Overview of Metabolism

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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.

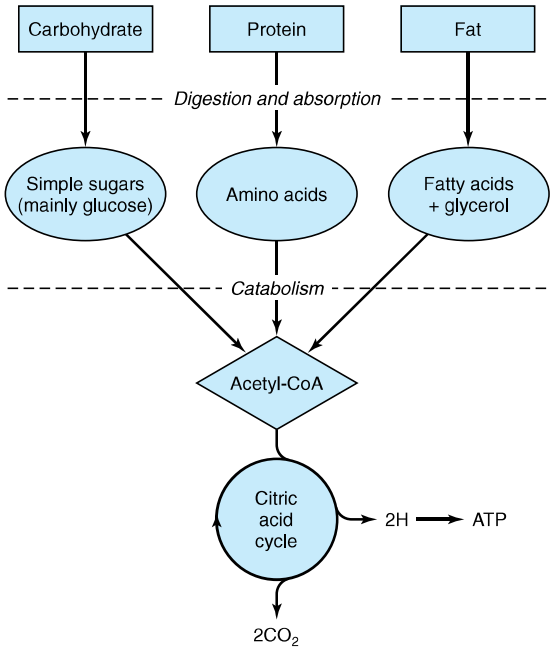

Figure 15–1. Outline of the pathways for the catabolism of dietary carbohydrate, protein, and fat. All the pathways lead to the production of acetyl-CoA, which is oxidized in the citric acid cycle, ultimately yielding ATP in the process of oxidative phosphorylation.

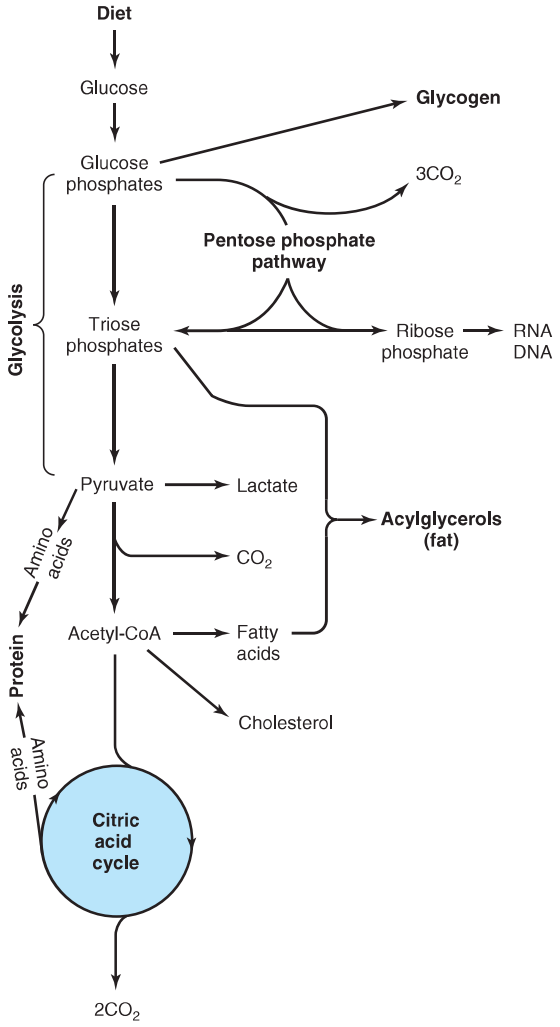

Figure 15–2. Overview of carbohydrate metabolism showing the major pathways and end products. Gluconeogenesis is not shown.

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.**

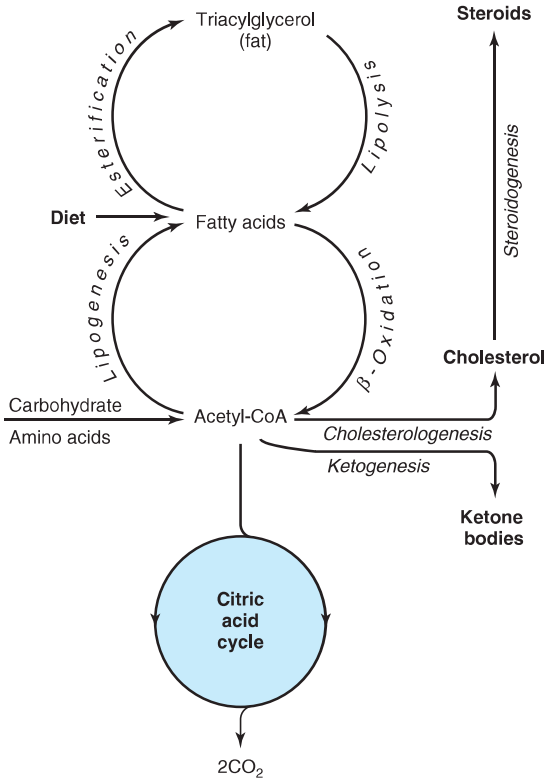

Figure 15–3. Overview of fatty acid metabolism showing the major pathways and end products. Ketone bodies comprise the substances acetoacetate, 3-hydroxybutyrate, and acetone.

(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-**

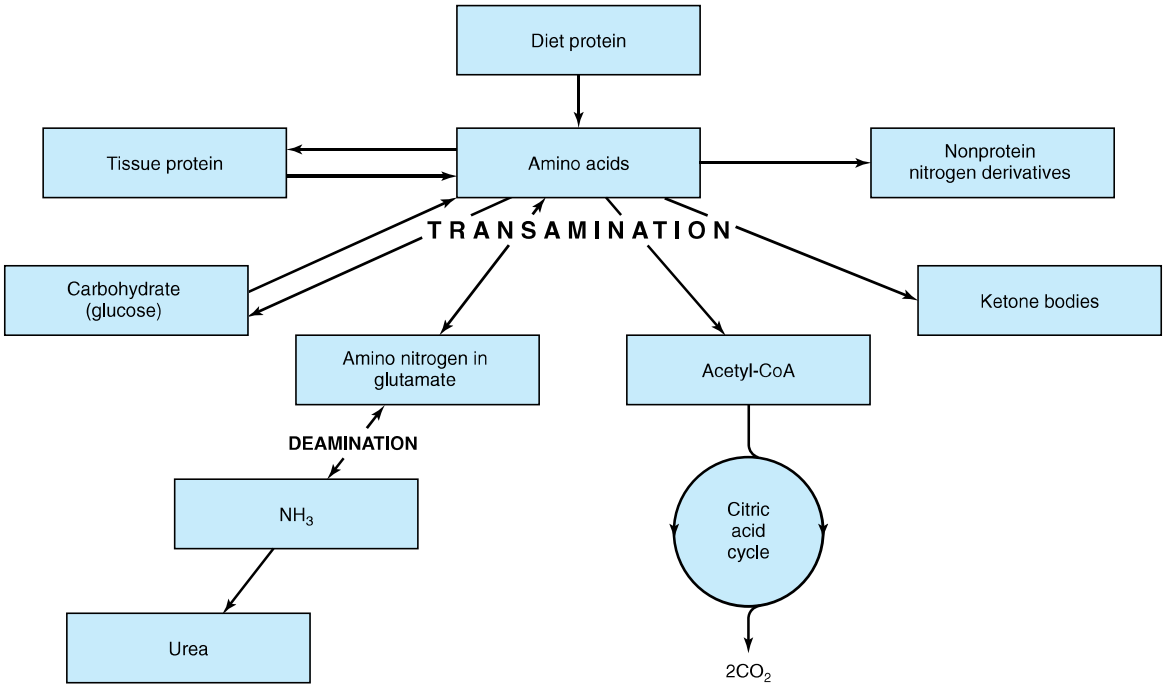

Figure 15–4. Overview of amino acid metabolism showing the major pathways and end products.

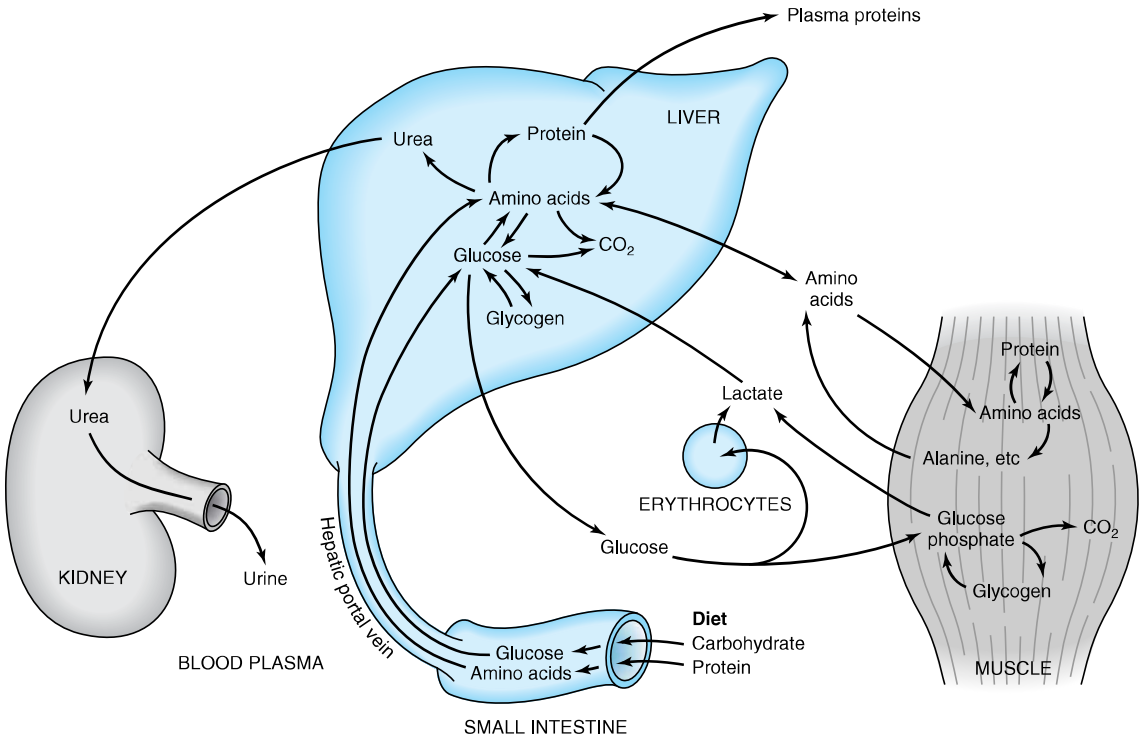

Figure 15–5. Transport and fate of major carbohydrate and amino acid substrates and metabolites. Note that there is little free glucose in muscle, since it is rapidly phosphorylated upon entry.

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-

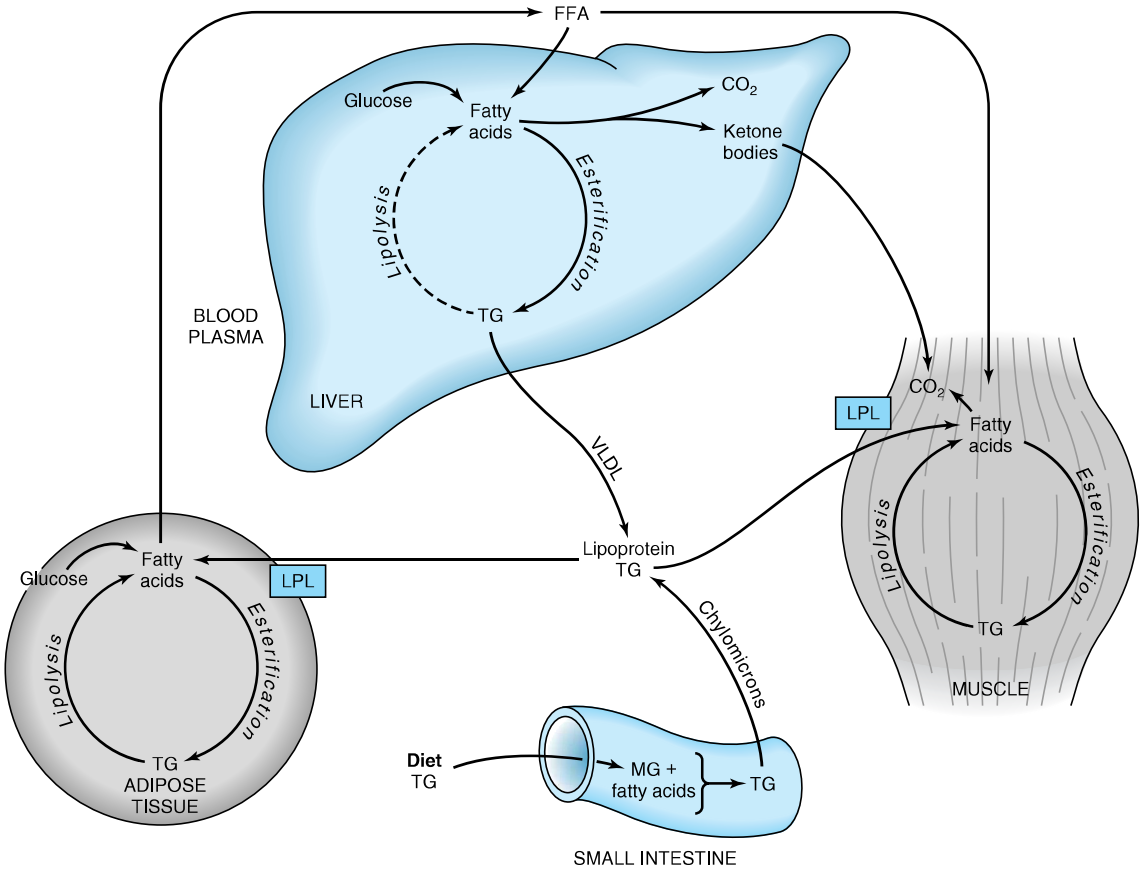

Figure 15–6. Transport and fate of major lipid substrates and metabolites. (FFA, free fatty acids; LPL, lipoprotein lipase; MG, monoacylglycerol; TG, triacylglycerol; VLDL, very low density lipoprotein.)

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

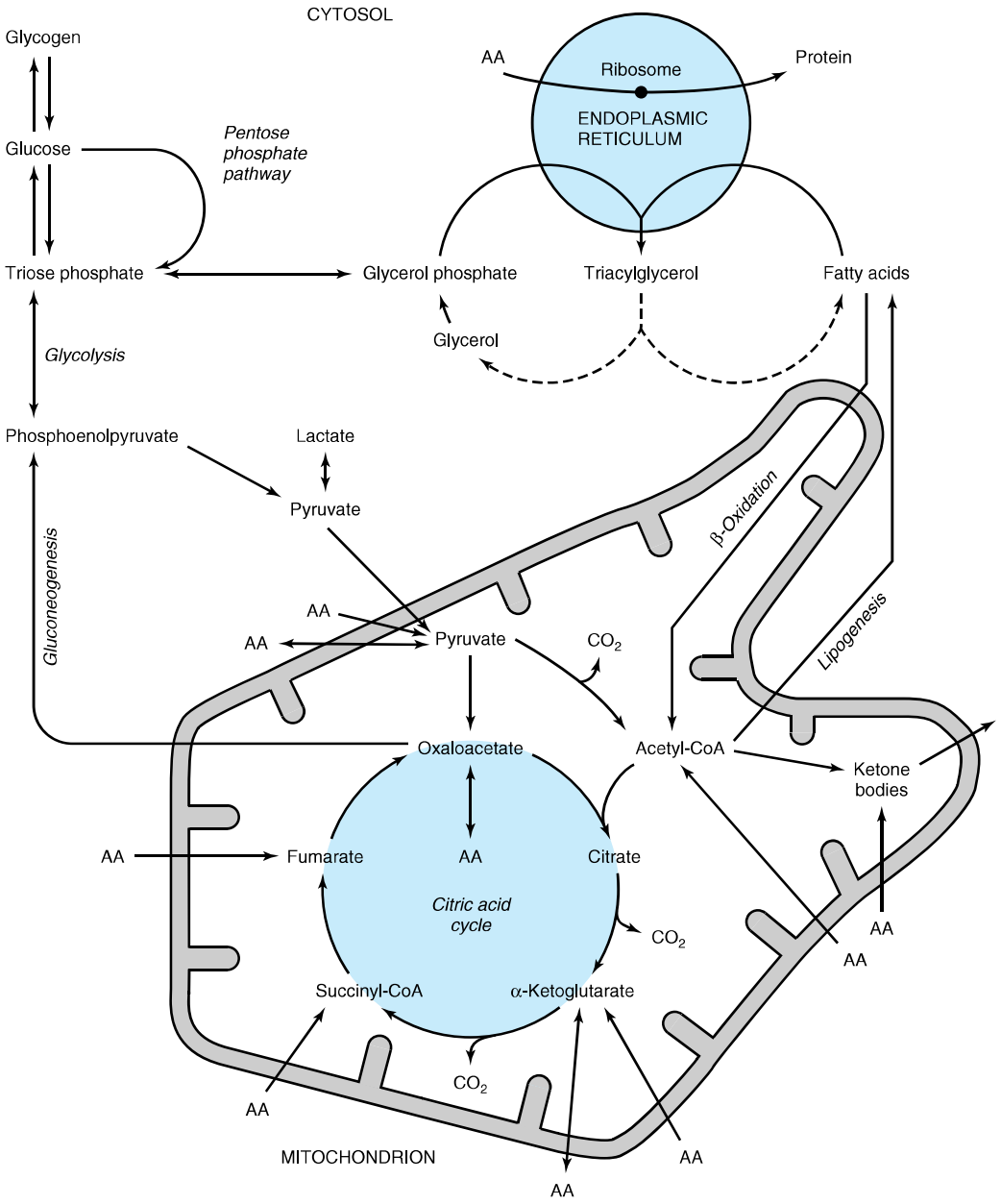

Figure 15–7. Intracellular location and overview of major metabolic pathways in a liver parenchymal cell. (AA →, metabolism of one or more essential amino acids; AA ↔, metabolism of one or more nonessential amino acids.)

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,

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

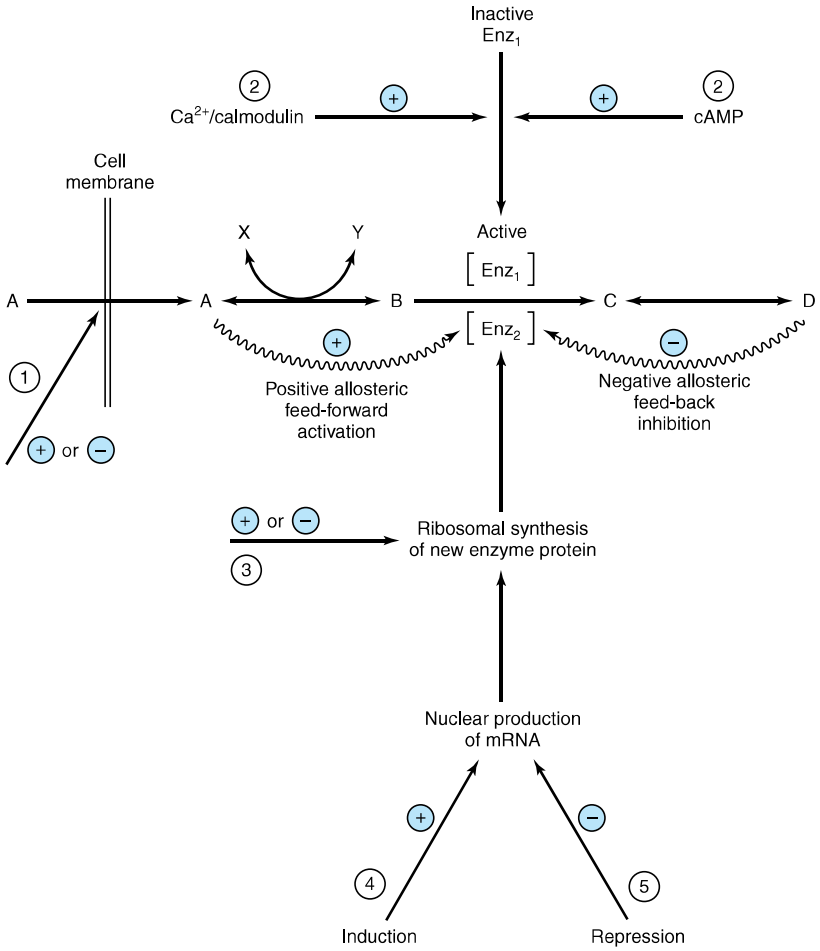

Figure 15–8. Mechanisms of control of an enzyme-catalyzed reaction. Circled numbers indicate possible sites of action of hormones. ①, Alteration of membrane permeability; ②, conversion of an inactive to an active enzyme, usually involving phosphorylation/dephosphorylation reactions; ③, alteration of 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

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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.

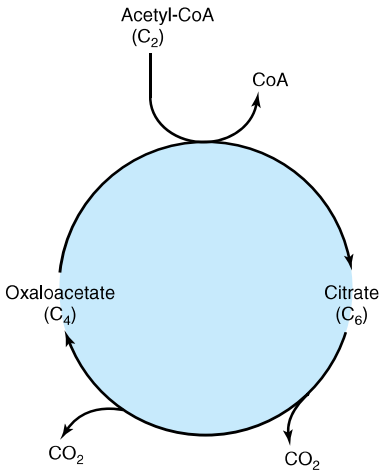

Figure 16–1. Citric acid cycle, illustrating the catalytic role of oxaloacetate.

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.

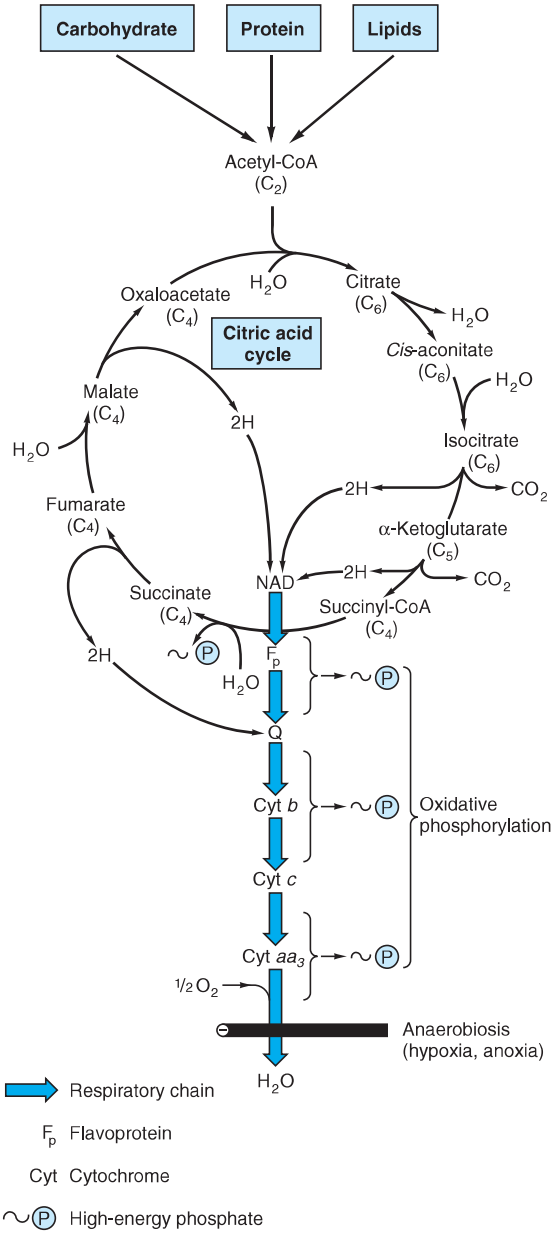

Figure 16–2. The citric acid cycle: the major catabolic pathway for acetyl-CoA in aerobic organisms. Acetyl-CoA, the product of carbohydrate, protein, and lipid catabolism, is taken into the cycle, together with H_2O, and oxidized to CO_2 with the release of reducing equivalents (2H). Subsequent oxidation 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.

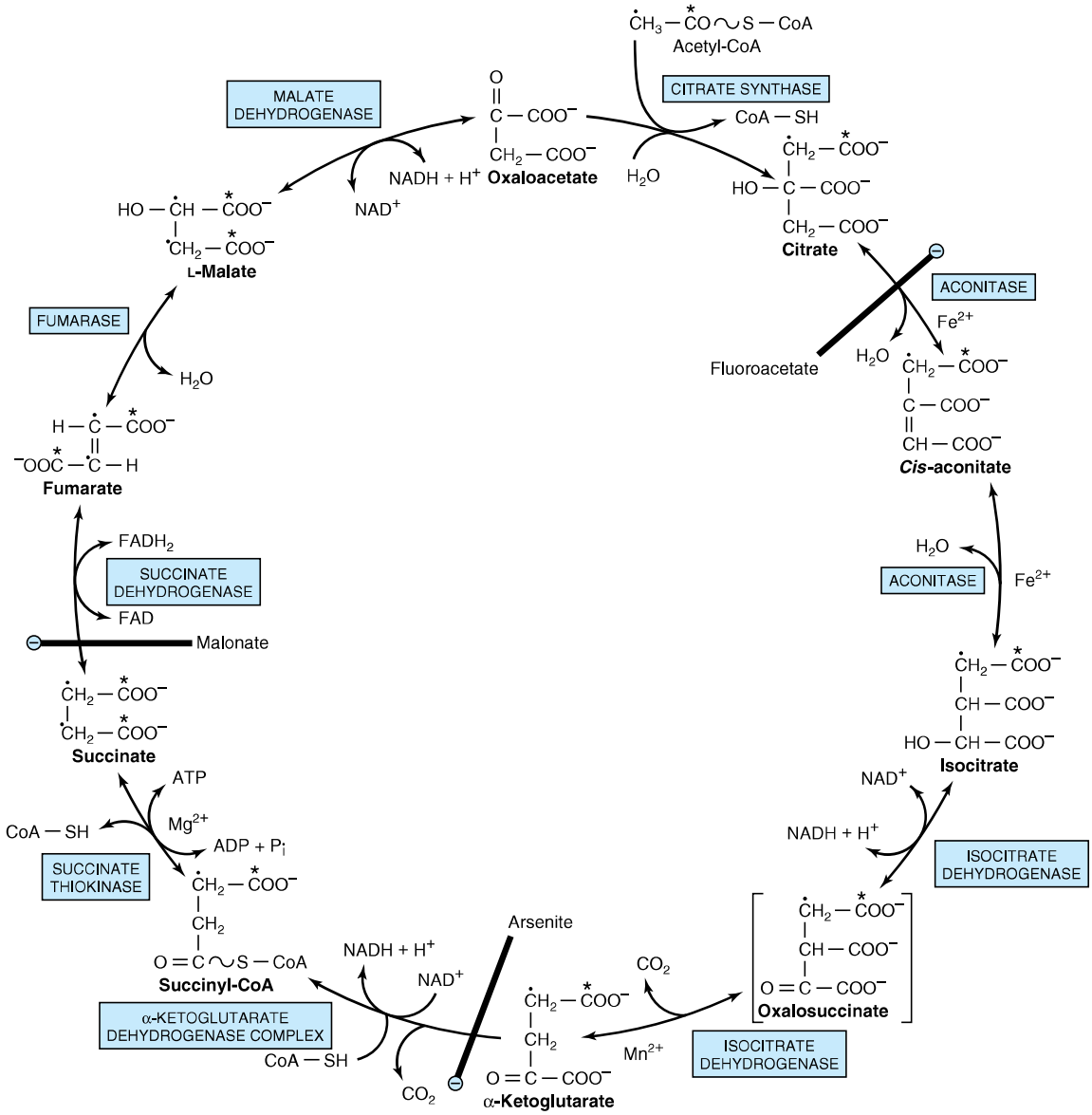

Figure 16–3. Reactions of the citric acid (Krebs) cycle. Oxidation of NADH and $FADH_2$ in the respiratory chain leads to the generation of ATP via oxidative phosphorylation. In order to follow the passage of acetyl-CoA through the cycle, the two carbon atoms of the acetyl radical are shown labeled on 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

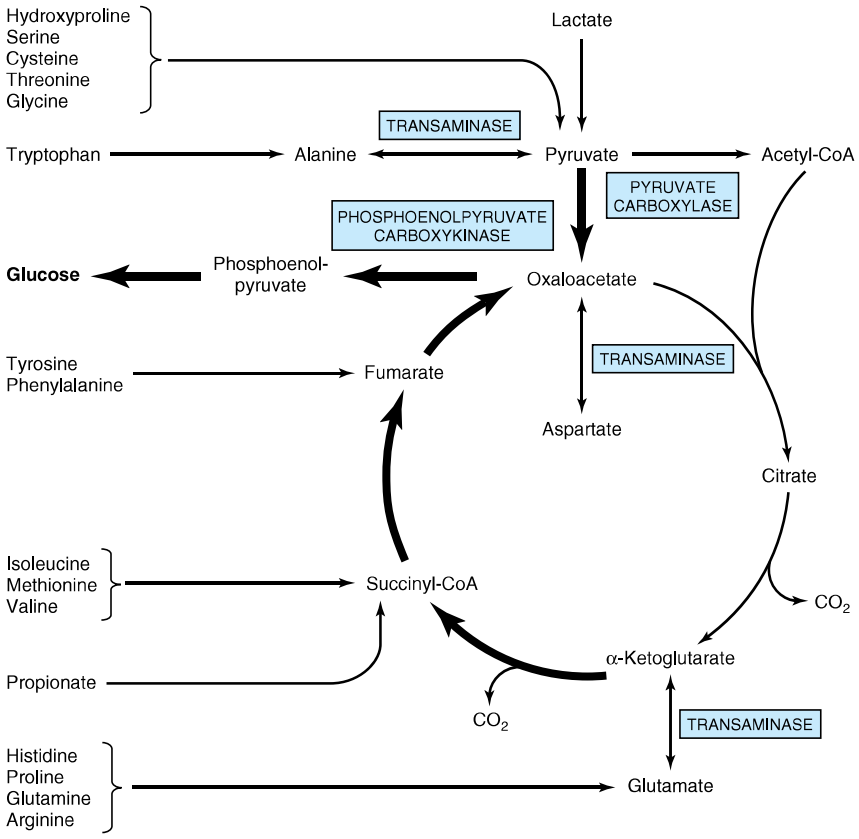

Figure 16–4. Involvement of the citric acid cycle in transamination and gluconeogenesis. The bold arrows indicate the main pathway of gluconeogenesis.

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-

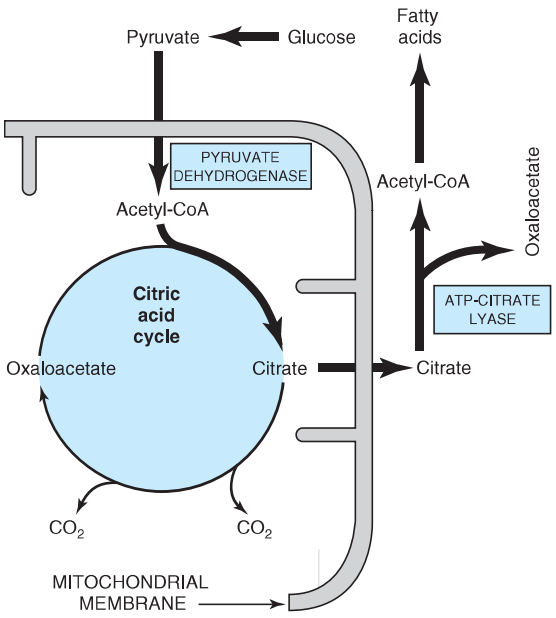

Figure 16–5. Participation of the citric acid cycle in fatty acid synthesis from glucose. See also Figure 21–5.

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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