Bioenergetics and Biologic oxidation

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper’s Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

By

Dr. Muthanna Owaid Hussein

Bioenergetics, or biochemical thermodynamics, is study of the energy changes associated with the biochemical reactions **or** is a field in biochemistry and cell biology that concerns energy flow through living systems.

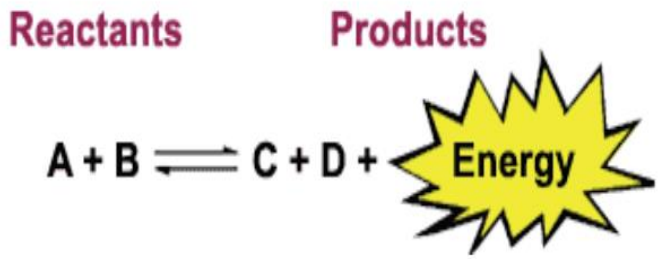
Biologic systems are essentially isothermic and use chemical energy to power living processes

Some problems related with the energy:

A. Death from **starvation** occurs when available energy reserves are depleted
B. Certain forms of **malnutrition** are associated with energy imbalance (**marasmus**).
C. Thyroid hormones control the metabolic rate (rate of energy release) and disease results if they malfunction Excess storage of surplus energy causes **obesity**

THERMODYNAMICS OF METABOLISM

- Overall process of catabolism RELEASES energy
- Overall process of anabolism REQUIRES energy input



How do we define amount of energy?

The quantity of usable energy (chemical potential) in a reaction is called the Gibbs Free Energy (ΔG).

ΔG is the difference between the energy contained in the products of a reaction and the reactants:

ΔG = (energy of products) - (energy of reactants)

FREE ENERGY IS THE USEFUL ENERGY IN A SYSTEM

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

Gibbs free energy, G, expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure.

Enthalpy, H, is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products.

Entropy, S, is a quantitative expression for the randomness or disorder in a system .

Biologic Systems Conform to the General Laws of Thermodynamics

The first law of thermodynamics states that the total energy of a system, including its surroundings, remains constant.

- It implies that within the total system, energy is neither lost nor gained during any change.

- The energy may be transferred from one part of the system to another or may be transformed into another form of energy.

In living systems, chemical energy may be transformed into heat or into electrical, radiant, or mechanical energy.

The second law of thermodynamics states that the total entropy of a system must increase if a process is to occur spontaneously.

The relationship between the free energy change (ΔG) of a reacting system and the change in entropy (ΔS) is expressed by the following equation, which combines the two laws of thermodynamics

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

where ΔH is the change in enthalpy(heat) and T is the absolute temperature In biochemical reactions, because ΔH is approximately equal to ΔE, the total change in internal energy of the reaction, the above relationship may be expressed in the following way:

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

In biological system chemical reactions are classified as being either exergonic or endergonic. That just means that a reaction can either release energy useful for work (an exergonic reaction) or requires energy to proceed (an endergonic reaction)

The spontaneous reaction is an exergonic reaction and ΔG will be negative. Thus, a negative ΔG value tells you that the reaction is possible

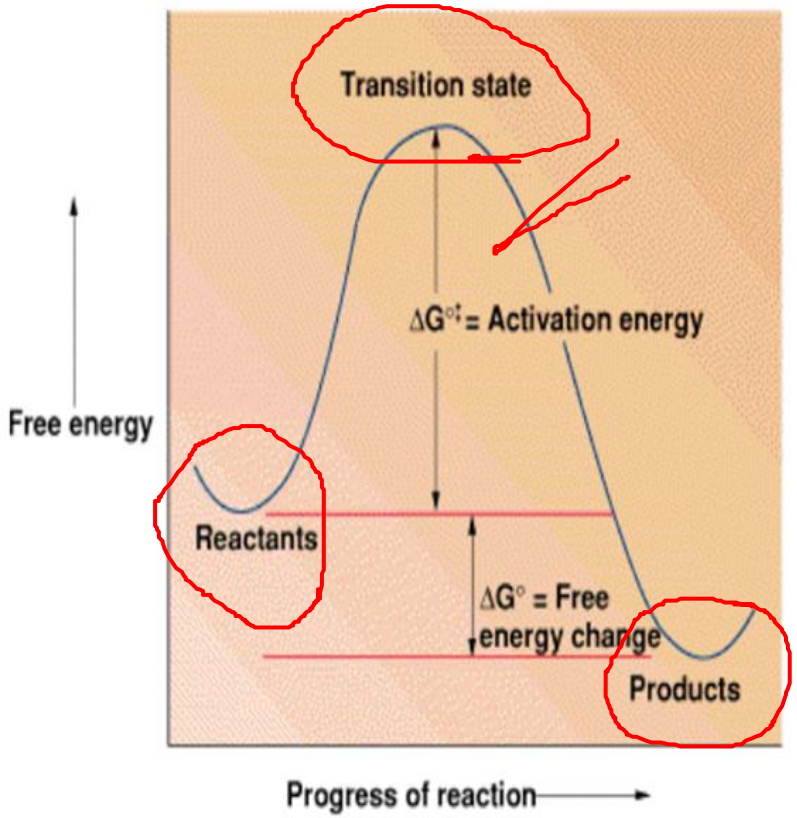


Table 14.5
Significance of $\Delta G^{\circ\prime}$ values

Value and Sign of $\Delta G^{\circ\prime}$	Thermodynamic Consequences
$\Delta G^{\circ\prime} = 0$	The reactants and the products are at the same energy level. The reaction under standard conditions is at equilibrium. No release of or requirement for energy.
$\Delta G^{\circ\prime} < 0$ (negative values)	The reaction releases energy as it approaches equilibrium. The reactants are at a higher energy level than products. Useful energy is released and available to do work.
$\Delta G^{\circ\prime} > 0$ (positive values)	The reactants are at a lower energy than products. The reaction requires an input of energy to proceed as written.

Table 14-5 Concepts in Biochemistry, 3/e

If ΔG is negative, the reaction proceeds spontaneously with loss of free energy, that is, it is exergonic. If, in addition, ΔG is of great magnitude, the reaction goes virtually to completion and is essentially irreversible.

On the other hand, if ΔG is positive, the reaction proceeds only if free energy can be gained, that is, it is endergonic. If, in addition, the magnitude of ΔG is great, the system is stable, with little or no tendency for a reaction to occur.

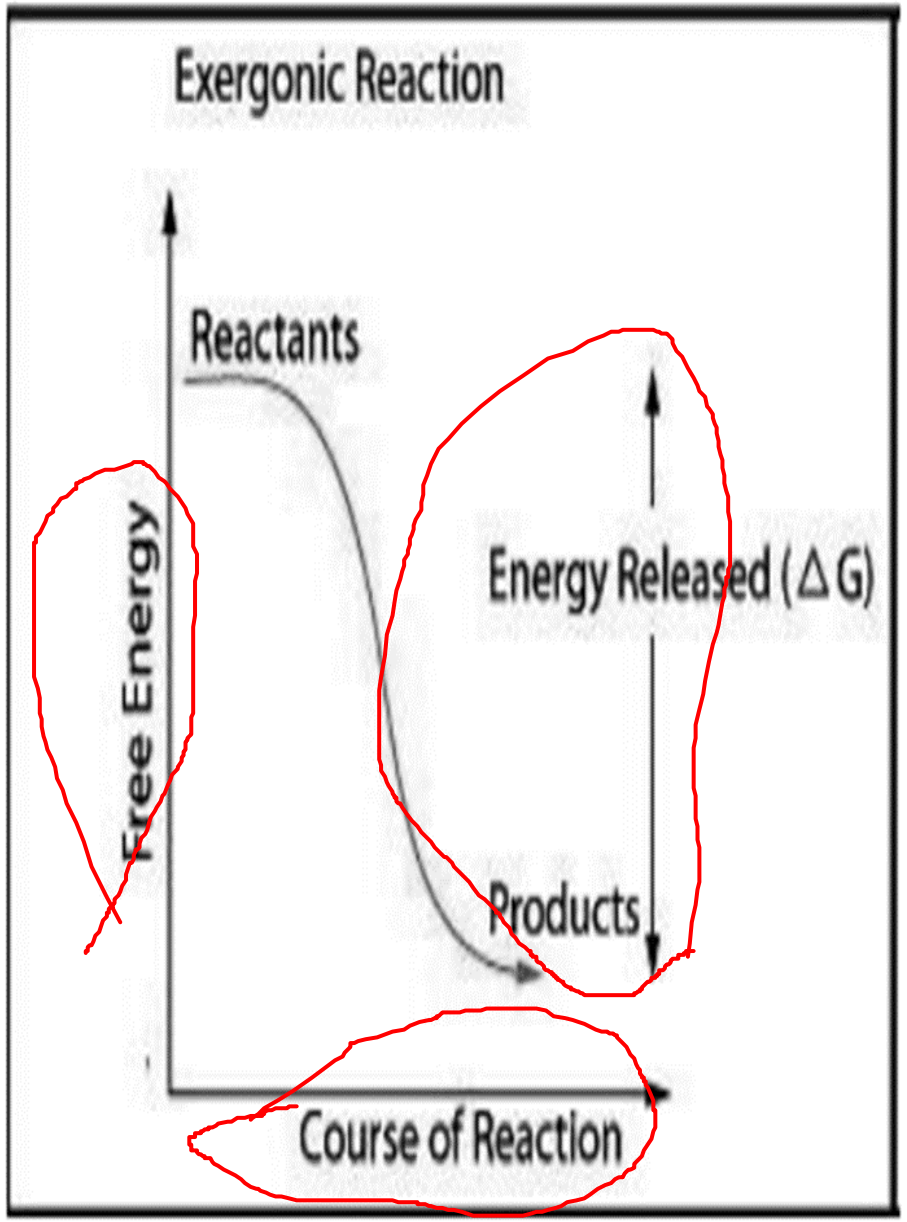
If ΔG is zero, the system is at equilibrium and no net change takes place.

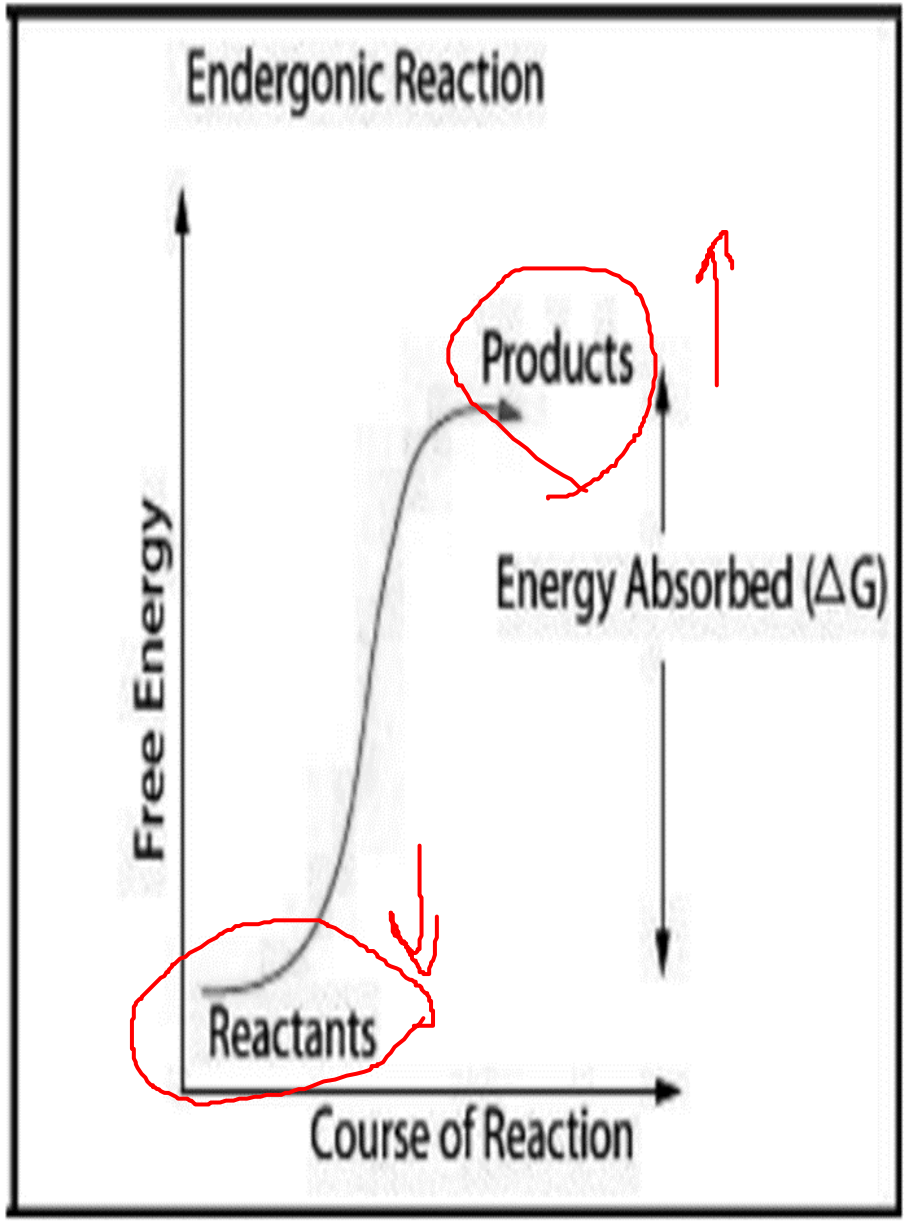
Exergonic Reaction

- Exergonic implies the release of energy from a spontaneous chemical reaction without any concomitant utilization of energy.
- The reactions are significant in terms of biology as these reactions have an ability to perform work and include most of the catabolic reactions in cellular respiration
- Most of these reactions involve the breaking of bonds during the formation of reaction intermediates as is evidently observed during respiratory pathways. The bonds that are created during the formation of metabolites are stronger than the cleaved bonds of the substrate
- $\Delta G = G_{products} - G_{reactants} < 0$..

Endergonic Reactions

- Endergonic in turn is the opposite of exergonic in being non-spontaneous and requires an input of free energy.
- Most of the anabolic reactions like photosynthesis , DNA, and protein synthesis are endergonic in nature.
- $\Delta G = G_{products} - G_{reactants} > 0$.


Exergonic Reaction
Reactants
Free Energy
Energy Released (ΔG)
Products
Course of Reaction


Endergonic Reaction
Products
Free Energy
Energy Absorbed (ΔG)
Reactants
Course of Reaction

Standard free energy change

The reactants are present in concentrations of 1.0 mol/L, ΔG^{o} is the standard free energy change. For biochemical reactions, a standard state is defined as having a pH of 7.0. The standard free energy change at this standard state is denoted by $\Delta G^{o\prime}$. The standard free energy change can be calculated from the equilibrium constant Keq.

$$\Delta G^{o\prime} = -RT \ln Keq$$

Thermodynamic constants

Boltzmann constant, $\mathbf{k} = 1.381 \times 10^{-23}$ J/K
Avogadro's number, $N = 6.022 \times 10^{23}$ mol^{-1}
Faraday constant, $\mathcal{F} = 96{,}480$ J/V · mol
Gas constant, $R = 8.315$ J/mol · K
$(= 1.987$ cal/mol · K)

Units of ΔG and ΔH are J/mol (or cal/mol)
Units of ΔS are J/mol · K (or cal/mol · K)
1 cal = 4.184 J

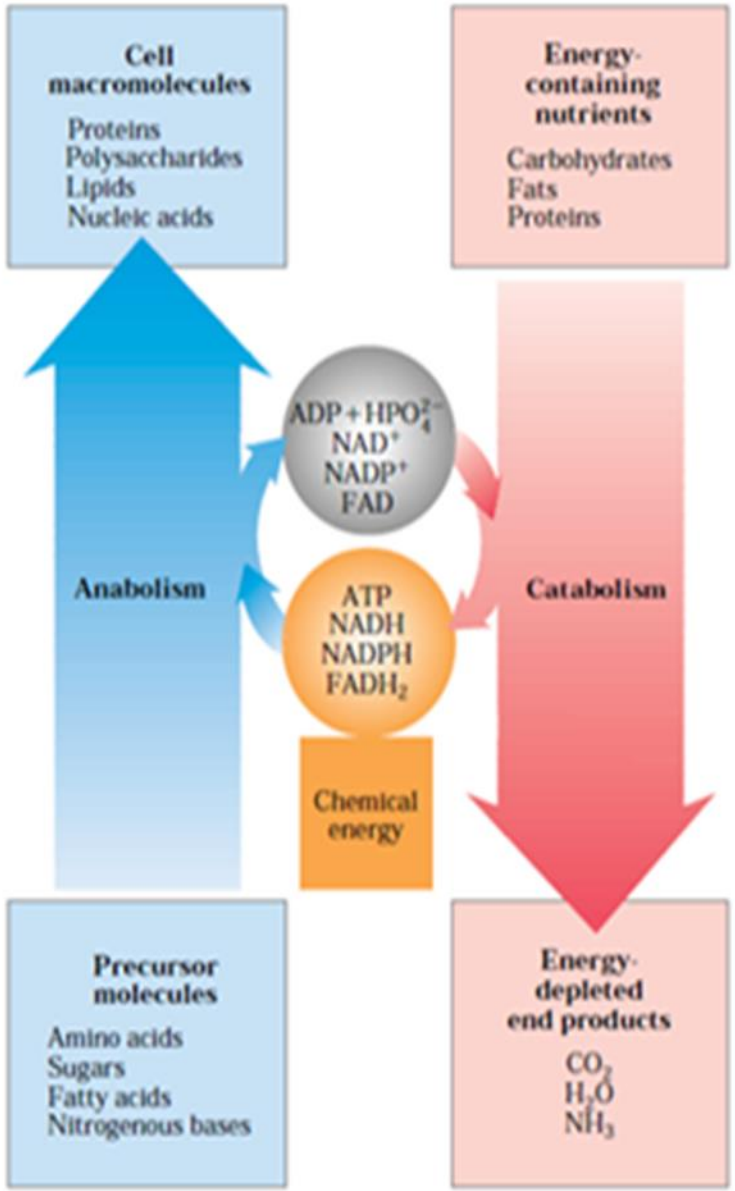
Units of absolute temperature, T, are Kelvin, K
25 °C = 298 K
At 25 °C, $RT = 2.479$ kJ/mol
$(= 0.592$ kcal/mol)

ENDERGONIC PROCESSES PROCEED BY COUPLING TO EXERGONIC PROCESSES

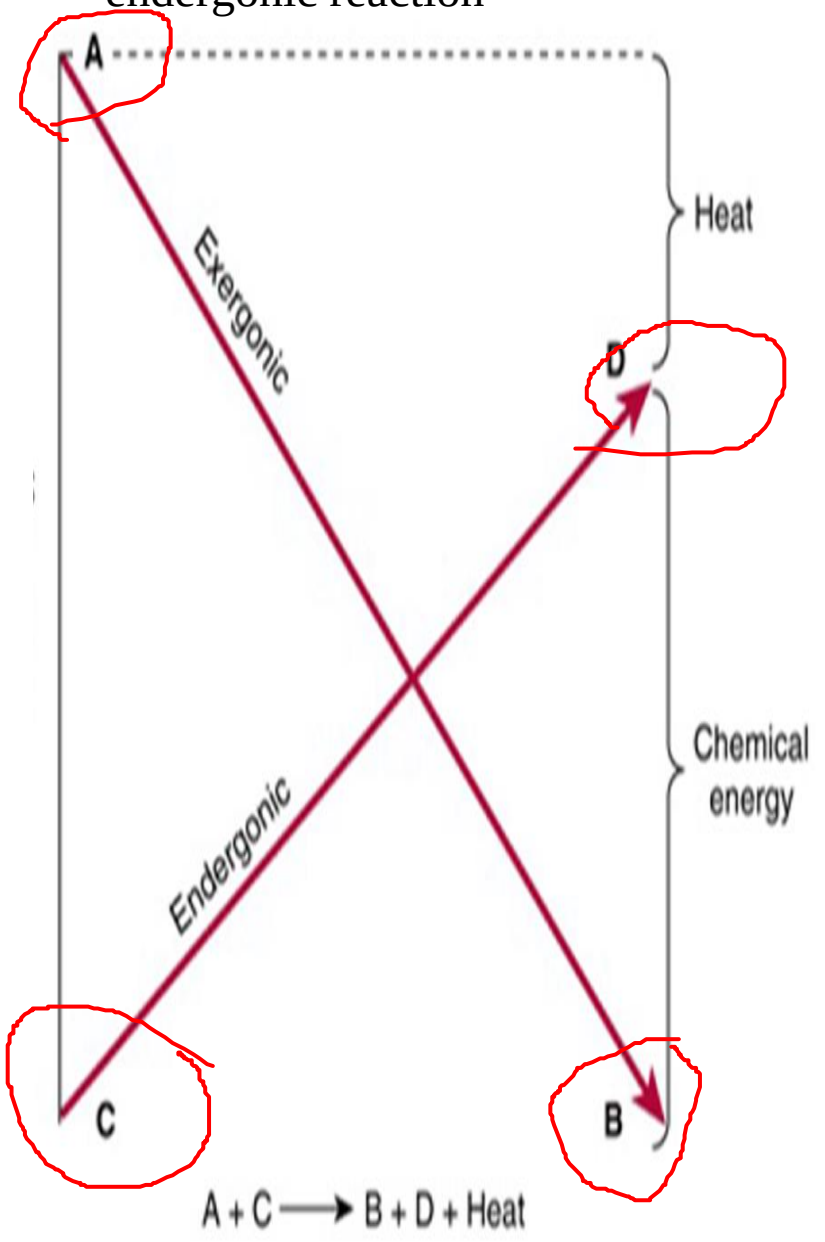
The vital processes, synthetic reactions, muscular contraction, nerve impulse conduction, and active transport—obtain energy by chemical linkage, or coupling, to oxidative reactions.

- The exergonic reactions are termed catabolism(generally, the breakdown or oxidation of fuel molecules)

- whereas the synthetic reactions that build up substances are termed anabolism. The combined catabolic and anabolic processes constitute metabolism.



Coupling of an exergonic to an endergonic reaction



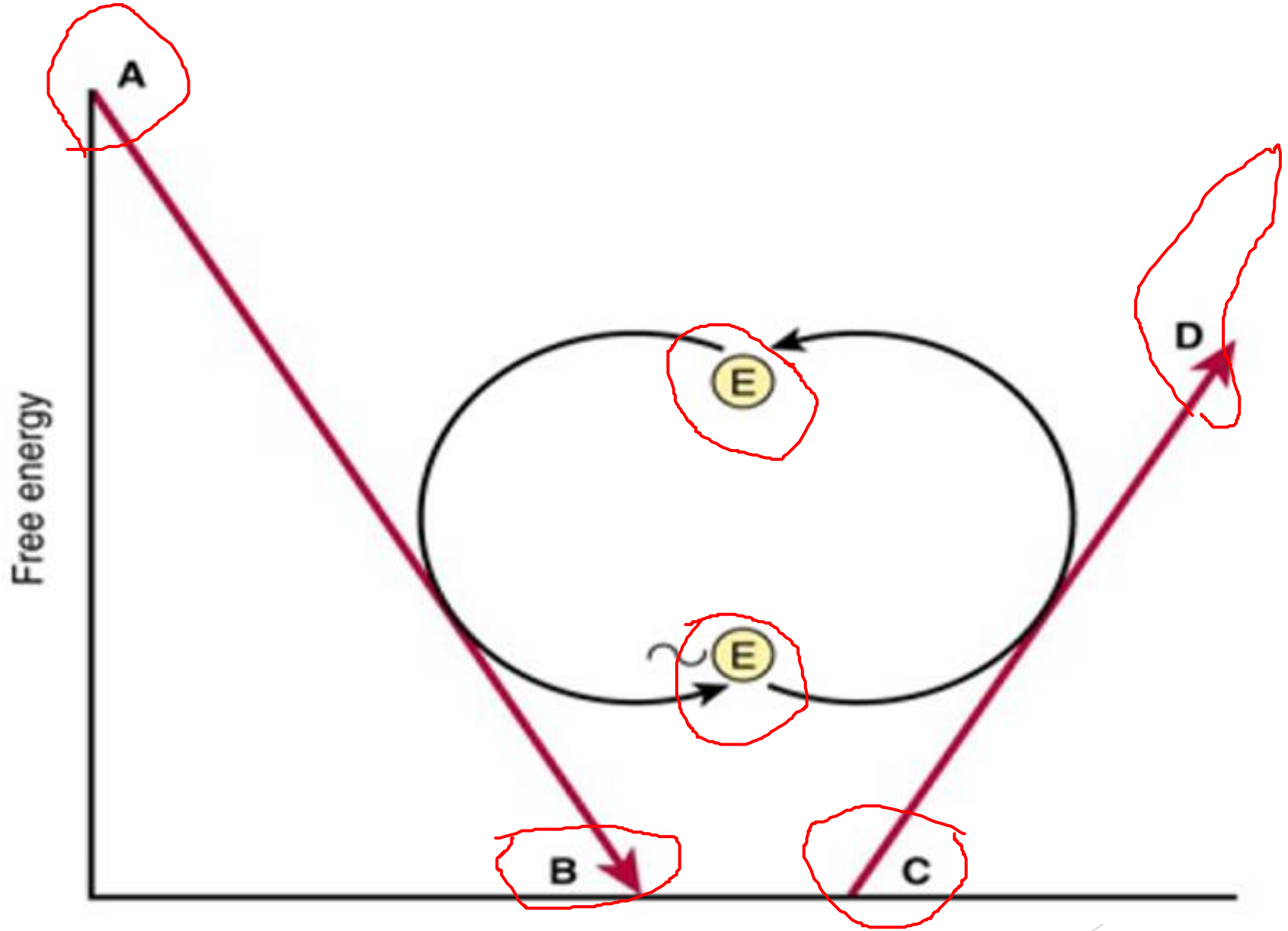
HIGH-ENERGY PHOSPHATES PLAY A CENTRAL ROLE IN ENERGY CAPTURE AND TRANSFER

In order to maintain living processes, all organisms must obtain supplies of free energy from their environment.

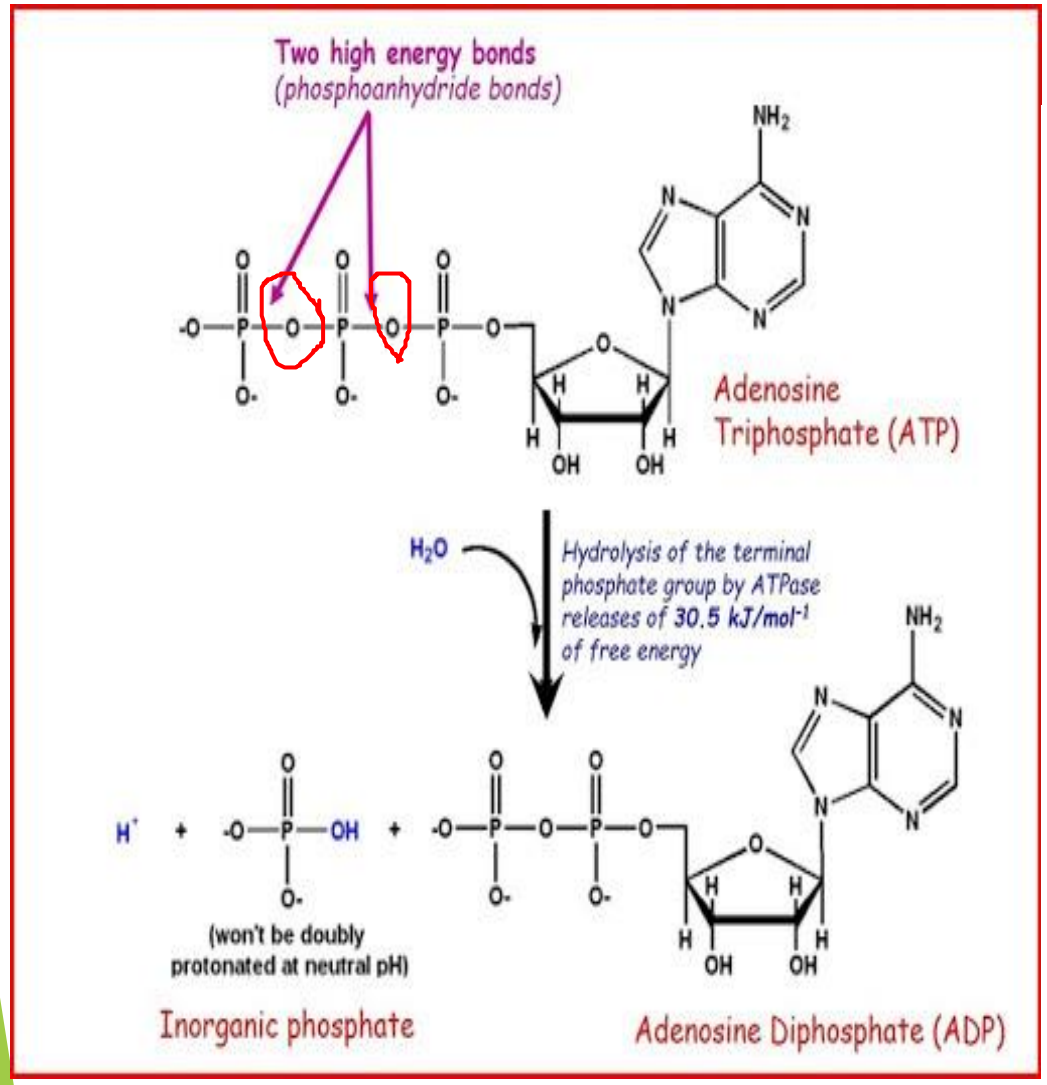
Autotrophic organisms utilize simple exergonic processes; eg, the energy of sunlight (green plants), the reaction Fe^{2+} Fe^{3+} (some bacteria).

Heterotrophic organisms obtain free energy by coupling their metabolism to the breakdown of complex organic molecules in their environment. In all these organisms, ATP plays a central role in the transference of free energy from the exergonic to the endergonic processes (Figure below). high-energy intermediate compound (~E).

Transfer of free energy from an exergonic to an endergonic reaction via a high-energy intermediate compound



ATP is a nucleotide containing of the nucleoside adenosine (adenine linked to ribose), and three phosphate groups in its reactions in the cell, it functions as the Mg^{2+} complex



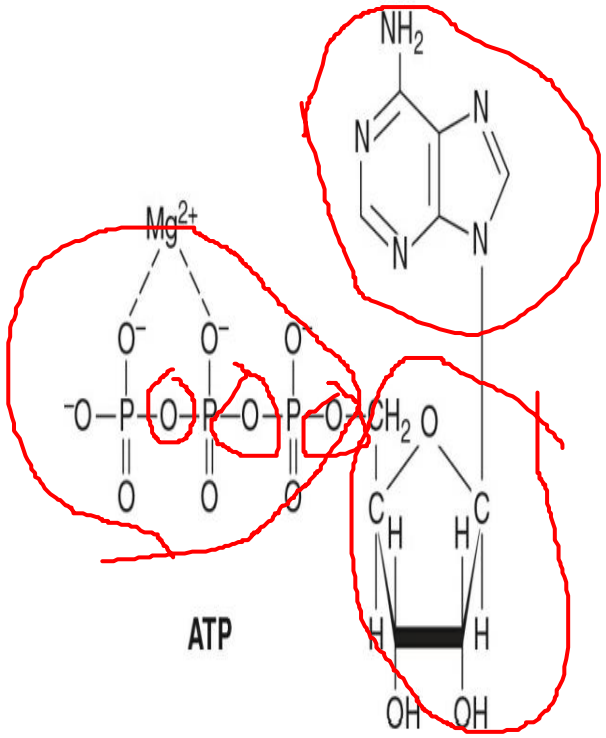


FIGURE 11–3 Adenosine triphosphate (ATP) is shown as the magnesium complex.

The standard free energy of hydrolysis of a number of biochemically important phosphates is shown in the Table

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

[1] P_i, inorganic orthophosphate.
[2] Values for ATP and most others taken from Krebs and Kornberg

Low-energy phosphates, exemplified by the ester phosphates found in the intermediates of glycolysis, have G′values smaller than that of ATP, while in high-energy phosphates the value is higher than that of ATP.

The symbol ~P indicates that larger quantity of free energy. For this reason, the term group transfer potential, rather than “high-energy Bond”. Thus, ATP contains two high-energy phosphate groups and ADP contains one, whereas the phosphate in AMP is of the low energy type since it is a normal ester link

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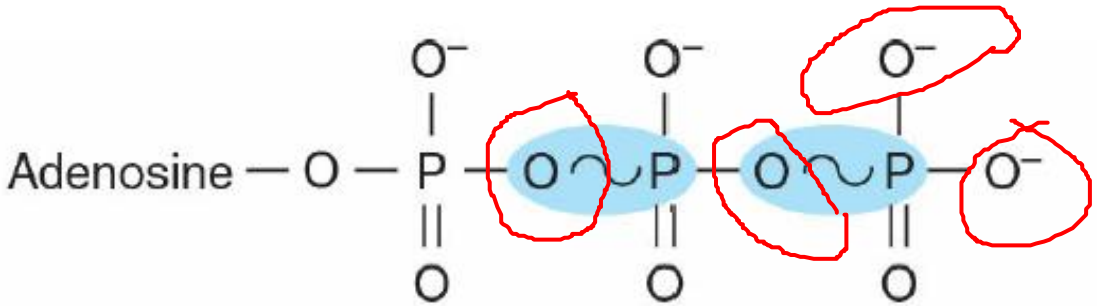
Reaction type	$\Delta G'^\circ$ (kJ/mol)	$\Delta G'^\circ$ (kcal/mol)
Hydrolysis reactions		
Acid anhydrides		
Acetic anhydride + $H_2O \longrightarrow$ 2 acetate	−91.1	−21.8
ATP + $H_2O \longrightarrow$ ADP + P_i	−30.5	−7.3
ATP + $H_2O \longrightarrow$ AMP + PP_i	−45.6	−10.9
PP_i + $H_2O \longrightarrow$ $2P_i$	−19.2	−4.6
UDP-glucose + $H_2O \longrightarrow$ UMP + glucose 1-phosphate	−43.0	−10.3
Esters		
Ethyl acetate + $H_2O \longrightarrow$ ethanol + acetate	−19.6	−4.7
Glucose 6-phosphate + $H_2O \longrightarrow$ glucose + P_i	−13.8	−3.3
Amides and peptides		
Glutamine + $H_2O \longrightarrow$ glutamate + NH_4^+	−14.2	−3.4
Glycylglycine + $H_2O \longrightarrow$ 2 glycine	−9.2	−2.2
Glycosides		
Maltose + $H_2O \longrightarrow$ 2 glucose	−15.5	−3.7
Lactose + $H_2O \longrightarrow$ glucose + galactose	−15.9	−3.8
Rearrangements		
Glucose 1-phosphate $\longrightarrow$ glucose 6-phosphate	−7.3	−1.7
Fructose 6-phosphate $\longrightarrow$ glucose 6-phosphate	−1.7	−0.4
Elimination of water		
Malate $\longrightarrow$ fumarate + H_2O	3.1	0.8
Oxidations with molecular oxygen		
Glucose + $6O_2 \longrightarrow 6CO_2 + 6H_2O$	−2,840	−686
Palmitate + $23O_2 \longrightarrow 16CO_2 + 16H_2O$	−9,770	−2,338

The Intermediate Value for the Free Energy of hydrolysis of ATP has Important Bioenergetic Significance

The high free-energy change on hydrolysis of ATP is due to relief of charge repulsion of adjacent negatively charged oxygen atoms and to stabilization of the reaction products, especially phosphate, as resonance hybrids Structure of ATP, ADP, and AMP showing the position and the number of high-energy phosphates (~ P).

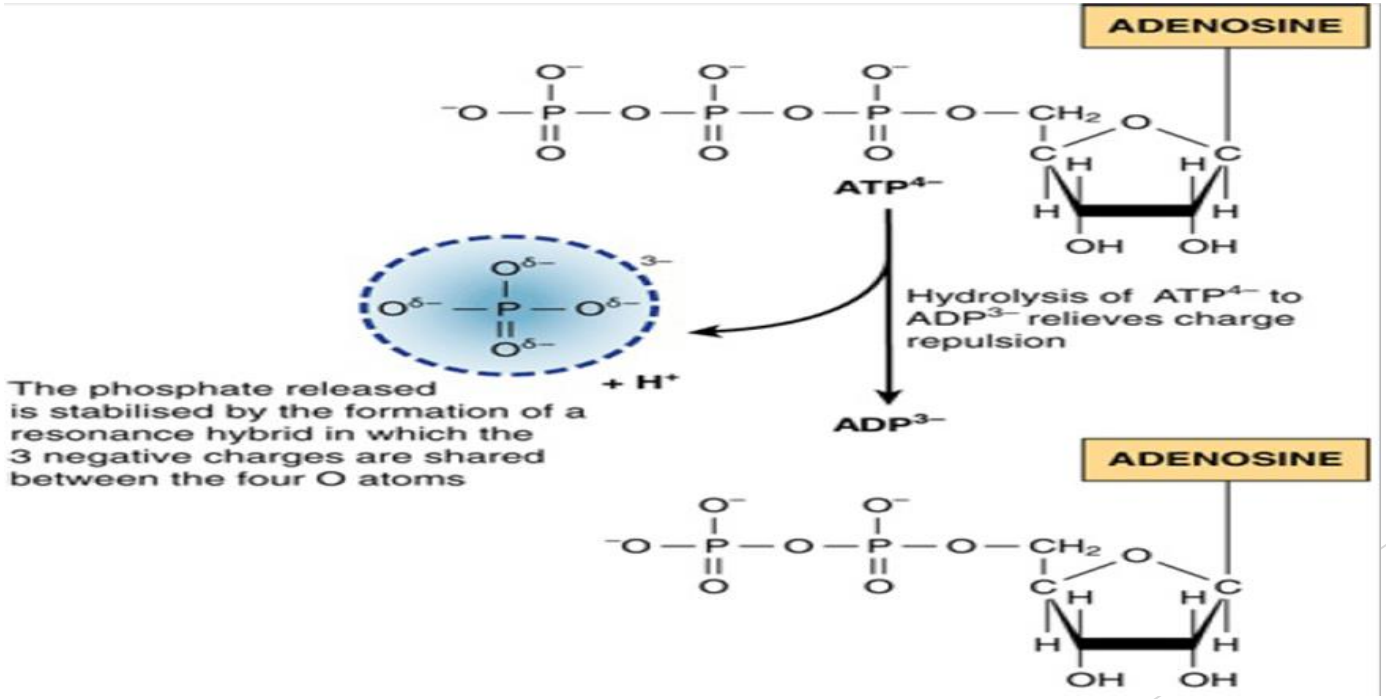


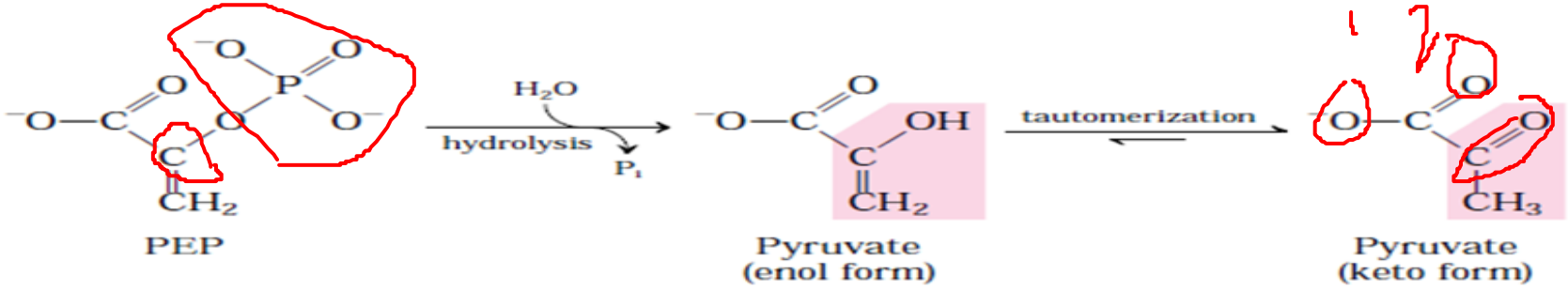
Adenosine triphosphate (ATP)

Adenosine diphosphate (ADP)

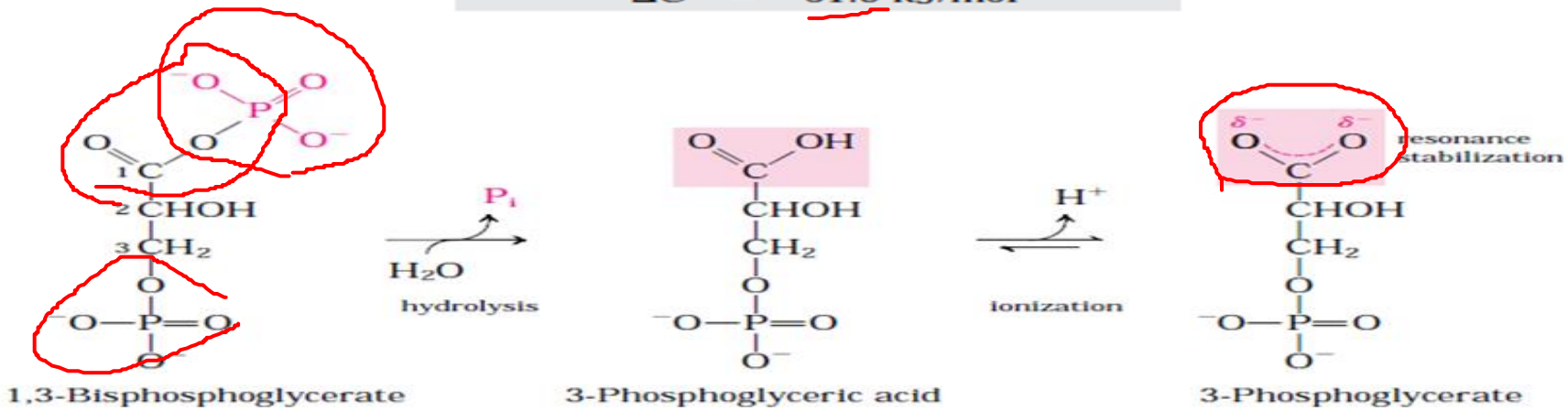
Adenosine monophosphate (AMP)

The value for the hydrolysis of the terminal phosphate of ATP divides the list into two groups. Low-energy phosphates, exemplified by the ester phosphates found in the intermediates of glycolysis, have $G^{o'}$ values smaller than that of ATP, while in high-energy phosphates the value is higher than that of ATP. The components of this latter group, including ATP, are usually anhydrides (eg, the 1-phosphate of 1,3-bisphosphoglycerate), enolphosphates (eg, phosphoenolpyruvate), and phosphoguanidines (eg, creatine phosphate, arginine phosphate).

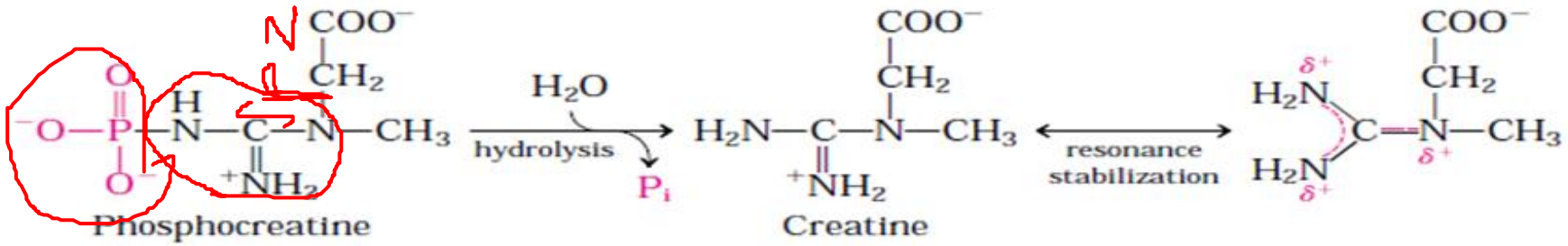




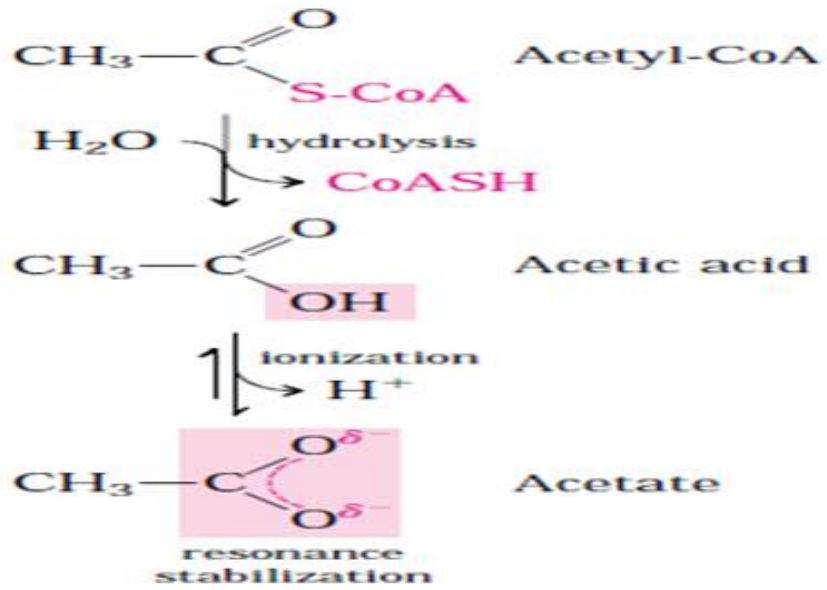
$$PEP^{3-} + H_2O \longrightarrow pyruvate^{-} + P_i^{2-}$$
$$\Delta G'^{\circ} = -61.9\ kJ/mol$$



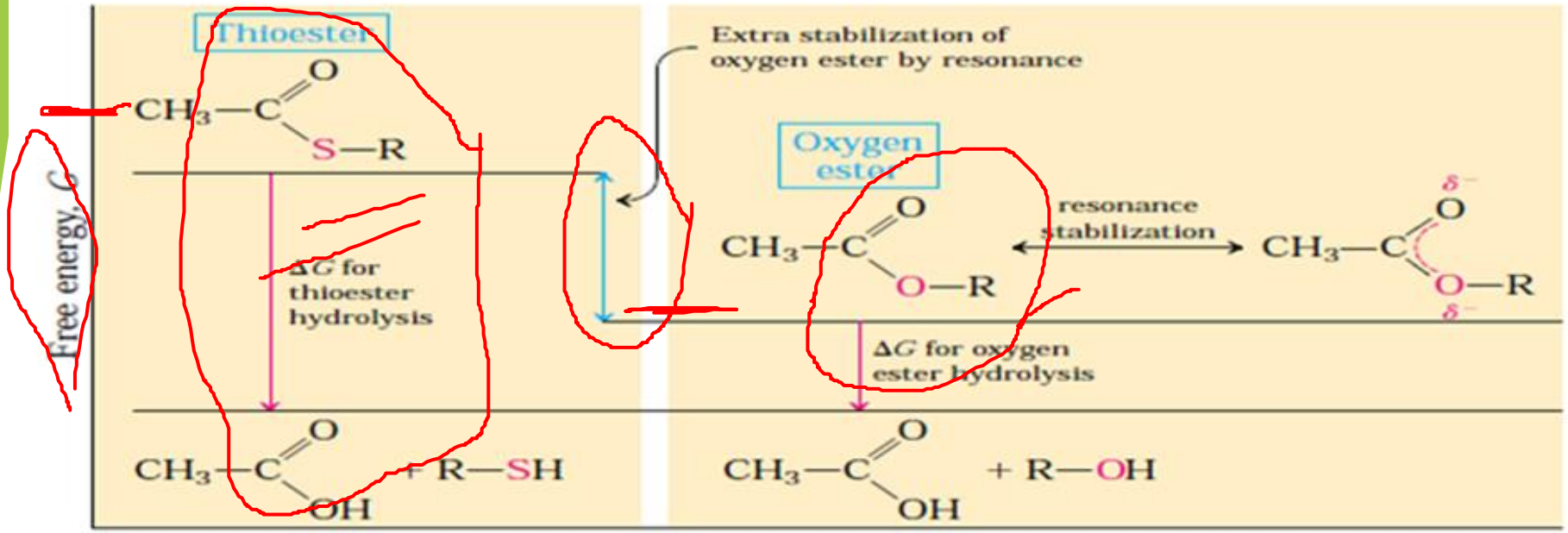
$$1,3\text{-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow 3\text{-phosphoglycerate}^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -49.3\ kJ/mol$$



$$Phosphocreatine^{2-} + H_2O \longrightarrow creatine + P_i^{2-}$$
$$\Delta G'^{\circ} = -43.0\ kJ/mol$$



$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$
$$\Delta G'^{\circ} = -31.4\ \text{kJ/mol}$$



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

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

1. Oxidative phosphorylation
2. Glycolysis.
3. The citric acid cycle.

Phosphagens act as storage forms of group transfer potential and include creatine phosphate, which occurs in vertebrate skeletal muscle, heart, spermatozoa, and brain, and arginine phosphate, which occurs in invertebrate muscle.

When ATP is rapidly being utilized as a source of energy for muscular contraction, phosphagens permit its concentrations to be maintained, but when the ATP/ADP ratio is high, their concentration can increase to act as an

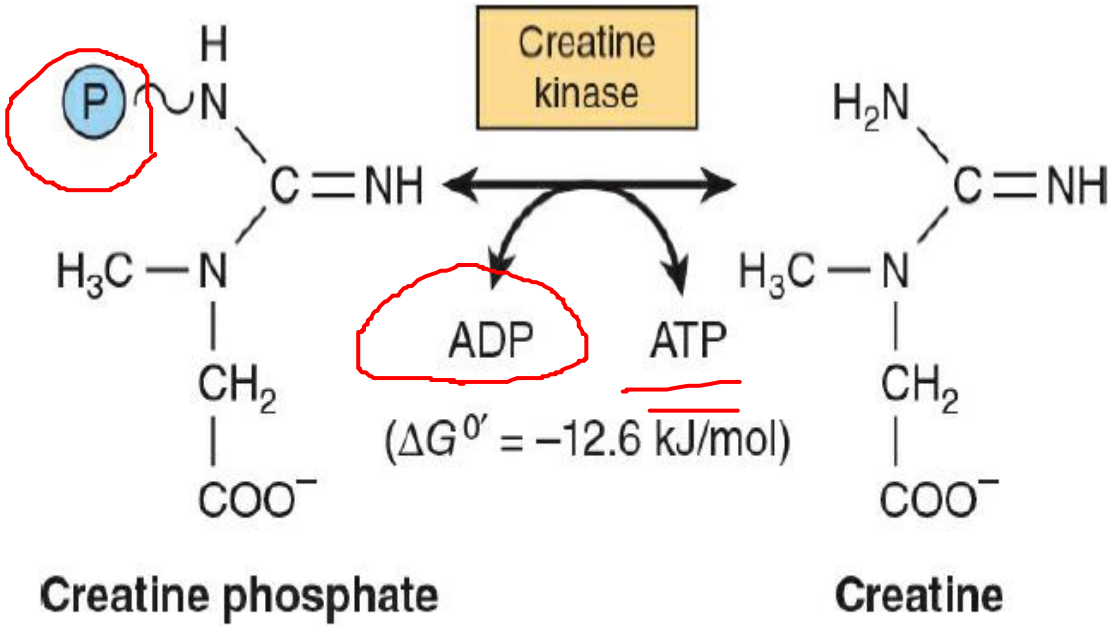
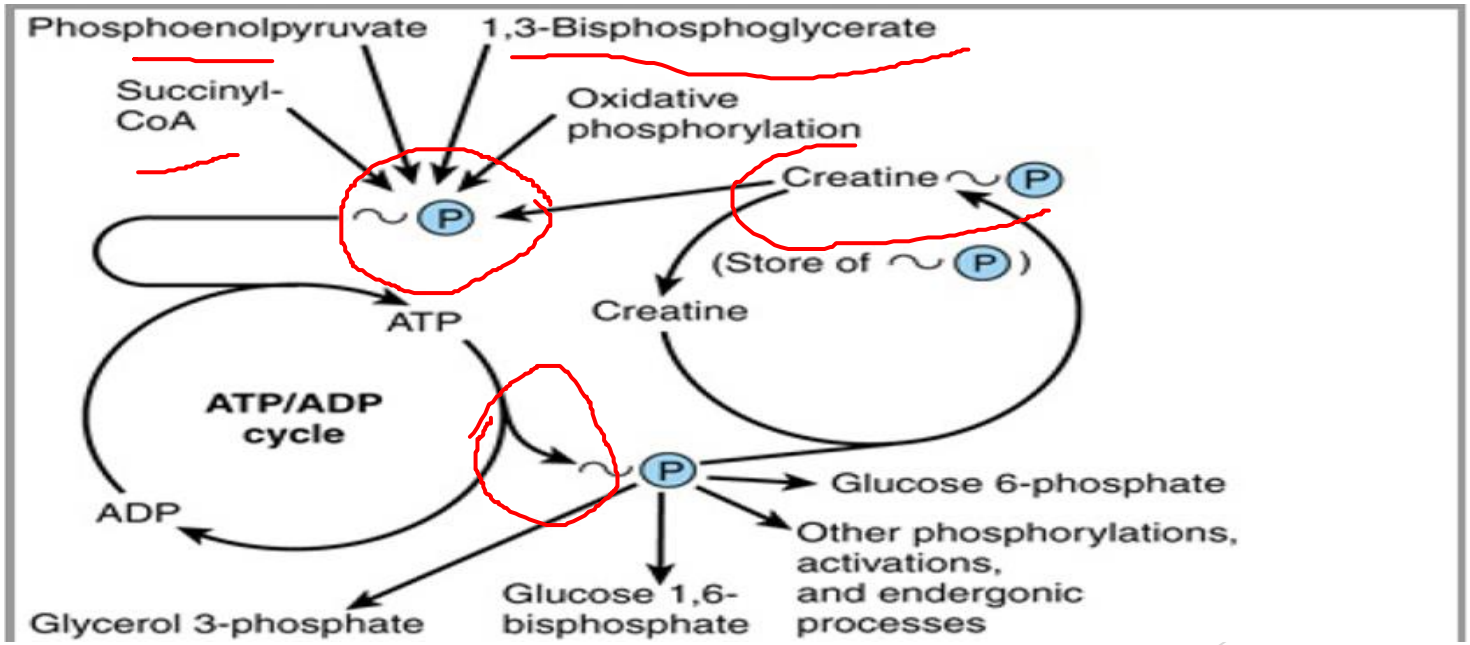


FIGURE 11–7 **Transfer of high-energy phosphate between ATP and creatine.**

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

ATP is able to act as a donor of high-energy phosphate to form those compounds below. The necessary enzymes, ADP can accept high-energy phosphate to form ATP . In effect, an **ATP/ADP cycle** connects those processes that generate ~ P to those processes that utilize ~P , continuously consuming and regenerating ATP. This occurs at a very rapid rate since the total ATP/ADP pool is extremely small and sufficient to maintain an active tissue for only a few seconds. In a cell, the ratio of ATP to ADP concentrations is known as the "energy charge" of the cell .



The cell can use this energy charge to relay information about cellular needs; if there is more ATP than ADP available, the cell can use ATP to do work, but if there is more ADP than ATP available, the cell must synthesize ATP via oxidative phosphorylation ATP allows the Coupling of thermodynamically Unfavorable reactions to Favorable Ones .

Adenylate Kinase (Myokinase) Interconverts adenine Nucleotides

Adenylate kinase is important for the maintenance of energy homeostasis in cells because it allows:

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

2. The AMP formed as a consequence of activating reactions involving ATP to rephosphorylated to ADP.

3. AMP to increase in concentration when ATP becomes depleted so that it is able to act as a metabolic (allosteric) signal to increase the rate of catabolic reactions, which in turn lead to the generation of more ATP

THANK YOU

Digestion of Lipids & Oxidation of Fatty Acids : Ketogenesis

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper’s Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

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BIOMEDICAL IMPORTANCE

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties.

They have the common property of being

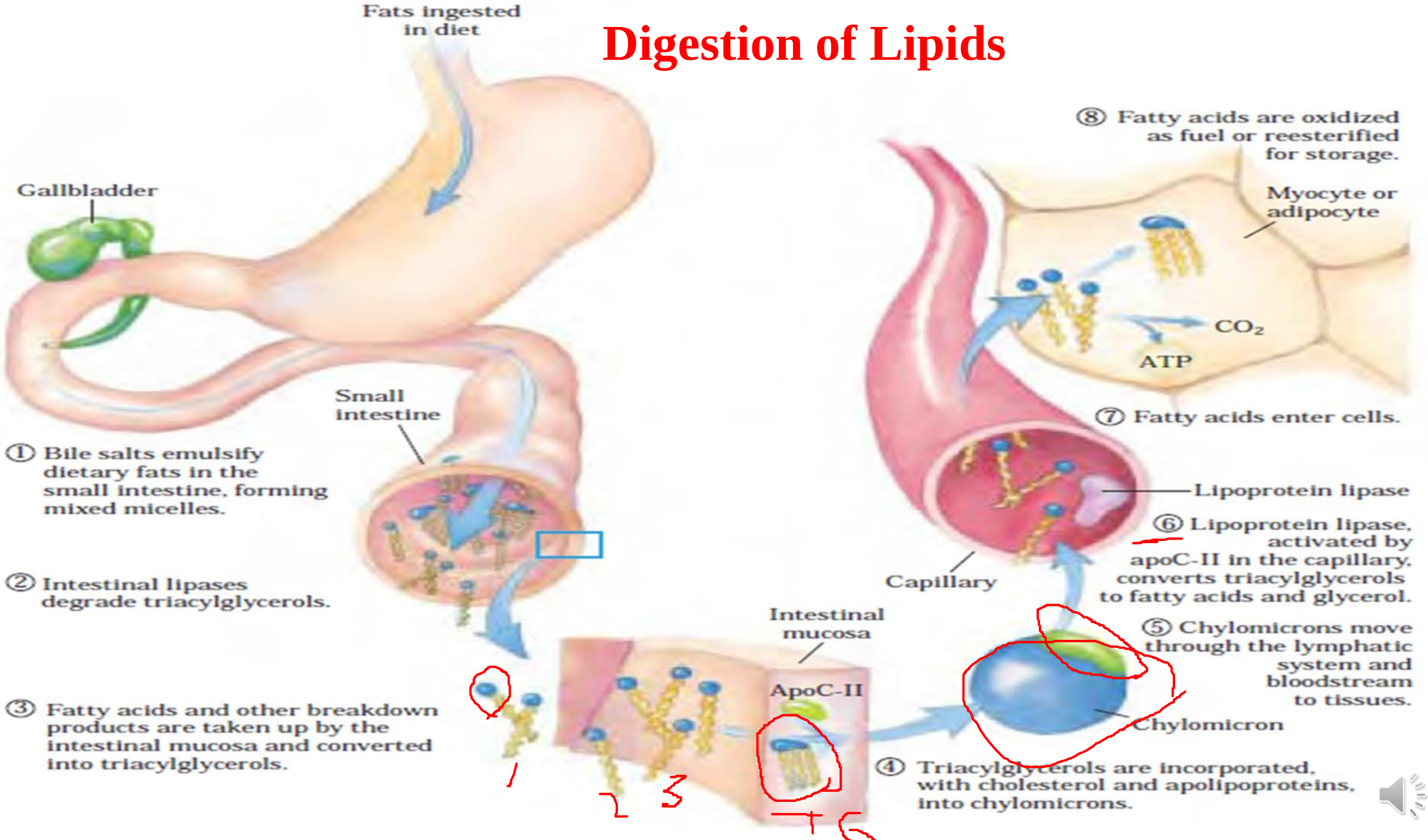
(1) Relatively insoluble in water

(2) Soluble in nonpolar solvents such as ether and chloroform.

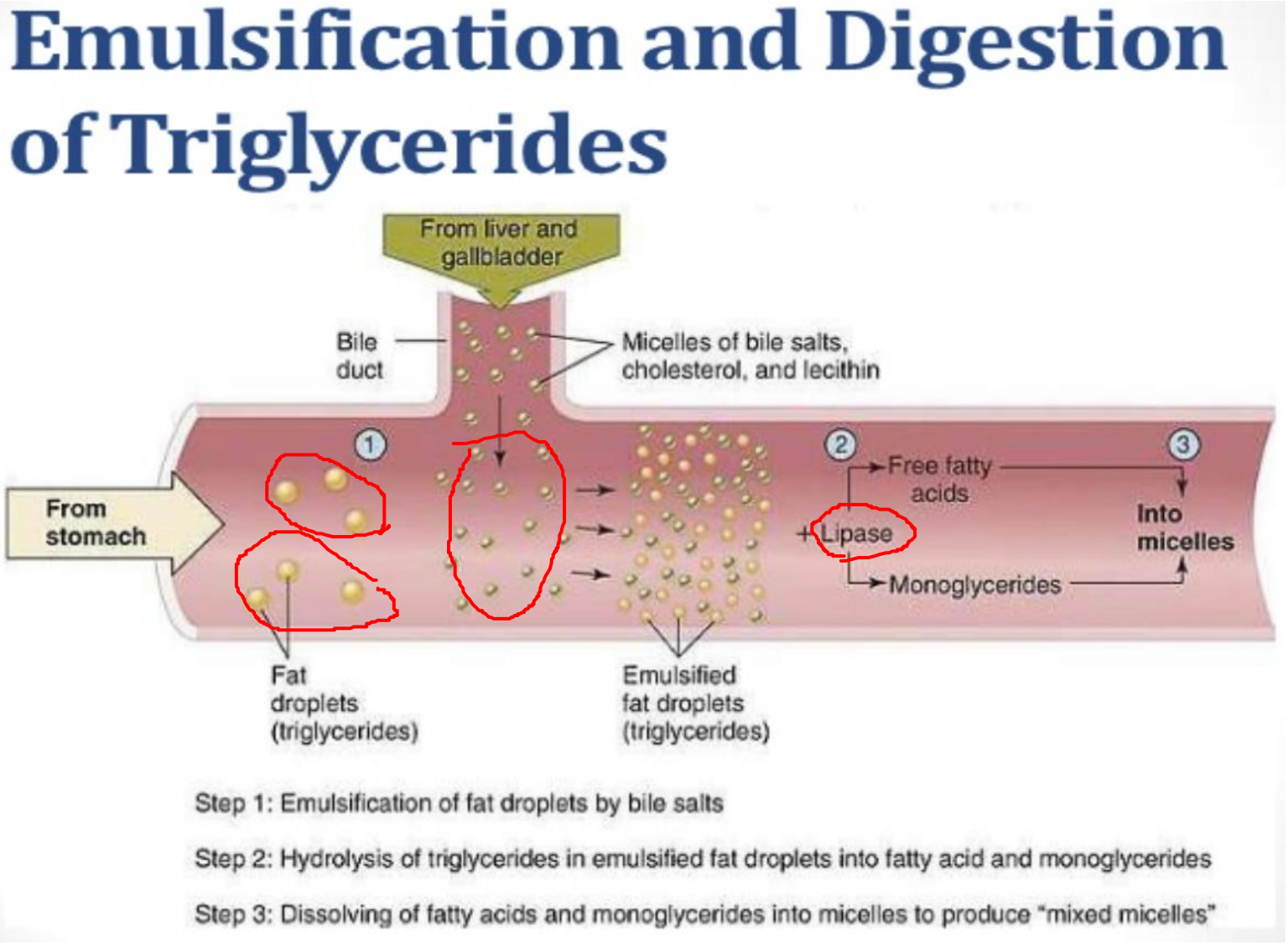
They are important dietary components not only because of the high energy value of fats but also because essential fatty acids, fat-soluble vitamins, and other lipophilic micronutrients are contained in the fat of natural foods. Dietary supplementation with long-chain ω3 fatty acids is believed to have beneficial effects in several chronic diseases, like cardiovascular disease.

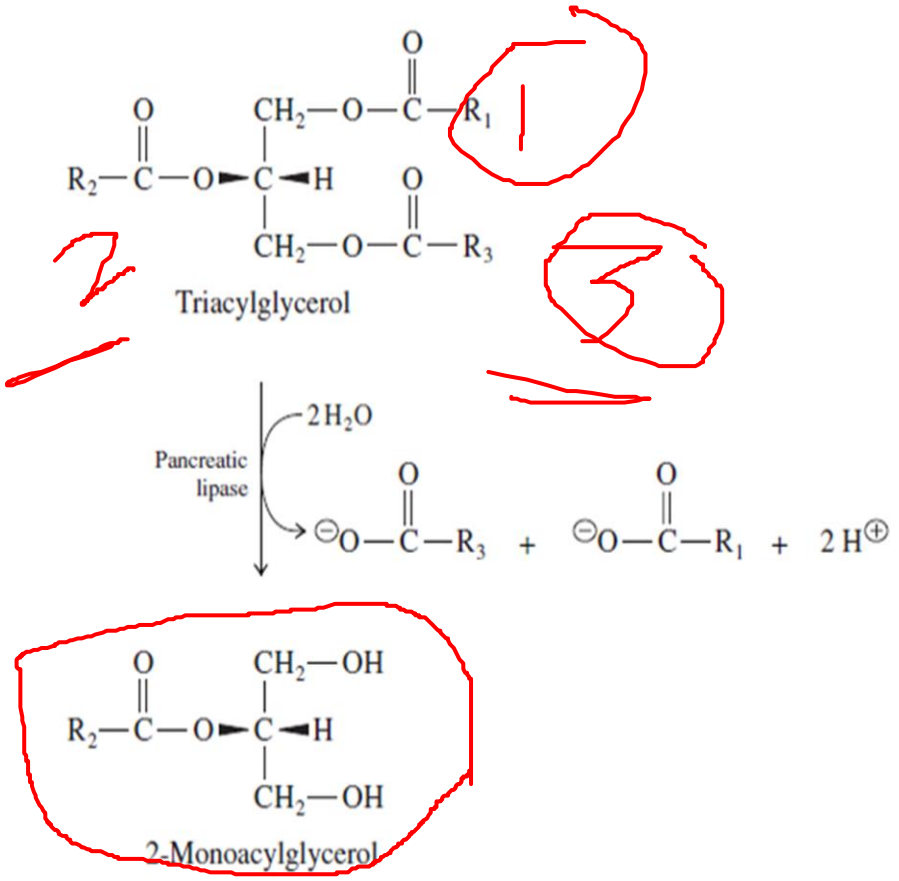
Fatty acids are broken down in mitochondria by oxidation to acetyl-CoA in a process that generates large amounts of energy. When this pathway is happening at a high rate, three compounds, acetoacetate, D-3- hydroxybutyrate, and acetone, known collectively as the ketone bodies, are produced by the liver. Acetoacetate and D-3-hydroxybutyrate are used as fuels by extrahepatic tissues in normal metabolism, but overproduction of ketone bodies causes ketosis. Increased fatty acid oxidation and consequently ketosis is a characteristic of starvation and of diabetes. Since ketone bodies are acidic, when they are produced in excess over long periods, as in diabetes, they cause ketoacidosis, which is ultimately fatal. Because gluconeogenesis is dependent on fatty acid oxidation, any impairment in fatty acid oxidation leads to hypoglycemia. This occurs in various states of carnitine deficiency or deficiency of essential enzymes in fatty acid oxidation, for example, carnitine palmitoyltransferase, or inhibition of fatty acid oxidation by toxins, for example, hypoglycin.

Digestion of Lipids



Pancreatic lipase acts to degrade triacylglycerols in the fat particles. This lipase catalyzes hydrolysis at the C-1 and C-3 positions of a triacylglycerol, producing free fatty acids and a 2-monoacylglycerol

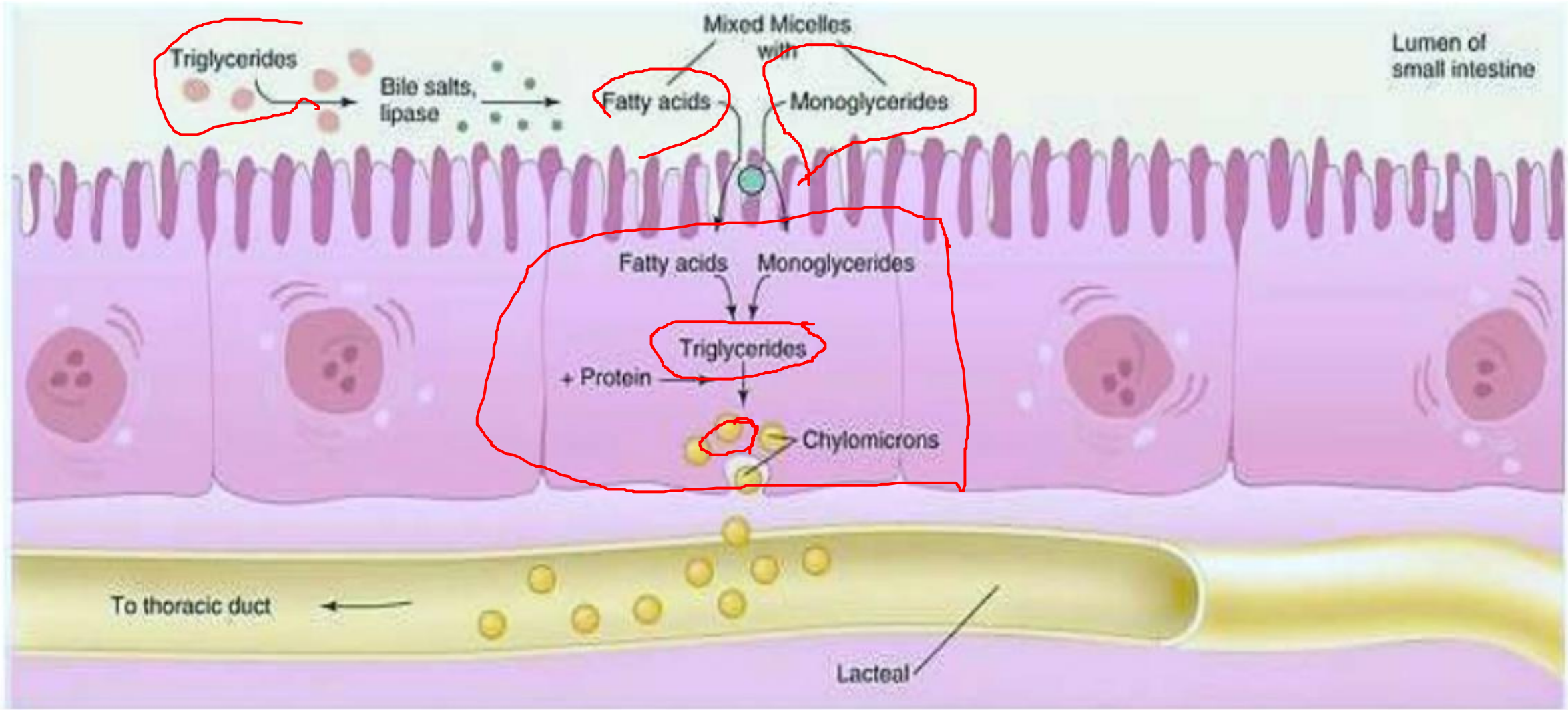




pancreatic lipases.

Note: The obesity-management / weightreduction prescription drug **Xenical** is an inhibitor of pancreatic lipase. It prevents absorption of dietary TG.

Formation and Transportation of Chylomicrons



Absorption of Dietary Lipids:

- On average, fat makes up 37% of calories in American diet
- Most diet lipids of mammals are TAGs
- 90% of the fat we eat is TAG; rest: cholesterol esters, phospholipids, essential unsaturated fatty acids (Linoleic acid (LA) (omega-6) $18{:}2\Delta^{9,12}$ and linolenic acid (omega-3) $18{:}3\Delta^{9,12,15}$), and fat soluble vitamins A, D, E, and K
- In normal individuals, 95% of fat consumed is absorbed and most transported to adipose for storage.
- General principle of dietary lipid assimilation is to hydrolyze large non-absorbable molecules into smaller units.

1. Emulsification starts in the stomach and continues in the lumen of the small Intestine.

2. In the small intestine, fat particles are coated with bile salts and digested

3. Free FA and cholesterol from bile salt mixed micelles are taken up through the intestinal wall

4. Once inside intestinal cells, cholesteryl acyl-CoA esters are formed and FA are resynthesized into triacylglycerols (TAGs).

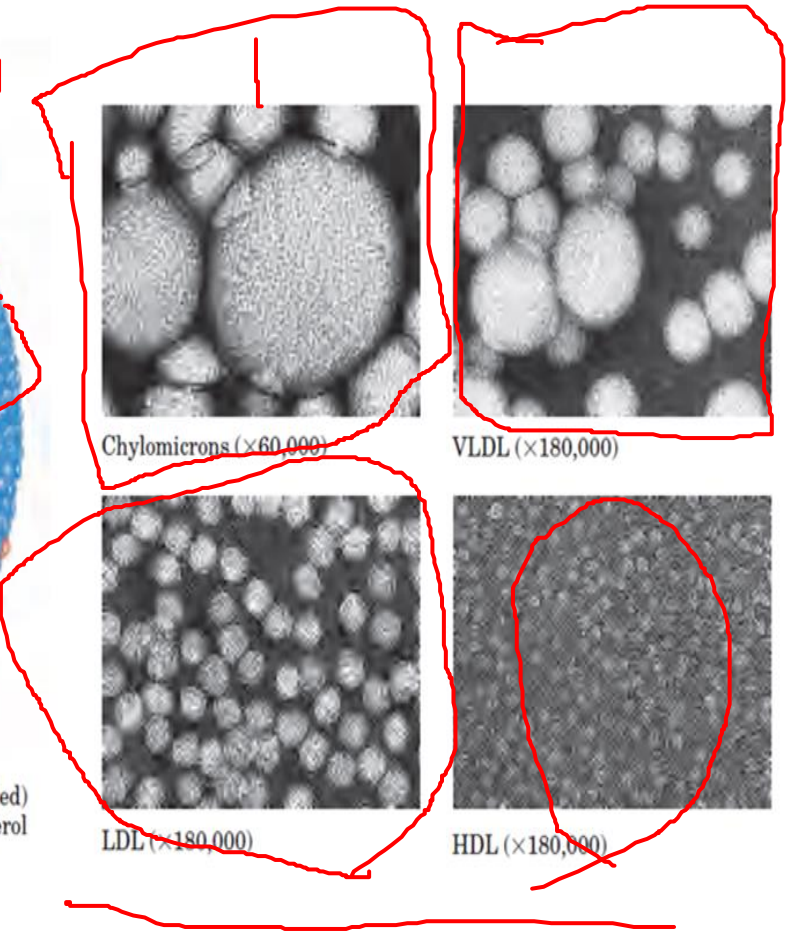
5. TAGs, cholesterol and apoproteins are packaged into a chylomicron (lipoprotein) and exported into blood

6. Lipoprotein lipase on cells lining capillary wall adjacent to ADIPOSE and MUSCLE tissue promotes release of fatty acids; The chylomicrons dock with lipoprotein lipases

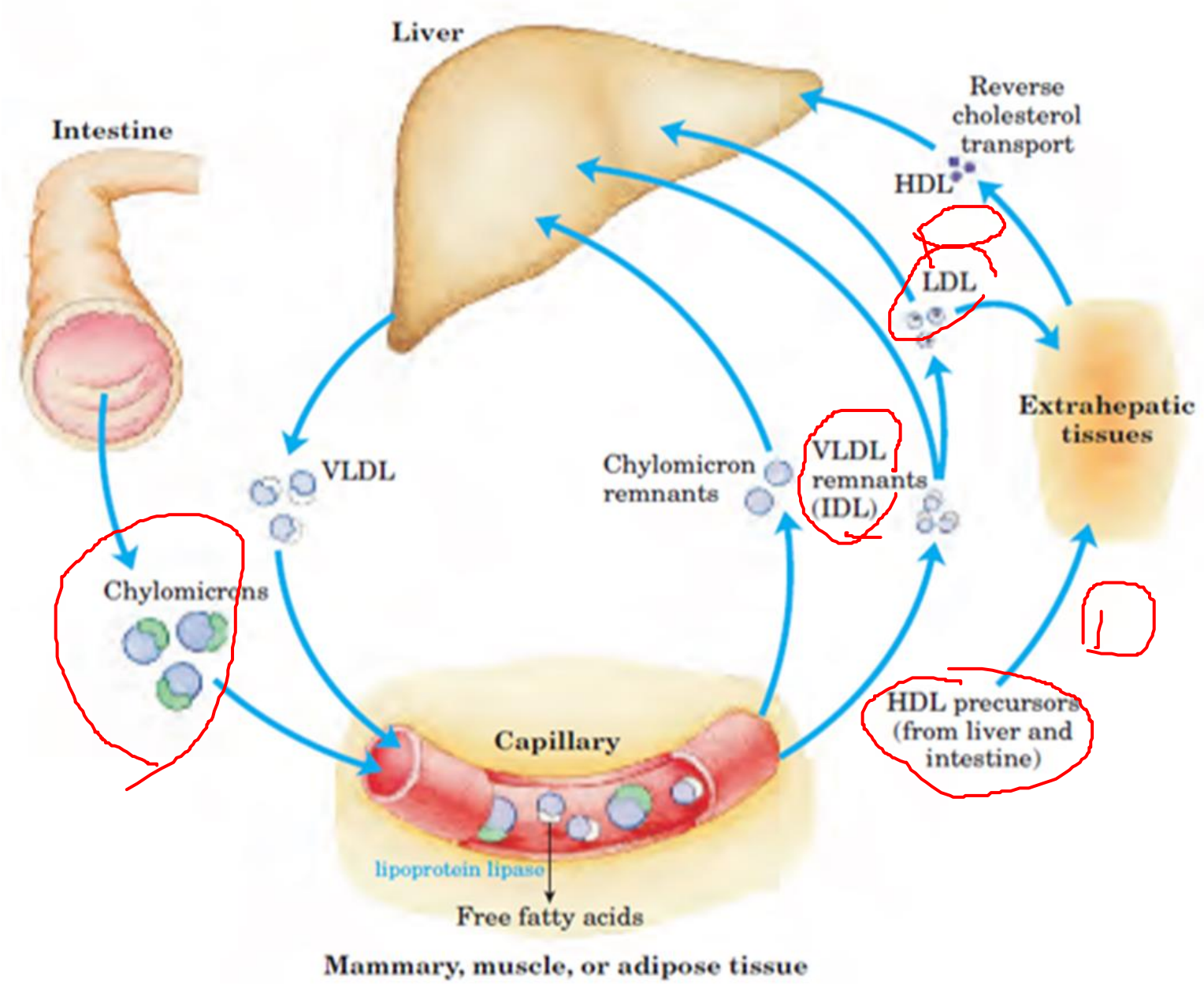
7. Fatty acids are taken up and degraded by β-oxidation in mitochondrial matrix

Lipoproteins

Triacylglycerols, cholesterol, and cholesteryl esters cannot be transported in blood or lymph as free molecules because they are insoluble in water. Instead, these lipids assemble with phospholipids and amphipathic lipid binding proteins to form macromolecular particles known as lipoproteins. A lipoprotein has a hydrophobic core containing triacylglycerols and cholesteryl esters and a hydrophilic surface consisting of a layer of amphipathic molecules such as cholesterol, phospholipids and proteins.



Blood plasma contains several other types of lipoproteins. They are classified according to their relative densities and types of lipid .



Bile salts: Biological Detergents

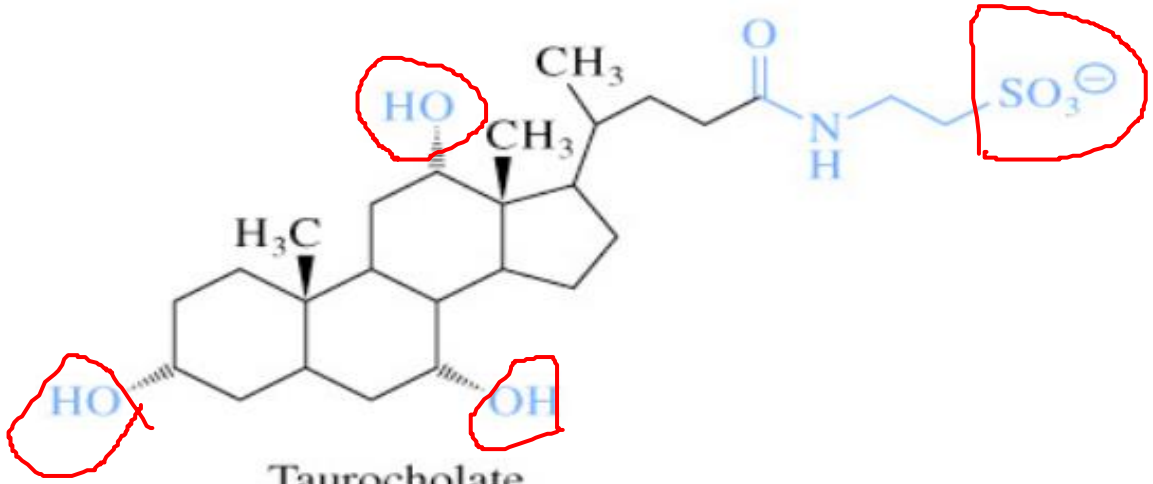
Bile salts are amphipathic: synthesized in liver, stored and secreted by gall bladder to intestine. Made from cholesterol: retain the ring structure but have more hydroxyl groups and a polar side chain – can act as DETERGENTS - Serve to convert water-insoluble lipids to dispersible micellar aggregates

• They emulsify fat globules into smaller micelles, increasing the surface area accessible to lipidhydrolyzing enzymes. Aid in lipid digestion and are essential for the absorption of lipid digestion products

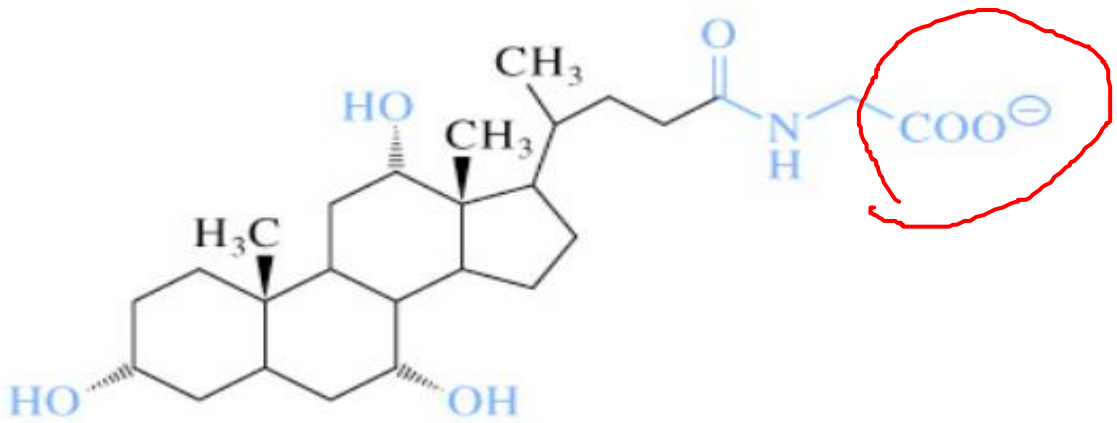
• Also required for efficient intestinal absorption of lipid-soluble vitamins A, D, E, and K

• Taurocholate and glycocholate (cholesterol derivatives) are the most abundant bile salts

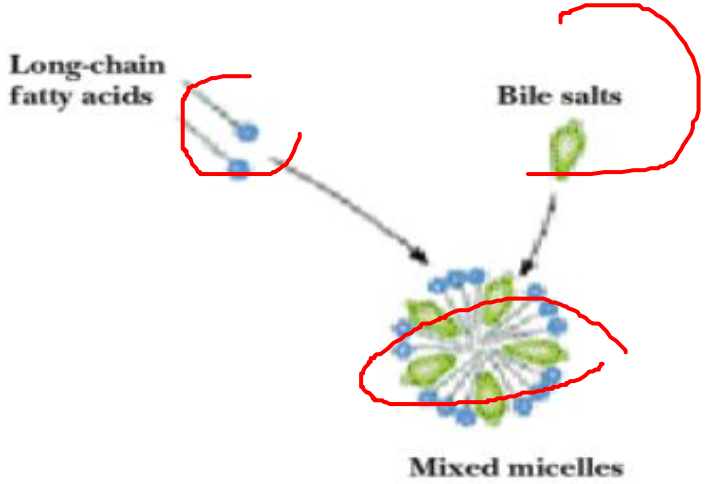
Amphipathic.



Taurocholate

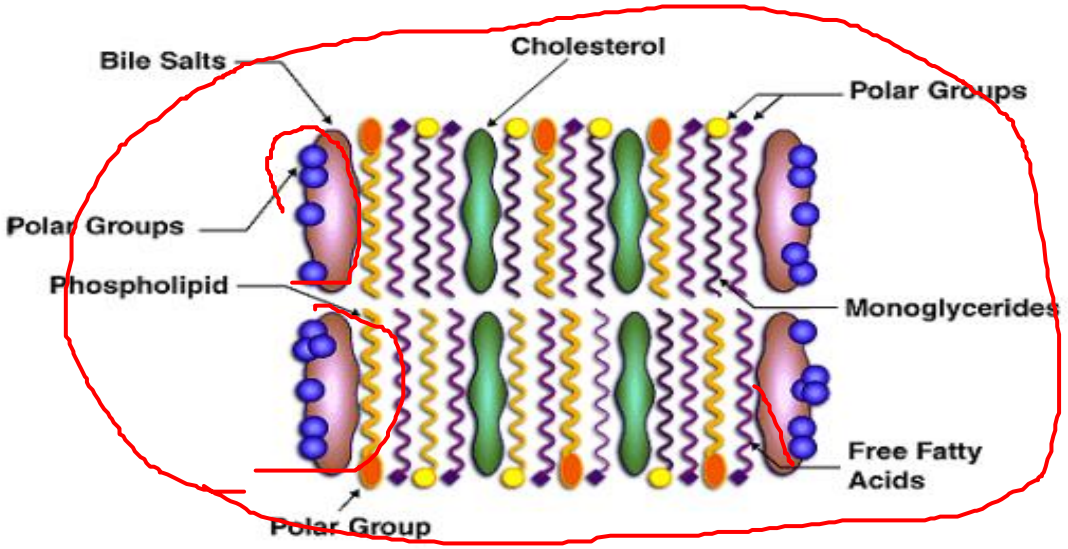


Glycocholate



Mixed micelles

Bile Salts Solubilize Products of Fat Digestion



Mixed Micelle
(bile salt + fat hydrolysis products)
longitudinal section

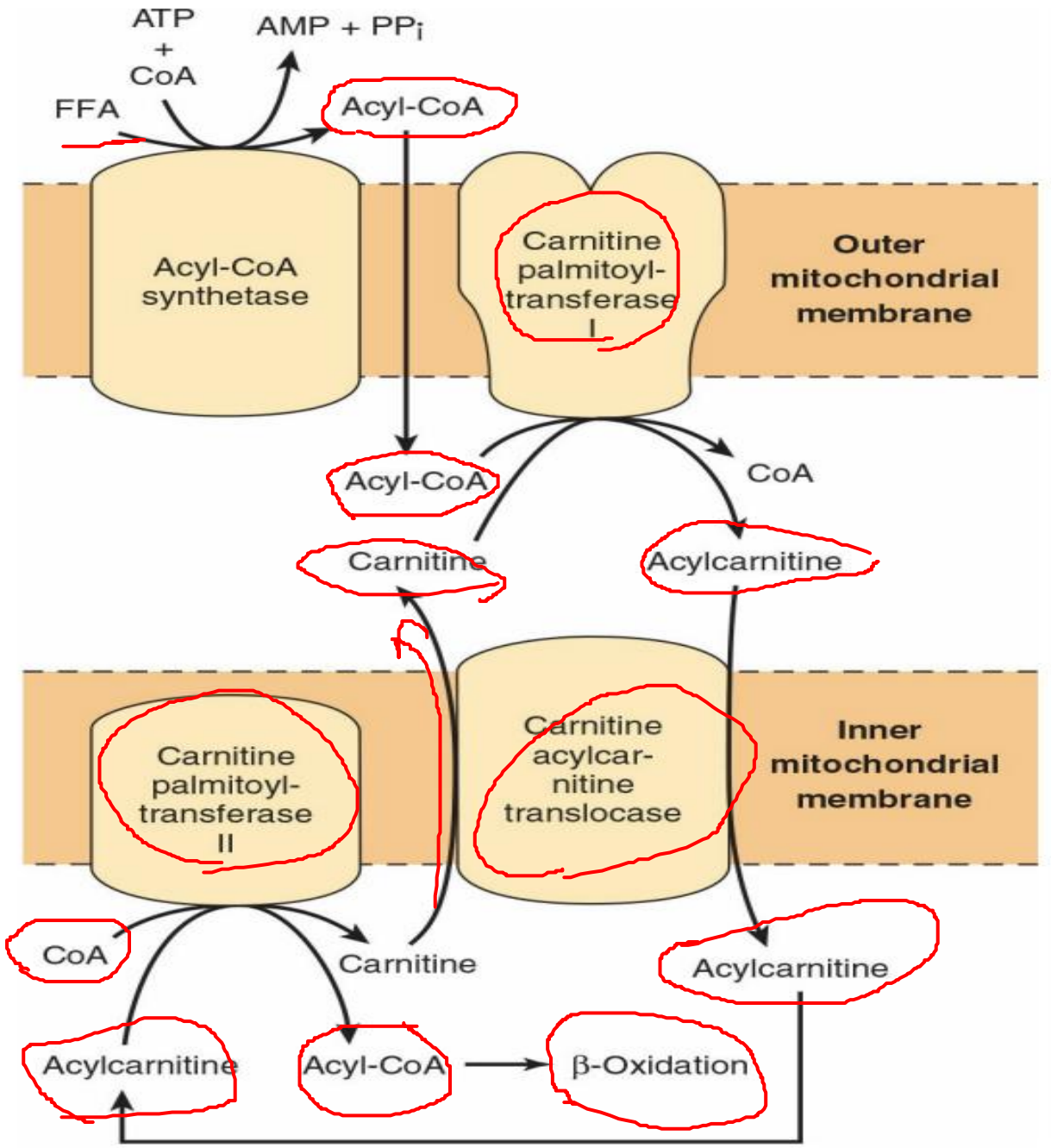
OXIDATION OF FATTY ACIDS OCCURS IN MITOCHONDRIA

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

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

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

FIGURE 22–1 Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane. Long-chain acylCoA formed by acyl-CoA synthetase enters the intermembrane space. For transport across the inner membrane, acyl groups must be transferred from CoA to carnitine by carnitine palmitoyltransferase-I. The acylcarnitine formed is then carried into the matrix by a translocase enzyme in exchange for a free carnitine and acyl-CoA is reformed by carnitine palmitoyltransferase-II.



CARNITINE: -

Diet: red meat, dairy, poultry, fish :

made in liver and kidneys
- Enters cells by specific transporter
- Carnitine deficiencies: §

Symptoms:
- Poor muscle tone
- Muscle weakness
- Brain dysfunction
- Heart dysfunction

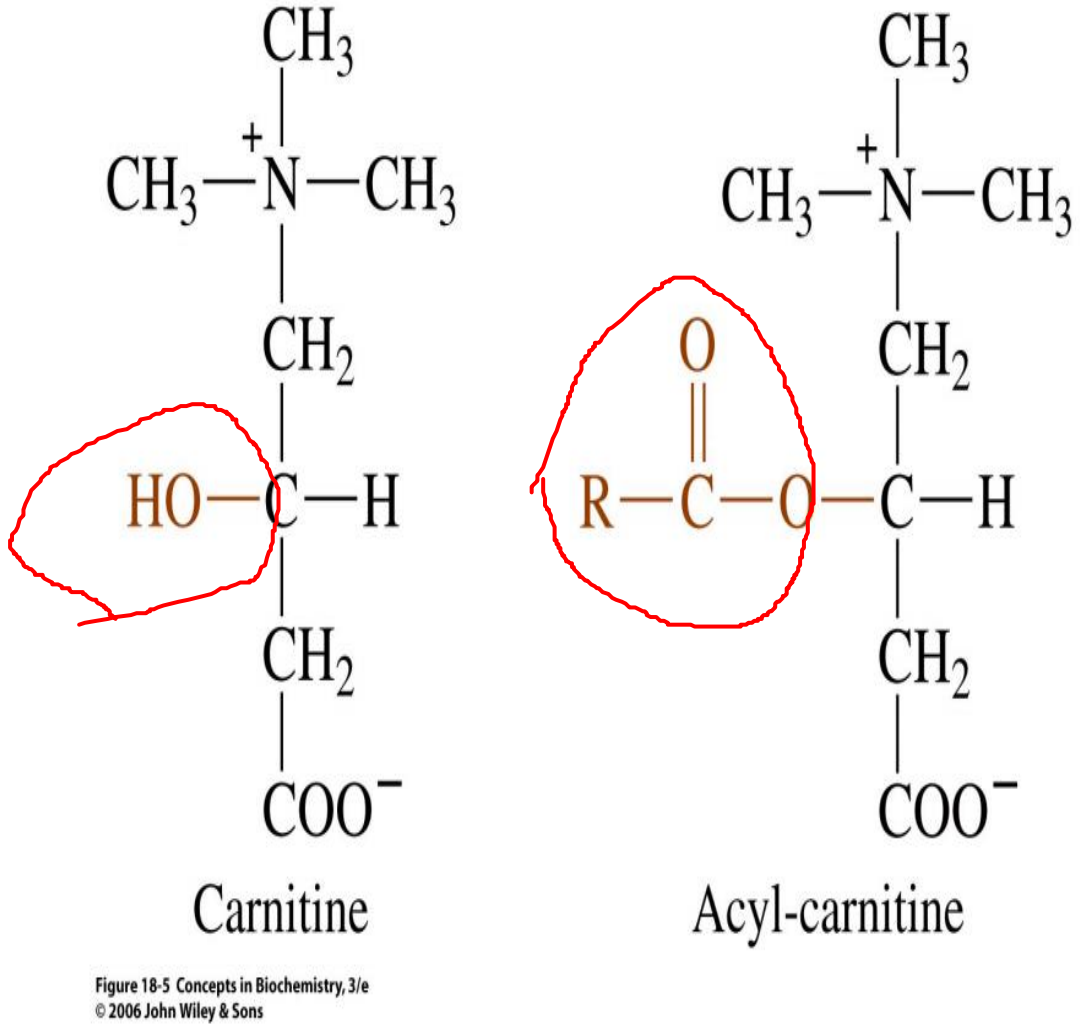


Figure 18-5 Concepts in Biochemistry, 3/e

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

1. Dehydrogenation of fatty acyl CoA to produce a trans double bond between the α and β carbons (or C-2 & C-3). the product is trans-Δ2-enoyl-CoA reaction catalyzed by acyl CoA dehydrogenase. the electron acceptor is FAD the reaction is analogous to succinate dehydrogenase.
2. Addition of water across double bond of trans-Δ2-enoyl-CoA. reaction catalyzed by enoyl-CoA hydratase product: L-β-hydroxyacyl CoA (3-hydroxyacyl CoA) reaction analogous to fumarase.
3. Dehydrogenation of L-β-hydroxyacyl CoA. product is to β-ketoacyl-CoA. enzyme: β-hydroxyacyl CoA dehydrogenase cofactor: NAD+ reduced to NADH + H+ reaction analogous to malate dehydrogenase.
4. The final step of β-oxidation cycle is thiolysis of C2-C3 (CαCβ) bond by nucleophilic attack on C2 (β carbon) by the –SH group of a new CoASH. products are acetyl CoA and acyl CoA (shorter by 2 C) Enzyme: Thiolase (acyl-CoA acetyltransferase) cofactor is CoASH.

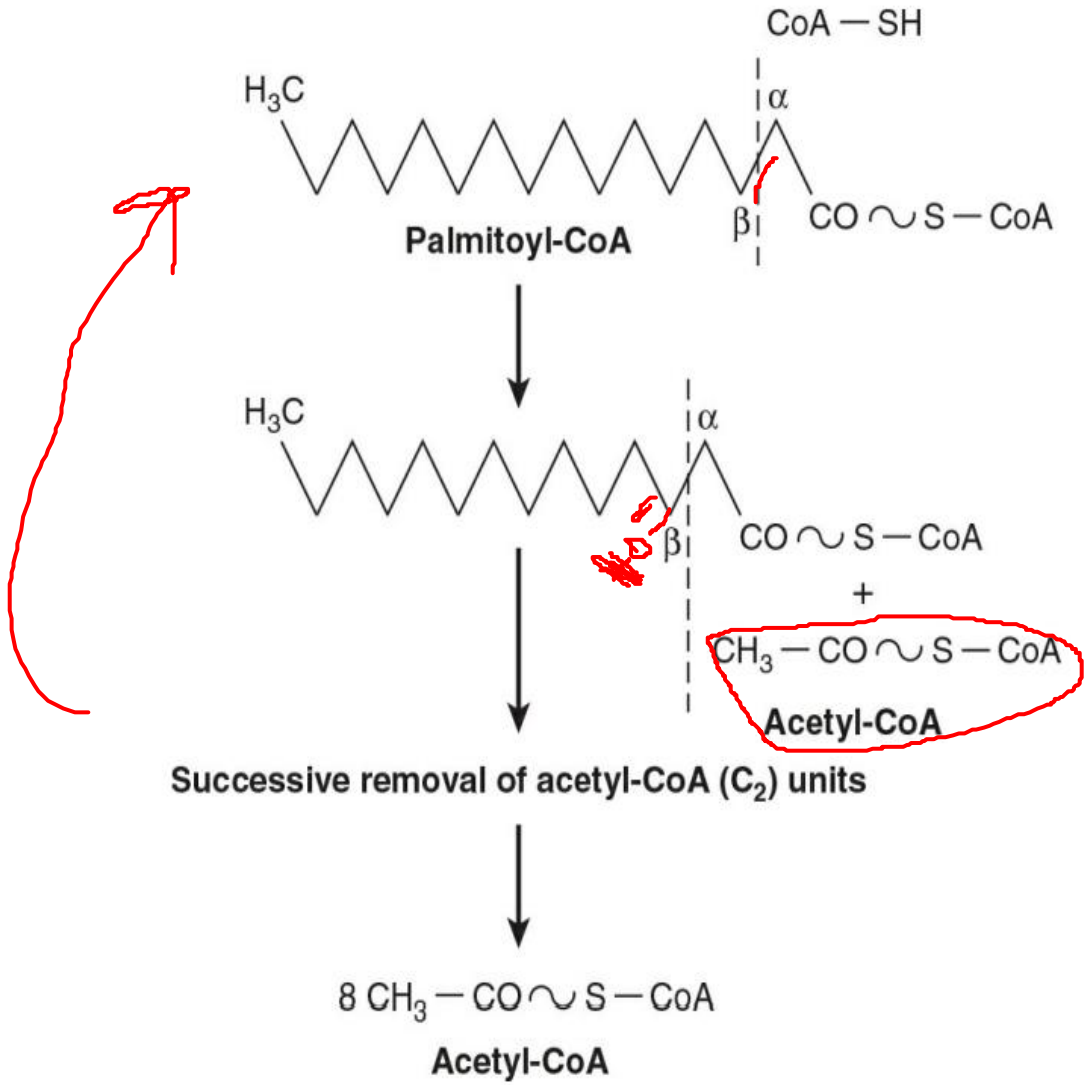


FIGURE 22–2 Overview of β-oxidation of fatty acids.

The β-Oxidation Cycle Generates FADH2 & NADH

Several enzymes, known collectively as “fatty acid oxidase,” are found in the mitochondrial matrix or inner membrane adjacent to the respiratory chain. These catalyze the oxidation of acyl-CoA to acetyl-CoA by the βoxidation pathway. The system proceeds in cyclic way which results in the degradation of long fatty acids to acetyl-CoA. In the process, large quantities of the reducing equivalents FADH2 and NADH are generated and are used to form ATP by oxidative phosphorylation (Figure 22–3).

Reactions for fatty acid activation, transport, and the β-oxidation spiral

Reaction Number	Reaction	Enzyme
1	Fatty acid + CoASH + ATP $\rightleftharpoons$ acyl-CoA + AMP + PP_i	Acyl-CoA synthetase
2	$PP_i + H_2O \rightleftharpoons 2\,P_i$	Pyrophosphatase
3	Carnitine + acyl-CoA $\rightleftharpoons$ acyl-carnitine + CoASH (intermembrane space)	Carnitine acyltransferase I
4	Acyl-carnitine + CoASH $\rightleftharpoons$ acyl-CoA + carnitine (mitochondria)	Carnitine acyltransferase II
5	Acyl-CoA + E—FAD $\rightleftharpoons$ *trans*-Δ^2-enoyl-CoA + E—$FADH_2$[b]	Acyl-CoA dehydrogenase
6	*trans*-Δ^2-Enoyl-CoA + $H_2O \rightleftharpoons$ L-3-hydroxyacyl-CoA	Enoyl-CoA hydratase
7	L-3-Hydroxyacyl-CoA + $NAD^+ \rightleftharpoons$ 3-ketoacyl-CoA + NADH + H^+	Hydroxyacyl-CoA dehydrogenase
8	3-Ketoacyl-CoA + CoASH $\rightleftharpoons$ acetyl-CoA + acyl-CoA[c]	β-Ketothiolase

[a] Reaction type: 1, oxidation–reduction; 2, group transfer; 3, hydrolysis; 4, nonhydrolytic cleavage (addition or elimination); 5, isomerization–rearrangement; 6, bond formation coupled to ATP cleavage.

[b] E—FAD and E—$FADH_2$ refer to the cofactor flavin adenine dinucleotide covalently linked to the enzyme.

[c] Acyl-CoA product is shortened by a C_2 unit.

The Reactions of β-oxidation

• One round of β-oxidation: 4 enzyme steps produce acetyl CoA from fatty acyl CoA

• Each round generates one molecule each of:

1 FADH2 – oxidative phosphorylation

1 NADH – oxidataive phosphorylation

1 Acetyl CoA – enters TCA cycle Fatty acyl CoA (2 carbons shorter each round)

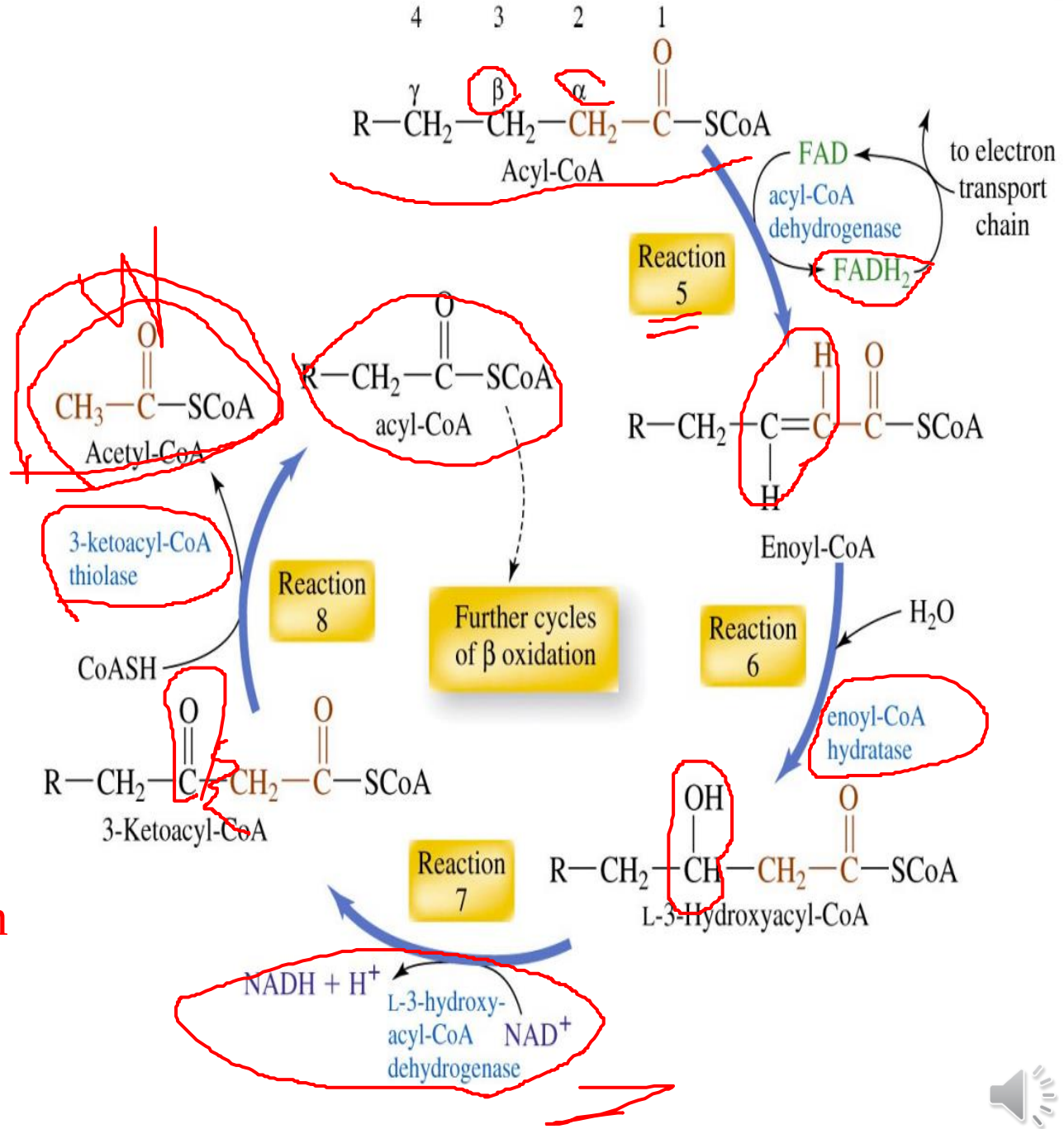
Example: §

Palmitic acid = 16:0

• 8 moles of acetyl-CoA (enter TCA cycle)

• 7 rounds of β-oxidation

Note: the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.



Oxidation of Fatty Acids Produces a Large Quantity of ATP

Transport of electrons from FADH2 and NADH via the respiratory chain leads to the synthesis of four high-energy phosphates for each of the seven cycles needed for the breakdown of the C16 fatty acid, palmitate, to acetyl-CoA . A total of 8 mol of acetyl-CoA is formed, and each gives rise to 10 mol of ATP on oxidation in the citric acid cycle. Two must be subtracted for the initial activation of the fatty acid, yielding a net gain of 106 mol of ATP per mole of palmitate. This represents 68% of the free energy of combustion of palmitic acid.

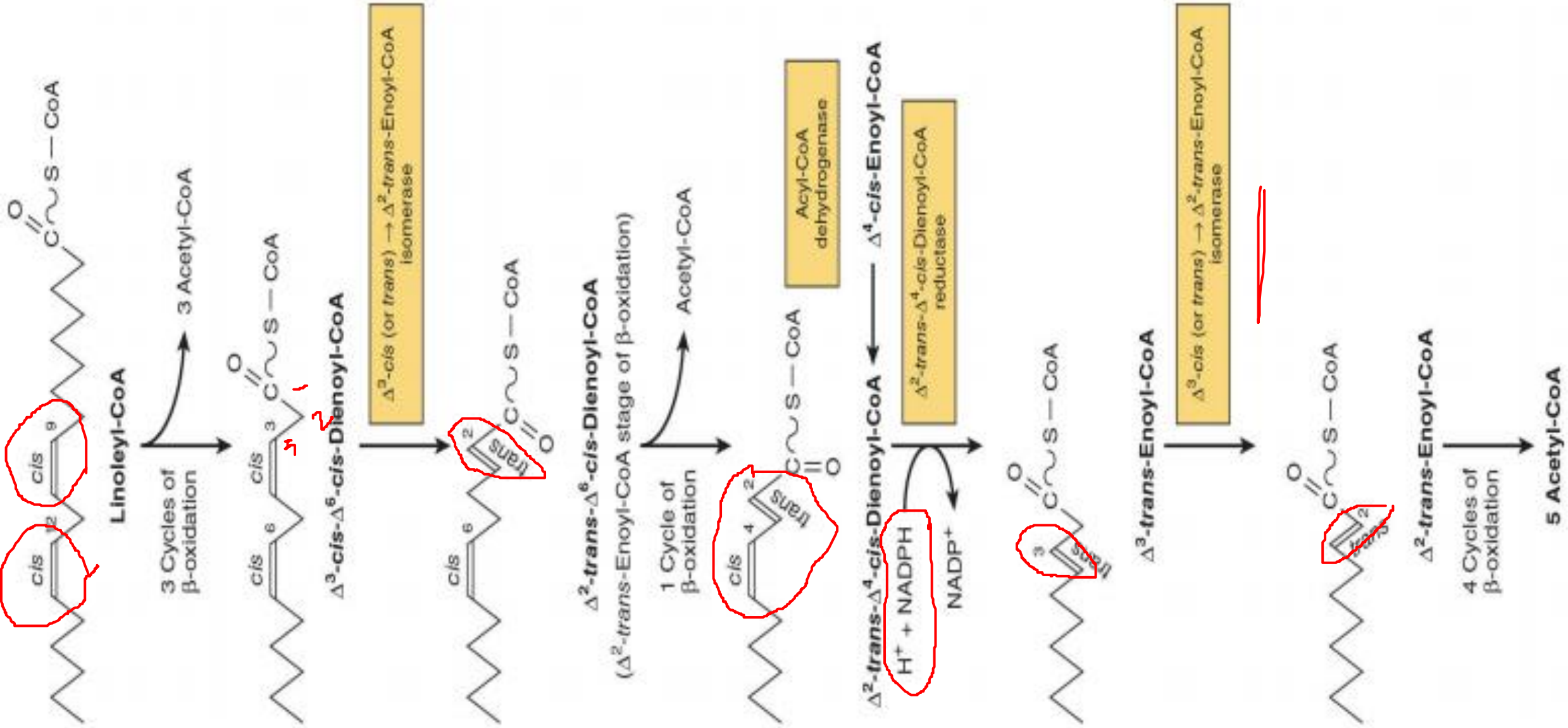
TABLE 22–1 Generation of ATP from the Complete Oxidation of a C16 Fatty Acid

Step	Product	Amount Product Formed (mol)/mol Palmitate	ATP Formed (mol)/ mol Product	Total ATP Formed (mol)/mol Palmitate	ATP Used (mol)/ mol Palmitate
Activation		–			2
β-Oxidation	$FADH_2$	7	1.5	10.5	–
β-Oxidation	NADH	7	2.5	17.5	–
Citric acid cycle	Acetyl-CoA	8	10	80	–
	Total ATP formed (mol)/mol palmitate			108	
	Total ATP used (mol)/mol palmitate				2

The table shows how the oxidation of 1 mol of the C16 fatty acid, palmitate, generates 106 mol of ATP (108 formed in total—2 used in the activation step).

Oxidation of Unsaturated Fatty Acids Occurs by a Modified β-Oxidation Pathway

Sequence of reactions in the oxidation of unsaturated fatty acids, for example, linoleic acid



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

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

the equilibrium is controlled by the mitochondrial [NAD+]/[NADH] ratio, that is, the redox stat

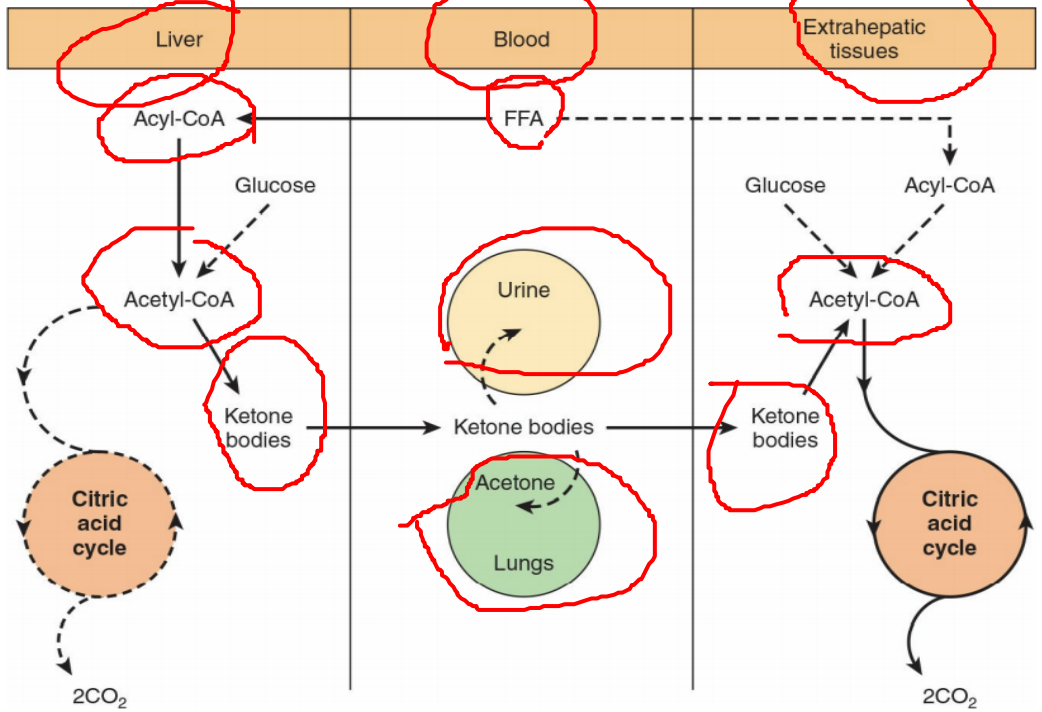


FIGURE 22–6 Formation, utilization, and excretion of ketone bodies. (The main pathway is indicated by the solid arrows.)

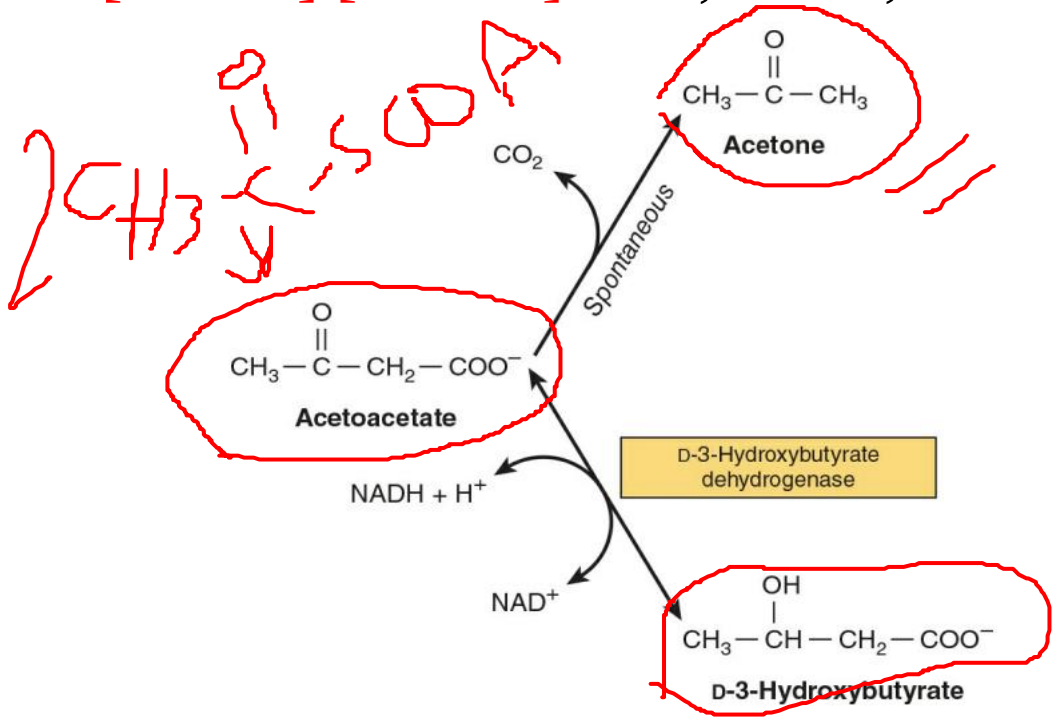


FIGURE 22–5 Interrelationships of the ketone bodies. D-3-Hydroxybutyrate dehydrogenase is a mitochondrial enzyme.

Acetoacetyl-CoA Is the Substrate for Ketogenesis & Use of Ketone Bodies as Fuel

- Ketone bodies are formed in the liver mitochondria
- Acetoacetate and β-hydroxybutyrate are transported by the blood to extrahepatic tissues
- Acetone is produced in smaller amounts and is exhaled.
- Ketone bodies are converted to acetyl CoA and oxidized by the TCA cycle for energy in skeletal and cardiac muscle and brain.
- The brain prefers glucose as fuel but can adapt to using ketones when glucose is not available.
- Oxidation of Ketones by other tissues facilitates the continued oxidation of FA in the liver.

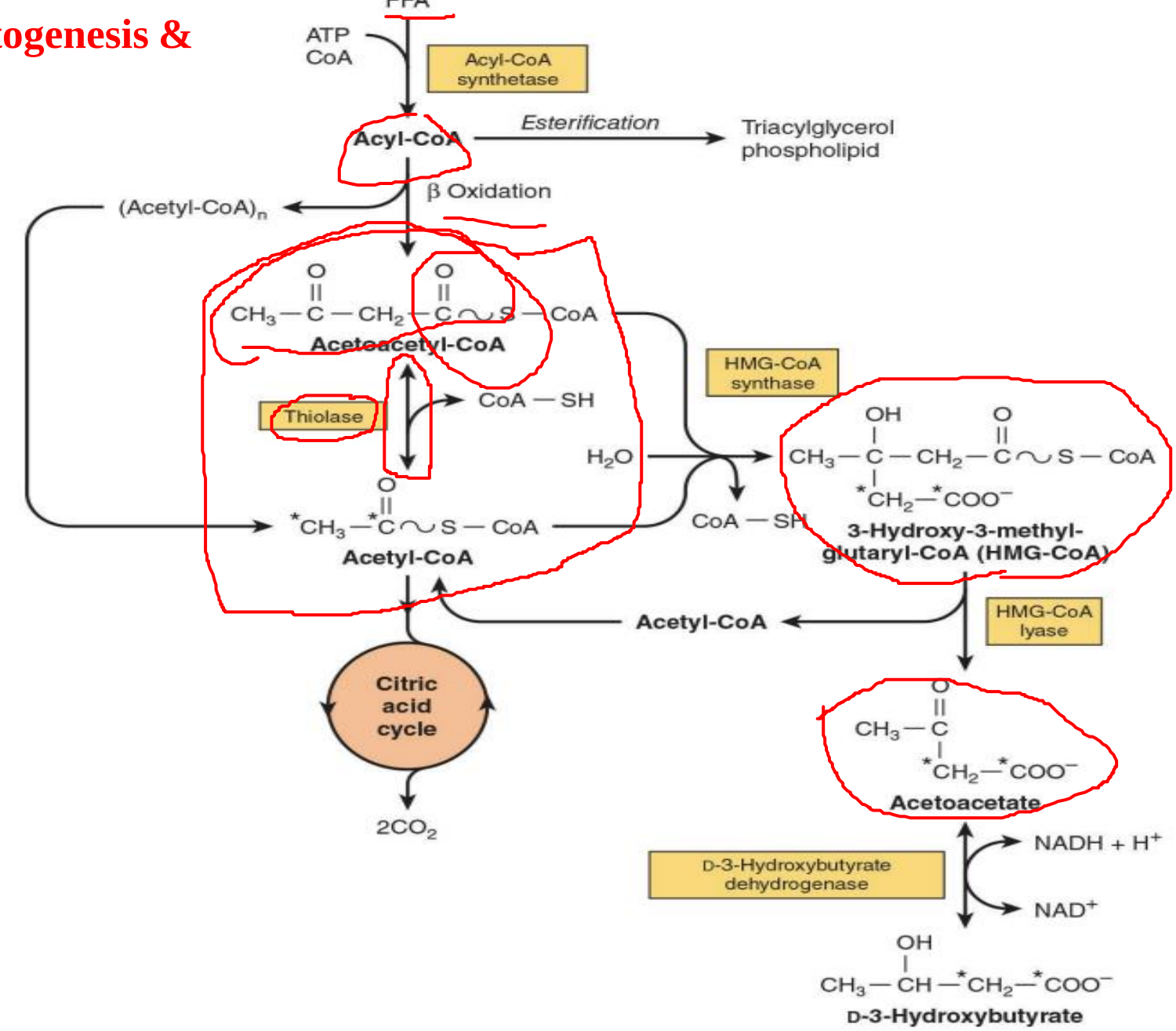


FIGURE 22–7 Pathways of ketogenesis in the liver. (FFA, free fatty

KETOGENESIS IS REGULATED AT THREE CRUCIAL STEPS

- Ketosis does not occur in vivo unless there is an increase in the level of circulating FFAs arising from lipolysis of triacylglycerol in adipose tissue. FFAs are the precursors of ketone bodies in the liver.

- After uptake by the liver, FFAs are either oxidized to CO2 or ketone bodies or esterified to triacylglycerol and phospholipid. There is regulation of entry of fatty acids into the oxidative pathway by carnitine palmitoyltransferase-I (CPT-I)

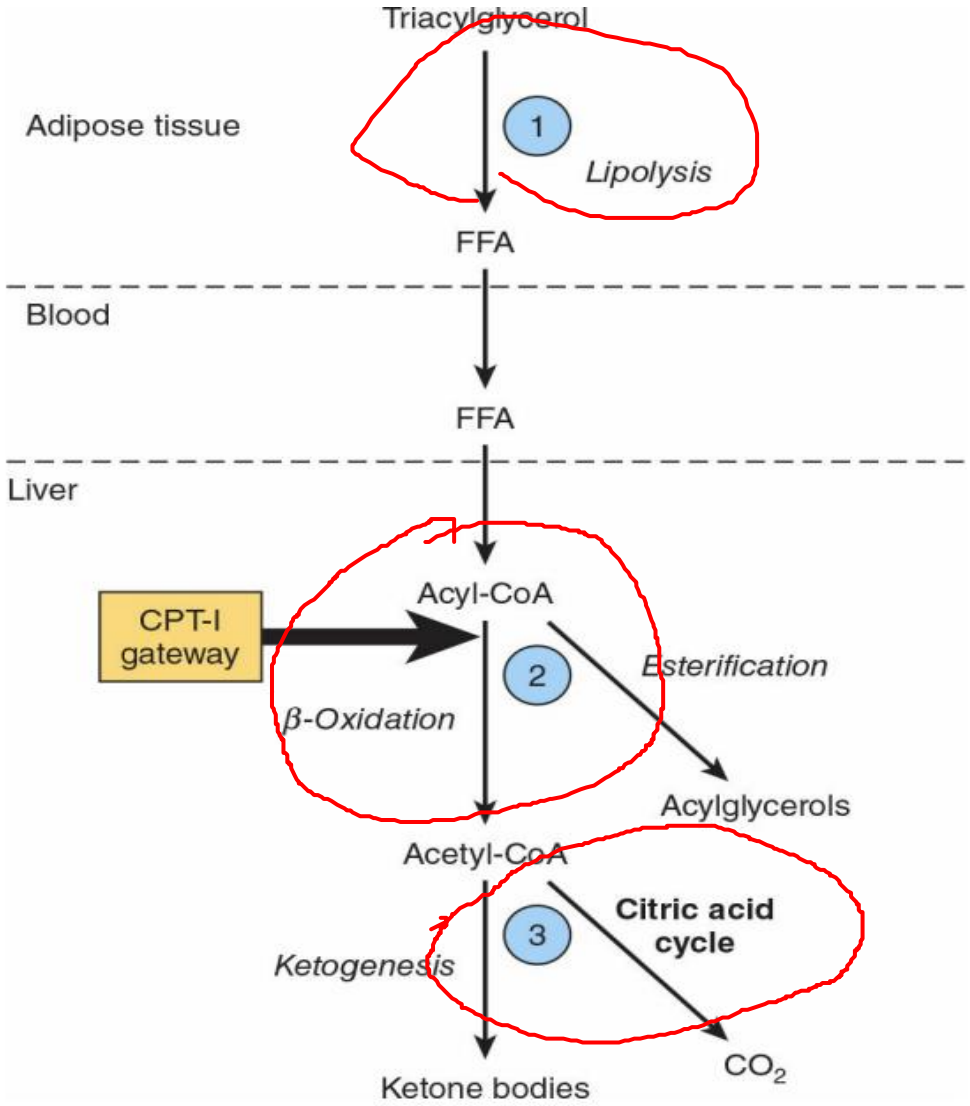


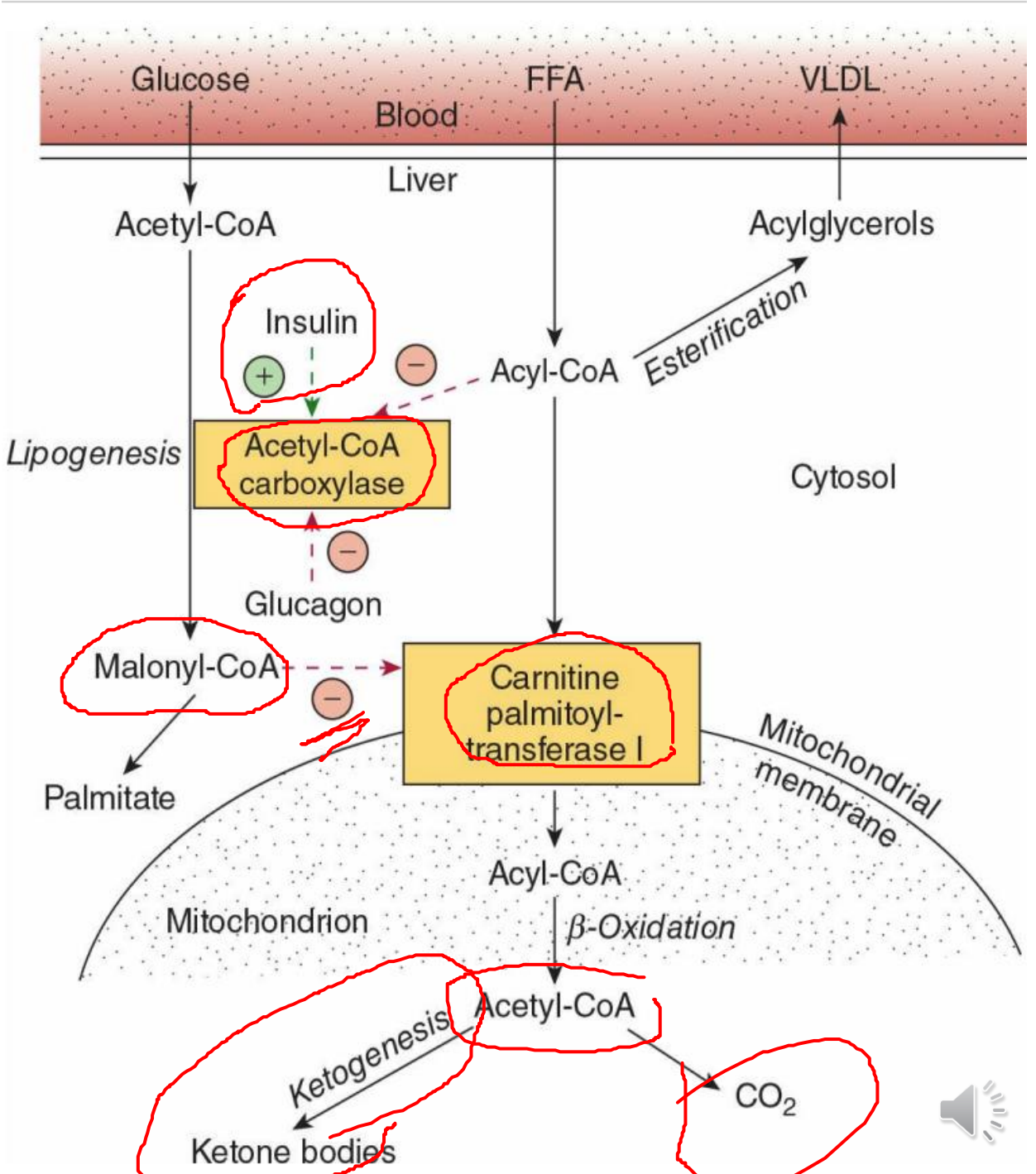
FIGURE 22–9 Regulation of ketogenesis. Symbol 1 to Symbol 3 show three crucial steps in the pathway of metabolism of free fatty acids (FFA) that determine the magnitude of ketogenesis. (CPT-I, carnitine palmitoyltransferase-I.)

Regulation of Fatty Acid Oxidation

FA metabolism is under hormonal regulation. When fuel levels are low, Epinephrine and Glucagon stimulate mobilization of fat and glycogen reserves. Insulin, which is secreted during the fed-state, is anti-lipolytic (it inhibits βoxidation).

The transport of FA into mitochondria is allosterically regulated. This is the rate-limiting step in β-oxidation. Carnitine Palmitoyl Transferases I and II are inhibited by malonyl-CoA, an intermediate of fatty acid synthesis. Thus fatty acid oxidation is reduced under conditions favoring fatty acid synthesis.

The two final steps in the β-oxidation cycle are also regulated. 3-hydroxyacyl-SCoA dehydrogenase is inhibited by NADH. Thiolase is regulated by feed-back inhibition by acetyl CoA.



THANK YOU

Biosynthesis of fatty acids & Eicosanoids

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper’s Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

By

Dr. Muthanna Owaid Hussein

BIOMEDICAL IMPORTANCE

Fatty acids are synthesized by an extramitochondrial system, which is responsible for the complete synthesis of palmitate from acetyl-CoA in the cytosol. In most mammals, glucose is the primary substrate for lipogenesis, the main fuel molecule they obtain from the diet.

Unsaturated fatty acids in phospholipids of the cell membrane are important in maintaining membrane fluidity. A high ratio of polyunsaturated fatty acids to saturated fatty acids (P:S ratio) in the diet is considered to be beneficial in preventing coronary heart disease.

Animal tissues have limited capacity for desaturating fatty acids, and require certain dietary polyunsaturated fatty acids derived from plants.

These essential fatty acids are used to form eicosanoic (C20) fatty acids, which give increase to the eicosanoids prostaglandins, thromboxanes, leukotrienes, and lipoxins.

Prostaglandins mediate inflammation, pain, induce sleep, and also regulate blood coagulation and reproduction. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen act by inhibiting prostaglandin synthesis.

Leukotrienes have muscle contractant and chemotactic properties and are important in allergic reactions and inflammation.

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

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

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

Bicarbonate as a source of CO_2 is required in the initial reaction for the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP and acetyl-CoA carboxylase. This enzyme has a major role in the regulation of fatty acid synthesis. Acetyl-CoA carboxylase has a requirement for the B vitamin biotin and is a multienzyme protein containing biotin, biotin carboxylase, biotin carboxyl carrier protein, and a carboxyl transferase, as well as a regulatory allosteric site.

The reaction takes place in two steps:

(1)Carboxylation of biotin involving ATP

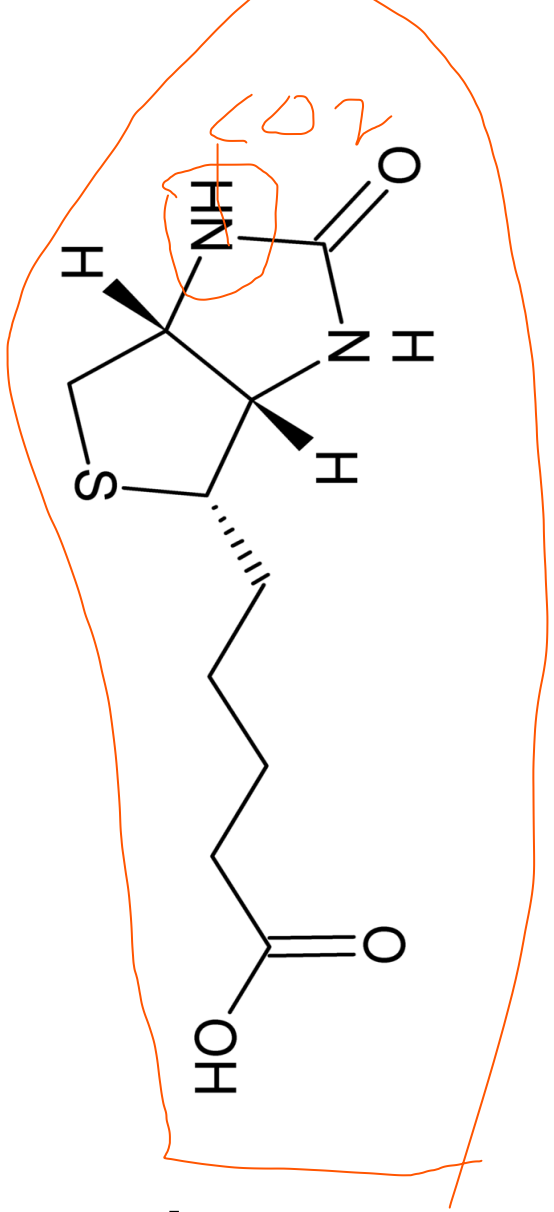
(2) Transfer of the carboxyl group to acetyl-CoA to form malonyl-CoA.

Acetyl carboxylase is a multienzyme complex containing two enzymes, biotin carboxylase (E1) and a carboxyltransferase (E2) and the biotin carrier protein (BCP). Biotin is covalently linked to the BCP.

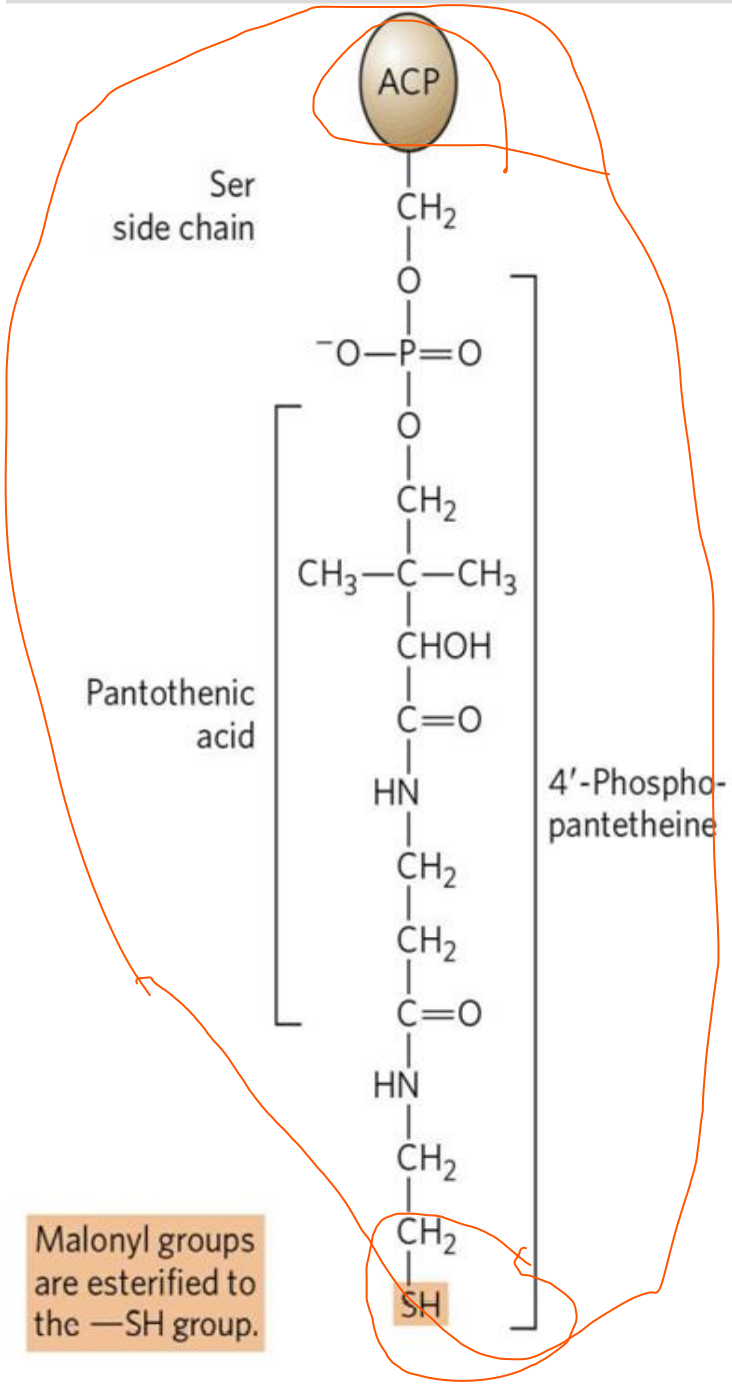
The reaction proceeds in two steps.

step 1, catalysed by E1, biotin is carboxylated as it accepts a COO^- group from HCO_3^- and ATP is used.

step 2, catalyzed by E2, the COO^- is transferred to acetyl-CoA forming malonylCoA.



biotin



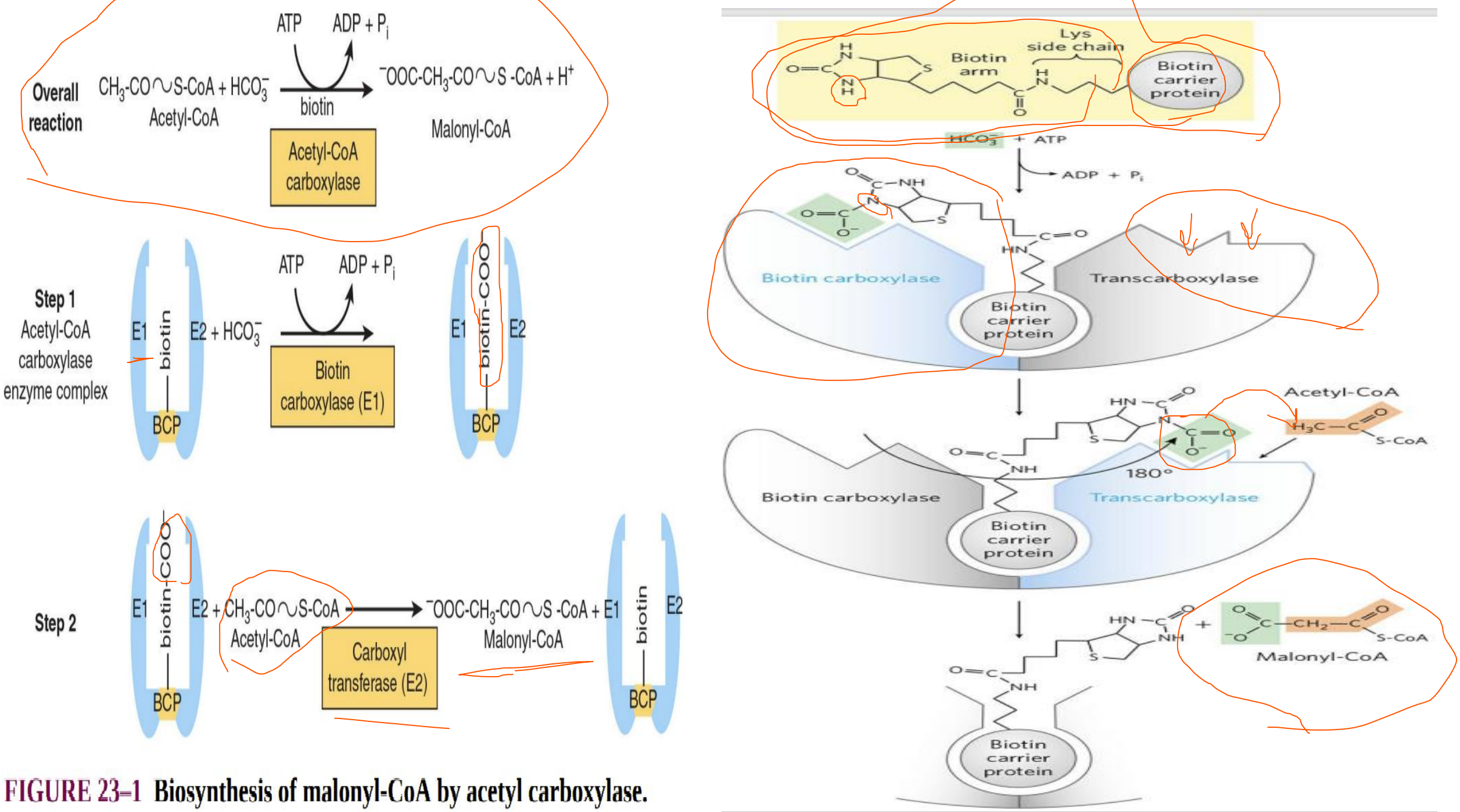
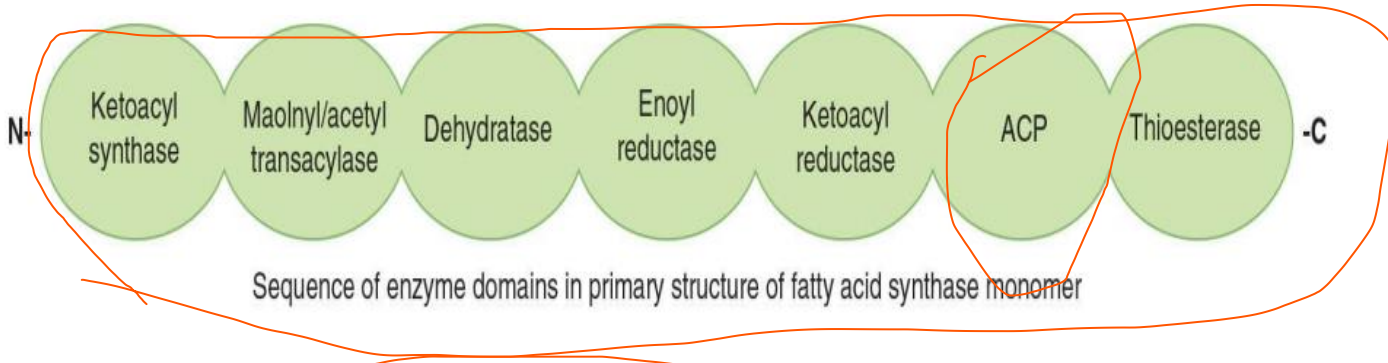


FIGURE 23–1 Biosynthesis of malonyl-CoA by acetyl carboxylase.

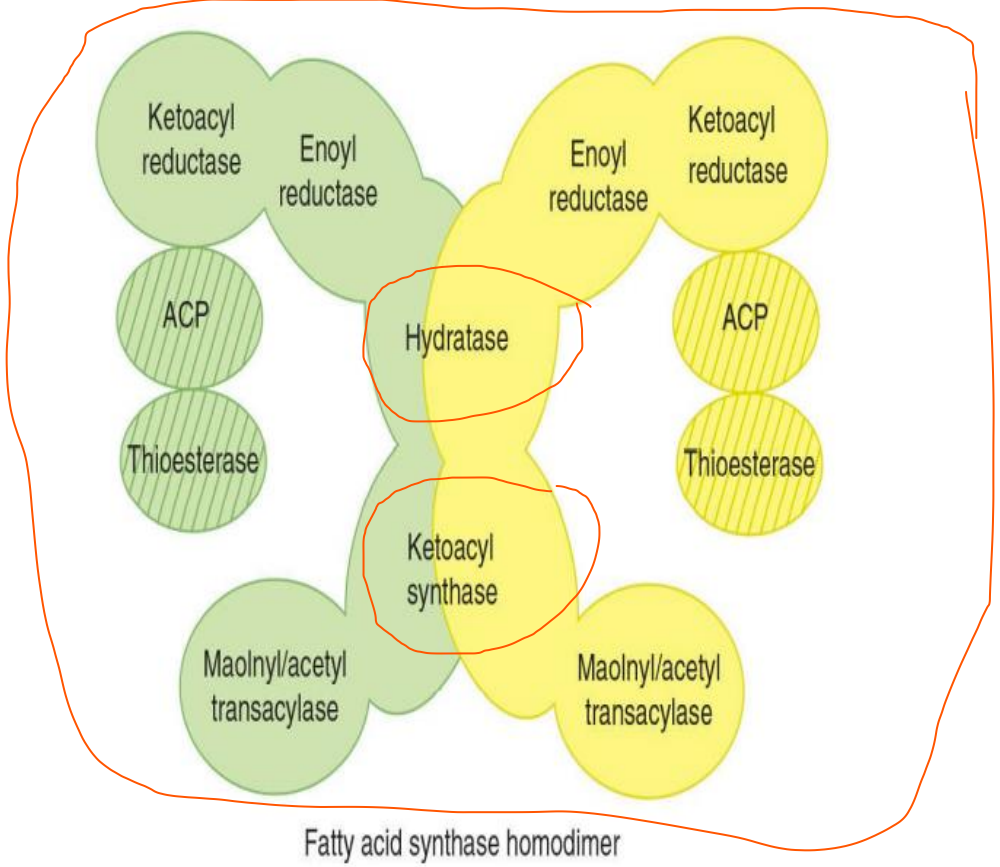
The Fatty Acid Synthase Complex Is a Homodimer of Two Polypeptide Chains Containing Six Enzyme Activities and the Acyl Carrier Protein

$$CH_3CO{-}S{-}CoA + 7HOOCCHCO{-}S{-}CoA + 14NADPH + 14H^+ \rightarrow CH_3(CH_2)_{14}COOH + 7CO_2 + 6H_2O + 8CoA{-}SH + 14NADP^+$$



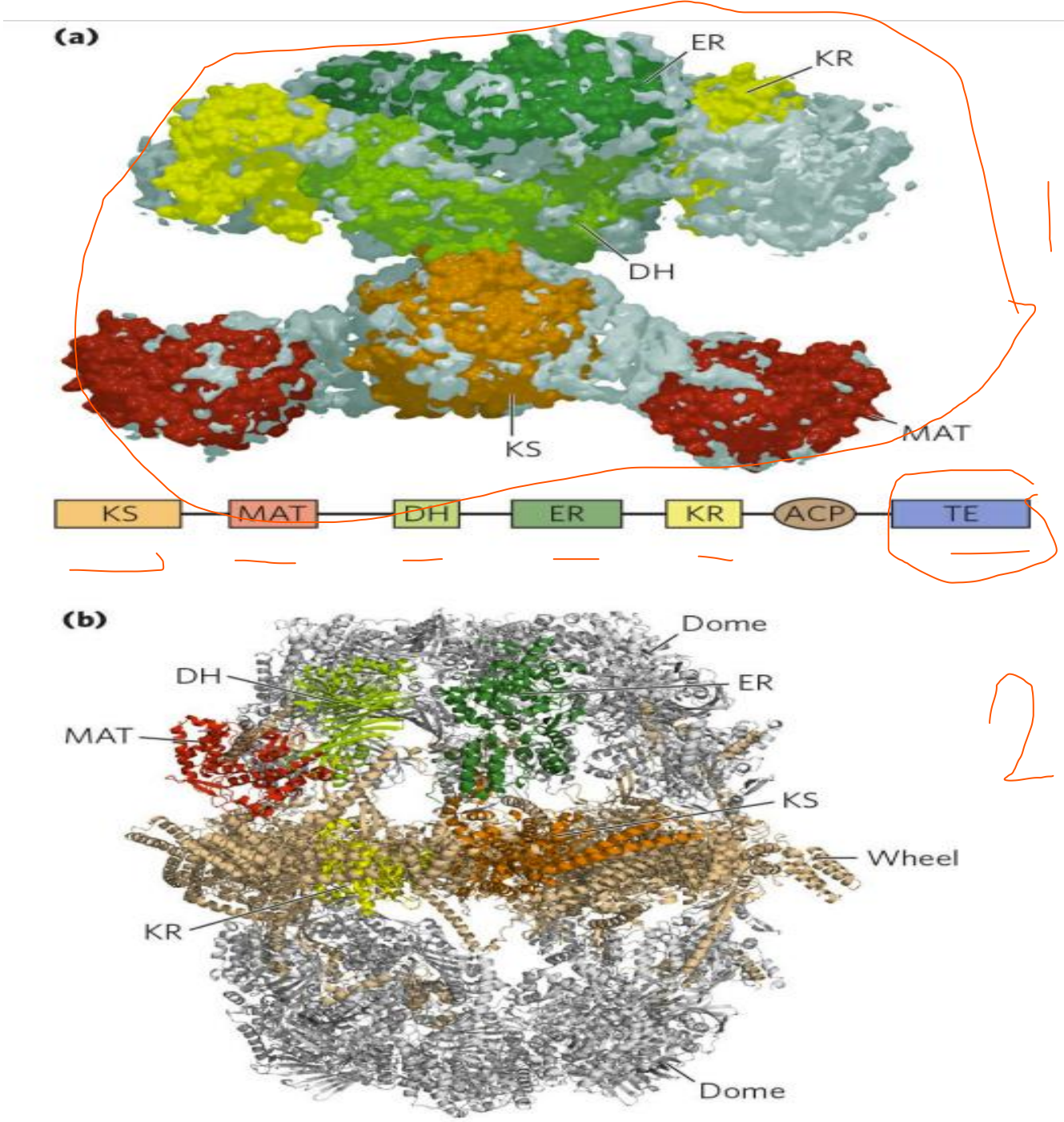
Sequence of enzyme domains in primary structure of fatty acid synthase monomer

After the formation of malonyl-CoA, fatty acids are formed by the fatty acid synthase enzyme complex. This individual enzymes required for fatty acid synthesis are linked in this multienzyme polypeptide complex that incorporates the acyl carrier protein (ACP), which has a similar function to that of CoA in the β-oxidation pathway It contains the vitamin pantothenic acid in the form of 4′- phosphopantetheine



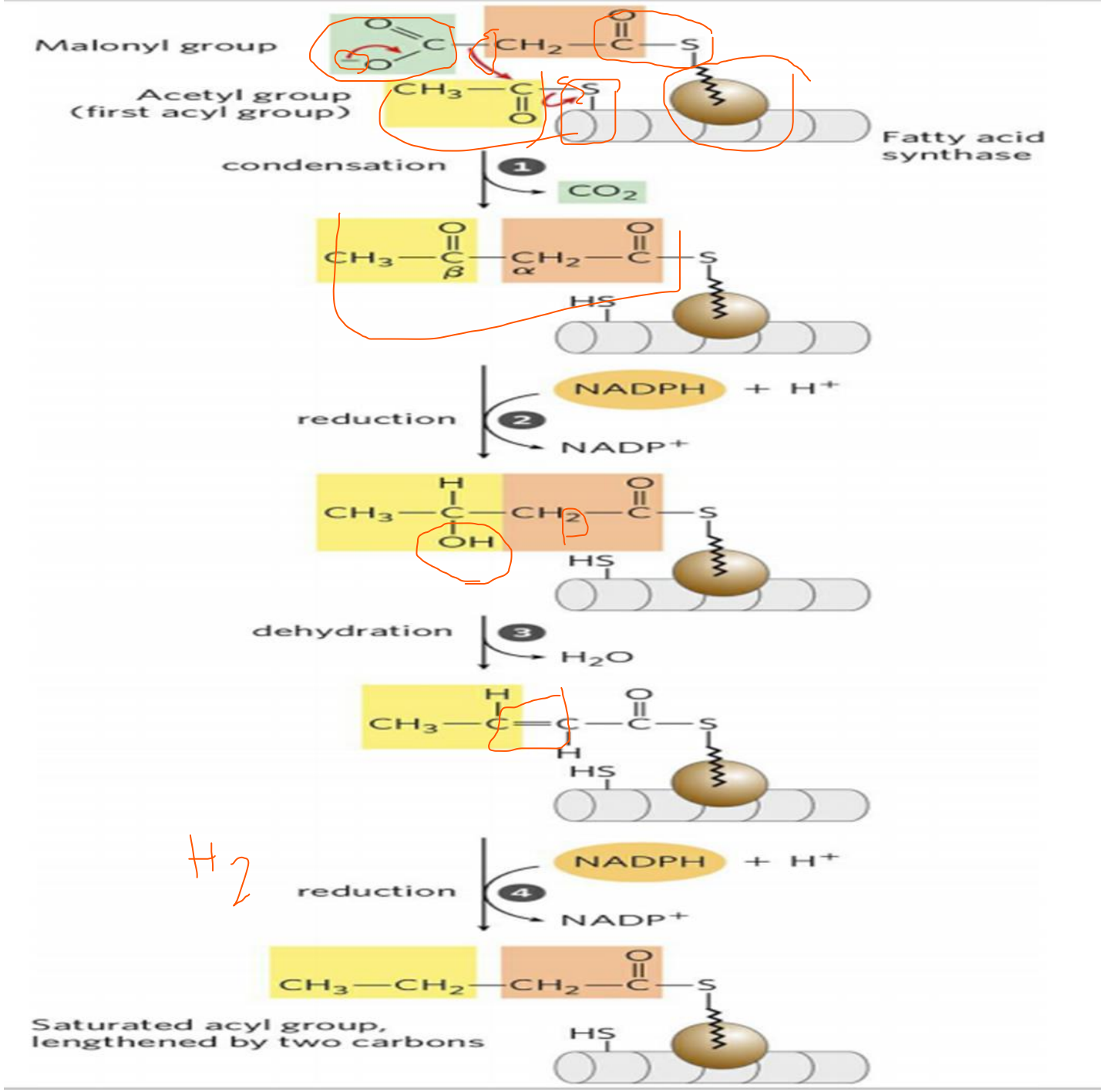
Fatty acid synthase homodimer

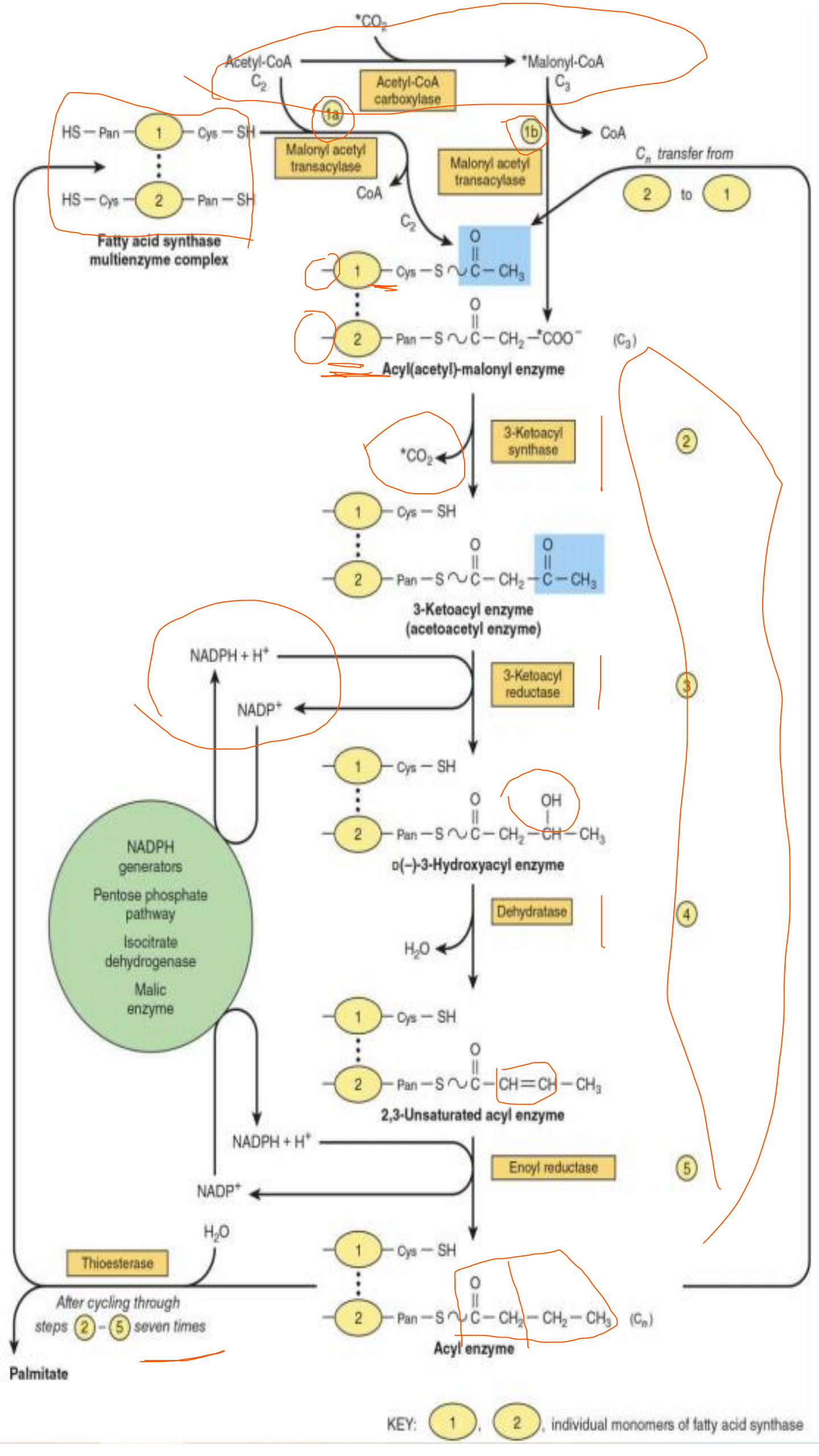
The structure of fatty acid synthase. Shown here are low-resolution structures of (a) the mammalian (porcine) and (b) fungal enzyme systems. (a) All of the active sites in the mammalian system are located indifferent domains within a single large polypeptide chain. The different enzymatic activities are:
β-ketoacyl-ACP synthase (KS)
malonyl/acetyl-CoA–ACP transferase (MAT)
β-hydroxyacyl-ACP dehydratase (DH)
enoyl-ACP reductase (ER)
β-ketoacyl-ACP reductase (KR)
ACP is the acyl carrier protein.
The linear arrangement of the domains in the polypeptide is shown the structure. The seventh domain is a thioesterase (TE) that releases the palmitate product from ACP when synthesis is completed. The ACP and TE domains are disordered in the crystal and are therefore not shown in the structure

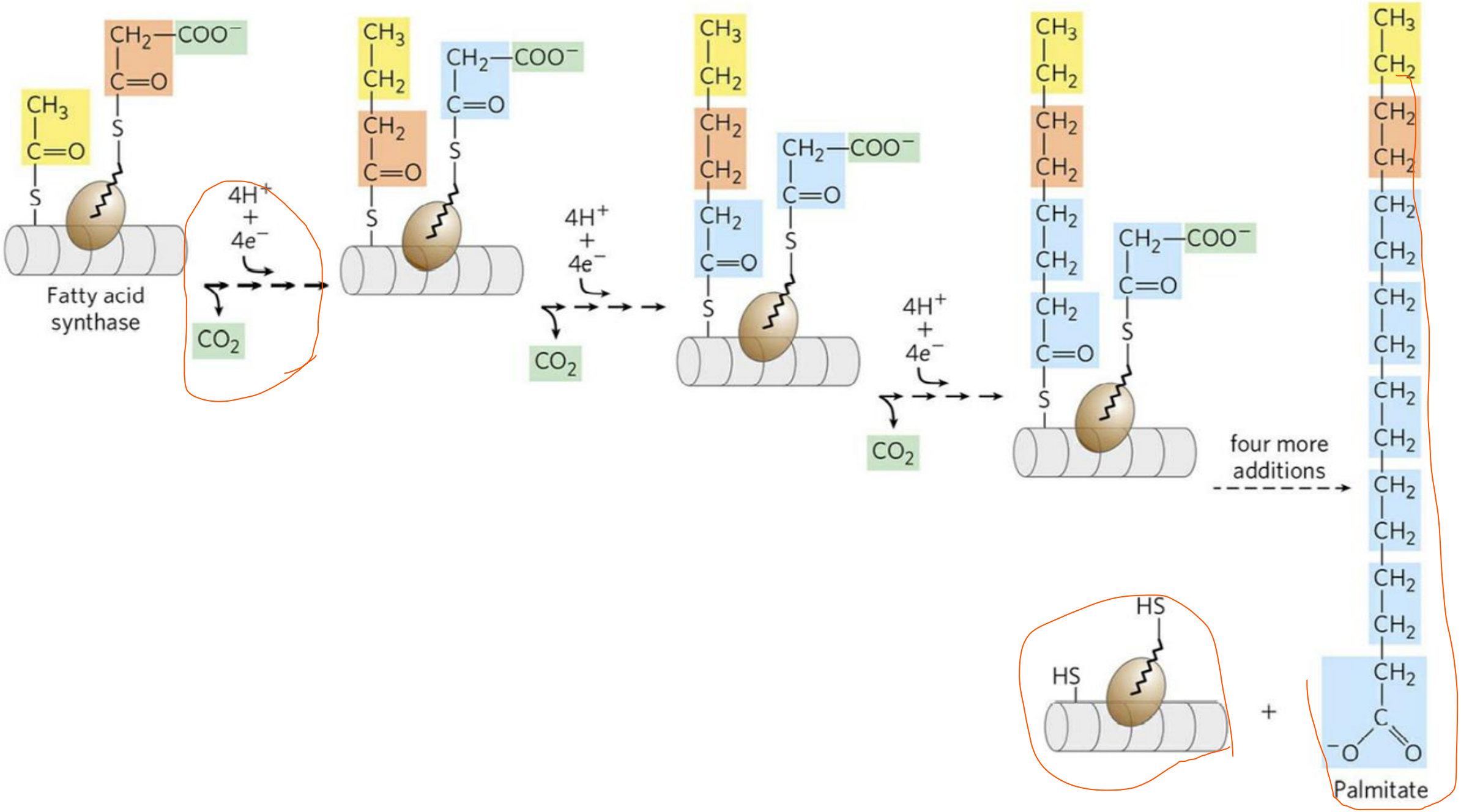


Addition of two carbons to a growing fatty acyl chain: a four-step sequence.

- Condensation of an activated acyl group and two carbons derived from malonyl-CoA, with elimination of CO_2 from the malonyl group, extends the acyl chain by two carbons.
- β-keto group is reduced to an alcohol
- Elimination of H_2O creates a double bond
- The double bond is reduced to form the corresponding saturated fatty acyl group

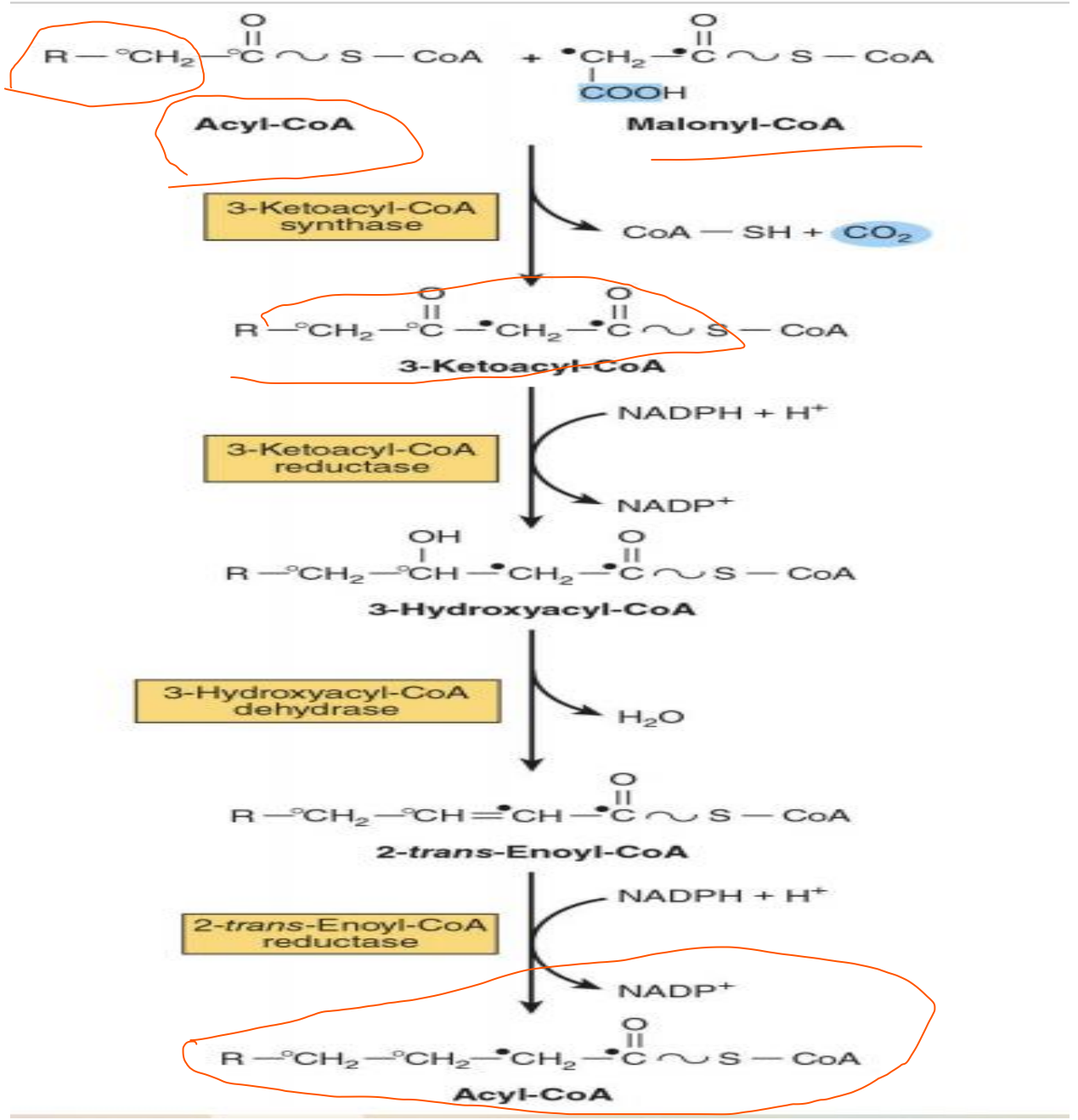



*CO2
Acetyl-CoA
C2
*Malonyl-CoA
C3
Acetyl-CoA carboxylase
1a
1b
CoA
Malonyl acetyl transacylase
Malonyl acetyl transacylase
Cn transfer from
2 to 1
HS — Pan — 1 — Cys — SH
HS — Cys — 2 — Pan — SH
Fatty acid synthase multienzyme complex
C2
Cys — S
CH3
Pan — S
CH2 — *COO−
(C3)
Acyl(acetyl)-malonyl enzyme
*CO2
3-Ketoacyl synthase
Cys — SH
CH2
CH3
3-Ketoacyl enzyme (acetoacetyl enzyme)
NADPH + H+
NADP+
3-Ketoacyl reductase
OH
CH
D(−)-3-Hydroxyacyl enzyme
NADPH generators
Pentose phosphate pathway
Isocitrate dehydrogenase
Malic enzyme
H2O
Dehydratase
CH=CH
2,3-Unsaturated acyl enzyme
NADPH + H+
NADP+
Enoyl reductase
H2O
Thioesterase
After cycling through steps 2 – 5 seven times
CH2 — CH2 — CH3
(Cn)
Acyl enzyme
Palmitate
2
3
4
5
KEY: 1, 2, individual monomers of fatty acid synthase


Fatty acid synthase
4H+ + 4e−
CO2
four more additions
HS
HS
Palmitate

Elongation of Fatty Acid Chains Occurs in the Endoplasmic Reticulum

This pathway (the "microsomal system") elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons, using malonyl-CoA as the acetyl donor and NADPH as the reductant, and is catalyzed by the microsomal fatty acid elongase system of enzymes



THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, and to provide energy .

Lipogenesis converts excess glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle.

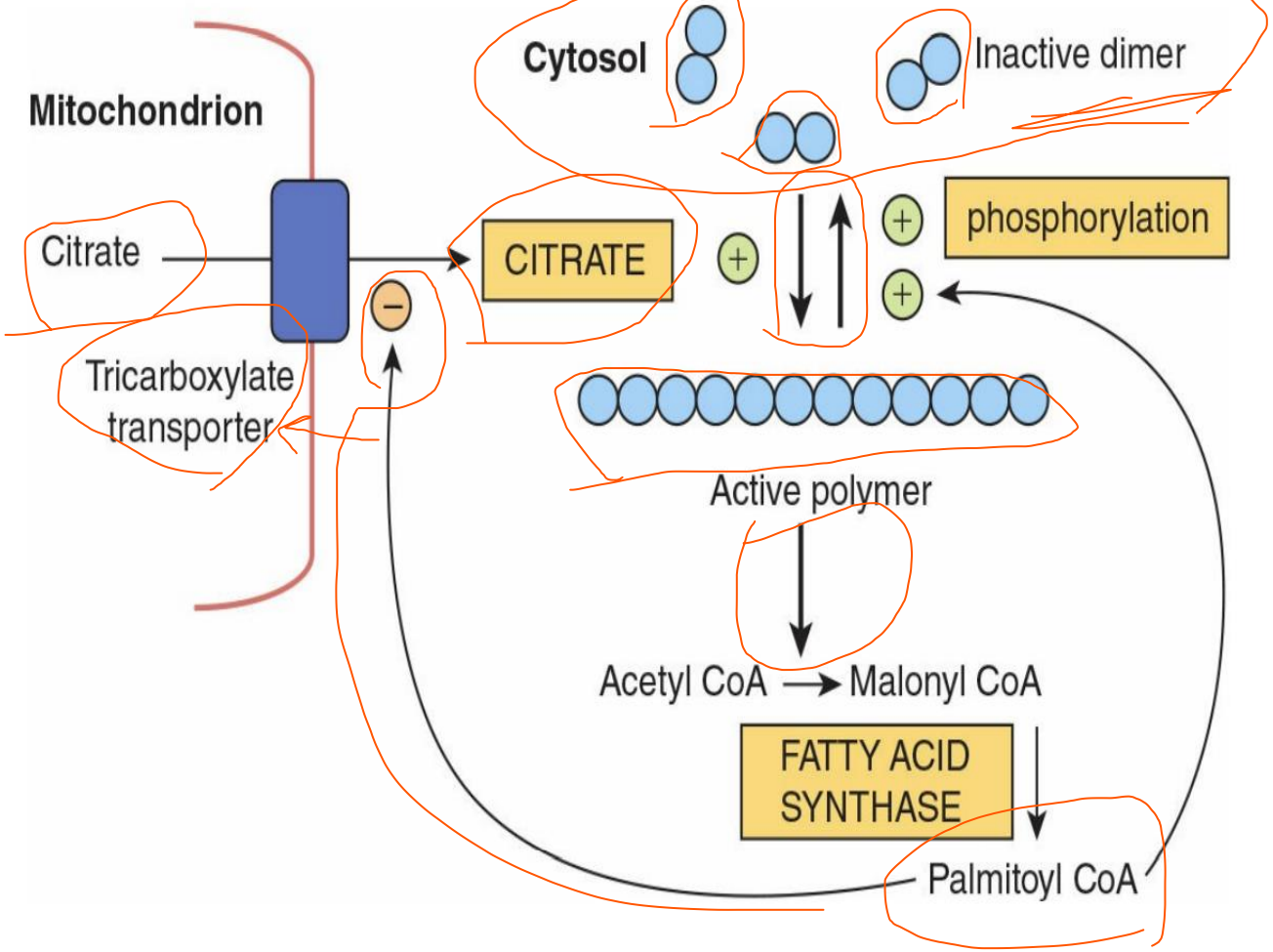
The nutritional state of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate.

Lipogenesis is increased when sucrose is fed instead of glucose because fructose bypasses the phosphofructokinase control point in glycolysis and floods the lipogenic pathway

SHORT- & LONG-TERM MECHANISMS REGULATE LIPOGENESIS

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

Regulation of acetyl-CoA carboxylase. Acetyl-CoA carboxylase is activated by citrate, which promotes the conversion of the enzyme from an inactive dimer to an active polymeric form. Inactivation is promoted by phosphorylation of the enzyme and by long-chain acyl-CoA molecules such as palmitoyl-CoA. In addition, acyl-CoA inhibits the tricarboxylate transporter, which transports citrate out of mitochondria into the cytosol, thus decreasing the citrate concentration in the cytosol and favoring inactivation of the enzyme.



Acetyl-CoA carboxylase is also regulated by hormones such as glucagon, epinephrine, and insulin via changes in its phosphorylation state

Regulation of acetyl-CoA carboxylase by phosphorylation/dephosphorylation. The enzyme is inactivated by phosphorylation by AMP-activated protein kinase (AMPK), which in turn is phosphorylated and activated by AMP-activated protein kinase kinase (AMPKK). Glucagon and epinephrine increase cAMP, and thus activate this latter enzyme via cAMP-dependent protein kinase. The kinase kinase enzyme is also believed to be activated by acyl-CoA. Insulin activates acetyl-CoA carboxylase via dephosphorylation of AMPK.

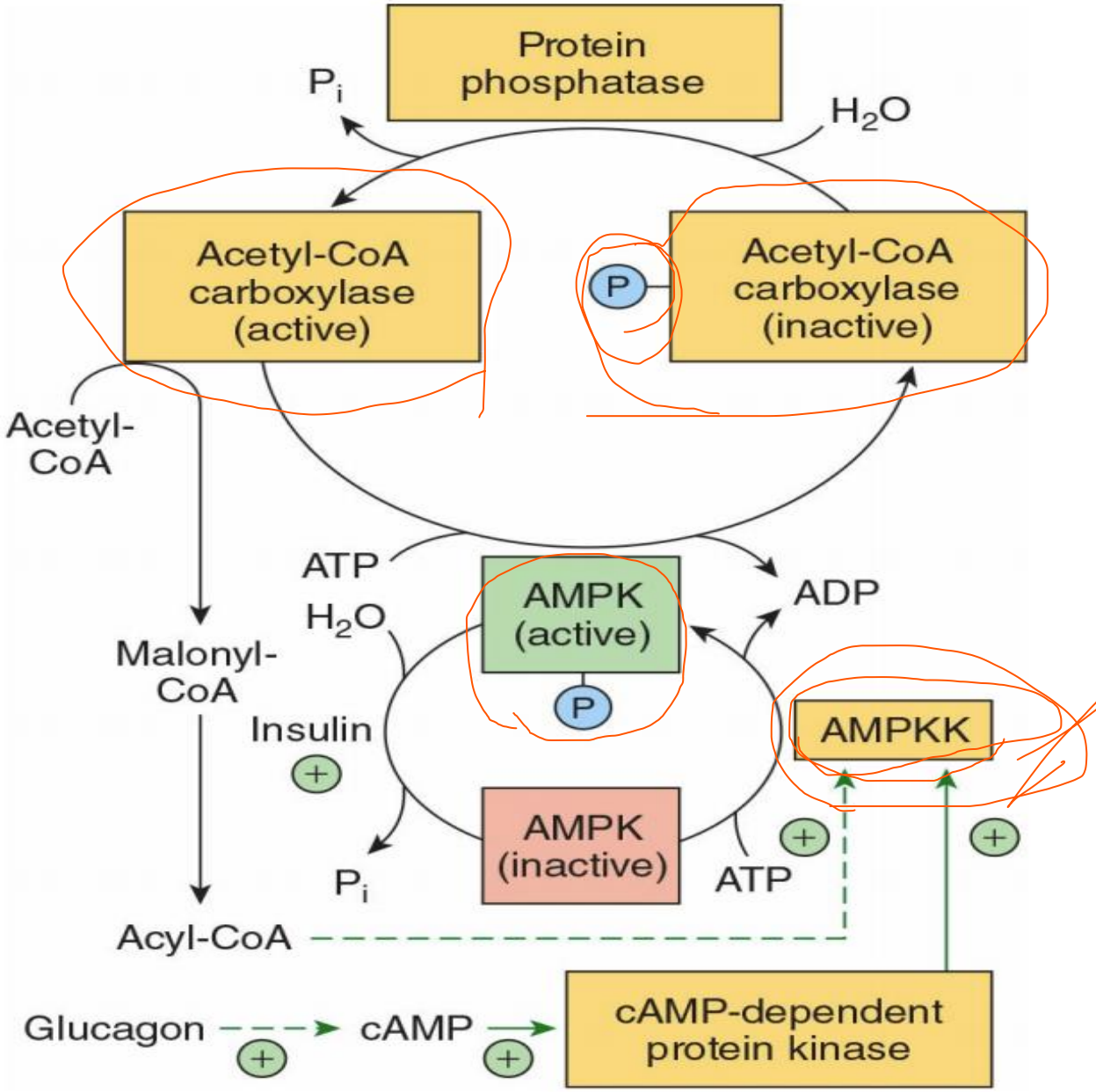


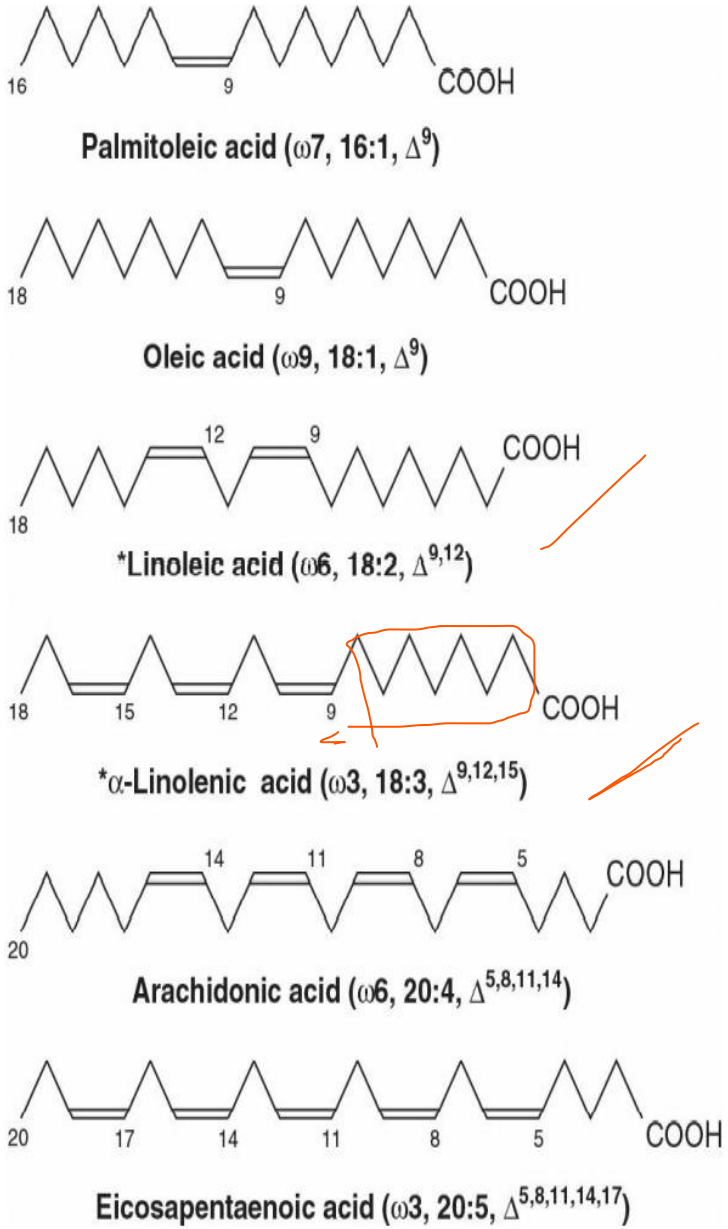
Table 14.3 Comparison of fatty acid synthesis and oxidation

		Fatty acid synthesis	*β-Oxidation*
1.	Major tissues	Liver, adipose tissue	Muscle, liver
2.	Subcellular site	Cytosol	Mitochondria
3.	Precursor/substrate	Acetyl CoA	Acyl CoA
4.	End product	Palmitate	Acetyl CoA
5.	Intermediates are bound to	Acyl carrier protein	Coenzyme A
6.	Coenzyme requirement	NADPH (supplying reducing equivalents)	FAD and NAD^+ (get reduced)
7.	Carbon units added/degraded	Malonyl CoA	Acetyl CoA
8.	Transport system	Citrate (mitochondria ⟶ cytosol)	Carnitine (cytosol ⟶ mitochondria)
9.	Inhibitor	Long chain acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA (inhibits carnitine acyltransferase I)
10.	The pathway increased	After rich carbohydrate diet	In starvation
11.	Hormonal status that promotes	High ratio of insulin/glucagon	Low ratio of insulin/glucagon
12.	Status of enzyme(s)	Multifunctional enzyme complex	Individual enzymes

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

Certain long-chain unsaturated fatty acids of metabolic significance in mammals. Other C20 , C22 , and C24 polyenoic fatty acids may be derived from oleic, linoleic, and α-linolenic acids by chain elongation. Palmitoleic and oleic acids are **not** essential in the diet because the tissues can introduce a double bond at the Δ 9 position of a saturated fatty acid. **Linoleic** and **α-linolenic acids** are the only fatty acids known to be essential for the complete nutrition of many species of mammals

In humans and most other mammals, arachidonic acid can be formed from linoleic acid. Double bonds can be introduced at the Δ 4 , Δ 5 , Δ 6 , and Δ 9 positions in most animals, but never beyond the Δ 9 position. In contrast, plants are able to synthesize the nutritionally essential fatty acids by introducing double bonds at the Δ 12 and Δ 15 positions.



MONOUNSATURATED FATTY ACIDS ARE SYNTHESIZED BY A Δ 9 DESATURASE SYSTEM

Several tissues including the liver are considered to be responsible for the formation of nonessential monounsaturated fatty acids from saturated fatty acids.

The first double bond introduced into a saturated fatty acid is nearly always in the Δ 9 position

An enzyme system Δ 9 desaturase in the endoplasmic reticulum catalyzes the conversion of palmitoyl-CoA or stearoyl-CoA to palmitoleoyl-CoA or oleoyl-CoA, respectively.

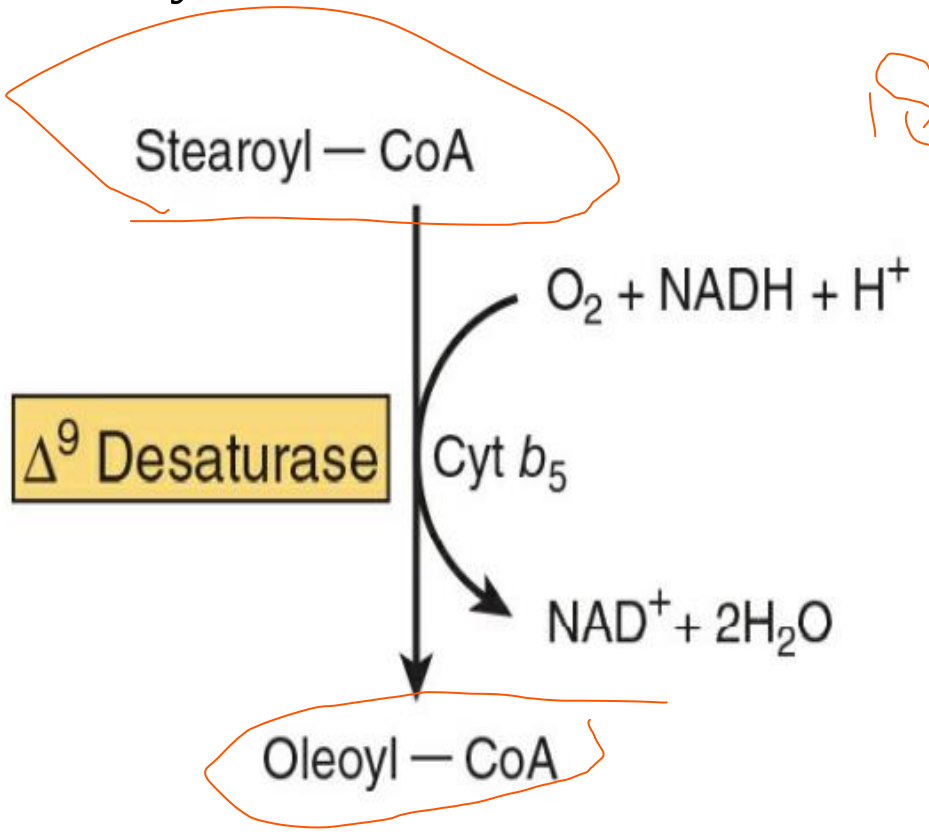
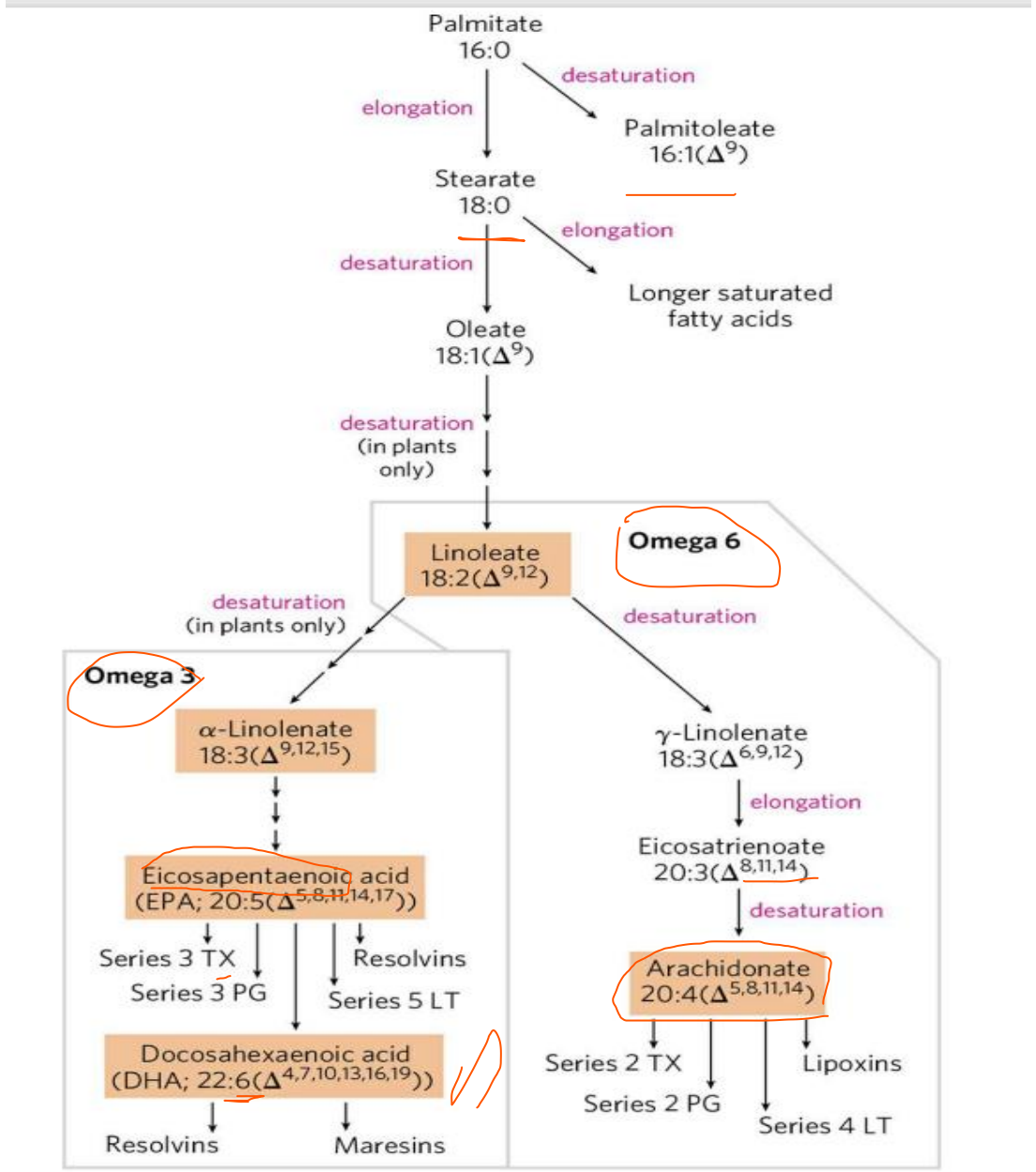


FIGURE 23–9 Microsomal δ9 desaturase.

SYNTHESIS OF POLYUNSATURATED FATTY ACIDS INVOLVES DESATURASE & ELONGASE ENZYME SYSTEMS



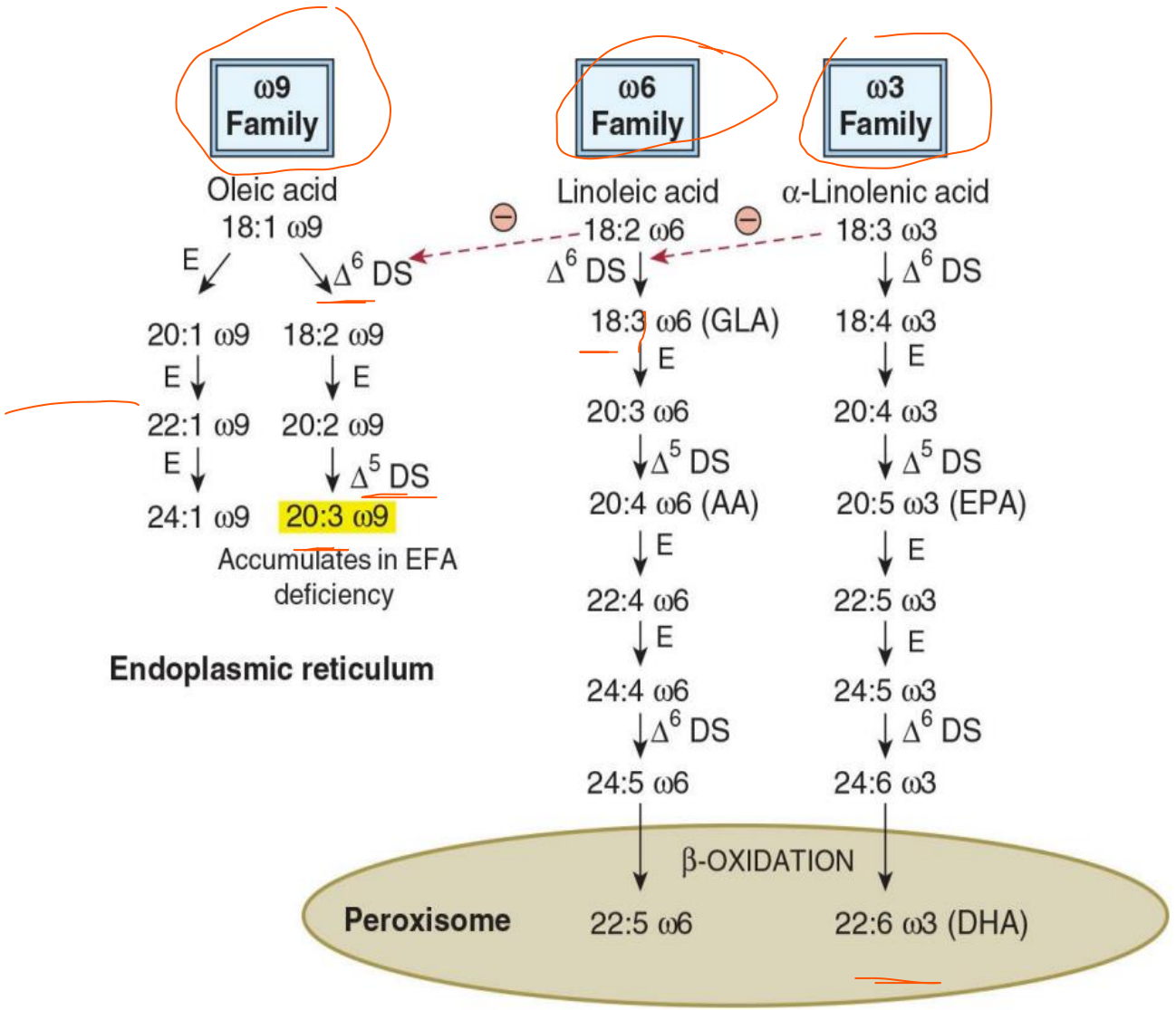


FIGURE 23–10 Biosynthesis of the ω9, ω6, and ω3 families of

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

Arachidonic acid is present in membranes and accounts for 5 to 15% of the fatty acids in phospholipids. Docosahexaenoic acid (DHA; ω3, 22:6), which is synthesized to a limited extent from α-linolenic acid or obtained directly from fish oils, is present in high concentrations in retina, cerebral cortex, testis, and sperm. DHA is particularly needed for development of the brain and retina and is supplied via the placenta and milk.

Patients with retinitis pigmentosa are reported to have low blood levels of DHA. In essential fatty acid deficiency, nonessential polyenoic acids of the ω9 family, particularly Δ 5,8,11 - eicosatrienoic acid (ω9 20:3), replace the essential fatty acids in phospholipids, other complex lipids, and membranes. The triene:tetraene ratio in plasma lipids can be used to diagnose the extent of essential fatty acid deficiency.

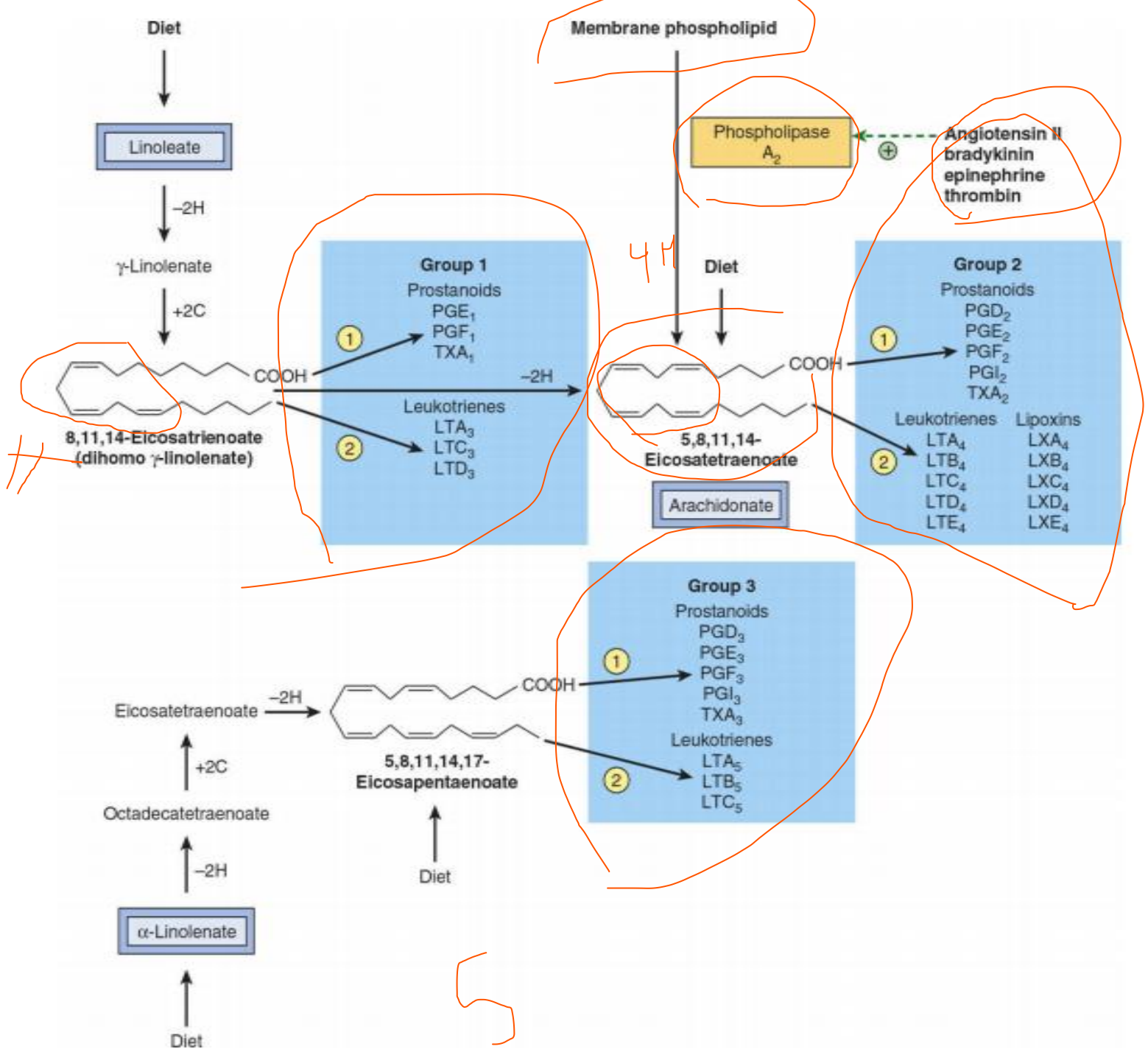
EICOSANOIDS ARE FORMED FROM C20 POLYUNSATURATED FATTY ACIDS

Arachidonate and some other C20 polyunsaturated fatty acids give increase to eicosanoids, physiologically and pharmacologically active compounds known as prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), and lipoxins (LX). Physiologically, they are considered to act as local hormones functioning

There are three groups of eicosanoids that are synthesized from C20 eicosanoic acids derived from the essential fatty acids linoleate and αlinolenate, or directly from dietary arachidonate and eicosapentaenoate.

- PG2 , TX2 series (prostanoids) by the cyclooxygenase pathway
- LT4 and LX4 series by the lipoxygenase pathway

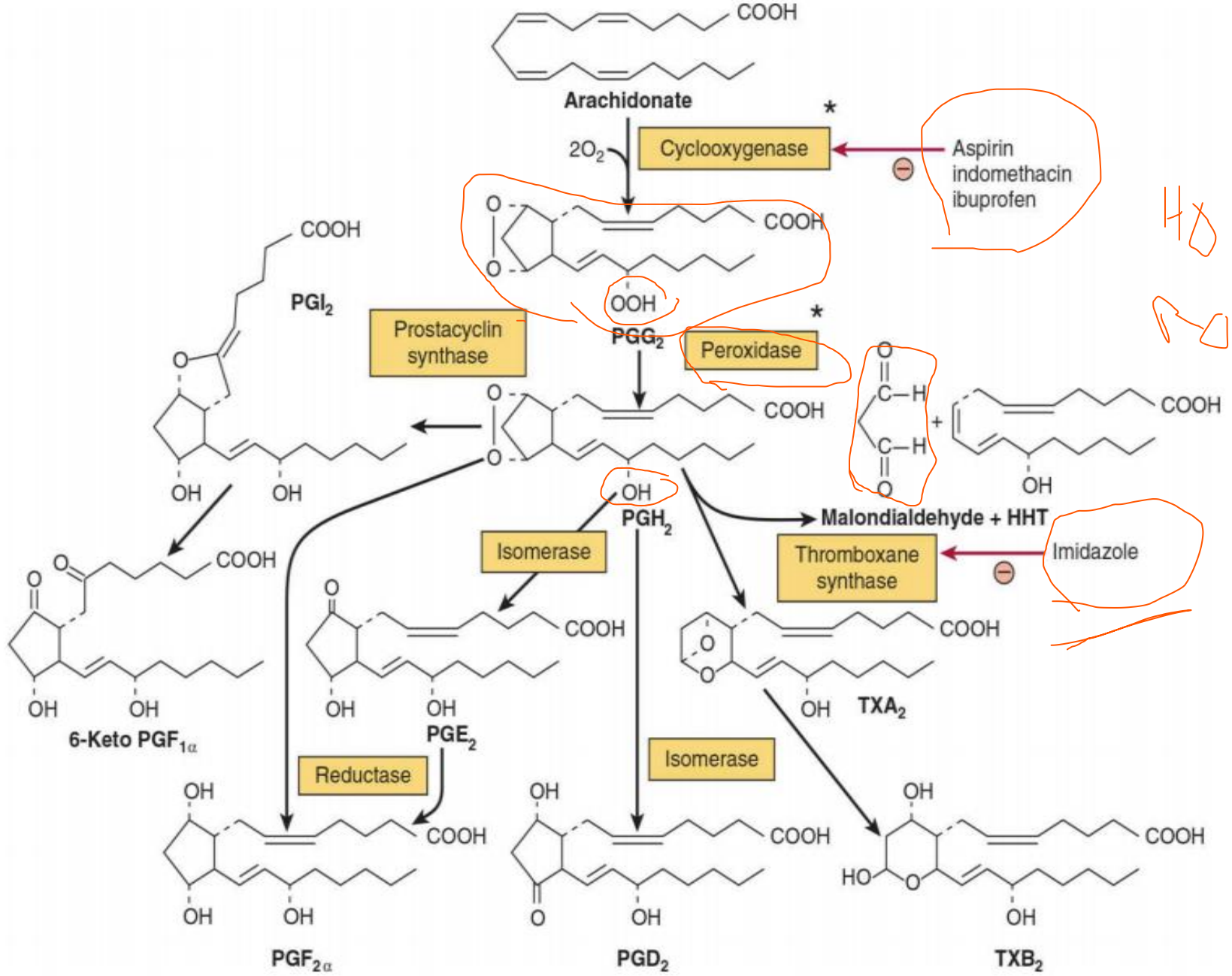
The three groups of eicosanoids and their biosynthetic origins. (, cyclooxygenase pathway; , lipoxygenase pathway; LT, leukotriene; LX, lipoxin; PG, prostaglandin; PGI, prostacyclin; TX, thromboxane.)



THE CYCLOOXYGENASE PATHWAY IS RESPONSIBLE FOR

Conversion of arachidonic acid to prostaglandins and thromboxanes of series 2

Note Aspirin is a nonsteroidal anti-inflammatory drug (NSAID) that inhibits COX-1 and COX-2. Other NSAIDs include indomethacin and ibuprofen, and these usually inhibit cyclooxygenases by competing with arachidonate



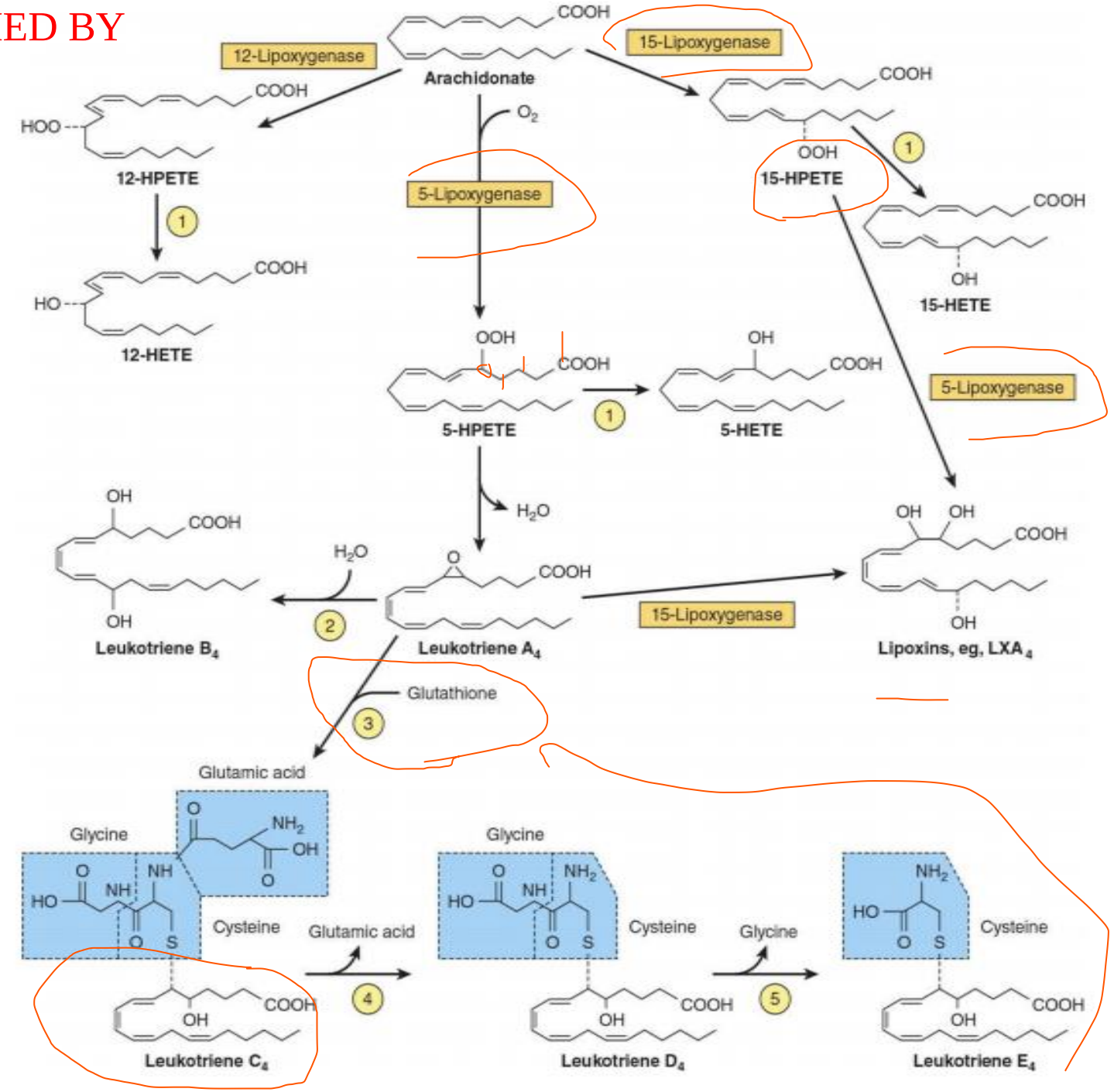
Prostanoids Are Potent, Biologically Active Substances

Thromboxanes are synthesized in platelets and upon release cause vasoconstriction and platelet aggregation. Their synthesis is specifically inhibited by low-dose aspirin. Prostacyclins (PGI2) are produced by blood vessel walls and are potent inhibitors of platelet aggregation.

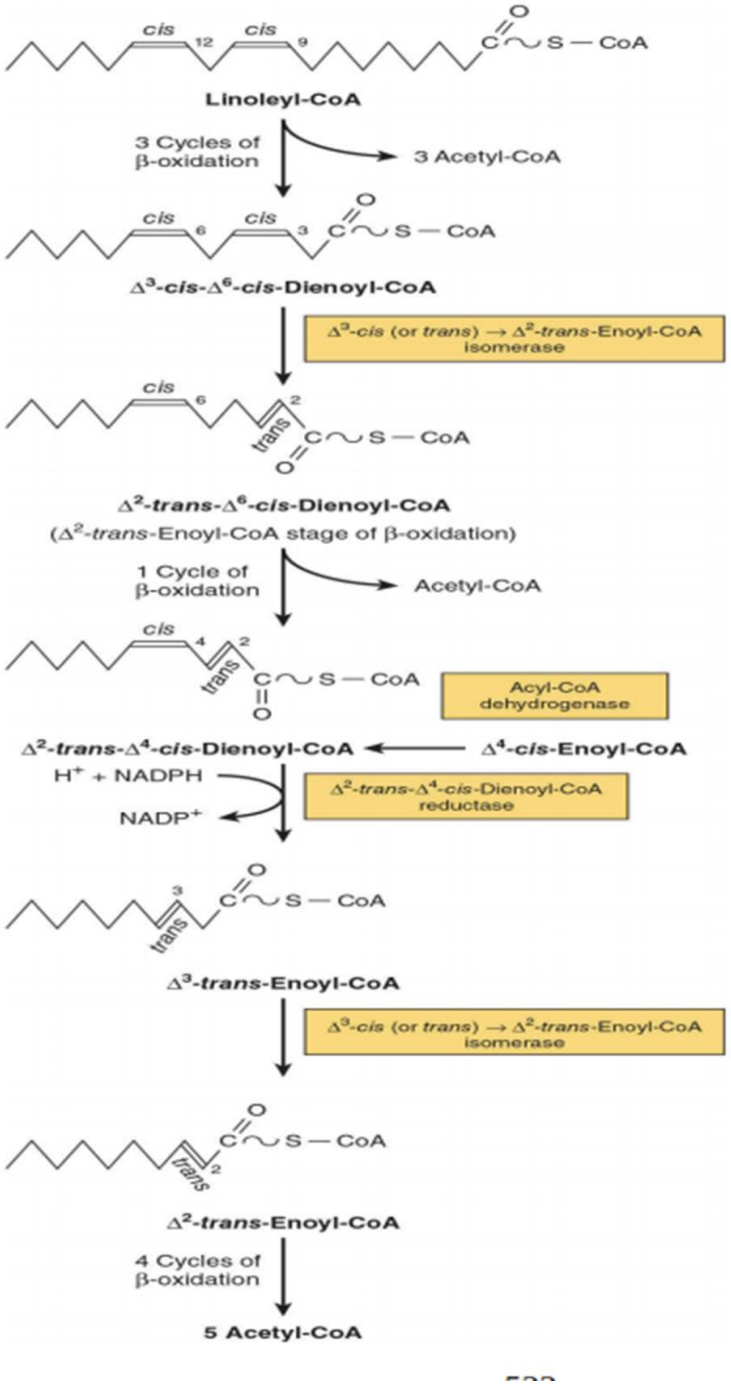
LEUKOTRIENES & LIPOXINS ARE FORMED BY THE LIPOXYGENASE PATHWAY

The leukotrienes are a family of conjugated trienes formed from eicosanoic acids in leukocytes, mastocytoma cells, platelets, and macrophages by the lipoxygenase pathway in response to both immunologic and nonimmunologic stimuli.

Conversion of arachidonic acid to leukotrienes and lipoxins of series 4 via the lipoxygenase pathway. Some similar conversions occur in series 3 and 5 leukotrienes. (1 peroxidase; 2 leukotriene A4 epoxide hydrolase; 3 glutathione S-transferase; 4 γglutamyltranspeptidase; 5 cysteinyl-glycine dipeptidase; HETE, hydroxyeicosatetraenoate; HPETE, hydroperoxyeicosatetraenoate.)



Oxidation of Unsaturated Fatty Acids Occurs by a Modified β-Oxidation Pathway / lecture 3



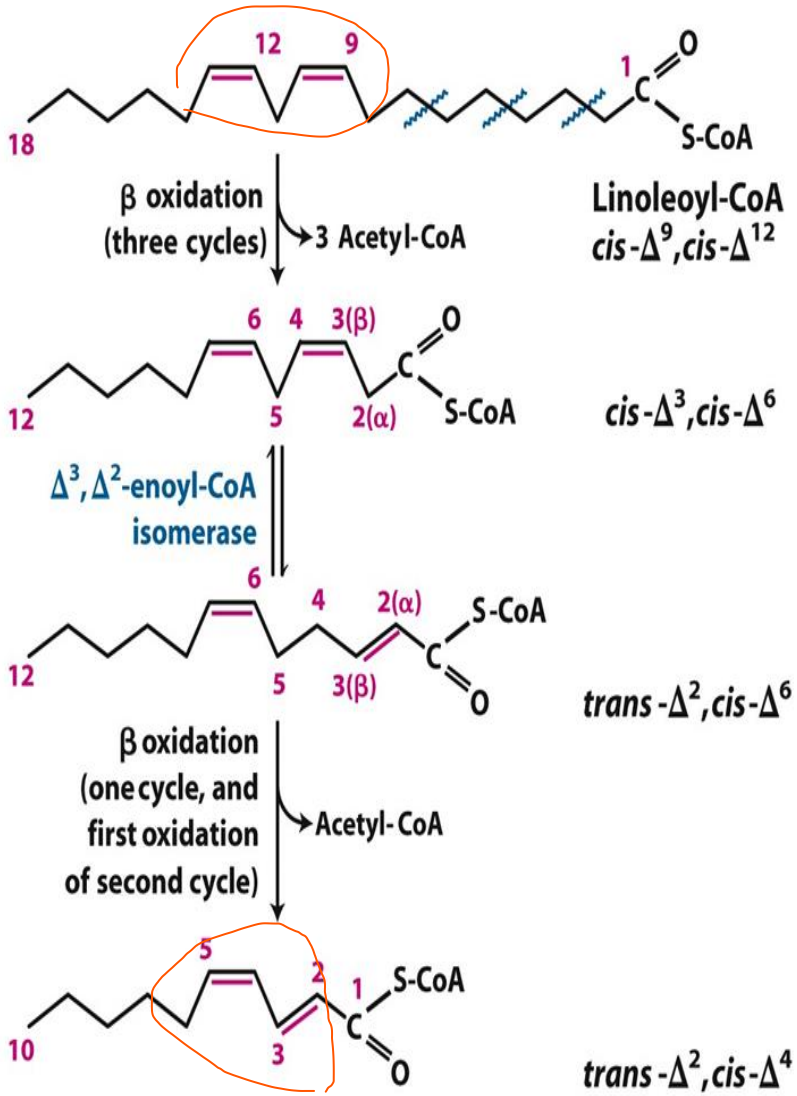


Figure 17-10 part 1
Lehninger Principles of Biochemistry, Fifth Edition

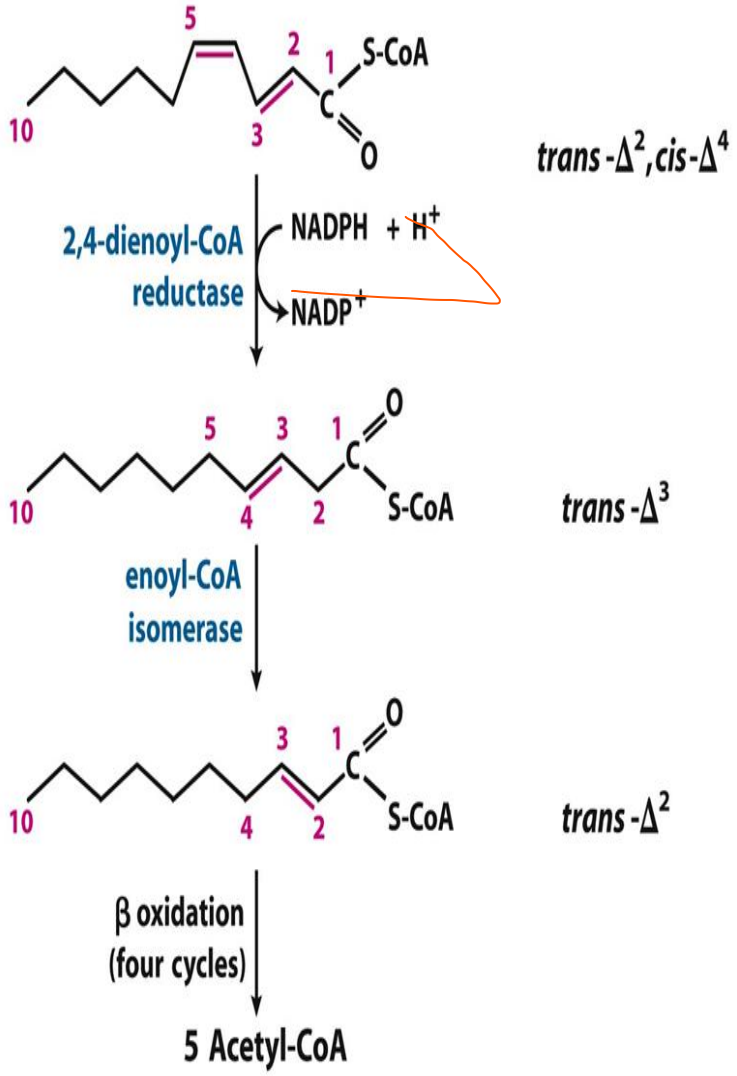


Figure 17-10 part 2
Lehninger Principles of Biochemistry, Fifth Edition

THANK YOU

Lipid Transport, Storage & Cholesterol synthesis

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper’s Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

By

Dr. Muthanna Owaid Hussein

BIOMEDICAL IMPORTANCE

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

Lipoproteins mediate this cycle by transporting lipids from the intestines as chylomicrons—and from the liver as very-low-density lipoproteins (VLDL)—to most tissues for oxidation and to adipose tissue for storage. Lipid is mobilized from adipose tissue as free fatty acids (FFAs) bound to serum albumin.

Abnormalities of lipoprotein metabolism cause various (hypo- or hyper) lipoproteinemias. The most common of these is in diabetes mellitus, where insulin deficiency causes excessive mobilization of FFA and underutilization of chylomicrons and VLDL, leading to hypertriacylglycerolemia. Most other pathologic conditions affecting lipid transport are due primarily to inherited defects, some of which cause hypercholesterolemia and premature atherosclerosis

LIPIDS ARE TRANSPORTED IN THE PLASMA AS LIPOPROTEINS

Four Major Lipid Classes Are Present in Lipoproteins

Plasma lipids consist of triacylglycerols (16%), phospholipids (30%), cholesterol (14%), and cholesteryl esters (36%) and a much smaller fraction of unesterified long-chain fatty acids (or FFAs) (4%).

Four Major Groups of Plasma Lipoproteins Have Been Identified

(1) Chylomicrons, derived from intestinal absorption of triacylglycerol and other lipids;

(2) VLDL, derived from the liver for the export of triacylglycerol;

(3) low-density lipoproteins (LDL), representing a final stage in the catabolism of VLDL.

(4) high-density lipoproteins, (HDL), involved in cholesterol transport and also in VLDL and chylomicron metabolism.

Composition of the Lipoproteins in Plasma of Humans

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

TABLE 21-2 Apolipoproteins of the Human Plasma Lipoproteins

Apolipoprotein	Polypeptide molecular weight	Lipoprotein association	Function (if known)
ApoA-I	28,100	HDL	Activates LCAT; interacts with ABC transporter
ApoA-II	17,400	HDL	Inhibits LCAT
ApoA-IV	44,500	Chylomicrons, HDL	Activates LCAT; cholesterol transport/clearance
ApoB-48	242,000	Chylomicrons	Cholesterol transport/clearance
ApoB-100	512,000	VLDL, LDL	Binds to LDL receptor
ApoC-I	7,000	VLDL, HDL	
ApoC-II	9,000	Chylomicrons, VLDL, HDL	Activates lipoprotein lipase
ApoC-III	9,000	Chylomicrons, VLDL, HDL	Inhibits lipoprotein lipase
ApoD	32,500	HDL	
ApoE	34,200	Chylomicrons, VLDL, HDL	Triggers clearance of VLDL and chylomicron remnants
ApoH	50,000	Possibly VLDL, binds phospholipids such as cardiolipin	Roles in coagulation, lipid metabolism, apoptosis, inflammation

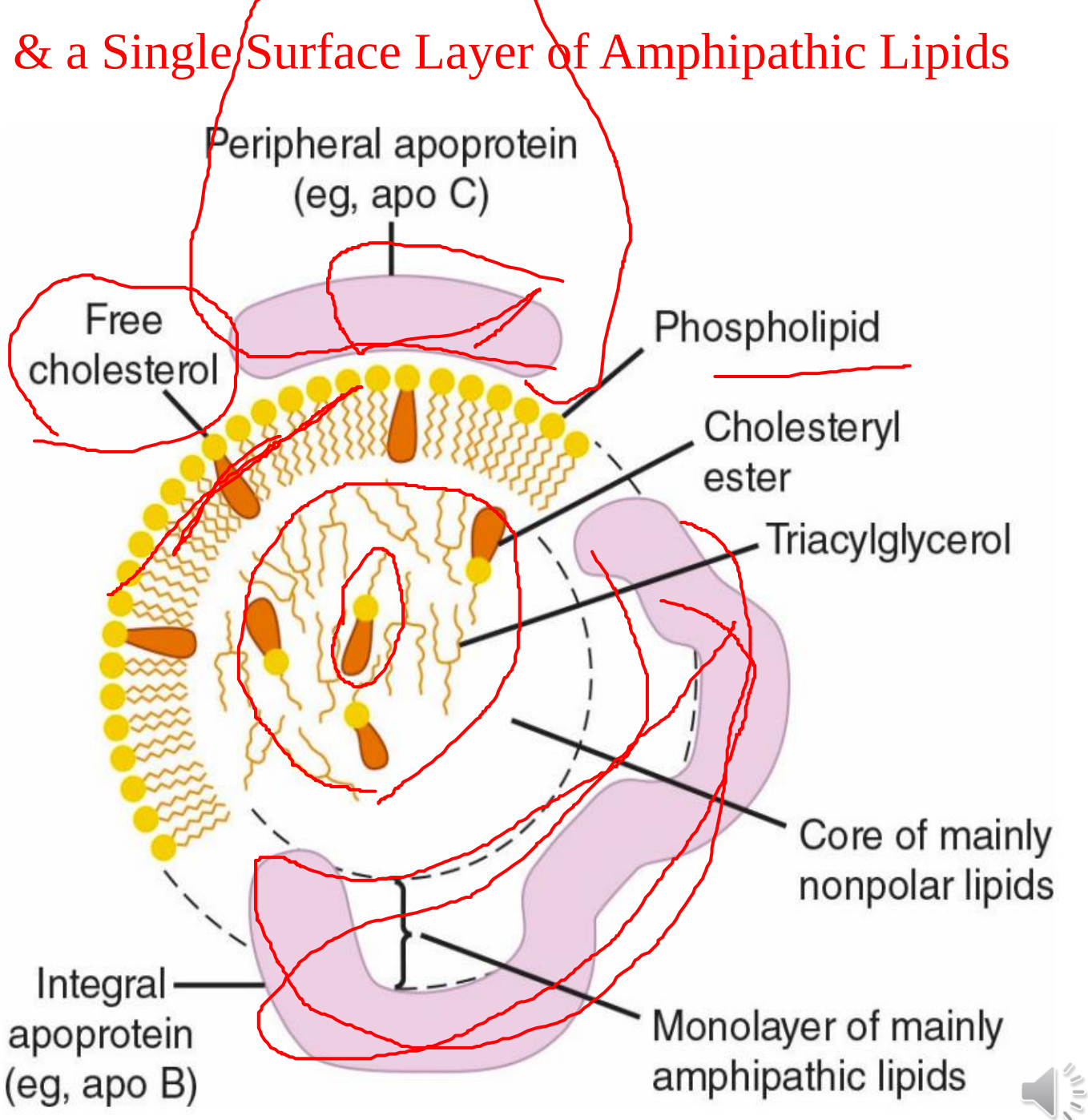
Source: Information from D. E. Vance and J. E. Vance (eds), *Biochemistry of Lipids and Membranes* , 5th edn,

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

The nonpolar lipid core consists of mainly triacylglycerol and cholesteryl ester and is surrounded by a single surface layer of amphipathic phospholipid and cholesterol molecules.

These are oriented so that their polar groups face outward to the aqueous medium, as in the cell membrane.

The protein moiety of a lipoprotein is known as an apolipoprotein or apoprotein, constituting nearly 70% of some HDL and as little as 1% of chylomicrons.



FREE FATTY ACIDS (FFAs) ARE RAPIDLY METABOLIZED

The FFAs arise in the plasma from the breakdown of triacylglycerol in adipose tissue or as a result of the action of lipoprotein lipase on the plasma triacylglycerols.

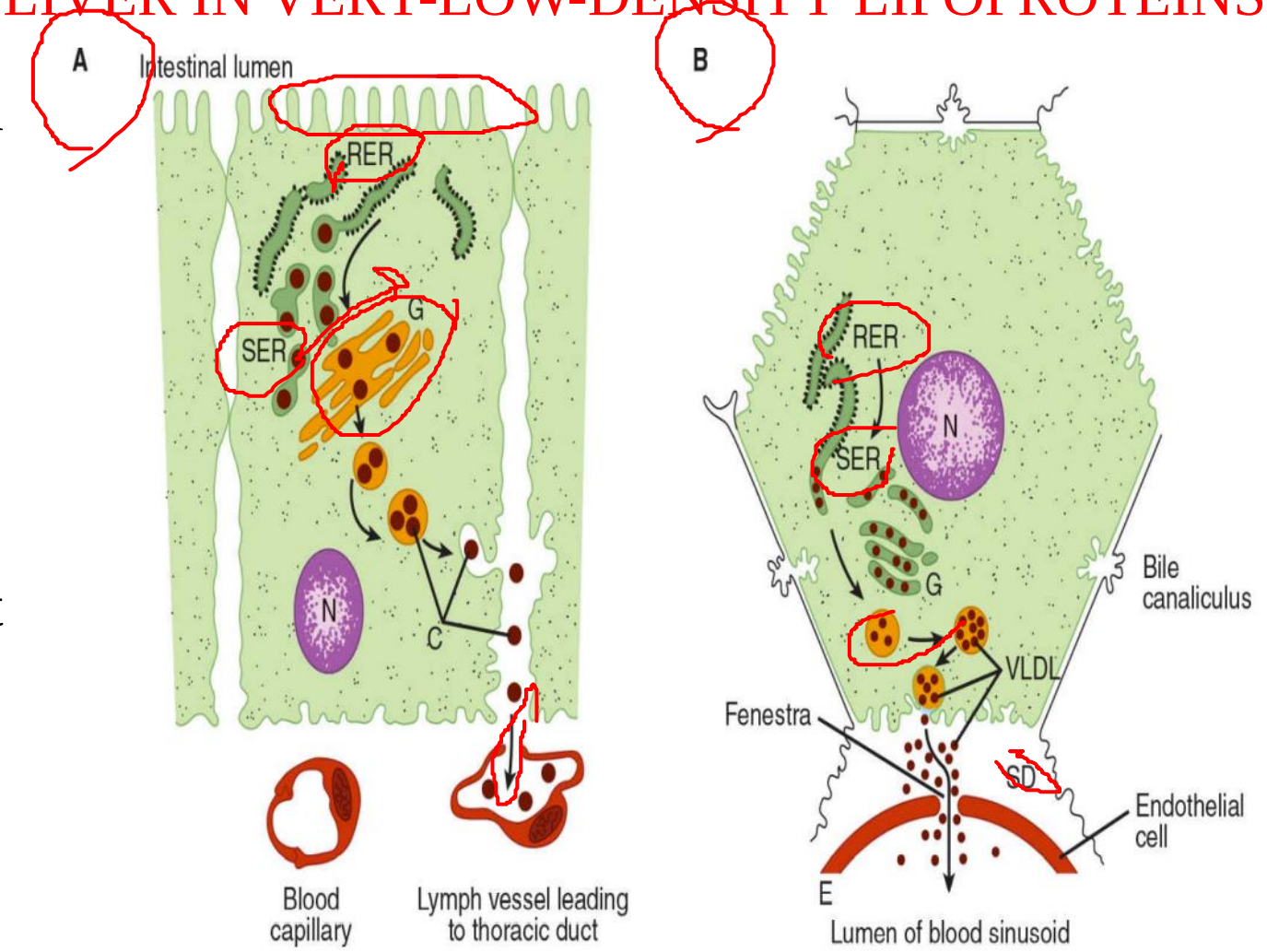
They are found in **combination with albumin**, a very effective solubilizer. FFAs are removed from the blood extremely rapidly by the tissues and oxidized (25-50% of energy requirements in starvation) or esterified to form triacylglycerol.

The FFA uptake by tissues is related directly to the plasma-FFA concentration, which in turn is determined by the rate of lipolysis in adipose tissue. After dissociation of the fatty acid–albumin complex at the plasma membrane, fatty acids bind to a membrane fatty acid transport protein that acts as a transmembrane cotransporter with Na+. On entering the cytosol, FFAs are bound by intracellular fatty acid–binding proteins.

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

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

abetalipoproteinemia (a rare disease), lipoproteins containing apo B are not formed and lipid droplets accumulate in the intestine and liver



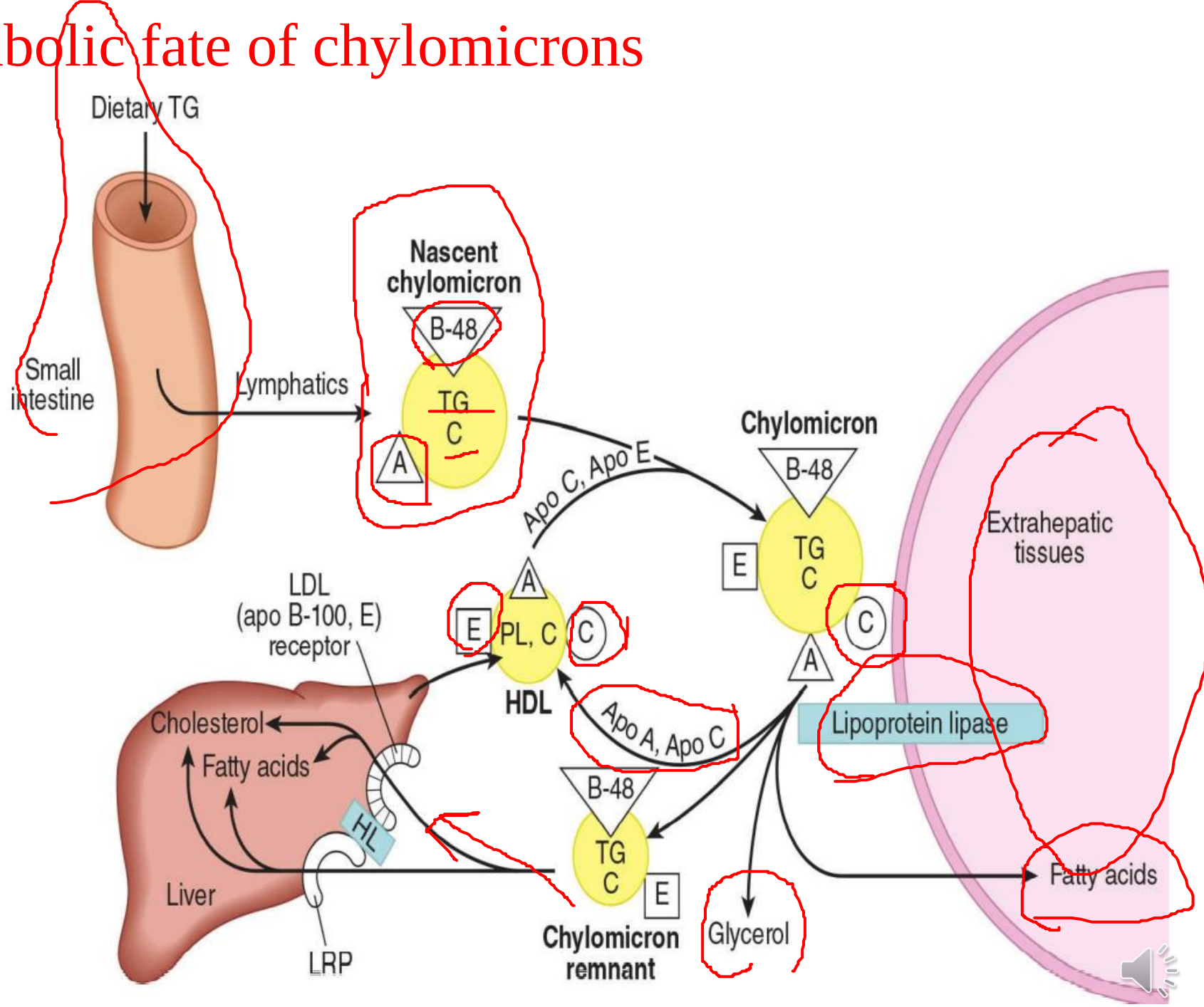
The formation and secretion of (A) chylomicrons by an intestinal cell and (B) very-low-density lipoproteins by a hepatic cell.

Metabolic fate of chylomicrons

The clearance of chylomicrons from the blood is rapid, the half-time of disappearance being under 1 hour in humans. Larger particles are catabolized more quickly than smaller ones.

Fatty acids originating from chylomicron triacylglycerol are delivered mainly to adipose tissue, heart, and muscle (80%), while ~20% goes to the liver.

the liver does not metabolize native chylomicrons or VLDL significantly; thus, the fatty acids in the liver must be secondary to their metabolism in extrahepatic tissues.



Metabolic fate of very-low-density lipoproteins (VLDL) and production of low-density lipoproteins (LDL)

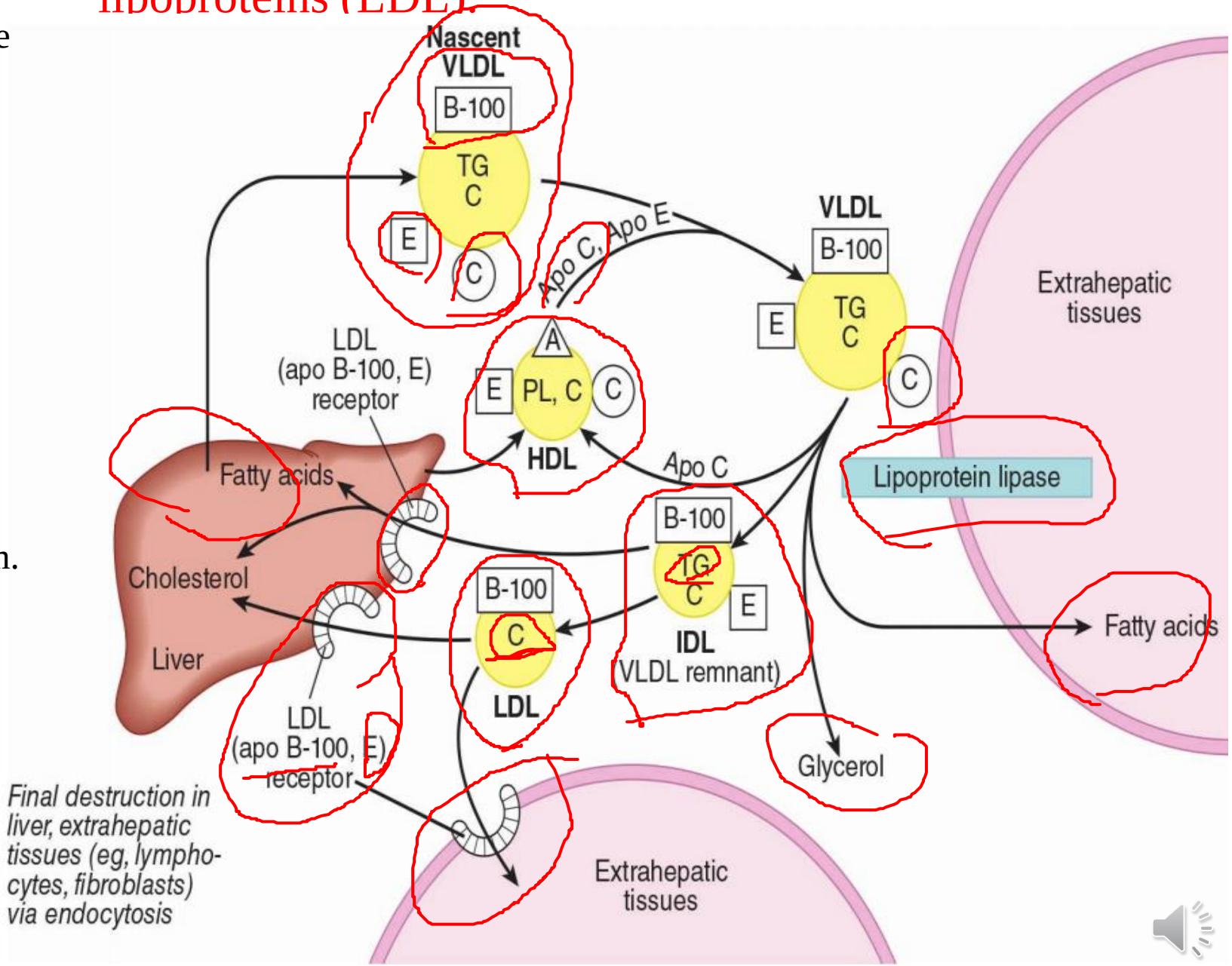
Lipoprotein lipase is an enzyme located on the walls of blood capillaries, anchored to the endothelium by negatively charged proteoglycan chains of heparan sulfate.

Hepatic lipase is bound to the sinusoidal surface of liver cells and is also released by heparin.

phospholipids and apo C-II are required as cofactors for lipoprotein lipase activity, while apo A-II and apo C-III act as inhibitors. Hydrolysis takes place while the lipoproteins are attached to the enzyme on the endothelium.

Heart lipoprotein lipase has a low Km for triacylglycerol, about one-tenth of that for the enzyme in adipose tissue.

In adipose tissue, insulin enhances lipoprotein lipase synthesis in adipocytes and its translocation to the luminal surface of the capillary endothelium.



The Liver Is Responsible for the Uptake of Remnant Lipoproteins

Chylomicron remnants are taken up by the liver by receptor-mediated endocytosis, and the cholesteryl esters and triacylglycerols are hydrolyzed and metabolized. Uptake is mediated by apo E, via two apo E-dependent receptors, the LDL (apo B-100, E) receptor and LDL receptor–related protein-1 (LRP-1).

Hepatic lipase has a dual role:

(1) It acts as a ligand to facilitate remnant uptake and (2) it hydrolyzes remnant triacylglycerol and phospholipid.

THE LIVER PLAYS A CENTRAL ROLE IN LIPID TRANSPORT & METABOLISM

The liver carries out the following major functions in lipid metabolism:

1. Facilitation of the digestion and absorption of lipids by the production of bile.
2. Active synthesis, oxidation of fatty acids, synthesis of triacylglycerols and phospholipids.
3. Conversion of fatty acids to ketone bodies (ketogenesis)
4. Synthesis and metabolism of plasma lipoproteins.

1- Chylomicrons are synthesized from dietary fats in the ER of enterocytes, epithelial cells that line the small intestine. The chylomicrons then move through the lymphatic system and enter the bloodstream via the left subclavian vein. The apolipoproteins of chylomicrons include apoB-48 , apoE, and apoC-II.

2- ApoC-II activates lipoprotein lipase in the capillaries of adipose, heart, skeletal muscle, and lactating mammary tissues, allowing the release of free fatty acids (FFA) to these tissues. Chylomicrons thus carry dietary fatty acids to tissues where they will be consumed or stored as fuel.

3- The remnants of chylomicrons, depleted of most of their triacylglycerols but still containing cholesterol, apoE, and apoB-48, move through the bloodstream to the liver. Receptors in the liver bind to the apoE in the chylomicron remnants and mediate uptake of these remnants by endocytosis.

4- In the liver, the remnants release their cholesterol and are degraded in lysosomes. This pathway from dietary cholesterol to the liver is the exogenous pathway.

5- They are converted to triacylglycerols or cholesteryl esters in the liver and packaged with specific apolipoproteins into very-low-density lipoprotein (VLDL). Excess carbohydrate in the diet can also be converted to triacylglycerols in the liver and exported as VLDL. In addition to triacylglycerols and cholesteryl esters, VLDL contains apoB-100, apoC-I, apoC-II, apoC-III, and apoE. VLDL is transported in the blood from the liver to muscle and adipose tissue.

6- In the capillaries of these tissues, apoC-II activates lipoprotein lipase, which catalyzes the release of free fatty acids from triacylglycerols in the VLDL. Adipocytes take up these fatty acids, reconvert them to triacylglycerols, and store the products in intracellular lipid droplets; myocytes, in contrast, primarily oxidize the fatty acids to supply energy. When the insulin level is high (after a meal), VLDL serves primarily to convey lipids from the diet to adipose tissue for storage. The loss of triacylglycerol converts some VLDL to VLDL remnants, also called intermediatedensity lipoprotein (IDL). Further removal of triacylglycerol from IDL (remnants) produces lowdensity lipoprotein (LDL). Rich in cholesterol and cholesteryl esters, and containing apoB-100 as
its major apolipoprotein,

7- LDL carries cholesterol to extrahepatic tissues such as muscle, adrenal glands, and adipose tissue. These tissues have plasma membrane LDL receptors that recognize apoB100 and mediate uptake of cholesterol and cholesteryl esters.

8- LDL also delivers cholesterol to macrophages, sometimes converting them into foam cells.

9- LDL not taken up by peripheral tissues and cells returns to the liver and is taken up via LDL receptors in the hepatocyte plasma membrane. Cholesterol that enters hepatocytes by this path may be incorporated into membranes, converted to bile acids, or reesterified by ACAT for storage within cytosolic lipid droplets.

HORMONES REGULATE FAT MOBILIZATION

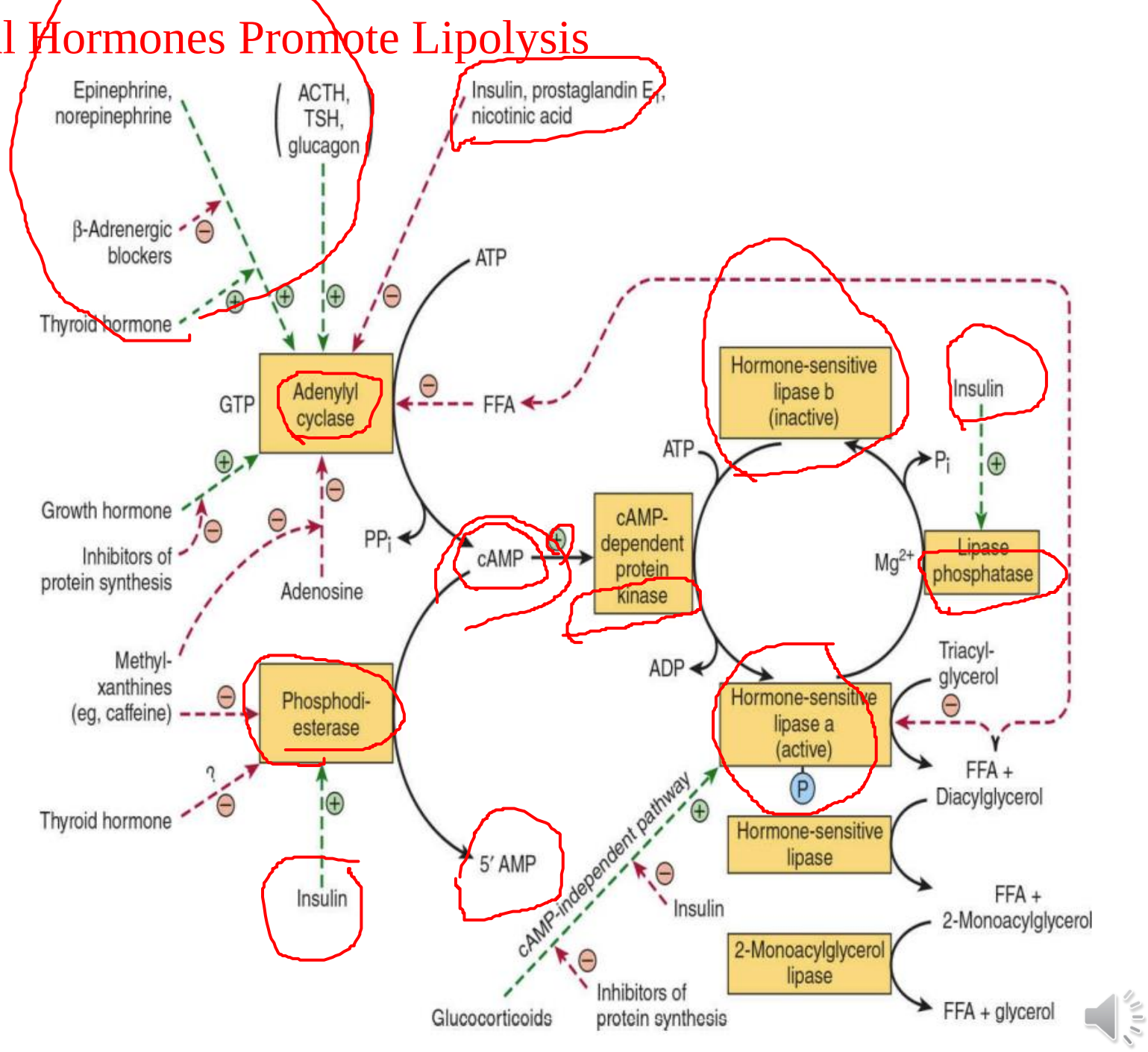
Adipose Tissue Lipolysis Is Inhibited by Insulin the rate of release FFA from adipose tissue is affected by many hormones that influence either the rate of esterification or the rate of lipolysis. Insulin inhibits the release of FFA from adipose tissue, which results in a fall in circulating plasma-free fatty acids. Insulin also enhances lipogenesis and the synthesis of acylglycerol and increases the oxidation of glucose to CO_2 via the pentose phosphate pathway.

Another principal action of insulin in adipose tissue is to inhibit the activity of hormone-sensitive lipase, reducing the release not only of FFA but also of glycerol.

Several Hormones Promote Lipolysis

Other hormones accelerate the release of FFA from adipose tissue and raise the plasma-free fatty acid concentration by increasing the rate of lipolysis of the triacylglycerol stores. These include epinephrine, norepinephrine, glucagon, adrenocorticotropic hormone (ACTH), α- and β-melanocyte-stimulating hormones (MSH), thyroid stimulating hormone (TSH), growth hormone (GH), and vasopressin.

Many of these activate hormone-sensitive lipase. For an optimal effect, most of these lipolytic processes require the presence of glucocorticoids and thyroid hormones.



Cholesterol Synthesis

Cholesterol is present in tissues and in plasma either as free cholesterol or combined with a long-chain fatty acid as cholesteryl ester, the storage form. In plasma, both forms are transported in lipoproteins. Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes, where it is important for the maintenance of the correct permeability and fluidity, and of the outer layer of plasma lipoproteins. Plasma low-density lipoprotein (LDL) is the vehicle that supplies cholesterol and cholesteryl ester to many tissues. Free cholesterol is removed from tissues by plasma highdensity lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion to bile acids in the process known as reverse cholesterol transport . Cholesterol is a major constituent of gallstones. However, its chief role in pathologic processes is as a factor in the development of atherosclerosis .

CHOLESTEROL IS BIOSYNTHESIZED FROM ACETYL-COA

Acetyl-CoA is the source of all carbon atoms in cholesterol.

Cholesterol is a 27-carbon compound consisting of four rings and a side chain. It is synthesized from acetyl-CoA by a lengthy pathway that may be divided into five steps.

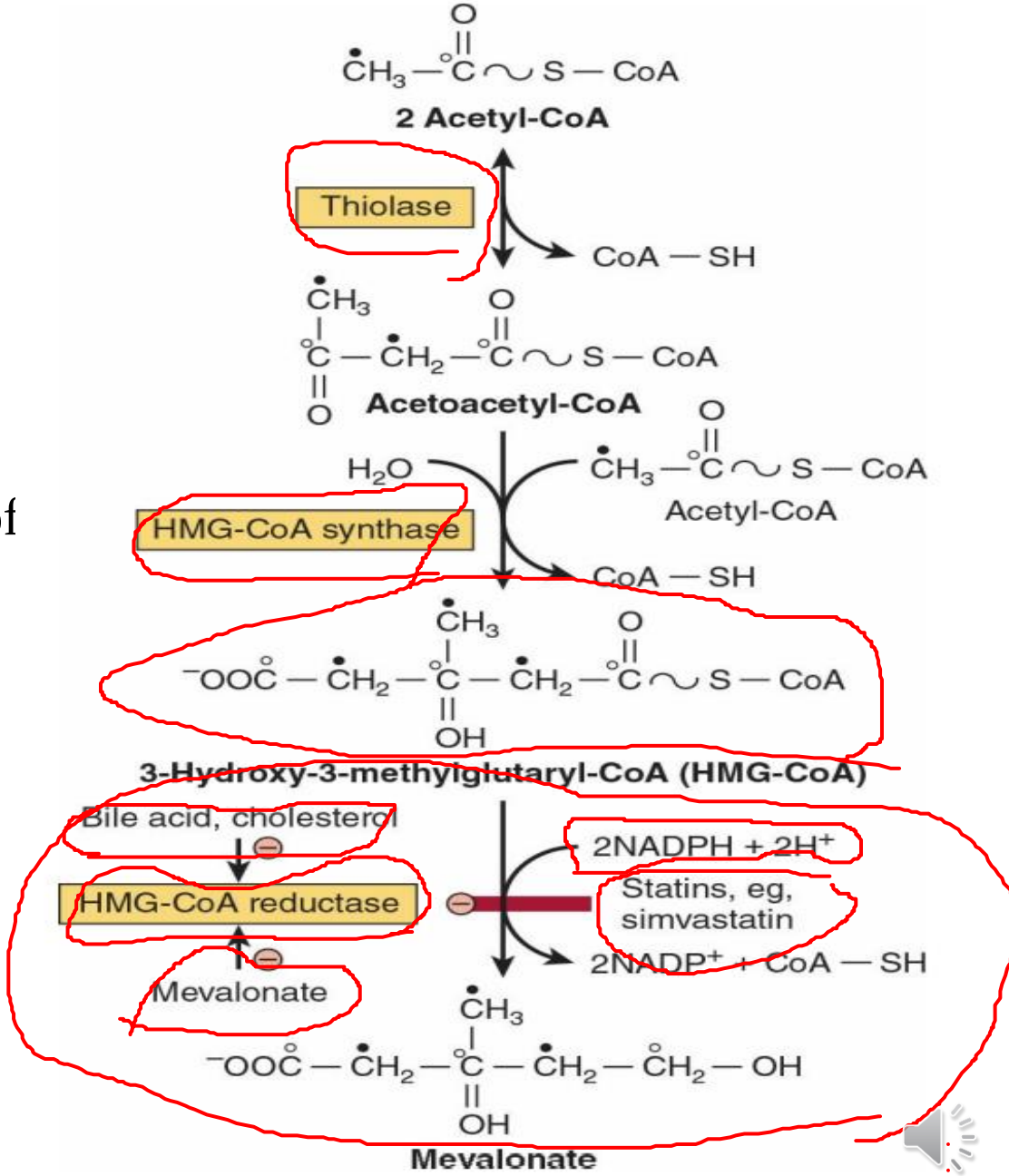
(1) synthesis of mevalonate from acetyl-CoA

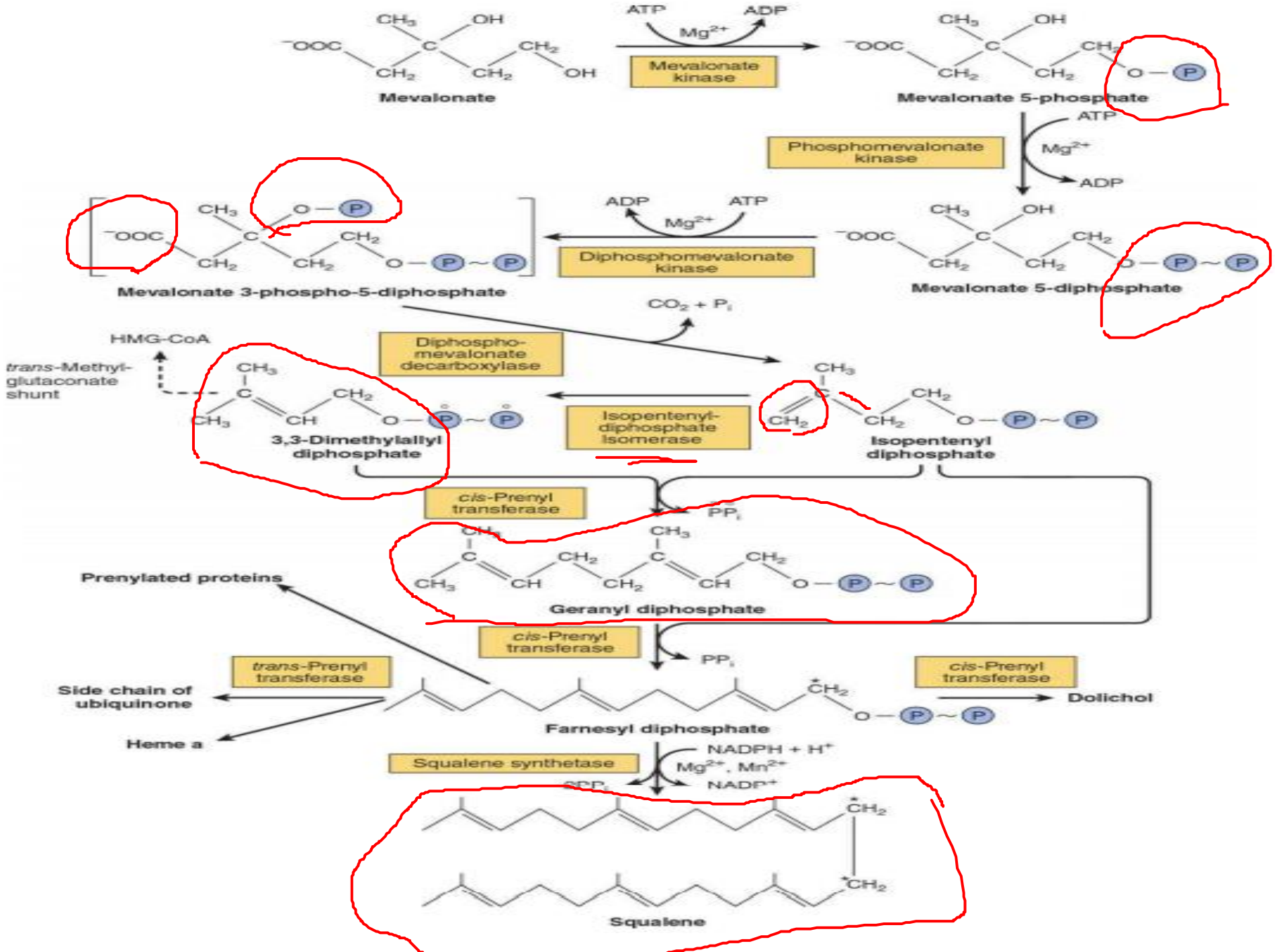
(2) formation of isoprenoid units from mevalonate by loss of CO_2

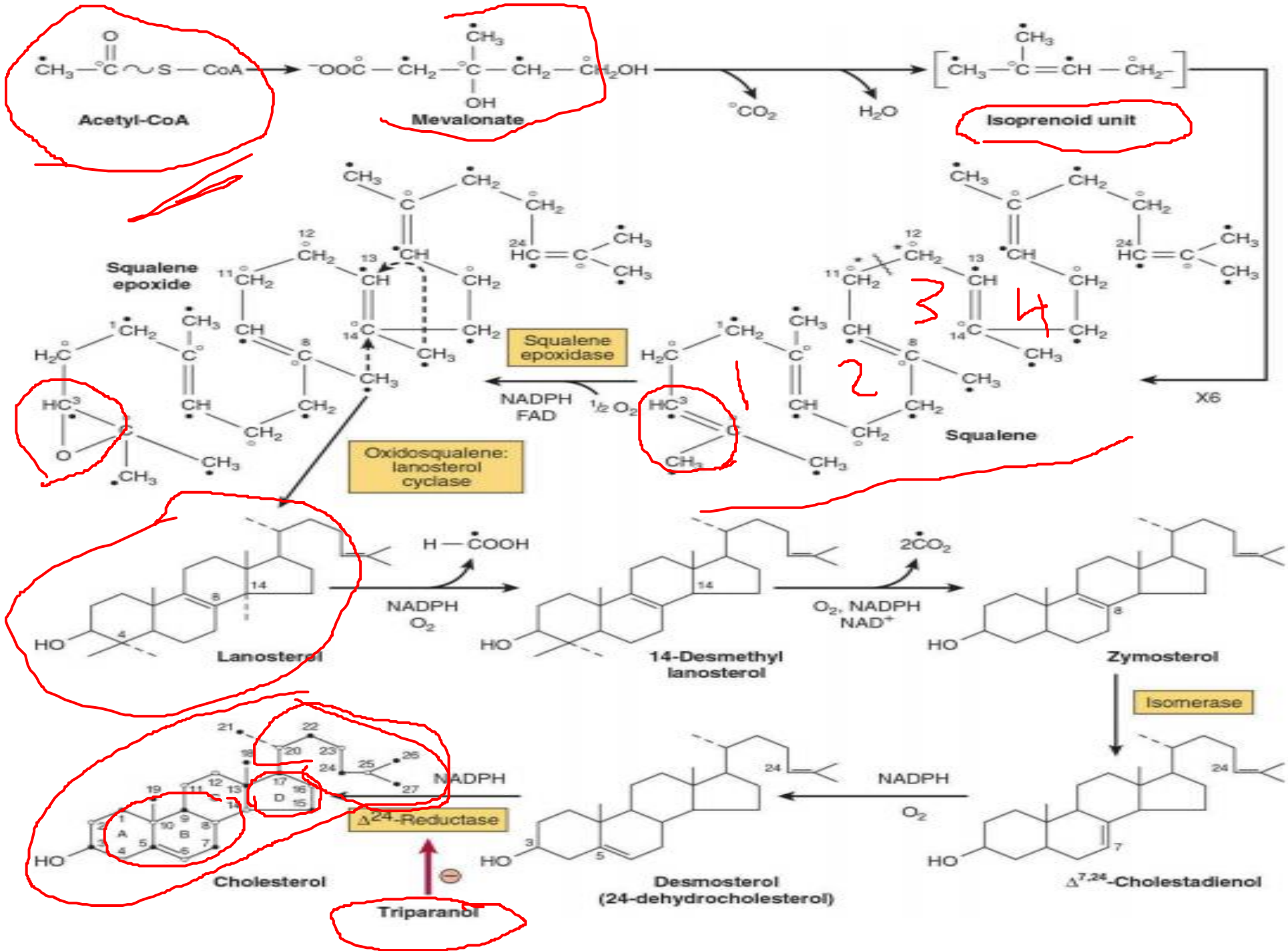
(3) condensation of six isoprenoid units form squalene

(4) cyclization of squalene gives increase to the parent steroid, lanosterol

(5) formation of cholesterol from lanosterol



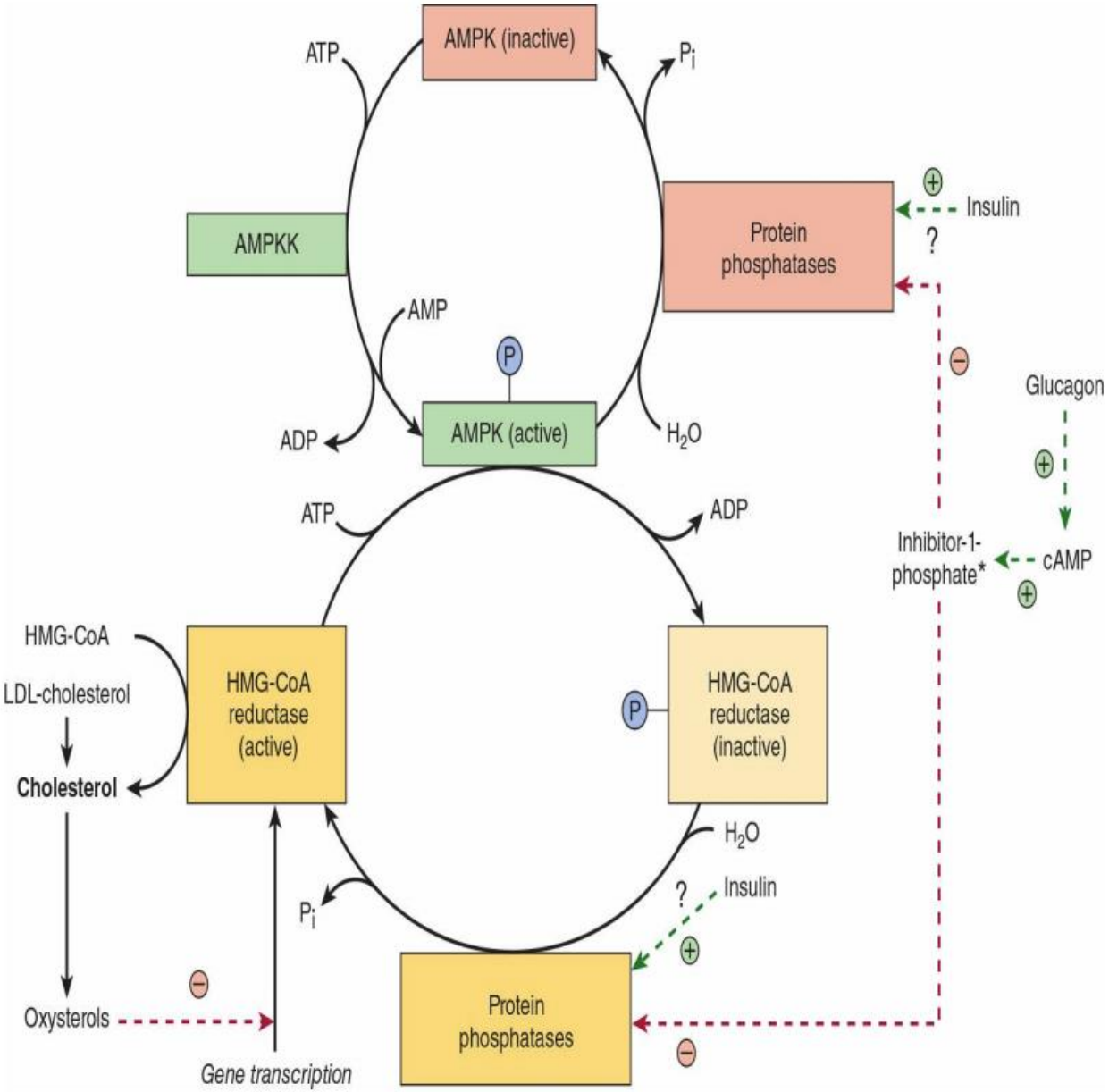

Mevalonate
ATP
ADP
Mg2+
Mevalonate kinase
Mevalonate 5-phosphate
Phosphomevalonate kinase
ATP
Mg2+
ADP
Mevalonate 3-phospho-5-diphosphate
ADP
ATP
Mg2+
Diphosphomevalonate kinase
Mevalonate 5-diphosphate
CO2 + Pi
Diphospho-mevalonate decarboxylase
HMG-CoA
trans-Methyl-glutaconate shunt
3,3-Dimethylallyl diphosphate
Isopentenyl-diphosphate isomerase
Isopentenyl diphosphate
cis-Prenyl transferase
PPi
Geranyl diphosphate
Prenylated proteins
cis-Prenyl transferase
PPi
trans-Prenyl transferase
cis-Prenyl transferase
Side chain of ubiquinone
Dolichol
Farnesyl diphosphate
Heme a
Squalene synthetase
NADPH + H+
Mg2+, Mn2+
NADP+
Squalene


Acetyl-CoA
Mevalonate
$^{\circ}CO_2$
H_2O
Isoprenoid unit
X6
Squalene
Squalene epoxide
Squalene epoxidase
NADPH
FAD
$^1/_2\ O_2$
Oxidosqualene: lanosterol cyclase
Lanosterol
NADPH
O_2
14-Desmethyl lanosterol
$2CO_2$
O_2, NADPH
NAD^+
Zymosterol
Isomerase
$\Delta^{7,24}$-Cholestadienol
NADPH
O_2
Desmosterol
(24-dehydrocholesterol)
NADPH
Δ^{24}-Reductase
Cholesterol
Triparanol

CHOLESTEROL SYNTHESIS IS CONTROLLED BY REGULATION OF HMG-COA REDUCTASE

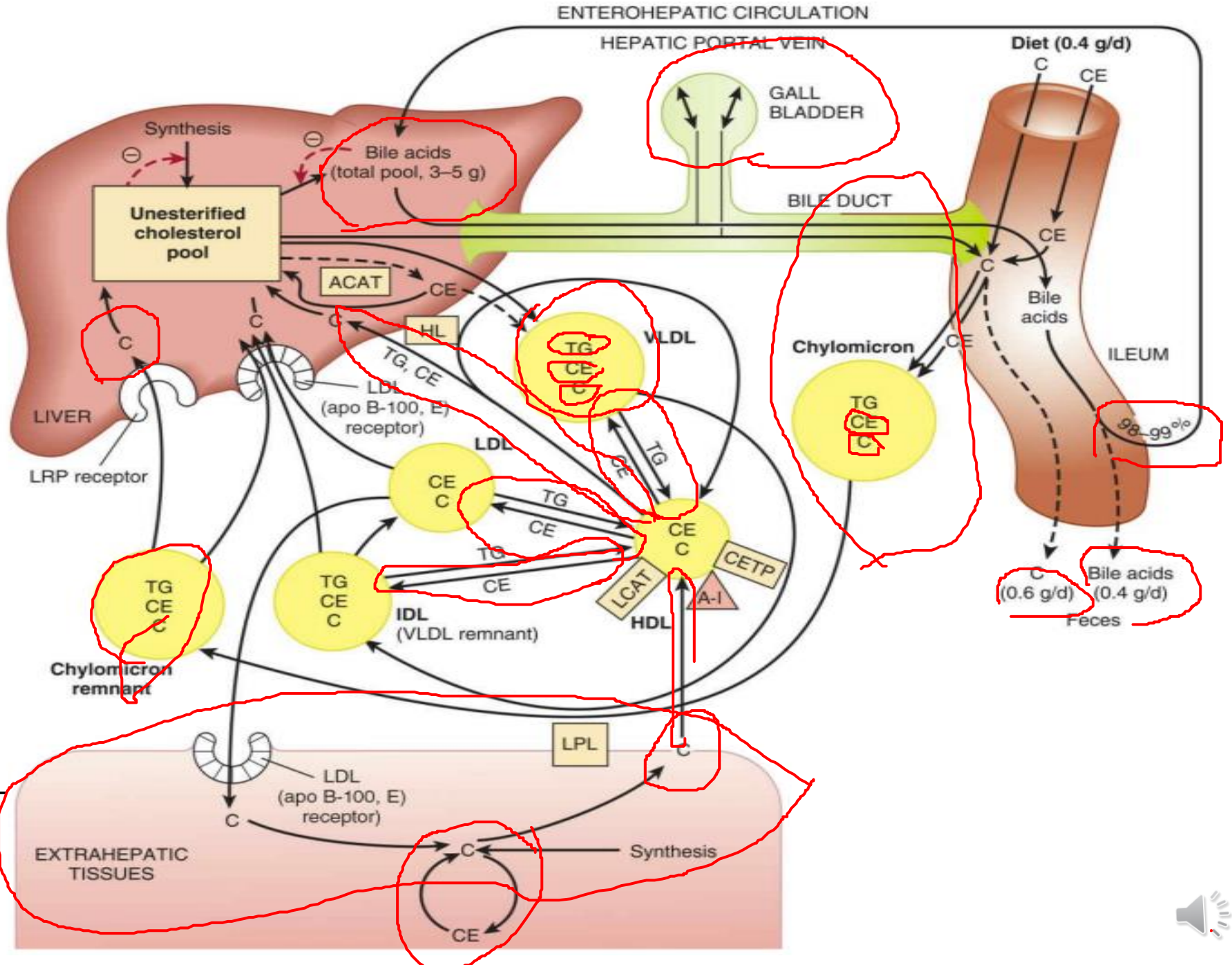
Cholesterol and metabolites repress transcription HMG-CoA reductase mRNA via inhibition of a sterol regulatory element-binding protein (SREBP) transcription factor.

Insulin has a dominant role compared with glucagon. (AMPK, AMP-activated protein kinase; AMPKK, AMP-activated protein kinase kinase.)

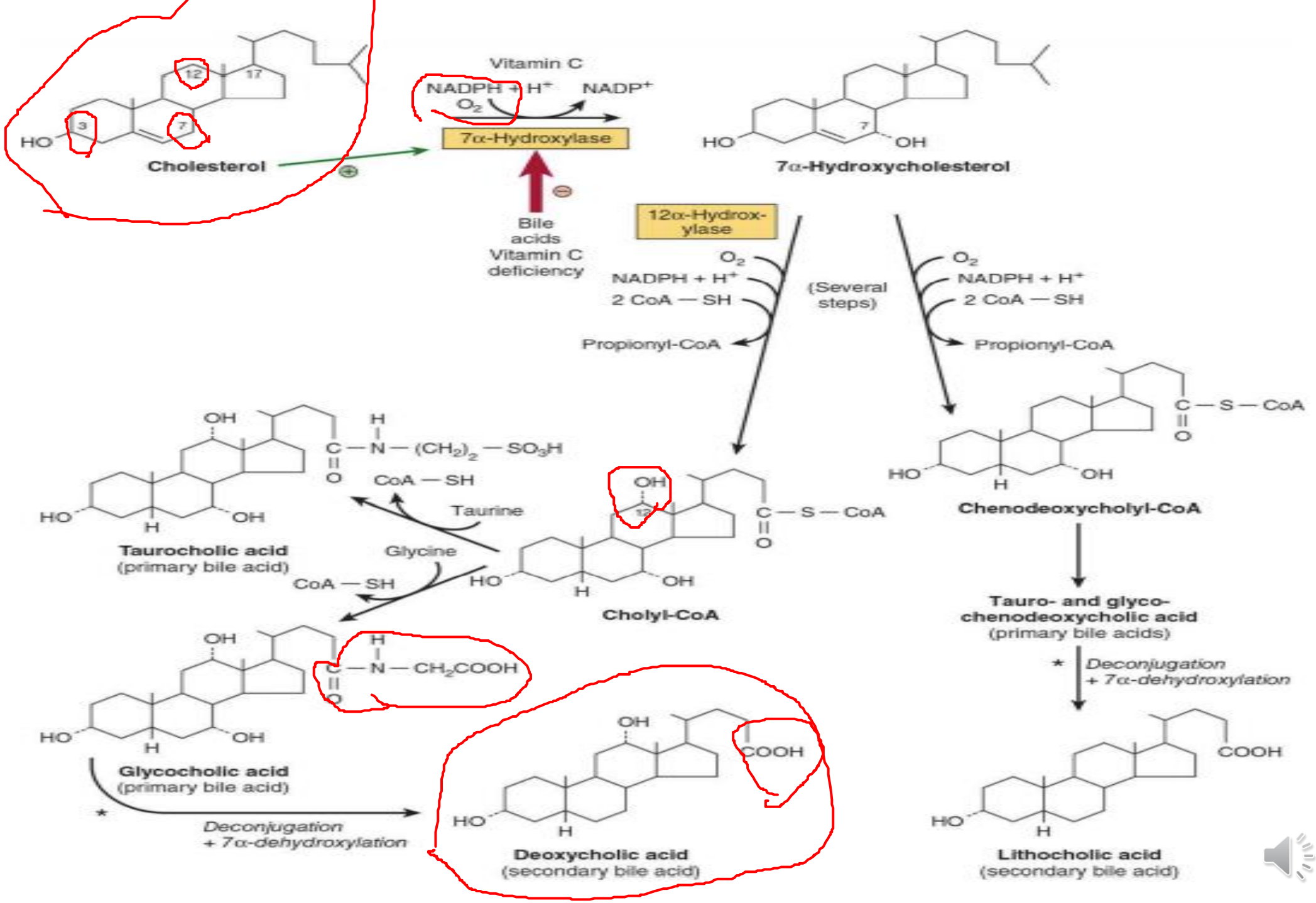


CHOLESTEROL IS TRANSPORTED BETWEEN TISSUES IN PLASMA LIPOPROTEINS

Transport of cholesterol between the tissues in humans. (ACAT, acyl-CoA:cholesterol acyltransferase; A-I, apolipoprotein A-I; C, unesterified cholesterol; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; HL, hepatic lipase; IDL, intermediate-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; LPL, lipoprotein lipase; LRP, LDL receptor–related protein-1; TG, triacylglycerol; VLDL, very-low-density lipoprotein.)



Bile Acids Are Formed From Cholesterol



THANK YOU

Catabolism of Proteins & Amino Acid Nitrogen

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper’s Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

By

Dr. Muthanna Owaid Hussein

Dietary Protein Is Enzymatically Degraded to Amino Acids

In humans, the degradation of eaten proteins to their component amino acids occurs in the gastrointestinal tract. Entry of dietary protein into the stomach stimulates the gastric mucosa to **secrete** the hormone gastrin, which in turn stimulates the secretion of hydrochloric acid by the parietal cells and pepsinogen by the chief cells of the gastric glands . The acidic gastric juice (pH 1.0 to 2.5) is both an antiseptic, killing most bacteria and other foreign cells, and a denaturing agent, unfolding globular proteins and rendering their internal peptide bonds more accessible to enzymatic hydrolysis. Pepsinogen (Mr 40,554), an inactive precursor, or zymogen , is converted to active pepsin (Mr 34,614) by an autocatalytic cleavage (a cleavage mediated by the pepsinogen itself) that occurs only at low pH. In the stomach, pepsin hydrolyzes ingested proteins at peptide bonds on the amino-terminal side of Leu and the aromatic amino acid residues Phe, Trp, and Tyr, cleaving long polypeptide chains into a mixture of smaller peptides

In animals, amino acids undergo oxidative degradation in three different metabolic circumstances:

1. During the normal synthesis and degradation of cellular proteins (protein turnover; some amino acids that are released from protein breakdown and are not needed for new protein synthesis undergo oxidative degradation.
2. When a diet is rich in protein and the ingested amino acids exceed the body's needs for protein synthesis, the surplus is catabolized; amino acids cannot be stored.
3. During starvation or in uncontrolled diabetes, when carbohydrates are either unavailable or not properly utilized, cellular proteins are used as fuel.

Proteins are converted to amino acids by digestive enzymes.

Many of the digestive proteases are produced and secreted as inactive zymogens. They are converted to their active forms by the **removal** of a peptide fragment in the lumen of the digestive tract.

The digestion of proteins begins in the stomach, where pepsin converts dietary proteins into smaller polypeptides.

In the lumen of the small intestine, proteolytic enzymes produced by the pancreas (trypsin, chymotrypsin, elastase, and the carboxypeptidases) cleave the polypeptides into oligopeptides and amino acids.

The digestive enzymes produced by the **intestinal epithelial cells** (aminopeptidases, dipeptidases, and tripeptidases) cleave the small peptides to amino acids.

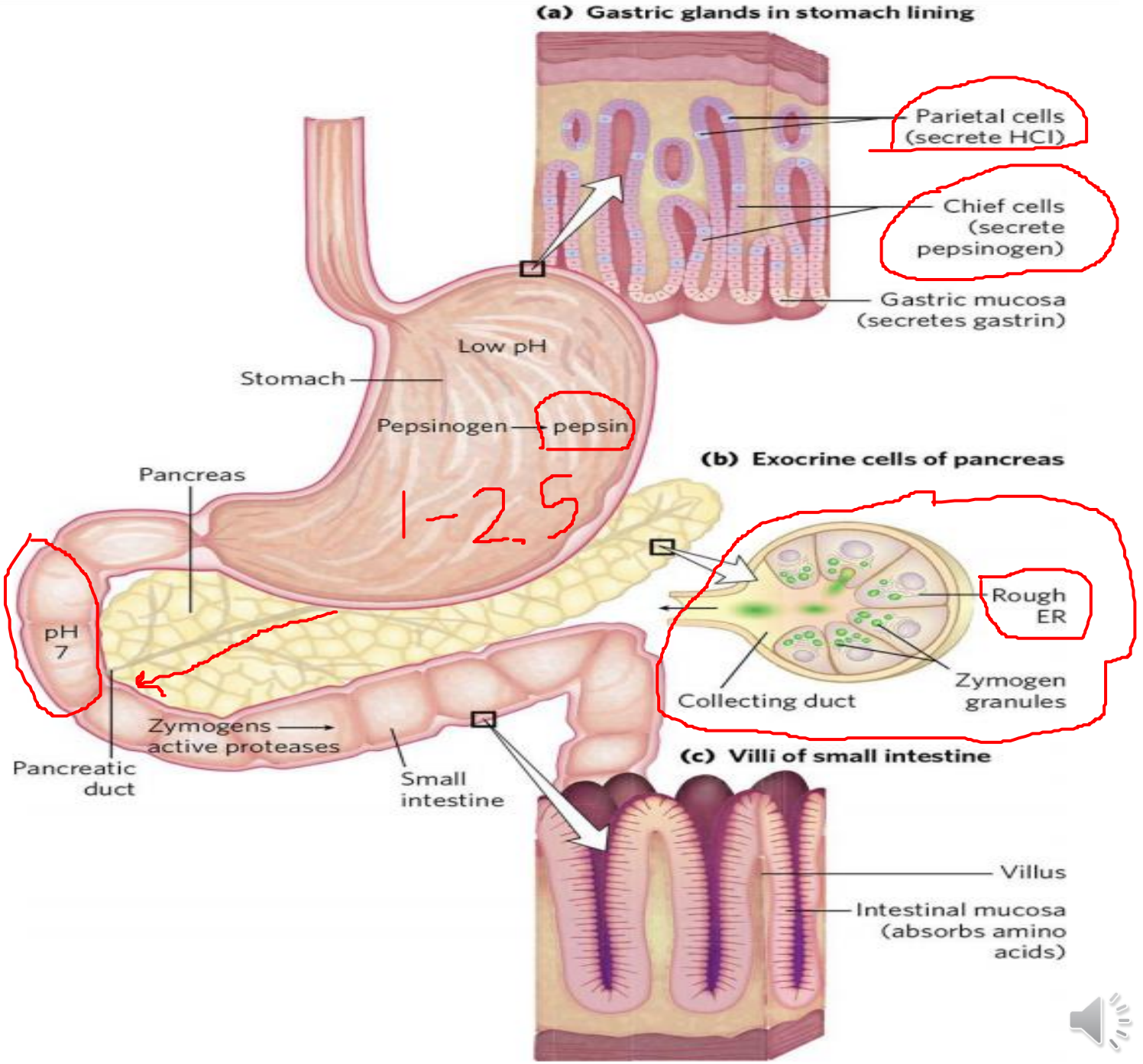
Amino acids, the final products of protein digestion, are absorbed through intestinal epithelial cells and enter the blood

Part of the human digestive (gastrointestinal) tract.

(a) The parietal cells and chief cells of the gastric glands secrete their products in response to the hormone gastrin. Pepsin begins the process of protein degradation in the stomach.

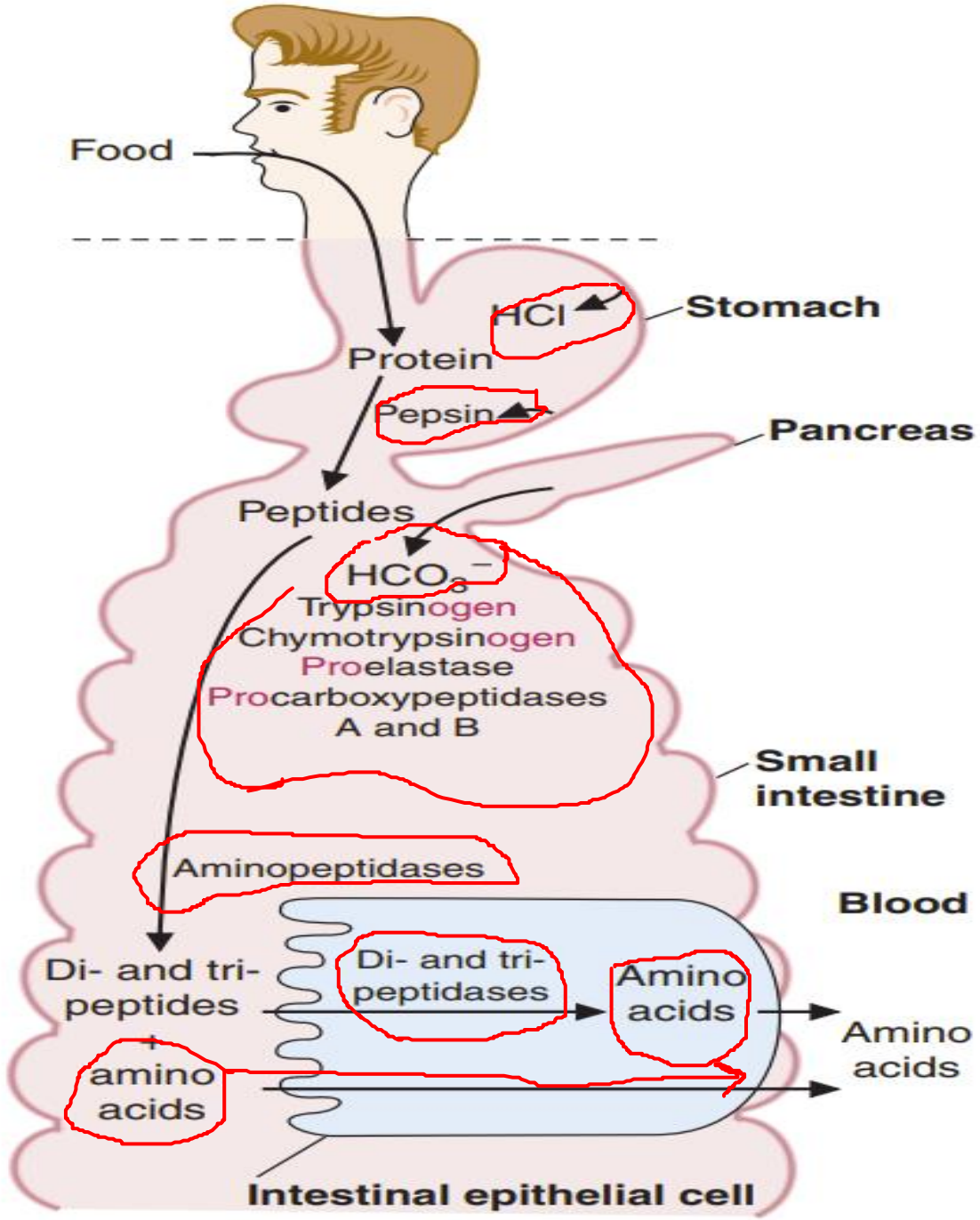
(b) The cytoplasm of exocrine cells of the pancreas is completely filled with rough endoplasmic reticulum, the site of synthesis of the zymogens of many digestive enzymes.

(c) In the small intestine, amino acids are absorbed through the epithelial cell layer (intestinal mucosa) of the villi and enter the capillaries.



The digestion of proteins

The proteolytic enzymes trypsin, chymotrypsin, elastase, and the carboxypeptidases are produced as zymogens (the [pro] and [ogen], in red, accompanying the enzyme name) that are activated by cleavage after they enter the gastrointestinal lumen.



Pepsin has a broad specificity but tends to **cleave** peptide bonds in which the amino group is contributed by the aromatic amino acids or by leucine.

Trypsin cleaves peptide bonds in which the carboxyl group is contributed by arginine or lysine

Chymotrypsin usually cleaves peptide bonds in which the carboxyl group is contributed by the aromatic amino acids or by leucine. Chymotrypsinogen, the inactive zymogen, is cleaved to form chymotrypsin by trypsin.

Elastase cleaves at the carboxyl end of amino acid residues with small, uncharged side chains such as alanine, glycine, or serine. Proelastase, the inactive zymogen, is cleaved to elastase by trypsin.

Carboxypeptidase A cleaves aromatic amino acids from the C-terminal end of peptides.

Carboxypeptidase B cleaves the basic amino acids, lysine and arginine, from the C-terminal end of peptides

Aminopeptidases are exopeptidases produced by the intestinal cells that **cleave** one amino acid at a time from the N-terminal end of peptides.

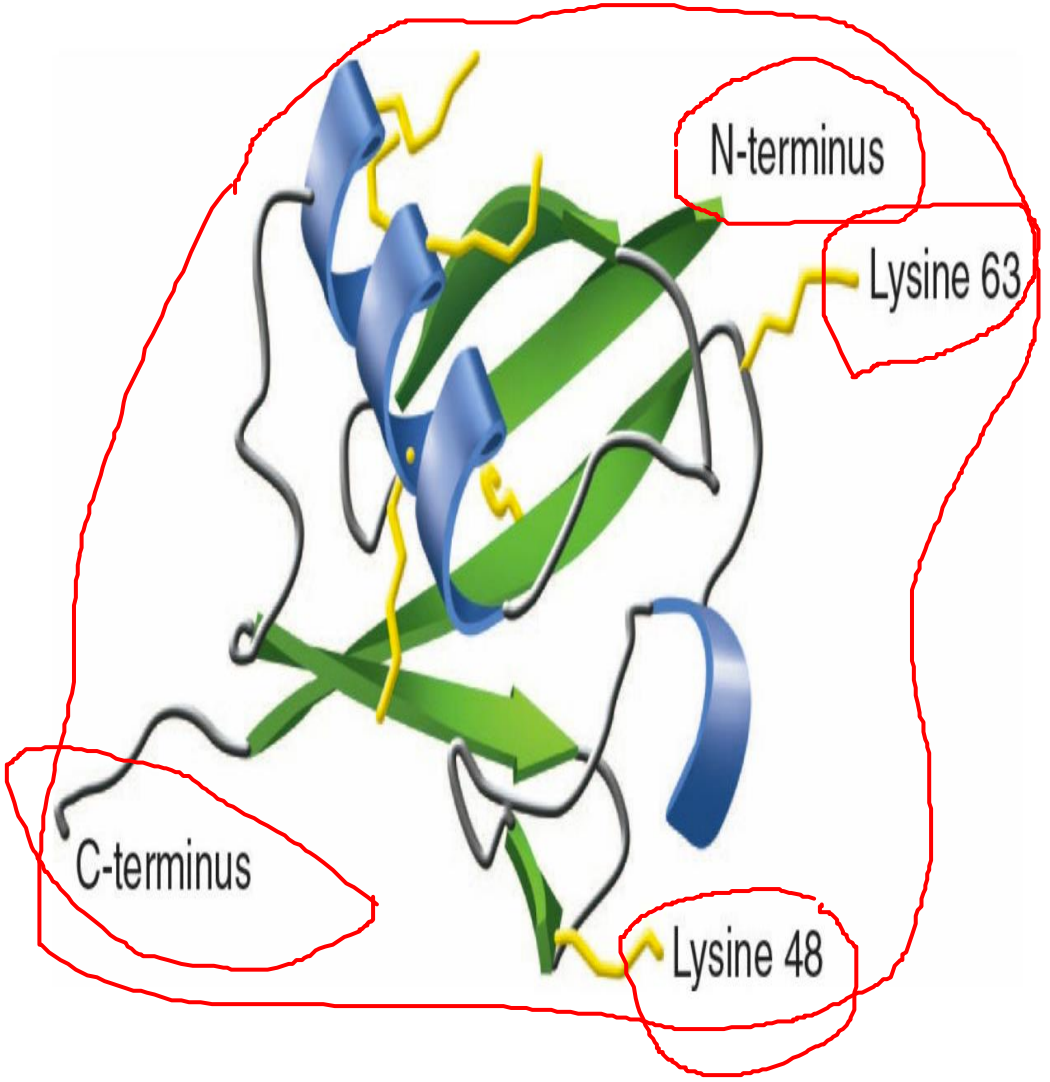
Dipeptidases and tripeptidases associated with the intestinal cells produce amino acids from dipeptides and tripeptides.

ATP & Ubiquitin-Dependent Degradation

Degradation of regulatory proteins with short half-lives and of abnormal or misfolded proteins occurs in the cytosol and requires ATP and ubiquitin. Named based on its presence in all eukaryotic cells, ubiquitin is a small.

Reactions involved in the attachment of ubiquitin (Ub) to proteins.

Three enzymes are involved. E1 is an activating enzyme, E2 a transferase, and E3 a ligase.



Ubiquitin-proteasome proteolytic pathway:

Proteins selected for degradation by this mechanism

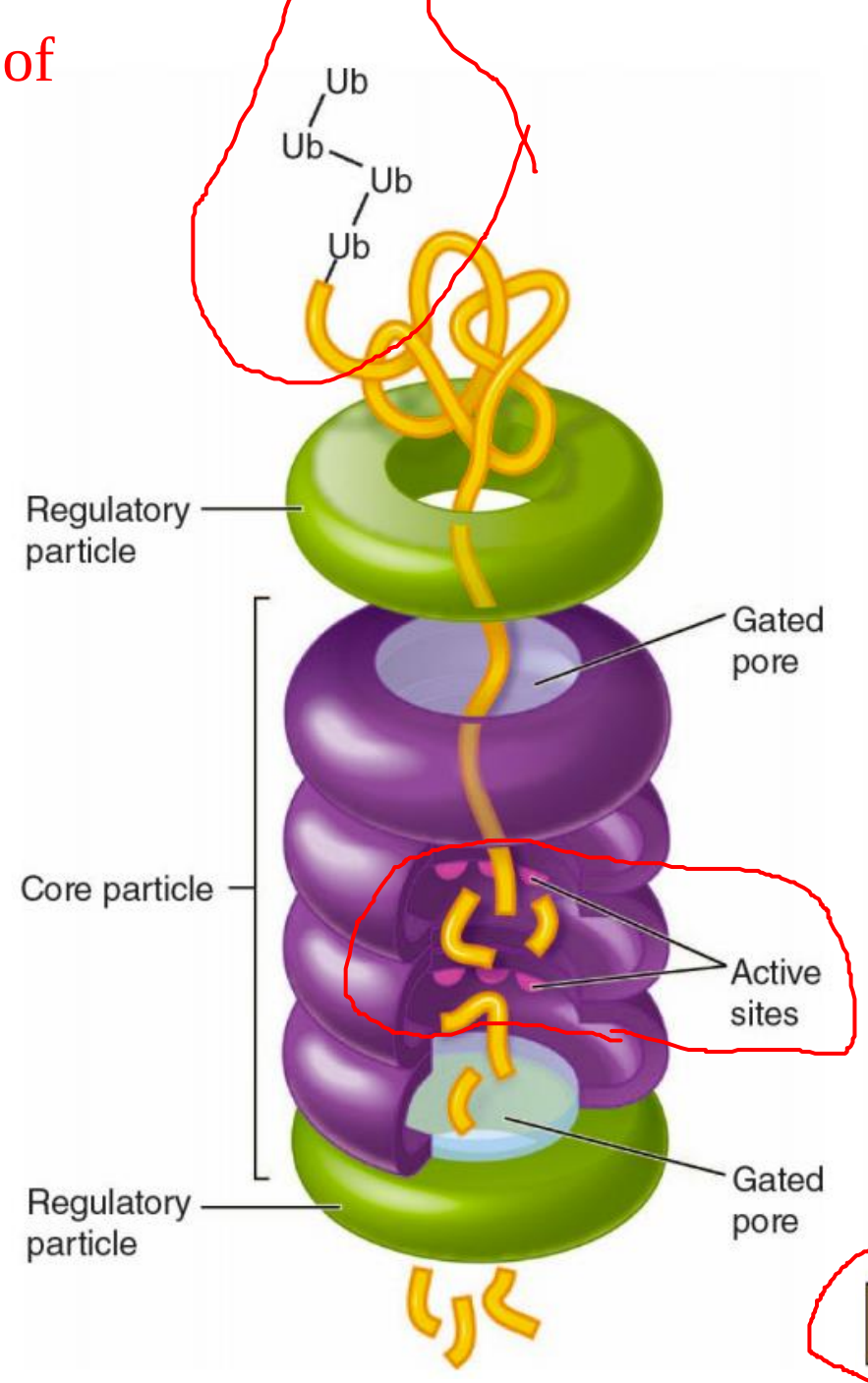
1. Ubiquitination: linkage of ubiquitin with target substrate (protein) take place in three step enzyme catalyzed process: The target protein first covalently attached to ubiquitin, the linkage of the α-carboxyl glycine of ubiquitin to a lysine$\in -amino\ group$ on protein.

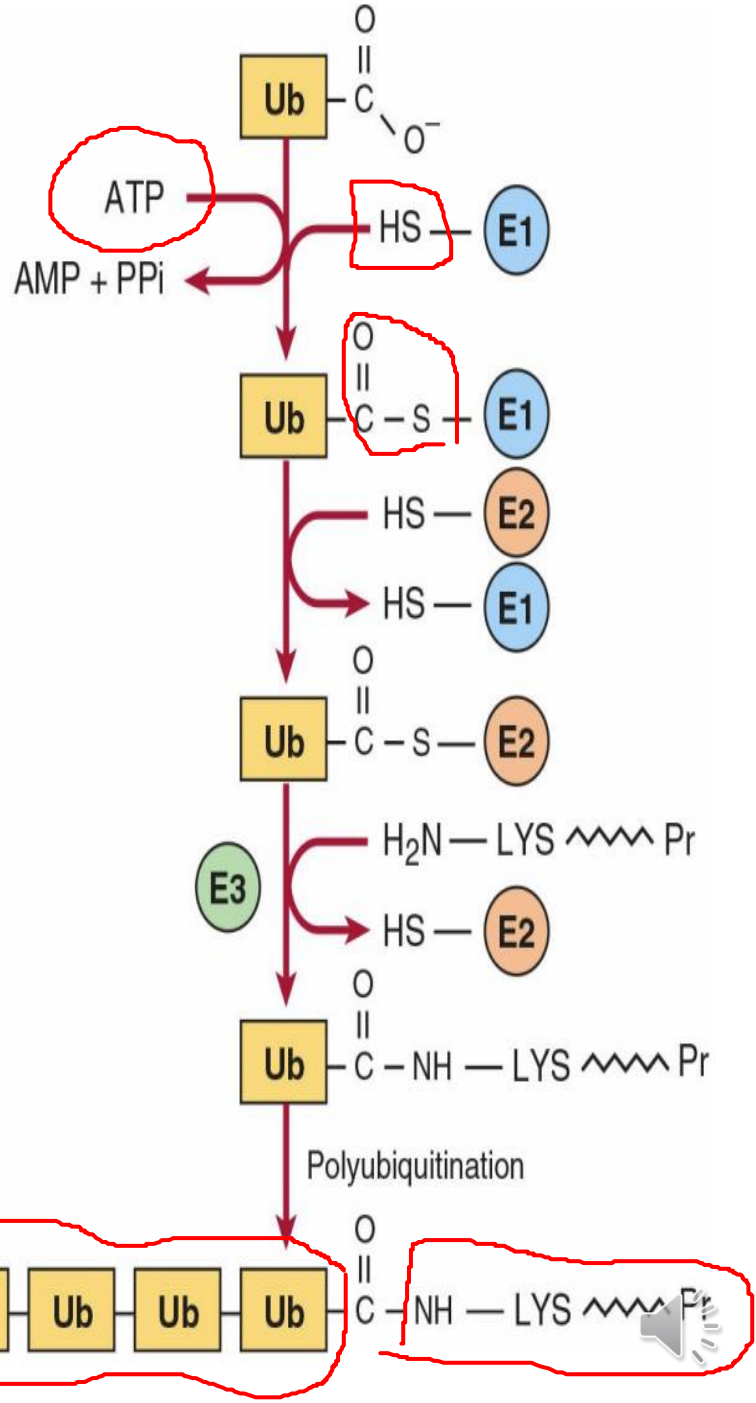
2. The following of addition of ubiquitin moieties generates a polyubiquitin chain.

3. Protein that labelled with ubiquitin are then recognized by a large barrel shaped macromolecular proteolytic complex called a proteasome which cuts the target protein into fragments that are then further degraded to amino acids, which enter the amino acid pool.

Representation of the structure of a proteasome.

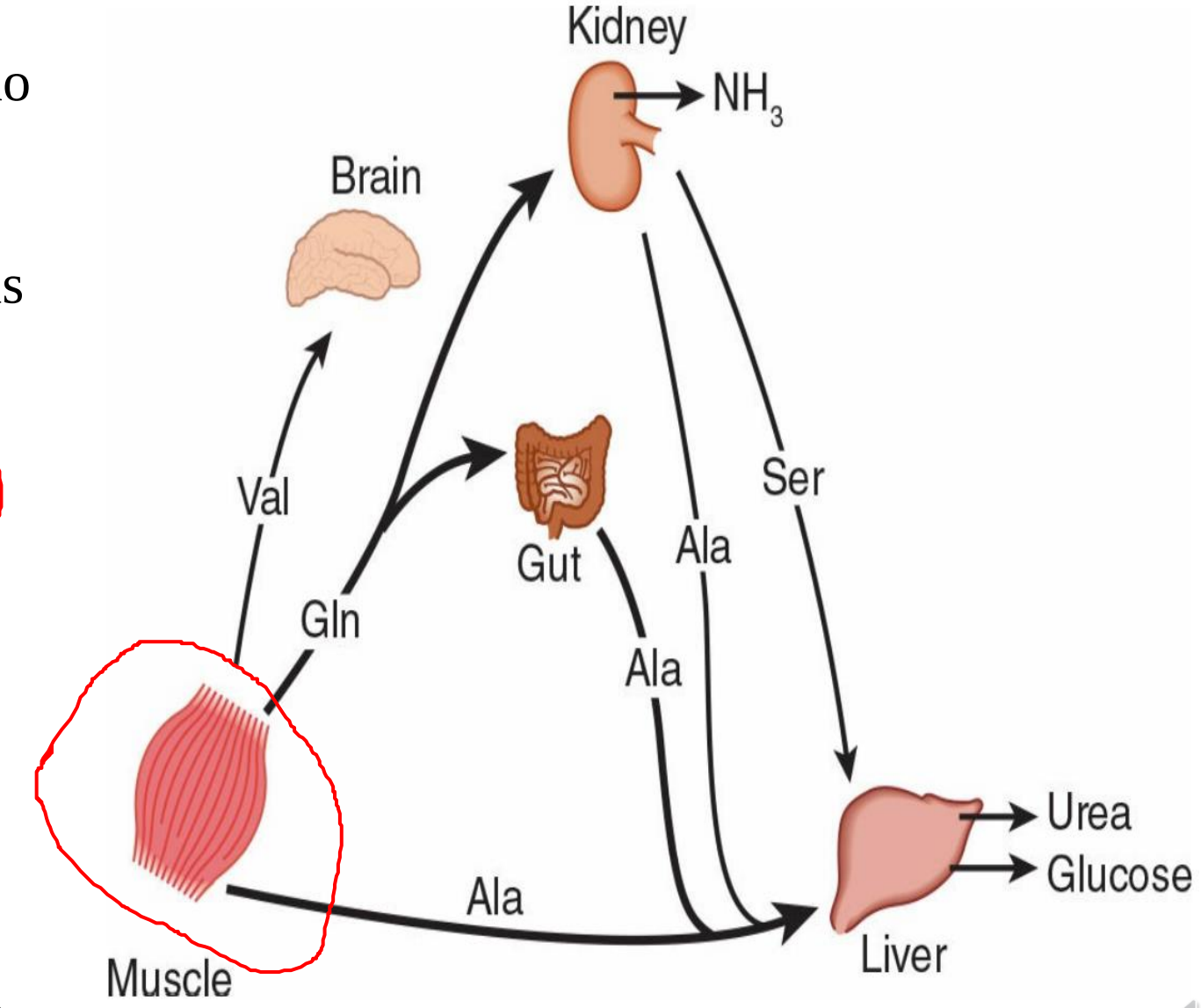
The upper ring is gated to permit only polyubiquitinated proteins to enter the proteosome, where immobilized internal proteases degrade them to peptides.



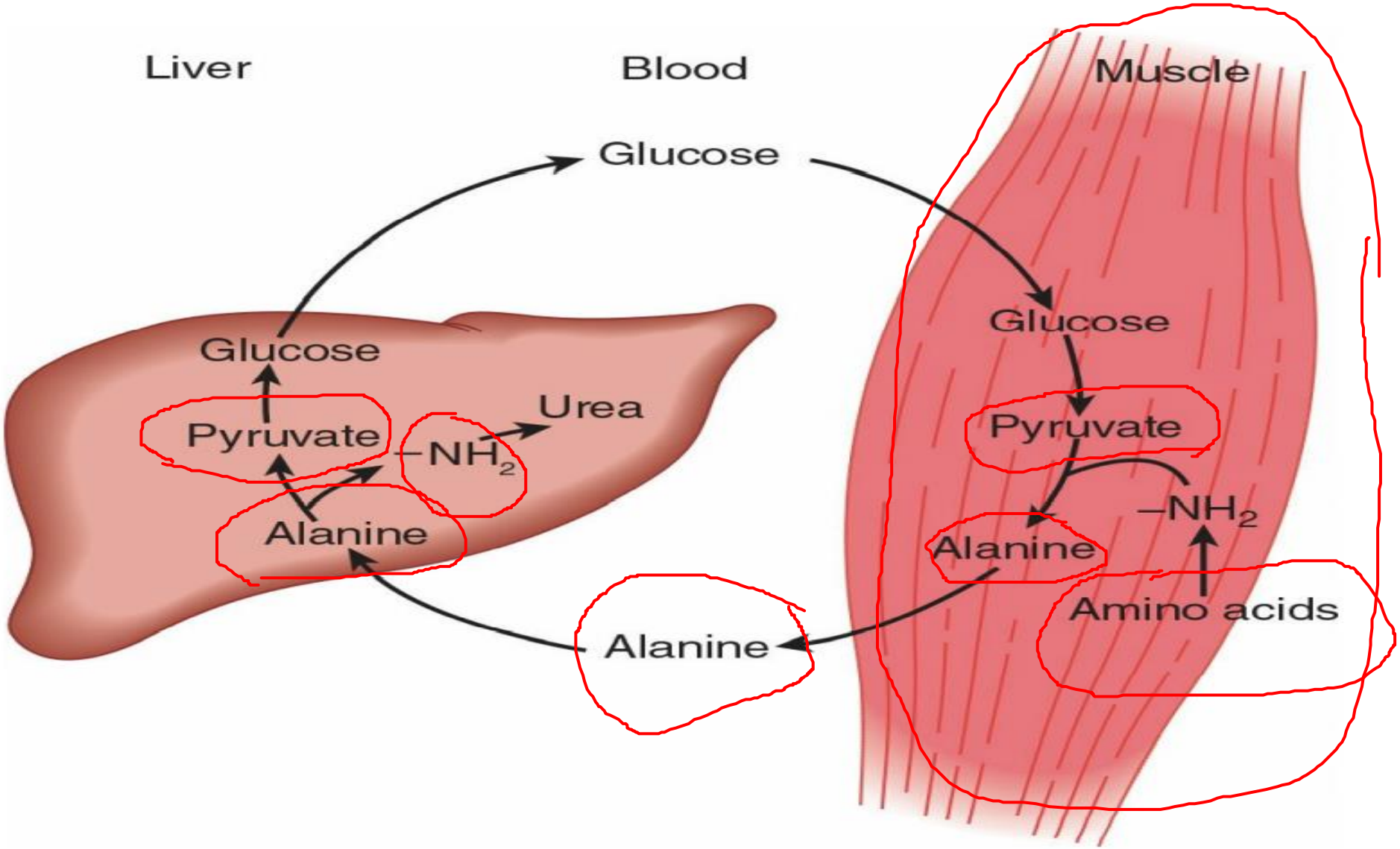


NTERORGAN EXCHANGE MAINTAINS CIRCULATING LEVELS OF AMINO ACIDS

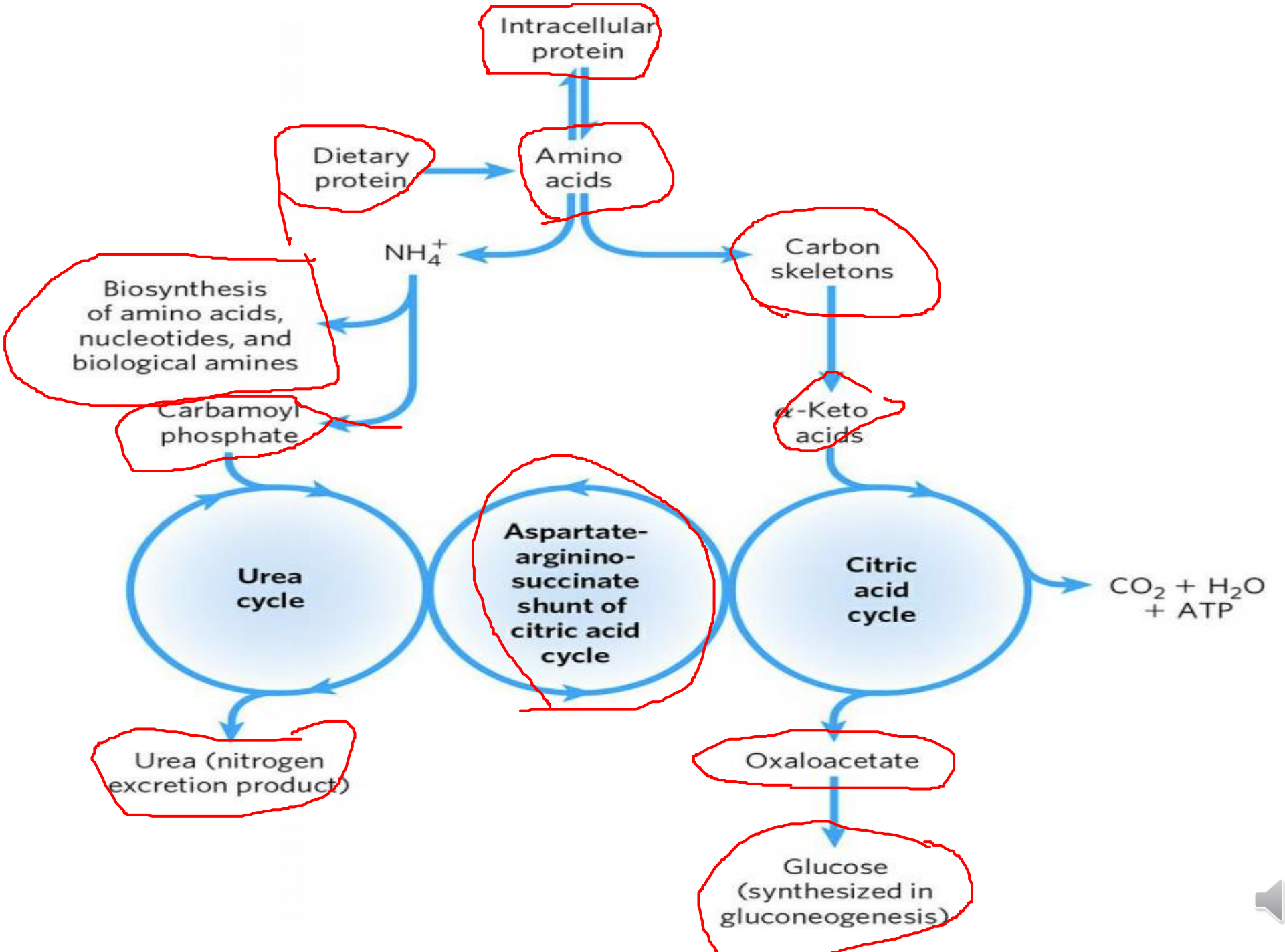
The maintenance of steady-state concentrations of circulating plasma amino acids between meals depends on the net balance between release from endogenous protein stores and utilization by various tissues. **Muscle** generates over half of the total body pool of free amino acids, and liver is the site of the urea cycle enzymes necessary for disposal of excess nitrogen. Muscle and liver thus play major roles in maintaining circulating amino acid levels.



Alanine is a key gluconeogenic amino acid. The rate of hepatic gluconeogenesis from alanine is far higher than from all other amino acids.



Overview of amino acid catabolism in mammals.



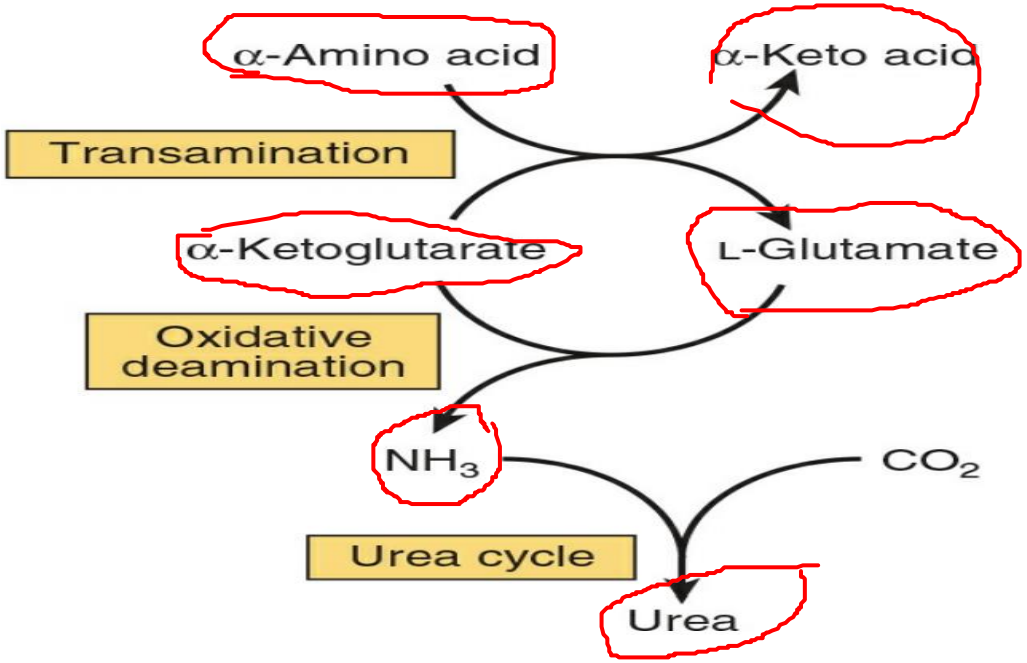
ANIMALS CONVERT α-AMINO NITROGEN TO VARIOUS END PRODUCTS

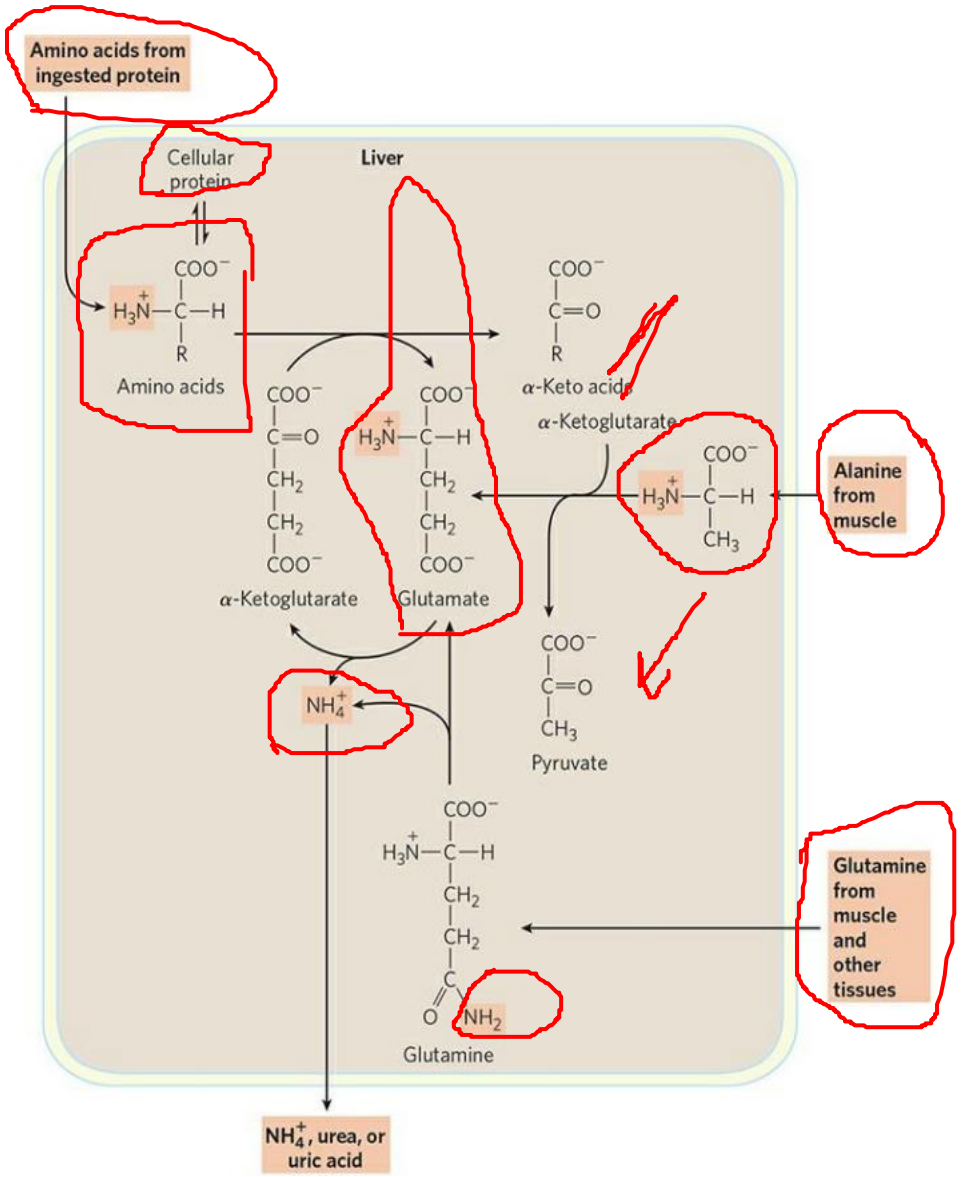
Depending on their ecological role and physiology, different animals excrete excess nitrogen as ammonia, uric acid, or urea.

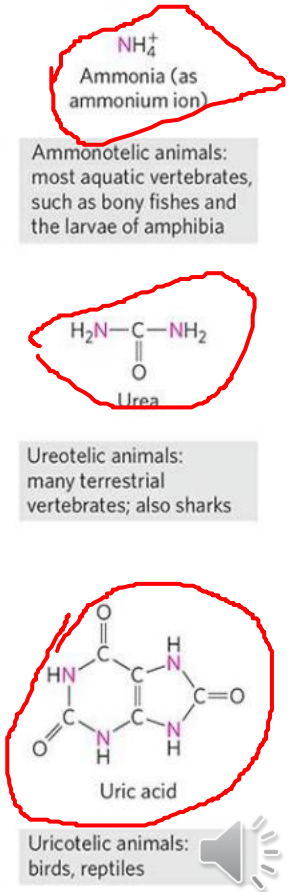
BIOSYNTHESIS OF UREA

Urea biosynthesis occurs in four stages

(1) Transamination
(2) oxidative deamination of glutamate
(3) ammonia transport
(4) reactions of the urea cycle

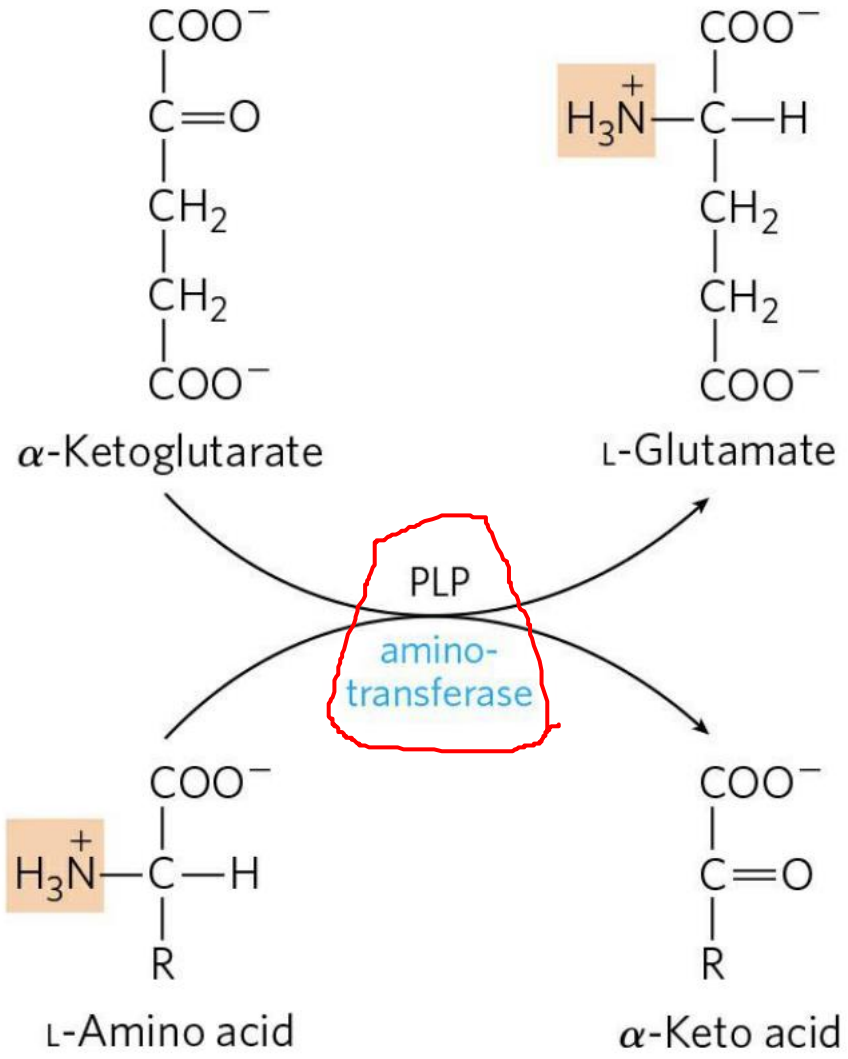


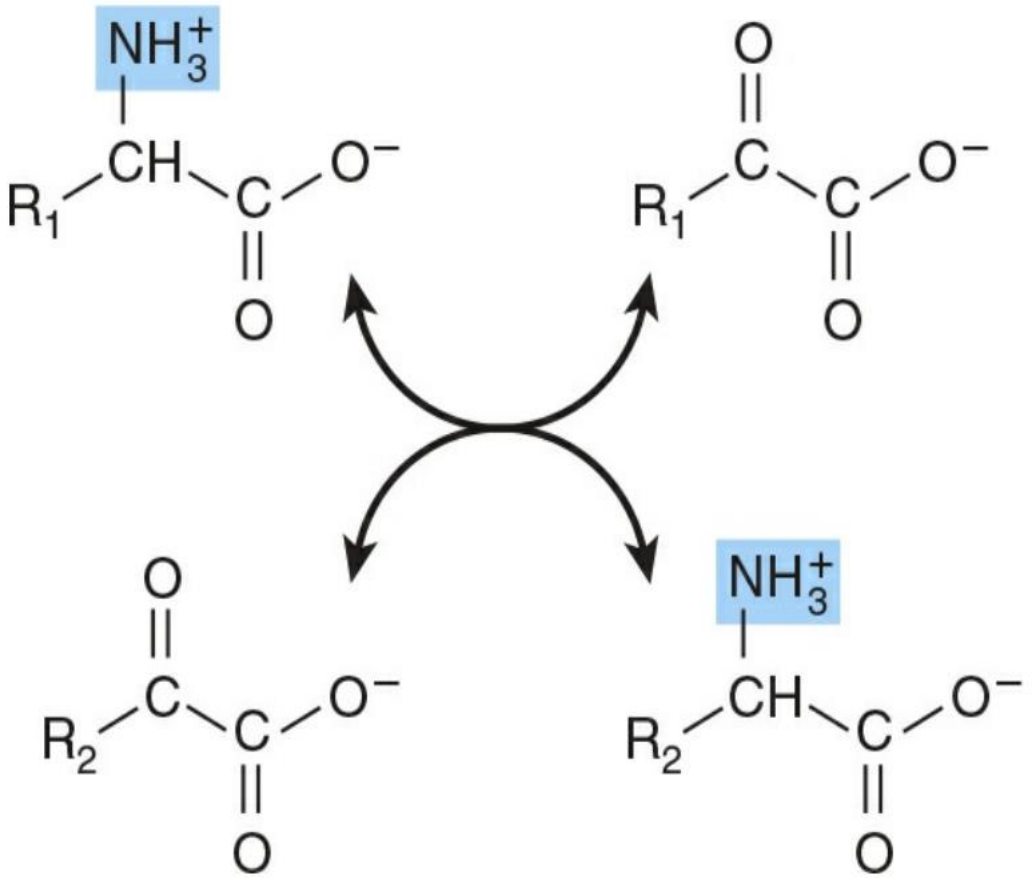




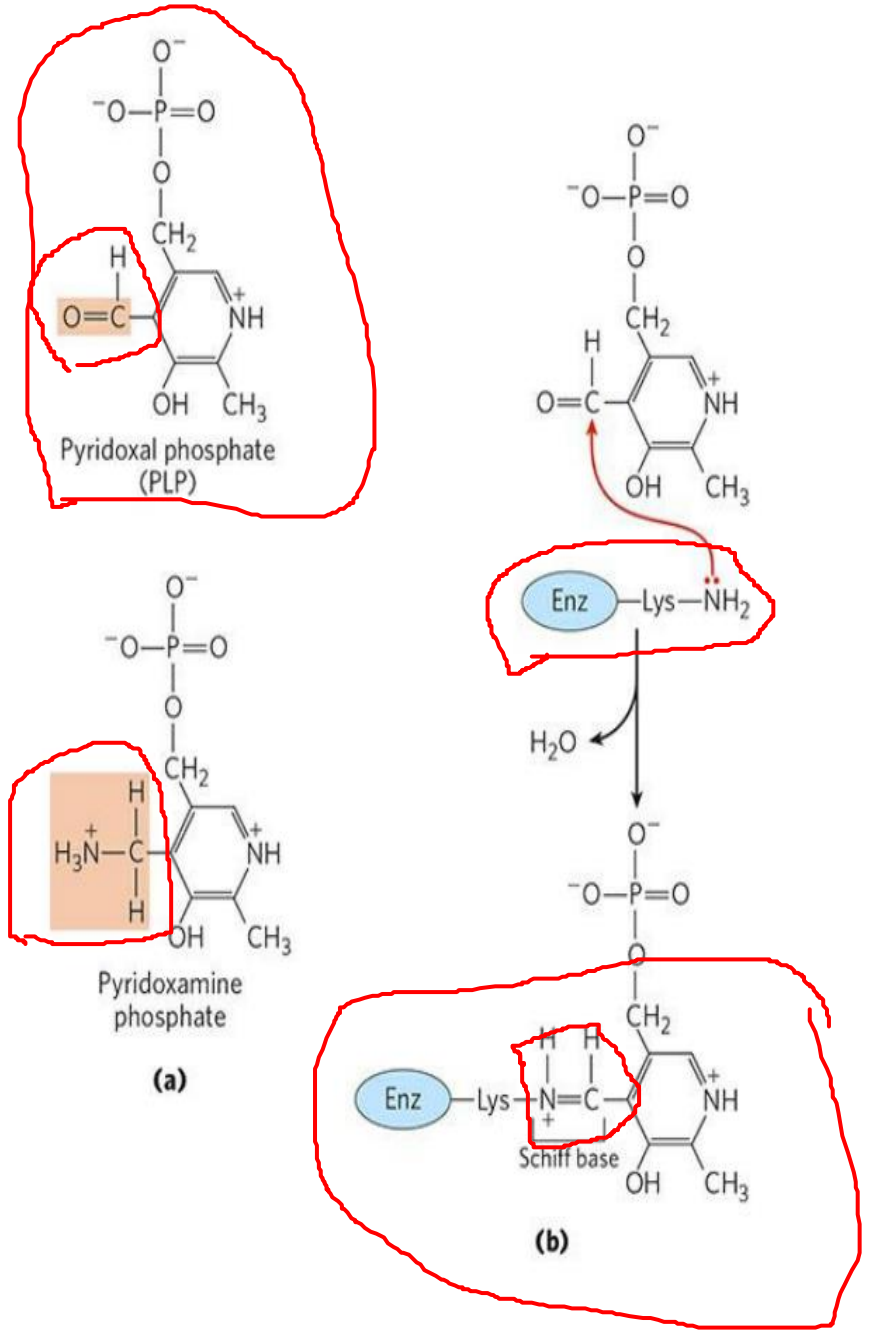
Transamination Transfers α-Amino Nitrogen to α-Ketoglutarate, Forming Glutamate

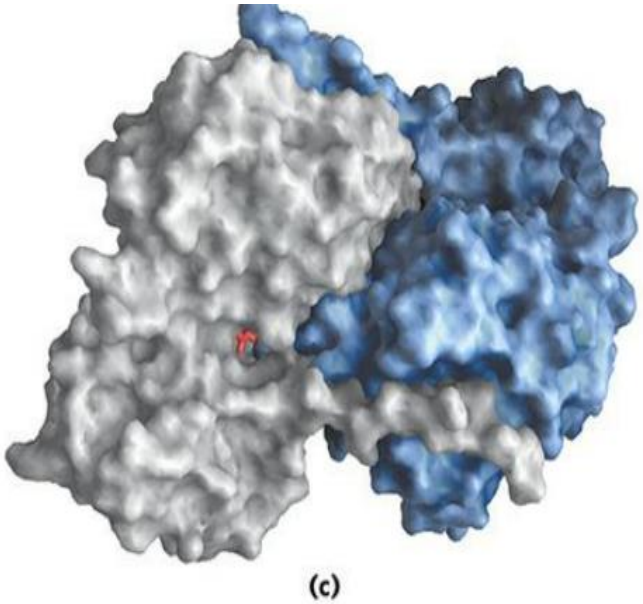
Transamination reactions interconvert pairs of α-amino acids and α-keto acids

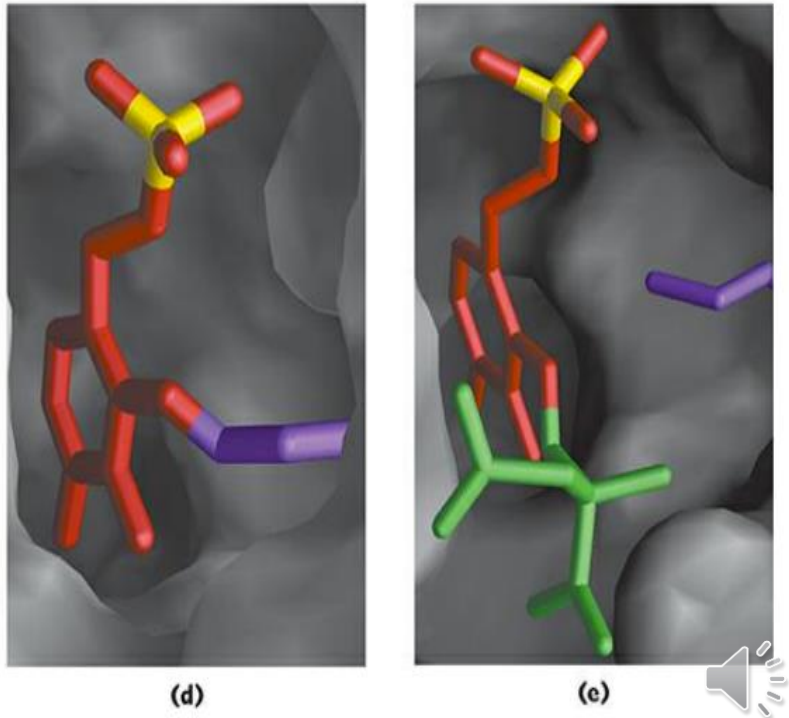




Pyridoxal phosphate, the prosthetic group of aminotransferases. (a) Pyridoxal phosphate (PLP) and its aminated form, pyridoxamine phosphate, are the tightly bound coenzymes of aminotransferases. The functional groups are shaded. (b) Pyridoxal phosphate is bound to the enzyme through noncovalent interactions and a Schiff-base (aldimine) linkage to a Lys residue at the active site.







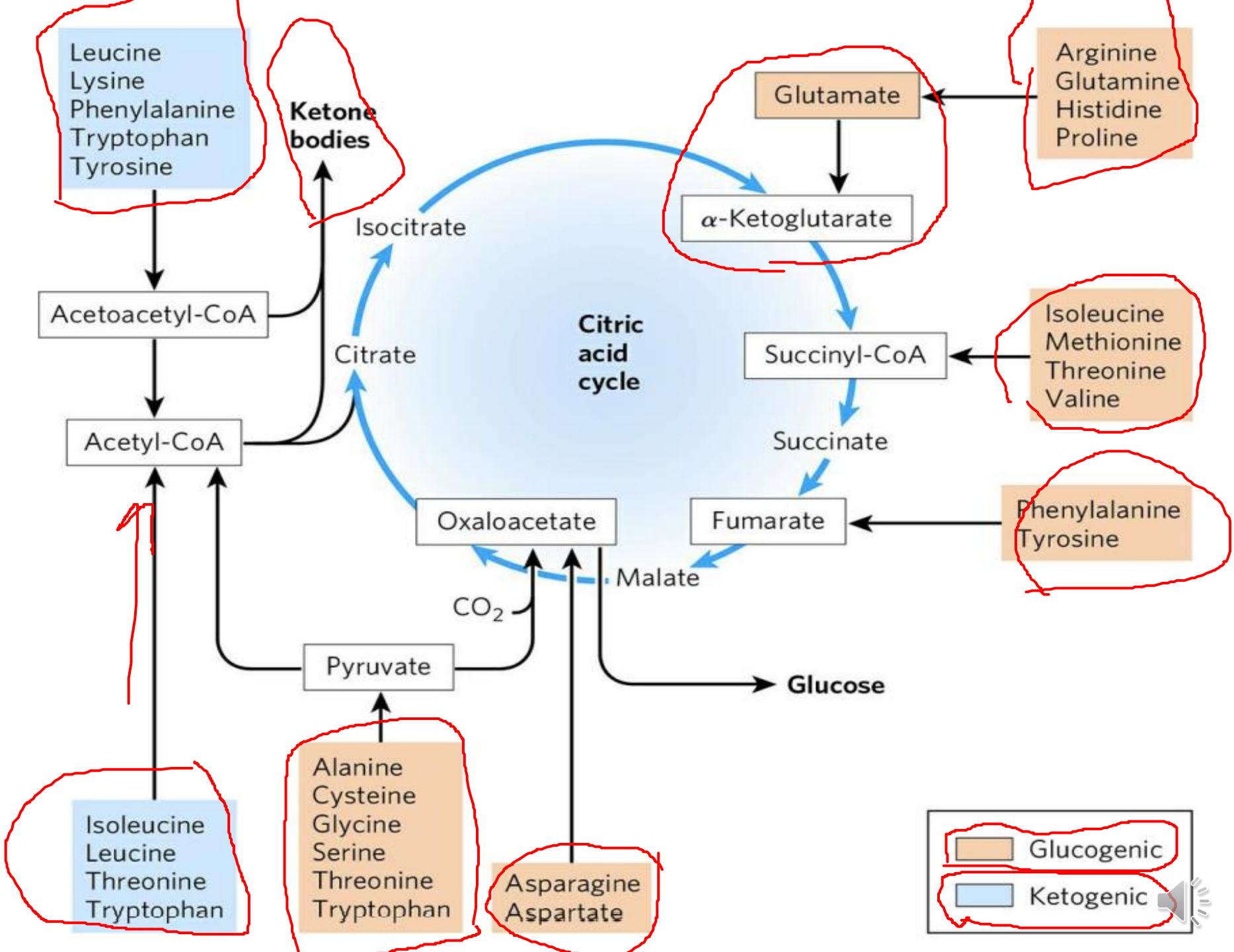
Mechanism action of aminotransferases:

- All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme.

* Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme.

Pathways of Amino Acid Degradation

Summary of amino acid catabolism. Amino acids are grouped according to their major degradative end product.



Reactions of the urea cycle

NH4 + and aspartate provide the nitrogen that is used to produce urea, and CO_2 provides the carbon. Ornithine serves as a carrier that is regenerated by the cycle.

Carbamoyl phosphate is synthesized in the first reaction from NH4+, CO2, and two ATP. Inorganic phosphate and two ADP are also produced. Enzyme: carbamoyl phosphate synthetase I, which is located in mitochondria and is activated by N-acetylglutamate.

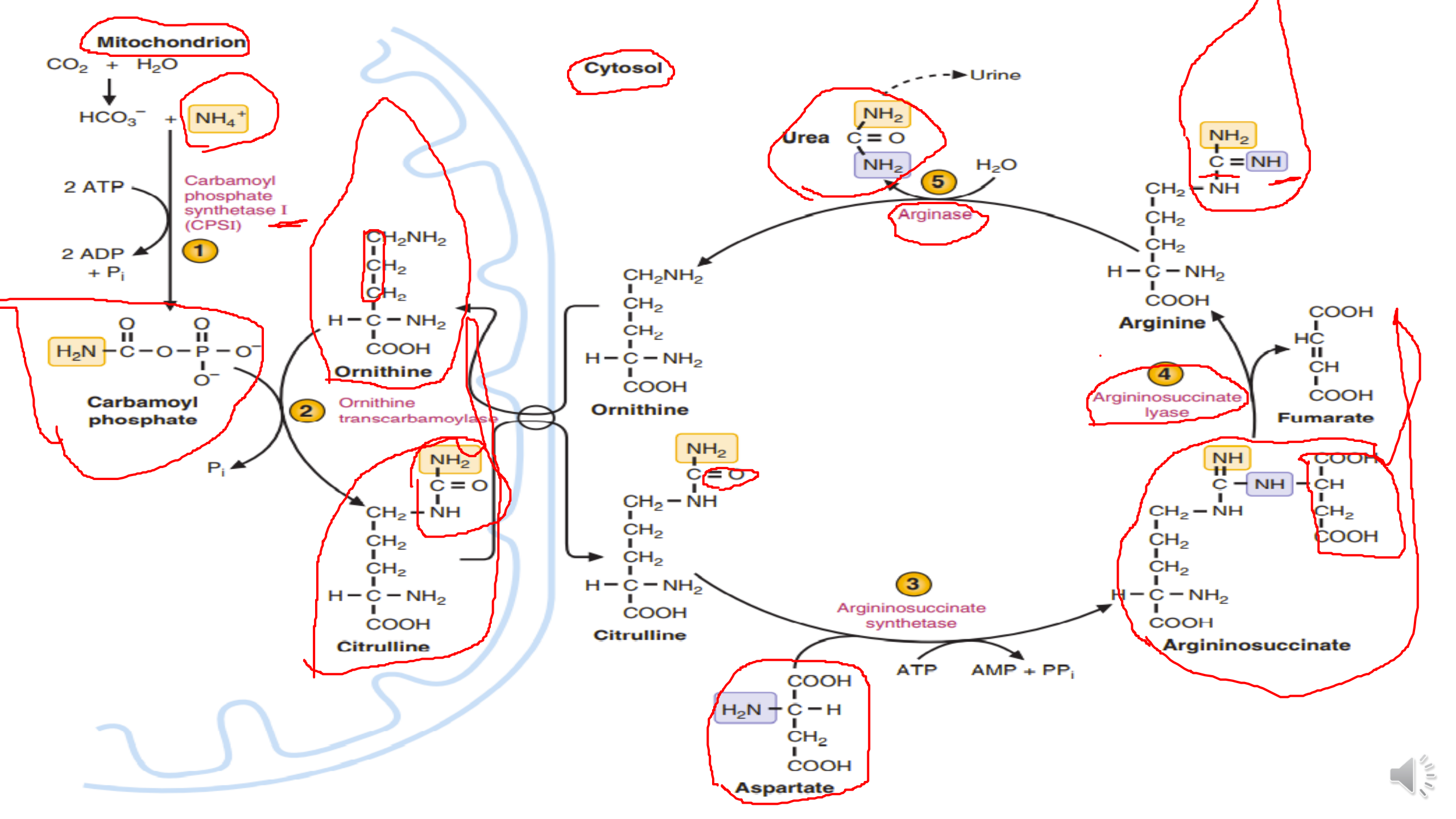
Ornithine reacts with carbamoyl phosphate to form citrulline. Inorganic phosphate is released. Enzyme: ornithine transcarbamoylase, which is found in mitochondria. The product, citrulline, is transported to the cytosol in exchange for cytoplasmic ornithine.

Citrulline combines with aspartate to form argininosuccinate in a reaction that is driven by the hydrolysis of ATP to AMP and inorganic pyrophosphate. Enzyme: argininosuccinate synthetase

Argininosuccinate is cleaved to form arginine and fumarate. Enzyme: argininosuccinate lyase. This reaction occurs in the cytosol.

Arginine is cleaved to form urea and regenerate ornithine. Enzyme: arginase, which is located primarily in the liver and is inhibited by ornithine.

Urea passes into the blood and is excreted by the kidneys. The urea excreted each day by a healthy adult (about 30 g) accounts for about 90% of the nitrogenous excretory products.


Mitochondrion
CO_2 + H_2O
HCO_3^- + NH_4^+
2 ATP
2 ADP + P_i
Carbamoyl phosphate synthetase I (CPSI)
1
Carbamoyl phosphate
2
Ornithine transcarbamoylase
P_i
Ornithine
Citrulline
Cytosol
Ornithine
Citrulline
3
Argininosuccinate synthetase
ATP
AMP + PP_i
Aspartate
Argininosuccinate
4
Argininosuccinate lyase
Fumarate
Arginine
5
Arginase
Urea
Urine
H_2O

THANK YOU