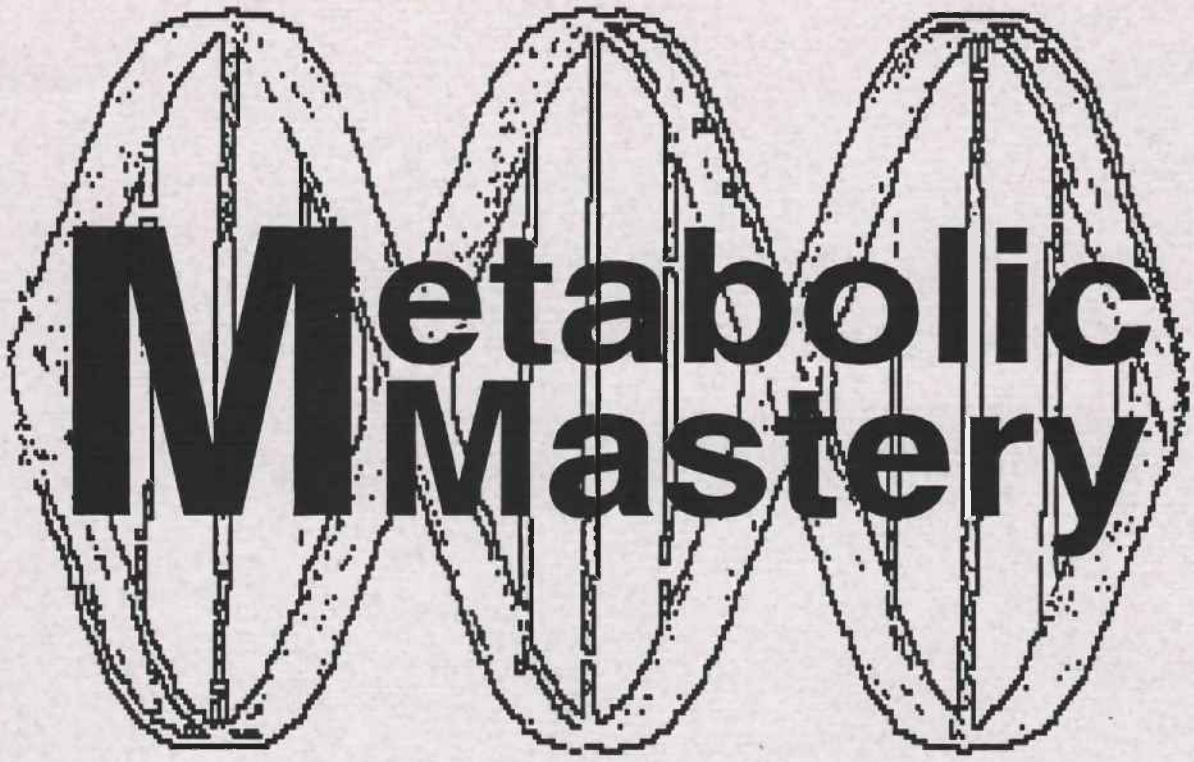


# A New Approach

# To

# Learn And Review

# Metabolism

RJ Innovations

# Metabolic Mastery

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— RJ Innovations —

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NOTES

**I. INTRODUCTION**: Metabolic Mastery is an educational tool to help the student learn and review metabolic pathways. Metabolic Mastery was developed by a professor (Ph.D., Biochemistry, University of California at Berkeley) with 18 years of teaching experience and by a student (B.S., Health Science, State University of New York College at Brockport). We saw the need for an easier, more entertaining way to learn the complex concepts of intermediary metabolism. This format is suitable for individuals and groups. It offers a useful adjunct to standard texts and provides a novel way to teach biochemistry.

The following information and instructions apply to the complete Metabolic Mastery game. If you only have the Metabolic Mastery manual, you have three options:

1. Just enjoy the manual by itself. It is complete and self-contained. The manual stands by itself or makes an excellent supplement to the standard textbooks.
2. Make the other game components. By following the information in this section, Overview 1.1, and Appendix 3, you can construct the game board and the playing cards.
3. By using the order form in the back of this manual, order the boxed set of the Metabolic Mastery game parts (excludes the manual).

The complete Metabolic Mastery game consists of a game board, flash (playing) cards, an instruction manual, and a packet with 1 die and 4 player's pawns.

Game board: The game board presents "The Big Picture," an overview diagram interlinking twelve major pathways. Rectangular blank spaces represent compounds which participate in reactions, as well as products of individual pathways. Each rectangle has a two-letter code.

Arrows interconnect the rectangles. Blue lines indicate anabolic (biosynthetic, energy consuming) pathways and red lines indicate catabolic (degradative, energy yielding) pathways. Numbers indicate enzymes or enzyme complexes. Numbers with asterisks indicate more complex steps. ATP and GTP production is indicated by diamonds. Hexagons indicate the production of the reducing equivalents, NADH and $FADH_2$.

Flash cards: The pull apart flash cards (playing cards) fit onto appropriate rectangles on the game board. The name of a reaction product is given on the front of each card. On the back of each card are terms and phrases identifying the essential structure and function of the compound named on the front. A two-letter code in the lower right-hand corner corresponds to the same code on the blank rectangle it occupies on the game board.

Instruction Manual: The Metabolic Mastery manual provides in-depth descriptions to help establish a sound understanding of twelve metabolic pathways. It also contains highlights featuring enzymes, energy relationships, carbohydrates, lipids, and amino acids. These sections review basic vocabulary and concepts.

The manual includes full-page figures to complement the overview of the game board. These summarize important pathways and regulatory schemes relevant to human metabolism. The figures include names and structures of intermediates, as well as names of enzymes. The text after each figure contains the following information about the pathway: input and output, location, function, characteristics, and individual enzymes and reactions. Supplemental notes at the end of each section point out important features. As an additional tool, the manual provides review questions and answers.

**II. INSTRUCTIONS**: How to use Metabolic Mastery. Metabolic Mastery can help you understand functional aspects of each pathway and, at the same time, help you visualize metabolic interrelationships. In general, you will not be required to memorize all structures and enzymes, nor will you be required to reproduce a particular pathway. Key enzymes, key intermediates and products, and regulatory mechanisms are important, however. You may wish to focus your attention on those steps indicated by a check mark (✓) in the manual, as well as "Input/Output" and "Functions" sections dealing with the pathways.

The following instructions are guidelines. The flash cards and game board can be combined in stages with varying degrees of complexity. There is no right or wrong way to use this learning aid. We encourage

you to be creative and to adapt it to your own learning style.

A. Studying from the game board and flash cards:

1. Begin by comparing the game board with Figure 1.1 (Overview), which contains the names of intermediates and products. Scan the manual to familiarize yourself with the format. "Highlight" sections provide general information, and the individual pathways are the focus of the remaining sections.
2. Lay out all of the flash cards, front (product name) side up on the appropriate rectangles on the game board to get a sense of the "lay of the land." Become familiar with the layout so that you can visualize the game board. Even though the metabolic pathways are not physically associated in this way, the spatial representation will make it easier to grasp interrelationships. You will find it easier to remember details when they are placed in a context of the "Big Picture."
3. With the game board and flash cards laid out as above, do your best to describe a given reaction product in terms of its structure and function, as given in sections [A] and [B] on the reverse side of the cards.
4. Lay out all the flash cards, back (descriptive) side up on the game board, pathway by pathway (refer to Figs. 1.1, 1.2, and the respective section in the manual). Study the information on the card backs and do your best to identify the name of the reaction product given on the front. Your course syllabus and outlines will indicate the steps to you need to emphasize.
5. Select one of the following review problems. They ask you to trace different pathways. Use the playing cards and the game board to construct your answer, or make copies of Figure 1.4 (Blank Overview) and write in your answers. Compare your answers with Figures 1.1, 1.2, and the appropriate Sections.
   (a) A molecule of glucose in glycogen is completely oxidized in skeletal muscle to $CO_2$ to produce ATP. (Refer to Sections 8.II, 8.III, 5, 6, 7.)
   (b) A molecule of glucose is oxidized under anaerobic conditions. (Refer to Section 5.)
   (c) A fatty acid in a triacylglycerol is completely oxidized in skeletal muscle to $CO_2$ to produce ATP. (Refer to Sections 12.I, 12.II, 6, 7.)
   (d) A molecule of glucose is oxidized to ribose 5-phosphate and NADPH. (Refer to Sections 5, 9.)
   (e) The amino group of aspartate is converted to urea. (Refer to Section 16.)
   (f) Acetyl CoA is converted to cholesterol. (Refer to Section 17.I.)
   (g) Acetyl CoA is converted to ketone bodies. (Refer to Section 12.III.)
   (h) Acetoacetate is converted to $CO_2$ to produce ATP. (Refer to Sections 12.III, 6, 7.)
   (i) Glucose is converted to glycerol 3-phosphate, and acetyl CoA is converted to fatty acids, to synthesize triacylglycerols. (Refer to Sections 5, 13.I, 13.II.)
   (j) Succinate is converted to glucose. (Refer to Sections 6, 10.)

   Can you devise other review problems?

B. Using Metabolic Mastery as a game: This can be done in a variety of ways. Here are three possibilities. The game can be played by a single individual or in a small group.

1. Choose a route from CARBOHYDRATES (or FATS & OILS) to $H_2O$ (or HORMONES/BILE SALTS), or choose your own starting and ending points.
   (a) The player or players place their pawns at CARBOHYDRATES (or FATS & OILS). Spread out the flash cards with the "name" side up. The die is thrown at each turn and the number on the die is the number of steps (blank rectangles) a player moves. For a more detailed progress along a pathway, choose three steps as the maximum number a player can take. (For example, on the die as usual, 1 spot = 1 step, 2 spots = 2 steps, 3 spots = 3 steps, but make 4 spots = 1 step, 5 spots = 2 steps, and 6 spots = 3 steps.)

(b) When a player lands on an unoccupied rectangle, the player has to pick the correct flash card (name side up) and place it onto the blank rectangle. The letter code is checked. If the choice is incorrect, the flash card is put back with the unused cards. The player's pawn stays on the blank rectangle and the player must try again the next turn. If the player is correct this time, he/she can roll the die on the following turn.

For an additional degree of complexity: If the choice is correct, and energy has been released (ATP, GTP, NADH, $FADH_2$), the player can then try to name the enzyme(s) that made the reaction(s) possible. If they are again correct, for each correctly named enzyme they get one "energy credit" and/or a free turn (decide before starting). There are no penalties if the player is incorrect in identifying the enzyme. Refer to Appendix 2 for the names of the numbered enzymes.

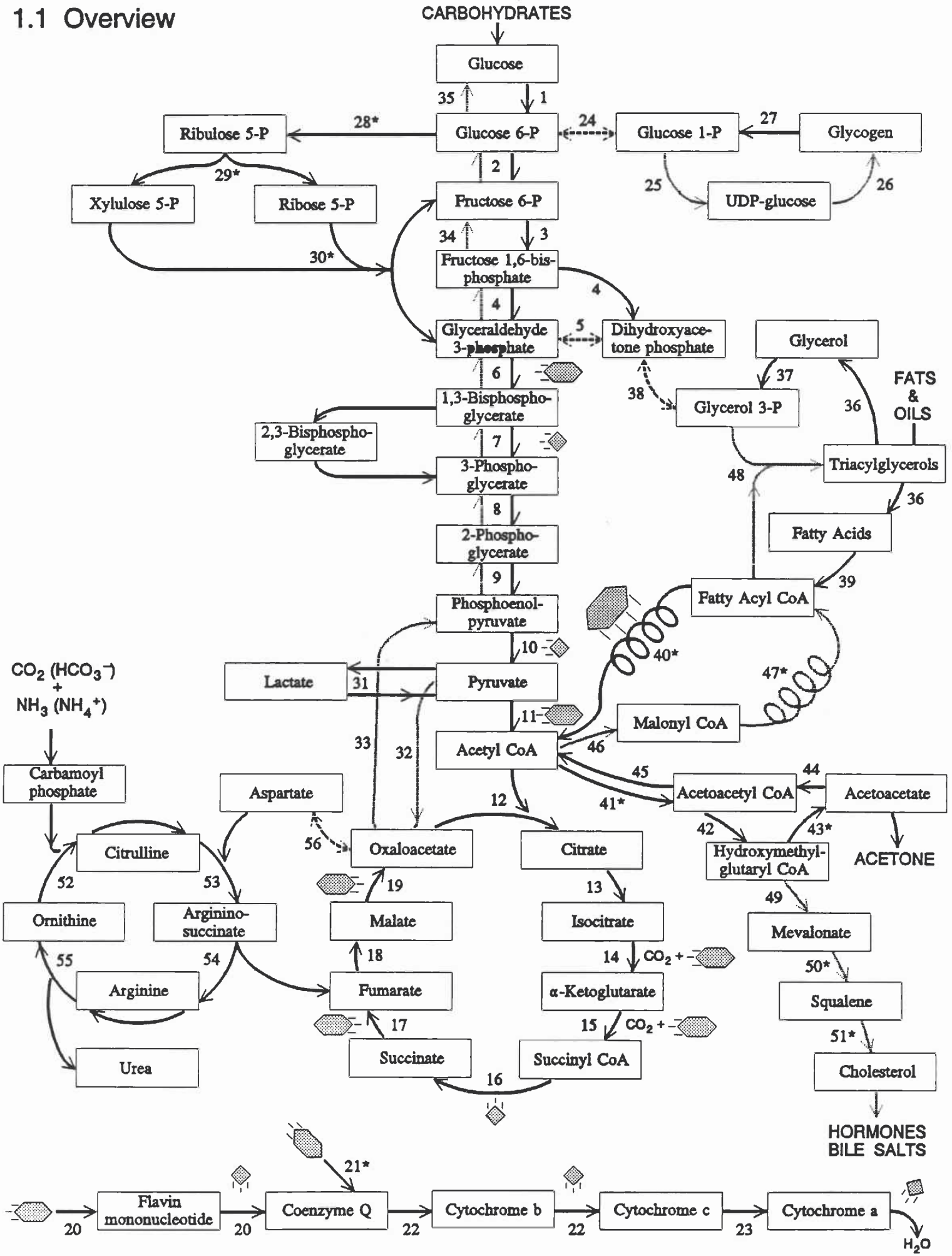
(c) If a player lands on a rectangle that already has a flash card (name side up) on it, the player can try to identify an important function of the reaction product (section [B] of the back side). If the player is correct, the flash card is turned over (back side up) and she/he remains on the occupied rectangle. If the preceding player, who placed the flash card onto this rectangle, did not choose to identify the enzyme (or incorrectly identified the enzyme), then the current player can try this. If the current player doesn't want to guess the reaction product's function, the player can instead move backwards, until an unoccupied rectangle space is reached and then follow the instructions in paragraph (b).

(d) Players continue the process, following the appropriate metabolic pathways all the way, until reaching the end point chosen at the beginning of the game. The first player to complete the route wins.

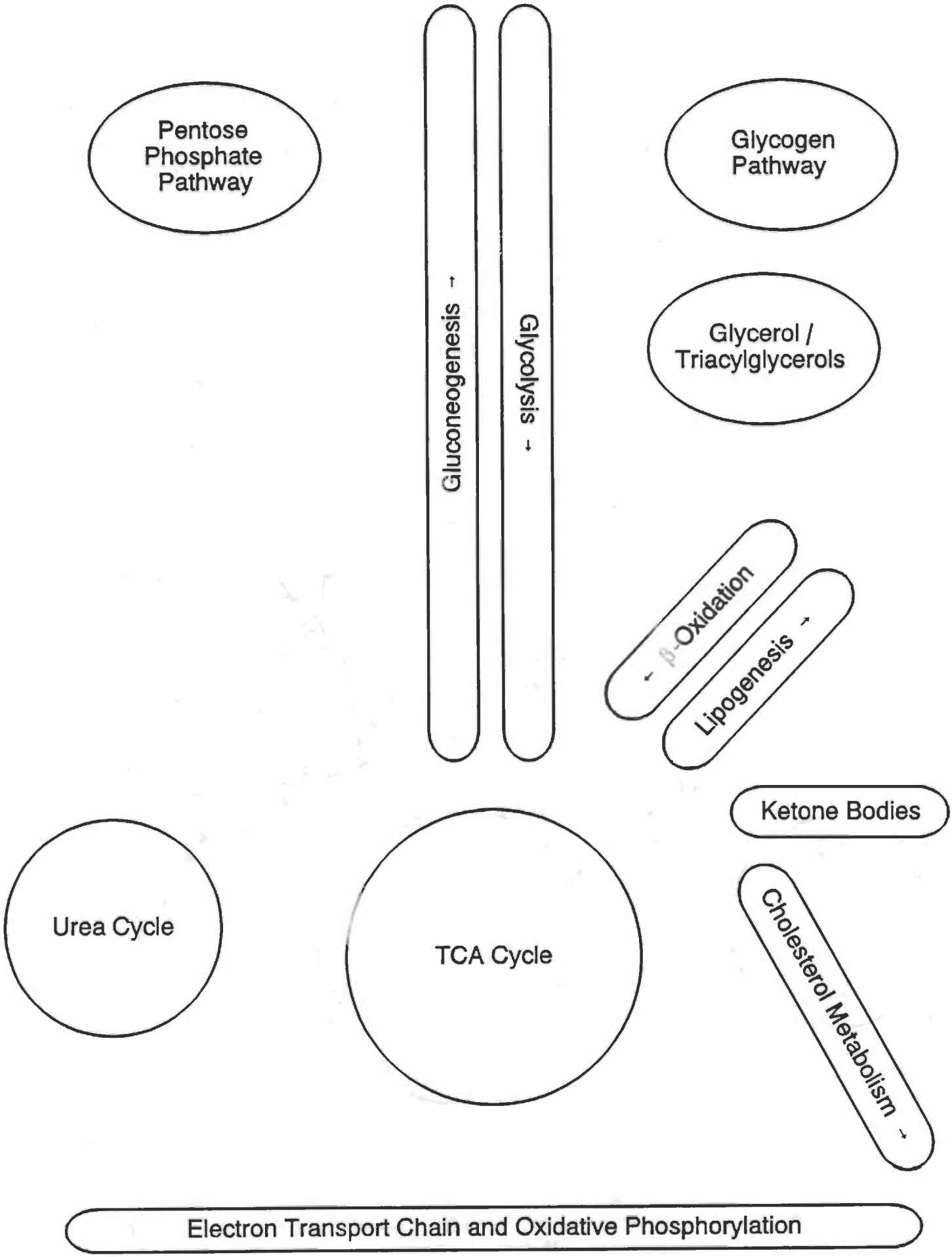
If the "energy credit" option is chosen, the winner is the player who has collected the most "energy credits" and has completed the route.

2. Evenly divide all the flash cards (front side up) among the players. The players take turns placing flash cards onto the appropriate rectangle spaces, but not necessarily following a particular pathway. If a player makes a mistake, he loses his turn. The first player to correctly place all her flash cards wins.
3. Challenge play: Divide the players into two teams. Spread out all the flash cards, name side up. The game starts with one team pointing to any unoccupied rectangle. The opposing team must correctly (1) choose the reaction product and (2) describe an important function. Score a point for each correct name and function. Alternate challenges until all the flash cards are used.

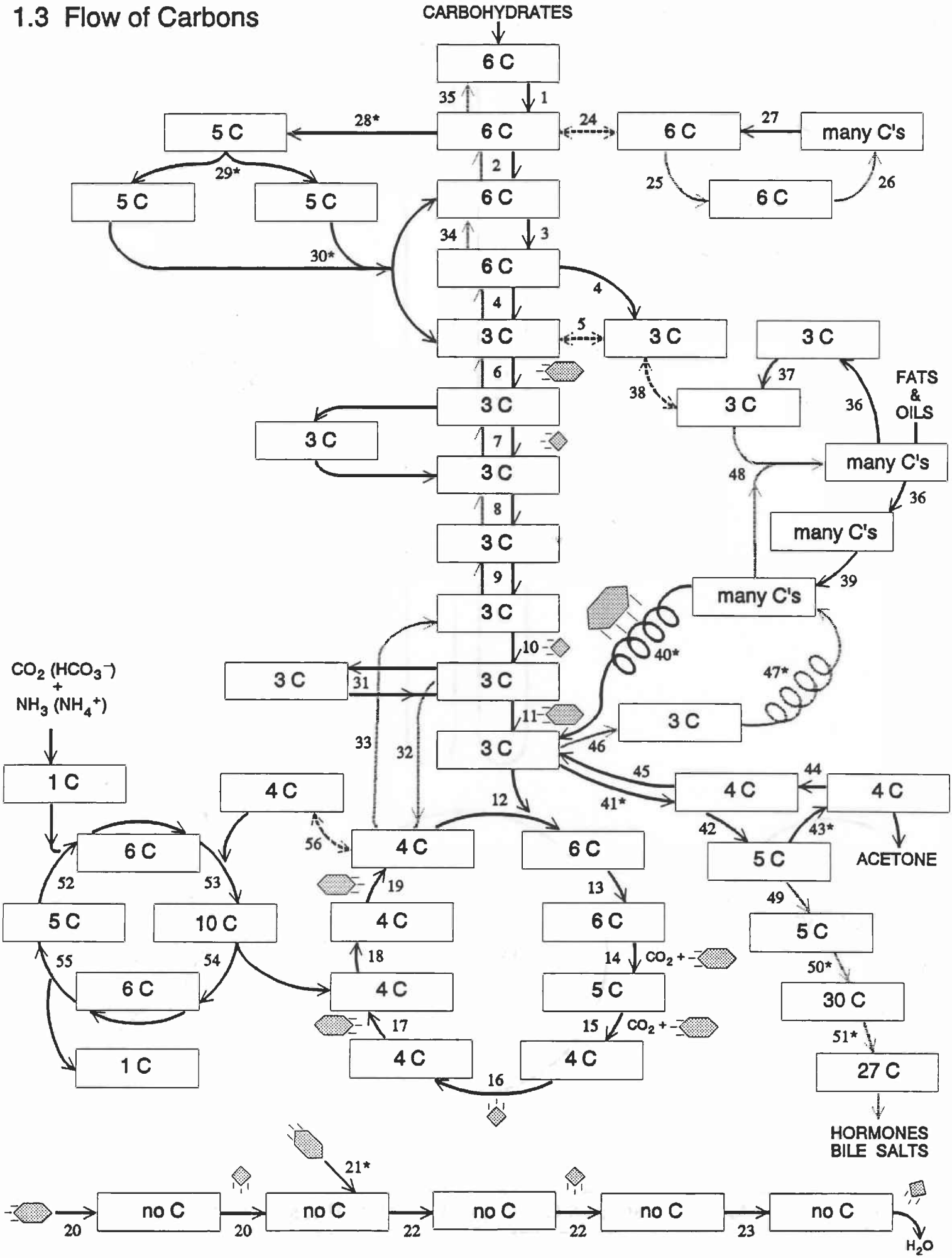
# 1.1 Overview


CARBOHYDRATES
Glucose
Glucose 6-P
Glucose 1-P
Glycogen
UDP-glucose
Ribulose 5-P
Xylulose 5-P
Ribose 5-P
Fructose 6-P
Fructose 1,6-bis-phosphate
Glyceraldehyde 3-phosphate
Dihydroxyace-tone phosphate
Glycerol
Glycerol 3-P
FATS & OILS
Triacylglycerols
Fatty Acids
Fatty Acyl CoA
1,3-Bisphospho-glycerate
2,3-Bisphospho-glycerate
3-Phospho-glycerate
2-Phospho-glycerate
Phosphoenol-pyruvate
Pyruvate
Lactate
Malonyl CoA
Acetyl CoA
Acetoacetyl CoA
Acetoacetate
ACETONE
Hydroxymethyl-glutaryl CoA
Mevalonate
Squalene
Cholesterol
HORMONES BILE SALTS
$CO_2$ ($HCO_3^-$) + $NH_3$ ($NH_4^+$)
Carbamoyl phosphate
Citrulline
Ornithine
Arginino-succinate
Arginine
Urea
Aspartate
Oxaloacetate
Malate
Fumarate
Succinate
Citrate
Isocitrate
α-Ketoglutarate
Succinyl CoA
Flavin mononucleotide
Coenzyme Q
Cytochrome b
Cytochrome c
Cytochrome a
$H_2O$

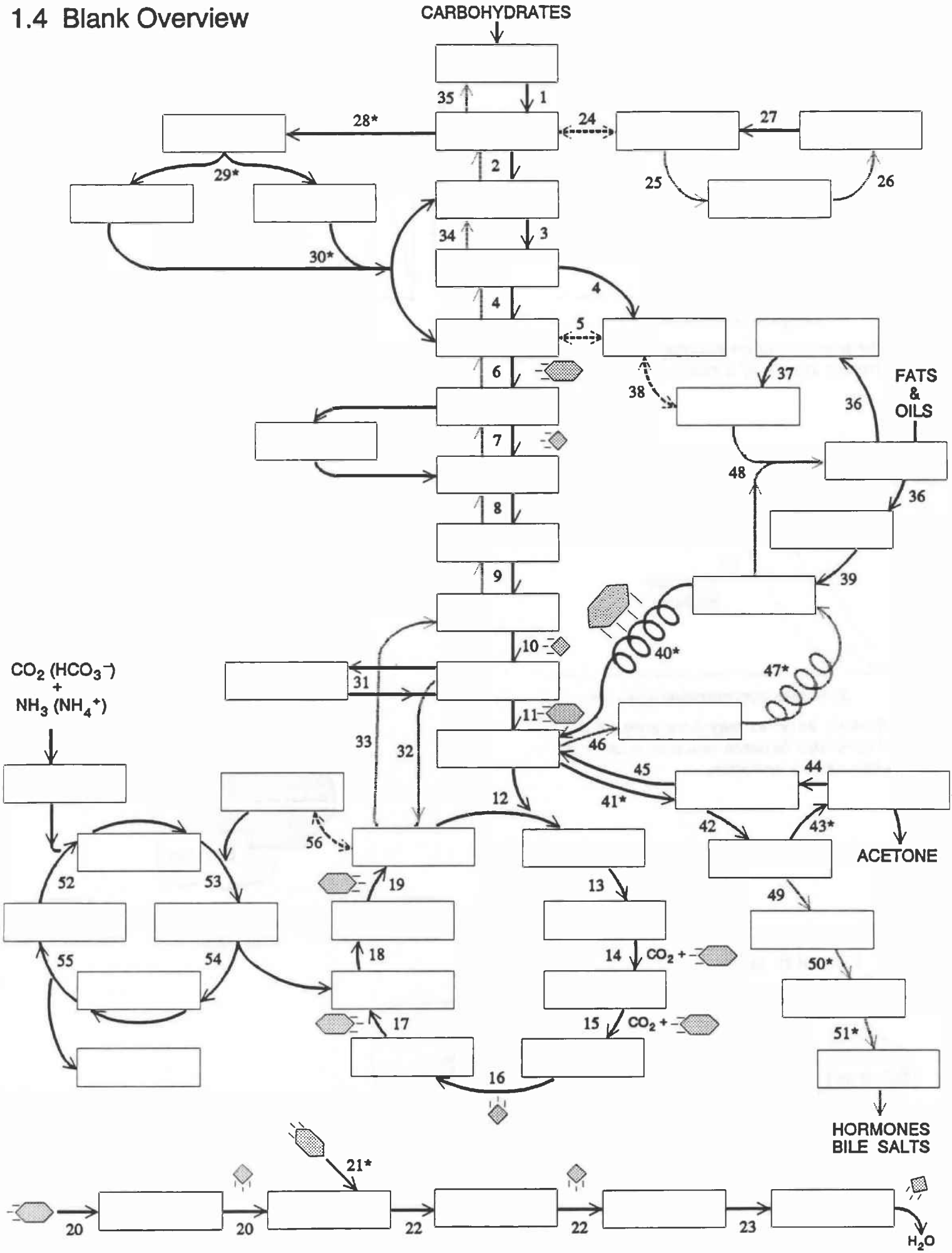
## 1.2 Organization of Pathways on the Game Board



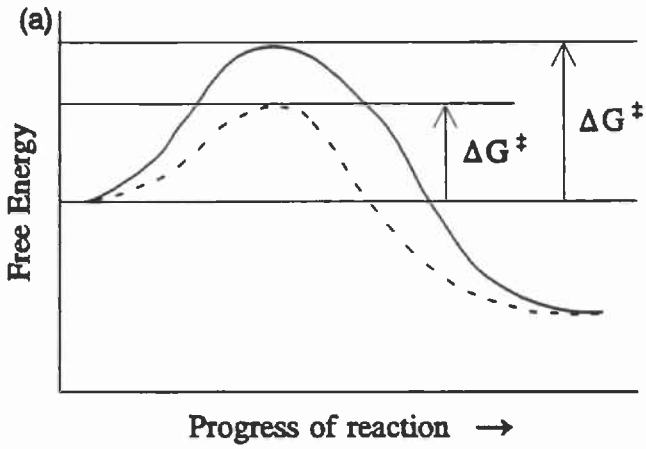
## 1.3 Flow of Carbons



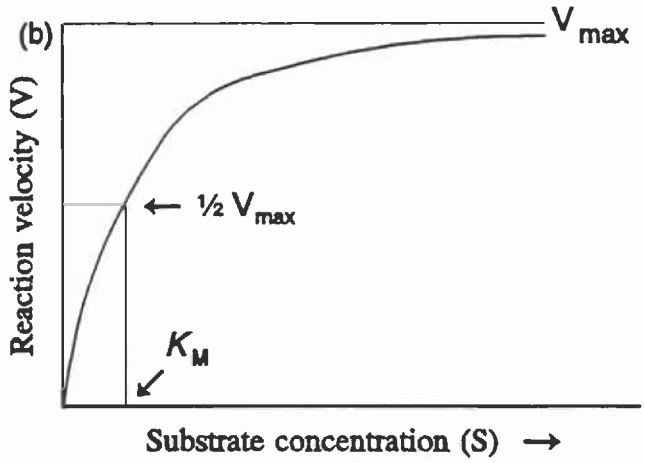
# 1.4 Blank Overview



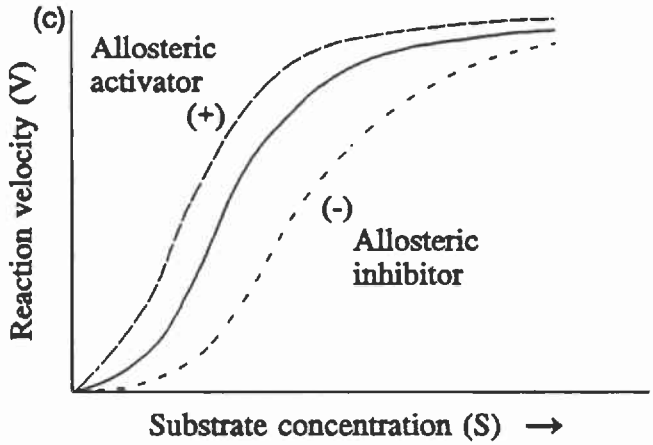
## 2.1 Enzyme Highlights




*In the presence of an enzyme (dotted line), the activation energy of a reaction is lowered.*

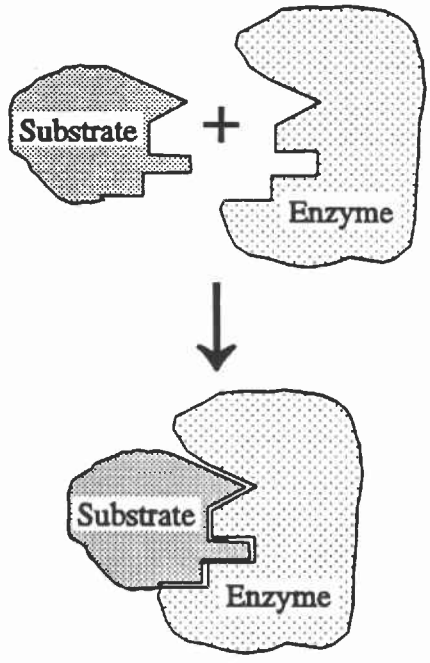



*The velocity of an enzyme-catalyzed reaction varies with the substrate concentration.*



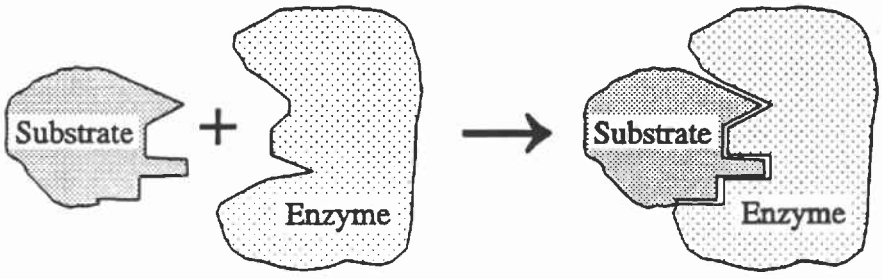

*Allosteric enzymes may have sigmoidal relationships between reaction velocity & substrate concentration.*

(d) Lock-and-key model




Enzyme-Substrate (ES) Complex

(e) Induced-fit model




Enzyme-Substrate (ES) Complex

ENZYMES ARE PROTEINS: Enzymes are polypeptides, chains of amino acids. Each type of enzyme exhibits a unique amino acid sequence which determines shape and function. Localized folding, or "secondary" structure, includes the $\alpha$-helix and $\beta$-pleated sheet. In general, enzymes are globular ("tertiary" structure) with hydrophilic amino acid side chains facing outward to interact with water, and hydrophobic side chains facing inward away from water. "Oligomeric" protein molecules are aggregates of polypeptide chains. Such proteins with subunits possess "quaternary" structure.

Enzymes are fragile. They can be inhibited by chemicals. They can be unfolded (denatured) by heat, extreme pH, and nonspecific agents such as detergents, and chemically modified (e.g. oxidized or hydrolyzed).

ENZYMES ARE CATALYSTS: Enzymes speed up chemical reactions of the cell. They are not consumed; instead they are used repeatedly. Enzymes do not change the direction of the reaction, nor do they supply energy. The direction of a chemical reaction is determined by the net change in energy of the system ($\Delta G$) and by the relative concentrations of reactants and products, not by the catalyst. An enzyme cannot make a chemical reaction proceed unless that reaction is able to proceed on its own accord, however slowly that may be.

Enzymes are classified according to the types of reactions they catalyze. The many thousands of enzymes carry out just six classes of chemical reactions:

1. Oxido-reductases carry out oxidation-reduction reactions. These are called *dehydrogenases* and *oxidases*. *Lactate dehydrogenase* and *cytochrome oxidase* are examples.
2. Transferases transfer functional groups. *Aminotransferases*, such as *aspartate aminotransferase*, transfer amino groups.
3. Hydrolases break bonds by adding water. Digestive enzymes, such as *lipase* (fat digestion), *proteases* like *trypsin* (protein digestion), and *amylase* (starch digestion) are examples.
4. Lyases cleave carbon-carbon bonds, carbon-nitrogen bonds, and carbon-oxygen bonds, without hydrolysis or oxidation-reduction. *Citrate lyase* is an example.
5. Isomerases catalyze intramolecular rearrangements. For example, *phosphoglucose isomerase* converts glucose 6-phosphate to fructose 6-phosphate.
6. Ligases create carbon-nitrogen, carbon-sulfur, and carbon-carbon bonds, coupled with the cleavage of a pyrophosphate (high energy) bond of ATP. *Fatty acyl CoA synthetase* is an example of this class. Fatty acids are coupled to CoA to form fatty acyl CoA's, with the cleavage of ATP to AMP and $PP_i$.

ENZYME KINETICS: Substrates are reactants of enzyme-catalyzed reactions. The rate of reaction for a substrate is controlled by the energy barrier, or activation energy. Enzymes lower the activation energy, $\Delta G^{\ddagger}$, as shown in Figure 2.1a. Enzymes lower the activation energy by guiding the collision of reactants at a cavity or cleft on the enzyme surface. This docking site for substrates is the "active site" of the enzyme. The classic model of a key (substrate) fitting into a precisely designed keyhole of a lock (enzyme) is illustrated by Figure 2.1d. The active sites of some enzymes differ in shape from the intended substrate. After the substrate is bound, the active site of the enzyme becomes modified to complement the substrate. This process is called induced fit (Fig. 2.1e).

Enzymes can be inhibited by a variety of mechanisms. In competitive inhibition, the inhibitor mimics the substrate, allowing it to penetrate the active site. By blocking the active site, the inhibitor prevents catalysis. Increasing the concentration of the substrate allows substrate molecules to compete effectively and to displace the competitive inhibitor from the active site. In other words, the maximum velocity is unaltered (see allosteric enzymes, below).

Kinetic analysis of enzymes reveals important features. The $K_M$ is a kinetic parameter which reflects

the affinity of an enzyme for its substrate. In general terms, a small $K_M$ suggests a high affinity of an enzyme for a substrate. Its active site can be saturated at low substrate concentrations. An enzyme with a large $K_M$ is likely to have a low affinity for its substrate. A higher substrate concentration is needed to saturate such an enzyme. In practical terms, the $K_M$ is the substrate concentration at which the rate of reaction is 50% of the maximum rate attainable for a particular enzyme concentration ($V_{max}$). Figure 2.1b illustrates the typical relationship when velocity is plotted as a function of increasing substrate concentration.

SOME ENZYMES NEED HELPERS: Cofactors are metal atoms and organic molecules which assist enzymes. Typical metals include trace mineral nutrients: iron, manganese, copper, and zinc. Coenzymes are organic molecules which complete active sites. For enzymes which require coenzymes, there can be no reaction without the appropriate coenzyme. The body transforms most of the B-complex vitamins to coenzymes. The diet must supply vitamins because the body cannot manufacture them. Each type of coenzyme plays a different role. Thus, a deficiency of vitamin $B_6$ (pyridoxine) cannot be remedied by a surplus of vitamin $B_3$ (niacin).

ENZYMES OFTEN PARTICIPATE IN METABOLIC PATHWAYS: In a functional sequence of reactions, the product of one enzyme becomes the substrate for the following enzyme, much like a bucket brigade, or an assembly line. The following represents a typical pathway: A → B → C → D, in which enzyme 1 catalyzes A → B; enzyme 2 catalyzes B → C; enzyme 3 catalyzes C → D and so on. In some pathways, enzymes exist as physically-associated multienzyme complexes. In others, enzymes are not bound to each other. Double-headed arrows indicate the reaction proceeds in both directions.

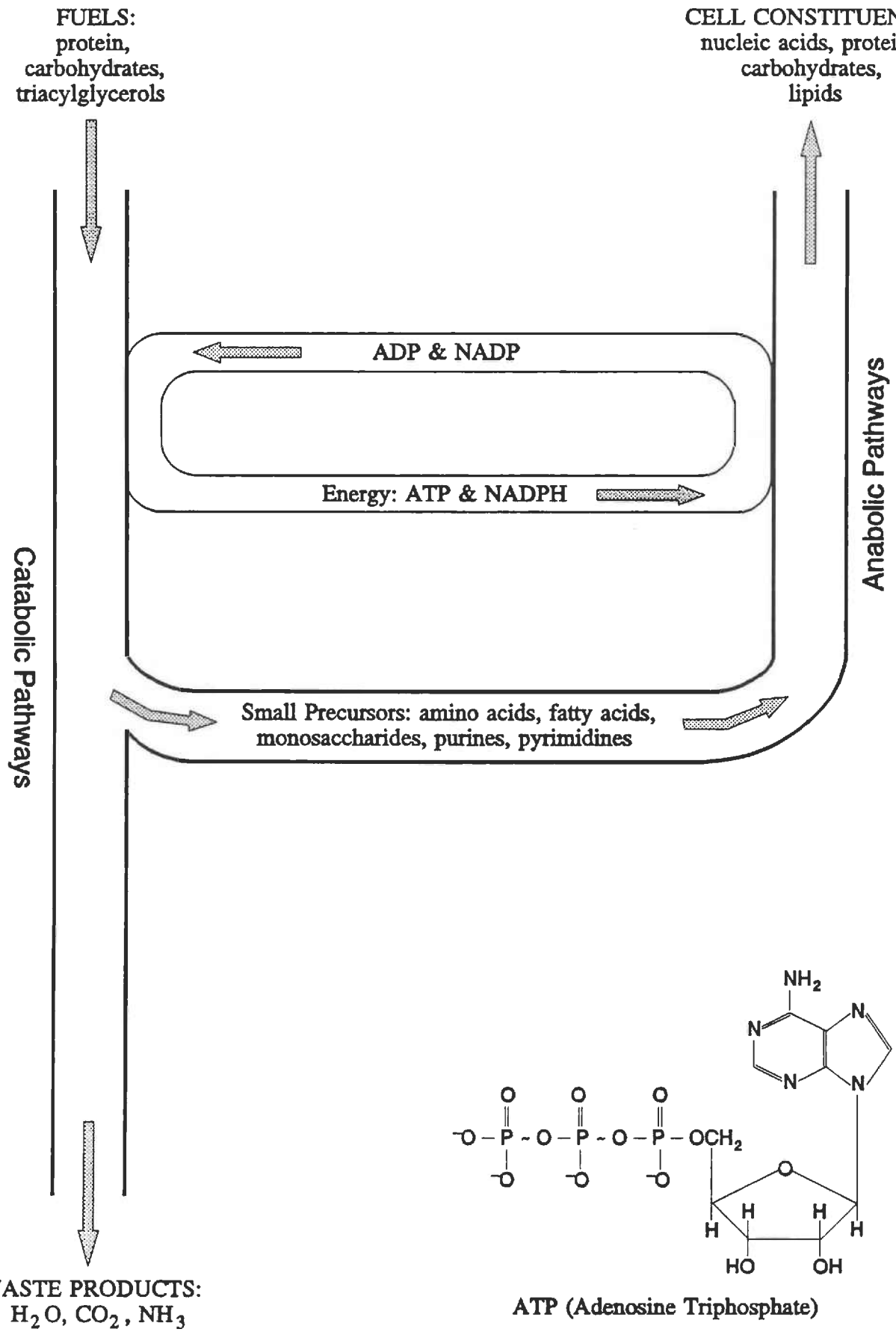
ENZYMES ARE OFTEN REGULATED: The first enzyme committed to the pathway is especially important. It catalyzes an irreversible reaction. This step is a bottleneck, limiting the flow of material through the pathway. Such rate-limiting enzymes are regulatory points either for activation or for inhibition. Entire pathways can be turned on or off by regulating the activity of such key enzymes. Important enzymes and steps are flagged (✓) in the text.

Typical mechanisms of regulating enzymes in intermediary metabolism:

1. Product inhibition: A key enzyme may be inhibited by the build-up of its immediate product. *Hexokinase* is inhibited by glucose 6-phosphate, its product (refer to Sect. 5).
2. Allosteric enzymes: These enzymes, at metabolic control points in pathways, contain multiple subunits and possess two or more different binding sites. Activators and inhibitors bind at sites distinct from the catalytic site of a subunit. Allosteric modifiers can lower an apparent $K_M$ (allosteric activators) or can raise an apparent $K_M$ (allosteric inhibitors). Figure 2.1c illustrates this typical behavior. Subunits communicate with each other. An allosteric activator or inhibitor binding to one subunit can affect adjacent subunits. Consequently, the net enzyme activity can be modulated by small changes in the concentration of modifiers. The modifiers can come from other pathways. Such molecular communication relies on the build-up of key intermediates, e.g. citrate, malonyl CoA, or alterations in ratios of NADH to $NAD^+$, or ATP to ADP. "Feedback inhibition" relies on allosteric enzymes. In such cases, the last product of the pathway feeds back on the first committed enzyme of the pathway, inhibiting it.
3. Covalent modification by phosphorylation/dephosphorylation: Some hormones can regulate key enzymes through covalent modification. Glucagon and epinephrine stimulate a chain of events culminating in the attachment of a phosphate to a target enzyme. Enzyme phosphorylation can either activate or inhibit, depending upon specific enzyme properties. For example, phosphorylation activates *phosphorylase b* for glycogen breakdown, and inhibits *glycogen synthase* (Sect. 8.III).

# NOTES

# 3.1 ATP/ADP Cycle



METABOLISM: Metabolism consists of degradative and biosynthetic pathways. It refers collectively to the many thousands of enzyme-catalyzed chemical reactions which occur in the body. For convenience, metabolism can be categorized as anabolism and catabolism (Fig. 3.1).

Anabolism refers to biosynthetic enzyme systems engaged in manufacturing cellular building blocks, such as amino acids, fatty acids, and monosaccharides, and assembling them into macromolecules, such as proteins, carbohydrates, lipids, and nucleic acids, and specialized molecules for carrying out specific functions. ATP and NADPH drive these reactions.

Catabolism refers to degradative enzyme systems required to extract energy from fuel molecules and the environment, with the production of smaller, simpler end products, such as ammonia, water, and carbon dioxide. Catabolism traps free energy in the form of energy-carrying molecules as ATP and NADPH.

Catabolism can be conveniently divided into four stages. In stage I, digestion occurs. Large biomolecules are hydrolyzed to amino acids, glucose, or fatty acids and glycerol. In stage II, these small molecules are converted to acetyl CoA. In stage III, acetyl CoA is converted to carbon dioxide. In stage IV, electrons from preceding oxidations, plus protons, combine with oxygen to form water. Oxidative phosphorylation traps energy from oxidations as ATP. This process creates the bulk of ATP used by the body.

ATP SUPPLIES ENERGY FOR CELLULAR PROCESSES: ATP (Sect. 19 & 20) is the energy currency of life. It supplies energy for biosynthesis and for energy-dependent processes, including muscle contraction, transport across membranes, nerve transmission, cell division, and motility of flagella. The pounds of protein, fat, and bone, which make up the body, are all products of ATP-driven processes.

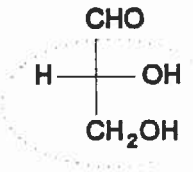
ATP CARRIES PHOSPHATE GROUPS: Potential energy is locked in the two terminal phosphate (pyrophosphate) bonds. Each pyrophosphate bond, indicated by a wavy line (~), releases a large amount of energy upon hydrolysis. Recall that the change in free energy, under standard conditions ($\Delta G°$), indicates the degree of spontaneity under those conditions. The $\Delta G°'$, in going from ATP to ADP + $P_i$ ( or to AMP + $PP_i$), is about -7,300 kcal/mole. Thus, each pyrophosphate bond is considered a "high energy bond." In contrast, esters are low energy bonds. The change in free energy of hydrolysis of typical phosphate esters, such as glucose 6-phosphate, is about half that of ATP's pyrophosphate bonds.

ATP IS RECYCLED: ATP is a carrier of phosphate groups. It accepts phosphate groups from compounds with a higher free energy of hydrolysis and donates them to create compounds of lower energy of hydrolysis. ATP traps the potential energy of oxidation/reduction of electron carriers.

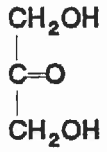
The amount of ATP required daily for a typical adult is about 40 kg, yet the amount of ATP in the body at a given time is only about 6 g. To supply the huge needs for immediate free energy, ATP is resynthesized after its conversion to ADP. ATP is regenerated from ADP by oxidation of fuels, especially oxidative phosphorylation in mitochondria (animals), or by photosynthesis (plants). Oxidation of a mole of glucose yields 686 kcal. Catabolism traps 38% of the energy released in this process as ATP, an extremely high efficiency. Figure 3.1 illustrates the ATP/ADP cycle and its relationship to energy-yielding pathways (catabolism) and biosynthetic pathways (anabolism).

# 4.1 Carbohydrate Highlights

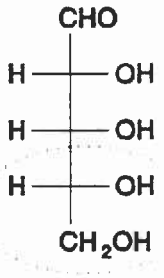
## Fischer Projections



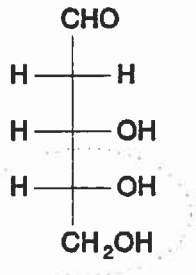

D-glyceraldehyde
(an aldotriose)



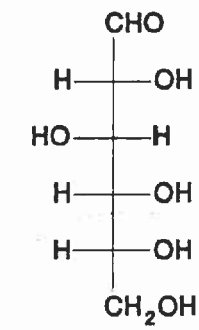

Dihydroxyacetone
(a ketotriose)



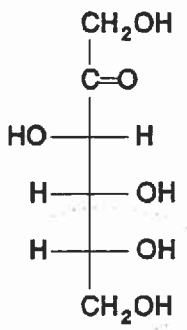

D-ribose
(an aldopentose)




2-deoxy-D-ribose
(an aldopentose)

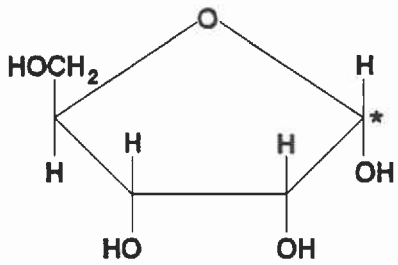



D-glucose
(an aldohexose)

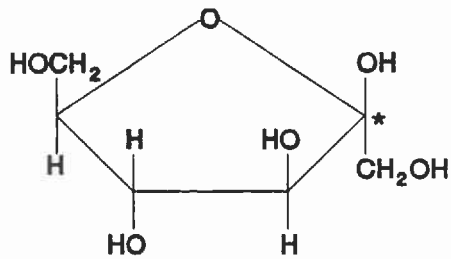



D-fructose
(a ketohexose)

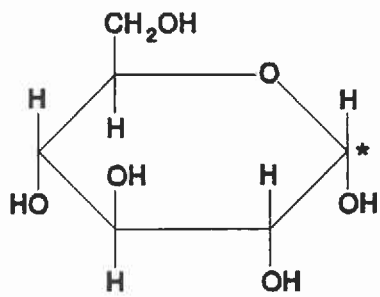
## Haworth Structures




D-ribose
(α-D-ribofuranose)
(a cyclic hemiketal)




D-fructose
(β-D-fructofuranose)
(a cyclic hemiketal)




D-glucose
(α-D-glucopyranose)
(a cyclic hemiacetal)

**I. CLASSIFICATION**: Carbohydrates are polyhydroxy aldehydes (aldoses) and polyhydroxy ketones (ketoses). The suffix "-ose" generally indicates an aldose (e.g. D-glucose) and "-ulose" indicates a ketose (e.g. D-xylulose). D-fructose, a ketose, is one exception. A commonly used abbreviation for carbohydrate is CHO.

Classification of simple carbohydrates by carbon chain length is also helpful (Fig. 4.1):

1. Trioses: 3 carbons. There are only two trioses; D-glyceraldehyde represents the aldose family and dihydroxyacetone represents the ketoses. Each is formed in glycolysis.
2. Tetroses: 4 carbons. These are not often encountered in metabolism. Erythrose is an example of an aldotetrose from the Pentose Phosphate pathway.
3. Pentoses: 5 carbons. D-ribose (RNA, many coenzymes, ATP) and 2-deoxy-D-ribose (DNA) are the most common aldopentoses. Xylulose and ribulose are ketoses from the Pentose Phosphate pathway.
4. Hexoses: 6 carbons. These are among the most common carbohydrates encountered in metabolism. Examples of aldohexoses are D-glucose, D-galactose, and D-mannose. The most common ketohexose is D-fructose.

Classification in terms of sugar composition:

1. Monosaccharides are simple sugars. They cannot be hydrolyzed to simpler structures. All of the above examples are monosaccharides.
2. Disaccharides contain two linked monosaccharides. For example, sucrose (table sugar) contains D-glucose and D-fructose, and lactose (milk sugar) contains D-galactose and D-glucose.
3. Oligosaccharides are short chains of 4 to 10 monosaccharides. Maltodextrins from partial starch digestion contain short chains made up of D-glucose. Glycoproteins contain oligosaccharide chains of various lengths and compositions.
4. Polysaccharides are extensive carbohydrate chains. They may be made of a single monosaccharide. For example, cellulose, starch, and glycogen are D-glucose polymers. Dietary fiber is a complex mixture of polysaccharides. They can contain pentoses, hexoses besides glucose, and derivatives of monosaccharides, for example sugar amines (e.g. D-glucosamine), and sugar acids (e.g. glucuronic and galacturonic acids).

## II. STRUCTURAL ASPECTS

1. Optical isomers: In Figure 4.1, note that carbohydrates possess one or more asymmetric carbons. Glyceraldehyde has two optical isomers (enantiomers). For historic reasons, the absolute configuration of the reference sugar, D-glyceraldehyde, represented as the "R" form, is designated as "D" while the "S" isomer is "L."

   Most of the carbohydrates in mammalian metabolism are D-sugars. In other words, the body metabolizes only D-glucose and D-fructose, not the "L" forms. All "D" sugars are related to the structure of D-glyceraldehyde, the reference point. The asymmetric carbon most distant from the carbonyl carbon of a "D" sugar has the same absolute configuration as D-glyceraldehyde (compare the encircled portions in the Fischer Projections).
2. Epimers are monosaccharides which differ in configuration around a single carbon atom, e.g. D-glucose and D-galactose.
3. Hemiacetal and hemiketal rings: Most monosaccharides containing five or more carbons form hemiacetal and hemiketal rings. If the ring has five carbons and one oxygen, the structure is a pyranose. If it has four carbons and one oxygen, it is a furanose. If the carbon at the ring closure (the anomeric carbon) is not attached to another group (e.g. a hemiacetal), it is classified as a "reducing

sugar." The ring is in equilibrium with a small fraction of the straight chain form, which can react with oxidizing agents (remember oxidizing agents are reduced).

Anomeric carbons are asymmetric. This new asymmetric center, indicated by an asterisk, can have two forms. Regarding its position relative to the ring, "$\alpha$-" indicates the new -OH group points down; "$\beta$-" indicates it points up, shown by the Haworth projection of the ring.

4. *O*-Glycosides form from the reaction of monosaccharides (cyclic hemiacetals or cyclic hemiketals) with an alcohol group (usually from another saccharide). The bond from the anomeric carbon to the -OR group is called an *O*-glycoside bond. The *O*-glycoside bond joins monosaccharides together to form various disaccharides, oligosaccharides, and polysaccharides.

   If the oxygen of the anomeric carbon is in the $\alpha$-configuration, the bond is an $\alpha$-glycosidic bond. (For example, all of the bonds in starch are $\alpha$-glycosidic bonds.) If it is in the $\beta$- configuration, the bond is a $\beta$-glycosidic bond. All links in cellulose are in the $\beta$- configuration. Linkages can occur at different positions on the ring. The carbon closest to the carbonyl carbon is designated as carbon #1. Hence, carbon #1 is the anomeric carbon in aldohexoses and aldopentoses.
5. *N*-Glycosides: If the anomeric carbon of a cyclic hemiacetal or hemiketal reacts with an NH group, instead of an R-OH group, an *N*-glycoside forms, almost always in the $\beta$-configuration.

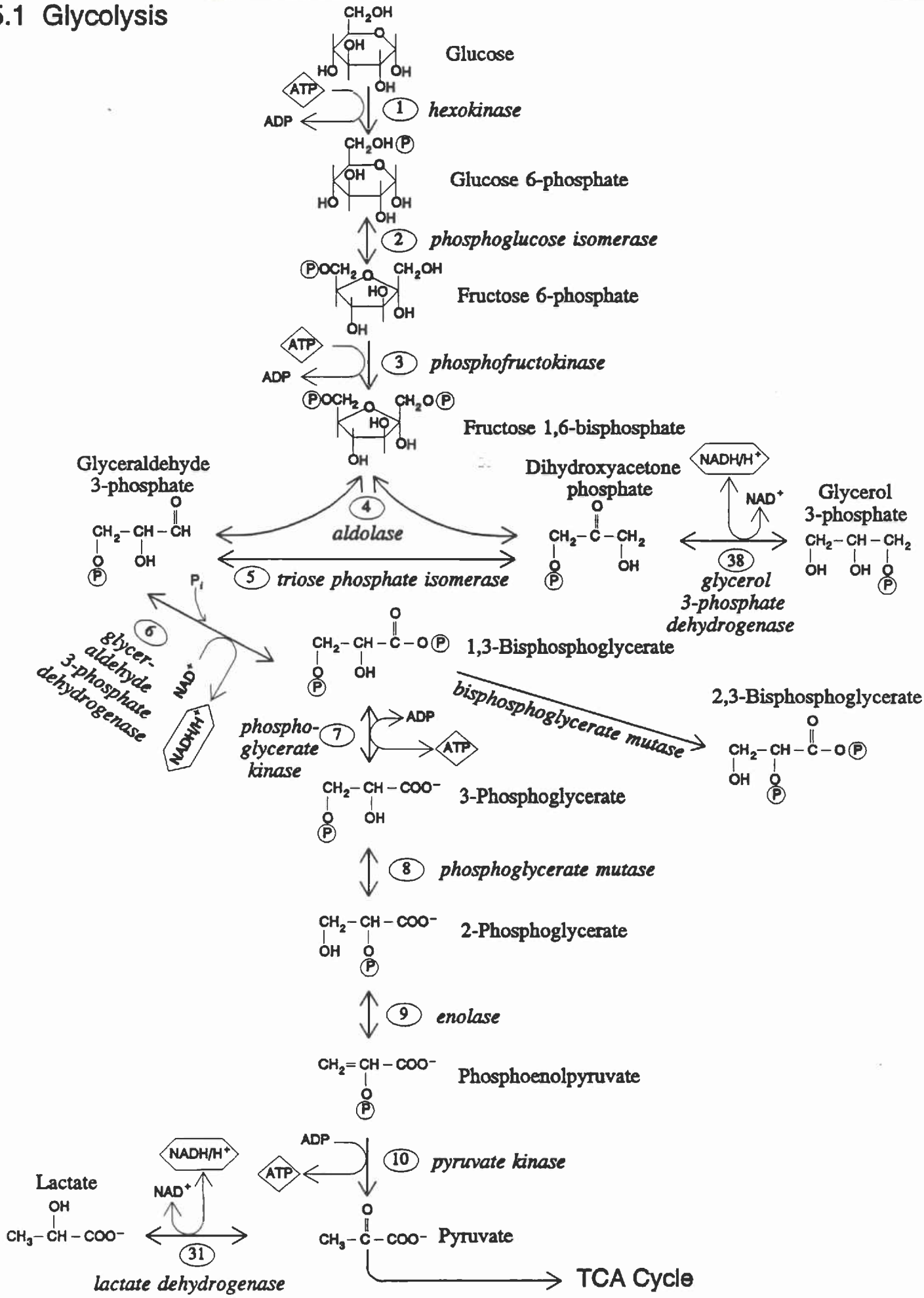
   Nucleic acids (*N*-glycosides) are formed between hemiketals (D-ribose and 2-deoxy-D-ribose) and heterocyclic, aromatic amines (uracil, cytosine, thymine, adenine, and guanine). See Section 19.

## III. DIGESTION

1. Glucose monomers in glycogen and starch are chained together by $\alpha$-1,4 glycosidic bonds. In cellulose, the linkage is $\beta$-1,4. This subtle distinction has profound consequences. Humans cannot digest cellulose because the pancreas does not produce enzymes specific for this glucose polymer linkage.
2. *Amylase*, from saliva and the pancreas, hydrolyzes alternate $\alpha$-1,4 linkages of starch to produce the disaccharide, maltose. Intestinal *maltase* hydrolyses maltose to glucose. Other fragments of starch digestion contain branch points ($\alpha$-1,6 glycosidic linkages). Intestinal *dextrinases* hydrolyze these links to yield glucose.
3. The intestine produces other *disaccharidases*. *Sucrase* hydrolyzes sucrose to glucose and fructose, and *lactase* hydrolyzes lactose to glucose and galactose. Milk intolerance is often due to a deficiency of *lactase*.

# NOTES

# 5.1 Glycolysis



## I. PREPARATORY STEP: Formation of glucose 6-phosphate.

Input: glucose + ATP
Output: glucose 6-phosphate + ADP

LOCATION: Cytoplasm of all cells.

FUNCTION: *Hexokinase* or *glucokinase* phosphorylates glucose in order to enter metabolic pathways. This step is irreversible.

CHARACTERISTICS:

1. Most tissues contain Enzyme 1, ✓*hexokinase*. Its low $K_M$ (0.1mM glucose) indicates that *hexokinase* is fully saturated, even at low blood glucose concentrations. This assures tissues like the brain of efficient glucose phosphorylation for entry into glycolysis. The product, glucose 6-phosphate, inhibits *hexokinase*.
2. ✓*Glucokinase* is localized in the liver. It possesses a much higher $K_M$ (10mM) and is not saturated at usual levels of blood glucose. Therefore, *glucokinase* activity fluctuates with the level of blood glucose. *Glucokinase* effectively removes glucose from the blood after a carbohydrate meal by phosphorylating large amounts of glucose. The product, glucose 6-phosphate, does not inhibit *glucokinase*. The liver can either store excess glucose as glycogen or convert the surplus to fat.

## II. GLYCOLYSIS (Glycolytic pathway)

Input: glucose 6-phosphate + 2 $NAD^+$ + ADP + 2 $P_i$
Output: 2 pyruvate + 2 ATP + 2 NADH + 2 $H^+$ + $H_2O$ (aerobic conditions)
Output: 2 lactate + 2 ATP + 2 $H_2O$ (anaerobic conditions)

LOCATION: Cytoplasm of all cells.

FUNCTION:

1. Though ancient, this pathway is far from obsolete. Glycolysis plays a central role in energy production from carbohydrates in organisms from bacteria to primates. Glucose (6C) forms pyruvate (3C) and ATP without involving $O_2$. In animal tissue, pyruvate is subsequently oxidized by the TCA cycle in the presence of oxygen.
2. Lactate can be formed from pyruvate in muscle when the oxygen supply becomes limiting, e.g. the anaerobic conditions of strenuous exercise.

CHARACTERISTICS:

1. The many glycolytic enzymes function in a linear sequence. Only *phosphofructokinase* and *pyruvate kinase* catalyze irreversible steps. The remaining steps are reversed during glucose synthesis in the liver (gluconeogenesis).
2. Glucose is cleaved to two 3C sugars. Each is processed. Therefore, multiply the products by a factor of 2 for correct stoichiometry.
3. Two intermediates are "high energy" compounds. 1,3-Bisphosphoglycerate and phosphoenolpyruvate yield one ATP each.
4. Oxygen does not participate in the oxidation of glucose in this pathway.

5. $NAD^+$ must be regenerated by reducing pyruvate to lactate, or by the reducing carriers in the Electron Transport Chain (respiration).

STEPS:

1. Isomerization of glucose 6-phosphate: Enzyme 2, *phosphoglucose isomerase*, isomerizes glucose 6-phosphate to fructose 6-phosphate (aldose to ketose).
2. The first committed step: Enzyme 3, ✓*phosphofructokinase (PFK I)*, catalyzes the first irreversible step of glycolysis and is the rate-limiting enzyme. It phosphorylates carbon #1 of fructose 6-phosphate with ATP to yield fructose 1,6-bisphosphate. ATP and citrate inhibit, and AMP activates the enzyme allosterically. In the liver, the most powerful activator is fructose 2,6-bisphosphate, an isomer of fructose 1,6-bisphosphate.
3. Cleavage of hexose bisphosphate to two triose phosphates: Enzyme 4, *aldolase*, cleaves fructose 1,6-bisphosphate (6C) to glyceraldehyde 3-phosphate (3C) and dihydroxyacetone phosphate (3C), phosphate esters of the simplest aldose and ketose.
4. Glyceraldehyde 3-phosphate leads into the following steps: Enzyme 5, *triose phosphate isomerase*, converts dihydroxyacetone phosphate to glyceraldehyde 3-phosphate. Thus, two molecules of glyceraldehyde 3-phosphate flow into subsequent steps.
5. The first example of substrate level phosphorylation: Enzyme 6, ✓*glyceraldehyde 3-phosphate dehydrogenase*, catalyzes the first oxidation of the pathway. $NAD^+$ oxidizes the aldehyde group of glyceraldehyde to a carboxylic acid, producing NADH. Concomitant attachment of $P_i$ creates a high energy phosphate anhydride, 1,3-bisphosphoglycerate.
6. The first ATP synthesis: Enzyme 7, *phosphoglycerate kinase*, uses the energy trapped in the anhydride linkage of 1,3-bisphosphoglycerate to form ATP. These ATP molecules replace the two ATP's consumed by *hexokinase* and *PFK I* to initiate glycolysis.
7. Isomerization: Enzyme 8, *phosphoglycerate mutase*, shifts the phosphate of 3-phosphoglycerate to the carbon #2 of 2-phosphoglycerate.
8. Creation of the second ATP-yielding product: Enzyme 9, *enolase*, removes water from 2-phosphoglycerate to produce phosphoenolpyruvate, the second high energy phosphate of glycolysis.
9. The second ATP synthesis: Enzyme 10, ✓*pyruvate kinase*, forms pyruvate and ATP from phosphoenolpyruvate. This is an irreversible step. *Pyruvate kinase* is inhibited by ATP.

NOTES:

1. Lactate and the Cori Cycle: Glycolysis requires an adequate supply of $NAD^+$. Therefore, NADH must be reoxidized for glycolysis to continue. When the oxygen supply is limited, oxidative phosphorylation cannot adequately regenerate $NAD^+$. Muscle then uses Enzyme 31, *lactate dehydrogenase (LDH)*, to regenerate $NAD^+$ by reducing pyruvate to lactate. Lactate is also the final product of anaerobic glycolysis in tissues lacking mitochondria, such as red blood cells. In the Cori cycle, lactate formed by exercising muscle travels in the bloodstream to the liver. The liver oxidizes lactate to pyruvate via *LDH*, and pyruvate is converted to glucose via gluconeogenesis (Sect. 10).
2. Regulation by glucagon:
   (a) Note, *PFK II* differs from *PFK I*. Elevated glucagon blocks glucose utilization by the liver. It decreases the concentration of the *PFK I* (Enzyme 3) activator, fructose 2,6-bisphosphate. Glucagon inhibits *PFK II*, the enzyme responsible for the synthesis of the 2,6 isomer. Glucagon also activates *fructose 2,6-bisphosphate phosphatase* which hydrolyzes fructose 2,6-bisphosphate.
   (b) Glucagon inhibits *pyruvate kinase* by stimulating a cyclic AMP-driven *protein kinase* cascade, culminating in the phosphorylation (inactivation) of *pyruvate kinase*. On the other hand, *pyruvate kinase* is activated by fructose 1,6-bisphosphate, thereby allowing it to keep up with *PFK I*.
3. Regulation by epinephrine: In the liver, this hormone activates glycogenolysis and inhibits glycolysis (providing glucose for muscle), and in muscle, stimulates glycolysis (generating ATP for contractile activity).

4. Glycerol metabolism: Glycerol is synthesized from dihydroxyacetone phosphate (refer to Sect. 13.II for triacylglycerol formation).
5. Regulation of hemoglobin: In erythrocytes, *bisphosphoglycerate mutase* isomerizes the phosphate group of 1,3-bisphosphoglycerate to form millimolar concentrations of 2,3-bisphosphoglycerate, which decreases the affinity of hemoglobin for oxygen.
6. Entry of fructose: The majority of dietary fructose is metabolized by the liver, using the fructose 1-phosphate pathway. Fructose is phosphorylated by *fructokinase* to fructose 1-phosphate. Next, *fructose 1-phosphate aldolase* splits fructose 1-phosphate into glyceraldehyde and dihydroxyacetone phosphate. Glyceraldehyde enters glycolysis when *triose kinase* phosphorylates it to glyceraldehyde 3-phosphate.

**Fructose** → Fructose 1-phosphate → **Dihydroxyacetone phosphate**
+
Glyceraldehyde → **Glyceraldehyde 3-phosphate**

In the liver, little fructose is phosphorylated to fructose 6-phosphate by *hexokinase* because of its low affinity with *hexokinase* and subsequent competitive inhibition by glucose.

Since adipose tissue contains much more fructose than glucose, there is little competitive inhibition with glucose and most of the fructose will be converted to fructose 6-phosphate by *hexokinase*.

6. Entry of galactose: In four steps, galactose (from dietary lactose) is converted into glucose 6-phosphate.
   (a) Galactose is phosphorylated to galactose 1-phosphate by *galactokinase*.

**Galactose** → Galactose 1-phosphate

   (b) *Galactose 1-phosphate uridyl transferase* adds a uridyl group from UDP-glucose, forming UDP-galactose and glucose 1-phosphate.

Galactose 1-phosphate + UDP-glucose → UDP-galactose + Glucose 1-phosphate

   (c) UDP-galactose is epimerized to UDP-glucose by *UDP-galactose-4-epimerase*. Because of this step, UDP-glucose is not consumed in the conversion of galactose to glucose.

UDP-galactose → UDP-glucose

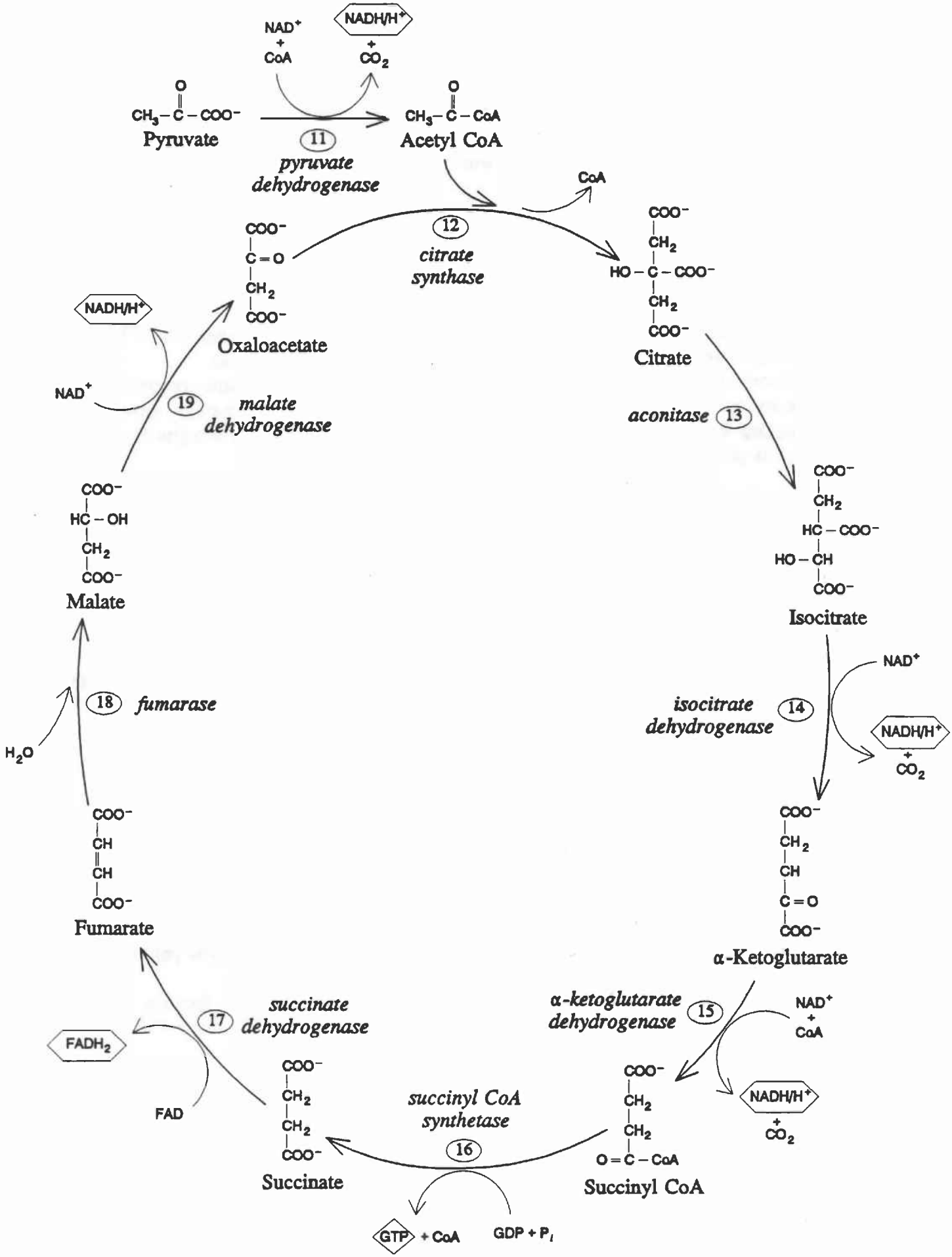
   (d) *Phosphoglucomutase* isomerizes glucose 1-phosphate to glucose 6-phosphate.

Glucose 1-phosphate → **Glucose 6-phosphate**

REVIEW QUESTIONS:

1. Name the products of aerobic glycolysis from glucose.
2. Describe the effects of increasing concentrations of the following on the glycolytic pathway: (a) AMP, (b) citrate, (c) glycogen. Identify the steps which are regulated.
3. Lactic acid is a major end product of glucose metabolism in erythrocytes. Explain why pyruvate production is not favored.
4. Define substrate level phosphorylation.
5. *Hexokinase* is not strictly a glycolytic enzyme. Demonstrate this by listing the possible routes for using glucose 6-phosphate.

# 6.1 Tricarboxylic Acid Cycle (Krebs Cycle)



## Section 6: TRICARBOXYLIC ACID CYCLE (TCA Cycle)
(Also called Krebs Cycle or Citric Acid Cycle)

### I. PYRUVATE DEHYDROGENASE

Input: pyruvate + CoA + $NAD^+$
Output: acetyl CoA + NADH + $H^+$ + $CO_2$

LOCATION: Mitochondrial matrix (soluble fraction of mitochondria).

FUNCTION: The preparatory step for the TCA cycle: formation of Acetyl CoA.

CHARACTERISTICS: Enzyme 11, ✓*pyruvate dehydrogenase (PDH)*, oxidizes pyruvate to acetyl CoA, which is the entry point of the TCA cycle. This step is irreversible: acetyl CoA cannot reform to pyruvate in animals. *PDH* is a multienzyme complex containing three different polypeptides:

1. *Dehydrogenase* removes the carboxyl group of pyruvate as $CO_2$ with a 2C fragment attached to thiamin pyrophosphate. The fragment is oxidized to an acetyl group as it is transferred to a sulfur group of lipoic acid (an endogenous disulfide cofactor bound to the *transacetylase).*
2. *Transacetylase* transfers the acetyl group to the -SH of CoA, forming acetyl CoA.
3. *Lipoyl dehydrogenase* oxidizes lipoic acid back to the disulfide, forming $FADH_2$. $NAD^+$ receives the hydrogens ultimately.

NOTES:

1. Regulation: *Pyruvate dehydrogenase* is inhibited by acetyl CoA, GTP, and NADH. It is also inactivated by phosphorylation by an endogenous *kinase*. The *kinase* is activated by elevated NADH, but not cyclic AMP. The *kinase* is blocked by elevated ADP. Dephosphorylation by an endogenous *phosphatase* reactivates *PDH*.
2. NADH funnels electrons into the ETC and generates three ATP.
3. Vitamins of the B-complex support *PDH*: Niacin, thiamin, riboflavin, and pantothenic acid form the following cofactors: $NAD^+$, thiamin pyrophosphate, FAD, and CoA.

### II. THE TCA CYCLE

Input: acetyl CoA + FAD + 3 $NAD^+$ + GDP + $P_i$ + 2 $H_2O$
Output: CoA + $FADH_2$ + 3 NADH + GTP + 2 $H^+$ + 2 $CO_2$

LOCATION: Mitochondrial matrix (soluble fraction of mitochondria).

FUNCTIONS:

1. The TCA cycle is a key catabolic pathway, working in conjunction with oxidative phosphorylation. This versatile pathway oxidizes acetyl CoA derived from amino acids, glucose, fatty acids, and ketone bodies, to $CO_2$.
2. Oxidations for one turn of the cycle require the transfer of four pairs of electrons (three NADH and a single $FADH_2$). These carriers feed the Electron Transport Chain to generate much of the ATP needed by the body.
3. The TCA cycle provides building blocks for biosynthesis.
4. An input of fumarate, α-ketoglutarate, or succinate can be converted to oxaloacetate for gluconeogenesis.

CHARACTERISTICS: The TCA cycle begins and ends with the same intermediate, oxaloacetate. The cycle does not consume any intermediate carboxylic acids. The input of two carbons as acetyl CoA corresponds to the loss of two carbons as $CO_2$.

STEPS:

1. Formation of citrate: Enzyme 12, ✓*citrate synthase*, joins acetyl CoA with oxaloacetate via an aldol condensation. Citrate is the first tricarboxylic acid of the cycle. *Citrate synthase* is inhibited by elevated ATP, NADH, succinyl CoA (from fourth step of the cycle), and fatty acyl CoA.
2. Isomerization of citrate: Enzyme 13, *aconitase*, isomerizes citrate to isocitrate.
3. First oxidation of the cycle: Enzyme 14, ✓*isocitrate dehydrogenase*, converts isocitrate (6C) to $\alpha$-ketoglutarate (5C) by oxidative decarboxylation. This step produces the first $CO_2$ and the first of three NADH. This is a key regulatory step. *Isocitrate dehydrogenase* is inhibited by elevated ATP and NADH, and activated by elevated ADP.
4. Second oxidation produces succinyl CoA: Enzyme 15, ✓*$\alpha$-ketoglutarate dehydrogenase*, converts $\alpha$-ketoglutarate (5C) to the CoA ester of succinate (4C) yielding the second $CO_2$ and the second NADH of the cycle. It is a multienzyme complex resembling *PDH* and requires the same cofactors: thiamin pyrophosphate, FAD, $NAD^+$, CoA, and lipoic acid. This enzyme is inhibited by elevated ATP, NADH, GTP, and succinyl CoA.
5. Substrate level phosphorylation produces GTP: Enzyme 16, *succinyl CoA synthetase*, generates high energy GTP by splitting the thioester bond of succinyl CoA. (GTP and ATP are interconverted and energetically are equivalent.)
6. Oxidation of succinate: Enzyme 17, *succinate dehydrogenase*, transfers two hydrogen atoms from succinate (oxidation) to produce $FADH_2$ and the unsaturated acid, fumarate. *Succinate dehydrogenase* is linked directly to the Electron Transport Chain via Coenzyme Q (Fig. 7.1).
7. Hydration of fumarate: Enzyme 18, *fumarase*, adds $H_2O$ to the double bond of fumarate to produce the hydroxy acid, malate.
8. Regeneration of oxaloacetate: Enzyme 19, *malate dehydrogenase*, oxidizes malate to oxaloacetate, the starting point of the cycle. It also produces the third and last NADH of the cycle.

NOTES:

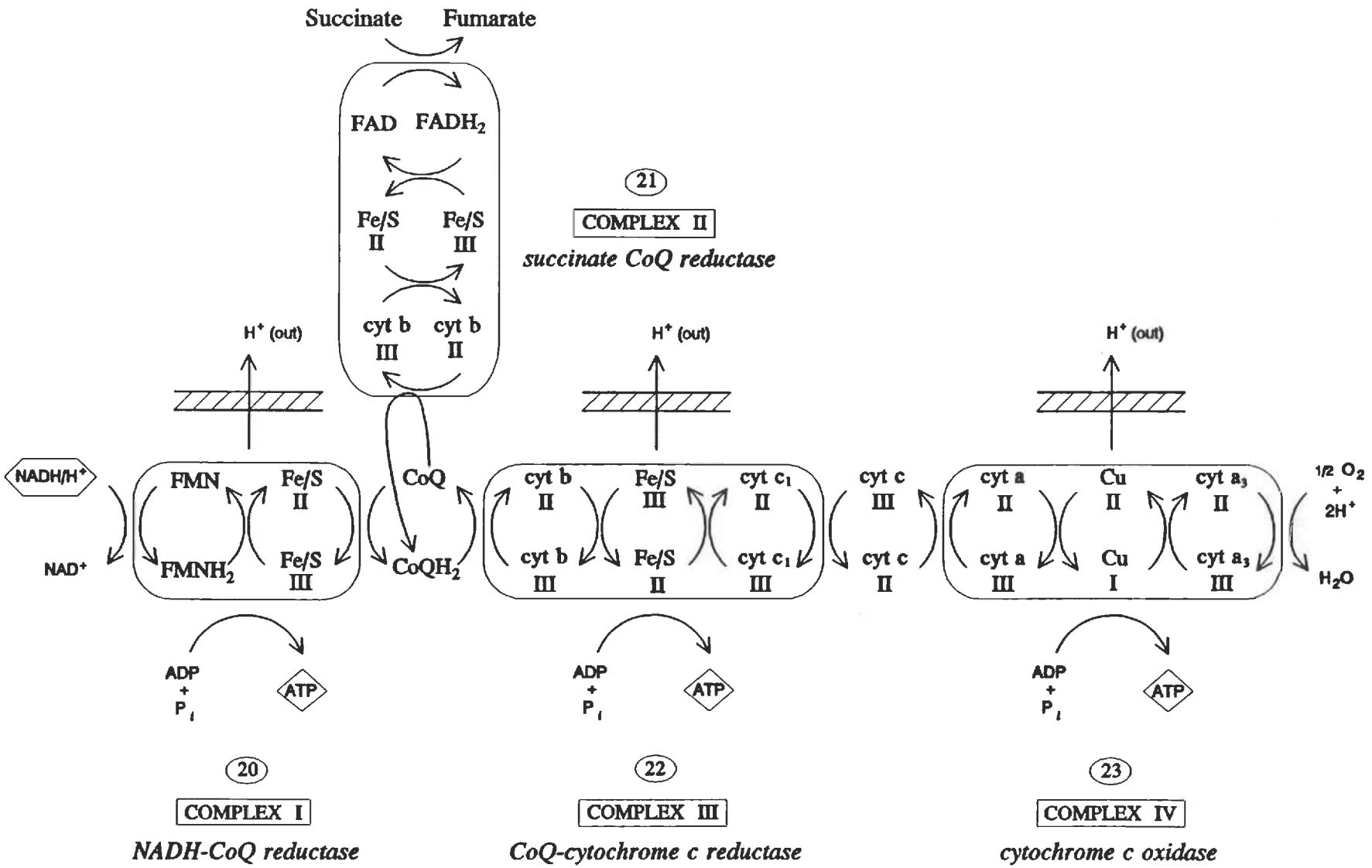
1. Regulation overview: This pathway is carefully regulated. Surplus end products (ATP, NADH, and succinyl CoA), which indicate an energy rich state, generally inhibit the cycle. An energy depleted state, indicated by elevated ADP, activates the cycle. Refer to the individual enzymes.
2. "Fat cannot be converted to carbohydrate." Acetyl CoA cannot flow backward into pyruvate (*PDH* step is irreversible). An output of two $CO_2$ molecules always balances the input of the two carbons from acetyl CoA. Hence, the cycle cannot provide a net synthesis of oxaloacetate from acetyl CoA.
3. Thiamin deficiency can increase blood levels of keto acids upstream from *PDH* and *$\alpha$-ketoglutarate dehydrogenase* because these enzymes require thiamin pyrophosphate. Beriberi is the result of severe thiamin deficiency.
4. Three keto acids form amino acids: Pyruvate forms alanine; oxaloacetate forms aspartate and related amino acids; $\alpha$-ketoglutarate forms glutamate and other 5C amino acids (Sect. 15).
5. Heme: Succinyl CoA is the precursor of porphyrins (heme).
6. Oxaloacetate can be removed to synthesize glucose: Fasting can deplete the cycle of this key intermediate in the liver. On the other hand, oxaloacetate can be synthesized from pyruvate by gluconeogenesis (Sect. 10). This is an example of anaplerotic reactions that fill up the TCA cycle when depleted of intermediates.
7. Oxaloacetate has four possible fates:
   (a) Condensation with acetyl CoA to form citrate.
   (b) Conversion into glucose via the gluconeogenic pathway.
   (c) Conversion into pyruvate.

(d) Transamination to aspartate.

REVIEW QUESTIONS:

1. Thiamin, niacin, riboflavin, and pantothenic acid, members of the B-complex, are required for effective energy production. Locate the steps which require coenzymes formed from these vitamins.
2. Carbon dioxide is the carbon end product of metabolism. Locate the steps carrying out oxidative decarboxylation of substrates ($CO_2$ production).
3. Describe the effects of increased concentrations of the following on the consumption of pyruvate by *PDH* and the TCA cycle: (a) ADP, (b) ATP, (c) NADH, (d) acetyl CoA. Name the enzymes which are regulated.
4. Yeast cells, previously cultivated anaerobically, are exposed to oxygen. It is observed that the rate of glucose utilization declines dramatically. Explain.
5. Acetyl CoA plays a pivotal role in metabolism. Demonstrate by describing the precursors of acetyl CoA Describe the fates of acetyl CoA.

# Electron Transport Chain and Oxidative Phosphorylation



Input: $ADP + P_i + H^+ + electrons + O_2$
Output: $ATP + H_2O$

LOCATION: The inner mitochondrial membrane surface (cristae).

FUNCTION: The Electron Transport Chain (ETC) drives the enzymatic machinery for efficient ATP production. It transfers electrons ultimately to oxygen to produce water.

CHARACTERISTICS:

(1) Electron Transport Chain: The ETC is a linear array of three large multienzyme complexes (I, III, IV), CoQ, cytochrome c, and Complex II (which feeds into CoQ). Each subsequent electron carrier has a higher affinity for electrons, consequently this pathway is unidirectional. Electrons are passed from one carrier to the next, much like a bucket brigade.

(2) Oxidative Phosphorylation: ATP formation is coupled to electron transfer via a proton gradient, according to the chemiosmotic hypothesis. As electrons flow through Complexes I, III, and IV, protons are pumped out of the mitochondrial matrix to establish a proton gradient at the intermembrane space.

Transport of protons back into the mitochondrial matrix creates one ATP per Complex (except for Complex II). *ATP synthetase* contains a proton channel that permits protons to reenter mitochondria. The precise mechanism by which proton flow links $P_i$ and ADP to synthesize ATP is not well understood.

STEPS:

1. Oxidation of NADH: NADH enters the pathway at Step 20, Complex I, or ✓*NADH-CoQ reductase*. The first electron acceptor of Complex I, FMN (flavin mononucleotide, also called riboflavin 5-phosphate), forms $FMNH_2$. The regenerated $NAD^+$ can participate in oxidations of other pathways. Complex I employs iron-sulfur proteins as intermediate carriers. Complex I reduces Coenzyme Q and pumps protons, generating one ATP.
2. Coenzyme Q is a versatile lipid electron carrier: Coenzyme Q (CoQ, $CoQ_{10}$, or ubiquinone) receives electrons from Complex I.

   CoQ also receives electrons from the oxidation of succinate via Step 21, Complex II or *succinate CoQ reductase*. Complex II reduces FAD while oxidizing succinate to fumarate from the TCA cycle. Electrons from the resulting $FADH_2$ pass via iron-sulfur proteins to cytochrome $B_{560}$, then to CoQ to form $CoQH_2$. Figure 7.1 illustrates the relative position of Complex II.
3. $CoQH_2$ reduces the third complex of the ETC: Cytochrome b is the first electron acceptor of Step 22, Complex III or ✓*CoQ-cytochrome c reductase*. Cytochromes are enzymes containing iron as heme. They catalyze electron transfers in all aerobic organisms. Complex III contains several cytochromes. Electrons flow first to cytochrome $b_{562}$, then to a second, cytochrome $b_{566}$, then to an iron sulfur protein, then to cytochrome $c_1$, and ultimately to cytochrome c. Protons are pumped out and one ATP is synthesized at this complex.
4. *Cytochrome c* is a mobile electron carrier: *Cytochrome c*, between Complex III and IV, is a small protein that is loosely membrane bound. It carries electrons from cytochrome $c_1$ of Complex III to the terminal Complex IV.
5. *Cytochrome c oxidase* reduces molecular oxygen: Step 23, Complex IV or ✓*cytochrome c oxidase*, is the last complex of the ETC. It transfers electrons from cytochrome c to $O_2$ to form $H_2O$. Electrons are thought to flow from cytochrome c to cytochrome a, then to copper proteins ($Cu^{2+} \rightarrow Cu^+$), then to cytochrome $a_3$ which interacts directly with oxygen molecules. Protons are pumped out and one ATP is synthesized.

NOTES:

1. Electrons from fatty acid oxidation: $\beta$-oxidation of fatty acids produces $FADH_2$, which also reduces CoQ (Sect. 12.II, Enzyme 40b, *fatty acyl CoA dehydrogenase*).
2. Transport of ATP and NADH:
   (a) The entry of ADP from the cytoplasm is coupled to the exit of ATP.
   (b) Glycerol 3-phosphate shuttles electrons from cytoplasmic NADH into mitochondria in tissues other than the liver and heart. Cytoplasmic *glycerol 3-phosphate dehydrogenase* reduces dihydroxyacetone phosphate using NADH. The product, glycerol 3-phosphate, readily crosses mitochondrial membranes. It is reoxidized to produce mitochondrial NADH.
3. Respiratory control: The term "respiratory control" refers to the fact that fuel oxidation and ATP production are coupled. Thus, electron transfer to $O_2$ by *cytochrome c oxidase* occurs only when ADP is available for ATP synthesis. If $NAD^+$ and FAD are not recycled by the ETC, the TCA cycle, glycolysis, and $\beta$-oxidation, slow down.
4. Uncouplers vs inhibitors:
   (a) Uncouplers of oxidative phosphorylation (dicumarol, 2,4-dinitrophenol, bilirubin, free fatty acids) interrupt the proton gradient. This effectively blocks ATP production, while allowing fuels to be oxidized. Surplus energy is released as heat, rather than being stored as ATP.
   (b) Physiologic uncoupling of brown fat mitochondria by free fatty acids in the newborn and marine mammals creates heat (non-shivering thermogenesis).
   (c) Inhibitors of oxidative phosphorylation (cyanide, antimycin A) are poisons. They interrupt the flow of electrons and thus block both fuel oxidation and ATP formation.
5. Energy yield from glucose: For each glucose consumed, an equivalent of 36 ATP are produced via oxidative phosphorylation (see chart below). Thus, aerobic ATP production is much more efficient than anaerobic ATP production of glycolysis (2 ATP per glucose). All cells containing mitochondria extensively use the TCA cycle, the ETC, and oxidative phosphorylation, for energy production.

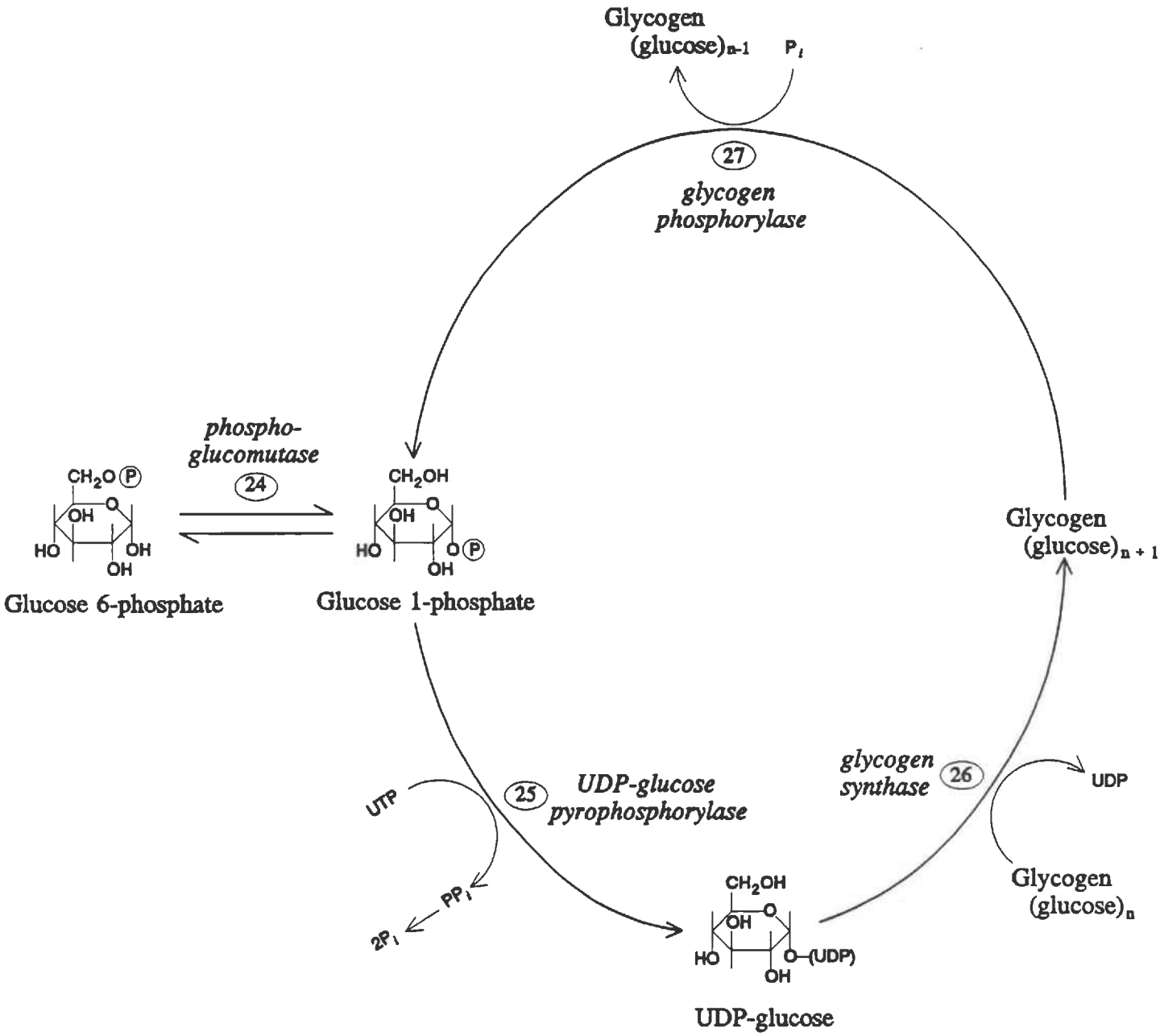
**ATP yield per glucose molecule**

| Step | ATP | NADH | $FADH_2$ |
|---|---|---|---|
| Glycolysis | 2 | 2 | |
| Pyruvate dehydrogenase | | 2 | |
| TCA cycle: 2 GTP = 2 ATP | 2 | 6 | 2 |
| Cytoplasm/mitochondria transport | -2 | | |
| Oxidative phosphorylation:<br>10 NADH = 30 ATP<br>2 $FADH_2$ = 4 ATP | <br>30<br>4 | | |
| Net ATP gain | 36 | | |

REVIEW QUESTIONS:

1. Cyanide inhibits *cytochrome c oxidase*. Predict the effect on oxidative phosphorylation and the TCA cycle. Identify the steps that are regulated by the accumulation of NADH.
2. How many ATP's are generated from NADH and $FADH_2$? What is the maximum number of ATP's generated by the complete oxidation of pyruvate; acetyl CoA.
3. Thyroxine can uncouple oxidative phosphorylation. Explain the effect of uncoupling on electron transport and upon ATP formation.
4. Name the ultimate electron acceptor of respiration.

NOTES


Glycogen (glucose)n-1
Pi
27
glycogen phosphorylase
phospho-glucomutase
24
Glucose 6-phosphate
Glucose 1-phosphate
Glycogen (glucose)n + 1
UTP
25
UDP-glucose pyrophosphorylase
PPi
2Pi
glycogen synthase
26
UDP
Glycogen (glucose)n
UDP-glucose

## I. GLYCOGEN SYNTHESIS

Input: glucose 1-phosphate + UTP + glycogen $(glucose)_n$
Output: glycogen $(glucose)_{n+1}$ + UDP + $PP_i$ (to 2 $P_i$)

LOCATION: Most tissues can make some glycogen. However, major reserves are the liver and skeletal muscle.

FUNCTION: Glycogen is the storage form of glucose in animals. It is synthesized when the cell has a surplus of glucose. In muscle, glycogen supplies glucose for energy production. In the liver, glucose is taken up and stored as glycogen when blood sugar rises after a meal. Glycogen is degraded to help maintain blood glucose levels between meals.

CHARACTERISTICS: Glycogen biosynthesis and degradation are separate pathways. The polymerization of glucose requires energy in the form of UTP. UTP is energetically equivalent to ATP.

STEPS:

1. Entry: The entry point is glucose 1-phosphate, created by Enzyme 24, *phosphoglucomutase*, from glucose 6-phosphate. This *isomerase* reaction is readily reversible and is used to convert glucose 1-phosphate back to glucose 6-phosphate during glycogen breakdown.
2. Glucose activation: Glucose is activated, prior to polymerization, as UDP-glucose, formed by Enzyme 25, *UDP-glucose pyrophosphorylase*. This enzyme attaches the uridine monophosphate portion of UTP to the phosphate of glucose 1-phosphate. Rapid hydrolysis of pyrophosphate ($PP_i$), the other product, prevents the reverse reaction.
3. Glycogen elongation: Enzyme 26, ✓*glycogen synthase*, adds glucose to a pre-existing glycogen chain. It attaches the carbon #1 of incoming glucose to the 4-position (non-reducing end) of a terminal glucose residue of a glycogen chain. The new glycosidic bond is $\alpha$-1,4.

NOTES:

1. Glycogen structure:
   (a) The highly branched, "bushy" structure of glycogen is created by a *"branching" enzyme*. The enzyme transfers a block of 5 - 8 glucosyl residues from a non-reducing end of a growing glycogen chain, to the 6-position of a glucosyl residue further back in the chain. The branch point glycosidic bonds are $\alpha$-1,6.
   (b) Branching increases glycogen solubility and creates multiple growing points. This accelerates the rate at which glycogen can be degraded through the simultaneous release of glucose from each branch.
2. Hormones regulate glycogen synthesis and breakdown: (See Sect. 8.III, for the regulation of glycogen metabolism).

## II. GLYCOGEN DEGRADATION (GLYCOGENOLYSIS)

Input: glycogen $(glucose)_n$ + orthophosphate ($P_i$)
Output: glycogen $(glucose)_{n-1}$ + glucose 1-phosphate

FUNCTION: Glycogen degradation yields glucose 1-phosphate, which can enter glycolysis for energy production (muscle), or can be hydrolyzed to glucose (liver) and released into the circulation.

CHARACTERISTICS: The $\alpha$-1,4 glycosidic bonds of glycogen are cleaved by phosphorolysis, to form a glucose phosphate ester.

STEPS:

1. Glycogenolysis: Glycogen is degraded by Enzyme 27, ✓*glycogen phosphorylase*. *Glycogen phosphorylase a* is the active (+) form. It cleaves $\alpha$-1,4 linkages of non-reducing ends of glycogen using orthophosphate, rather than water. The product is glucose 1-phosphate.
2. The fate of glucose 1-phosphate: Enzyme 24, *phosphoglucomutase*, converts glucose 1-phosphate to glucose 6-phosphate. Glucose 6-phosphate can enter the glycolytic pathway, or it can be hydrolyzed in the liver by Enzyme 35, *glucose 6-phosphatase*, to glucose, which provides energy for the nervous system (Sect. 10). *Glucose 6-phosphatase* is absent in the brain and muscle, and since phosphorylated glucose can't easily diffuse out of cells, it is retained and used for the generation of ATP.

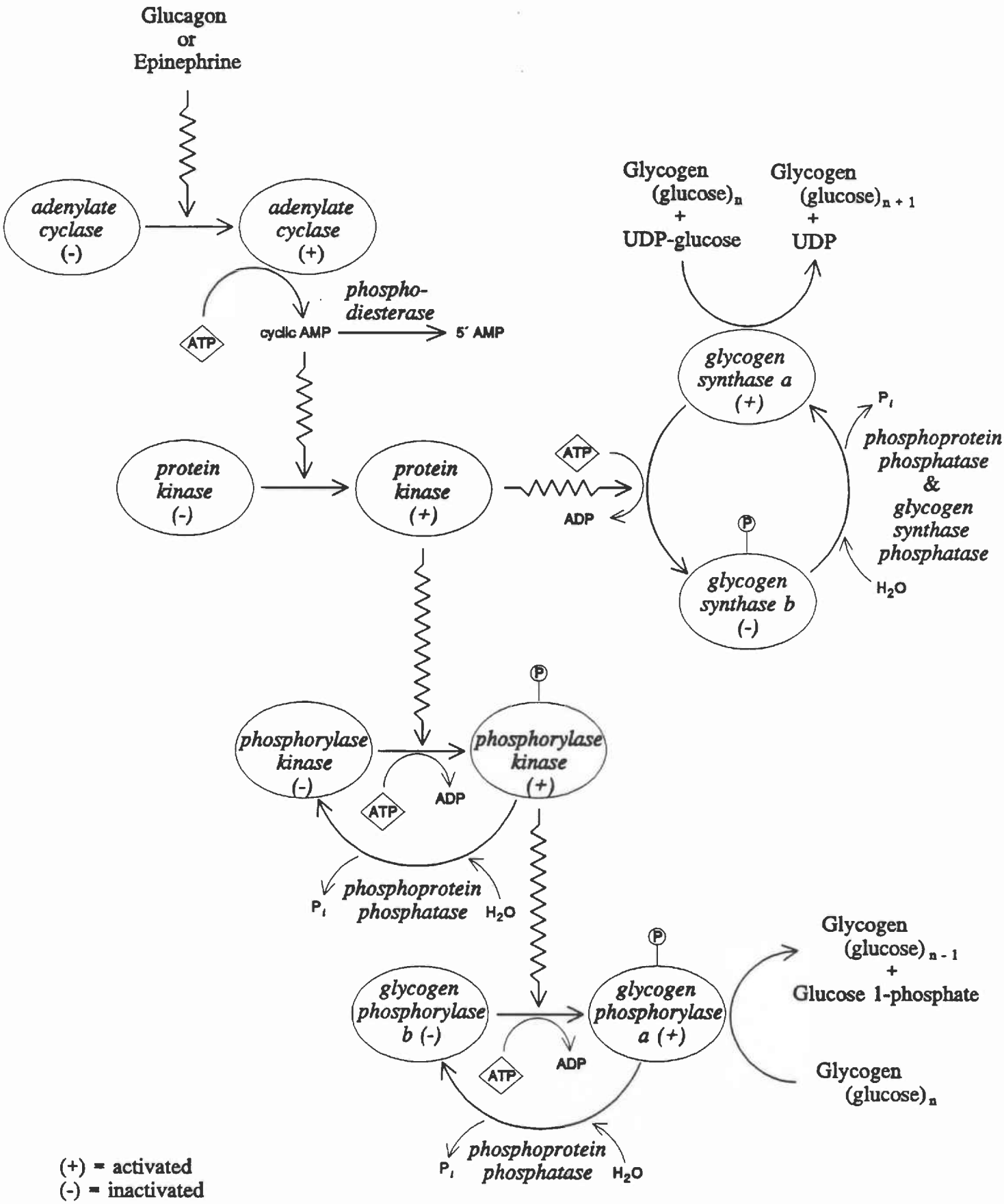
NOTES:

1. Glucose is efficiently stored as glycogen: The cost of storing a single molecule of glucose as glycogen is one high energy phosphate bond. By using phosphate to cleave glycogen, 90% of the resulting glucose molecules are phosphorylated without the participation of *hexokinase* and ATP.
2. Removal of branch points: *Glycogen phosphorylase* cannot clip glycogen chains any closer than four glucose units from a branch point. A second enzyme, *"debranching enzyme,"* degrades the $\alpha$-1,6 branch points. Elimination of $\alpha$-1,6 branch points converts the branched structure into a linear one and allows further glycogen degradation by *glycogen phosphorylase*.
3. Regulation: Glycogenolysis is activated by glucagon and epinephrine, which initiate a cyclic AMP/*protein kinase* cascade (Fig. 8.2). Glycogenolysis is blocked by insulin.
4. Pyridoxal 5´-phosphate (PLP), a derivative of pyridoxine (vitamin $B_6$), is required by *glycogen phosphorylase* in the phosphorolysis of glycogen.

GLYCOGEN METABOLISM continues with Sect. 8.III, following Figure 8.2.

# NOTES

# 8.2 Regulation of Glycogen Degradation



## III. REGULATION OF GLYCOGEN METABOLISM

Because of the complexity of glycogen regulation, for clarity we use (+) for activated and (-) for inactivated enzymes.

**A. GLYCOGEN DEGRADATION:** Glucagon and epinephrine stimulate glycogen breakdown (glycogenolysis). Glucagon acts primarily on the liver. This organ releases glucose to maintain blood glucose levels. Glucagon blocks glycolysis and stimulates gluconeogenesis as well. In skeletal muscle, epinephrine blocks glycogen synthesis and promotes glycogen degradation to provide glucose 6-phosphate for muscle energy.

CHARACTERISTICS: The cyclic AMP cascade is a mechanism by which enzymes activate other enzymes sequentially. This elaborate system of enzyme regulation features a very rapid amplification of the initial hormonal signal. Glycogen degradation and synthesis are regulated reciprocally. The two pathways do not compete.

STEPS:

1. Hormone binding: Both glucagon and epinephrine bind to cell surfaces at specific receptor sites. This perturbs proteins on the inner plasma membrane surface, leading to the activation of membrane-bound *adenylate cyclase*.
2. *Adenylate cyclase*: Cyclic 3´,5´-adenosine monophosphate (cyclic AMP or cAMP) is formed by hormone activated ✓*adenylate cyclase* from ATP. Cyclic AMP is an intracellular messenger.
3. *Protein kinase* activation: Increased cyclic AMP activates *protein kinase* by binding to its regulatory subunit, releasing the active catalytic subunit.
4. Activation of *phosphorylase kinase*: *Protein kinase* covalently modifies target proteins by transferring a phosphate group from ATP. In the activation of glycogenolysis, the general *protein kinase* activates *phosphorylase kinase (-)* by phosphorylation.
5. Activation of *glycogen phosphorylase* Second protein phosphorylation: Activated ✓*phosphorylase kinase (+)* next phosphorylates inactive *glycogen phosphorylase b (-)*, converting it to the active form, *glycogen phosphorylase a (+)*. *Glycogen phosphorylase a (+)* then degrades glycogen to glucose 1-phosphate.
6. Additional mechanisms for *glycogen phosphorylase* activation:
   (a) In muscle, calcium/calmodulin can activate *phosphorylase kinase (-)* without the intervention of cyclic AMP.
   (b) *Glycogen phosphorylase b (-)* can be activated by high levels of AMP allosterically, and is inhibited by ATP and glucose 6-phosphate. Elevated AMP occurs during strenuous exertion.

**B. INHIBITION OF GLYCOGEN DEGRADATION:** *Phosphoprotein phosphatase* inactivates both *phosphorylase kinase (+)* and *glycogen phosphorylase a (+)* by hydrolyzing the esterified phosphate groups. *Glycogen phosphorylase's* susceptibility to *phosphoprotein phosphatase* is a function of glucose concentration.

*Glycogen phosphorylase a (+)* is the liver's glucose sensor. How does this work? (1) *Glycogen phosphorylase a (+)* binds *phosphoprotein phosphatase*. (2) With high glucose concentrations, glucose binds to *glycogen phosphorylase a (+)*. (3) This repetitive change alters conformation of *glycogen phosphorylase a (+)* and exposes the phosphate bonds to *phosphoprotein phosphatase* for inactivation. (4) Dephosphorylation then frees *phosphoprotein phosphatase* from *glycogen phosphorylase b (-)*, allowing it to attack other susceptible phosphoproteins, such as *glycogen synthase* (see below).

Glycogen degradation can also be inhibited by *phosphodiesterase*. This enzyme hydrolyzes cyclic AMP

to form AMP. *Phosphodiesterase* is inhibited by methylxanthines like caffeine and theophylline.

**C. ACTIVATION OF GLYCOGEN SYNTHESIS**: This process is quite complex. Insulin increases the capacity to synthesize glycogen. The mechanisms by which insulin stimulate protein phosphorylation and dephosphorylation are unclear. *Phosphoprotein phosphatase* activates *glycogen synthase b (-)* by hydrolyzing its phosphate bonds. *Phosphoprotein phosphatase* can also act indirectly in muscle. There, *glycogen phosphorylase a (+)* inhibits *glycogen synthase phosphatase*, which can specifically activate *glycogen synthase b (-)* by removing its phosphate groups. *Phosphoprotein phosphatase* removes such a phosphorylase-induced inhibition of *glycogen synthase phosphatase*.

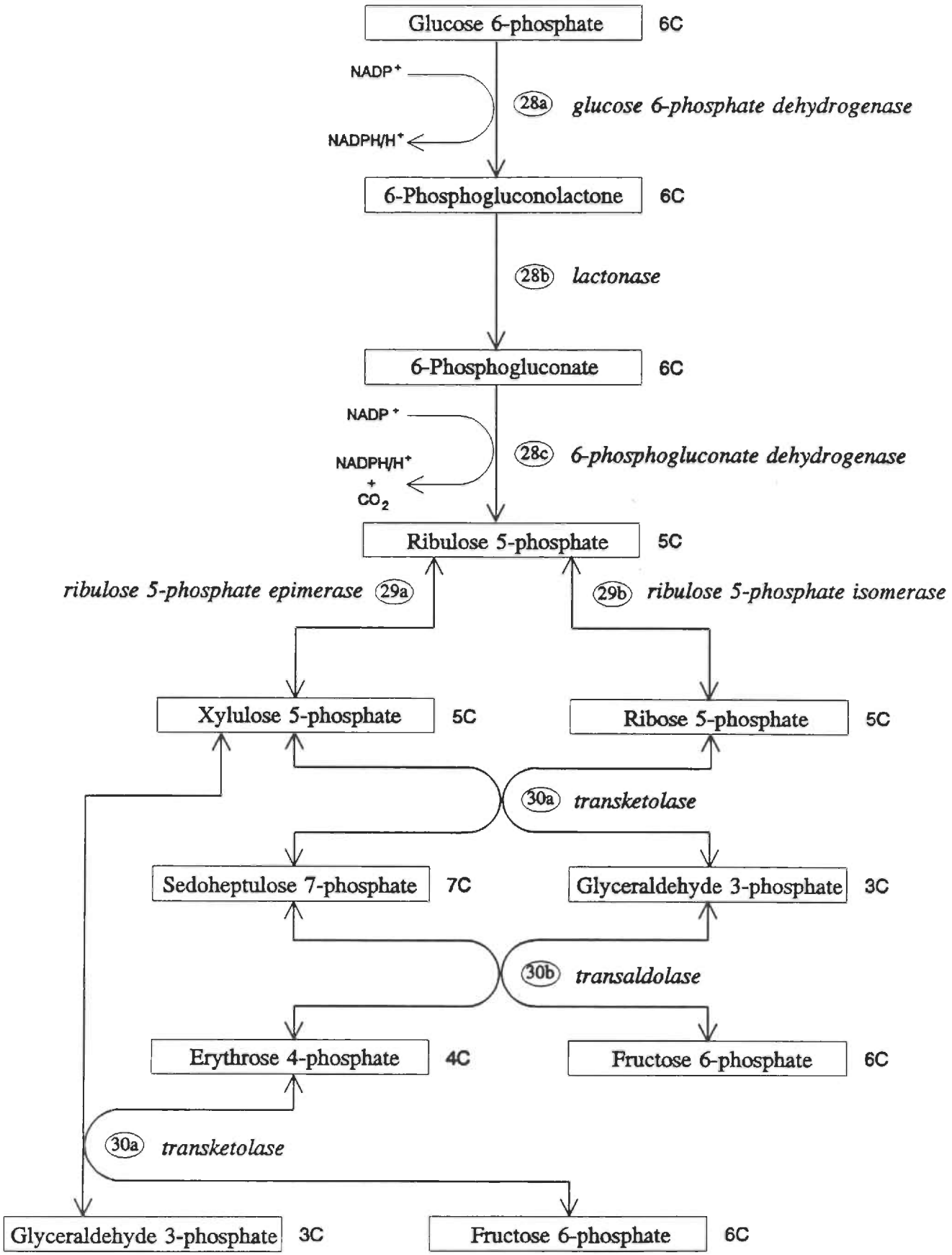
**D. INHIBITION OF GLYCOGEN SYNTHESIS** by epinephrine and glucagon: After the activation of *adenylate cyclase* and the accumulation of cyclic AMP, cyclic AMP-dependent *protein kinase* and *phosphorylase kinase* phosphorylate active (non-phosphorylated) *glycogen synthase a (+)*. The resulting inactive (phosphorylated) enzyme is *glycogen synthase b (-)*. *Glycogen synthase a (+)* in muscle is activated by glucose 6-phosphate.

REVIEW QUESTIONS:

1. Write the reaction sequence (a) for converting glucose to glycogen, and (b) for converting glycogen to glucose 6-phosphate. Identify the steps catalyzed by *glycogen phosphorylase* and *glycogen synthase*.
2. Demonstrate that glycogen breakdown is "cost effective" (compared to *hexokinase*) in forming glucose 6-phosphate.
3. Describe the effects of elevated levels of the following on glycogen degradation: (a) calcium ions in skeletal muscle, (b) glucagon, (c) glucose.
4. Describe the effects of elevated levels of the following on *glycogen synthase*: (a) cAMP-dependent *protein kinase*, (b) activated *phosphoprotein phosphatase*, (c) blood glucose.

# NOTES

# 9.1 Pentose Phosphate Pathway



## Section 9: PENTOSE PHOSPHATE PATHWAY (Hexose Monophosphate Shunt)

Input: glucose 6-phosphate + 2 $NADP^+$
Output: ribose 5-phosphate + $CO_2$ + 2 NADPH + 2 $H^+$

LOCATION: The Pentose Phosphate pathway is cytoplasmic. This pathway is especially important in the adrenal cortex (steroid synthesis), and in mammary glands, liver, and adipose, where fatty acid synthesis is prominent.

FUNCTIONS:
1. Supplies ribose 5-phosphate for nucleotide, RNA, and DNA synthesis.
2. Supplies reducing equivalents for biosynthesis via the electron carrier, NADPH.
3. Provides an alternative pathway for oxidizing glucose 6-phosphate completely to $CO_2$ when more NADPH, than ribose 5-phosphate, is required.
4. Provides a way for using dietary pentoses.

CHARACTERISTICS: Steps 1 and 3 represent irreversible oxidations with NADP, in which carbon #1 is lost as $CO_2$. ATP is neither produced nor consumed. The flow of intermediates through subsequent steps is not fixed, rather supply and demand direct the flow and direction of the pathway. It can produce ribose 5-phosphate. Alternatively, the pathway offers a route for converting pentoses to glyceraldehyde 3-phosphate and fructose 6-phosphate.

STEPS:
1. Oxidation of glucose 6-phosphate: Enzyme 28a, ✓*glucose 6-phosphate dehydrogenase*, oxidizes glucose 6-phosphate to a cyclic intermediate (6-phosphogluconolactone), with $NADP^+$. This first committed step is regulated: a high NADPH to $NADP^+$ ratio inhibits the enzyme. Oxidized glutathione (see Note 2.b) partially reverses this inhibition.
2. 6-Phosphogluconolactone is hydrolyzed: Enzyme 28b, *lactonase*, hydrolyzes the lactone to the sugar acid, 6-phosphogluconate.
3. Oxidative decarboxylation: Enzyme 28c, *6-phosphogluconate dehydrogenase*, oxidizes the gluconate to the first pentose of the pathway, ribulose 5-phosphate. Again, $NADP^+$ is the required cofactor.

Non-oxidative stage of the pathway involves steps 4-7:

4. Ribulose 5-phosphate is isomerized: Ribulose 5-phosphate is isomerized by two different enzymes to either another aldopentose or a ketopentose. Enzyme 29a, *ribulose 5-phosphate epimerase*, produces xylulose 5-phosphate (ketose). Enzyme 29b, *ribulose 5-phosphate isomerase*, produces ribose 5-phosphate (aldose).
5. Ribose and xylulose phosphates are joined: Enzyme 30a, *transketolase*, combines ribose 5-phosphate and xylulose 5-phosphate to form a novel 7C sugar, sedoheptulose 7-phosphate, plus glyceraldehyde 3-phosphate.
6. Enzyme 30b, *transaldolase*, combines the 7C sugar with the triose phosphate to form the 4C sugar, erythrose 4-phosphate, and fructose 6-phosphate. Fructose 6-phosphate can enter glycolysis or it can be converted back to glucose 6-phosphate for a repetition of the steps.
7. *Transketolase* combines the 4C sugar-phosphate (erythrose 4-phosphate) with one of the 5C sugars (xylulose 5-phosphate). The rearrangement yields glyceraldehyde 3-phosphate and a second molecule of fructose 6-phosphate.

NOTES:

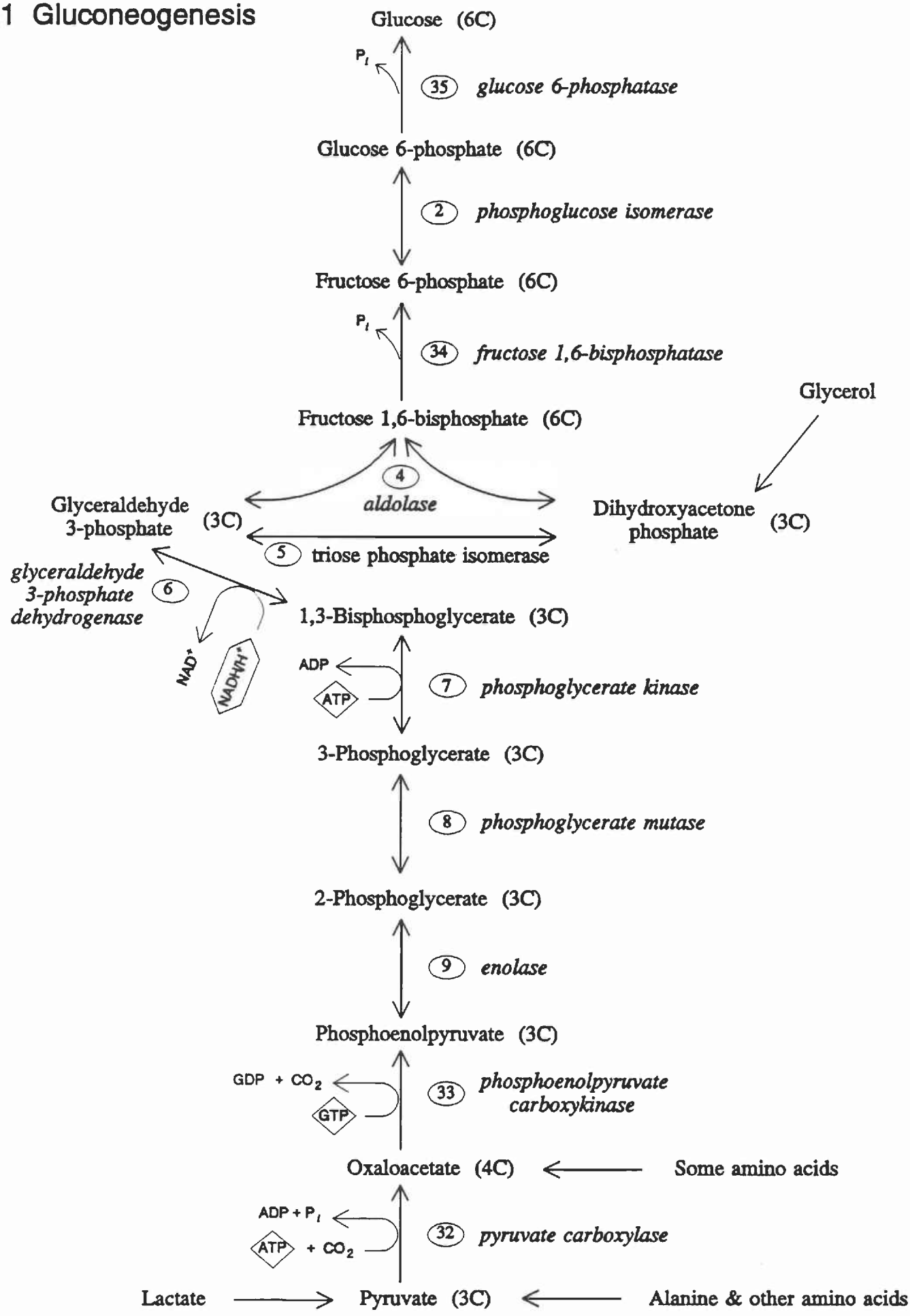
1. Cofactor requirements: *Transketolase* requires thiamin pyrophosphate as the cofactor. The *dehydrogenases* require $NADP^+$.
2. Uses of NADPH:
   (a) NADPH is required to synthesize long chain fatty acids, cholesterol, and steroid hormones.
   (b) It also is used to maintain the sulfhydryl antioxidant pool of the cell. The tripeptide, glutathione (GSH), is kept in the reduced state by *glutathione reductase*, which requires NADPH. Glutathione protects the cell against free radical damage. If the supply of NADPH is inadequate, as in *glucose 6-phosphate dehydrogenase* deficiency, free radical damage to cell membranes can lead to hemolytic anemia.
   (c) NADPH is the cofactor of mixed function *oxidases* (microsomal cytochrome $P_{450}$ monooxygenase systems). In the liver, these enzymes hydroxylate compounds such as aromatic compounds, steroids, and drugs, prior to their conjugation and excretion.

REVIEW QUESTIONS:

1. List the oxidative products of the Pentose Phosphate pathway.
2. List the possible non-oxidative products.
3. Describe major uses of NADPH.
4. Identify the rate limiting step.

# NOTES

# 10.1 Gluconeogenesis



Input: 2 pyruvate + 2 GTP + 4 ATP + 2 NADH + 6 $H_2O$
Output: glucose + 2 GDP + 4 ADP + 6 $P_i$ + 2 $NAD^+$ + 2 $H^+$

LOCATION: Predominantly a liver pathway. The first enzyme is mitochondrial. The remaining enzymes are cytoplasmic.

FUNCTION: Converts non-carbohydrate compounds to blood glucose. The first enzyme can also synthesize oxaloacetate from pyruvate to replenish TCA cycle intermediates. Any intermediate which can be converted to pyruvate or oxaloacetate, can form glucose. Thus, glucogenic precursors include lactate (Cori cycle), glycerol (fat hydrolysis), and dicarboxylic acids from glucogenic amino acids (amino acid catabolism).

CHARACTERISTICS: Glucose synthesis is not simply the reversal of glucose breakdown. Four unique enzymes bypass the irreversible reactions of glycolysis. The remaining steps use glycolytic enzymes.

STEPS:

1. Formation of phosphoenolpyruvate (PEP) from pyruvate: Gluconeogenesis requires two enzymes to go up the energy hill to PEP. Enzyme 32, ✓*pyruvate carboxylase*, attaches a carboxyl group to 3C pyruvate to form 4C oxaloacetate. This enzyme is activated by acetyl CoA. Next, oxaloacetate is indirectly shuttled to the cytoplasm. There, Enzyme 33, ✓*phosphoenolpyruvate carboxykinase*, uses GTP for a decarboxylation and a phosphorylation. Elevated ADP inhibits both enzymes.
2. From PEP to fructose 1,6-bisphosphate: Reversible glycolytic reactions return PEP to fructose 1,6-bisphosphate (Enzymes 9,8,7,6,5,4).
3. Conversion of fructose 1,6-bisphosphate to fructose 6-phosphate. Enzyme 34, ✓*fructose 1,6-bisphosphatase*, hydrolyzes the bisphosphate ester to fructose 6-phosphate. This step is inhibited by AMP (indicative of energy depleted state) and fructose 2,6-bisphosphate, and is activated with high citrate. Recall that the opposite is true for *phosphofructokinase*, *PFK I* (Sect. 5).
4. From fructose 6-phosphate to glucose 6-phosphate: This is a reversal of Enzyme 2, *phosphoglucose isomerase*, of glycolysis.
5. Release of glucose: The last uniquely gluconeogenic enzyme, Enzyme 35, ✓*glucose 6-phosphatase*, hydrolyzes glucose 6-phosphate. This enzyme bypasses *glucokinase* of liver. Muscle cannot convert glucose 6-phosphate to glucose because it lacks *glucose 6-phosphatase*.

NOTES:

1. Gluconeogenesis requires biotin: Enzymes that carry out carboxylations, including *pyruvate carboxylase*, require biotin as the cofactor to transfer $CO_2$.
2. Regulation: (a) Glycolysis and gluconeogenesis are regulated reciprocally. Activators of *PFK I* inhibit *fructose 1,6-bisphosphatase* and vice versa. (b) *Pyruvate kinase* is activated by fructose 1,6-bisphosphate and inhibited by ATP. (c) *Pyruvate carboxylase* is activated by acetyl CoA and inhibited by ADP. (d) Glucagon decreases gluconeogenesis and activates glycolysis. This hormone activates a cyclic AMP cascade leading to increased synthesis of fructose 2,6-bisphosphate, which inhibits *fructose 1,6-bisphosphatase*, while activating *phosphofructokinase*, of glycolysis (Sect. 5).
3. Gluconeogenesis consumes six high energy bonds, four more than produced by glycolysis. The difference is the input of energy required to make the pyruvate → phosphoenolpyruvate conversion energetically possible.

REVIEW QUESTIONS:

1. List typical gluconeogenic substrates. Where do these originate?
2. List the steps unique to gluconeogenesis.
3. Describe conditions which favor gluconeogenesis.
4. Describe the effects of elevated levels of the following on gluconeogenesis: (a) acetyl CoA, (b) ADP, (c) fructose 2,6-bisphosphate.

# NOTES

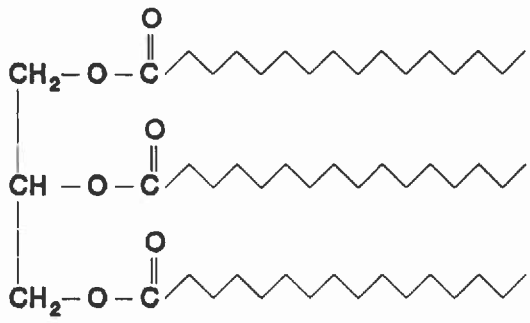
# 11.1 Lipid Highlights

## Common Saturated Fatty Acids

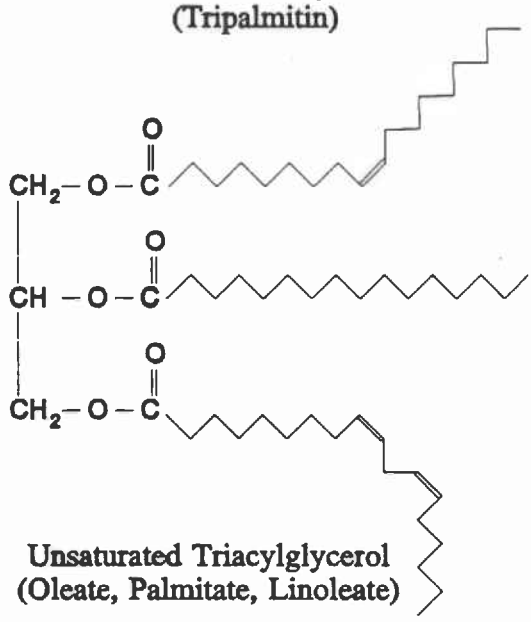
| | | |
|---|---|---|
| C16 | Palmitate | $CH_3(CH_2)_{14}COO^-$ |
| C18 | Stearate | $CH_3(CH_2)_{16}COO^-$ |
| C20 | Arachidate | $CH_3(CH_2)_{18}COO^-$ |

## Common Unsaturated Fatty Acids

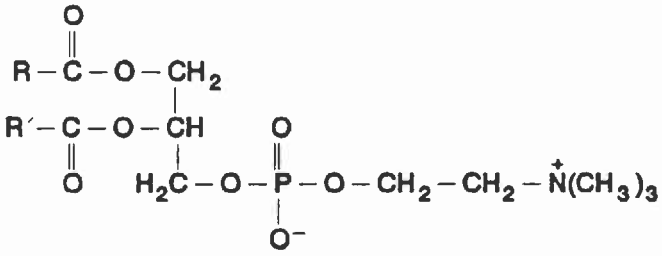
| | | |
|---|---|---|
| C16 | Palmitoleate | $CH_3(CH_2)_5CH=CH(CH_2)_7COO^-$ |
| C18 | Oleate | $CH_3(CH_2)_7CH=CH(CH_2)_7COO^-$ |
| C18 | Linoleate | $CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COO^-$ |
| C18 | Linolenate | $CH_3CH_2(CH=CHCH_2)_3(CH_2)_6COO^-$ |
| C20 | Arachidonate | $CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COO^-$ |



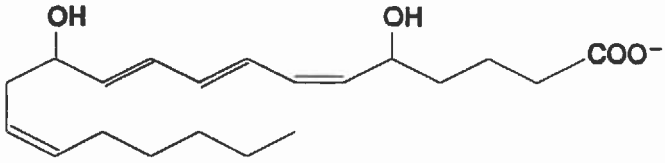
Saturated Triacylglycerol
(Tripalmitin)



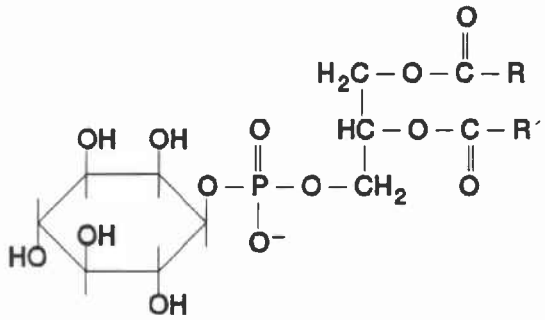
Unsaturated Triacylglycerol
(Oleate, Palmitate, Linoleate)



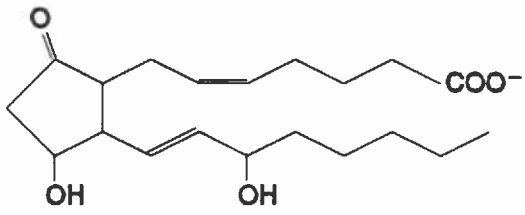
Phosphatidylcholine



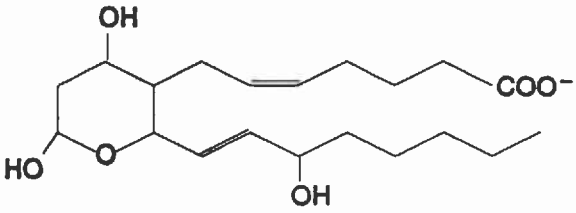
Leukotriene $B_4$



Phosphatidylinositol



Prostaglandin $PGE_2$



Thromboxane $B_2$

Lipids represent a broad class of compounds. They can be defined operationally as organic compounds which are generally insoluble in water (hydrophobic) and soluble in non-polar solvents (lipophilic). Three important classes are triacylglycerols (triglycerides), phospholipids, and cholesterol and its derivatives.

## I. FATTY ACIDS

STRUCTURE:

1. Saturated fatty acids (SFA): The most common saturated fatty acids are palmitate (16C) and stearate (18C). Short chain fatty acids are not esterified as triacylglycerols.
2. Monounsaturated fatty acids (MUFA): Fatty acids with one double bond are classified as monounsaturated. Most naturally occurring MUFA's contain *cis* double bonds. The most common C18 monounsaturated fatty acid in the body is oleate.
3. Polyunsaturated fatty acids (PUFA): The two most common 18C polyunsaturated fatty acids are linoleate (double bonds beginning at C9 and C12) and linolenate (double bonds at C9, C12, C15). Fatty acids are also categorized by counting from the terminal methyl carbon of the chain (the omega carbon) to the first double bond. Thus, linoleate belongs to the "Omega-6" family of fatty acids, in which the first double bond occurs at the $6^{th}$ carbon from the methyl-terminus. Linolenate belongs to the "Omega-3" family. These two families are not metabolically interchangeable.
4. Essential fatty acids (EFA): The body cannot form double bonds in fatty acids beyond carbon #9. Other polyunsaturated fatty acids can be synthesized from linoleate and linolenate. Consequently, these two polyunsaturated fatty acids are essential in the diet.

## II. TRIACYLGLYCEROLS

STRUCTURE: Triacylglycerols (fats and oils) consist of the polyhydroxy alcohol, glycerol, esterified to three long chain fatty acids. One or more of these fatty acids can be unsaturated. Simple triacylglycerols contain a single type of fatty acid. The more common mixed triacylglycerols contain different types of fatty acid residues.

FUNCTIONS:

1. Energy source: Triacylglycerols stored in adipose tissue comprise the major energy reserves of the body. They yield 9 kcal/g upon oxidation, more than twice the caloric yield of carbohydrates and protein.
2. Digestion: Dietary triacylglycerols are digested by *pancreatic lipase* (Fig. 1.1, step 36; also Sect. 12.1) to 2-monoacylglycerols and free fatty acids. These are taken up by the intestinal mucosa. Bile constituents, including bile salts, efficiently emulsify lipids prior to hydrolysis by *lipase*. Colipase, a protein secreted with *lipase*, helps promote hydrolysis of emulsified lipids.
3. Transportation of dietary triacylglycerols: Triacylglycerols are reassembled within intestinal mucosal cells. Fatty acids are converted to fatty acyl CoA's, then esterified to the 2-monoacylglycerols. The mucosa package the resulting triacylglycerols and cholesterol esters as lipoprotein complexes: lipid droplets surrounded by phospholipids and proteins, including apolipoprotein B-100. The resulting chylomicrons are micelles specialized for lipid transport through the circulatory system (Sect. 17.II).

   After transport to their destinations, triacylglycerols are degraded to free fatty acids and glycerol. Free fatty acids can be oxidized for fuel or they can reform triacylglycerols if absorbed by adipose tissue.

## III. PHOSPHOLIPIDS

STRUCTURE: Phospholipids are structurally related to triacylglycerols. However, they are polar at one end of the molecule, while maintaining their non-polar character at the other end. Such molecules are called "amphipathic."

The polar head group consists of a small polar alcohol (such as serine, ethanolamine, choline, or inositol) linked to phosphatidate (diacylglycerol esterified to phosphate). The most common phospholipids are phosphatidylcholine (a lecithin) and phosphatidylethanolamine (a cephalin). Other phospholipids, such as sphingomyelins and gangliosides, represent more complex structures.

FUNCTIONS: Phospholipids form membranes of all cells. The lipid bilayer structure of membranes depends upon the ability of these molecules to orient themselves so that the hydrophobic fatty acid tails are arranged opposite each other.

In micelles, such as lipoproteins used to transport lipids in the blood (chylomicrons, LDL, HDL), the fatty acid tails align themselves parallel to each other. The resulting shell of phospholipid can interact with other hydrophobic molecules, such as cholesterol and cholesterol esters, and triacylglycerols.

Also shown is phosphatidylinositol. This phospholipid stores arachidonate in membranes prior to its use in prostaglandin, leukotriene, and thromboxane synthesis. Phosphatidylinositol bisphosphate participates in a second messenger system responding to many hormones and other external stimuli.

**IV. CHOLESTEROL**: This is the most common sterol. Cholesterol is very hydrophobic, more so than fatty acids. Cholesterol metabolism and transport are discussed in Section 17.

STRUCTURE: See Figure 17.1 for the structure of cholesterol. Its major identifying feature is the four fused carbon ring structure (cyclopentanoperhydroanthrene).

FUNCTIONS: Cholesterol is an essential animal membrane building block. It modulates membrane fluidity. Cholesterol is the precursor for all steroid hormones. It is converted to bile salts by the liver for digestion (Sect. 17.III). Finally, cholesterol forms the precursor of vitamin D.

SYNTHESIS: The synthesis of cholesterol is regulated in part by the amount of cholesterol taken up by cells during lipoprotein metabolism. Internalization of LDL by peripheral tissues causes an increase in cholesterol concentration, inhibiting *de novo* cholesterol synthesis.

Glucagon leads to the inhibition of the key regulatory step of the pathway, Enzyme 49, *HMG CoA reductase* (Sect. 17.1). Insulin favors its activation and subsequent cholesterol formation. Dietary saturated fatty acids, such as palmitate, favor cholesterol formation in the liver, while dietary unsaturated fatty acids tend to diminish cholesterol synthesis.

## V. BIOLOGICAL MEMBRANES

STRUCTURE: The current model for membranes is called the fluid-mosaic model. In this model, membrane phospholipids form a lipid bilayer, and membrane proteins are embedded in this layer.

Phospholipids are the predominate class of membrane lipids composing the bilayer. Through noncovalent hydrophobic and electrostatic interactions, the phospholipid molecules spontaneously self-assemble in bilayers when in the presence of water. In the bilayer, as with micelles, the phospholipids are oriented with the polar heads (hydrophilic phosphate ester groups) on the outside and the nonpolar tails (hydrophobic hydrocarbon chains) pointed inward. Glycolipids, glycoproteins, and cholesterol are other important constituents found in many animal cell membranes.

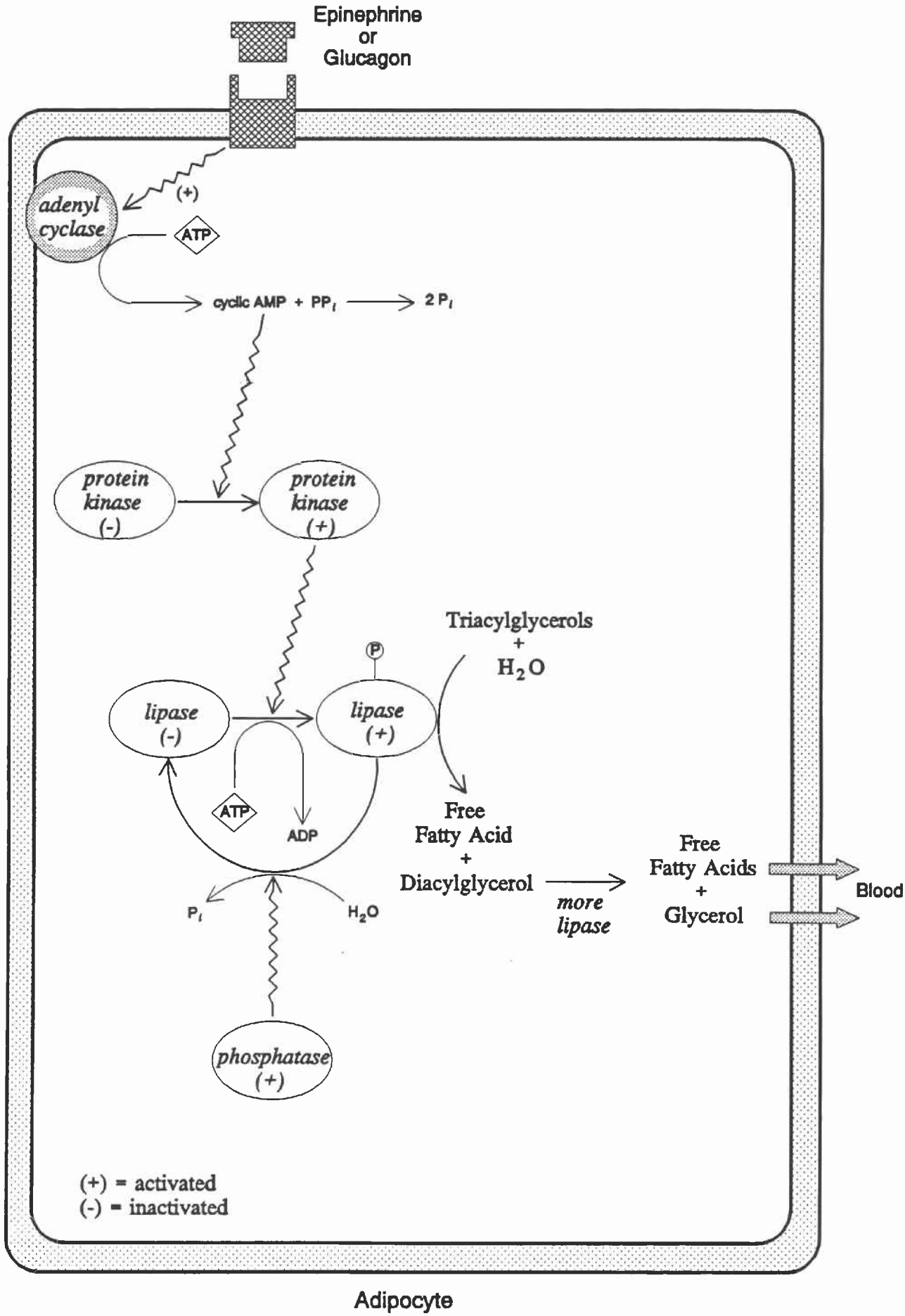
Membrane proteins can be on the inner or outer surfaces, or they can penetrate the entire thickness of the membrane. Some membrane proteins lie within the membrane, not in contact with the inner or outer

surfaces. The protein content of membranes varies, from a low of 18% (myelin) to a high of 75% (mitochondria). While the lipids form the permeable barrier, the embedded proteins mediate most of the other membrane functions. Membranes containing different proteins will perform different functions. The proteins can act as pumps, gates, receptors, and enzymes.

FUNCTIONS: The cell membrane provides structural support, acts as a mechanical barrier, and controls the passage of ions and molecules into and out of the cell.

Subcellular membranes comprise organelles that perform many functions, depending upon the proteins (enzymes) that they contain. For example: respiration (inner mitochondria membrane), protein synthesis (rough endoplasmic reticulum), steroid synthesis (smooth endoplasmic reticulum), intracellular digestion (lysosomes).

# 12.1 Mobilization of Fatty Acids: Hormonal Regulation of Lipase



## I. MOBILIZATION OF FATTY ACIDS (Lipolysis): Hormonal Regulation of *Lipase*

Input: triacylglycerols + $H_2O$
Output: free fatty acids + glycerol

LOCATION: Cytoplasm of adipocytes.

FUNCTION: Releases long chain fatty acids from stored triacylglycerols in adipose tissue.

CHARACTERISTICS: Glucagon and epinephrine trigger a cyclic AMP/*protein kinase* cascade which activates *hormone-sensitive lipase*. This process is resembles the activation of *phosphorylase* (Sect. 8.III).

STEPS:

1. Hormone binding: The activating hormone binds reversibly to its receptor on the exterior of the cell.
2. Cyclic AMP synthesis: Hormone binding activates ✓*adenylate cyclase* located on the interior cell membrane. The product of the enzyme is cyclic AMP, the intracellular hormone mediator.
3. Activation of *protein kinase*: Elevated cyclic AMP levels activate ✓*protein kinase* by binding to its regulatory subunits and releasing the catalytic subunits.
4. Phosphorylation of *lipase*: Activated *protein kinase* can then phosphorylate an inactive (non-phosphorylated) Enzyme 36, ✓*lipase (hormone-sensitive)*. This covalent modification activates *lipase*.
5. Hydrolysis of triacylglycerols: *Hormone-sensitive lipase* hydrolyzes a single fatty acid from depot triacylglycerols. Other *lipases* are able to remove the two remaining fatty acids from the diacylglycerol. Free fatty acids and glycerol enter the bloodstream.
6. *Phosphatase* reverses *lipase* activation by hydrolyzing its phosphate group.

   Elevated blood glucose and insulin levels favor lowered cyclic AMP levels and the inactivation of *hormone-sensitive lipase*. The detailed mechanisms are not known.

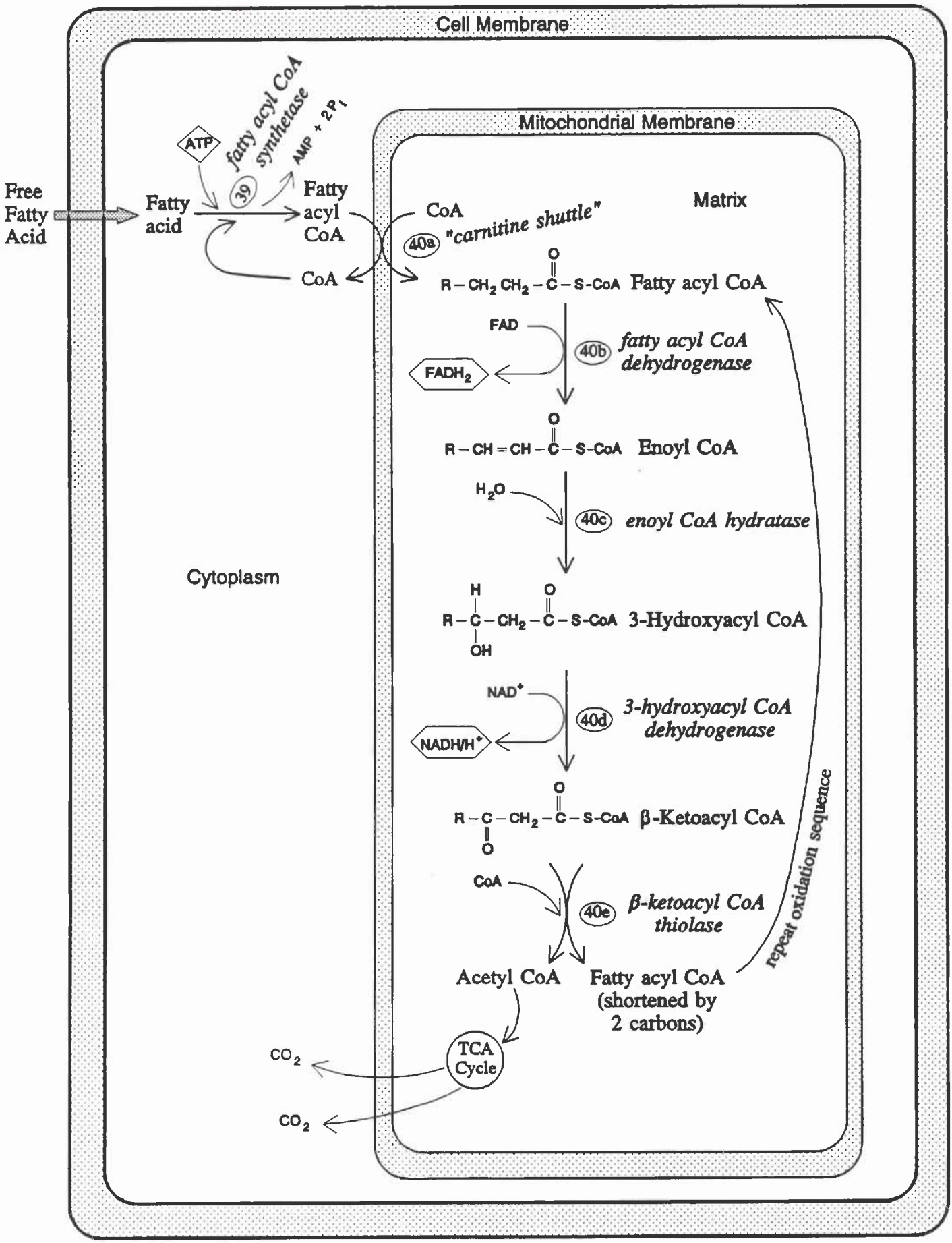
NOTES:

1. Transportation in the blood stream: Free fatty acids bind to serum albumin and are transported to tissues, such as muscle, for ATP production by $\beta$-oxidation. Tissues which use free fatty acids as fuel include skeletal and heart muscle. Those which cannot use fatty acids include nerves and the brain, red blood cells, and the adrenal medulla. Glycerol, produced by lipolysis, is absorbed by the liver where it is converted to dihydroxyacetone phosphate to enter glycolysis (Sect.5) or gluconeogenesis (Sect. 10).
2. A contrast with *pancreatic lipase*: In the context of digestion, Enzyme 36, *lipase (pancreatic)*, hydrolyzes dietary triacylglycerols to 2-monoacylglycerols and free fatty acids in the presence of bile salts. These digestion products are absorbed and reassembled into triacylglycerols by intestinal epithelia (Sect. 13.II). Dietary lipids are exported as chylomicrons (Sect. 17.II).

REVIEW QUESTIONS:

1. Distinguish between the functions of *pancreatic lipase* and *hormone-sensitive lipase*.
2. Compare the activation of *hormone-sensitive lipase* with the activation of *glycogen phosphorylase*.

# 12.2 β-Oxidation of Fatty Acids



## II. β-OXIDATION OF FATTY ACIDS

Input: palmitoyl CoA + 7 FAD + 7 $NAD^+$ + 7 CoA
Output: 8 acetyl CoA + 7 $FADH_2$ + 7 NADH

LOCATION: Mitochondrial matrix.

FUNCTION: Key pathway for energy production from triacylglycerols. Oxidizes fatty acyl CoA to acetyl CoA with the production of NADH and $FADH_2$. The oxidation of palmitoyl CoA yields a total of 131 ATP, but because two high energy phosphate bonds are consumed for fatty acyl CoA formation, the net yield is 129 ATP.

CHARACTERISTICS: This oxidative pathway cleaves two-carbon fragments from fatty acyl CoA as acetyl CoA. The cyclic process requires an oxidation, hydration of a double bond, oxidation of 3-hydroxyl group to a keto group, and thiolysis with CoA.

STEPS:

1. The formation of fatty acyl CoA: On the outer mitochondrial membrane, free fatty acids are coupled to CoA by Enzyme 39, *fatty acyl CoA synthetase*. ATP supplies the energy for this activation.
2. Entry into mitochondria: The ✓*carnitine shuttle*, Enzymes 40a, includes *carnitine acyltransferases I & II*. *Carnitine acyltransferase I* creates fatty acyl carnitine, which then is translocated across the membrane by a carrier protein. Once inside the mitochondrial matrix, *carnitine acyltransferase II* reforms fatty acyl CoA from acyl carnitine. Free carnitine is shuttled back out, like a revolving door.
3. Fatty acid oxidation with FAD: Enzyme 40b, ✓*fatty acyl CoA dehydrogenase*, carries out the first oxidation of the saturated chain by removing two hydrogen atoms to form a *trans* double bond. This creates $FADH_2$. The *dehydrogenase* uses an *iron-sulfur reductase* to transfer electrons to CoQ. Therefore, half the electrons from β-oxidation enter the Electron Transport Chain at CoQ via Complex II (Sect. 7.1).
4. Hydration of the double bond: Enzyme 40c, *enoyl CoA hydratase*, forms a 3-hydroxyacyl CoA derivative.
5. Oxidation of the 3-hydroxyacyl CoA: Enzyme 40d, ✓*3-hydroxyacyl CoA dehydrogenase*, requires $NAD^+$ to produce the β-ketoacyl CoA.
6. Release of acetyl CoA: The last step in the cycle, carried out by Enzyme 40e, *β-ketoacyl CoA thiolase*, requires CoA to split off acetyl CoA, leaving the acyl chain shortened by two carbons.
7. Steps 3-6 are repeated to remove the next two carbons: Complete oxidation of C16 palmitoyl CoA thus requires 7, not 8, repetitions of the above sequence.

NOTES:

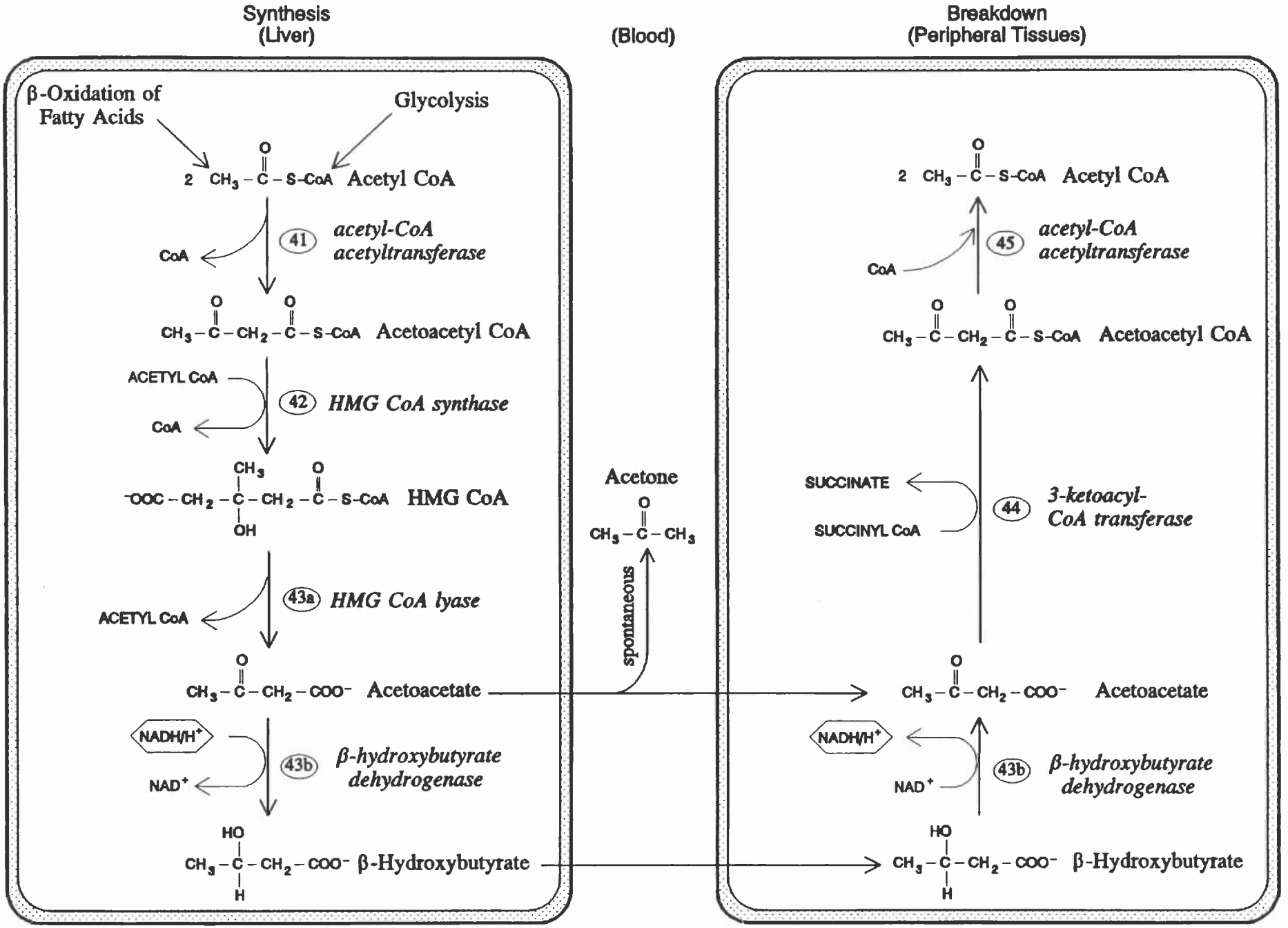
1. Unsaturated fatty acids: Oxidation of unsaturated fatty acids yields somewhat fewer ATP because they are more oxidized than saturated fatty acids. Accessory enzymes isomerize *cis* double bonds of these fatty acids to *trans* double bonds and create the appropriate stereoisomer of enoyl CoA.
2. Metabolism of propionyl CoA: Odd-numbered chain-length fatty acids yield a 3C fragment, propionyl CoA. The oxidation of several amino acid carbon skeletons also yields propionyl CoA (Sect. 15). This does not form acetyl CoA, rather a separate path allows these carbons to enter the TCA cycle. Propionyl CoA is carboxylated to form 4C methylmalonyl CoA. Coenzyme $B_{12}$ rearranges the carbon skeleton to produce succinyl CoA.

3. Regulation of β-oxidation: Malonyl CoA, the first product of fatty acid synthesis, blocks *carnitine acyltransferase I*. This prevents entry of fatty acids into mitochondria while fatty acids are being synthesized in the cytoplasm.

REVIEW QUESTIONS:

1. Describe the mechanisms by which fatty acid synthesis blocks the oxidation of free fatty acids.
2. Describe role of carnitine in fatty acid metabolism.
3. Describe the products of fatty acid oxidation.
4. How many acetyl CoA's are produced from stearoyl CoA?
5. Explain why fatty acids cannot be converted to glucose.

# Ketone Body Metabolism



## III. KETONE BODY METABOLISM

### A. SYNTHESIS

Input: 2 acetyl CoA + $H_2O$
Output: acetoacetate + 2 CoA + $H^+$

LOCATION: Liver mitochondria.

FUNCTION: Ketone bodies, acetoacetate and β-hydroxybutyrate, can be considered as water-soluble fatty acid equivalents to be used as fuel.

CHARACTERISTICS: Ketone body formation takes place only in the liver, when fat breakdown predominates. The major ketone body is acetoacetate. Acetoacetate spontaneously decarboxylates to acetone, a metabolic dead-end that is released in urine and in the breath. When there is surplus NADH, acetoacetate is reduced to β-hydroxybutyrate.

STEPS: (liver)
1. Enzyme 41, *acetyl-CoA acetyltransferase (thiolase)*, condenses two acetyl CoA's to form acetoacetyl CoA.
2. Enzyme 42, *HMG CoA synthase*, attaches the third acetyl CoA, to form HMG CoA (3-hydroxy-3-methylglutaryl CoA). This step is also the rate-limiting step.
3. Enzyme 43a, *HMG CoA lyase*, releases acetoacetate and acetyl CoA. In this way, acetoacetate is formed without the hydrolysis of a CoA ester bond.
4. Enzyme 43b, *β-hydroxybutyrate dehydrogenase*, reduces acetoacetate with NADH, forming β-hydroxybutyrate.

NOTE: Ketoacidosis: Ketone bodies are normally made in small amounts. The usual level in the blood is less than 3 mg/dl for a healthy individual consuming a balanced diet. During prolonged fasting or uncontrolled diabetes, the level of ketone bodies can reach 90 mg/dl (ketonemia). The accumulation of acetoacetate and β-hydroxybutyrate also leads to acidosis. Excretion in the urine (ketonuria) can lead to dehydration.

### B. UTILIZATION

Input: acetoacetate + succinyl CoA + CoA
Output: 2 acetyl CoA + succinate

LOCATION: Peripheral tissues, including cardiac and skeletal muscle, brain, and adrenal cortex. The liver cannot use acetoacetate.

FUNCTION: To supply peripheral tissues with acetyl CoA from a water-soluble, fatty acid derivative.

CHARACTERISTICS: Tissues require succinyl CoA to use acetoacetate. They oxidize β-hydroxybutyrate with $NAD^+$.

STEPS: (non-liver)

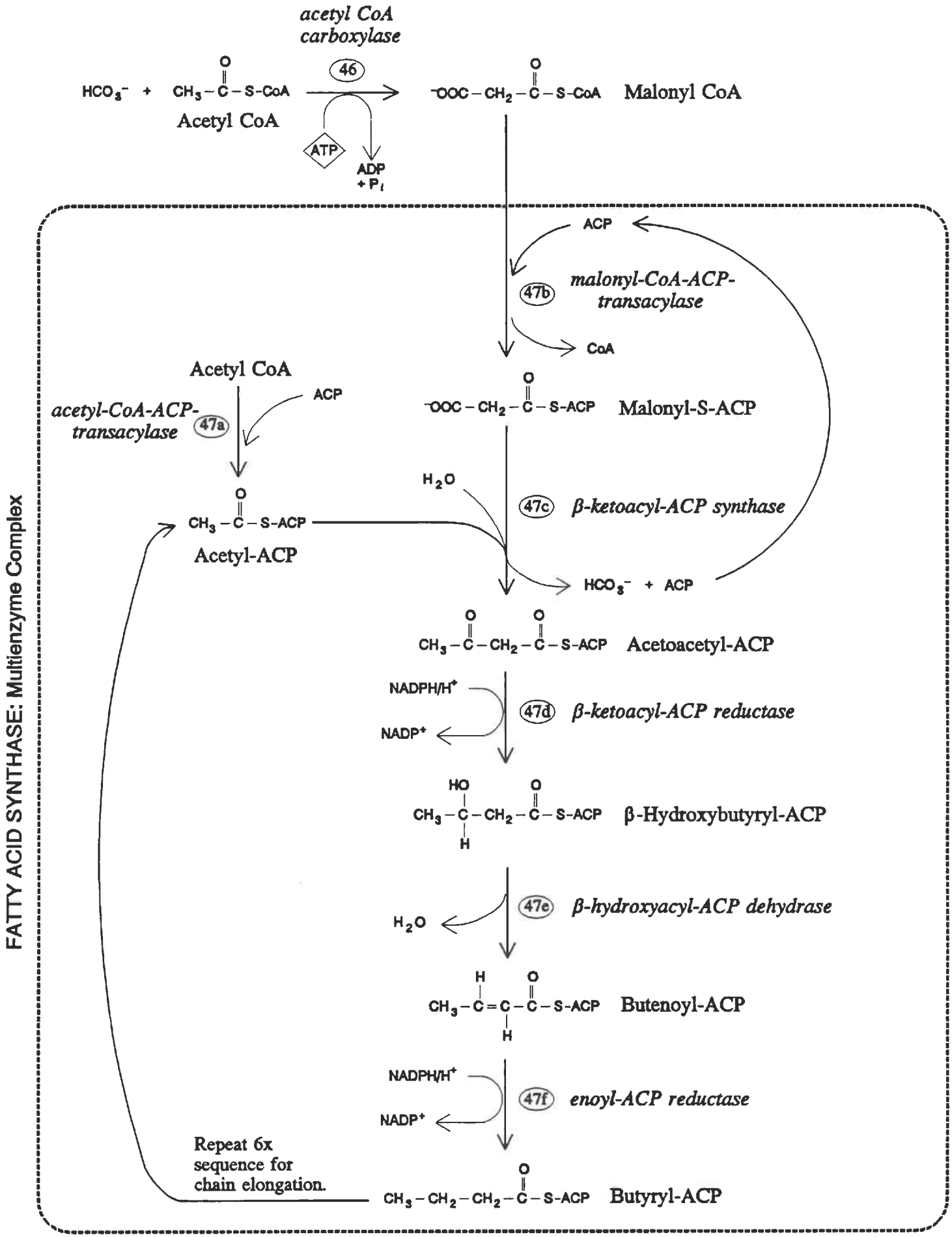
1. Oxidation β-hydroxybutyrate: Enzyme 43b, *β-hydroxybutyrate dehydrogenase*, uses $NAD^+$ to oxidize β-hydroxybutyrate, reforming acetoacetate.
2. Formation of acetoacetyl CoA: Enzyme 44, *3-ketoacyl-CoA transferase*, employs succinyl CoA as the CoA donor to form acetoacetyl CoA. The liver lacks this enzyme, so it cannot degrade acetoacetate.
3. Formation of acetyl CoA: Enzyme 45, *acetyl-CoA acetyltransferase (thiolase)*, cleaves acetoacetyl CoA into two acetyl CoA's.

NOTE: Use of ketone bodies: Many tissues, including cardiac and skeletal muscle, efficiently use ketone bodies as fuels. When the blood level is sufficiently high, ketone bodies penetrate the blood-brain barrier and are oxidized. This lowers the brain's requirement for glucose as a fuel.

REVIEW QUESTIONS:

1. Name the compounds classified as ketone bodies.
2. Describe where ketone bodies are formed.
3. Describe conditions favoring ketone body formation.
4. Name the precursor of acetoacetyl CoA.
5. Describe the fate of ketone bodies.

NOTES


acetyl CoA carboxylase
46
$HCO_3^-$ + $CH_3-C(=O)-S-CoA$
Acetyl CoA
$^-OOC-CH_2-C(=O)-S-CoA$
Malonyl CoA
ATP
ADP + $P_i$
FATTY ACID SYNTHASE: Multienzyme Complex
ACP
47b
malonyl-CoA-ACP-transacylase
CoA
Acetyl CoA
ACP
acetyl-CoA-ACP-transacylase
47a
$^-OOC-CH_2-C(=O)-S-ACP$
Malonyl-S-ACP
$H_2O$
47c
β-ketoacyl-ACP synthase
$CH_3-C(=O)-S-ACP$
Acetyl-ACP
$HCO_3^-$ + ACP
$CH_3-C(=O)-CH_2-C(=O)-S-ACP$
Acetoacetyl-ACP
$NADPH/H^+$
$NADP^+$
47d
β-ketoacyl-ACP reductase
$CH_3-C(HO)H-CH_2-C(=O)-S-ACP$
β-Hydroxybutyryl-ACP
$H_2O$
47e
β-hydroxyacyl-ACP dehydrase
$CH_3-C(H)=C(H)-C(=O)-S-ACP$
Butenoyl-ACP
$NADPH/H^+$
$NADP^+$
47f
enoyl-ACP reductase
Repeat 6x sequence for chain elongation.
$CH_3-CH_2-CH_2-C(=O)-S-ACP$
Butyryl-ACP

## I. FATTY ACID SYNTHESIS

Input: 8 acetyl CoA + 14 NADPH + 7 ATP
Output: palmitate + 14 $NADP^+$ + 7 ADP + 7 $P_i$ + 8 CoA + $H_2O$

LOCATION: Predominantly in adipose, liver, and lactating mammary gland. It is a cytoplasmic pathway.

FUNCTION: *Fatty acid synthase*, a multienzyme complex, synthesizes the 16C saturated fatty acid, palmitate, from activated acetyl CoA.

CHARACTERISTICS: Acetyl CoA must first be decarboxylated to malonyl CoA. Decarboxylation provides the driving force for coupling 2C units. Fatty acid synthesis is a sequence of seven steps. Fatty acids are assembled stepwise with two-carbon additions. The first acetyl group comes from acetyl CoA. The seven subsequent 2C additions use malonyl CoA. The reducing agent in biosynthetic reactions is NADPH, not NADH.

STEPS:
1. Activation of acetyl CoA as malonyl CoA: Enzyme 46, ✓*acetyl CoA carboxylase*, uses ATP to carboxylate acetyl CoA. Its cofactor is biotin. This is the control point.
2. Priming fatty acid synthesis: Enzyme 47a, *acetyl-CoA-ACP-transacylase*, transfers the acetyl group of acetyl CoA, to a carrier protein designated as ACP, acyl carrier protein. This first step is used only once for each palmitate chain synthesized. ACP is a sulfhydryl protein carrier which incorporates pantothenic acid.
3. Addition of malonyl CoA: Enzyme 47b, *malonyl-CoA-ACP-transacylase*, attaches malonyl to the carrier protein, ACP. Enzyme 47c, *β-ketoacyl-ACP synthase*, is also called *acyl-malonyl-ACP condensing enzyme*. It condenses malonyl and acetyl groups to form acetoacetyl-ACP.
4. Reduction of keto group of acetoacetyl-ACP: Enzyme 47d, *β-ketoacyl-ACP reductase*, reduces the β-keto group to a hydroxy group using the first NADPH, which is supplied by the Pentose Phosphate pathway.
5. Removal of hydroxyl group by dehydration: Enzyme 47e, *β-hydroxyacyl-ACP dehydrase*, dehydrates the β-hydroxybutyryl-ACP and produces an unsaturated chain, butenoyl-ACP.
6. Reduction of a double bond: Enzyme 47f, *enoyl-ACP reductase*, reduces the double bond of butenoyl-ACP with the second NADPH to produce butyryl-ACP (a 4C saturated acyl chain).
7. Repetition of steps 3 through 6 (six times) produces palmitoyl-ACP.
8. Hydrolysis of palmitoyl ACP: *Palmitoyl thioesterase* produces palmitate, and frees ACP to be recycled.

NOTES:
1. Citrate-malate shuttle supplies cytoplasmic acetyl CoA: Acetyl groups, synthesized by *pyruvate dehydrogenase* in mitochondria, are shuttled out to the cytoplasm via citrate (from condensation of acetyl CoA with oxaloacetate). Once in the cytoplasm, *citrate lyase* cleaves citrate back to acetyl CoA and oxaloacetate. The oxaloacetate is reduced by NADH to malate. Then malate is oxidatively decarboxylated by $NADP^+$ to pyruvate, which diffuses back into the mitochondrion. There, pyruvate is carboxylated back to oxaloacetate.
2. Regulation: *Acetyl CoA carboxylase* regulates fatty acid synthesis. It is activated by citrate, and inhibited by its product (malonyl CoA) and by the end product, palmitoyl CoA. Glucagon blocks fatty acid synthesis while mobilizing fatty acids. This hormone initiates a cyclic AMP/*protein kinase* cascade (see Sect. 12.I, for the regulation of *lipase*). *Protein kinase* inactivates *acetyl CoA carboxylase* by

phosphorylating it.

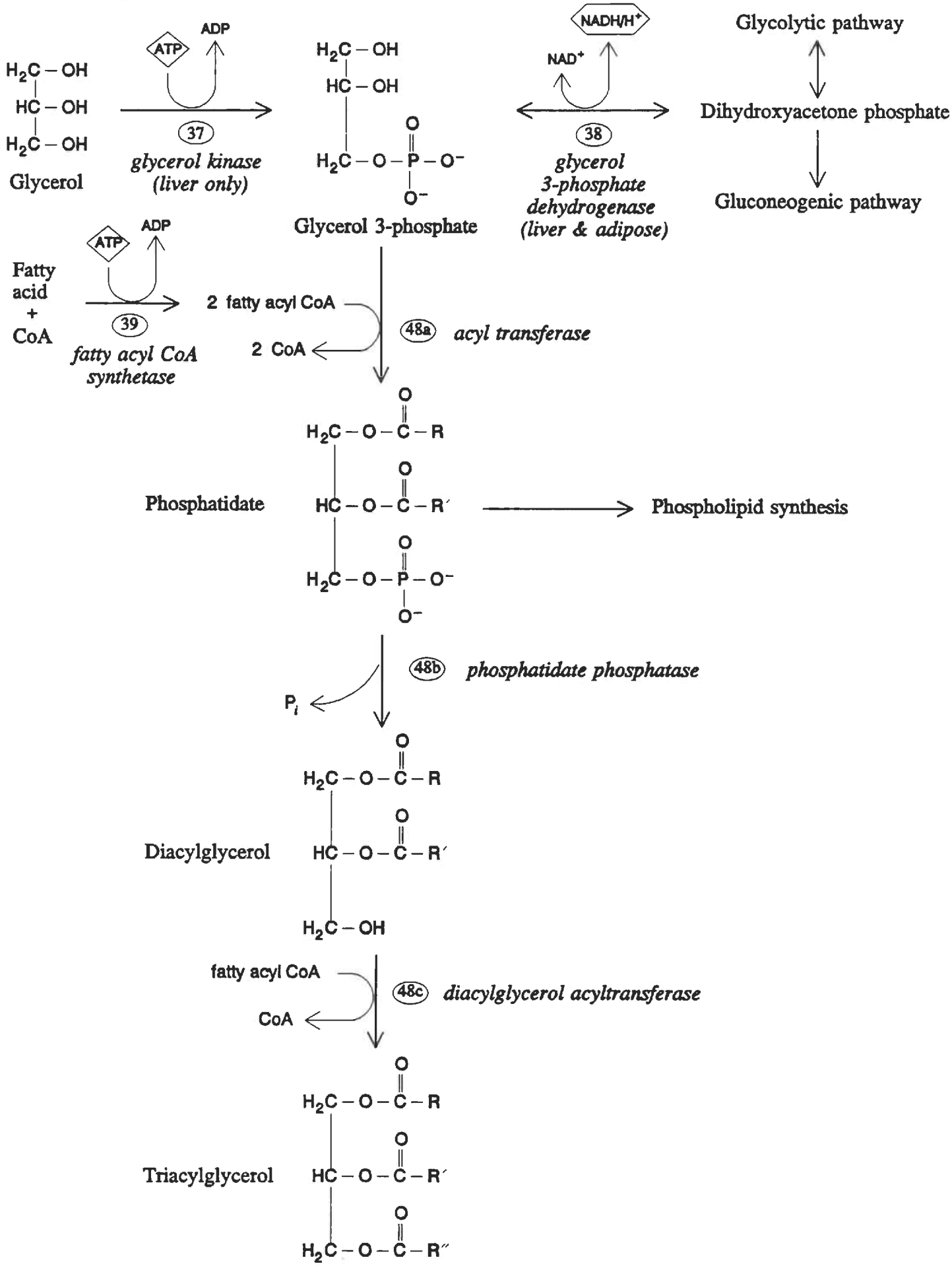
3. Elongation of fatty acyl CoA: Palmitoyl CoA can be elongated by the endoplasmic reticulum. Malonyl CoA is added via reactions similar to those described above.
4. Unsaturated fatty acids: Monounsaturated fatty acids are synthesized at the endoplasmic reticulum. Stearoyl CoA (saturated 18C) is converted to oleoyl CoA (monounsaturated 18C) by a *desaturase*. This cytochrome-containing enzyme requires NADH and oxygen.
5. 18C polyunsaturated fatty acids cannot be synthesized by mammalian enzymes: Linoleate and linolenate must be supplied by the diet and are designated "essential fatty acids." However, these fatty acids can be elongated and further desaturated by the endoplasmic reticulum. For example, linoleate forms arachidonate (20C with 4 double bonds) required for synthesis of $PGE_2$ family of prostaglandins, as well as leukotrienes and thromboxanes (Fig. 11.1).

REVIEW QUESTIONS:

1. List the precursors required for fatty acid synthesis.
2. Describe the rate-limiting step in this process.
3. Describe the effect of increased levels of the following on fatty acid synthesis: (a) citrate, (b) malonyl CoA, (c) palmitoyl CoA, (d) glucagon.
4. Contrast the requirements and subcellular location of fatty acid synthesis and fatty acid oxidation.

# NOTES

# 13.2 Triacylglycerol Synthesis



## II. TRIACYLGLYCEROL SYNTHESIS

Input: glycerol 3-phosphate + 3 fatty acyl CoA
Output: triacylglycerol + 3 CoA

LOCATION: Most triacylglycerol synthesis occurs in the liver and in adipose tissue, especially after a high carbohydrate meal.

FUNCTION: Triacylglycerols are the storage form of fatty acids, which represent the greatest percentage of fuel reserves in the body.

CHARACTERISTICS: Fatty acyl CoA's are activated derivatives. No input of ATP is required for triacylglycerol synthesis. A multienzyme complex carries out this process. Glycolysis is required to supply glycerol 3-phosphate.

STEPS:

1. Formation of glycerol 3-phosphate:
   (a) Enzyme 38, *glycerol 3-phosphate dehydrogenase* reduces dihydroxyacetone phosphate (from glucose), forming glycerol 3-phosphate.
   (b) In the liver only, Enzyme 37, *glycerol kinase*, phosphorylates glycerol released, from adipose tissue, to produce glycerol 3-phosphate.
2. The formation of fatty acyl CoA: Free fatty acids are coupled to CoA by Enzyme 39, *fatty acyl CoA synthetase*. ATP supplies the energy for this activation.
3. Synthesis of triacylglycerols: Enzyme 48, *triacylglycerol synthetase*, is a complex bound to the endoplasmic reticulum. It consists of three polypeptide species.
   (a) Formation of phosphatidate: Enzyme 48a, *acyl transferase*, adds two fatty acyl chains to glycerol 3-phosphate.
   (b) Formation of diacylglycerol: Enzyme 48b, *phosphatidate phosphatase*, hydrolyzes the phosphate group from phosphatidate.
   (c) Formation of triacylglycerol: Enzyme 48c, *diacylglycerol acyltransferase*, adds the third fatty acyl chain.

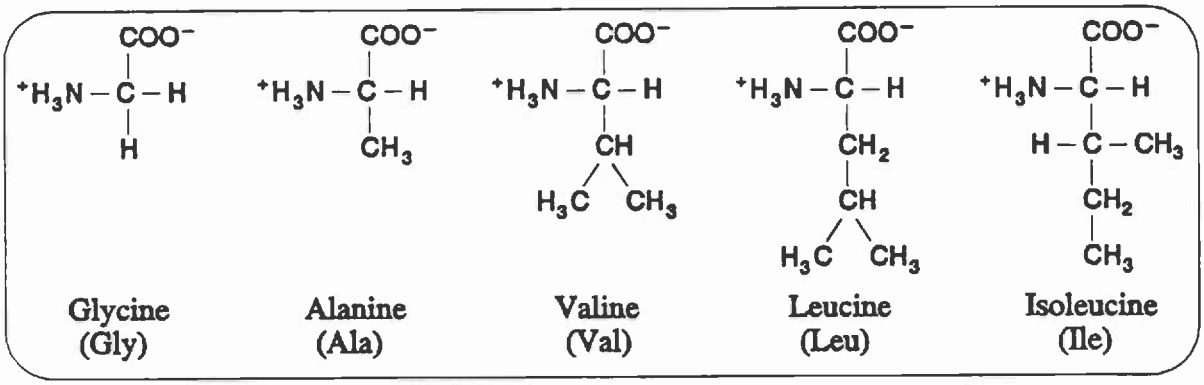
NOTES:

1. The specificity of the *acyl transferases* overlap in terms of chain length and degree of unsaturation of fatty acids. In humans, adipose tissue generates triacylglycerols enriched in palmitate at carbon #1 and oleate at carbon #2.
2. The liver can also convert glycerol released during lipolysis to glucose. In other words, glycerol can be a glucogenic substrate.

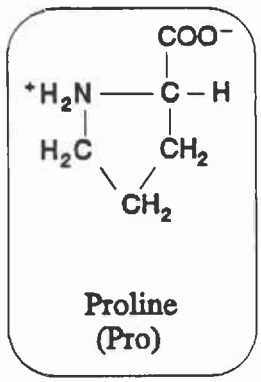
REVIEW QUESTIONS:

1. List the tissues involved in triacylglycerol synthesis.
2. Explain why glycolysis is required in triacylglycerol synthesis.
3. Describe the steps by which glycerol is converted to triacylglycerol.
4. Predict the effect of elevated insulin of this pathway. Predict the effect of elevated glucagon and epinephrine on triacylglycerol metabolism.

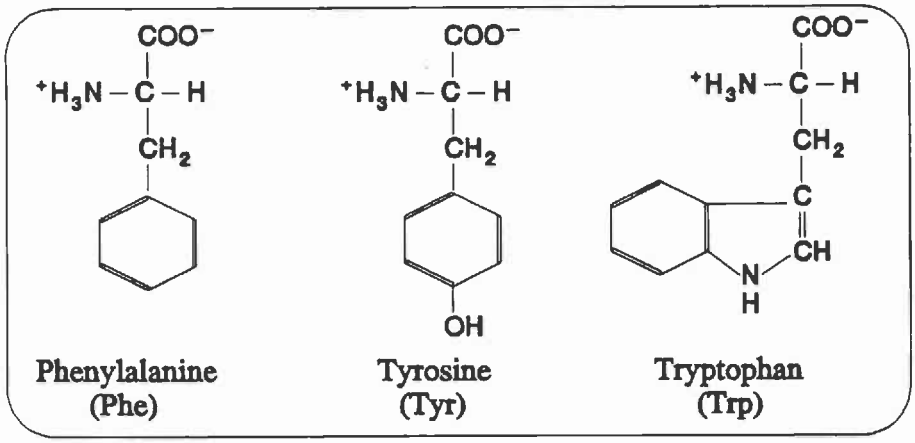
# 14.1 Amino Acids (pH 7.0)



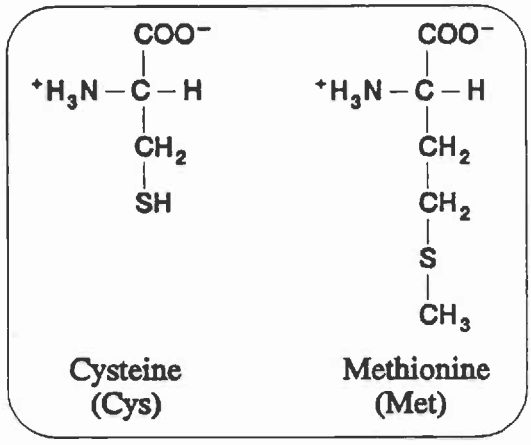
Glycine (Gly) | Alanine (Ala) | Valine (Val) | Leucine (Leu) | Isoleucine (Ile)



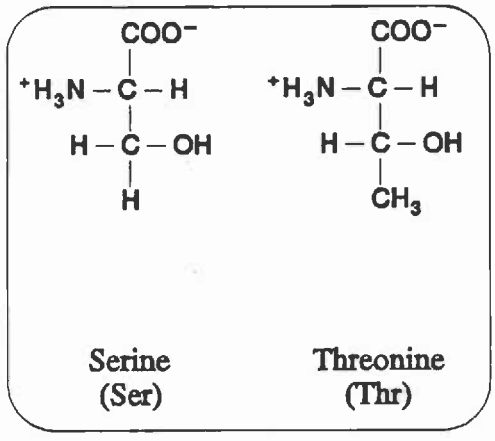
Proline (Pro)



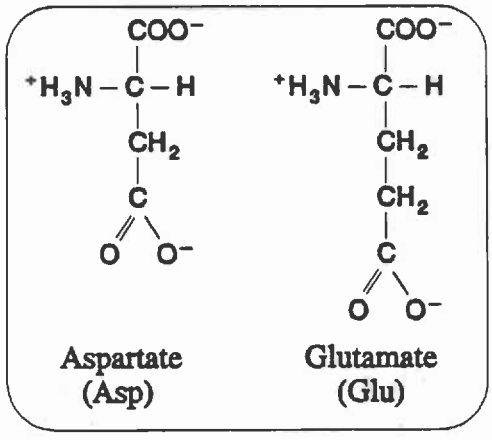
Phenylalanine (Phe) | Tyrosine (Tyr) | Tryptophan (Trp)



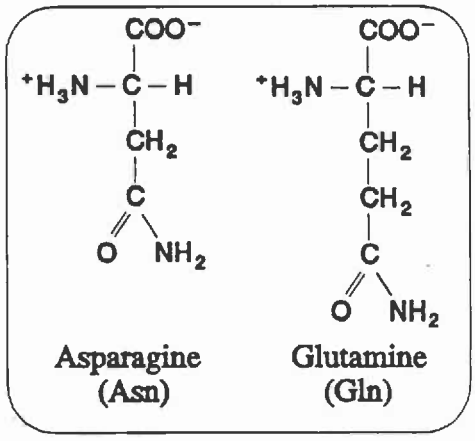
Cysteine (Cys) | Methionine (Met)



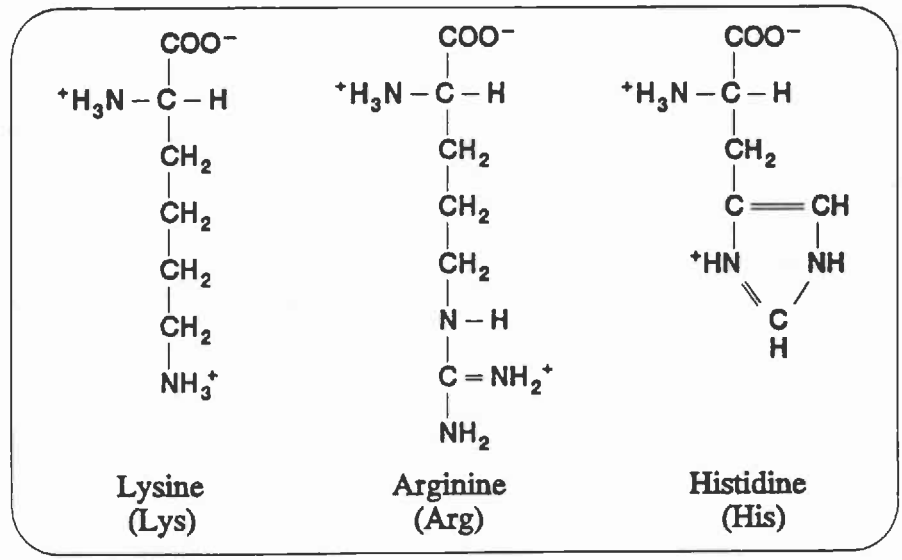
Serine (Ser) | Threonine (Thr)



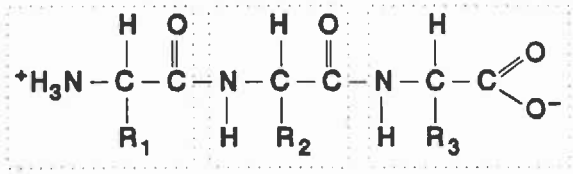
Aspartate (Asp) | Glutamate (Glu)



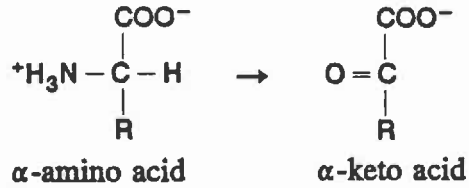
Asparagine (Asn) | Glutamine (Gln)



Lysine (Lys) | Arginine (Arg) | Histidine (His)



A tripeptide
(three residue polypeptide)



α-amino acid → α-keto acid

## I. AMINO ACIDS

STRUCTURE: The many thousands of proteins in cells are constructed from just twenty amino acids (AA), coded by DNA. Other amino acids which appear in proteins result from modifications occurring after protein synthesis.

The twenty amino acids are $\alpha$-amino acids, in which an amino group and a carboxyl group are attached to the $\alpha$-carbon. Each amino acid, except proline, also has a hydrogen atom and a unique side chain or "R-group" attached to the $\alpha$-carbon.

CLASSIFICATION: In polypeptides, the $\alpha$-amino and $\alpha$-carboxyl groups are linked in peptide bonds. The unbound R-groups are accessible and affect properties of the polypeptide. Therefore, it is useful to classify amino acids in terms of the properties of their R-groups.

1. Aliphatic (hydrophobic) R-groups: As shown in Figure 14.1, five amino acids have R-groups containing only hydrogen and carbon in the side chains. Glycine, with a single hydrogen as its R-group, is placed here for convenience. Alanine has a simple methyl R-group. Valine has an isopropyl R-group, leucine has an isobutyl R-group, and isoleucine has a *sec*-butyl R-group. Valine, leucine, and isoleucine are called "branched chain" amino acids (BCAA). It is worth noting that these three amino acids are dietary essential amino acids (EAA), and are not extensively metabolized by the liver. Instead, skeletal muscle selectively metabolizes the branched chain amino acids in the presence of insulin.
2. Imino acid: Though proline's side chain is aliphatic, it is not strictly an $\alpha$-amino acid because the carbon chain is attached to the nitrogen, creating a secondary amine. Proline is synthesized from glutamate.
3. Aromatic R-groups: Phenylalanine, tryptophan, and tyrosine are aromatic amino acids, as is histidine. Since histidine can also accept protons, it is usually classified as a basic amino acid. Phenylalanine and tryptophan are non-polar and are also dietary essential amino acids. Tyrosine is synthesized from phenylalanine.
4. Sulfur-containing R-groups: Only cysteine and methionine contain sulfur. Methionine, with a thioether linkage, is an essential amino acid. Cysteine possesses a sulfhydryl (-SH) group, which forms disulfide bridges in proteins. Cysteine is synthesized from methionine. Neither R-group is considered polar.
5. R-groups with hydroxyl groups: Serine, threonine , and tyrosine contain hydroxyl groups and therefore may hydrogen-bond with water molecules. Only threonine is an essential amino acid.
6. Acidic R-groups: Aspartate and glutamate contain side chains with carboxyl groups, which give up protons at physiologic pH and contribute a negative charge. Each can be synthesized from TCA cycle intermediates.
7. R-groups which contain amides: The terminal carboxyl groups of aspartate and glutamate can be replaced by amides, creating asparagine and glutamine. Both amino acid side chains are neutral.
8. Basic R-groups: Lysine, arginine, and histidine possess R-groups which acquire protons and become positively charged at physiologic pH. Although arginine can be synthesized in the body, its synthesis may be inadequate for optimal health in several conditions.

   Histidine, a heterocyclic aromatic amino acid, can be partially protonated at physiologic pH. In contrast, the side chains of lysine and arginine carry full positive charges. In children, histidine is an EAA needed to support periods of rapid growth.

NOTES:

1. Ionizable groups of amino acids: In free amino acids, each $\alpha$-amino group (pKa $\sim$9) is a weak base and each $\alpha$-carboxyl group (pKa $\sim$2) is a weak acid. Hence, at pH 7, the carboxyl group bears a

negative charge and the amino group bears a positive charge. This double ion form of an amino acid is designated a "zwitterion."

At pH 7, R-groups of acidic amino acids contribute negative charges and R-groups of basic amino acids contribute positive charges. Only the imidazole R-group of histidine has a pKa within one pH unit of 7. Consequently, histidine, in proteins, can serve as a buffer at physiologic pH.

2. "L" amino acids are required for protein synthesis: The $\alpha$-amino acids are chiral compounds. The $\alpha$-carbon, with four different groups attached, is an asymmetric carbon. Therefore, "D" and "L" optical isomers exist for all AA's except glycine. The amino acids found in proteins are the "L" isomers.
3. Peptide bonds: Amino acids can be considered monomers which can be polymerized to form long chains. To link amino acids, the $\alpha$-carboxyl group of one amino acid forms an amide bond with the $\alpha$-amino group of the next. This linkage is a peptide bond and amino acid chains are designated peptides or polypeptides. Constituent amino acids are "amino acid residues."

   The peptide bond does not accept or donate protons from pH 2 - 10. Therefore, charges of the polypeptide chain are contributed only by ionized R-groups, the uncombined terminal amino group, and the uncombined terminal carboxyl group.

## II. POLYPEPTIDES AND PROTEINS

STRUCTURE: Polypeptides are ten or more amino acids linked by peptide bonds. Proteins consist of one or more polypeptide chains, and possess molecular weights over 5000.

1. Primary (1°) structure: This is the amino acid sequence in a polypeptide chain, and it is a complete description of all the covalent bonding in a polypeptide or protein. Primary structure is coded by DNA. The primary structure, once completed, determines 2°, 3°, and 4° structures.
2. Secondary (2°) structure: The spatial arrangements (conformations) of amino acid residues in localized regions of a polypeptide or protein. The $\alpha$-helix and $\beta$-pleated sheet are examples of secondary structure.
3. Tertiary (3°) structure: The overall folding pattern and arrangement in space of all the atoms in a polypeptide chain. Disulfide bonds are important in stabilizing the structure.
4. Quaternary (4°) structure: Exhibited by proteins that contain two or more linked polypeptide chains. Hemoglobin, containing four chains (subunits), is an example.

DENATURATION: Denaturation occurs when a protein undergoes a change in its normal three-dimensional shape (2°, 3°, and 4° structures), resulting in a loss of biological activity. Excessive temperatures (50° C to 60° C), heavy metal ions, detergents, organic solvents, and large changes in pH can cause proteins to become denatured. Denaturation can be partial or complete, and reversible or irreversible.

FIBROUS PROTEINS: These proteins are tough macromolecules composed of insoluble rodlike polypeptide chains. Important fibrous proteins are keratin, collagen, and elastin.

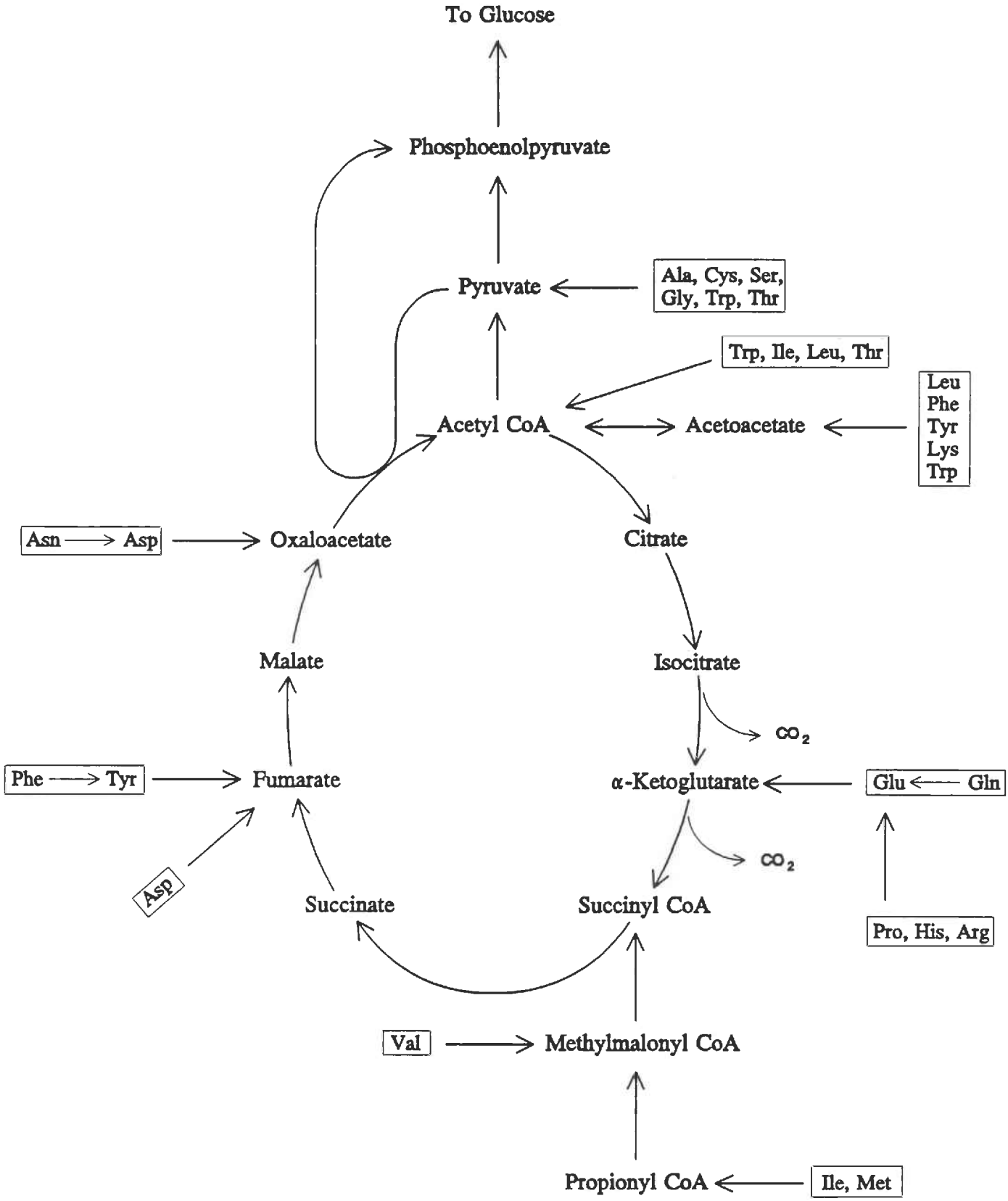
Keratin proteins are the main constituents of skin, wool, claws, horns, scales, and feathers. Keratin is composed of bundles of $\alpha$-helices. The sulfur containing amino acid, cysteine, is an important component of keratin, allowing for disulfide cross-linking.

Collagen, the most abundant protein found in mammals, is a family containing five types of polypeptides. Collagen is the major fibrous component of skin, bone, tendon, cartilage, blood vessels, and teeth. The basic structural unit of collagen is tropocollagen, a triple-stranded helical rod that differs from an $\alpha$-helix. Important amino acids found in collagen are glycine, proline, 4-hydroxyproline, and 5-hydroxylysine.

Elastin, a rubberlike protein in blood vessels and ligaments, is found in conjunction with collagen in most connective tissues. The $\beta$-spiral is a unique structural feature of elastin. Glycine is the predominate amino acid. Elastin is also rich in proline, but unlike collagen, it has little hydroxyproline and no hydroxylysine.

# NOTES

# 15.1 Amino Acid Catabolism



# I. REMOVAL OF NITROGEN

## A. TRANSAMINATION

Input: amino acid + α-ketoglutarate
Output: α-keto acid of donor amino acid + glutamate

LOCATION: Most tissues carry out transamination.

FUNCTION: Usually the first step in amino acid catabolism is the removal of the amino nitrogen by transamination. Lysine and threonine pathways do not use transamination.

CHARACTERISTICS: In transamination, an amino group is transferred to a receiver, generally α-ketoglutarate. The α-keto acid (from the donor) and glutamate (from α-ketoglutarate) are produced by this exchange. Enzymes catalyzing these reactions are designated *transaminases* or *aminotransferases*. They require the coenzyme, pyridoxal 5-phosphate, which is derived from vitamin $B_6$. As a result of this battery of transaminases, amino groups are funneled to make glutamate.

*Aminotransferases* catalyze reversible reactions: The direction often depends upon supply and demand for a given amino acid and its α-keto acid. For example, transamination of pyruvate supplies alanine. Oxaloacetate yields aspartate, and α-ketoglutarate yields glutamate.

A typical panel of diagnostic tests includes assays of these serum *aminotransferases*: (1) Enzyme 56, ✓*ASpartate aminoTransferase (AST)*, also known as *Serum Glutamate-Oxaloacetate Transaminase (SGOT)* and (2) *ALanine amino Transferase (ALT)*, also known as *Serum Glutamate-Pyruvate Transaminase (SGPT)*.

AST catalyzes the synthesis of aspartate: oxaloacetate + glutamate ⟷ aspartate + α-ketoglutarate

ALT catalyzes the synthesis of alanine: pyruvate + glutamate ⟷ alanine + α-ketoglutarate

NOTE: Dietary essential amino acids: Many amino acids can be synthesized at rates adequate to meet the body's requirements. This partially explains why many amino acids are not dietary essentials. Dietary essential amino acids either cannot be made in the body, or the synthetic rate is inadequate for optimal growth. The essential amino acids are: phenylalanine, tryptophan, histidine (for children), methionine, threonine, leucine, isoleucine, valine, lysine, and arginine (in special conditions, e.g. liver disease).

## B. OXIDATIVE DEAMINATION

Input: glutamate + $NAD^+$ (or $NADP^+$) + $H_2O$
Output: α-ketoglutarate + $NH_4^+$ + NADH (or NADPH) + $H^+$

LOCATION: In most tissues, but especially important in liver mitochondria.

FUNCTION: In the liver, deamination produces ammonia for urea synthesis (Sect. 16).

CHARACTERISTICS: Glutamate is the terminal nitrogen product of amino group transfer. In the liver, ammonia is freed from glutamate to synthesize urea. In this reaction, *glutamate dehydrogenase* oxidizes glutamate to α-ketoglutarate.

NOTE: Alternative origin of glutamate: *Glutamate dehydrogenase* can synthesize glutamate from ammonia and α-ketoglutarate. This enzyme is inhibited by elevated GTP and ATP, while it is activated by the dinucleotides, GDP and ADP.

## II. UTILIZATION OF CARBONS FROM AMINO ACIDS

Input: 20 amino acids
Output: ammonia + one or more of the following:
pyruvate, oxaloacetate, α-ketoglutarate, succinate and fumarate (from glucogenic amino acids - see notes 1 & 2), acetyl CoA, acetoacetate (from ketogenic amino acids - see notes 2 & 3).

LOCATION: Especially important in skeletal muscle and the liver.

FUNCTION: To use the carbon skeletons of amino acids for energy production and for gluconeogenesis.

CHARACTERISTICS: Some carbon skeletons, freed of α-amino groups, can funnel into the TCA cycle as acetyl CoA. These carbons cannot contribute a net synthesis of oxaloacetate or glucose. Other carbons from amino acids enter as α-keto acid intermediates of the TCA cycle, or as pyruvate. Each of these can contribute to glucose synthesis (see Sect. 10 for Gluconeogenesis).

SUMMARY OF AMINO ACID CATABOLISM:

1. Amino acids yielding pyruvate:
   Alanine (via transamination).
   Cysteine (via loss of sulfur).
   Serine (via a loss of $H_2O$ and $NH_3$).
   Glycine (via conversion to serine).
   Threonine (via glycine and acetyl CoA).
   Tryptophan (via alanine).
2. Amino acids yielding succinate:
   Isoleucine, Methionine (via oxidation to 3C propionyl CoA, carboxylation to 4C methylmalonyl CoA which is transformed to succinyl CoA).
   Valine (oxidation to methylmalonyl CoA).
   Threonine (loss of $H_2O$ and oxidation to propionyl CoA or conversion to glycine).
3. Amino acids yielding fumarate:
   Phenylalanine → Tyrosine (via oxidation to fumarate and acetoacetate).
   Aspartate → Fumarate (via argininosuccinate from urea cycle)
4. Amino acids yielding oxaloacetate:
   Asparagine → Aspartate (via hydrolysis) → oxaloacetate (via transamination).
5. Amino acids yielding α-ketoglutarate:
   Glutamine → Glutamate (via hydrolysis) → α-ketoglutarate (via transamination or oxidative deamination).
   Proline (via oxidation to glutamate).
   Arginine → Ornithine → α-ketoglutarate (via transamination, oxidation, and another transamination).
   Histidine (via deamination, oxidation to glutamate).
6. Amino acids forming acetyl CoA and/or acetoacetate:
   Leucine (via acetyl CoA + acetoacetate).
   Isoleucine (via acetyl CoA + propionyl CoA).
   Lysine (via acetoacetyl CoA). Neither amino group is transaminated.
   Tryptophan (via acetoacetyl CoA + formate + alanine).

NOTES:

1. Glucogenic amino acids: Alanine, arginine, asparagine, aspartate, cysteine, glutamine, glutamate, glycine, histidine, methionine, proline, serine, threonine, valine.
2. Glucogenic and ketogenic amino acids: Isoleucine, phenylalanine, tryptophan, tyrosine.
3. Ketogenic amino acids: Leucine, lysine.
4. Other uses of carbon skeletons:

   (a) Metabolism of propionyl CoA: In the conversion of 3C propionyl CoA to succinyl CoA, a *carboxylase* first forms 4C methylmalonyl CoA. Its carbon chain is straightened to form succinyl CoA by a $B_{12}$-requiring enzyme, *methylmalonyl CoA mutase*. This is one of the two known $B_{12}$-dependent steps in humans.

   (b) Methionine: Methionine is activated by ATP to form the universal donor of methyl groups, *S*-adenosylmethionine. Methionine is regenerated by methyl tetrahydrofolate and methylcobalamin coenzyme in the second $B_{12}$-requiring step of animal metabolism. Methionine also forms cysteine.

   (c) Tryptophan: About 3% of tryptophan is converted to niacin (vitamin $B_3$) in the body. However, this amount is inadequate to meet metabolic needs and so niacin stays classified as a vitamin. Tryptophan also is the precursor of the neurotransmitter, serotonin.

   (d) Tyrosine: Tyrosine is the precursor of the catecholamines dopa, dopamine, norepinephrine, and epinephrine. It is also the precursor of thyroid hormones and melanin.

   (e) Heme: Glycine and succinyl CoA are heme building blocks.

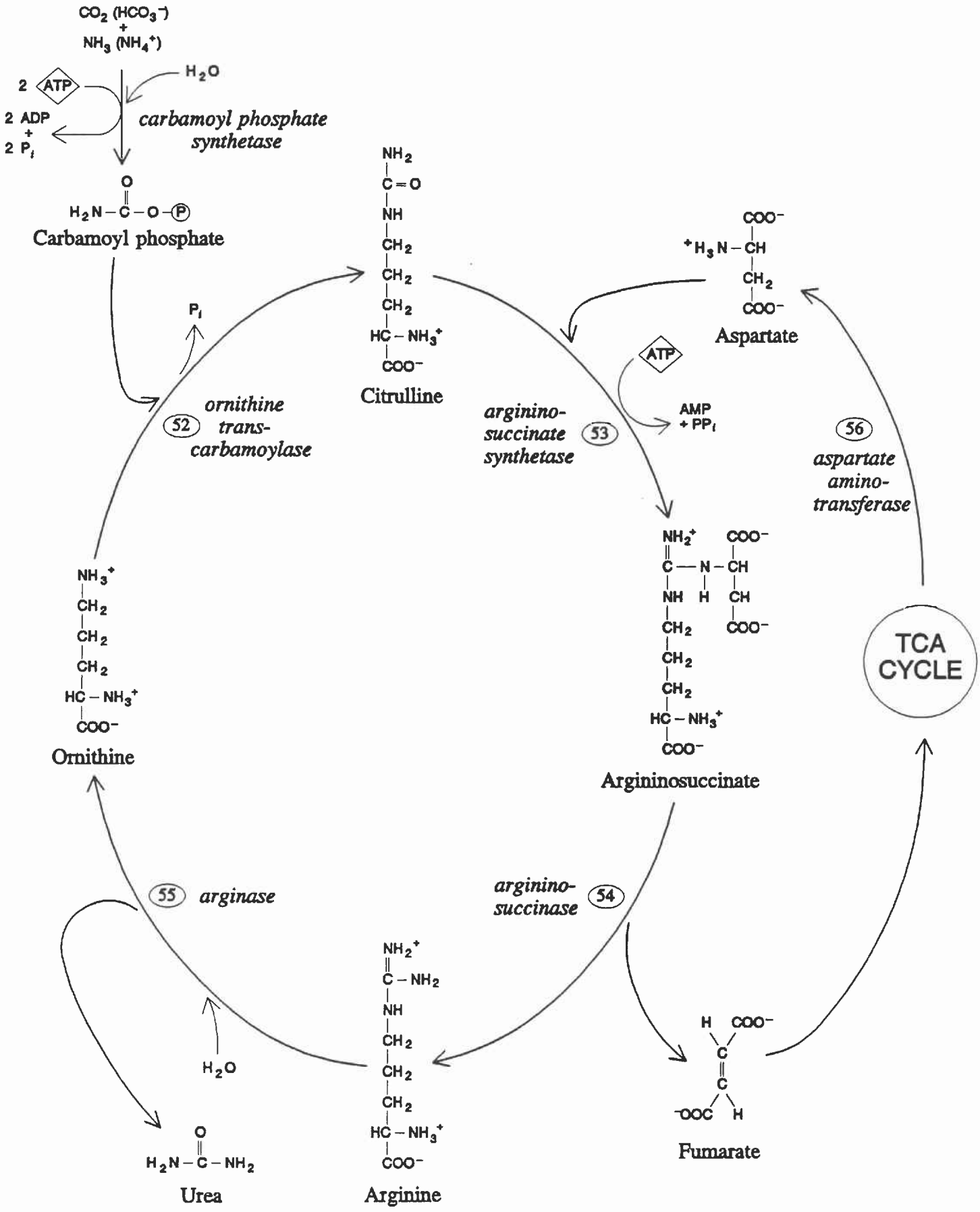
   (f) Folic acid: Tetrahydrofolate carries single carbon fragments from serine, glycine, histidine, and tryptophan metabolism to synthesize purines and thymine, as well as to regenerate methionine.

   (g) Carnitine: Lysine forms its backbone and methionine *S*-methyl group supplies the *N*-methyl groups (refer to *carnitine acyltransferase*, Sect. 12.II).

REVIEW QUESTIONS:

1. Explain why proline is a glucogenic amino acid.
2. Explain why leucine is a ketogenic amino acid. The aromatic amino acids tryptophan, tyrosine, and phenylalanine are both ketogenic and glucogenic. True or false?
3. Distinguish between essential and non-essential amino acids.
4. Describe precursors which yield aspartate, glutamate, and alanine by transamination.
5. Describe in general terms the reactants, products, and cofactor requirements of aminotransferases.
6. Under what conditions could tyrosine and cysteine become dietary essential amino acids?

# 16.1 Urea Cycle (Liver)



## Section 16: UREA CYCLE

Input: $CO_2$ + $NH_4^+$ + aspartate + 3 ATP + 2 $H_2O$
Output: urea + fumarate + 2 ADP + 2 $P_i$ + AMP + $PP_i$

LOCATION: The liver. Predominantly a cytoplasmic process.

FUNCTION: To convert ammonia to non-toxic urea. Ammonia is produced by oxidative deamination of glutamate (Sect. 15.I.B).

CHARACTERISTICS: In terrestrial vertebrates, urea is the nitrogenous end product of amino acid metabolism. It is both water-soluble and nontoxic. Urea represents 90% of the nitrogen excreted in urine. The nitrogen atoms of urea are derived from two sources: ammonia and aspartate. The carbon comes from $HCO_3^-$ (bicarbonate).

STEPS:

1. Formation of aspartate: Enzyme 56, ✓*aspartate aminotransferase*, synthesizes aspartate from oxaloacetate (Sect. 15.I.A).
2. Formation of carbamoyl phosphate: In mitochondria, ammonia and bicarbonate form an activated intermediate, carbamoyl phosphate, in a reaction catalyzed by *carbamoyl phosphate synthetase*. Two high energy bonds are hydrolyzed to drive the reaction.
3. Formation of citrulline: Enzyme 52, *ornithine transcarbamoylase*, catalyzes the transfer of the carbamoyl group to ornithine. Cleavage of the high energy phosphate group as $P_i$, drives this reaction to completion. Citrulline, the product, is transported to the cytoplasm where subsequent reactions occur.
4. Formation of argininosuccinate: Enzyme 53, *argininosuccinate synthetase*, catalyzes the condensation of citrulline and aspartate. The $\alpha$-amino group of aspartate supplies the second nitrogen for urea synthesis. This reaction is ATP-driven.
5. Cleavage of argininosuccinate: Enzyme 54, *argininosuccinase*, releases arginine and fumarate from argininosuccinate. Arginine is the immediate precursor of urea. Fumarate joins the TCA cycle and is hydrated to malate.
6. Cleavage of arginine: Enzyme 55, *arginase*, hydrolyzes arginine to ornithine and urea. *Arginase* is a liver-specific enzyme. Regeneration of the cycle is assured by ornithine formation.

NOTES:

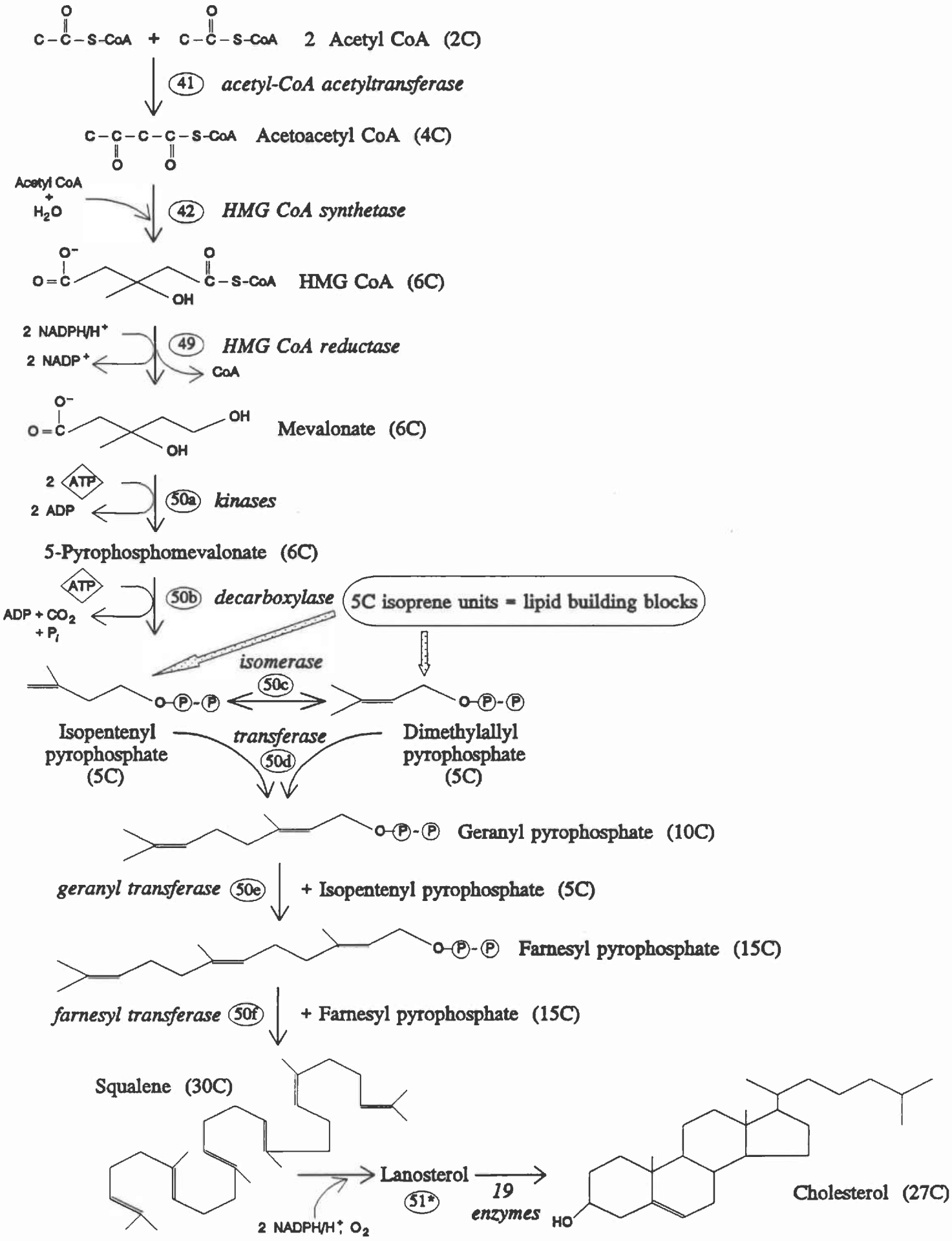
1. Regulation: After a high protein meal, or when amino acid breakdown increases, *N*-acetyl glutamate is synthesized from glutamate and acetyl CoA. This compound activates *carbamoyl phosphate synthetase* and stimulates urea synthesis. Other enzymes of the urea cycle are regulated by substrate availability.
2. Ornithine is an analog of the basic amino acid, lysine: Unlike lysine, ornithine is not incorporated into proteins. It serves as a carrier for the assembly of the constituents of urea. Ornithine is the workhorse of the urea cycle, much like oxaloacetate in the TCA cycle.
3. Linkage to the TCA cycle: Fumarate (output) links the urea cycle to the TCA cycle, as does oxaloacetate via aspartate (input).
4. Other sources of arginine: The kidney, intestinal mucosa, and liver, synthesize arginine, but only the liver contains *arginase*.

REVIEW QUESTIONS:

1. Describe the nitrogen precursors of urea.
2. Name the amino acid which is regenerated in the urea cycle, making it a cyclic process.
3. Name the amino acid which is both a protein building block and an integral part of the urea cycle.
4. Outline the steps in transforming ammonia, produced in skeletal muscle, to urea produced in the liver.

# NOTES

# 17.1 Cholesterol Synthesis



## I. CHOLESTEROL SYNTHESIS

Input*: acetyl CoA + ATP + NADPH + $O_2$ + $H_2O$
Output: cholesterol + ADP + $P_i$ + $NADP^+$ + CoA + $CO_2$
*This very complex biosynthetic pathway requires many of these building blocks.

LOCATION: Many tissues, especially the liver, intestine, adrenal cortex, ovaries, testes, and placenta. The pathway is located in the cytoplasm.

FUNCTIONS: Cholesterol is a membrane building block, and the precursor of steroid hormones, vitamin D, and bile salts (quantitatively the major end product of cholesterol metabolism).

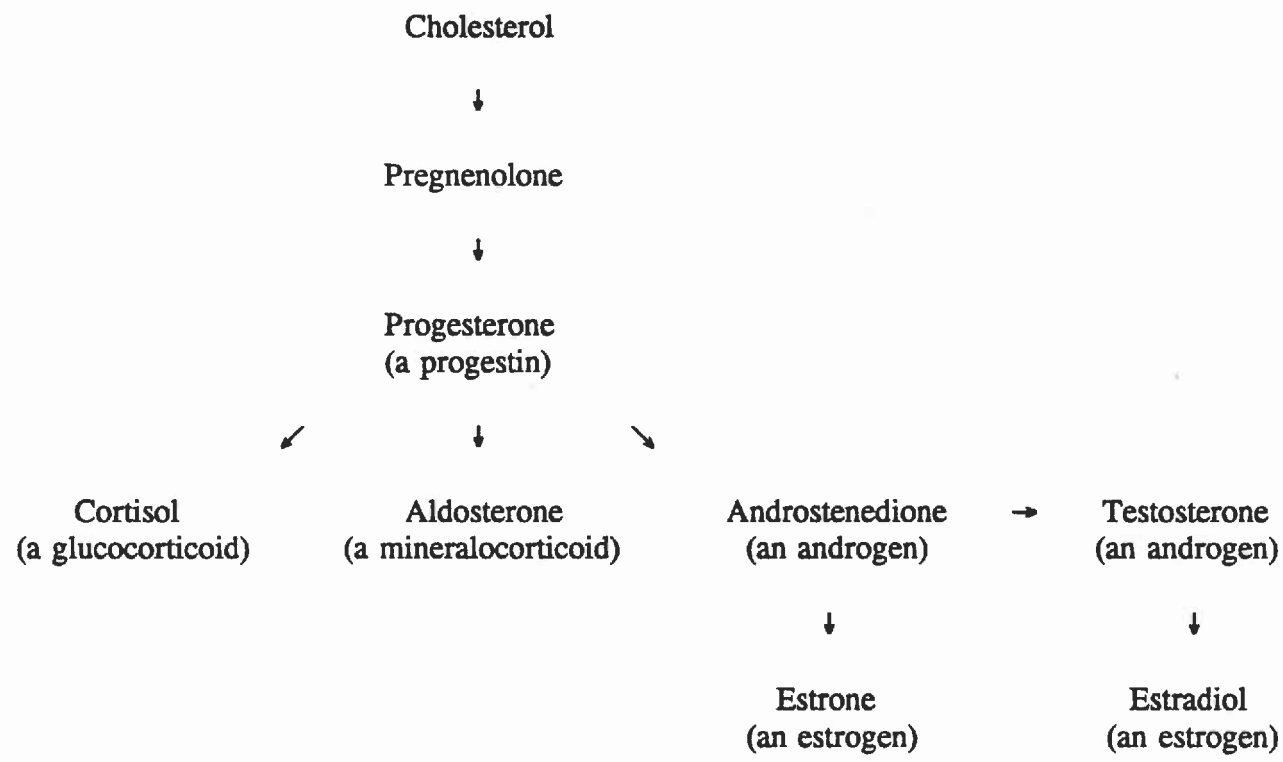
CHARACTERISTICS: All carbons come from acetyl CoA. Reduction requires NADPH. The first two steps of the pathway, leading to synthesis of HMG CoA, resemble ketone body synthesis of liver mitochondria. The initial steps of the pathway synthesize a universal 5C lipid building block, an activated isoprene. Two 5C units combine to form a 10C intermediate. Addition of another 5C unit creates a 15C intermediate. Two 15C units combine to form 30C squalene. Squalene is cyclized, in steps requiring $O_2$ and NADPH, to cholesterol.

STEPS:

1. Conversion of acetyl CoA to HMG CoA (3-hydroxy-3-methylglutaryl CoA): This requires Enzyme 41, *acetyl-CoA acetyltransferase*. This *thiolase* is cytoplasmic, in contrast to liver mitochondrial *acetyl-CoA acetyltransferase* of ketone body synthesis. Enzyme 42, *HMG CoA synthetase*, adds acetyl CoA to acetoacetyl CoA.
2. Reduction of HMG CoA to mevalonate: Enzyme 49, ✓*HMG CoA reductase*, is the key regulatory enzyme of cholesterol synthesis (refer to NOTES). The product is 6C mevalonate.
3. Formation of isoprene building blocks: Step 50a, two *kinases*, generate 5-pyrophosphomevalonate, an activated intermediate which is decarboxylated by Enzyme 50b, a *decarboxylase*, to produce isopentenyl pyrophosphate. This unsaturated isoprene building block is isomerized by Enzyme 50c, an *isomerase*, to a second isoprenoid. Subsequent steps are condensations of these units.
4. Formation of geranyl pyrophosphate (10C): Enzyme 50d, *transferase*, combines two 5C isoprene units, isopentenyl pyrophosphate and dimethylallyl pyrophosphate.
5. Formation of farnesyl pyrophosphate (15C): Enzyme 50e, *geranyl transferase*, adds isopentenyl pyrophosphate (5C) to geranyl pyrophosphate (10C).
6. Formation of squalene (30C): Enzyme 50f, *farnesyl transferase (squalene synthase)*, joins two molecules of farnesyl pyrophosphate tail-to-tail. Presqualene pyrophosphate is an intermediate of this molecular rearrangement.
7. Sterol synthesis: Step 51a represents a complex sequence in which straight-chain squalene forms the four fused rings of sterols. Oxidation of squalene by *squalene monooxygenase*, a mixed-function *oxidase* requiring NADPH and $O_2$, creates a highly reactive epoxide. This promotes a series of electron shifts resulting in ring closures. Following ring closure, hydrogen and methyl group shifts create lanosterol, an intermediate sterol.
8. Cholesterol synthesis from lanosterol: Step 51b represents a complex series of reactions whose details are unclear. By means of about nineteen different steps, three methyl groups are lost and a double bond is reduced by NADPH. The maturation of cholesterol occurs on the endoplasmic reticulum.

NOTES:

1. Regulation: *HMG CoA reductase* is tightly regulated.
   (a) Glucagon favors the inactivation of this enzyme, shutting down cholesterol synthesis. On the other hand, insulin favors the active form of the *reductase* and increases cholesterol synthesis. Increased levels of cyclic AMP activate *protein kinase*, which in turn activates *HMG CoA reductase kinase*. This *kinase* inactivates the non-phosphorylated *reductase* by transferring a phosphate group from ATP. *Phosphoprotein phosphatase* reverses these processes. The process is analogous to the activation/inhibition of *glycogen phosphorylase* (Sect. 8.III).
   (b) Dietary cholesterol suppresses *HMG CoA reductase* in the liver, a primary site of cholesterol synthesis. An increased level of cholesterol also leads to *protein kinase* activation.
   (c) Cholesterol uptake by cells also regulates cholesterol synthesis (Sect. 17.II).
2. Certain drugs block cholesterol synthesis: The second generation of drugs designed to lower blood cholesterol, such as Mevacor (lovastatin), inhibit *HMG CoA reductase*.
3. Hormone production: Cholesterol is the precursor of the five major classes of steroid hormones: progestins, glucocorticoids, mineralocorticoids, androgens, and estrogens.

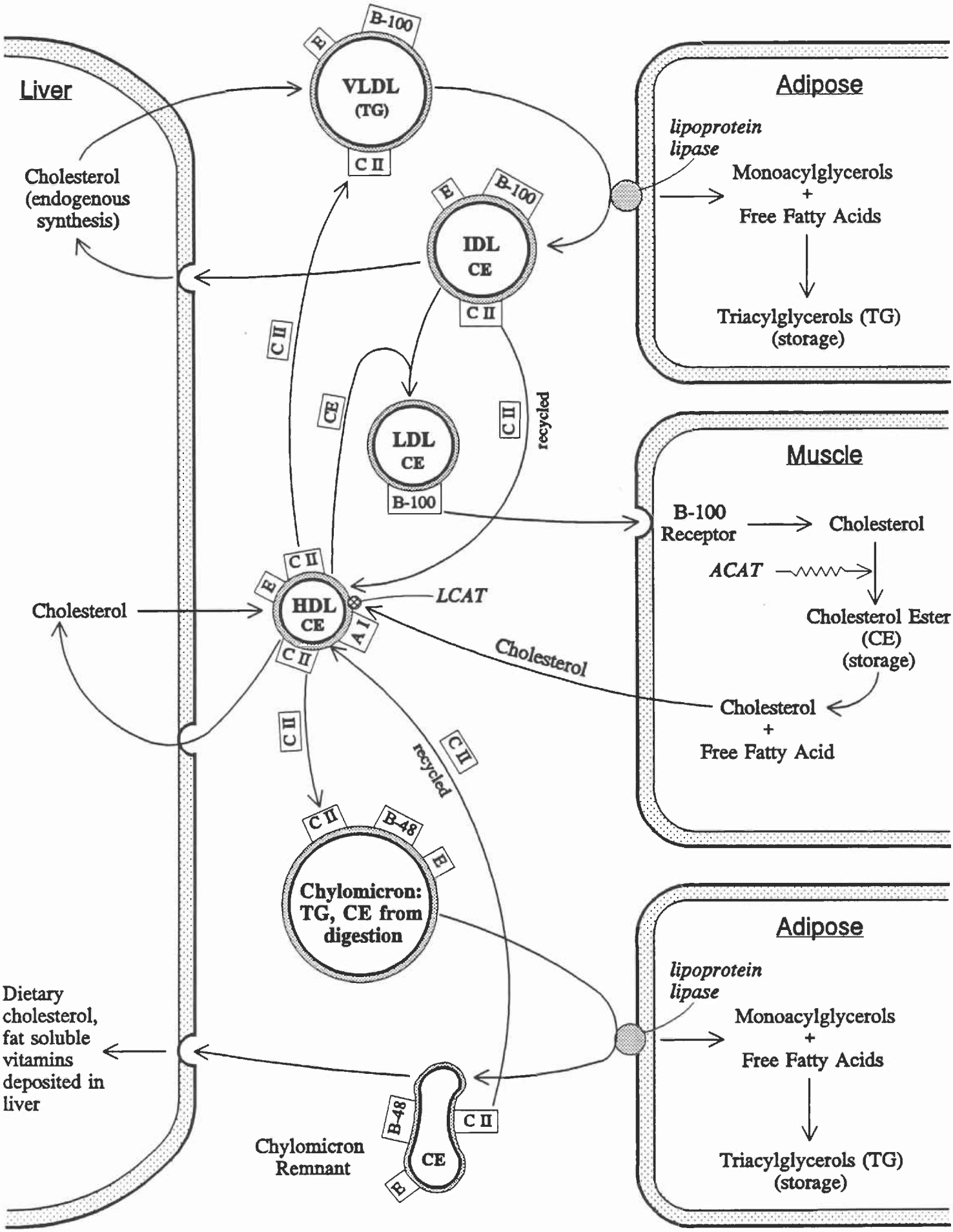



REVIEW QUESTIONS:

1. Name the initial precursors of cholesterol.
2. List classes of compounds formed from cholesterol.
3. Name the intermediate common to cholesterol synthesis and ketone body formation.
4. Describe the effects of elevated levels of the following on cholesterol synthesis: (a) cholesterol, (b) glucagon, (c) insulin.
5. Identify the key regulatory step in cholesterol synthesis.
6. Arrange the following intermediates according to increasing size: mevalonate, squalene, farnesyl pyrophosphate, lanosterol, acetoacetyl CoA.

# NOTES

# 17.2 Lipoprotein Metabolism



## II. LIPOPROTEIN METABOLISM

### A. BASIC FACTS

LIPOPROTEINS: To transport lipids, which are essentially hydrophobic, in an aqueous environment like blood, lipids are emulsified. These micelles are designated lipoproteins. All lipoproteins have the same general composition: an oily core of triacylglycerols and cholesterol ester. The shell is a monolayer of phospholipid emulsifiers and unesterified cholesterol. In addition to lipids, these complexes contain one or more "apolipoproteins," which determine the fate of each type of lipoprotein.

APOLIPOPROTEINS: Major families include: Apo A, Apo B, Apo C and Apo E. Apo A-I activates ✓*LCAT (Lecithin-cholesterol acyltransferase)*. Apo B-48 is a ligand for binding chylomicron remnants to liver cells. Apo B-100 and Apo E are ligands for the LDL receptor responsible for LDL uptake by peripheral tissues. Apo C-II activates *lipoprotein lipase*.

CLASSIFICATION: Three major classes of lipoproteins are named according to their different densities. Lipids have a lower density than proteins. The greater the lipid content, the lower the density. The greater the protein content, the higher the density. Thus, VLDL (Very Low-Density Lipoproteins) and LDL (Low-Density Lipoproteins) contain more lipid (and less protein) than HDL (High-Density Lipoproteins).

### B. CHYLOMICRONS

ORIGIN: Chylomicrons originate in the intestinal mucosa. Intestinal epithelia reform triacylglycerols from the digestion products, fatty acids and 2-monoacylglycerols. Some of the dietary cholesterol is converted to cholesterol ester. Chylomicrons are exocytosed into the lymph system, which carries them to the bloodstream via the thoracic duct. In their maturation, chylomicrons receive Apo C-II from HDL.

FUNCTION: Chylomicrons contain 80 - 95% triacylglycerols and 2 - 4% cholesterol ester. They are the largest of the lipoproteins and have the greatest triacylglycerol content. Chylomicrons carry dietary triacylglycerols, fat-soluble vitamins, and cholesterol to peripheral tissues and to the liver. The half-life of chylomicrons is very short, about five minutes.

FATE: Refer to Figure 17.2 to visualize the following:

1. Adipose tissue: With Apo C-II, chylomicrons can interact with Enzyme 36, ✓*lipase (lipoprotein)*, in the capillary bed. This *lipase* hydrolyzes triacylglycerols to free fatty acids and 2-monoacylglycerols, which are taken up and converted back to triacylglycerols for storage.
2. Liver: Cholesterol-rich chylomicron remnants recycle Apo C-II back to HDL. Apo B-48 binds to receptors on liver cells, which engulf chylomicron remnants. Cholesterol ester (and fat-soluble vitamins) is then either processed or exported from the liver.

### C. VLDL (Very Low-Density Lipoprotein)

ORIGIN: VLDL originates in the liver. Maturation requires receiving Apo C-II from HDL.

FUNCTION: VLDL contains 45 - 65% triacylglycerols, 16 - 22% cholesterol ester, and 4 - 8% free cholesterol. It transports endogenously synthesized triacylglycerols to adipose and other tissues. VLDL is transformed to LDL. The half-life of VLDL and remnants (IDL) is about six hours.

FATE: Much like chylomicrons, VLDL requires Apo C-II to activate capillary *lipoprotein lipase*. *Lipase* releases free fatty acids, which are then taken up by adipose tissue and stored as triacylglycerols. The remnant is IDL (Intermediate-Density Lipoprotein). IDL is either taken up by the liver or is converted to LDL (as described below).

D. **LDL** (Low-Density Lipoprotein)

ORIGIN: LDL is derived from the VLDL remnant (IDL). Formation of LDL from IDL requires the removal of apolipoproteins, leaving only Apo B-100. HDL also donates cholesterol esters to fill up the LDL micelle.

FUNCTION: LDL contains 45 - 50% cholesterol ester and 6 - 8% free cholesterol. Linoleate is the most common fatty acyl chain in cholesterol ester. It transports cholesterol to peripheral tissues. Most of their cholesterol is supplied by LDL.

FATE: LDL is engulfed by cells via receptor-mediated endocytosis. Target cells possess receptors which specifically bind Apo B-100. The engulfed LDL is hydrolyzed by lysosomes. Freed cholesterol can partake in membrane biosynthesis. It is also converted to cholesterol ester for storage by ✓*ACAT (acyl CoA-cholesterol acyltransferase)*.

Regulation: Excess cholesterol in target cells slows endogenous cholesterol synthesis by blocking *HMG CoA reductase* (enzyme 49, Sect. 17.1), and repressing Apo B-100 receptor synthesis.

Familial hypercholesterolemia, a high concentration of LDL-cholesterol in the plasma, arises from a deficiency of LDL receptors or because of various problems in function or location of the receptors. Because of the elevated levels of cholesterol, nodules of cholesterol (xanthomas) form in the skin and other areas. The excess cholesterol can also form arterial plaques, producing atherosclerosis, leading to heart attacks, strokes, and peripheral vascular disease.

E. **HDL** (High-Density Lipoprotein)

ORIGIN: Hepatic synthesis.

FUNCTION: HDL plays a dynamic role in cholesterol homeostasis. It takes up surface material, such as Apo C-II, from IDL and chylomicron remnants, and picks up cholesterol from peripheral tissues.

FATE: HDL acquires cholesterol and stores it as cholesterol ester. Apo A-I on HDL activates *LCAT* to convert free cholesterol to esterified cholesterol, which moves to the oily core of HDL. In this process, a smaller precursor form is converted to larger, cholesterol-rich HDL. HDL also delivers cholesterol ester to the liver. *Hepatic lipase* can hydrolyze cholesterol esters received from HDL. Cholesterol ester is also unloaded to IDL to form mature LDL. HDL shuttles Apo C-II to chylomicrons and VLDL. HDL receives Apo C-II from remnants (IDL and chylomicron remnants).

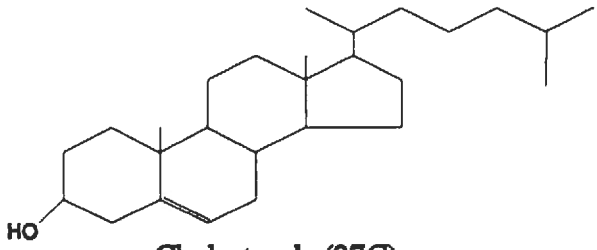
REVIEW QUESTIONS:

1. Identify the lipoproteins which are responsible for transporting triacylglycerols.
2. Describe the function of *lipoprotein lipase*.
3. Describe the function of *LCAT (lecithin-cholesterol acyltransferase)*.
4. Describe the fate of LDL.
5. Describe the functions of HDL.

# NOTES

# 17.3 Bile Salt Formation (Liver)

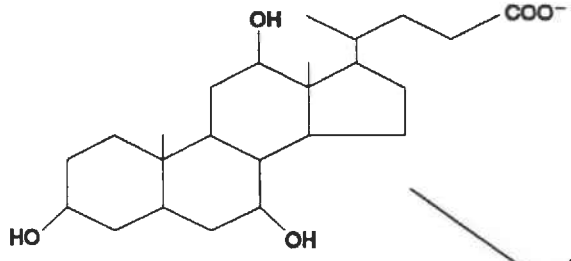
*NOTE: For simplicity, only the cholate pathway is shown. A similar pathway exists for chenodeoxycholate.




Cholesterol (27C)

NADPH

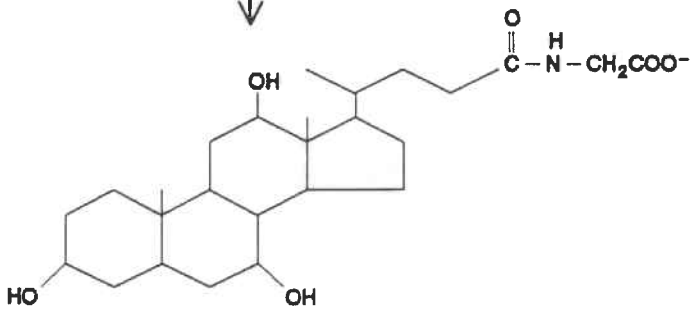
Several Steps



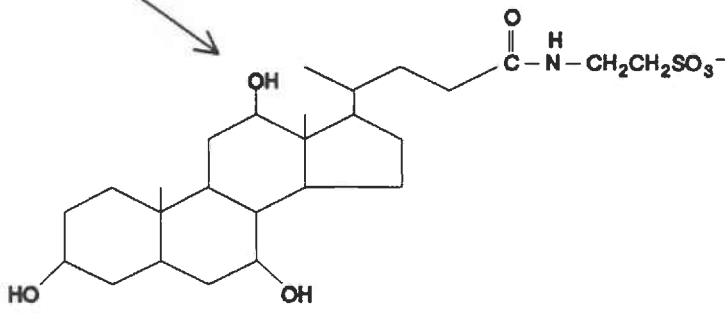

Cholate*
(a primary bile acid)

'Conjugation' with taurine

'Conjugation' with glycine




Glycocholate (a bile salt)




Taurocholate (a bile salt)

Microbial modification

Microbial modification

Deoxycholate
(a secondary bile acid)

Lithocholate
(a secondary bile acid)

## III. BILE SALT FORMATION

LOCATION: The liver.

FUNCTION: Bile salts are the main degradative metabolic end products of cholesterol metabolism. Steroid hormones account for only a small percentage of cholesterol usage.

CHARACTERISTICS: Bile salts are amphipathic molecules, i.e. they have a polar, hydrophilic face, and a hydrophobic back-surface. They act as detergents to emulsify dietary triacylglycerols, fat-soluble vitamins, and dietary cholesterol. Emulsification is essential for digestion by *pancreatic lipases* and subsequent absorption of these nutrients.

### A. PRIMARY BILE ACIDS

Input: cholesterol + $O_2$ + NADPH
Output: cholate and chenodeoxycholate (primary bile acids)

CHARACTERISTICS: Degradation of cholesterol to cholate and chenodeoxycholate involves several enzymes responsible for hydroxylations and oxidations to create a carboxyl group.

### B. BILE SALTS

Input: cholate & chenodeoxycholate + ATP + glycine (or taurine)
Output: glycocholate & glycochenodeoxycholate (or taurocholate & taurochenodeoxycholate) + ADP + $P_i$

CHARACTERISTICS: Before leaving the liver, the carboxyl group of cholate is conjugated with either glycine (forming glycocholate) or taurine (forming taurocholate). These additions create a more acidic (ionizable) form and increase the amphipathic nature of the bile salt. In a similar way, chenodeoxycholate is conjugated to form either glycochenodeoxycholate or taurochenodeoxycholate.

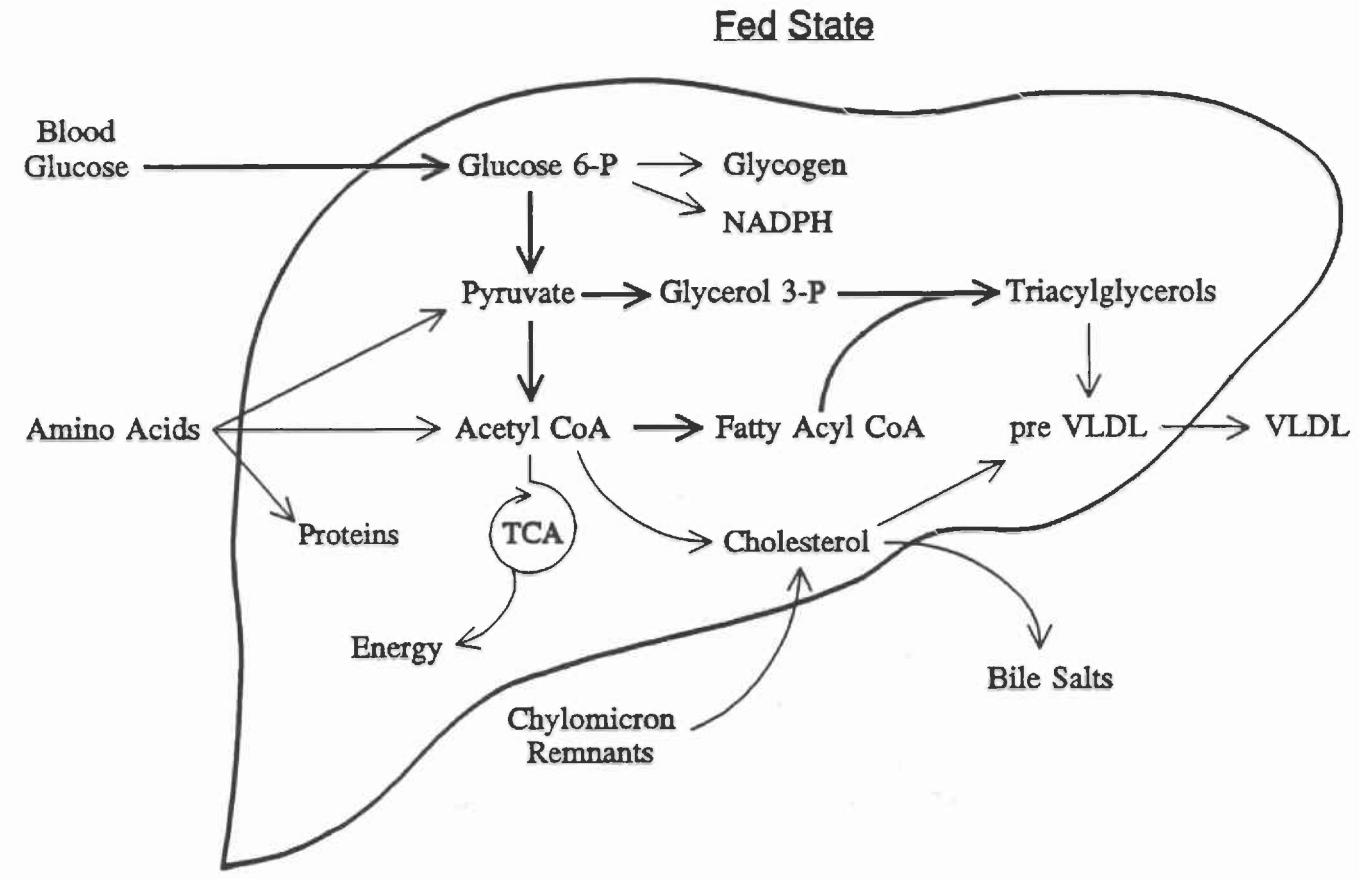
NOTES:

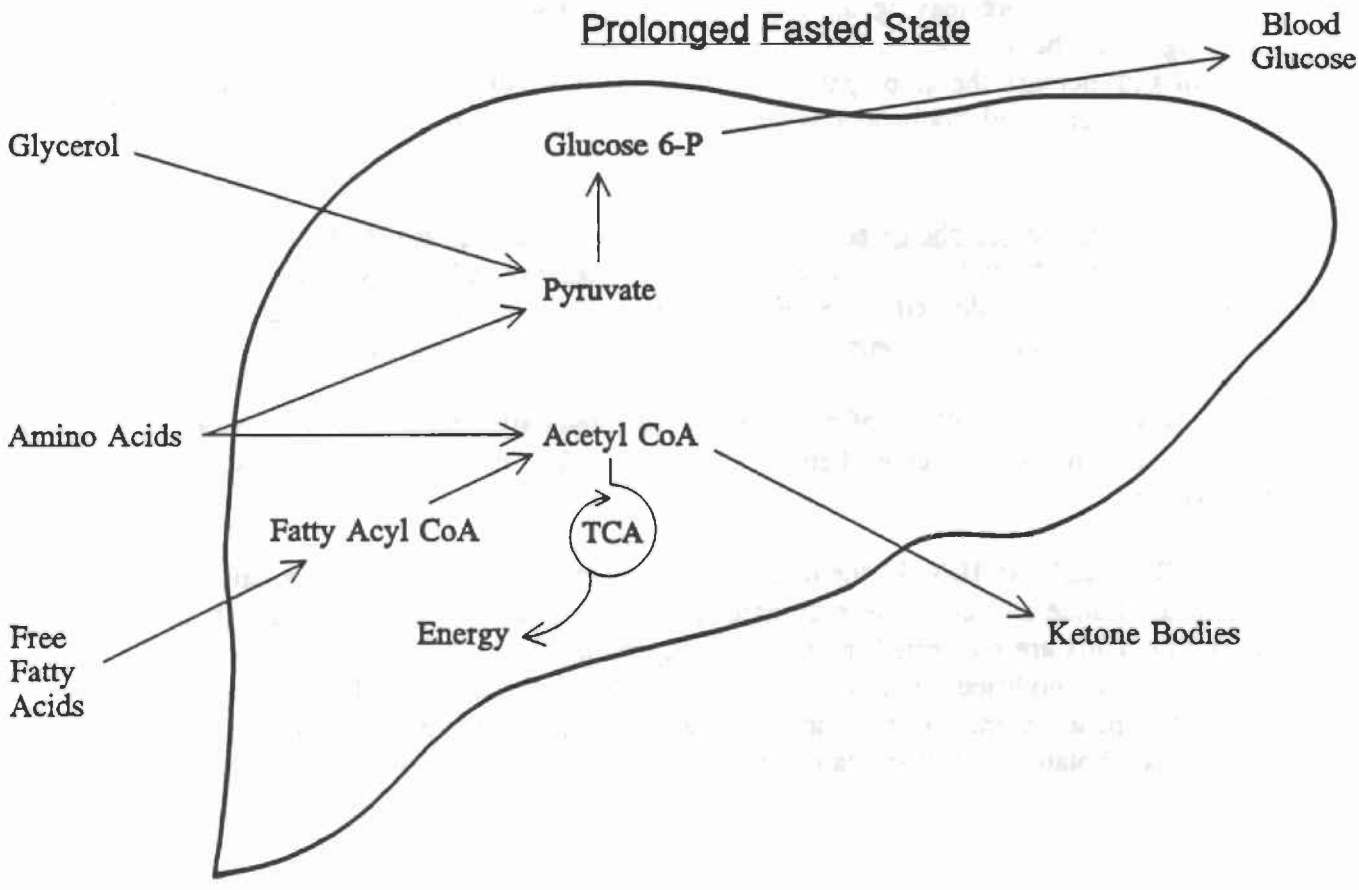
1. Conjugation of cholate (or chenodeoxycholate): This process requires ATP and CoA to form cholyl CoA (or chenodeoxycholyl CoA). This activated intermediate condenses with either glycine or taurine. Conjugation is a general detoxification mechanism used by the liver to increase water solubility of lipophilic end products and environmental pollutants. Other groups used for conjugation include sulfate and glucuronic acid.
2. Secretion: Between 15 and 30 gm of bile salts are secreted daily. Most of this is absorbed, transported via portal blood, and reused (enterohepatic circulation). The daily loss of 300 mg of bile salt is met by liver synthesis.

C. **SECONDARY BILE ACIDS**: In the distal ileum and colon, the bile salts are partially deconjugated by bacteria to cholate and chenodeoxycholate (primary bile acids). By bacterial dehydroxylation, the secondary bile acids are converted mainly to deoxycholate and lithocholate (secondary bile acids).

Bile salts are modified by intestinal bacteria. In forming deoxycholate and lithocholate, the conjugated groups leave and some of the hydroxyl groups are removed from the rings. Formed outside the liver, deoxycholate and lithocholate are therefore classified as secondary bile acids.

# 18.1 Overview of Liver Metabolism



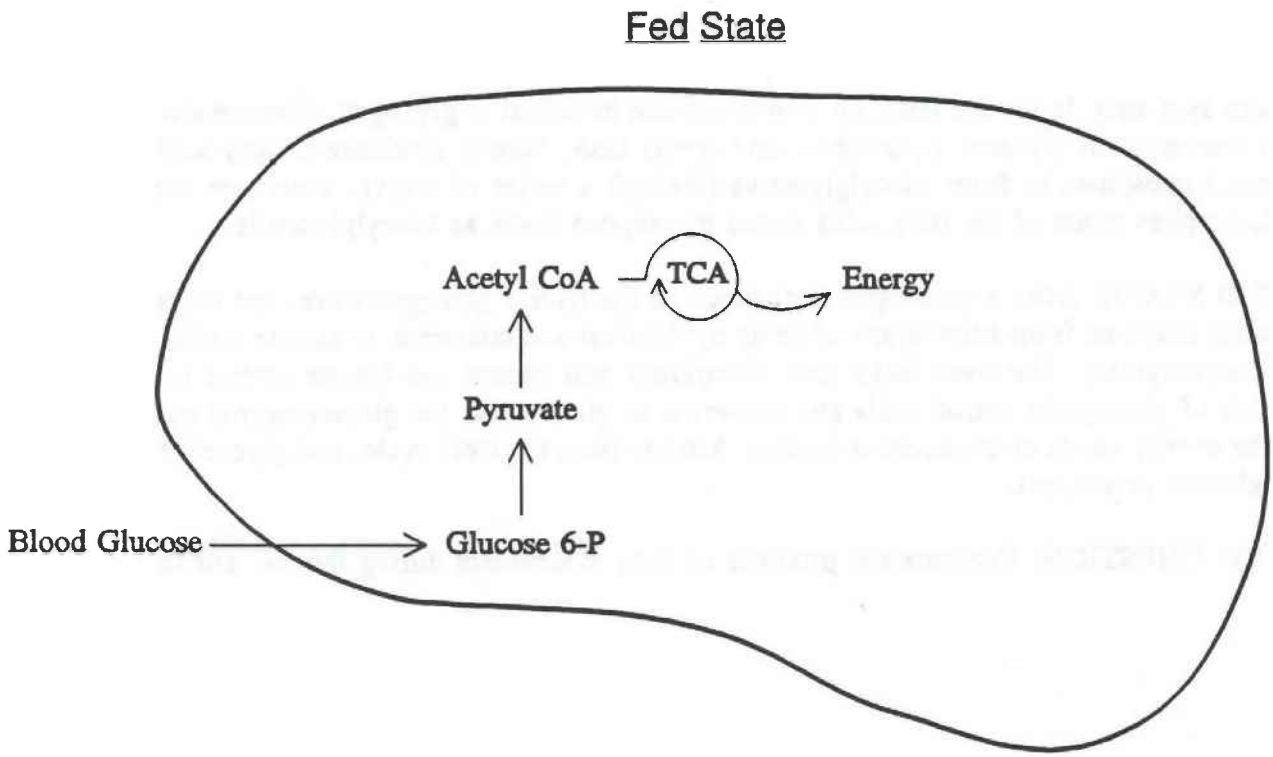
## I. LIVER METABOLISM

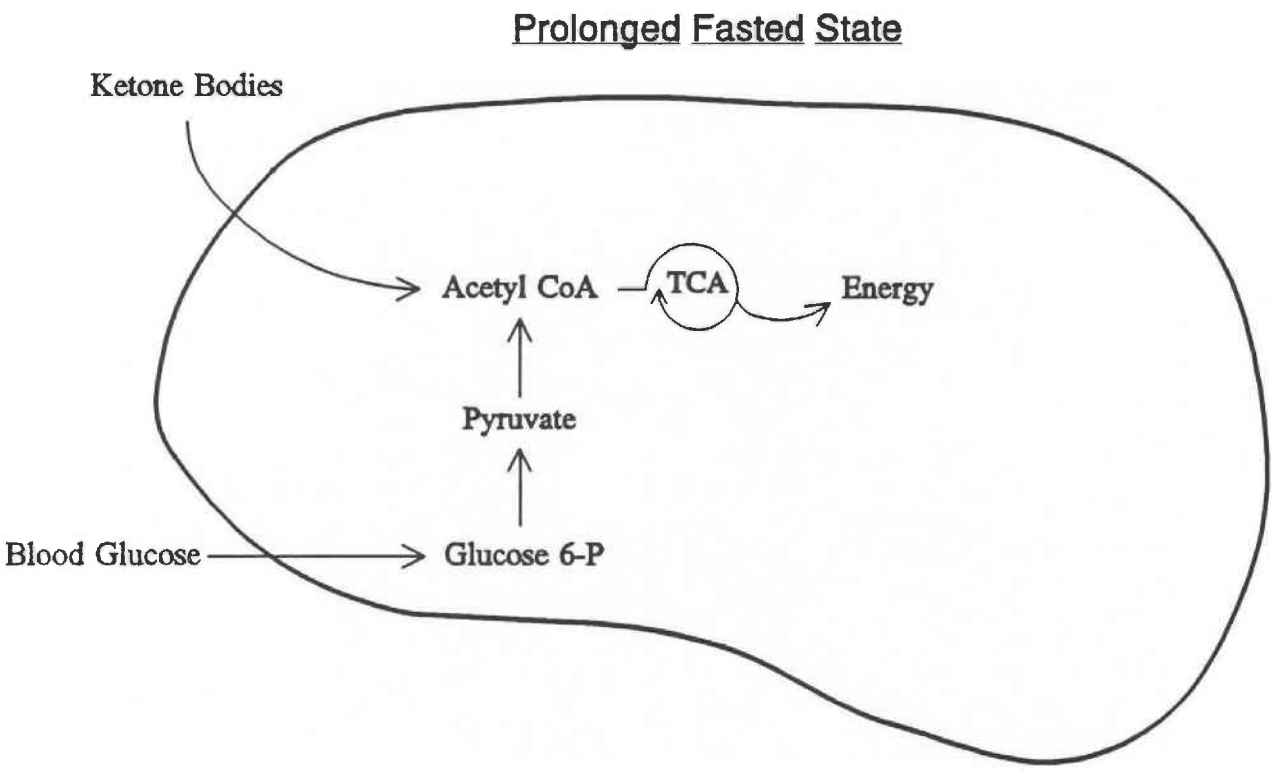
FED STATE: Recall that a key liver function is to provide fuel for the brain and other tissues. The liver meets its energy needs through oxidation of keto acids from amino acid metabolism, rather than by burning fatty acids, glucose, or ketone bodies. Glucose readily enters the liver without insulin, but insulin stimulates glycogen synthesis. In the fed state, up to 400 kcal can be stored as glycogen. Glucose also enters glycolysis and is converted to glycerol 3-phosphate and acetyl CoA. Newly synthesized fatty acyl CoA's link with glycerol 3-phosphate to form triacylglycerols (through a series of steps), which are exported as VLDL. VLDL supplies much of the fatty acids stored by adipose tissue as triacylglycerols.

FASTED STATE: After a prolonged fast, much of the liver's glycogen stores are depleted. Surplus free fatty acids liberated from adipose are taken up by the liver and converted to ketone bodies (acetoacetate and $\beta$-hydroxybutyrate). The liver lacks *CoA transferase* and cannot use ketone bodies for energy. Carbon skeletons of glucogenic amino acids are converted to glucose via the gluconeogenic pathway in order to meet the energy needs of the nervous system. Alanine from the Cori cycle, and glycerol from lipolysis, are other glucose precursors.

REVIEW QUESTION: Compare the products of liver metabolism during the fed and fasted states.

## 18.2 Overview of Brain Metabolism



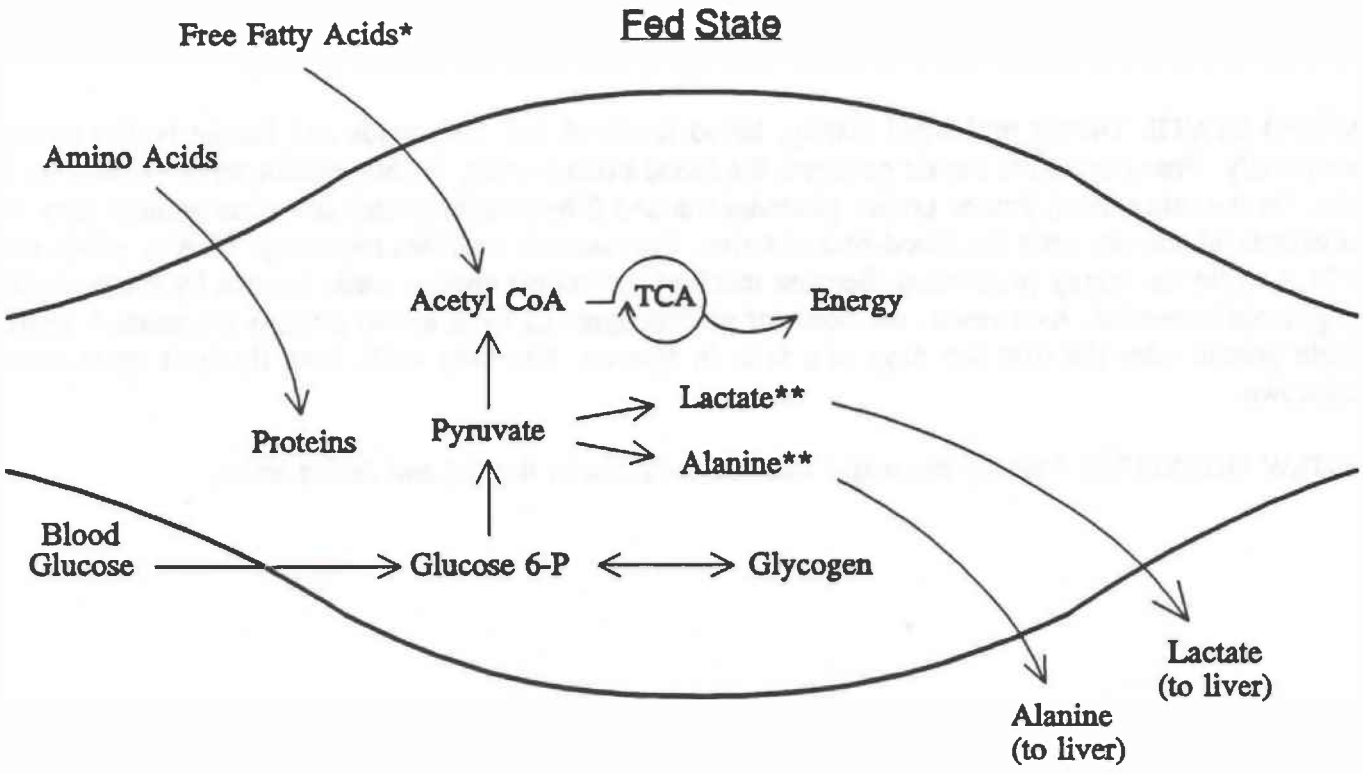
## II. BRAIN METABOLISM

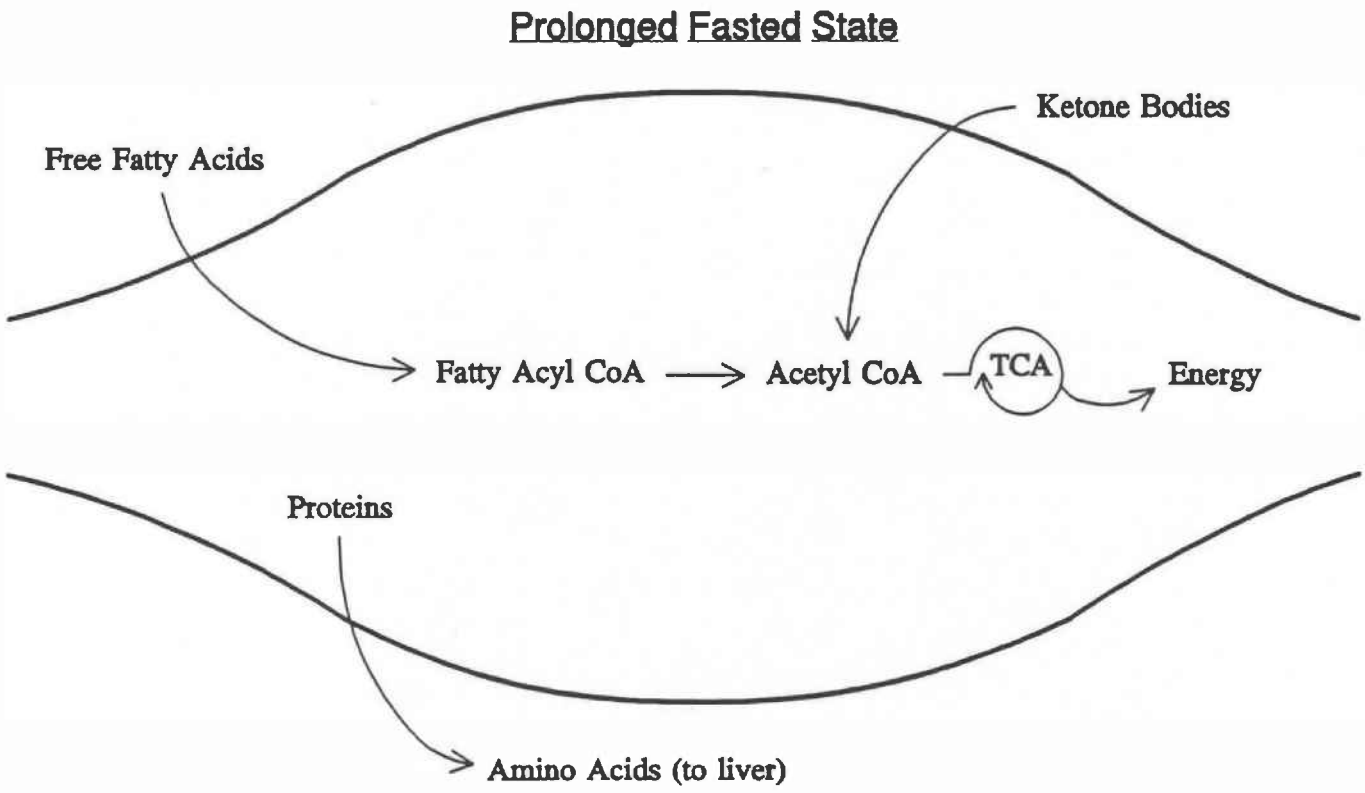
FED STATE: Glucose is the predominant fuel of the brain, even during a prolonged fast. The brain does not store glycogen or fat, and consequently requires adequate blood glucose at all times. The brain consumes about 120 grams of glucose daily. Glucose uptake does not require insulin.

FASTED STATE: During prolonged fasting, blood levels of free fatty acids and ketone bodies increase dramatically. Free fatty acids cannot penetrate the blood-brain barrier, so they cannot serve as fuels for the brain. On the other hand, ketone bodies (acetoacetate and $\beta$-hydroxybutyrate) are water-soluble fatty acid equivalents which can cross the blood-brain barrier. Acetoacetate provides two acetyl CoA's, which enter the TCA cycle for energy production. Because much of the brain's energy needs are met by ketone bodies, less glucose is needed. As a result, the need for gluconeogenesis from amino acids is diminished, sparing muscle protein after the first two days of a fast. In essence, free fatty acids from lipolysis spare muscle breakdown.

REVIEW QUESTION: Identify the major fuels of the brain in the fed and fasted states.

# 18.3 Overview of Muscle Metabolism




*Usage depends on degree of exertion. **Production and export depend on degree of exertion.

## III. SKELETAL MUSCLE METABOLISM

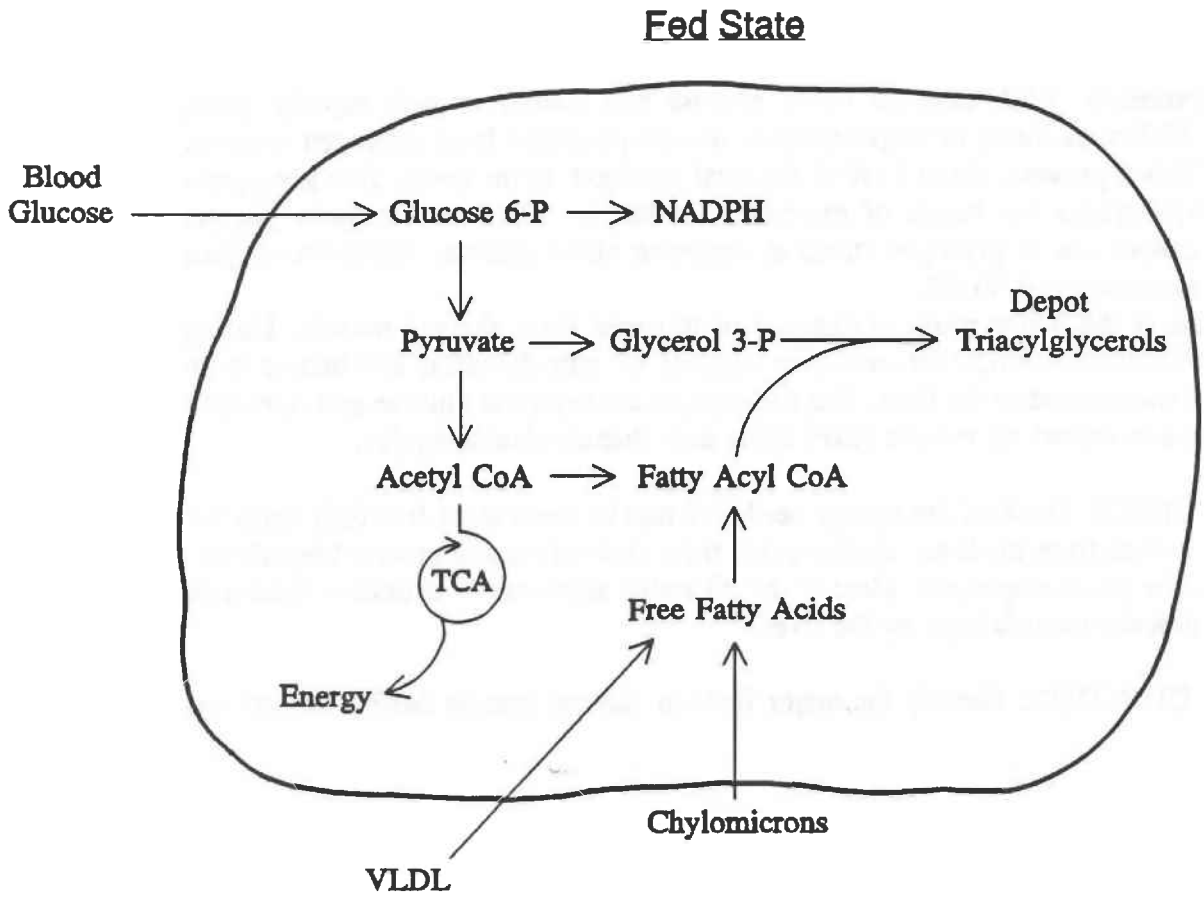
FED STATE: The major fuels for muscle are glucose and free fatty acids (depending upon the degree of physical exertion). With elevated blood glucose and insulin, muscle rapidly incorporates glucose into glycogen. Unlike the brain or adipose tissue, muscle possesses large glycogen reserves, up to 1200 kcal for an adult. This represents about 75% of the total glycogen in the body. This glycogen serves as a source of glucose 6-phosphate for bursts of exertion. Unlike the liver, muscle lacks *glucose 6-phosphatase* and therefore cannot use its glycogen stores to replenish blood glucose. *Lipoprotein lipase* releases fatty acids from chylomicrons and VLDL.

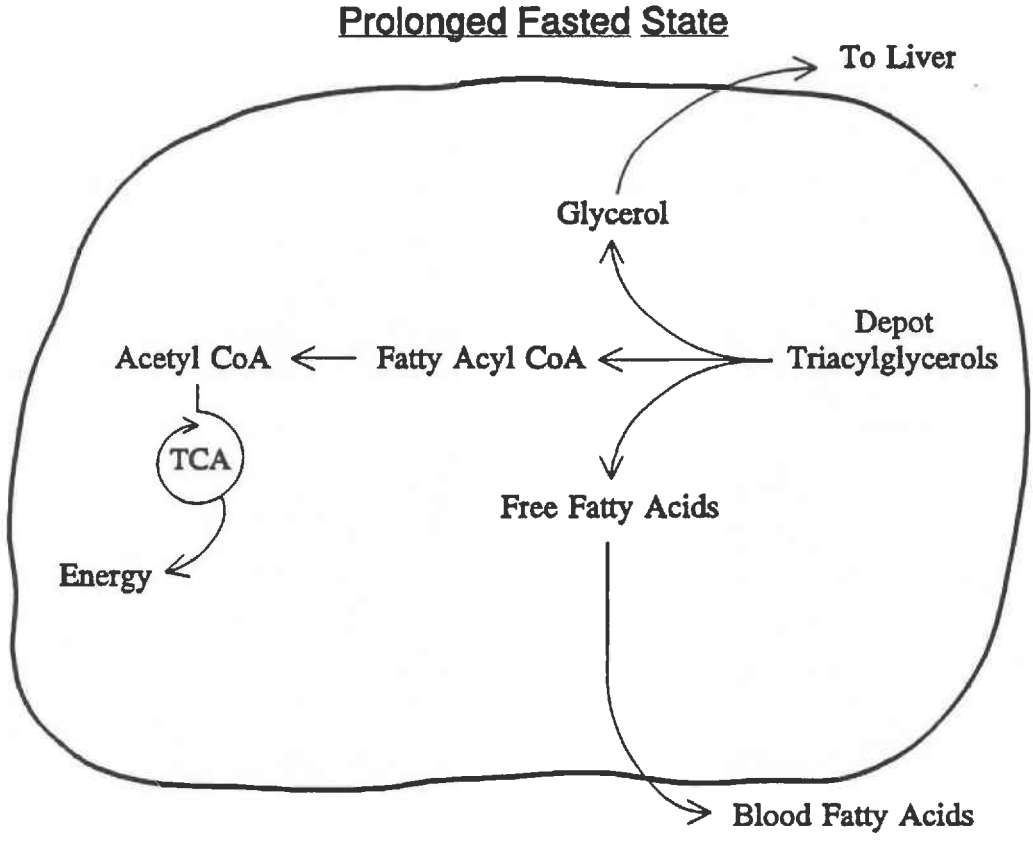
Alanine is the major route of disposal of nitrogen from skeletal muscle. During strenuous exertion, glucose utilization outstrips the oxidative capacity of mitochondria, and lactate is produced. Lactate and alanine are transported to the liver. The carbons are converted to glucose and released into the bloodstream. This glucose is reused by muscle (Cori cycle and glucose-alanine cycle).

FASTED STATE: Much of the energy needs are met by circulating free fatty acids from adipose tissue and by ketone bodies from the liver. Amino acids from skeletal muscle protein breakdown are the major source of carbons for gluconeogenesis. Most of the 20 amino acids can contribute at least a portion of their carbon chains to glucose manufacture by the liver.

REVIEW QUESTION: Identify the major fuels in skeletal muscle during the fed and fasted states.

## 18.4 Overview of Adipose Tissue Metabolism



## IV. ADIPOSE TISSUE METABOLISM

FED STATE: Huge amounts of energy are stored in adipose tissue. Typically 15 - 20% of the total body weight of an adult male may be adipose stores, equivalent to 100,000 - 150,000 kcal. Adult females store a higher percentage of triacylglycerols, typically 20 - 25% of total body weight.

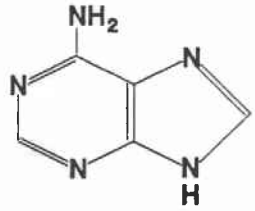
Surplus fuel is stored, not wasted. Free fatty acids are received from chylomicrons and VLDL via the action of *lipoprotein lipase*. They are reassembled as triacylglycerols for storage. With elevated blood glucose and insulin, adipose tissue also synthesizes triacylglycerols from glucose. Glucose must be available as a source of glycerol 3-phosphate for reesterification. Otherwise, turnover of triacylglycerols leads to the release of fatty acids into the bloodstream.

FASTED STATE: Under the influence of epinephrine and cortisol, depot triacylglycerols are hydrolyzed to free fatty acids and glycerol. Free fatty acids are rapidly taken up by other tissues, except the brain, and are used for fuel. Glycerol is converted to glucose by the liver. Surplus fatty acids are converted to ketone bodies in the liver.

REVIEW QUESTION: Compare the products of adipose metabolism during the fed and fasted states.

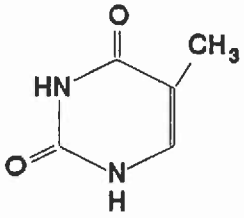
# 19.1 Nucleotide Highlights

Base

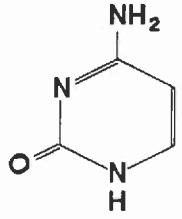


Adenine (A)
(RNA & DNA Purine)

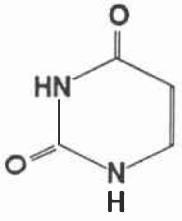
Guanine (G)
(RNA & DNA Purine)



Thymine (T)
(Primarily DNA Pyrimidine)

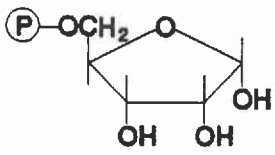


Cytosine (C)
(RNA & DNA Pyrimidine)

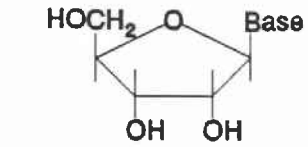


Uracil (U)
(RNA Pyrimidine)

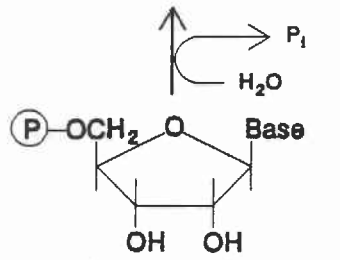
+



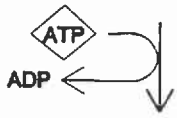
Ribose 5′-phosphate



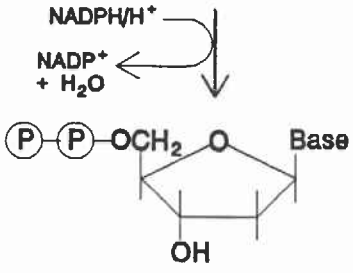
Ribonucleoside



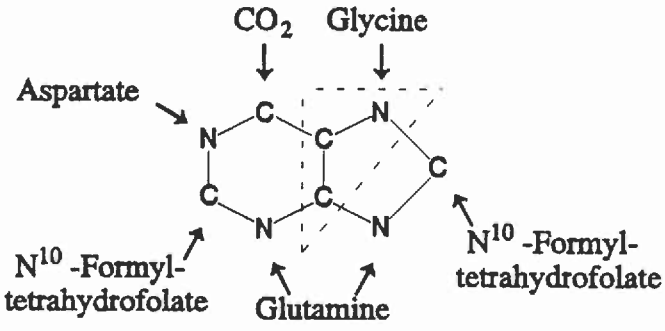
Ribonucleoside
5′-monphosphate (NMP)



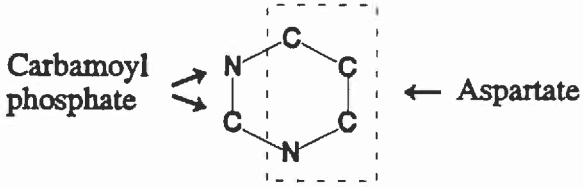
Ribonucleoside
5′-diphosphate (NDP)



2′-Deoxyribonucleoside
5′-diphosphate (dNDP)



Sources of the atoms in the purine ring.



Sources of the atoms in the pyrimidine ring.

**I. FUNCTIONS:** Nucleotides play important roles in metabolism. They are building blocks of RNA and DNA. They form activated intermediates in biosynthesis of polysaccharides. For example, UDP-glucose (uridine diphosphate glucose) is the precursor of glycogen. *S*-adenosylmethionine transfers methyl groups to synthesize choline, epinephrine, and other important compounds. ATP (adenosine triphosphate) is the "energy currency" of the cell. GTP (guanosine triphosphate) is an energy source for protein synthesis and for membrane-bound coupling systems, such as used in hormonal regulation. 3′, 5′ cyclic AMP is a ubiquitous intracellular messenger. Nucleotides are parts of the essential parts of Coenzyme A, FAD, $NAD^+$, and $NADP^+$.

When denoting the position of a carbon of the sugar in a nucleotide, a prime "′" is used, thus distinguishing it from the ring carbon of the base.

## II. CLASSIFICATION

1. Bases: The five major bases found in nucleotides are heterocyclic, aromatic amines. The purines are adenine and guanine. Inosine is the precursor. The pyrimidines are cytosine and thymine (DNA) or uracil (RNA). The purines (short name) are larger bases with two rings. In contrast, the pyrimidines (long name) represent the simpler structures with only one ring.
2. Nucleosides: Purines and pyrimidines are linked to ribose or 2-deoxyribose as *N*-glycosides at the 1′ carbon of the sugar. Ribose forms the ribonucleosides (RNA): adenosine, guanosine, cytidine, thymidine (only rRNA & tRNA), and uridine. If the sugar is deoxyribose, the deoxyribonucleosides (DNA) are formed: deoxyadenosine, deoxyguanosine, deoxycytidine, and deoxythymidine.
3. Nucleotides: Typically a phosphate group is esterified at the 5′ carbon of the pentose in nucleoside monophosphates (nucleotides). AMP is a typical example. In nucleoside diphosphates, a second phosphate group is attached to the first via an anhydride (pyrophosphate) linkage as in ADP. Nucleoside triphosphates, such as ATP (adenosine triphosphate), contain two pyrophosphate linkages.

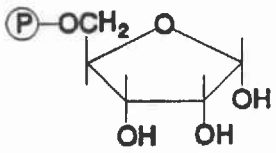
   Energetically, GTP, CTP, UTP, TTP, and ATP are equivalent. Each contains two highly active pyrophosphate bonds. They are used in biosynthetic processes, and they are RNA precursors. Their deoxyribose equivalents are designated as dGTP, dCTP, dTTP, and dATP. These triphosphates serve only as DNA precursors.
4. Nucleotide interconversion: ATP (and other nucleoside triphosphates) can react with nucleoside diphosphates (NDP) to form the equivalent triphosphate (NTP).

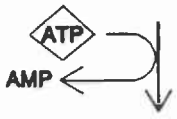
$$ATP + NDP \longleftrightarrow ADP + NTP$$

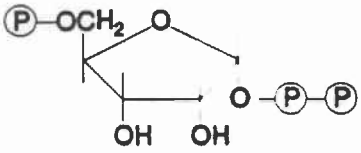
| Base | Ribonucleoside | Ribonucleotide (RNA) |
|---|---|---|
| Adenine (A), purine | Adenosine | Adenosine 5′-monophosphate (AMP) |
| Guanine (G), purine | Guanosine | Guanosine 5′-monophosphate (GMP) |
| Uracil (U), pyrimidine | Uridine | Uridine 5′-monophosphate (UMP) |
| Cytosine (C), pyrimidine | Cytidine | Cytidine 5′-monophosphate (CMP) |
| **Base** | **Deoxyribonucleoside** | **Deoxyribonucleotide (DNA)** |
| Adenine (A), purine | 2′-Deoxyadenosine | 2′-Deoxyadenosine 5′-monophosphate (dAMP) |
| Guanine (G), purine | 2′-Deoxyguanosine | 2′-Deoxyguanosine 5′-monophosphate (dGMP) |
| Thymine (T), pyrimidine | 2′-Deoxythymidine | 2′-Deoxythymidine 5′-monophosphate (dTMP) |
| Cytosine (C), pyrimidine | 2′-Deoxycytidine | 2′-Deoxycytidine 5′-monophosphate (dCMP) |

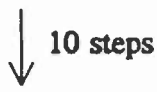
# 20.1 Nucleotide Synthesis

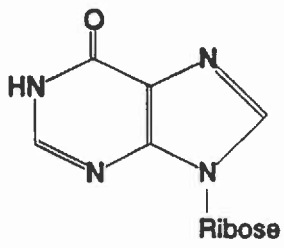
## Purine Synthesis

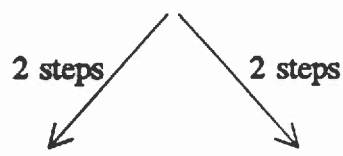


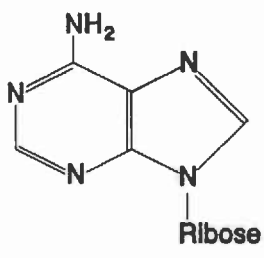



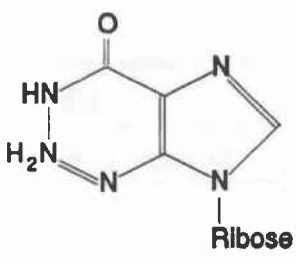



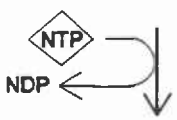



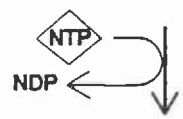

Adenosine
5′-monophosphate
(AMP)



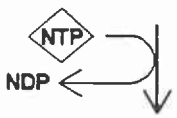

Guanosine
5′-monophosphate
(GMP)



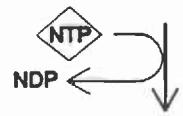

ADP




GDP

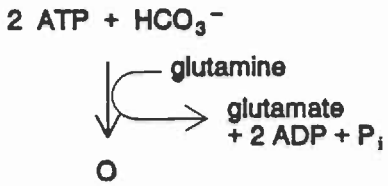


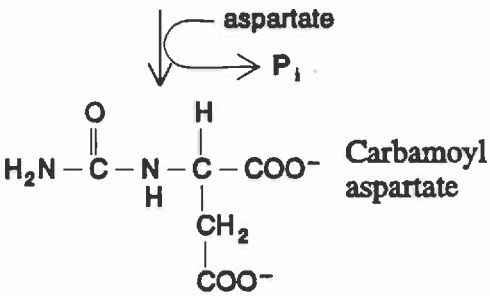

ATP

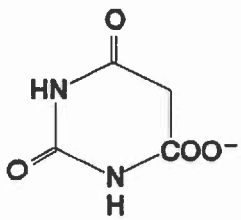


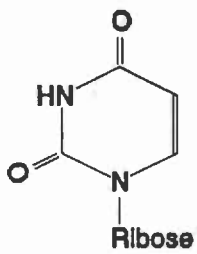

GTP

## Pyrimidine Synthesis

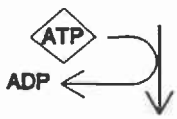


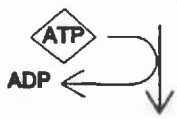




Uridine 5′-monophosphate
(UMP)




UDP

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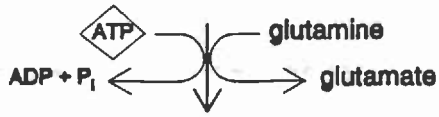


CTP

# I. PURINES

**A. PURINE *DE NOVO* SYNTHESIS**: Purines are assembled stepwise beginning with activated ribose 5´-phosphate in a complex series of reactions. It is important to realize that glutamine and aspartate supply the ring nitrogens, and carbon dioxide and tetrahydrofolate derivatives supply single carbon units, and glycine provides two carbons and a nitrogen (Fig. 19.1).

STEPS:

1. Preparatory step: Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP). This is the starting point for purine synthesis, the last step in pyrimidine synthesis, and is required in the purine salvage pathway. Inhibitors include ADP and AMP. Activators include $P_i$ and $Mg^{2+}$.
   Input: ATP + ribose 5´-phosphate
   Output: PRPP + AMP
2. Ring formation: Ten steps are required (the first of which is the committed step in purine synthesis) to form the parent purine, inosine 5´-monophosphate (IMP).
   Input: PRPP + $CO_2$ + aspartate + glycine + 2 glutamine + 2 $N^{10}$-formyltetrahydrofolate + 4 ATP
   Output: inosine 5´-monophosphate + $PP_i$ + 2 glutamate + 2 tetrahydrofolate + 4 ADP + 4 $P_i$ + fumarate.
3. Conversion of inosine 5´-monophosphate to AMP and GMP: Aspartate, with GTP, provides the amino group for AMP synthesis. AMP is a feedback inhibitor. In GMP synthesis, inosine 5´-monophosphate is oxidized with $NAD^+$, then glutamine provides the amino group. In this case, GMP is the feedback inhibitor of the pathway.
   Input for AMP: inosine 5´-monophosphate + GTP + aspartate
   Output for AMP: AMP + GDP + $P_i$ + fumarate
   Input for GMP: inosine 5´-monophosphate + $NAD^+$ + ATP + glutamine
   Output for GMP: GMP + NADH + $H^+$ + AMP + $PP_i$ + glutamate
4. Formation of dADP and dGDP: *Ribonucleotide reductase* converts ribonucleoside diphosphates to deoxyribonucleoside diphosphates.
   Input: ADP (or GDP) + NADPH + $H^+$
   Output: dADP (or dGDP) + $NADP^+$ + $H_2O$

NOTES:

1. Regulation: In the first committed step of the pathway (the second step of ring formation), *amidophosphoribosyl transferase* transfers the amide nitrogen from glutamine to PRPP to form 5-phosphoribosyl-1-amine. This step is inhibited by end products of the pathway, AMP and GMP.
2. Basis of cancer chemotherapy: The *amidotransferase's* steps, where glutamine donates a nitrogen atom, are blocked by glutamine analogs, azaserine and 6-diazo-5-oxo-aminohexanoic acid, used in cancer chemotherapy. Purine synthesis diminishes, hence DNA synthesis is limited. These drugs are toxic to cancer cells and to normal, rapidly dividing cells, as found in bone marrow.
3. $NAD^+$ synthesis:
   (a) First, nicotinate ribonucleotide is formed from nicotinate (niacin) and PRPP.
   (b) Next, desamido-$NAD^+$ is formed by the addition of an AMP moiety (from ATP).
   (c) Finally, $NAD^+$ is formed with the transfer of the amide group from glutamine.
4. $NADP^+$ synthesis: *$NAD^+$ kinase* catalyzes the transfer of a phosphoryl group from ATP to an adenine ribose moiety.

5. FAD synthesis: First, riboflavin 5´-phosphate is formed by ATP phosphorylating riboflavin. Then, an AMP moiety is transferred from ATP.
6. Coenzyme A synthesis: This is a five step process, beginning with the phosphorylation of pantothenate (vitamin $B_5$), then with an addition of an amino group from cysteine, an addition of an AMP moiety from ATP, and a second phosphorylation.

**B. SALVAGE PATHWAYS**: Hydrolysis of nucleotides yield purines and pyrimidines. The free purine bases can be coupled to PRPP. This is especially important in non-hepatic tissues. The destruction of the released $PP_i$ makes the reaction irreversible.

*Adenine phosphoribosyl transferase* incorporates adenine with PRPP to form AMP. *Hypoxanthine-guanine phosphoribosyl transferase (HGPRT)* incorporates hypoxanthine and guanine with PRPP to form inosine 5´-monophosphate and GMP, respectively.

NOTE: Lesch-Nyhan Syndrome, an inherited disorder resulting from a deficiency of *HGPRT*, causing a reduced synthesis of purines by the salvage pathway. This leads to elevated PRPP and to increased purine synthesis by the *de novo* pathway. This causes an excessive production of urate and severe neurological symptoms, such as mental retardation, involuntary movements, and self-mutilation. The brain relies upon the salvage pathways for purines.

**C. DEGRADATION**: Purines are oxidized to uric acid, which ionizes to urate. In this process, adenosine is deaminated to the nucleoside, inosine, which in turn is hydrolyzed to the free base, hypoxanthine. Hypoxanthine is oxidized to xanthine by *xanthine oxidase*. Guanine is deaminated to xanthine. Xanthine is oxidized by *xanthine oxidase* to uric acid (urate).

NOTES:
1. *Xanthine oxidase* requires molybdenum, FAD, and iron.
2. Gout: Gout is a form of arthritis from excessive blood urate due to either increased urate production or decreased renal excretion.

   In primates, urate is excreted without further modification. (Other mammals may oxidize it further.) The concentration of urate in the blood is close to the saturation point. It acts as a potent free radical scavenger.

   Primary gout is inherited. There are probably several different forms of primary gout. Hypoglycemia can lead to ketoacidosis with inhibition of tubular urate secretion and precipitation of urate in joints of the extremities. Lesch-Nyhan syndrome also leads to a huge overproduction of purines.

   Secondary gout has a variety of causes. Certain chemotherapies for leukemia can cause gout. Dietary factors promoting gout include a sustained intake of foods rich in nucleic acids, such as liver and other organ meats, yeast, anchovies, and sardines. Obesity and dehydration contribute to the risk. Alcohol consumption can lead to lactate production which can block urate excretion and promote precipitation.

   Allopurinol, a drug used to treat gout, is an analog of hypoxanthine. Allopurinol inhibits *xanthine oxidase*, thus decreasing urate production.

## II. PYRIMIDINES

**A. SYNTHESIS**: The ring is synthesized before attachment to ribose. The ring carbon and nitrogen atoms are derived from aspartate and carbamoyl phosphate.

STEPS:

1. Formation of carbamoyl phosphate: Catalyzed by *carbamoyl synthase*.
   Input: ATP + $HCO_3^-$ + glutamine
   Output: carbamoyl phosphate + glutamate + ADP + $P_i$
2. Formation of UMP:
   Input: ATP + carbamoyl phosphate + aspartate + $NAD^+$ + PRPP
   Output: UMP + $CO_2$ + $PP_i$ + NADH + $P_i$ + glutamate + ADP
   (a) The committed step is the condensation of aspartate and carbamoyl phosphate, catalyzed by *aspartate transcarbamoylase*.
   (b) Formation of orotate: Two steps yield orotate, the first pyrimidine. Orotate resembles uracil, except that the former possesses a carboxyl group.
   (c) Formation of UMP: Orotate condenses with PRPP to form a nucleotide, which is decarboxylated to UMP.
3. Formation of CTP: Catalyzed by *CTP synthase*, glutamine supplies nitrogen to aminate UTP.
   Input: UTP + glutamine + ATP + $H_2O$
   Output: CTP + glutamate + ADP + $P_i$ + 2 $H^+$
4. Formation of dTMP:
   (a) *Ribonucleotide reductase* converts UDP to dUDP.
   (b) dUDP is hydrolyzed to dUMP.
   (c) *Thymidylate synthetase* transfers a methyl group to dUMP from $N^5$,$N^{10}$-methylenetetrahydrofolate, forming dTMP and dihydrofolate.

NOTES:

1. Regulation: Pyrimidine synthesis is inhibited by UMP, UTP, and CTP (feedback inhibition), as well as by AMP. It is activated by ATP and PRPP.
2. Chemotherapy: Drugs used in chemotherapy, such as methotrexate and aminopterin, block folate reduction to tetrahydrofolate, thus limiting its ability to form purines, as well as dTMP. This inhibits the DNA synthesis of rapidly dividing cells.
3. Carbamoyl phosphate: The carbamoyl phosphate used for pyrimidine synthesis is formed in the cytosol, while the carbamoyl phosphate used in urea synthesis (Sect. 16) is made in mitochondria.

**B. CATABOLISM**: Pyrimidine rings are cleaved by human tissues to freely soluble end products, as $\beta$-aminoisobutyrate (thymine) and $\beta$-alanine (uracil). $\beta$-aminoisobutyrate is converted to methylmalonyl CoA, and then enters the TCA cycle when it's converted to succinyl CoA (Sect's. 6 & 15). There are no gout-like disorders resulting from pyrimidine catabolism.

## Section 5: Glycolysis

1. 2 ATP + 2 pyruvate per glucose.
2. a) Increased.
   b) Decreased.
   c) Increased. PFK I (phosphofructokinase I) is the major regulatory enzyme. Pyruvate kinase is the secondary control point.
3. Erythrocytes lack mitochondria. Therefore, pyruvate must be reduced to lactate in order to regenerate $NAD^+$, required to continue glycolysis.
4. High energy phosphate bonds (capable of being used to form ATP) are formed simultaneously with oxidation of a substrate, without the participation of molecular oxygen.
5. Glucose 6-P can enter glycolysis, it can be converted to glycogen, and it can be converted to ribose 5-P.

## Section 6: Tricarboxylic Acid Cycle

1. Thiamin pyrophosphate, FAD, Coenzyme A, and NADH are required by pyruvate dehydrogenase and $\alpha$-ketoglutarate dehydrogenase.
2. Carbon dioxide is produced by oxidative decarboxylation by pyruvate dehydrogenase (pyruvate to acetyl CoA), isocitrate dehydrogenase (isocitrate to $\alpha$-ketoglutarate), and $\alpha$-ketoglutarate dehydrogenase ($\alpha$-ketoglutarate to succinyl CoA).
3. ADP activates pyruvate dehydrogenase and isocitrate dehydrogenase. Elevated ATP and NADH indicate that the cell's energy supply is adequate, and that the TCA cycle should slow. These inhibit pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and $\alpha$-ketoglutarate dehydrogenase.
4. Cells deriving their ATP from glucose anaerobically must consume large amounts of glucose because the energy yield of glycolysis is only two ATP per glucose. Under aerobic conditions, glucose is oxidized completely to $CO_2$. The NADH and $FADH_2$ produced, efficiently yield ATP by oxidative phosphorylation. Consequently, less glucose is required to meet needs of cellular metabolism.
5. Acetyl CoA can enter the TCA cycle, it can be converted to ketone bodies (liver), and it can be converted to fatty acids. Sources of acetyl CoA include oxidative decarboxylation of pyruvate (pyruvate dehydrogenase), $\beta$-oxidation of fatty acids, breakdown of ketone bodies, and oxidation of several amino acid carbon skeleton (e.g. leucine, isoleucine, lysine, and tryptophan).

## Section 7: Electron Transport and Oxidative Phosphorylation

1. Electron flow is blocked. Electron carriers of Complexes I, II, III, and IV cannot be reoxidized. NADH build-up inhibits pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, $\alpha$-ketoglutarate dehydrogenase.
2. Three ATP from NADH, two ATP from $FADH_2$. Oxidation of pyruvate yields a maximum of 15 ATP; acetyl CoA yields 12 ATP (GTP is equivalent to ATP).
3. NADH and $FADH_2$ are oxidized. Electrons flow through complexes I, III, and IV. However, the proton gradient across the inner mitochondrial membrane is diminished and ATP synthesis is reduced.
4. Molecular oxygen.

## Section 8: Glycogen Metabolism

1. (a) Glucose → glucose 6-P → glucose 1-P → UDP-glucose → glycogen
   Glycogen phosphorylase catalyzes the last step.

   (b) Glycogen → glucose 1-P → glucose 6-P
   Glycogen synthase catalyzes the first step.

2. Glycogen phosphorylase leads to the formation of glucose 6-P without the expenditure of additional ATP. Glucose conversion to glucose 6-P requires ATP.
3. a) Calcium bound to calmodulin in skeletal muscle activates protein kinase, leading to the activation of phosphorylase kinase and the activation of phosphorylase b.
   b) Glucagon activates adenylate cyclase, leading to increased cyclic AMP, which activates protein kinase, which in turn activates phosphorylase kinase leading to the conversion of phosphorylase b to a.
   c) Elevated glucose binds to activated glycogen phosphorylase, making it more susceptible to phosphatase attack. The end result is inactivation of phosphorylase.
4. a) These protein kinases phosphorylate glycogen synthase, thereby inhibiting it.
   b) Activated phosphoprotein phosphatase removes phosphate groups from glycogen synthase, thereby activating it.
   c) Elevated blood glucose implies elevated insulin. Insulin reduces cyclic AMP production and leads to activation of glycogen synthesis.

Section 9: Pentose Phosphate Pathway

1. Ribulose 5-P and NADPH
2. Ribulose 5-P is isomerized to ribose 5-P. Further rearrangements lead to glyceraldehyde 3-P and fructose 6-P.
3. NADPH is used for biosynthesis, including fatty acid and cholesterol synthesis. It is used to reduce glutathione, the cellular antioxidant. It is the coenzyme for cytochrome $P_{450}$ monooxygenases.
4. The rate limiting step is glucose 6-P dehydrogenase (inhibited by elevated NADPH).

Section 10: Gluconeogenesis

1. Gluconeogenic substrates include: lactate (from anaerobic glycolysis in muscle, red blood cells); TCA cycle intermediates (oxaloacetate, pyruvate) and propionyl CoA (these may originate from carbon skeletons of glucogenic amino acids); and glycerol (from lipolysis).
2. Retrace the gluconeogenic pathway.
   (a) Pyruvate to oxaloacetate via pyruvate carboxylase.
   (b) Oxaloacetate to phosphoenolpyruvate via phosphoenolpyruvate carboxykinase.
   (c) Fructose 1,6-bisphosphate to fructose 6-phosphate via fructose 1,6-bisphosphatase.
   (d) Glucose 6-phosphate to glucose via glucose 6-phosphatase.
3. Conditions which favor gluconeogenesis include starvation, fasting, alcoholism, and uncontrolled diabetes.
4. Acetyl CoA activates pyruvate carboxylase. ADP activates both pyruvate carboxylase and PEP carboxykinase. Fructose 2,6-bisphosphate inhibits fructose 1,6-bisphosphatase.

Section 12.I: Mobilization of Triacylglycerols

1. Pancreatic lipase is responsible for digesting triacylglycerols. Hormone-sensitive lipase located in adipose tissue breaks down depot triacylglycerols to free fatty acids and glycerol.
2. Both hormone-sensitive lipase and glycogen phosphorylase require a cyclic AMP-protein kinase cascade for activation. These steps require:
   (a) Hormone binding to receptors on the cell surface.
   (b) Activation of adenylate cyclase.
   (c) Elevation of intracellular cyclic AMP.
   (d) Activation of cyclic AMP-dependent protein kinase, which can activate the lipase or glycogen phosphorylase kinase.

Section 12.II: β-Oxidation of Fatty Acids

1. The first intermediate in fatty acid synthesis is malonyl CoA. This inhibits carnitine acyltransferase I.
2. Carnitine is responsible for transporting fatty acids into the mitochondria.
3. Fatty acid oxidation yields NADH, $FADH_2$, and acetyl CoA.
4. Stearate is an 18C fatty acid. Cleaving this into 2-carbon fragments yields nine acetyl CoA's.
5. Fatty acids yield acetyl CoA. Because pyruvate dehydrogenase is irreversible, acetyl CoA cannot reform pyruvate. The stoichiometry of the TCA cycle requires two carbons leave as $CO_2$ for each two carbon (acetyl CoA) input. There can be no net synthesis of oxaloacetate for acetyl CoA.

Section 12.III: Ketone Body Metabolism

1. Acetoacetate, β-hydroxybutyrate, and acetone are traditionally classified as ketone bodies.
2. Under conditions of massive fatty acid metabolism, the liver funnels surplus acetyl CoA's into water-soluble ketone bodies.
3. Starvation, crash dieting, alcoholism, and uncontrolled diabetes mellitus favor ketone body production.
4. Acetoacetyl CoA is synthesized from acetyl CoA. It is also an end product of catabolism of the carbon skeletons of several amino acids. Acetoacetyl CoA is a terminal intermediate of fatty acid oxidation.
5. Acetoacetate and β-hydroxybutyrate are taken up by many tissues, including some regions of the nervous system, and converted to acetyl CoA to enter the TCA cycle. The liver cannot break down ketone bodies. Acetone is not metabolized.

Section 13.I: Synthesis of Fatty Acids

1. Fatty acids are assembled from malonyl CoA, which is derived from acetyl CoA. NADPH supplies reducing equivalents.
2. Acetyl CoA carboxylase, which synthesizes malonyl CoA, is rate-limiting.
3. Citrate activates, while malonyl CoA and palmitoyl CoA inhibit fatty acid synthesis. Elevated glucagon leads to the inactivation of acetyl CoA carboxylase.
4. Fatty acid synthase is a cytoplasmic, multienzyme complex. β-Oxidation is a mitochondrial process. It is not carried out by a physically-associated complex. Fatty acid synthase uses reductions with NADPH. β-Oxidation carries out oxidations producing NADH and $FADH_2$. Fatty acid synthase uses a protein bound pantetheine derivative of pantothenic acid as a carrier. β-oxidation uses CoA as a carrier. Fatty acids synthesis requires acetyl CoA, activated as malonyl CoA. β-Oxidation produces acetyl CoA.

Section 13.II: Synthesis of Triacylglycerols and Glycerol Metabolism

1. Liver and adipose tissues are the major sites of triacylglycerol synthesis.
2. Glycolysis provides dihydroxyacetone P, which is reduced to glycerol 3-P.
3. Glycerol kinase phosphorylates glycerol in the liver, not adipose. Glycerol 3-phosphate reacts with two fatty acyl CoA's to form phosphatidate. Phosphate is removed from phosphatidate to form diacylglycerol, which is finally esterified with the third fatty acyl CoA.
4. Insulin is required for glucose uptake by adipose tissue. Glucose is converted to glycerol 3-P and acetyl CoA. Glucagon and epinephrine are catabolic hormones, favoring glucose release from the liver, and lipolysis in adipose tissue.

Section 15: Amino Acid Catabolism

1. The oxidation of proline leads to glutamate, which transaminates to α-ketoglutarate. An input of five carbons can lead to a net synthesis of oxaloacetate for gluconeogenesis.

2. The oxidation of leucine leads to acetoacetate and to acetyl CoA. False, neither of these is a precursor for gluconeogenesis.
3. Dietary essential amino acids cannot be synthesized in the body. These include methionine, threonine, phenylalanine, leucine, isoleucine, valine, tryptophan, and lysine. Histidine cannot be synthesized at rates adequate for optimal growth in children. Arginine may not be synthesized in adequate amounts in patients with liver disease.
4. Oxaloacetate yields aspartate. α-Ketoglutarate yields glutamate. Pyruvate yields alanine.
5. Aminotransferases require pyridoxal 5-P derived from pyridoxine. These enzymes require an amino acid and a keto acid, usually α-ketoglutarate. Products are the corresponding keto acid and glutamate.
6. Phenylalanine yields tyrosine, and methionine yields cysteine. If the diet is deficient in phenylalanine and methionine, tyrosine and cysteine become dietary essentials.

Section 16: Urea Cycle

1. The nitrogen atoms of aspartate and ammonia form urea.
2. Ornithine is regenerated. It plays a role similar to oxaloacetate in the TCA cycle.
3. Arginine is an intermediate of the urea cycle.
4. a) Transamination with various amino acids and α-ketoglutarate forms glutamate.
   b) Glutamate transaminates with pyruvate to form alanine.
   c) Alanine is released into the bloodstream and is taken up by the liver.
   d) Alanine transaminates with α-ketoglutarate to form pyruvate and glutamate in the liver.
   e) Pyruvate forms glucose which is released into the bloodstream.
   f) Glutamate is oxidized to ammonia.
   g) Glutamate transaminates with oxaloacetate to form aspartate.
   h) Aspartate and ammonia enter the urea cycle.

Section 17.I: Cholesterol Synthesis

1. Cholesterol is synthesized from acetyl CoA and NADPH.
2. Cholesterol is the precursor of steroid hormones, vitamin D, and bile salts.
3. HMG CoA.
4. a) Elevated cholesterol inhibits its synthesis.
   b) Glucagon blocks cholesterol formation.
   c) Insulin activates cholesterol synthesis.
5. HMG CoA reductase is the key regulatory enzyme.
6. Acetoacetyl CoA → mevalonate → farnesyl pyrophosphate → squalene → lanosterol → cholesterol

Section 17.II: Lipoprotein Metabolism

1. Chylomicrons, from the intestinal mucosa, transport dietary triacylglycerols. VLDL transports triacylglycerols synthesized by the liver.
2. Lipoprotein lipase lines the capillary bed of peripheral tissues. This enzyme hydrolyzes triacylglycerols of VLDL and chylomicrons to produce free fatty acids. These are absorbed by tissues.
3. LCAT is located on HDL. It esterifies free cholesterol to cholesterol esters which are then sequestered within the HDL.
4. LDL binds to specific receptors on cell membranes. Once bound, these particles are endocytosed. Hydrolysis of transported cholesterol esters yields free cholesterol. If the cholesterol is to be stored, it is re-esterified by ACAT (acyl CoA:cholesterol acyltransferase).
5. HDL scavenges cholesterol from peripheral tissues. It carries cholesterol back to the liver for disposal. HDL also transfers cholesterol esters to LDL. It recycles Apoprotein C-II for VLDL and chylomicrons.

I. During the fed state, the liver converts glucose to glycogen, triacylglycerols and cholesterol. The lipids are released as VLDL. Cholesterol exits from the liver as bile salts. During a fast, glycogen is broken down to replenish blood glucose. With starvation, excess free fatty acids are converted to ketone bodies. Amino acids from muscle breakdown are converted to glucose via gluconeogenesis.

II. During the fed state the brain relies upon glucose. In starvation, ketone bodies build up in the blood. They supply a significant fraction of the brain's fuel requirements. Consequently, less glucose is required.

III. After a meal, muscle converts glucose to glycogen. Free fatty acids and glucose supply energy needs. During a prolonged fast, free fatty acids from lipolysis and ketone bodies from the liver, are the major fuels.

IV. In the fed state, the adipose tissue synthesizes triacylglycerols from blood glucose in response to insulin. In contrast, during starvation, hormones such as epinephrine stimulate lipolysis. Free fatty acids and glycerol are released into the bloodstream.

1. ✓***Hexokinase***: First step in glycolysis. *Hexokinase* is found in most tissues, including brain and muscle. Hexokinase has a low $K_M$ for glucose. It phosphorylates hexoses, in addition to glucose. It is inhibited by glucose 6-phosphate, its product.

   *Glucokinase* is an additional enzyme found only in the liver. It is specific for glucose, though it has a high $K_M$. There is no product inhibition.

2. ***Phosphoglucose isomerase***: Second step in glycolysis.

3. ✓***Phosphofructokinase (PFK I)***: Third step in glycolysis. Catalyzes a phosphoryl transfer. The most important control point in glycolysis. This reaction is rate-limiting for the pathway. The enzyme is designated *PFK I* to distinguish it from *PFK II*, responsible for synthesizing fructose 2,6-bisphosphate, the most powerful activator of *PFK I*. Fructose 2,6-bisphosphate levels increase when glucagon concentration is low.

   *PFK I*, an allosteric enzyme, is activated by AMP and fructose 2,6-bisphosphate, and inhibited by elevated ATP and citrate levels, which indicate adequate cellular energy stores.

4. ***Aldolase***: Fourth step in glycolysis. Catalyzes an aldol cleavage.

5. ***Triose phosphate isomerase***: Fifth step in glycolysis. Catalyzes an isomerization.

6. ✓***Glyceraldehyde 3-phosphate dehydrogenase***: Sixth step in glycolysis. Catalyzes a phosphorylation coupled to oxidation. Note that this reaction requires $NAD^+$, linking niacin to energy production. Oxidation of the substrate is accompanied by creation of a high energy phosphate bond in this example of substrate level phosphorylation.

7. ***Phosphoglycerate kinase***: Seventh step in glycolysis. Catalyzes a phosphoryl transfer.

8. ***Phosphoglycerate mutase***: Eighth step in glycolysis. Catalyzes a phosphoryl shift.

9. ***Enolase***: Ninth step in glycolysis. Catalyzes a dehydration.

10. ✓***Pyruvate kinase (PK)***: Tenth step in glycolysis. Catalyzes a phosphoryl transfer.
    A secondary control point of glycolysis. *PK* is activated by fructose 1,6-bisphosphate (feed forward control). Increased *phosphofructokinase* activity leads to increased *PK* activity.

    *PK* is inactivated by phosphorylation when glucagon concentration is elevated (low blood sugar). Instead of producing pyruvate, the liver shunts phosphoenolpyruvate to gluconeogenesis.

11. ✓***Pyruvate dehydrogenase (PDH)***: The link between glycolysis and the tricarboxylic acid cycle (TCA cycle; also called Krebs or citric acid cycle). Catalyzes an oxidative decarboxylation.A multienzyme complex which feeds acetyl CoA into the TCA cycle. This enzyme, and the other enzymes of the cycle, are mitochondrial.

    *PDH* requires thiamin pyrophosphate, $NAD^+$, FAD, and CoA, thus linking thiamin, niacin, riboflavin, and pantothenic acid to energy production. A fifth coenzyme, lipoic acid, is a disulfide made by the body.

12. ✓***Citrate synthase***: First step in the TCA cycle. Catalyzes a condensation. Note that oxaloacetate, an intermediate of the TCA cycle, produces another intermediate. Therefore, entry of acetyl CoA does not lead to a net production of intermediates of the cycle.

    A key control point for the TCA cycle. *Citrate synthase* is inhibited by ATP, NADH, succinyl CoA, and fatty acyl CoA's.

13. ***Aconitase***: Second and third steps in the TCA cycle. Catalyzes first a dehydration and then a hydration.

14. ✓***Isocitrate dehydrogenase***: Fourth step in the TCA cycle. Catalyzes a decarboxylation and an oxidation.

Requires $NAD^+$ (niacin) for the first oxidative decarboxylation of the TCA cycle. It produces the first of three NADH's of the cycle.

A major control point. This *dehydrogenase* is activate by ADP, and inhibited by ATP and NADH.

15. ✓***α-Ketoglutarate dehydrogenase***: Fifth step in the TCA cycle. Catalyzes a decarboxylation and an oxidation. The second oxidative decarboxylation of the TCA cycle. This is a multienzyme complex resembling enzyme 11, *PDH*. It requires thiamin pyrophosphate, $NAD^+$, FAD, and CoA, linking thiamin, niacin, riboflavin, and pantothenic acid to energy production.

    A secondary control point of the cycle. This enzyme is inhibited by ATP, GTP, NADH, and succinyl CoA.

16. ***Succinyl CoA synthetase***: Sixth step in the TCA cycle. Catalyzes a substrate-level phosphorylation.

17. ***Succinate dehydrogenase***: Seventh step in the TCA cycle. Catalyzes an oxidation. This enzyme is bound to the inner mitochondrial membrane, where it functions as part of Complex II of the ETC, *succinate CoQ reductase*, and reducing CoQ (see enzyme 21).

18. ***Fumarase***: Eighth step in the TCA cycle. Catalyzes a hydration.

19. ***Malate dehydrogenase***: Ninth step in the TCA cycle. Catalyzes an oxidation.

20. ✓***NADH-CoQ reductase***: Complex I of the electron transport chain (ETC; also called the respiratory chain). Transfers electrons from NADH to Coenzyme Q. This is the first proton pump of the ETC and generates the first ATP of oxidative phosphorylation.

21. ✓***Succinate CoQ reductase***: Complex II of the ETC. Transfers electrons from $FADH_2$ to coenzyme Q. Electrons from the oxidation of succinate are transferred sequentially to FAD, to iron-sulfur proteins, and ultimately to CoQ. No ATP is produced by this *reductase*. The resulting reduced CoQ does participate in oxidative phosphorylation.

22. ✓***CoQ-cytochrome c reductase***: Complex III of the ETC. Transfers electrons from $QH_2$ to cytochrome c. Second proton pump of the ETC.

23. ✓***Cytochrome oxidase***: Complex IV of the ETC. Transfers electrons from reduced cytochrome c to molecular oxygen. Third proton pump of the ETC.

    This enzyme is the only component of the ETC which interacts directly with molecular oxygen. Two cytochromes, a and $a_3$, and copper proteins are reversibly reduced and reoxidized.

    This complex is inhibited by cyanide, which explains why this substance is so toxic.

24. ***Phosphoglucomutase***: Isomerase class enzyme important in the formation and utilization of glycogen.

25. ***UDP-glucose pyrophosphorylase***: Transferase class enzyme used in the production of activated glucose (glycogen synthesis).

26. ✓***Glycogen synthase***: Transferase class enzyme, along with a branching enzyme, used in the synthesis of glycogen. This enzyme and *glycogen phosphorylase* are reciprocally regulated by hormones (see also enzyme 27). Epinephrine and glucagon inhibit the *synthase* (*glycogen synthase a*). They activate a *protein kinase* via cAMP. Activated kinase transfers phosphate from ATP to the *synthase*, thereby inactivating it (*glycogen synthase b*). Insulin leads to the activation of glycogen phosphatase, which clips off phosphate from phosphorylated *synthase*, returning it to the active form.

27. ✓***Glycogen phosphorylase***: Transferase class enzyme, along with a debranching enzyme, used in glycogen degradation. It cleaves glycosidic bonds of glycogen with phosphate ions.

Glucagon and epinephrine activate this enzyme via cAMP-mediated activation of *protein kinases*. Ultimately, *phosphorylase kinase* transfers phosphate from ATP to the active *phosphorylase* (*glycogen phosphorylase a*), thereby inactivating it (*glycogen phosphorylase b*).

Insulin inhibits *glycogen phosphorylase* by activating phosphatases which remove the phosphate group from *glycogen phosphorylase b*.

In muscle, calcium-calmodulin can activate *phosphorylase kinase*, thus inhibiting *phosphorylase* independently of cAMP-driven steps. *Glycogen phosphorylase b* can be activated by high levels of AMP in muscle.

28a. ✓***Glucose 6-phosphate dehydrogenase***: This enzyme specifically requires $NADP^+$ as a cofactor, rather than $NAD^+$. This step is the major control point for the Pentose Phosphate pathway. It is inhibited by the product, NADPH. Thus, the ratio of $NADP^+$/NADPH is the regulator of the pathway.

NADPH is requires to maintain glutathione, a major cellular antioxidant, in the reduced state. In *G6P dehydrogenase* deficiency, erythrocytes have an impaired ability to reduce oxidized glutathione. Reduced glutathione is required to repair membrane-lipid free-radical damage, and to destroy hydrogen peroxide, which otherwise could oxidize cellular constituents. Infection and exposure to drugs, such as the antimalarial primaquine, promote lysis of erythrocyte membranes, leading to hemolytic anemia.

28b. ***Lactonase***: In the Pentose Phosphate pathway, *lactonase* hydrolyzes 6-phosphogluconolactone to 6-phosphogluconate.

28c. ***6-Phosphogluconate dehydrogenase***: In the Pentose Phosphate pathway, this enzyme oxidizes 6-phosphogluconate to ribulose 5-phosphate.

29a. ***Ribulose 5-phosphate epimerase***: In the Pentose Phosphate pathway, this enzyme isomerizes ribulose 5-phosphate to xylulose 5-phosphate.

29b. ***Ribulose 5-phosphate isomerase***: In the Pentose Phosphate pathway, this enzyme isomerizes ribulose 5-phosphate to ribose 5-phosphate.

30a. ***Transketolase***: Noteworthy for several reasons. It is the only step in the Pentose Phosphate pathway which requires thiamin pyrophosphate. Its mechanism has a component resembling an aldol condensation.

30b. ***Transaldolase***: In the Pentose Phosphate pathway, this enzyme combines sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate to form erythrose 4-phosphate and fructose 6-phosphate.

31. ✓***Lactate dehydrogenase (LDH)***: The direction of this reversible reaction is determined in part by the ratio of NADH/$NAD^+$. In tissues such as heart and muscle, a lowered ratio favors the oxidation of lactate to pyruvate. In skeletal muscle, the ratio is higher and reduction of pyruvate is favored.

*LDH* represents a class of isozymes, enzymes which are structurally related and which carry out the same reaction mechanism, but exhibit a different kinetic parameter, e.g. $K_M$. *LDH* consists of four subunits made up of either "H" or "M" types. $H_4$ predominates in tissues with a high oxidative capacity, e.g. heart and liver. Striated muscle fibers and other tissues contain $M_4$, which is regulated by concentrations of reactants.

32. ✓***Pyruvate carboxylase***: This enzyme, like all *carboxylases*, requires biotin as a cofactor. By generating oxaloacetate, it can replenish the TCA cycle when intermediates are drawn off for synthetic pathways.

This enzyme is worth remembering because it is the first step of the gluconeogenic pathway and is a key regulatory step. It is activated by ADP.

33. ***Phosphoenolpyruvate carboxykinase***: In gluconeogenesis, this enzyme uses GTP to catalyze the conversion of oxaloacetate to phosphoenolpyruvate.

34. ✓***Fructose 1,6-bisphosphatase***: This enzyme is activated by elevated ATP, and is inhibited by elevated AMP. It is also inhibited by fructose 2,6-bisphosphate. Glucagon lowers the level of this allosteric effector (an activator of *PFK I* - see #3).

35. ✓***Glucose 6-phosphatase***: This enzyme is restricted to the liver, and to a lesser extent, the kidney. It is absent from skeletal muscle and heart muscle, consequently these tissues cannot release glucose to augment blood sugar levels.

36. ✓***Lipases***: Three lipases are worth noting:
(1) ✓*Pancreatic lipase*: During digestion, lipase hydrolyzes dietary triacylglycerols to free fatty acids and 2-monoacylglycerols, which are readily absorbed by the intestine.
(2) ✓*Hormone-sensitive lipase*: In adipose tissue, depot triacylglycerols are hydrolyzed to free fatty acids and 2-monoacylglycerols. This enzyme is activated by phosphorylation due to a cyclic AMP-protein kinase cascade. Glucagon, epinephrine, and others trigger this sequence. Insulin blocks lipolysis. Inactivation by removal of the lipase-bound phosphate is caused by phosphatase.
(3) ✓*Lipoprotein lipase*: This enzyme adheres to the endothelium of capillaries. In conjunction with apoprotein C-II, is able to hydrolyze triacylglycerols transported by chylomicrons and VLDL. The resulting free fatty acids and 2-monoacylglycerols are reconverted to triacylglycerols by adipose tissue.

37. ***Glycerol kinase***: Liver *glycerol kinase* phosphorylates glycerol released from adipose tissue by hydrolysis of triacylglycerols. The product, glycerol 3-phosphate, can enter glycolysis or it can be a gluconeogenic substrate.

38. ***Glycerol 3-phosphate dehydrogenase***: This enzyme, in the liver, can oxidize glycerol 3-phosphate to dihydroxyacetone phosphate. During fatty acid synthesis, this *dehydrogenase* carries out the reverse reaction, to create glycerol 3-phosphate from dihydroxyacetone phosphate.

39. ***Fatty acyl CoA synthetase***: This enzyme is required to convert fatty acids to their activated (CoA ester) forms prior to oxidation and prior to synthesis of triacylglycerols.

40. This multistep process (40a - 40e below) is responsible for the conversion of fatty acids to acetyl CoA.

40a. ✓***Carnitine shuttle***: This refers to a transport system by which fatty acids can enter mitochondria for $\beta$-oxidation.
(1) In this first step, cytoplasmic fatty acyl CoA is coupled to carnitine by *carnitine acyltransferase I*. This enzyme is blocked by malonyl CoA from fatty acid synthesis. This ensures that fatty acids will not be degraded at the same time they are being made.
(2) In the next step, the fatty acid is carried into the mitochondrial matrix.
(3) Finally, *carnitine acyltransferase II* reattaches the fatty acid to reform the fatty acyl CoA, and regenerates carnitine.

40b. ***Fatty acyl CoA dehydrogenase***: This enzyme carries out the first oxidation in $\beta$-oxidation, producing $FADH_2$.

40c. ***Enoyl CoA hydratase***: In $\beta$-oxidation, this enzyme forms a 3-hydroxy acyl CoA derivative.

40d. ***3-Hydroxyacyl CoA dehydrogenase***: In $\beta$-oxidation, this enzyme uses $NAD^+$ to produce a 3-keto acyl CoA.

40e. ***$\beta$-Ketoacyl CoA thiolase***: See enzyme 41.

41. ***Thiolase***: This activity is involved in four processes:
(1) *$\beta$-ketoacyl CoA thiolase* (enzyme 40e): The thiolytic cleavage of ketoacyl CoA's in $\beta$-oxidation of fatty acids can condense two molecules of acetyl CoA.
(2) *Acetyl-CoA acetyltransferase* (enzyme 41): The first step of ketone body formation in the liver requires

formation of acetoacetyl CoA from acetyl CoA. (mitochondrial)
(3) *Acetyl-CoA acetyltransferase* (enzyme 41): The first step in cholesterol metabolism requires the formation of acetoacetyl CoA from the condensation of acetyl CoA with *thiolase* (cytoplasmic).
(4) *Acetyl-CoA acetyltransferase* (enzyme 45): The utilization of acetoacetate by muscle for energy requires cleavage of acetoacetyl CoA by the reverse reaction.

42. ***Hydroxymethylglutaryl (HMG) CoA synthetase***: The liver contains two isozymes of *HMG CoA synthetase*. The mitochondrial enzyme is involved in ketone body formation and is the rate limiting enzyme. The cytoplasmic enzyme is involved in cholesterol synthesis.

43a. ***Hydroxymethylglutaryl (HMG) CoA lyase***: In ketone body metabolism, this enzyme produces acetyl CoA and acetoacetate from HMG CoA.

43b. ***β-Hydroxybutyrate dehydrogenase***: This enzyme reduces acetoacetate to β-hydroxybutyrate with NADH. This process is reversed to reform acetoacetate in peripheral tissues.

44. ***CoA transferase***: In ketone body metabolism, this enzyme uses succinyl CoA to form acetoacetyl CoA.

45. ***Acetyl-CoA acetyltransferase***: This enzyme catalyzes the cleavage of acetoacetyl CoA to two molecules of acetyl CoA, which allows acetoacetate to be used as an energy source. See also ketone body formation (41a), the last step of β-oxidation of fatty acids (40e), and the first step in cholesterol synthesis (41).

46. ***✓Acetyl CoA carboxylase***: Biotin is the required coenzyme. This enzyme regulates fatty acid synthesis. It is activated by citrate and is inhibited by its product, malonyl CoA, as well as the end product of the pathway.

47a. ***Acetyl-CoA-ACP-transacylase***: In fatty acid synthesis, this enzyme is used only once for each palmitate chain synthesized. It transfers the acetyl group of acetyl CoA to ACP.

47b. ***Malonyl-CoA-ACP-transacylase***: In fatty acid synthesis, this enzyme attaches malonyl to ACP.

47c. ***β-Ketoacyl-ACP synthase (acyl-malonyl-ACP condensing enzyme)***: In fatty acid synthesis, this enzyme condenses malonyl and acetyl groups to form acetoacetyl-ACP.

47d. ***β-Ketoacyl-ACP reductase***: In fatty acid synthesis, this enzyme uses the first NADPH (from Pentose Phosphate pathway) of the cycle to reduce the 3-keto group of acetoacetyl-ACP.

47e. ***β-Hydroxyacyl-ACP dehydrase***: In fatty acid synthesis, this enzyme dehydrates β-hydroxybutyryl-ACP and produces an unsaturated chain.

47f. ***Enoyl-ACP reductase***: In fatty acid synthesis, this enzyme uses the second NADPH to reduce butenoyl-ACP to produce butyryl-ACP.

48. ***Triacylglycerol synthetase***: This is a multienzyme complex with three activities:
(1) *Acyl transferase* (enzyme 48a) catalyzes the following reaction:

glycerol 3-phosphate + 2 fatty acyl CoA → phosphatidate

(2) *Phosphatidate phosphatase* (enzyme 48b) hydrolyzes phosphatidate to diacylglycerol and $P_i$ in the reaction:

phosphatidate + $H_2O$ → diacylglycerol + $P_i$

(3) *Diacylglycerol acyltransferase* (enzyme 48c) catalyzes the following reaction:

diacylglycerol + fatty acyl CoA → triacylglycerol + CoA

49. ***✓Hydroxymethylglutaryl (HMG) CoA reductase***: In cholesterol synthesis, this enzyme is the key regulatory enzyme. It uses NADPH to reduce HMG CoA.

50a. ***Kinases***: A pair of *kinases* are required to add two phosphate groups to mevalonate to form mevalonate pyrophosphate.

50b. ***Decarboxylase***: This enzyme requires ATP to convert 5-pyrophosphomevalonate to isopentenyl pyrophosphate (IPP).

50c. ***Isomerase***: *Isopentenyl pyrophosphate isomerase* reversibly forms the second isoprenoid building block, dimethylallyl pyrophosphate (DPP).

50d. ***Transferase***: *Dimethylallyl transferase* combines the two isoprenoid precursors, IPP and DPP, to form geranyl pyrophosphate.

50e. ***Geranyl transferase***: In cholesterol synthesis, this enzyme adds 5C dimethylallyl pyrophosphate to 10C geranyl pyrophosphate, forming 15C farnesyl pyrophosphate.

50f. ***Farnesyl transferase (squalene synthase)***: This enzyme joins two molecules of farnesyl pyrophosphate tail-to-tail in a reductive condensation requiring NADPH. Presqualene pyrophosphate is an intermediate in this reaction.

51. The formation of cholesterol from squalene entails a very complex series of reactions:
Step 51a: Oxidation of squalene by *squalene monooxygenase*, a mixed-function oxidase, requires NADPH, FAD, and $O_2$. The product is squalene 2,3-epoxide. A second enzyme, *oxidosqualene lanosterol cyclase* converts the epoxide to lanosterol.
Step 51b: This step involves approximately nineteen different enzymes. They catalyze ring closures, demethylations, and NADPH-dependent reductions of double bonds.

52. ***Ornithine transcarbamoylase***: This enzyme is regulated by *N*-acetyl glutamate. It is synthesized in response to elevated glutamate.

53. ***Argininosuccinate synthetase***: In the urea cycle, this enzyme catalyzes the condensation of citrulline and aspartate, forming argininosuccinate.

54. ***Argininosuccinase***: Releases arginine and fumarate from argininosuccinate.

55. ✓***Arginase***: Hydrolyzes arginine to ornithine and urea.

56. ✓***Aspartate aminotransferase***: In the urea cycle, this enzyme synthesizes aspartate from oxaloacetate.

1. *Hexokinase*
2. *Phosphoglucose isomerase*
3. *Phosphofructokinase (PFK I)*
4. *Aldolase*
5. *Triose phosphate isomerase*
6. *Glyceraldehyde 3-phosphate dehydrogenase*
7. *Phosphoglycerate kinase*
8. *Phosphoglycerate mutase*
9. *Enolase*
10. *Pyruvate kinase (PK)*
11. *Pyruvate dehydrogenase (PDH)*
12. *Citrate synthase*
13. *Aconitase*
14. *Isocitrate dehydrogenase*
15. *α-Ketoglutarate dehydrogenase*
16. *Succinyl CoA synthetase*
17. *Succinate dehydrogenase*
18. *Fumarase*
19. *Malate dehydrogenase*
20. *NADH-CoQ reductase*
21. *Succinate CoQ reductase*
22. *CoQ-cytochrome c reductase*
23. *Cytochrome oxidase*
24. *Phosphoglucomutase*
25. *UDP-glucose pyrophosphorylase*
26. *Glycogen synthase*
27. *Glycogen phosphorylase*

28a. *Glucose 6-phosphate dehydrogenase*
28b. *Lactonase*
28c. *6-Phosphogluconate dehydrogenase*
29a. *Ribulose 5-phosphate epimerase*
29b. *Ribulose 5-phosphate isomerase*
30a. *Transketolase*
30b. *Transaldolase*

31. *Lactate dehydrogenase (LDH)*
32. *Pyruvate carboxylase*
33. *Phosphoenolpyruvate carboxykinase*
34. *Fructose 1,6-bisphosphatase*
35. *Glucose 6-phosphatase*
36. *Lipases*
37. *Glycerol kinase*
38. *Glycerol 3-phosphate dehydrogenase*
39. *Fatty acyl CoA synthetase*
40. This is a multistep process (40a - 40e below)

40a. *Carnitine shuttle*
40b. *Fatty acyl CoA dehydrogenase*
40c. *enoyl CoA hydratase*
40d. *3-Hydroxy acyl CoA dehydrogenase*
40e. *β-Ketoacyl CoA thiolase*

41. *Thiolase*
42. *Hydroxymethylglutaryl CoA synthetase*

43a. *Hydroxymethylglutaryl CoA lyase*
43b. *β-Hydroxybutyrate dehydrogenase*
44. *CoA transferase*
45. *Acetyl-CoA acetyltransferase*
46. *Acetyl CoA carboxylase*

47a. *Acetyl-CoA-ACP-transacylase*
47b. *Malonyl-CoA-ACP-transacylase*
47c. *β-Ketoacyl-ACP synthase*
47d. *β-Ketoacyl-ACP reductase*
47e. *β-Hydroxyacyl-ACP dehydrase*
47f. *Enoyl-ACP reductase*

48. *Triacylglycerol synthetase*
49. *Hydroxymethylglutaryl CoA reductase*

50a. *Kinases*
50b. *Decarboxylase*
50c. *Isomerase*
50d. *Transferase*
50e. *Geranyl transferase*
50f. *Farnesyl transferase (squalene synthase)*

51. Step 51a: *squalene monooxygenase*
Step 51b: ~nineteen enzymes

52. *Ornithine transcarbamoylase*
53. *Argininosuccinate synthetase*
54. *Argininosuccinase*
55. *Arginase*
56. *Aspartate aminotransferase*

**Acetoacetate** / WP: [A] 4C ketoacid. Ketone body. [B] From acetyl CoA (liver). Fuel source for muscle and brain. Reforms acetyl CoA in peripheral tissues. Excess: ketonuria, ketoacidosis.

**Acetoacetyl CoA** / IR: [A] Thioester of acetoacetate. [B] From 2 acetyl CoA's. To acetoacetate (ketone body). To cholesterol via HMG CoA.

**Acetyl CoA** / WD: [A] Thioester of acetic acid. [B] From pyruvate by pyruvate dehydrogenase (PDH). From $\beta$-oxidation. To $CO_2$ by TCA cycle. Forms ketone bodies (liver), fatty acids, and cholesterol.

**Arginine** / UF: [A] Basic amino acid. [B] Protein building block. In urea cycle, forms urea and regenerates ornithine by arginase.

**Argininosuccinate** / GT: [A] 3rd non-protein amino acid of urea cycle. [B] Forms arginine and fumarate (to TCA cycle).

**Aspartate** / NZ: [A] Non-essential, acidic amino acid. [B] Protein building block. Forms oxaloacetate (transamination). Donates amino nitrogen for urea, purine, and pyrimidine synthesis.

**1,3-Bisphosphoglycerate** / DW: [A] High energy phosphate anhydride of phosphoglycerate. [B] From glyceraldehyde 3-phosphate by dehydrogenase. Yields 1st ATP of glycolysis.

**Carbamoyl phosphate** / QJ: [A] First precursor of urea. [B] From $CO_2$ and ammonia. Requires ATP.

**Cholesterol** / PX: [A] Most abundant sterol. 4 fused rings, 27C. [B] From acetyl CoA and NADPH. Transported in blood as LDL and HDL. Forms membranes, steroid hormones, bile salts, vitamin D.

**Citrate** / FU: [A] 1st 6C tricarboxylic acid in TCA cycle. [B] From oxaloacetate and acetyl CoA by synthase. To cytoplasmic acetyl CoA. Inactivates (-) glycolysis. Activates (+) fatty acid synthesis.

**Citrulline** / EI: [A] 2nd non-protein amino acid of urea cycle. [B] Combines with aspartate to form argininosuccinate.

**Coenzyme Q** / VQ: [A] Isoprenoid quinone. [B] Oxidizes $FMNH_2$ (Complex I of ETC). Oxidizes $FADH_2$ from fatty acids and from succinate oxidations. Reduces cytochrome b (Complex III of ETC).

**Cytochrome a** / MN: [A] Complex IV, last electron carrier of ETC. Contains Cu and heme for redox. [B] Reacts directly with $O_2$. Forms $H_2O$. Pumps $H^+$ to generate 3rd ATP (oxidative phosphorylation).

**Cytochrome b** / ZA: [A] Electron carrier. Contains heme and Fe-S proteins for redox. [B] Complex III of ETC. Oxidizes $CoQH_2$. Reduces cytochrome c. $H^+$ to generate 2nd ATP (oxidative phosphorylation).

**Cytochrome c** / TG: [A] Heme for redox. [B] Electron carrier between Complexes III and IV of ETC. Oxidizes cytochrome b. Reduces cytochrome a.

**Dihydroxyacetone phosphate** / FH: [A] 3C ketose. [B] From fructose 1,6-bisphosphate (FBP) by aldolase. Isomerizes to glyceraldehyde 3-phosphate to continue glycolysis. Reduced to glycerol 3-phosphate (triacylglycerol synthesis).

**Fatty Acids** / OL: [A] Often C16 or C18. May be saturated or unsaturated (*cis* double bonds). [B] Released from fat by lipase. Three are esterified with glycerol 3-phosphate in triacylglycerol synthesis.

**Fatty Acyl CoA / SH:** [A] Activated fatty acid. Thioester. [B] To acetyl CoA, NADH, $FADH_2$ by $\beta$-oxidation. End product of fatty acid synthesis. With glycerol 3-phosphate, to triacylglycerols by acyl transferase.

**Flavin mononucleotide / XC:** [A] Electron carrier. Complex I of ETC. Contains FMN, plus Fe-S proteins for redox. [B] Oxidizes NADH. Reduces CoQ. Pumps $H^+$ to generate 1st ATP (oxidative phosphorylation).

**Fructose 6-phosphate / JC:** [A] Phosphate ester of major ketohexose. Isomer of glucose 6-phosphate. [B] To fructose 1,6-bisphosphate (FBP) by phosphofructokinase (PFK). From FBP by phosphatase (gluconeogenesis). Possible product of pentose phosphate pathway.

**Fructose 1,6-bisphosphate / PK:** [A] Hexose phosphate diester. [B] To triose phosphates (glycolysis). From fructose 6-phosphate by phosphofructokinase (PFK). Inactivated (-) by citrate. To fructose 6-phosphate by phosphatase (gluconeogenesis).

**Fumarate / LO:** [A] 4C, unsaturated dicarboxylic acid. [B] Forms malate by adding $H_2O$ to double bond in TCA cycle. Also from urea cycle.

**Glucose 6-phosphate / SU:** [A] Hexose phosphate ester. First glucose intermediate. [B] By hexokinase (muscle, brain) and by glucokinase (liver). To pyruvate (glycolysis). To glucose 1-phosphate (glycogen synthesis).

**Glucose 1-phosphate / QW:** [A] Isomer of glucose 6-phosphate. [B] From glycogen by phosphorylase. Activated (+) by glucagon and epinephrine. To glycogen by UDP-glucose pyrophosphorylase and glycogen synthase.

**Glucose / NM:** [A] Major aldohexose. [B] Blood sugar. Building block of starch and glycogen. From carbohydrate (CHO) digestion. End product of gluconeogenesis.

**Glyceraldehyde 3-phosphate / ZT:** [A] 3C aldose phosphate. [B] From fructose 1,6-bisphosphate (FBP) by aldolase. Oxidized to high energy 1,3-bisphosphoglycerate by dehydrogenase (substrate level phosphorylation).

**Glycerol 3-phosphate / CX:** [A] Phosphate ester of 3C polyhydroxy alcohol. [B] Esterified with fatty acyl CoA's by acyl transferase (triacylglycerol synthesis).

**Glycerol / IA:** [A] 3C polyhydroxy alcohol. Esterified with 3 fatty acids in triacylglycerols. [B] From fat digestion. To glyceraldehyde 3-phosphate for glycolysis and gluconeogenesis.

**Glycogen / VE:** [A] Branched polymer. D-glucose linked $\alpha$-1,4 and $\alpha$-1,6 branches. [B] Fuel reserve in muscle and liver. Glycogen synthase inactivated (-) and phosphorylase activated (+) by glucagon and epinephrine.

**Hydroxymethylglutaryl CoA / HE:** [A] Condensation product of 3 acetyl CoA's. [B] Forms acetoacetate and cholesterol. Substrate for HMG CoA reductase (controls cholesterol synthesis).

**Isocitrate / OY:** [A] 2nd tricarboxylic acid. Isomer of citrate. [B] Oxidized to $\alpha$-ketoglutarate, $CO_2$, and NADH by dehydrogenase. Activated (+) by ADP. Inactivated (-) by ATP and NADH.

**$\alpha$-Ketoglutarate / ID:** [A] 5C ketoacid. [B] From isocitrate by oxidative decarboxylation. To succinyl CoA and $CO_2$ by dehydrogenase. Inactivated (-) by NADH.

**Lactate / RI:** [A] 3C hydroxyacid. [B] From pyruvate by lactate dehydrogenase (LDH). From muscle during strenuous exercise. Reforms pyruvate in liver (Cori cycle).

**Malate / UR:** [A] 4C hydroxy dicarboxylic acid. [B] To oxaloacetate by dehydrogenase (last oxidation of TCA cycle).

**Malonyl CoA** / BL: [A] Activated (carboxylated) acetyl CoA. [B] Product of first step of fatty acid synthesis by carboxylase. Activated (+) by citrate.

**Mevalonate** / XO: [A] 5C cholesterol precursor. [B] Forms isoprene building blocks of lipids. From HMG CoA by HMG CoA reductase (which controls cholesterol synthesis).

**Ornithine** / KP: [A] 1st non-protein, basic amino acid of urea cycle. [B] Combines with carbamoyl phosphate in 1st step of cycle. Carries nitrogen through urea cycle. Regenerated from arginine.

**Oxaloacetate** / BY: [A] 4C ketoacid. [B] With acetyl CoA, forms citrate by synthase in 1st step in TCA cycle. To phosphoenolpyruvate (PEP - gluconeogenesis). To aspartate (transamination).

**Phosphoenolpyruvate** / CK: [A] High energy enol phosphate. [B] 2nd ATP-yielding compound in glycolysis. To pyruvate by pyruvate kinase. Liver kinase inactivated (-) by glucagon. From oxaloacetate (gluconeogenesis).

**2-Phosphoglycerate** / AZ: [A] 3C phosphorylated acid. Isomer of 3-phosphoglycerate. [B] Loses $H_2O$ to form the high energy enol phosphate, phosphoenolpyruvate (PEP), by enolase.

**3-Phosphoglycerate** / RV: [A] 3C phosphorylated acid. [B] From 1,3-bisphosphoglycerate by kinase.

**Pyruvate** / YN: [A] 3C ketoacid. [B] End product of glycolysis. From phosphoenolpyruvate (PEP) by kinase. To acetyl CoA by pyruvate dehydrogenase (PDH). To lactate by lactate dehydrogenase (LDH). To oxaloacetate (gluconeogenesis). To alanine.

**Ribose 5-phosphate** / TS: [A] Aldopentose. Ribulose 5-phosphate isomer. [B] Product of pentose phosphate pathway. From fructose 6-phosphate by reversal of pathway. To nucleotides, RNA, deoxyribose of DNA.

**Ribulose 5-phosphate** / EV: [A] 1st ketopentose of pentose phosphate pathway. [B] Formation also yields NADPH. To ribose 5-phosphate by isomerase. To fructose 6-phosphate by non-oxidative steps of pentose phosphate pathway.

**Squalene** / JQ: [A] 30C isoprenoid hydrocarbon. Straight chain. [B] Forms polycyclic lanosterol and cholesterol with $O_2$ and NADPH.

**Succinate** / GF: [A] 4C dicarboxylic acid. [B] From succinyl CoA. Oxidized to fumarate and $FADH_2$ by succinate dehydrogenase (Complex II of ETC, which reduces CoQ).

**Succinyl CoA** / DJ: [A] Thioester of 4C dicarboxylic acid of TCA cycle. [B] From $\alpha$-ketoglutarate by dehydrogenase. Forms guanosine triphosphate (GTP) by succinyl CoA synthetase.

**Triacylglycerols** / YB: [A] Glycerol ester, 3 fatty acids (saturated and/or unsaturated). [B] Effective fuel (9 kcal/g). Formed by liver and adipose (storage). Transported by chylomicrons and VLDL.

**UDP-glucose** / AM: [A] Glucose activated for glycogen synthesis. [B] From uridine triphosphate (UTP) and glucose 1-phosphate by UDP-glucose pyrophosphorylase.

**Urea** / VR: [A] Carbonyl diamide. [B] Nitrogenous waste. Disposal of amino groups from amino acids. Nitrogen from $NH_3$ (deamination) and from aspartate.

**Xylulose 5-phosphate** / HS: [A] 2nd ketopentose of pentose phosphate pathway. Isomer of ribulose 5-phosphate. [B] Combines with ribose 5-phosphate and rearranges to form fructose 6-phosphate and glyceraldehyde 3-phosphate.

| | |
|---|---|
| **AM** | UDP-glucose |
| **AZ** | 2-Phosphoglycerate |
| **BL** | Malonyl CoA |
| **BY** | Oxaloacetate |
| **CK** | Phosphoenolpyruvate |
| **CX** | Glycerol 3-phosphate |
| **DJ** | Succinyl CoA |
| **DW** | 1,3-Bisphosphoglycerate |
| **EI** | Citrulline |
| **EV** | Ribulose 5-phosphate |
| **FH** | Dihydroxyacetone phosphate |
| **FU** | Citrate |
| **GF** | Succinate |
| **GT** | Argininosuccinate |
| **HE** | Hydroxymethylglutaryl CoA |
| **HS** | Xylulose 5-phosphate |
| **IA** | Glycerol |
| **ID** | α-Ketoglutarate |
| **IR** | Acetoacetyl CoA |
| **JC** | Fructose 6-phosphate |
| **JQ** | Squalene |
| **KP** | Ornithine |
| **LO** | Fumarate |
| **MN** | Cytochrome a |
| **NM** | Glucose |
| **NZ** | Aspartate |
| **OL** | Fatty Acids |
| **OY** | Isocitrate |
| **PK** | Fructose 1,6-bisphosphate |
| **PX** | Cholesterol |
| **QJ** | Carbamoyl phosphate |
| **QW** | Glucose 1-phosphate |
| **RI** | Lactate |
| **RV** | 3-Phosphoglycerate |
| **SH** | Fatty Acyl CoA |
| **SU** | Glucose 6-phosphate |
| **TG** | Cytochrome c |
| **TS** | Ribose 5-phosphate |
| **UF** | Arginine |
| **UR** | Malate |
| **VE** | Glycogen |
| **VQ** | Coenzyme Q |
| **VR** | Urea |
| **WD** | Acetyl CoA |
| **WP** | Acetoacetate |
| **XC** | Flavin mononucleotide |
| **XO** | Mevalonate |
| **YB** | Triacylglycerols |
| **YN** | Pyruvate |
| **ZA** | Cytochrome b |
| **ZT** | Glyceraldehyde 3-phosphate |

| | |
|---|---|
| **WP** | **Acetoacetate** |
| **IR** | **Acetoacetyl CoA** |
| **WD** | **Acetyl CoA** |
| **UF** | **Arginine** |
| **GT** | **Argininosuccinate** |
| **NZ** | **Aspartate** |
| **DW** | **1,3-Bisphosphoglycerate** |
| **QJ** | **Carbamoyl phosphate** |
| **PX** | **Cholesterol** |
| **FU** | **Citrate** |
| **EI** | **Citrulline** |
| **VQ** | **Coenzyme Q** |
| **MN** | **Cytochrome a** |
| **ZA** | **Cytochrome b** |
| **TG** | **Cytochrome c** |
| **FH** | **Dihydroxyacetone phosphate** |
| **OL** | **Fatty Acids** |
| **SH** | **Fatty Acyl CoA** |
| **XC** | **Flavin mononucleotide** |
| **JC** | **Fructose 6-phosphate** |
| **PK** | **Fructose 1,6-bisphosphate** |
| **LO** | **Fumarate** |
| **SU** | **Glucose 6-phosphate** |
| **QW** | **Glucose 1-phosphate** |
| **NM** | **Glucose** |
| **ZT** | **Glyceraldehyde 3-phosphate** |
| **CX** | **Glycerol 3-phosphate** |
| **IA** | **Glycerol** |
| **VE** | **Glycogen** |
| **HE** | **Hydroxymethylglutaryl CoA** |
| **OY** | **Isocitrate** |
| **ID** | **α-Ketoglutarate** |
| **RI** | **Lactate** |
| **UR** | **Malate** |
| **BL** | **Malonyl CoA** |
| **XO** | **Mevalonate** |
| **KP** | **Ornithine** |
| **BY** | **Oxaloacetate** |
| **CK** | **Phosphoenolpyruvate** |
| **AZ** | **2-Phosphoglycerate** |
| **RV** | **3-Phosphoglycerate** |
| **YN** | **Pyruvate** |
| **TS** | **Ribose 5-phosphate** |
| **EV** | **Ribulose 5-phosphate** |
| **JQ** | **Squalene** |
| **GF** | **Succinate** |
| **DJ** | **Succinyl CoA** |
| **YB** | **Triacylglycerols** |
| **AM** | **UDP-glucose** |
| **VR** | **Urea** |
| **HS** | **Xylulose 5-phosphate** |

## Appendix 4: COMMONLY USED ABBREVIATIONS and CONVENTIONS

| | |
|---|---|
| **AA** | amino acid |
| **ACAT** | acyl CoA-cholesterol acyltransferase |
| **ACP** | acyl carrier protein |
| **(+)** | activated, stimulated |
| **ADP** | adenosine 5´-diphosphate |
| **Ala** | alanine |
| **ALT** | alanine aminotransferase |
| **AMP** | adenosine 5´-monophosphate |
| **cAMP** | cyclic AMP |
| **Arg** | arginine |
| **Asn** | asparagine |
| **Asp** | aspartate |
| **AST** | aspartate aminotransferase |
| **ATP** | adenosine 5´-triphosphate |
| **BCAA** | branched chain amino acid |
| **BPG** | 2,3-bisphosphoglycerate |
| **C** | carbon, e.g. 3C (3 carbons) |
| **CDP** | cytidine 5´-diphosphate |
| **CHO** | carbohydrate |
| **CMP** | cytidine 5´-monophosphate |
| **CTP** | cytidine 5´-triphosphate |
| **CoA** | coenzyme A |
| **CoQ** | coenzyme Q, ubiquinone |
| **$CoQH_2$** | reduced CoQ, ubiquinol |
| **cyclic AMP** | cyclic 3´,5´-adenosine monophosphate |
| **Cys** | cysteine |
| **d** | 2´-deoxyribo, e.g. dAMP, 2´-deoxyadenosine 5´-monophosphate |
| **DNA** | deoxyribonucleic acid |
| **DPP** | dimethylallyl pyrophosphate |
| **EAA** | essential amino acid |
| **EFA** | essential fatty acid |
| **ES** | enzyme-substrate |
| **ETC** | electron transport chain |
| **FA** | fatty acid |
| **FAD** | flavin adenine dinucleotide (oxidized) |
| **$FADH_2$** | flavin adenine dinucleotide (reduced) |
| **FBP** | fructose 1,6-bisphosphate |
| **FMN** | flavin mononucleotide (oxidized) |
| **$FMNH_2$** | flavin mononucleotide (reduced) |
| **Glc** | glucose |
| **Gln** | glutamine |
| **Glu** | glutamate |
| **GDP** | guanosine 5´-diphosphate |
| **GMP** | guanosine 5´-monophosphate |
| **GSH** | glutathione (reduced) |
| **GSSG** | glutathione (oxidized) |
| **GTP** | guanosine 5´-triphosphate |
| **Hb** | hemoglobin |
| **HDL** | high-density lipoprotein |
| **His** | histidine |
| **HMG CoA** | 3-hydroxy-3-methylglutaryl CoA |
| **$K_M$** | Michaelis constant (from enzyme kinetics) |
| **IDL** | intermediate-density lipoprotein |
| **Ile** | isoleucine |
| **IMP** | inosine 5´-monophosphate |
| **(-)** | inactivated, inhibited |
| **IPP** | isopentenyl pyrophosphate |
| **ITP** | inosine 5´-triphosphate |
| **✓** | key enzyme or step |
| **LCAT** | lecithin-cholesterol acyltransferase |
| **LDH** | lactate dehydrogenase |
| **LDL** | low-density lipoprotein |
| **Leu** | leucine |
| **Lys** | lysine |
| **Met** | methionine |
| **MUFA** | monounsaturated fatty acid |
| **$NAD^+$** | nicotinamide adenine dinucleotide (ox.) |
| **NADH** | nicotinamide adenine dinucleotide (red.) |
| **$NADP^+$** | nicotinamide adenine dinucleotide phosphate (oxidized) |
| **NADPH** | nicotinamide adenine dinucleotide phosphate (reduced) |
| **ox. phos.** | oxidative phosphorylation |
| **P** | phosphate, e.g. glucose 6-P |
| **PDH** | pyruvate dehydrogenase |
| **PEP** | phosphoenolpyruvate |
| **PFK** | phosphofructokinase |
| **PGE** | prostaglandin, E series |
| **Phe** | phenylalanine |
| **$P_i$** | orthophosphate |
| **PK** | pyruvate kinase |
| **PLP** | pyridoxal 5´-phosphate |
| **$PP_i$** | pyrophosphate |
| **Pro** | proline |
| **PRPP** | 5´-phosphoribosyl-1´-pyrophosphate |
| **PUFA** | polyunsaturated fatty acid |
| **RNA** | ribonucleic acid |
| **Q** | ubiquinone |
| **Redox** | oxidation-reduction |
| **Ser** | serine |
| **SFA** | saturated fatty acid |
| **TCA Cycle** | tricarboxylic acid cycle (Krebs cycle) |
| **TG** | triacylglycerol (triglyceride) |
| **Thr** | threonine |
| **TPP** | thiamine pyrophosphate |
| **Trp** | tryptophan |
| **TTP** | thymidine 5´-triphosphate |
| **Tyr** | tyrosine |
| **UDP** | uridine 5´-diphosphate |
| **UMP** | uridine 5´-monophosphate |
| **UTP** | uridine 5´-triphosphate |
| **Val** | valine |
| **VLDL** | very low-density lipoprotein |

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