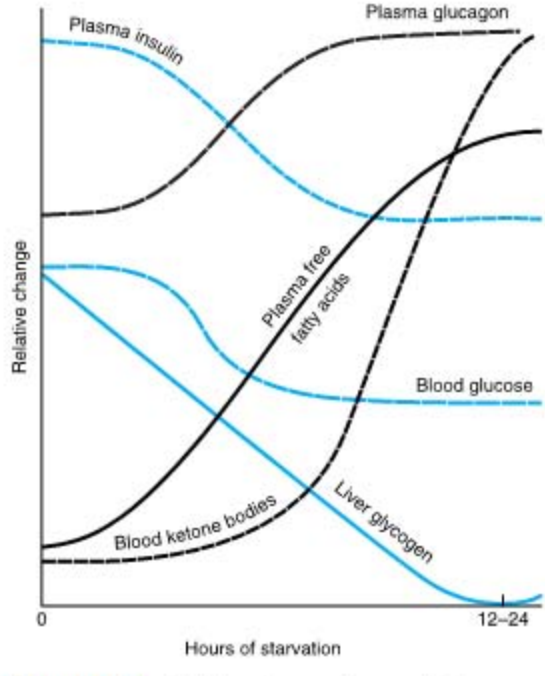

Figure 27–2. Relative changes in metabolic parameters during the onset of starvation.

Table 27–3. Summary of the major and unique features of metabolism of the principal organs.

Organ	Major Function	Major Pathways	Main Substrates	Major Products	Specialist Enzymes
Liver	Service for the other organs and tissues	Most represented, including gluconeogenesis; β-oxidation; ketogenesis; lipoprotein formation; urea, uric acid, and bile acid formation; cholesterol synthesis; lipogenesis[1]	Free fatty acids, glucose (well fed), lactate, glycerol, fructose, amino acids (Ethanol)	Glucose, VLDL (triacylglycerol), HDL, ketone bodies, urea, uric acid, bile acids, plasma proteins (Acetate)	Glucokinase, glucose-6-phosphatase, glycerol kinase, phosphoenolpyruvate carboxykinase, fructokinase, arginase, HMG-CoA synthase and lyase, 7α-hydroxylase (Alcohol dehydrogenase)
Brain	Coordination of the nervous system	Glycolysis, amino acid metabolism	Glucose, amino acid, ketone bodies (in starvation) Polyunsaturated fatty acids in neonate	Lactate	
Heart	Pumping of blood	Aerobic pathways, eg, β-oxidation and citric acid cycle	Free fatty acids, lactate, ketone bodies, VLDL and chylomicron triacylglycerol, some glucose		Lipoprotein lipase. Respiratory chain well developed.
Adipose tissue	Storage and breakdown of triacylglycerol	Esterification of fatty acids and lipolysis; lipogenesis[1]	Glucose, lipoprotein triacylglycerol	Free fatty acids, glycerol	Lipoprotein lipase, hormone-sensitive lipase
Muscle Fast twitch Slow twitch	 Rapid movement Sustained movement	 Glycolysis Aerobic pathways, eg, β-oxidation and citric acid cycle	 Glucose Ketone bodies, triacylglycerol in VLDL and chylomicrons, free fatty acids	Lactate	 Lipoprotein lipase. Respiratory chain well developed.
Kidney	Excretion and gluconeogenesis	Gluconeogenesis	Free fatty acids, lactate, glycerol	Glucose	Glycerol kinase, phosphoenolpyruvate carboxykinase
Erythrocytes	Transport of O_2	Glycolysis, pentose phosphate pathway. No mitochondria and therefore no β-oxidation or citric acid cycle.	Glucose	Lactate	(Hemoglobin)

[1]In many species but not very active in humans.

CLINICAL ASPECTS

In prolonged starvation, as adipose tissue reserves are depleted there is a very considerable increase in the net rate of protein catabolism to provide amino acids not only as substrates for gluconeogenesis but also as the main metabolic fuel of the tissues. Death results when essential tissue proteins are catabolized beyond the point at which they can sustain this metabolic drain. In patients with cachexia as a result of release of cytokines in response to tumors and a number of other pathologic conditions, there is an increase in the rate of tissue protein catabolism as well as a considerably increased metabolic rate, resulting in a state of advanced starvation. Again, death results when essential tissue proteins have been catabolized.

The high demand for glucose by the fetus and for synthesis of lactose in lactation can lead to ketosis. This may be seen as mild ketosis with hypoglycemia in women, but in lactating cattle and in ewes carrying twins there may be very pronounced ketosis and profound hypoglycemia.

In poorly controlled type 1 diabetes mellitus, patients may become hyperglycemic, partly as a result of lack of insulin to stimulate uptake and utilization of glucose and partly because of increased gluconeogenesis from amino acids in the liver. At the same time, the lack of insulin results in increased lipolysis in adipose tissue, and the resultant free fatty acids are substrates for ketogenesis in the liver. It is possible that in very severe diabetes utilization of ketone bodies in muscle (and other tissues) is impaired because of lack of oxaloacetate (most tissues have a requirement for some glucose metabolism to maintain an adequate amount of oxaloacetate for citric acid cycle activity). In uncontrolled diabetes, the magnitude of ketosis may be such as to result in severe acidosis (ketoacidosis) since acetoacetic acid and 3-hydroxybutyric acid are relatively strong acids. Coma results from both the acidosis and the considerably increased osmolarity of extracellular fluid (mainly due to the hyperglycemia).

A summary of the major and unique metabolic features of the principal tissues is presented in Table 27–3.

SUMMARY

- The body can interconvert the majority of foodstuffs. However, there is no net conversion of most fatty acids (or other acetyl-CoA-forming substances) to glucose. Most amino acids, arising from the diet or from tissue protein, can be used for gluconeogenesis, as can the glycerol from triacylglycerol.
- In starvation, glucose must be provided for the brain and erythrocytes; initially, this is supplied from liver glycogen reserves. To spare glucose, muscle and other tissues reduce glucose uptake in response to lowered insulin secretion; they also oxidize fatty acids and ketone bodies preferentially to glucose.
- Adipose tissue releases free fatty acids in starvation, and these are used by many tissues as fuel. Furthermore, in the liver they are the substrate for synthesis of ketone bodies.
- Ketosis, a metabolic adaptation to starvation, is exacerbated in pathologic conditions such as diabetes mellitus and ruminant ketosis.

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