Ministry of Health of Ukraine
Zaporizhzhya State Medical University
Biochemistry & Laboratory Diagnostics Department

Biological chemistry

A manual for independent work at home and in class preparation for licensing examination "KROK 1" on module 1 "General regularities of metabolism. Metabolism of carbohydrates, lipids, amino acids and their regulation"

for students of International Faculty (the second year of study)
speciality: 7.120 10001 «General Medicine»

Zaporizhzhya
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УДК 577.1(072)=111

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This manual is recommended for II year students of International Faculty of specialty 7.12010001 "General medicine" studying biological chemistry, as additional material to prepare for practical training module 1 and licensing exam “KROK 1: General medical training”.

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CONTENT

INTRODUCTION

The manual "Biological chemistry. A manual for independent work at home and in class preparation for licensing examination “KROK 1” on module 1 “General regularities of metabolism. Metabolism of carbohydrates, lipids, amino acids and their regulation” for students of International Faculty (the second year of study) speciality: 7.120 10001 «General Medicine»contains a summary of the theory, which facilitates finding the right answer test tasks.

Tests of this manual are similar in content and form to the test tasks, provided Testing Center of Ministry of Health of Ukraine. Each test task has only one either correct or more correct answer, that must be chosen among the available ones by a student. As a self-study students are invited to give rationale for the choice of the correct answer, identify key words for case described in a test task.

The authors hope that this special form of student work with test tasks, with detailed explanation described in these tasks mostly clinical situations allow foreign English-speaking students to prepare properly and pass licensing exam "KROK 1: General medical training".

CLASSIFICATION, PHYSICOCHEMICAL PROPERTIES AND FUNCTIONS OF SIMPLE PROTEINS IN HUMANS. THE METHODS FOR INDICATION, SEPARATION AND RELEASE OF PROTEINS FROM BIOLOGICAL FLUIDS

(Levich S.V.)

INFORMATIONAL MATERIAL

Simple proteins are complex nitrogen containing organic compounds (polymers) that are consisted of α-amino acid residues, connected by peptide bonds.

AMINO ACIDS AND THEIR CLASSIFICATION

Amino acids are biologically important organic compounds composed of *amine* ($-NH_2$) and *carboxylic* (-COOH) functional groups, along with a side-chain specific to each amino acid. In biochemistry, amino acids having both the amine and the carboxylic acid groups attached to the first (alpha-) carbon atom have particular importance. They are known as *2-*, *alpha-*, or *α-amino acids* with the general formula represented on the fig. 1. Amino acids can be related to a specific stereochemical lines (D- or L-) using D-Glyceraldehyde as a reference compound.

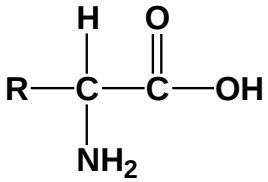
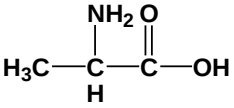


Figure 1. General structure of *α-amino acids,* where R is an organic substituent known as a "side-chain".

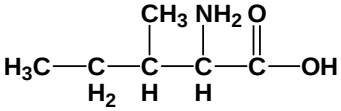
This group of amino acids includes the ***20 proteinogenic*** ("protein-building") amino acids, which combine into peptide chains ("polypeptides") to form the building-blocks of a vast array of proteins.

There are many ways to ***classify*** amino acids. These molecules can be assorted into *7 groups* on the basis of their ***structure*** and the ***general chemical characteristics*** of their ***side-chain radicals***:

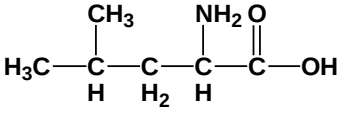
Amino Acids with Hydrophobic Side Chain – Aliphatic



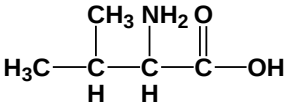
Alanine, Ala



Isoleucine, Ile

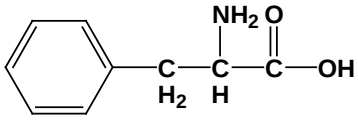


Leucine, Leu

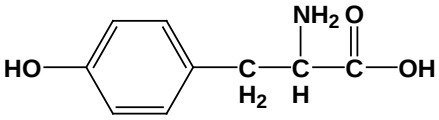


Valine, Val

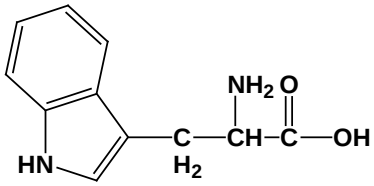
Amino Acids with Hydrophobic Side Chain - Aromatic



Phenylalanine, Phe

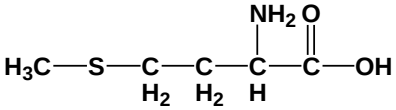


Tyrosine, Tyr



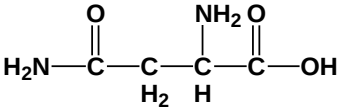
Tryptophan, Trp

Sulfur-containing Amino Acid with Hydrophobic Side Chain

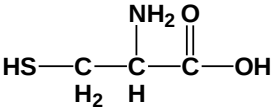


Methionine, Met

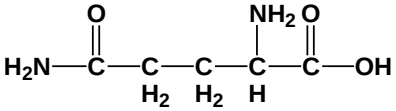
Amino Acids with Polar Neutral Side Chains



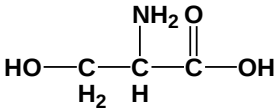
Asparagine, Asn



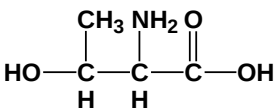
Cysteine, Cys



Glutamine, Gln

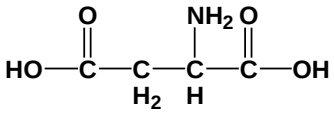


Serine, Ser

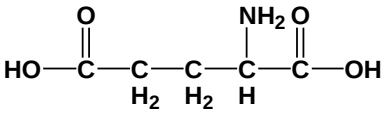


Threonine, Thr

Amino Acids with Negatively Charged Side Chains - Acidic

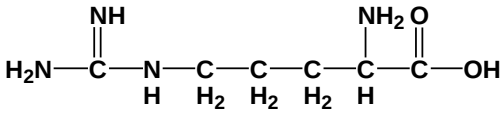


Aspartic acid, Asp

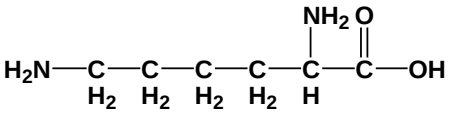


Glutamic acid, Glu

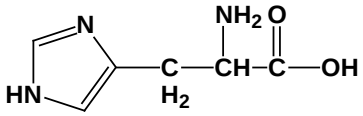
Amino Acids with Positively Charged Side Chains - Basic



Arginine, Arg

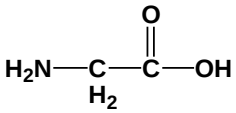


Lysine, Lys

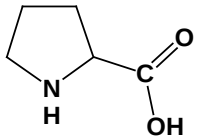


Histidine, His

Unique Amino Acids



Glycine, Gly



Proline, Pro

The other type of amino acids classification based on the abilityof organism to synthesized them de novo. By this classification, amino acid can be divided on ***essential***, ***non-essential*** and ***conditional amino acid.*** Essential amino acids cannot be synthesized by the human organism. As a result, they must come from food (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Nonessential amino acids are produced in human organism even if they don't come from food (alanine, asparagine, aspartic acid, glutamic acid and serine). Conditional amino acids are usually not essential, except in times of illness and stress (arginine, cysteine, glutamine, tyrosine, glycine, proline and serine).

QUALITATIVE REACTIONS FOR PROTEINS AND AMINO ACIDS

1. ***Piotrovsky's test*** or ***biuretic test.*** This reaction proves the peptide bond in

proteins and peptides (starting from tripeptides). The protein solution during the interaction with copper ions gets blue-violet colour in the alkaline environment.

2. *Ninhydrin reaction.* There is the formation of blue-violet product after the additional of ninhydrin to protein solution. This reaction is used to prove the presence of α-aminoacids residues.

3. *Sakaguchi's test.* Arginine is oxidized with sodium hypobromite and reaction with α-naphthol gives red colouring.

4. *Fole's test.* This test is used to prove the presence one amino acid residue, only, in the composition of proteins – cysteine.

5. *Millon's test.* Tyrosine, reacting with Milon's reagent, forms mercurial salt coloured red.

6. *Adamkiewicz's test.* Tryptophan can react with glyoxylic acid in acid environment. Red-violet coloured condensation products are formed.

7. *Reaction with formaldehyde.* Tryptophan, condensing with formaldehyde, forms with mineral acids blue-violet coloured salts.

8. *Pauli's test.* The test is used to prove the presence of histidine and tyrosine which react with diazobenzene-sulfonic acid, forming cherry-red coloured complex.

CLASSIFICATION OF PROTEINS

All proteins can be classified:

I. According to their function

1. *Catalytic (enzymes)* – more than 3000 proteins are enzymes.

2. *Nutritive (reserve)* – casein, ovalbumin etc.

3. *Transport* – blood serum proteins, which are capable to transport different compounds and substances to corresponding target organs (hemoglobin, blood plasma albumins, lipoproteins etc).

4. *Protective* – specific protective antibody proteins in response to the invasion of the organism by bacteria, toxins, or viruses. The coagulation of the blood by plasma protein fibrinogen prevents the organism from blood loss.

5. ***Contractile –*** a large number of proteinic substances are involved in the act of muscular contraction and relaxation (actin, myosin)

6. ***Structural –*** collagen in connective tissue, keratin in hair, skin, and nails, elastin in vascular wall etc.

7. ***Hormonal –*** a number of hormones are proteins or polypeptides (the hypophyseal and pancreatic hormones)

8. ***Receptor*** – rhodopscin, chemoreceptors etc.

9. ***Regulatory*** – histones, which stabilize structure of DNA, heat shock proteins etc.

10. Other vitally important functions "performed by proteins may be quoted-for example, the maintenance of oncotic pressure.

II. According to their three-dimensional structure

1. ***Globular proteins***, or ***spheroproteins*** are spherical ("globe-like") water-soluble proteins (they form colloids in saline solutions) that perform dynamic functions (enzymes, immunoglobulins, transport proteins). During the formation of globular proteins hydrophobic radicals of the polypeptide chain are located inside the structure, and hydrophilic one – on the surface of globular structure.

2. ***Scleroproteins,*** or ***fibrous proteins*** have an elongated form, insoluble in water, because they consist mostly from hydrophobic amino acids (proline, hydroxyproline, etc.), physically lasting, perform both structural and protective function: collagen, elastin, keratin.

III. According to their composition

1. ***Simple proteins*** consist from amino acid residues, only

2. ***Conjugated*** *or* ***complex proteins*** consist from polypeptide chains and non-protein part – ***prostetic group.***

CLASSIFICATION OF SIMPLE PROTEINS

1. Protamines. A group of the simplest water-soluble basic proteins, which consist mostly from arginine (60 %-85 %) Well known protamines: Salmin

– a protein of salmon sperm; Clupein – a protein of herring sperm. They take place in the structure of DNA-containing proteins.

2. Histones. A group of simple basic proteins that has high solubility in the water and saline solutions, and consist 20-30 % Arg and Lys. They are part of the DNP structure and has regulative role in the control of genome activity.

3. Prolamines and ***Glutelines.*** Simple proteins located in plants (vegetables). Prolamines contain 20-25% Glu and 10-15% Pro and are soluble in 60-80% aqueous ethanol without denaturation.

4. Albumins and ***Globulins.*** Simple proteins, that are abundant very widely (blood plasma, milk, egg white, muscles) and belong to globular proteins. They have different solubility in saline solutions. Solubility of albumins much higher, but they have the lesser mass in comparison with globulins.

SIMPLE PROTEINS STRUCTURE

Each protein in its native state has an unique tree-dimensional structure, which is referred to its conformation. This conformation determined by primary structure of Protein.

Primary structure of proteins (fig. 2) is an unique determined sequence of ά-amino acid residues connected by peptide bond. This sequence is coded by gene of DNA and determines the native state of protein molecule. Mechanism of peptide bond formation is represented on the fig. 3 and 4.

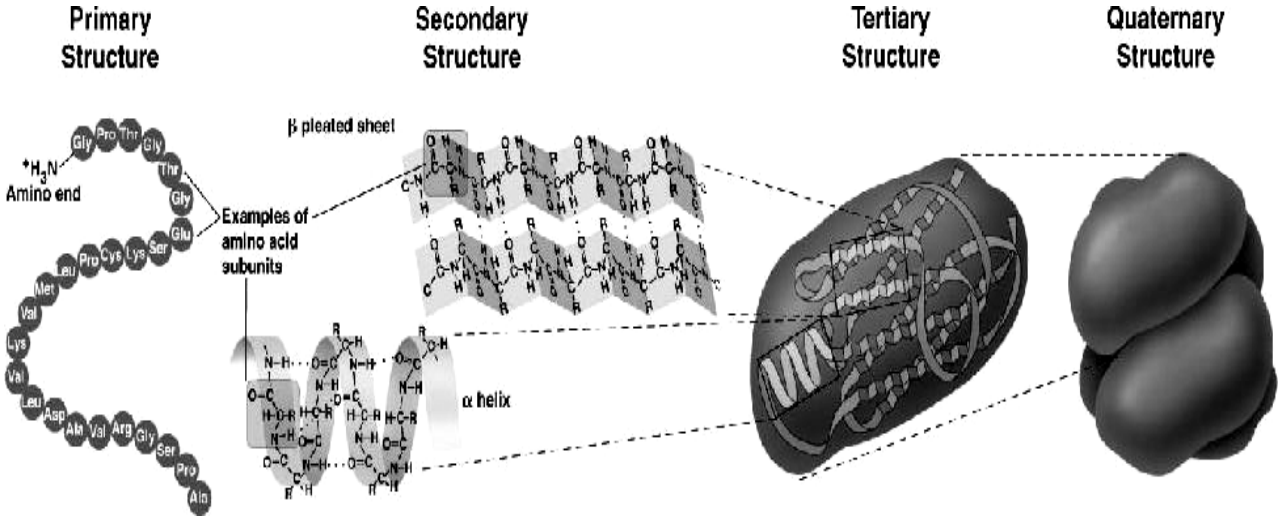


Figure 2. Simple proteins structure.

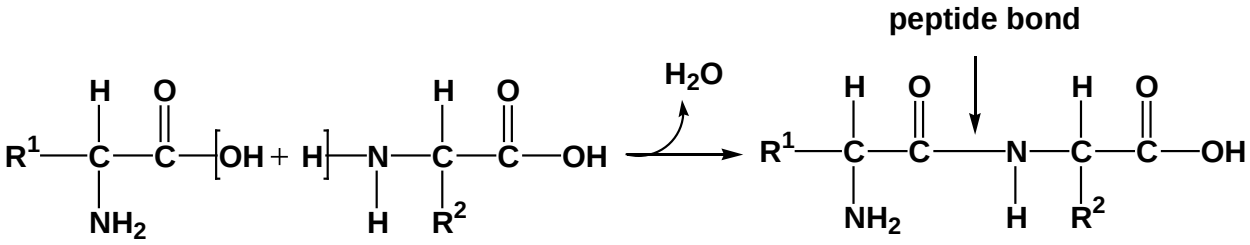


Figure 3. Mechanism of peptide bond formation.

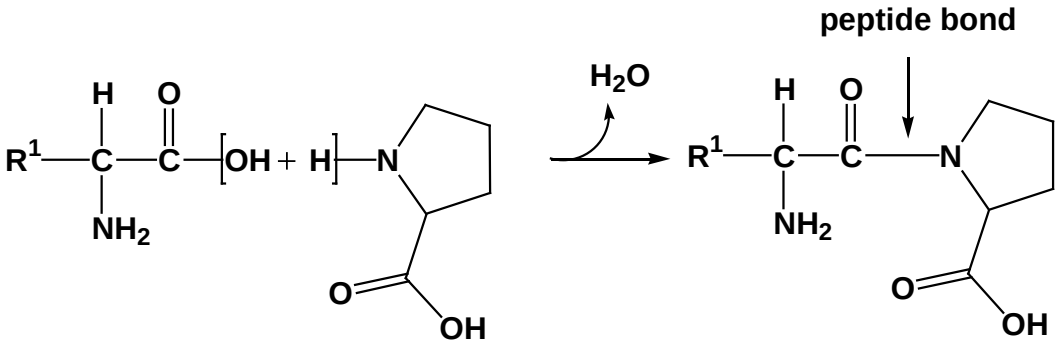


Figure 4. Mechanism of peptide bond formation of Proline is used

Secondary structure (fig. 2) is dictated by the primary structure. Secondary structure is a configuration of a polypeptide chain in space. It is formed due to *Hydrogen bonds* between peptide fragments of polypeptide chain. Each normal peptide fragment binds to each another one by two Hydrogen bonds.

1) If Hydrogen bonds are formed between peptide fragments in the same chain the ***α-helix*** turns. Characteristic of α-helix:

a) The polypeptide chain turns to the right.

b) There are 3,6 amino acid residues per turn of the helix.

c) The total length of the ά-helix in a globular protein can vary from almost 0 to more then 75% of the total chain length.

d) ά-Helix chains are much shorter in globular proteins then in fibrous ones.

2) If hydrogen bonds are formed between peptide fragments in different chains, extended structures are formed, such as ***β-pleated sheet***.

The chains lie side by side with the Hydrogen bonds forming between -CO group of one peptide fragment and the –NH group of another peptide fragment in the neighboring chain. The chains may run in the same direction, forming parallel β-sheet or they may run in opposite directions forming anti-parallel β-structure.

The most known protein with β-pleated sheet structure is silk fibroin.

It is impossible to form α-helix or β-pleated sheet structures if Pro residues are represented mostly in polypeptide chain because peptide fragments are without hydrogen.

Tertiary structure of Proteins (fig 2). The secondarily ordered polypeptide chain tends to fold into globular structure (like a ball) because this conformation represents a state of lowest energy and of greatest stability for this structure. The conformation results from various interactions between side chain radicals of amino acid residues in polypeptide chain: Hydrogen bonds, disulfide bonds, ester bonds, non-covalent bonds (electro-static interactions, Van-der-Waals forces – magnetic attraction forces). Disulfide bonds are the strongest among all these because they are covalent non-polar.

Quaternary Structure (fig. 2) refers to the spatial relationships between individual polypeptide chains in a multichain protein. Each chain is in tertiary conformation and known as protomer. Disulfide bonds are the most important for formation of Quaternary structure. But if one subunit has an overall charge negative and another subunit is positively charged, they can attract and result in multichain protein. Example of protein with quaternary level of structural organization is hemoglobin.

It should be noted that fibrous proteins organization is considered with missed tertiary level of organization. All fibrous proteins are with quaternary structure in native state.

PHYSICOCHEMICAL PROPERTIES OF GLOBULAR AND FIBROUS PROTEINS

The most characteristic physico-chemical properties inherent in proteins are: 1) high viscosity in solution; 2) low diffusion; 3) pronounced swelling ability; 4) optical activity; 5) mobility in electric field; 6) low osmotic and high oncotic pressures; 7) ability to absorb UV light at 280 nm wavelength (this property which is attributable to the occurrence of aromatic amino acids in proteins, is made use of

for protein quantitation).

Proteins, similar to amino acids, are amphoteric owing to the occurrence of free NH_2 and COOH groups in their structure and exhibit, accordingly, all properties characteristic of acids and bases. Depending on the pH medium and the percentage of constituent acidic and basic amino acids, proteins in solution develop either a positive, or a negative charge and tend to migrate, respectively, to the anode, or cathode. This property is profitably made use of in the electrophoretic purification of proteins.

Globular proteins solubility in aqueous solutions is due to the presence on their surface of polar amino acid residues

Globular and fibrous proteins have dissimilar physicochemical properties due to their structure formation differences (Fig. 5).

DENATURATION OF PROTEINS

The process of native protein molecule structure disruption to the primary level is named denaturation. Denaturation is not usually considered to include the breaking of peptide bonds. Depending on the degree of denaturation, the protein molecule may partially or completely lose its biological activity.

Denaturing conditions include the following:

1. ***Strong acids or bases.*** Changes in pH result in protonation of some protein side groups, which alters hydrogen bonds and salt bridge patterns. As a protein approaches its isoelectric point, it becomes insoluble and precipitates from solution. The structure degradation occurs too.

2. ***Organic solvents.*** Water-soluble organic solvents such as ethanol interfere with hydrophobic interactions because they interact with non-polar radicals and form hydrogen bonds with water and polar amino acid groups. Non-polar solvents also disrupt hydrophobic interactions.

3. ***Detergents.*** These amphipathic molecules disrupt hydrophobic interactions, causing proteins to unfold into extended polypeptide chains. (Amphipathic molecules contain both hydrophobic and hydrophilic fragments in structure.)

Figure 5. Differences of physicochemical properties of globular and fibrous proteins

Physicochemical properties	*Globular protein*	*Fibrous protein*
Molecular weight	6000-1000000 Da	6000-1000000 Da
Shape	Spherical, ellipsoidal	Elongated
The temperature of the existence of the native molecule	0-40 ºC	0-40 ºC
The temperature of complete denaturation	More than 70 ºC	More than 70 ºC
Time of thermal denaturation	1-2 minutes	More than 60 minutes
Relation to water	Formation of hydrate shell of the micelles	Swelling, only
Possibility of the formation of saline solution	Yes	No
Type of solution	Colloidal	No solution
Relation to: - mineral and organic acids - salts of heavy metals	 - complete denaturation - complete denaturation	 - complete or partial denaturation - complete or partial denaturation
Presence of isoelectric point	Determined	Absent
Conduct in electric field	Occurs in protein solution	Not considered
pH value, that does not cause denaturation	5≤pH≤10 (depend on structure, location and function of protein in the organism)	Neutral pH
Passive diffusion	Occurs	Not considered
Functions: - Structural - Nutrition - Transport - Regulatory - Contractile - Protective (antibody) - Protective (mechanical) - Enzymatic	 Yes Yes Yes Yes Yes Yes No Yes	 Yes After prolonged denaturation as rare exception No No Yes No Yes No

4. ***Reducing agents.*** In the presence of reagents such as urea, reducing agents such as β-mercaptoethanol convert disulfide bridges to sulfhydryl groups. Urea disrupts hydrogen bonds and hydrophobic interactions.

5. ***Heavy metal ions.*** Heavy metals such as mercury (Hg^{2+}) and lead (Pb^{2+}) affect protein structure in several ways. They may disrupt salt bridges by forming ionic bonds with negatively charged groups. Heavy metals also bond with sulfhydryl groups, a process that may result in significant changes in protein structure and function. For example, Pb^{2+} binds to sulfhydryl groups in two enzymes in the haem synthetic pathway. The resultant decrease in hemoglobin synthesis causes severe anemia. (In anemia the number of red blood cells or the hemoglobin concentration is lower than normal.) Anemia is one of the most easily measured symptoms of lead poisoning. This type of denaturation used to reduce the intoxication of the organism after poisoning by lead salts. In this case protein solution is used as a lead scavenger.

6. ***Temperature changes.*** As the temperature increases, the rate of molecular vibration increases. Eventually, weak interactions such as hydrogen bonds are disrupted and the protein unfolds. Some proteins are more resistant to heat denaturation and this fact can be used in purification procedures.

7. ***Mechanical stress.*** Stirring and grinding actions disrupt the delicate balance of forces that maintain protein structure. For example, the foam formed when egg white is beaten vigorously contains denatured protein.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	The patient was taken to the hospital in serious condition after being poisoned by lead salts. Which of these compounds can be used as a lead scavenger to reduce the intoxication of the organism? A. Multivitamins B. Water C. Protein solution D. A solution of sucrose	

№	Test:	Explanation:
	E. Analgesics	
2.	The structure of the protein includes proteinogenic amino acids. Find out the correct position of amine group in proteinogenic amino acid: A. δ-position B. ε-position C. α-position D. β-position E. γ-position	
3.	The irreversible changes of the spatial structure of the protein during heat treatment of food are observed. This process is named: A. Renaturation B. Salting-out C. Hydration D. Denaturation E. Dialysis	
4.	Membrane proteins, contacting with biological active substance and transmissing information within the cell are named: A. Protein Feeds B. Receptor-proteins C. Glycocalyx D. Enzyme protein E. Proteins-pumps	
5.	Alpha-helix is one of subtypes of secondary structure of the protein. Indicate bonds stabilize which this structure: A. Ionic bond B. Intermolecular interactions C. Hydrophobic attraction D. Hydrogen bond E. Peptide bond	
6.	One indicator of metabolism in the body is the level of total protein in serum. What reaction is usually used in clinical laboratories for the determination of protein content? A biuretic test B Ninhydrin reaction C Xanthotoprotein reaction D Foll's test E Nitroprusside reaction	
7.	The study of spatial conformation of proteins may be using specific method.	

№	Test:	Explanation:
	Specify it: A. Salting-out B. Electrophoresis C. X-ray analysis* D. Dialysis E. Isoelectric focusing	
8.	Specify the level of structural organization of the protein molecule, which remains after complete denaturation: A. Primary* B. Secondary C. Tertiary D. Quaternary E. Secondary and tertiary	
9.	Indicate the main type of bonds that is typical for primary structure of protein molecule: A. Peptide bond B. Hydrophobic attraction C. Hydrogen bond D. Disulfide bond E. Ionic interactions	
10.	Indicate, which of these amino acids is essential: A. Methionine B. Cysteine C. Alanine D. Serine E. Glycine	
11.	Structural feature of fibrous proteins is the presence of several parallel polypeptide chains. Name those protein that is component of hair, skin and nails. A. Keratin B. Albumin C. Prothrombin D. Globulin E. Histone	
12.	Solubility of most globular proteins in aqueous solutions is due to the presence on their surface of: A. Polar amino acid residues B. Non-polar amino acid residues C. Peptide groups D. Benzene radicals E. Heterocyclic radicals	
13.	Choose the correct continuation of the phrase: "Essential amino acids" are those that ...:	

№	Test:	Explanation:
	A. Are positively charged B. Are negatively charged C. Are synthesized in the body D. Are not synthesized in the body E. Have no charge	
14.	Amino acids which contain in the side-chain radical hydroxy group, are often included in the active center of enzymes. Name those amino acid: A. Cysteine B. Alanine C. Serine D. Phenylalanine E. Valine	
15.	Specify the principle which underlies the classification of simple proteins: A. The feature of the primary structure B. Thermal stability C. Thermolability D. The high molecular weight E. Physical and chemical properties	
16.	Several levels of structural organization may be recognized for protein molecules as biopolymers. Specify the highest level of structural organization for hemoglobin: A. Quaternary structure B. β-Spleated sheet C. Tertiary structure D. Primary structure E. Secondary structure	
17.	Specify a chemical compound used as a reference for amino acids relating to a specific stereochemical lines (D- or L-): A. Glycerol B. D-Glucose C. L-Glucose D. Galactose E. D-Glyceraldehyde	
18.	Name the proteins that are part of deoxyribonucleoprotein: A. Prolamines B. Glutelines C. Globulins D. Albumins E. Histones	

CONJUGATED PROTEINS. THE METHODS OF ALLOCATION AND QUANTITATIVE DETERMINATION OF PROTEINS IN BIOLOGICAL FLUIDS

(Levich S.V.)

INFORMATIONAL MATERIAL

Many proteins yield, on hydrolysis, some other chemical component in addition to amino acids and they are called ***conjugated proteins***. The non-protein part of a conjugated protein is usually named ***prosthetic group***. Protein part of conjugated protein has a name – ***apoprotein***. Prosthetic groups may be combined with the protein part by the different kinds of bond.

Conjugated proteins are classified on the basis of chemical nature of their prosthetic groups:

1. Chromoproteins

A non-protein component of this class of holoproteins has a special colour. They can be divided on several subgroups:

a) *Hemoproteins* keep a heme (a special prosthetic group), containing the iron ion (Fe^{2+} / Fe^{3+}) or copper ions (Cu^{+} / Cu^{2+}). ***Examples***: Hemoglobin. Its native conformation is a quaternary globular structure, composed from four subunits α_1, α_2, β_1, β_2. Each subunit keeps one heme and one polypeptide chain. Hemoglobin is a transfer of oxygen from lungs to any tissue and the transfer of carbon dioxide from tissue to lungs. Molecules of oxygen are connected with iron ions of four hemes, that are contained in hemoglobin.

Cytochromes. Their native conformation is tertiary structure (one polypeptide chain), except *Cytochrome oxidase (CChO)* (6 subunits in one molecule). The heme of cytochromes contains the iron- ion which can be in two forms: Fe^{2+} / Fe^{3+}. CChO keeps two subunits with Copper Cu^{+}/Cu^{2+}. Cytochromes b, c_1, c and CChO are used for electrons transfer to molecular oxygen in tissue respiration chain.

The presence of iron ion Fe^{2+}explains the colour of hemoproteins – some reddish or reddish – brown shade is found for their molecules.

b) ***Flavoproteins*** contain in the non-protein part an isoalloxazine fragment from vitamin B_2 (riboflavin). A majority of these proteins are enzymes, taking part in oxidation-reduction of some substrates. The prosthetic group of these proteins may be FMN (flavin adenine mononucleotide) or FAD (flavin adenine dinucleotide).

2. ***Metalloproteins***

The prosthetic group of these conjugated proteins is represented by metal ions. Depending on nature of ions metalloproteins can be divided to:

a) ***Non-heme iron-ions containing.*** *Ferritin* is located in spleen, liver, bone marrow and serves for storage of iron in the organism. *Transferrin* is a transfer of iron ions Fe^{3+} from the intestine wall to each tissue. It is indicated in blood plasma in β-globulin fraction. *Hemosiderin* is located in reticuloendotheliocytes of liver and spleen. Its function has been yet little studied.

b) ***Copper ions containing proteins.*** *Ceruloplasmin* (it is also glycoprotein) is an enzyme with weakly pronounced catalytic activity in oxidation of ascorbic acid, adrenalin, dihydroxyphenylalanine, and a number of compounds.

A lot of enzymes contain other metall-ions: alchohol dehydrogenase (Zn^{2+}), phosphotransferases or kinases (Mg^{2+}), catalase (Fe^{2+} / Fe^{3+}), ATPases (Ca^{2+}, Mg^{2+}) etc.

3. ***Glycoproteins***

This group of conjugated proteins contains carbohydrates and their derivatives as a non-protein part (glucose, mannose, galactose, xylose, arabinose, glucuronic acid derivatives, neuraminic acids, sialic acids, hyaluronic acid, chondroitin sulphuric acid and other glucose aminoglucans).

The last three types of prosthetic group are represented abundantly in proteins of connective tissue. Their function may be structural, protective. All the receptors for hormones and some hormones (gonadotropins, FSH) are glycoproteins. Some glycoproteins may be also enzymes. This type of proteins is also engaged in immune reactions, ion exchange, cellular adhesion.

4. ***Lipoproteins***

They are synthesized in many human tissues or organs: liver, an intestine

wall, kidneys, blood. The main function of them in the blood is to transfer lipids from one organ to another one. They are divided in four groups: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons (ChM). Lipoproteins found in nervous tissue are discussed as structural components of neurons comparments and as transporter of electric impulses.

5. *Phosphoproteins*

This group of very spread proteins is synthesized in cells in post-translational modification, using special enzymes – protein kinases. Phosphoproteins contain residues of phosphoric acid, which usually are connected to protein part due to serine or threonine side radicals. Phosphoproteins are widely represented in central nervous system (CNS), in the liver, kidneys, bone marrow. A majority of these phosphoproteins are key enzymes of many processes. Caseinogen is also phosphoprotein.

6. *Nucleoproteins*

This group of conjugated proteins contains nucleic acid as prosthetic group. Depending on the type of nucleic acid nucleoproteins are divided on: DNP (deoxyribonucleoproteins) and RNP (ribonucleoproteins). DNP are found in the nucleus and mitochondrions and RNP — in cytoplasma, endoplasmatic reticulum, in some cases: in nuclei and nucleoli (for high-molecular RNP). Function of these proteins is stipulated by the non-protein part. *DNA* is a keeper of hereditary information (or genetic information) in a cell. RNAs have such functions:are divided in three groups (types) according their functions:

Ribosomal RNA may be discussed as prosthetic group constantly linked with protein part, because these proteins are in need to create small and big subunits of ribosome.

tRNA may be in linkage with proteins short time during its function: to transfer amino acid residue to the place where the translation occurs.

mRNA messages the information about sequence of amino acid residues in the polypeptide chain that is produced due to translation.

Sharp RNA found in nucleus are enzymes which catalyze splicing (cutting of non-information parts in primary transcription).

METHODS OF CLEANING AND SEPARATION OF PROTEINS

Dialysis

This method works on the principles of the diffusion of solutes and ultrafiltration of fluid across a semi-permeable membrane. So that, low molecular weight impurities pass through the pores of the membrane, and macromolecular compounds (proteins) are retained. Thus, proteins are cleaned from impurities. This method is used in the department of "artificial kidney" to purify blood of its low molecular weight compounds.:

Salting-out

There is no denaturation of protein molecule due to salting-out. When large amount of neutral salt is added to a protein micella in solution, a precipitate forms. The large number of salt ions can effectively compete with the protein for water molecules, that is, the solvation spheres surrounding the protein ionized groups are removed. The net charge of protein molecule becomes zero an it aggregate and then precipitate. This process is referred to as salting out. Because salting out is usually reversible, it is often used as an early step in protein purification without denaturation.

This method is also used for separation of proteins. For example, globulins sediment is formed from 50 % solution of ammonium sulfate and albumins precipitate under the addition crystal form of ammonium sulfate. Difference in solubility give an opportunity to separate these proteins from each other, using salting-out.

Ultracentrifugation

Centrifuges of many sizes and speeds are used in the laboratory to remove debris as well as to collect precipitated proteins and other materials at various steps in a purification scheme. They may be used both for separation of molecules and for determination of molecular mass (M_r) (Fig. 6). When macromolecules in the

solution are subjected to an ultracentrifugal field they are accelerated rapidly to a constant velocity of sedimentation. This is expressed as a sedimentation constant S, which is the rate (cm/s) per unit of centrifugal force. The unit of S is the second but it is customary to give it in Svedberg units, S (1 S = 10^{-13}s). Sizes of particles are often cited by their S values. At a constant velocity the equilibrium will eventually be attained in which sedimentation is just balanced by diffusion and a smooth concentration gradient forms from the top to the bottom of the centrifuge cell or tube. After centrifugation, which is usually done in a plastic tube, a hypodermic needle is inserted through the bottom of the tube and the contents are pumped or allowed to flow by gravity into a fraction collector.

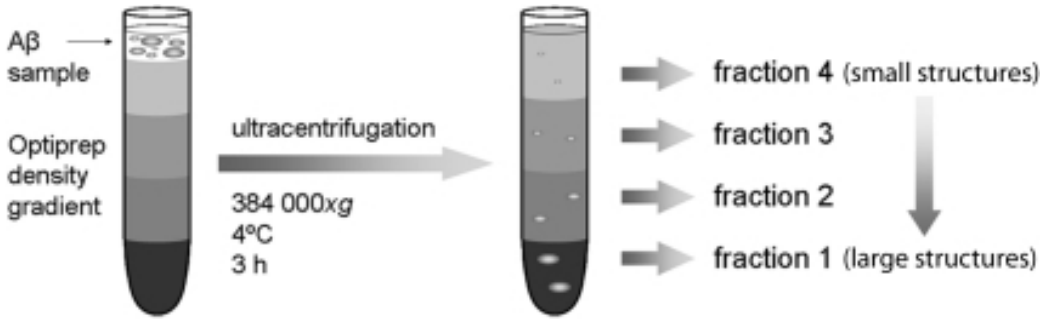


Figure 6. Ultracentrifugation.

Electrophoresis

Electrophoresis, the process of separating molecules, which based on the difference of their net charges, by migration in an electrical field, is conducted in many ways. In zone electrophoresis, a tiny sample of protein solution is placed in a thin line on a piece of paper or cellulose acetate. The sheet is moistened with a buffer and electrical current is passed through it. An applied voltage of a few hundred volts across a 20-cm strip suffices to separate serum proteins in an hour. To hasten the process and to prevent diffusion of low-molecular-weight materials, a higher voltage may be used. Two to three thousand volts may be applied to a sample cooled by water-chilled plates. Large-scale electrophoretic separations may be conducted in beds of starch or of other gels.

One of the most popular and sensitive methods for separation of proteins is ***electrophoresis in a column filled with polyacrylamide or agarose gel or on a***

thin layer of gel on a plate. The method depends upon both electrical charge and molecular size and has been referred to as electrophoretic molecular sieving. This method, which is often referred to as SDS–PAGE, has the advantage of breaking up complex proteins composed of more than one subunit and sorting the resultant monomeric polypeptide chains according to molecular mass (fig. 7, 8).

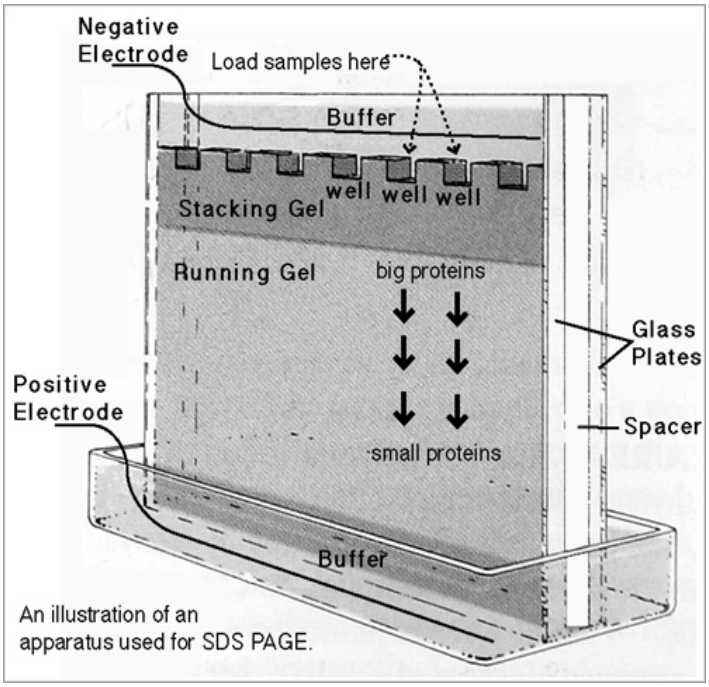


Figure 7. Electrophoresis in a column filled on a thin layer of gel on a plate.

Capillary electrophoresis is increasingly popular and can be used to separate attomole amounts (10^{-18} mole). It may be used not only for separation of proteins but also for rapid estimation of the net charge of a protein molecule.

Whereas in conventional ***zone electrophoresis*** most of the electrical current is carried by the buffer, in ***isotachophoresis*** the ions being separated carry most of the current. In ***isoelectric focusing***, a pH gradient is developed electrochemically in a vertical column or on a thin horizontal plate between an anode and a cathode. Proteins within the column migrate in one direction or the other until they reach the pH of the isoelectric point where they carry no net charge and are “focused” into a narrow band. As little as 0.01 pH unit may separate two adjacent protein bands which are located at positions corresponding to their isoelectric points. The isoelectric point (pI), is the pH at which a particular molecule carries no net

electrical charge and their electrophoretic mobility are absent. . If pH value more than pI protein particle will move to anode, if less – to cathode.

Such two-dimensional method in which proteins are separated by isoelectric focusing in the first dimension and by SDS-gel electrophoresis in the second has become a popular and spectacularly successful method for studying complex mixtures of proteins.

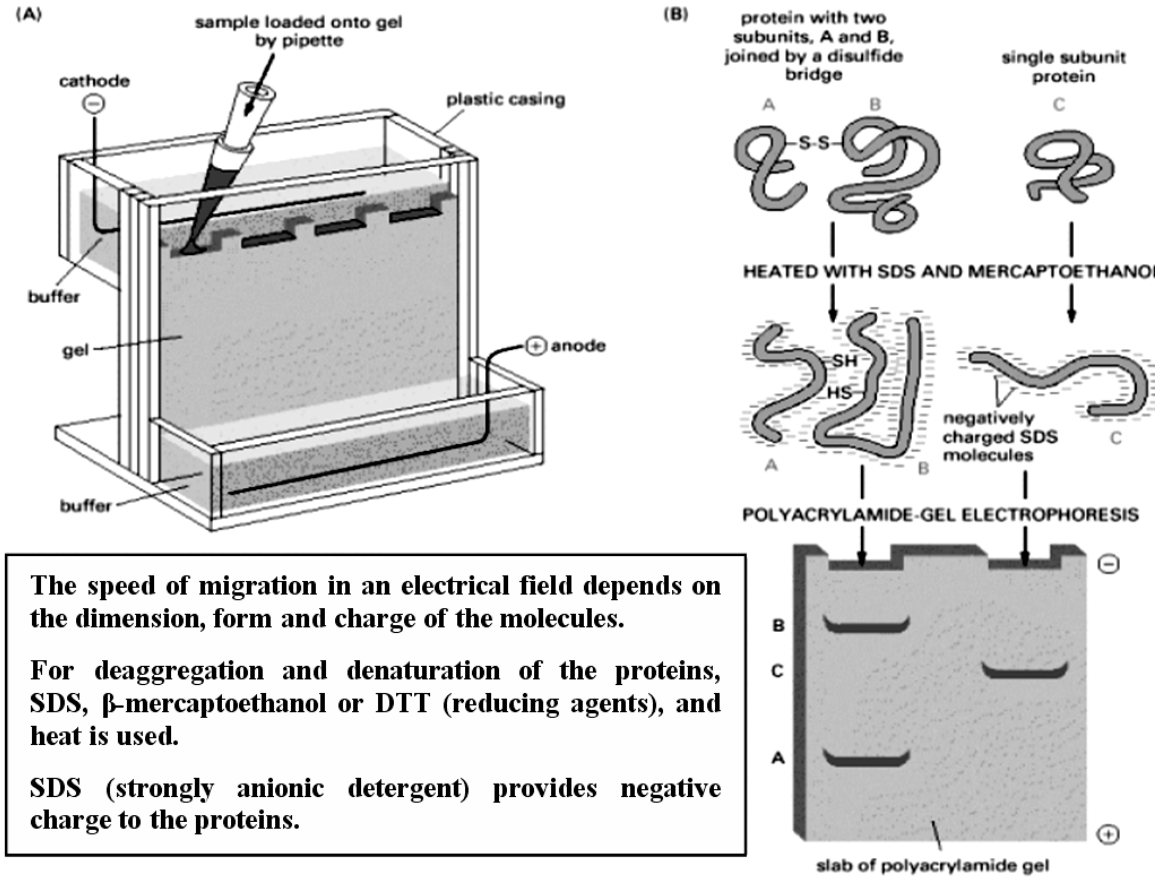


Figure 8. Polyacrylamide-gel electrophoresis (SDS-PAGE).

Affinity chromatography

Affinity chromatography is a method of separating biological mixtures based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand. The stationary phase is typically a gel matrix, often of agarose. Usually the starting point is an undefined heterogeneous group of molecules in solution, such as a cell lysate, growth medium or blood serum. The molecule of interest will have a well known and defined property, and can be exploited during the affinity purification process. The process itself can be thought

of as an entrapment, with the target molecule becoming trapped on a solid or stationary phase or medium. The other molecules in the mobile phase will not become trapped as they do not possess this property. The stationary phase can then be removed from the mixture, washed, and the target molecule is released from the entrapment in a process known as elution. Possibly the most common use of affinity chromatography is for the purification of recombinant proteins.

Affinity chromatography may be used to:

1. purify and concentrate a substance from a mixture into a buffering solution;
2. reduce the amount of a substance in a mixture;
3. discern what biological compounds bind to a particular substance;
4. purify and concentrate an enzyme solution.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	What group of the side-chain radical of amino acid residue will be joined with phosphoric acid in phosphoprotein? A. SH-group of cysteine B. NH- group of lysine C. COO-group of glutamine D. OH-group of the serine E. CH_3 group of methionine	
2.	In Wilson's disease copper transport is disturbed, leading to an accumulation of this metal ions in the brain and liver cells. The violation of what protein is observed at Wilson's disease? A. Metallothionein B. Haptoglobin C. Transcobalamin D. Transferrin E. Ceruloplasmin	
3.	Hemoglobin transports oxygen from lung to tissues the body and removes carbon dioxide from it. Indicate the class of complex proteins that it is belonged to. A. Metalloproteins B. Nucleoproteins C. Lipoproteins D. Glycoproteins	

№	Test:	Explanation:
	E. Chromoproteins	
4.	In order to prevent thrombosis the anticoagulant heparin was prescribet for the patient. This compound is selected to: A. Heteropolysaccharides B. Oligosaccharides C. Homopolysaccharides D. Monosaccharides E. Lipids	
5.	Specify a blood protein that contains copper ion in its composition: A. Ceruloplasmin B. Fibrinogen C. Thrombin D. Albumin E. Fibrinolysin	
6.	Specify the principle which underlies the classification of complex proteins: A. The chemical nature of the protein component B. Amino acid composition C. Solubility in water D. The chemical nature of the prosthetic group E. Ability to renaturatio	
7.	Most of the protein clotting factors by the chemical nature are: A. Glycoproteins B. Haemoproteins C. Flavoproteins D. Phosphoproteins E. Lipoproteins	
8.	From the above list, select a complex protein - Chromoprotein: A. Tobacco mosaic virus B. Hemoglobin C. Caseinogen D. Vitellin E. Ichthulin	
9.	Lipoproteins are complex proteins that are founded in biological membranes and blood plasma. Specify the basic function of plasma lipoproteins: A. Energy source B. Plastic function C. Transport of compounds D. Regulatory of process E. Catalytic function	

№	Test:	Explanation:
10.	Choose from the list Phosphoprotein: A. Catalase B. Hemosiderin C. Transferrin D. Interferon E. Caseinogen	
11.	Specify the substance that promotes saliva viscousity protects mucousa calls of oral cavity and prevents mechanical damage of the mucousal membrane, too: A. Mucin B. Glucose C. Kallikrein D. Amylase E. Lysozyme	
12.	Which electrode to the protein particle will move during electrophoresis, if its isoelectric point is 4.0, and the pH for buffer solution is 5.0? A. Anode B. Calomel electrode C. Silver electrode D. Cathode E. Platinum electrode	
13.	The patient is in the department of "artificial kidney". Specify the method that is used to purify blood of its low molecular weight compounds: A. Denaturation B. Salting-out C. Dialysis D. Hydrolysis E. Electrophoresis	
14.	The isoelectric point (pI) of the protein is 8.3. At what pH value the electrophoretic mobility of the protein macromolecule will be absent? A. 8,3 B. 4,7 C. 7,0 D. 11,5 E. 2,3	
15.	Doctor, before prescribing of parenteral protein nutrition made laboratory study electrophoretic of spectrum of blood serum proteins. What physicochemical properties of proteins are used in this method? A. Availability to be charged B. Viscosity	

№	Test:	Explanation:
	C. The inability to denaturate D. Hydrophility and swelling E. Optical activity	
16.	The method of salting-out is used for the fractionation of blood serum proteins in clinical practice. Name compounds which are used for this method? A. Detergents B. Salts of heavy metals C. Acids D. Salts of alkali metals E. Alkalis	
17.	In biochemical laboratories different methods are used for fractionation of protein mixtures. Specify the method that is based on the difference of net charge of the protein molecule: A. Gel filtration B. Affinity Chromatography C. Ion Chromatography D. Electrophoresis E. Ultracentrifugation	
18.	Specify serum proteins undergoing sedimentation at 50% saturation of ammonium sulfate: A. Histones B. Protamines C. Glutelins D. Albumins E. Globulins	
19.	Specify serum proteins undergoing salting out at 100% saturation of ammonium sulfate: A. Globulins B. Glutelins C. Albumins D. Histones E. Protamines	

ENZYMES: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES. CLASSIFICATION AND NOMENCLATURE OF ENZYMES

(Krisanova N.V.)

INFORMATIONAL MATERIAL

Enzymes are catalysts of protein nature. Enzymes share some properties with chemical catalysts.

Shared properties:

1. Enzymes are neither consumed nor produced during the course of a reaction.
2. Enzymes do not cause reactions to take place; they expedite reactions that would ordinarily proceed, but at a much slower rate, in their absence. They don't alter the equilibrium constants of reactions that they catalyze.

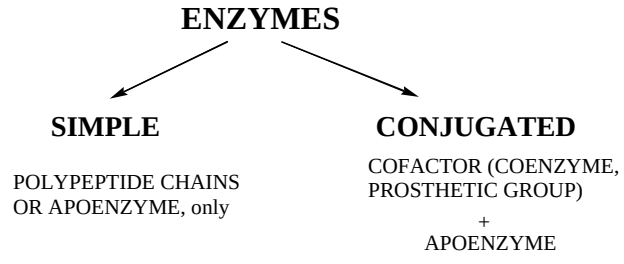
Differences between enzymes and chemical catalysts:

1. Enzymes are invariably proteins
2. Enzymes are highly specific for the reactions they catalyze and produce only the expected products from the given reactants (or substrates)
3. Enzymes often show a high specificity toward one substrate, although some enzymes have a broader specificity, using more then one substrate.
4. Enzymes function within a moderate pH and temperature range.

A majority of enzymes are globular proteins. So, all specified properties of globular proteins are introduced in enzymes.

Composition and structure of enzymes

Enzymes may be simple or conjugated, it depends upon the presence of the non-protein part (see below):



Cofactor (is the common term for non-protein part)

Prosthetic group

If the non-protein part is linked to polypeptide chains by covalent bonds, can`t dissociate

Coenzyme

If the non-protein part is linked to polypeptide chains weak bonds and can dissociate

Cofactors may be non-organic or organic compounds.

Non-organic cofactors:

1. Metal ions: Ca^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, K^{+}, Na^{+}, Cu^{2+}, selenium for glutathione peroxidase, etc.

2. Phosphoric acid residues: $H_2PO_4^{-}$, HPO_4^{2-}, PO_4^{3-}.

Organic compounds-cofactors:

1. Nucleotides: ATP, AMP, ADP, etc.

2. Carbohydrates: glucose, galactose, mannose, etc.

3. Vitamins and their derivatives (look Fig. 9)

4. Heme and its deravatives: 1) Cytochromes *b, c_1, c, aa_3,* P_{450}; 2) Catalase, Peroxidase

5. Short peptides: Glutathione (GSH/GS-SG), etc.

Figure 9. The use of some vitamins in the structure of enzymes catalyzed special type of the reaction.

Vitamin	**Coenzyme or prosthetic group**	**Type of the reaction catalyzed by the enzyme**
Thiamine	TPP (thiamine pyrophosphate)	Oxidative decarboxylation of keto acids; Transketolase reactions
Riboflavin	FMN (Flavin MonoNucleotide) FAD, Flavin Adenine Dinucleotide)	Oxidation-Reduction
Pantothenic acid	CoASH (Coenzyme A) ACP (Acyl carrier protein)	Activation of free acids Palmitate synthetase complex
Nicotinic acid or nicotin amide	NAD^{+}, $NADP^{+}$ (Nicotinamide Adenine Dinucleotide, Nicotinamide Adenine Dinucleotide Phosphate) and their reduced forms	Oxidation-Reduction Hydroxylation (NADPH mainly)
Pyridoxin	Pyridoxal phosphate, Pyridoxamine	Alpha-decarboxylation, Transamination of amino acids
Lipoic acid	Lipoamide	Oxidative decarboxylation of keto acids
Biotin	Carboxybiotin	Carboxylation of some acids

It should be noted that some medicines may be found in the formation of so named pseudo-coenzymes thus they can block activity of enzymes, for example: izoniazide is precursor for pseudo-coenzyme similar in structure to NAD^+.

Specific sites of enzyme

The most important part of any enzyme is ***the active centre***. ***It is a structural fragment of enzyme which attaches a substrate (one or more), and there is a conversion of substrates to the products of enzymatic reaction*** in this centre. There are two parts in each active centre of enzymes: a ***catalytic site*** and ***binding site*** for substrates.

Active centre of simple enzymes is composed from amino acid residues, only. The most frequently used amino acid residues in active centre of many enzymes are: Serine, Aspartic acid, Histidine, Lysine, Glutamic acid, Cysteine.

Active centre of conjugated enzymes usually keeps the non-protein part, for example:

a) Alcohol Dehydrogenase has NAD^+;

b) Cytochrome oxidase has heme-containing Fe^{2+}/Fe^{3+} and Cu^+/Cu^{2+}.

As a rule vitamin derivatives are in the active centre of conjugated enzymes. There are some amino acid residues in the active centre of conjugated enzymes, too. A conformation of active centre is formed only when a three-dimensional structure of enzymes is formed.

A majority of enzymes are synthesized as precursors of enzymes (***inactive form, proenzyme***). ***There are some ways of activation of proenzymes to form active enzymes:***

1. ***Non-complete proteolysis of precursor***: a part of polypeptide chain of precursor is eliminated by some another enzyme (protease). For example: 1) Enteropeptidase action on trypsinogen: N-terminal hexopeptide is eliminated from precursor to form active enzyme trypsin; 2) Trypsin produces chymotrypsin from its precursor chymotrypsinogen. The subtype of limited proteolysis is ***Autocatalysis: ability of active form of enzyme to produce itself from proenzyme.***

This way is discussed for pepsin, trypsin and chymotrypsin formation.

2. ***Allosteric activation*** of proenzyme. As a rule the key enzymes of process have **allosteric centers**. **Allosteric centre** is a site in the enzyme molecule structure which is able to adopt some organic or non-organic compounds. They are named effectors. The effector changes the conformation of enzyme (or proenzyme) after its linkage:

1) to form the active centre in the structure of proenzyme. In this case it is named allosteric activator;

2) to destroy the active centre of enzyme. In this case it is named allosteric inhibitor.

Phosphorylation–Dephosphorylation is this type of enzyme activation (or inhibition). As example, look in your textbook at two key enzymes regulation in glycogen metabolism: ***glycogen phosphorylase and glycogen synthetase.***

Properties of enzymes

1. Specificity of enzymes

Absolute specificity. This is specificity of enzyme action that is determined by its ability to act with only one substrate. For example: enzyme ***urease*** can destroy the urea, only, and can't react with any other substrate.

Relative group specificity. Many enzymes in nature have more then one substrate. This type of specificity may be named as ***relative group*** one. Term "relative" is used for the enzyme catalyzing the conversion of the same fragment in the structure of its substrate molecules. For example: A salivary amylase has the relative group specificity. It can destroy the α-1.4-glycosidic bond in the structure of polysaccharides such as starch, glycogen and their non-complete digestion products. But this enzyme can't react with disaccharides such as sucrose or maltose and monosaccharides as substrates. Second enzyme from saliva named lysozyme (or muramidase) can destroy proteoglycans in bacterial wall and has the same type of specificity.

Stereochemical specificity. For example: There are two types of alanine

oxidase in the liver: L-oxidase and D-oxidase. L-oxidase can react with L-alanine, only. D-oxidase can react with D-alanine, only.

Any type of specificity of enzyme is determined by:

1) The functional groups of the substrate (or product);

2) The functional groups in the active centre of enzyme and its cofactors (coenzymes)

3) The physical proximity of these various functional groups during the duration of the reaction.

2. Thermolability of enzymes

High temperature of environment (more then 60 ^{o}C) should be considered as a factor for denaturation of human enzymes. Optimal temperature for enzymatic action in human organism is about 38-40^{o}C. The low enzymatic activity is keeping at low temperature in region -8^{o}C-0^{o}C. So the curve of enzymatic activity (A) dependence on temperature is like this one (Fig. 10):

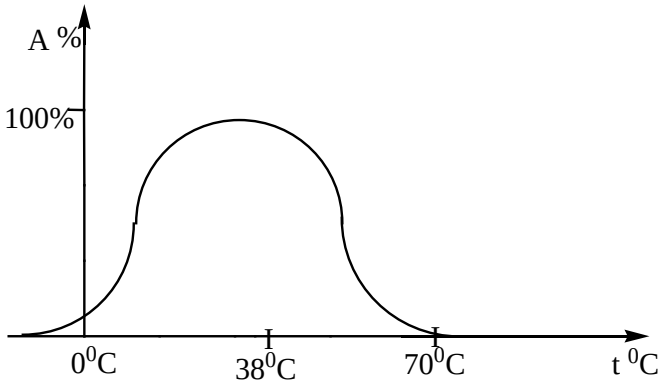


Figure 10. A temperature of environment influences the enzyme activity.

3. Effect of pH medium on enzymatic activity

Each enzyme-catalyzed reaction has its pH optimum. For majority tissue enzymes in humans pH optimum is about 7.2-7.4. Pepsin of gastric juice has very low pH optimum 1.5-2.5 at healthy adults. Enzymes of small intestine have the pH optimum about 8.0-8.4. So, the pH optimum of enzymes is very individual characteristic for them. The curve of the enzymatic activity (A) dependence on pH environment may be shown for tissue enzymes like this graph curve (Fig. 11):

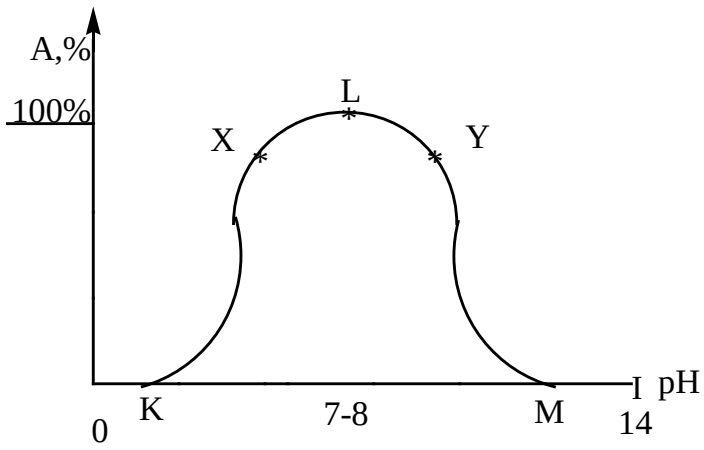


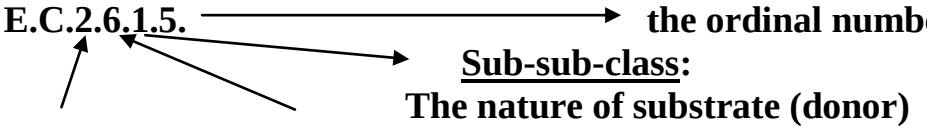
Figure 11. The influence of pH medium on enzyme activity.

There is the denaturation of tissue enzymes at points K and M because of strong acidic (point K) or strong alkalic (point M) medium around enzyme is found as denaturation factor. There is the lower enzyme activity in point X and Y in comparison with the point L because the charge of amino acid residues in active centre of enzyme is changed at pH values related to points X and Y. This change influences the rate of enzymatic reaction, and it is decreased.

Classification and nomenclature of enzymes

The International Union of Biochemistry recommended to introduce a decimal system of enzymes based on the nature of the catalyzed reaction. In 1972, the Commission for Biochemical Nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) published a new addition of enzyme nomenclature. Before this time the substrate name was usually taken and the suffix “ase” was attached. In other cases, the suffix was attached to the name of the catalyzed reaction. Some of the yearly described enzymes have special names, such as trypsin, pepsin, catalase. Today according to the classification there are six classes of enzymes. Each enzyme receives a four-part-number code and is also given a systematic name and recommended trivial name. For example: membrane carrier proteins that facilitate diffusion are named permeases, because it is difficult to estimate type of the reaction catalyzed by them.

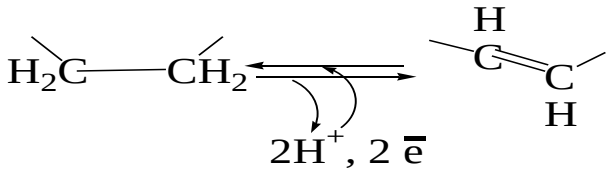
Example of enzyme class code:
Tyrosine amino transferase:



Classes of enzymes

1. Oxido reductases are involved in oxidation and reduction. The trivial names: dehydrogenases, oxidases, oxygenases, cytochromes. All the enzymes of this class are conjugated proteins. The cofactors of this class: FAD/$FADH_2$, FMN/$FMNH_2$, NAD^+/NADH, $NADP^+$/NADPH, heme (Fe^{2+}/Fe^{3+}), Cu^+/Cu^{2+}

Scheme of reactions, related to the oxidation / reduction:

1) 

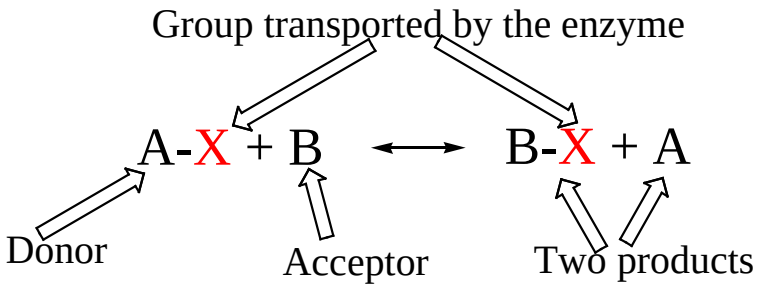
2) $A + 2\bar{e} \longleftrightarrow B$

3) $A + O_2 \longrightarrow AO_2$

4) $SH + O_2 \longrightarrow S\text{-}OH + H_2O$ ($2H^+$, 2 e)

2. Transferases transfer structural fragment from one substrate (donor) to another one (acceptor).

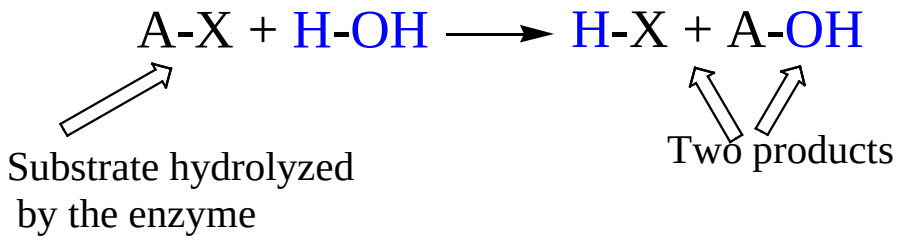
Scheme of this type of reaction:



Transferases catalyze usually reversible reactions. Fragments that may be transported: **Amino – NH_2; Methyl – CH_3; Acetyl – CH_3CO-; Phosphate –**

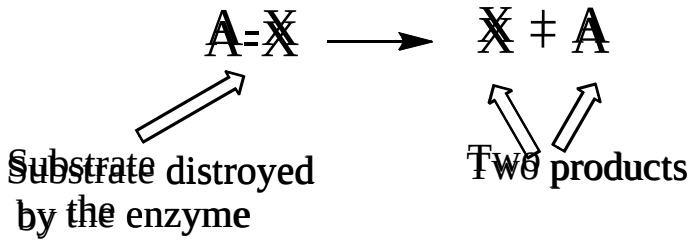
OPO_3H_2 and many others.

3. Hydrolases catalyze the hydrolysis of a substrate. The structural fragment (or bond) of a substrate is digested, water molecule is used in the formation of products. A scheme of this type of reaction:



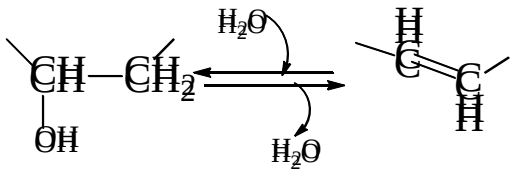
The digestion of proteins, polysaccharides, some lipids is carried out by this class of enzymes. Invasive properties of phytopathogenic microorganisms are due to this enzyme class.

4. Lyases add (or remove) the elements of water, ammonia, or carbon dioxide (CO_2) to (or from) double bonds. They can destroy the bond without water molecule utilization.



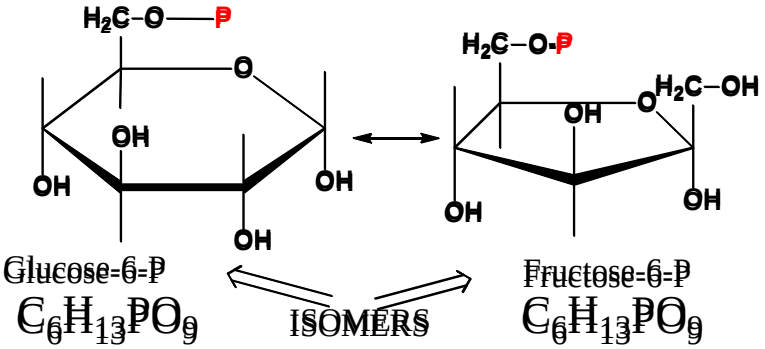
1) alpha-decarboxylation of amino acid

2) dehydration of beta-hydroxyacyl-CoA fragment:



5. Isomerases catalyze changes within one molecule; they include **racemases** and **mutases**, as well as **epimerases**. Isomers are different in structures, but quantitative composition is the same for both substances.

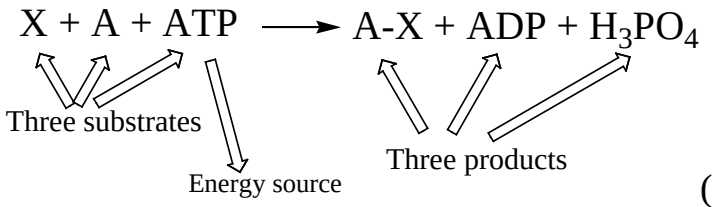
For example, reaction catalyzed by glucose-6-phosphate isomerase:



A sign for this type of reaction: the reaction is obligatory reversible!

6. Ligases (trivial: synthetases) join two or more molecules (substrates) together at the expense of energy released after degradation of high-energy bond of nucleoside triphosphate (ATP, GTP, UTP and others).

The schemes of this type of reaction are:



(1)

or:

$$A + B + ATP \rightarrow A - B + AMP + H_4P_2O_7 \qquad (2)$$

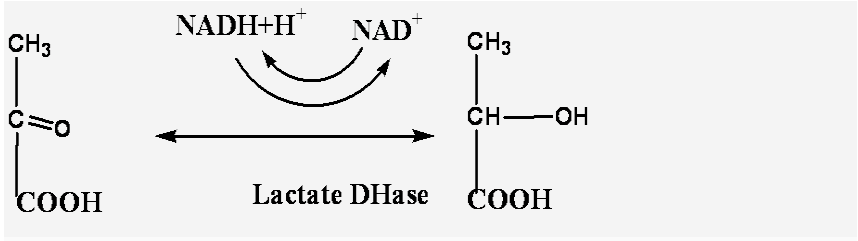
ATP may be used as the agent for phosphorylation (as a donor of phosphate group) catalyzed by phosphotransferase:

$$S + ATP \rightarrow S\text{-}OPO_3H_2 + ADP \quad (3)$$

Compare equation (1) and (3) and care for the transformation of ATP molecule in both reactions to differ them.

Isozymes: the definition and properties

The genetic information about the same enzyme may be represented in different tissues of human organism by variation of genes. In this case genetic forms of this enzyme may be differ partially in variation of subunits which are in creation of the native molecule of the enzyme. As example, let us consider those genetic forms for lactate dehydrogenase (LDH):



- **LDH1 LDH2 LDH3 LDH4 LDH5**
  H4 H3M H2M2 HM3 M4

H and M – subunits of the quaternary structure of enzyme

- **Myocardium** **LDH1 and LDH2**
- **Liver** **LDH3 < LDH4 < LDH5**
- **Skeletal muscle** **LDH3 > LDH4 > LDH5**
- **Kidneys** **LDH3**
- **Erythrocyte** **LDH1**

These genetic forms are named ***isozymes***. The active site in isozymes structure is the same, and any isozyme of LDH catalyzes the same reaction. Dut they are different in quaternary structures, physicochemical properties and location in tissues, that is because the determination of activity and concentration of each isozyme in the blood serum may be used in clinical diagnostics of diseases.

Multienzyme complexes

This is a complex of enzymes that are located together and carry out the same reaction or process.

For example: Pyruvate dehydrogenase complex (Fig. 12) is composed from three enzymes:

1) Pyruvate Dehydrogenase: E_1-TPP

2) Dihydrolipoyl transacetylase: E_2 (Lipoic Acid in two forms, CoA~SH)

3) Dihydrolipoyl dehydrogenase: E_3 (FAD, NAD^+)

The inhibition of any one enzyme from this complex causes the inactivation of the whole system. There are many Multienzyme complexes (MC) in cells: MC for High Fatty Acids Synthesis; MC for Oxidative decarboxylation of alpha–

ketoglutarate; MC for β–oxidation of HFA; a respiratory chain in the inner membrane of mitochondria, etc.

Figure 12. The composition of pyruvate dehydrogenase complex.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	In case of enterobiasis acrihine - the structural analogue of vitamin B2 - is administered for patient. Name enzyme whose synthesis disorder will be observed in microorganisms under the influence of this medicine: A FAD-dependent dehydrogenases B Cytochromeoxidases C Peptidases D NAD-dependet dehydrogenases E. Aminotransferases	
2.	In clinical practice tuberculosis is treated with izoniazide preparation – that is an anti-vitamin able to penetrate into the tuberculosis bacillus. Tuberculostatic effect is induced by the interference with replication processes and oxidation-reduction reactions due to the buildup of pseudo-coenzyme: A.FMN B.NAD^+ C.CoQ D.FAD E.TDP	
3.	Five isozymes of Lactate dehydrogenase were	

№	Test:	Explanation:
	allocated from the blood serum of human person and were investigated for their properties. What property of them can prove that these isoforms re related to one single enzyme? A. The same molecular mass B. They catalyze the same reaction C. The same electrophoretic mobility D. The same tissue location E. The same physicochemical properties	
4.	The deficit of microelement selenium in human organism is pronounced as cardiomyopathia state. The probable reason of this state development is the decrease of selenium-linked enzyme activity named: A. Lactate dehydrogenase B. Cytochrome oxidase C. Succinate dehydrogenase D. Catalase E. Glutathione peroxidase	
5.	There is enzyme in saliva that has strong antibacterial action due to ability to destroy proteoglycans of bacterial wall. Name this enzyme: A. Lysozyme (Muramidase) B. Alpha-amylase C. Trypsin D. Phosphatase E. Ribonuclease	
6.	A protective function of saliva is based on some mechanisms, one of them is promoted by the enzyme which has antibacterial activity to cause the lysis of polysaccharide complexes found in staphylococcus and streptococcus. Find out this enzyme: A. Lysozyme B. Alpha-amylase C. Oligo-1.6-glicosidase D. Collagenase E. Beta-glucuronidase	
7.	There is high activity of isozyme LDH1 in the blood serum of patient. Name the location (tissue, organ), where the pathology is in the development: A. Heart B. Liver C. Skeletal muscles D. Pancreas gland E. Kidneys	

№	Test:	Explanation:
8.	Affine chromatography method with the use of special ligand placed on the carrier proposed to obtain from pancreas of animals enzyme amylase preparation. Name substance that may be used as the ligand: A. Glucose B. Starch C. Sucrose D. Cellulose E. Lactose	
9.	Enzyme catalyzes the transfer of structural fragment from one substrate to other one to form two products. Name the class of this enzyme: A. Hydrolase B. Isomerase C. Transferase D. Oxidoreductase E. Ligase	
10.	Enzymes are frequently used as medical preparations produced by pharmaceutical industry. Name main difference of enzymes from non-biological catalysts: A. Small versatility B. High homogeneity C. High specificity of action and selectivity D. High versatility E. High dispersion	
11.	The entering of nutrients into the bacterial cell is by means of different mechanisms. One of them is facilitated diffusion, which is carried out by special membrane carrier proteins. Name them: A. Ligases B. Isomerases C. Permeases D. Lyases E. Oxidoreductases	
12.	Dehydrogenases are enzymes that remove the protons from a substrate. Name the enzyme class that Lactate dehydrogenase is related to: A. Transferase B. Isomerase C. Lipase D. Oxidoreductase E. Hydrolase	
13.	Enzymes (biocatalysts) are frequently used as medical preparations produced by pharmaceutical industry. Name the feature of	

№	Test:	Explanation:
	enzyme action that is found for any enzyme molecule in biochemical reaction: A. They decrease the energy for reaction activation B. They change the value for constant of the rate of reaction C. They change the order of the reaction D. They inhibit the reaction E. They increase the energy for reaction activation	
14.	Infection of medicinal plants by microorganisms eliminates their subsequent use by the pharmaceutical industry. Invasive properties of phytopathogenic microorganisms are due to those enzymes: A. Hydrolases B. Lyases C. Transferases D. Isomerases E. Oxidoreductases	
15.	Name the water-soluble vitamin whose derivative is used for creation of amino trasferase molecule: A. B1 B. B2 C. B3 D. B6 E. PP	
16.	Main biogenic amines are produced due to decarboxylation reaction. Name enzyme class to catalyze this type of reaction: A. Lyases B. Oxidoreductases C. Isomerases D. Hydrolases E. Transferases	
17.	Name class of enzyme for Glucokinase catalyzed the transfer of phosphate group from ATP to glucose: A. Lyases B. Oxidoreductases C. Isomerases D. Hydrolases E. Transferases	
18.	It is known that some membrane enzymes have ability to create multienzyme complexes which catalyze couple biochemical reactions. Name those complex: A. Pyruvate dehydrogenase	

№	Test:	Explanation:
	B. Hexokinase C. Lactate dehydrogenase D. Glycogen phosphorylase E. Phosphofructokinase 1	
19.	Biological oxidation is the main way to produce energy for living organisms. Name the class of enzymes which are the main to promote biological oxidation: A. Lyases B. Oxidoreductases C. Isomerases D. Hydrolases E. Transferases	
20.	Conversions of Proline into hydroxyl-proline and Lysine into hydroxyl-lysine in collagen molecules are catalyzed by enzymes: A.Hydroxylases B. Dehydratases C. Isomerases D. Hydrolases E. Transferases	
21.	Oxidase of D-amino acids catalyzes deamination for D-amino acids, only. What property is pronounced for this enzyme? A. Stereochemical specificity B. Thermolability C. Relative specificity D. Dependence from pH E. Absolute specificity	
22.	Name class of enzymes involved in digestion of proteins in gastrointestinal tract: A. Isomerases B. Lyases C. Hydrolases D. Transferases E. Oxidoreductases	
23.	Name the class of enzymes participated in anabolic pathways to produce new bonds in structure: A. Ligases B. Isomerases C. Transferases D. Hydrolases E. Oxidoreductases	
24.	Name the class of enzymes that is the helper to form reduced forms of coenzymes and of prosthetic groups which are donors of electrons: A. Ligases B. Isomerases	

№	Test:	Explanation:
	C. Transferases D. Hydrolases E. Oxidoreductases	
25.	Choose the substance that is unable to function as a substrate for enzyme in human body: A. HNO_3 B. High Fatty Acid C. Glucose D. Acetic acid E. Glycogen	
26.	Lipase is enzyme catalyzed the destruction of ester bonds in triacylglycerol molecules. Name the class of this enzyme: A. Hydrolase B. Transferase C. Isomerase D. Ligase E. Oxidoreductase	
27.	Choose the right continuation of the phrase: "Enzymes are proteins …": A. Regulating processes in a cell B. Increasing energy activation for the reaction C. Catalyzing conversion of substrates to products in biochemical reaction D. Promoting transport of substances across membrane E. Inhibiting duration of processes in a cell	
28.	Most enzymes are conjugated proteins. Find out a substance which is unable to function as the non-protein part of enzyme: A. AMP B. H_2SO_4 C. Biotin D. Ca^{2+} E. ATP	
29.	Salivary amylase cleaves α-1.4-glycosidic bonds in starch and its intermediate digestion products. Name type of specificity for this enzyme: A. Stereochemical B. Absolute C. Absolute group D. Relative group E. Classical	
30.	Pancreatic juice contains many enzymes represented as proenzymes (inactive forms) there. Choose those ones: A. Trypsinogen, chymotrypsinogen	

№	Test:	Explanation:
	B. Nuclease, pepsin C. Sucrase, amylase D. Amylase, lipase E. Nuclease, aminopeptidase	
31	The most important type of post-synthetic regulation of enzymes is covalent modification. Name a mechanism of Glycogen phosphorylase and Glycogen synthetase activities regulation: A. Phosphorylation-Dephosphorylation B. Methylation C. Adenylation D. Limited Proteolysis E. ADP-ribosylation	

THE MECHANISM OF ACTION AND KINETIC PROPERTIES OF ENZYMES. THE REGULATION OF ENZYMATIC ACTIVITY

(Krisanova N.V.)

INFORMATIONAL MATERIAL

A mechanism of Enzymes action

Enzymes decrease the energy of activation. A chemical reaction occurs when a certain proportion of the substrate molecules are sufficiently energized to reach a transition state in which there is high probability that a chemical bond will be made or to form the product. The effect of enzymes is to decrease the energy of activation (fig. 13).

$$E_{activation} = E_{transition\ state} - E_{initial\ state}$$

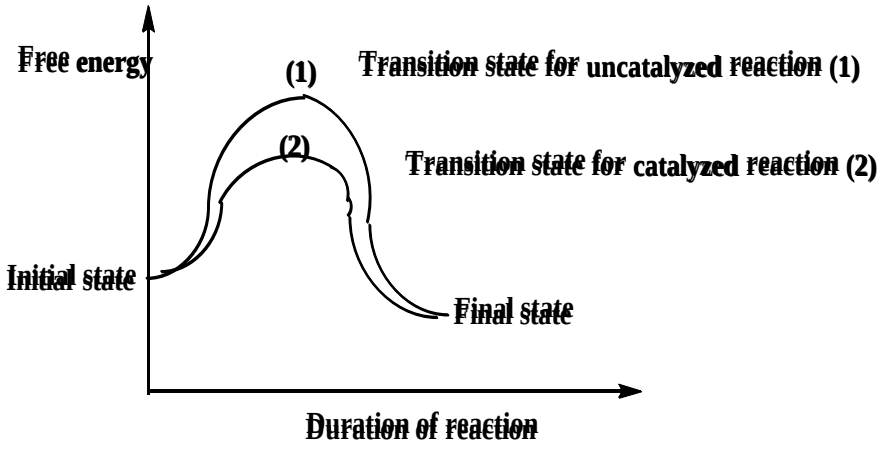


Figure 13. Free energy of chemical reaction for uncatalyzed reaction and catalyzed by enzyme. ***Energy activation for enzymatic reaction is lower!***

In 1913, L. Michaelis and M. Menten noted that an enzyme – substrate complex ES is formed which undergoes a chemical reaction and is broken down to free enzyme E and the product P.

So, the common equation of reversible enzymatic reaction must be:

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P \quad (1)$$

$$E + S \rightleftharpoons ES \rightarrow EP \rightarrow E + P \quad (2),$$

where case (1) – equation for reversible reaction; case (2) - equation for irreversible reaction.

The rate of both reactions is depended on the substrate, enzyme concentration, and the rate to reach transition state is promoted by ES complex concentration. Product concentration influences the rate of reaction (2), only.

The types of bonds for ES complex formation: Hydrogen bonds; Electro-static interactions; Covalent bonds; Magnetic attractions.

Two theories have been proposed to explain specificity of enzyme action:

a) The lock and the key theory (Fisher E., 1940th)

The active centre of the enzyme (the lock) is complementary in conformation to the substrate (the key), so that enzyme and substrate "recognize" one other.

b) The induced-fit theory (D.E. Koshland, 1950th)

The enzyme changes shape upon binding the substrate, so that the conformations of a substrate and enzyme protein are only complementary after binding reaction. The "enduced-fit" hypothesis presumes the existence between the enzyme and the substrate of not only spatial ore geometrical complanarity, but also electrostatic charge complementary: it means interactions of oppositely charged groups of the substrate and the active centre of the enzyme.

Today a majority of scientists agree with the second theory, because it can explain any type of specificity of enzymes, and the least level of energy activation for enzymatic reaction. Step by step whole mechanism of enzymatic reaction may be explained so:

- there is a moment of orientation and approach of enzyme and substrate relatively (may be at the expense of high-energy bond digestion) one to another in space;
- then it is a moment of an enzyme contact with the substrate – as the result ES complex is formed, and there is the induced fit of enzyme to substrate at this moment too. The attachment of a substrate provokes the spatial changes in the enzyme conformation. There is some strain in the conformation of active centre, and there is some deformation in substrate structure attached to the active centre.

All these changes promote quickly the reaching of the transition state of the reaction.

Enzymes catalyze reactions by utilizing the same general reactions as studied in organic chemistry:

- ***Covalent catalysis***
- ***Metal ion catalysis***
- ***Catalysis by alignment (approximation)***
- ***Acid-base catalysis***

• Additional free energy is obtained through the "Binding Energy" (binding of the substrate to the enzyme);

• Binding energy often helps stabilize the transition state, lowering energy for activation of enzyme.

Acid-base catalysis. There are some specific amino acid residues in active centre of enzymes that can be donors or acceptors of protons during the catalysis. Such as:

Donors	Acceptors
- COOH	- COO^-
- NH_3^+	- NH_2
- SH	- S^-

These groups take part in catalysis of many organic reactions in water phase.

Covalent catalysis (Fig. 14). In some cases enzyme (E-OH) can replace the functional group in a substrate RCO-X to form the covalent complex E-OCOR and first product HX (step A: acylation). This complex is not stable and is quickly hydrolyzed due to water use (step B: deacylation):

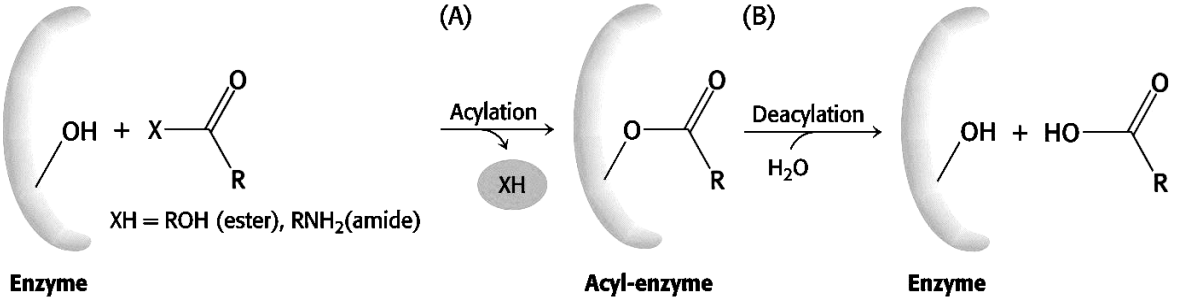


Figure 14. Covalent catalysis mechanism in steps (A, B) for chymptrypsin.

The hydroxylic group –OH in the enzyme active site may be from amino acid residues such as Serine or Threonine. This mechanism of enzymatic action is discussed for chymotrypsin and is named as covalent catalysis.

Kinetics of enzymatic reactions

Kinetic is the trend of enzymology that is concerned with study of all the factors which can influence the rate of enzymatic reaction. The determination of special indexes for each enzyme (Km and V) at normal condition (or in a case of some factors influence the rate of enzymatic reaction) is made. These indexes can help us to estimate the behavior of enzyme in living system.

Substrate concentration influences the rate of enzymatic reaction

Common equation of reversible enzymatic reaction is:

$$E + S \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} ES \rightleftharpoons EP \underset{K_{-2}}{\overset{K_{+2}}{\rightleftharpoons}} E + P \qquad (1)$$

K_{+1} – the rate constant for the formation of ES

$K\text{-}_1$ – the rate constant for dissociation of ES

K_{+2} – the rate constant for dissociation of ES to E plus P.

$K\text{-}_2$ – the rate constant of ES formation from E and P.

If the substrate concentration [S] equals zero, the rate of enzymatic reaction equals zero, too. The rate of enzymatic reaction depends upon the rate of saturation of active centers of enzyme by substrate molecules. The curve of reaction velocity (V) dependence on the substrate concentration [S] is this one (Fig. 15):

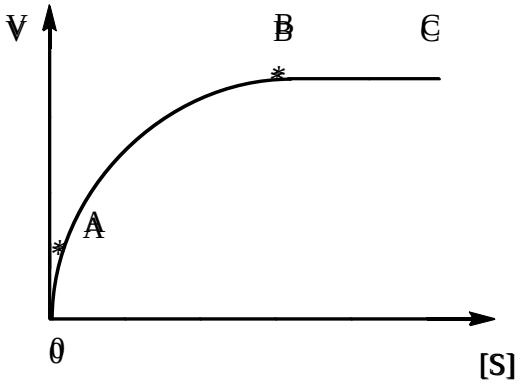


Figure 15. The curve of reaction velocity (V) dependence on the substrate concentration [S].

1) when the [S] is low, the reaction is first-order with respect to substrate: V ~ [S] → intercept 0A

2) in the middle of the curve (part AB) the reaction is mixed-order.

3) the part BC is discussed as a complete saturation of active centers of enzyme by substrate mole-cules. The velocity is maximal V = V_{max}. The [S] corresponding to the point B is named as the substrate concentration for saturation of active centers.

This curve may be described by mathematic equation (Michaelis-Menten equation):

$$V = Vmax \bullet [S]/Ks+[S] \quad (2),$$

where V_{max} - maximal reaction velocity; K_s - dissociation constant of enzyme-substrate complex ES.

Briggs and Haldeine later decided to replace the constant K_s by a new one → K_m (Michaelis constant), that may be calculated as:

$$K_m = K_s + \frac{K_{+2}}{K_{-1}}; \text{ and the new equation is } V = \frac{V_{max} \cdot [S]}{K_m + [S]} \qquad (3)$$

Physical sense of K_m: K_m equals to the substrate concentration at which the velocity is half-maximal, that is because it may found using the curve (fig. 16)

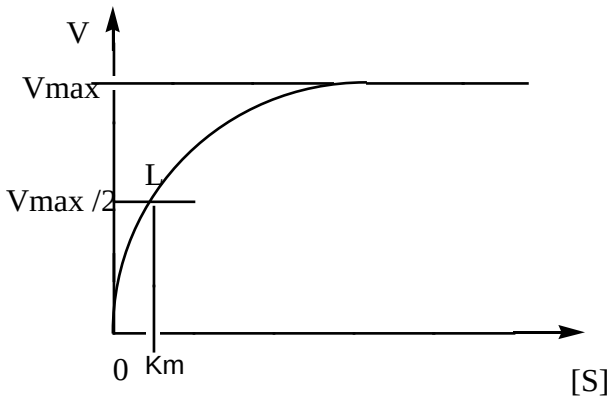


Figure 16. An example of Km determination for enzyme using the graph.

The affinity of an enzyme for its substrate is estimated by K_m: The lower the value of K_m the greater the affinity of the enzyme for its substrate

V_{max} and K_m are very important characteristics which are placed in special reference books for each enzyme.

Because it is difficult to estimate V_{max} from the position of an asymptote, as in the plot of a rectangular hyperbola (Michaelis-Menthen curve), linear transforms of the Michaelis-Menten equation are often used. The equation (3) is transformed into (4) and (5).

The reverse value to V are produced from equation (3):

$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max} \cdot [S]} \quad (4); \qquad \frac{1}{V} = \frac{K_m}{V_{\max}} \cdot \frac{1}{S} + \frac{1}{V_{\max}} \quad (5)$$

This method is named as ***Lineweaver-Burk method***. It shows the straight-line graph obtained by plotting of 1/V opposite 1/[S] (fig. 17), where the y-intercept equals $1/V_{max}$, and the x–intercept equals $-1/K_m$, and the slop equals K_m/V_{max}. This method is often used at the research of inhibitors` influence on the rate of enzymatic reaction.

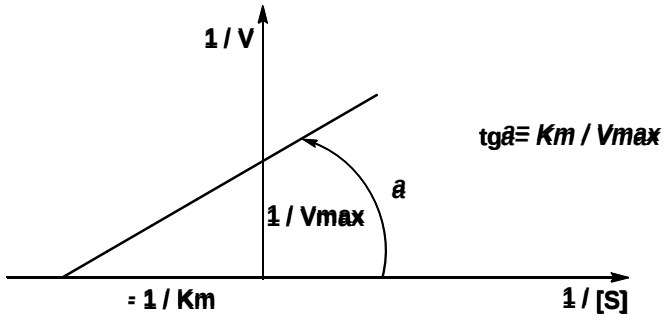


Figure 17. Lineweaver-Burk method graph.

Enzyme concentration

Enzyme activity is regulated by Enzyme concentration (fig.18). This dependence is considered only if:

pH, t^0C – optimal, [S] >> $[S]_{saturation}$

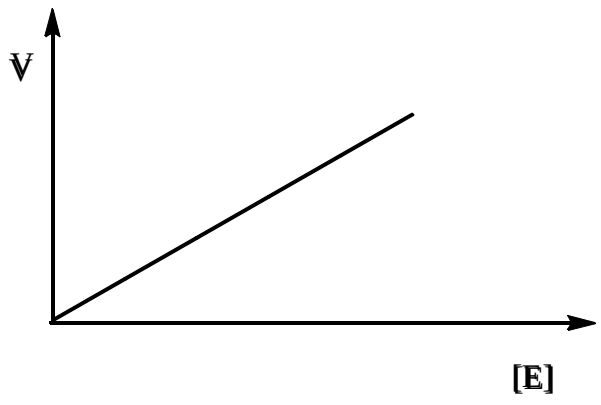


Figure 18. First-order dependence of V from [E].

Reversible inhibition of enzyme activity

Different types of reversible inhibition are possible, and they may be easily distinguished by analysis of Lineweaver-Burk plots.

Competitive inhibition features

a) Inhibitor (I) is similar in a structure to S.

b) I makes linkage only with active center of E. The inhibition is observed if [I] > [S].

c) If [I] << [S], I is displaced by substrate molecule from active center of E.

Example 1: Malonic acid HOOC – CH_2 – COOH is the competitive I for succinate dehydrogenase reaction in Krebs cycle. Thanks to two carboxylic groups in structure Malonic acid blocks active centre of E. It increases the K_m, but V_{max} is not changed in value. Lineweaver-Burk plots are as shown in fig.19.

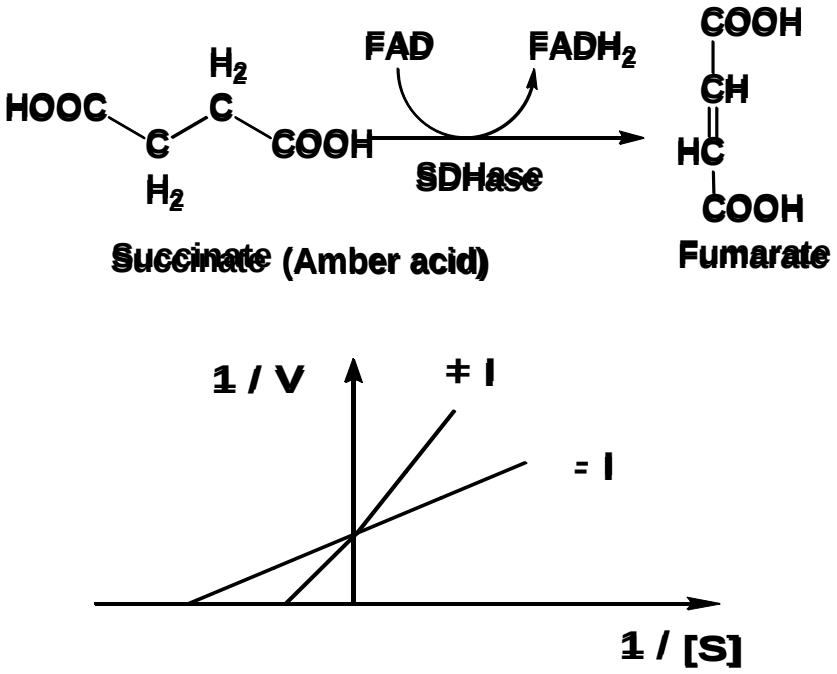


Figure 19. Lineweaver-Burk plots for competitive inhibition investigation and succinate dehydrogenase reaction.

Example 2: Proserin preparation influences the acetylcholine esterase activity: proserin competes with acetylcholine to attach active site of this enzyme, thus it decreases activity of acetylcholine esterase in treatment of myasthenia.

Example 3: Antimicrobial effect of Sulfonamide preparations is associated with the damage of folic acid (vitamin B_9) synthesis from para-amino benzoic acid,

and sulfonamide competes with para-amino benzoic acid to be linked to active site of the enzyme involved in production of this very important vitamin.

Example 4: Ethanol is used for treatment of patients with methanol poisoning (per os or intravenously) in a quantity that can cause separately toxicity for healthy person. The effect of ethanol use as drug in this case is explained so: affinity of ethanol to active site of alcohol dehydrogenase is much higher then for methanol, and it can replace methanol by itself under condition of ethanol excess intake.

Non-competitive inhibition features

a) I has another structure in a comparison with *S*
b) I may be attached not only with active centre of *E*.
c) The complex EIS is formed due to weak or covalent bonds.
d) I changes the V_{max} value, but K_m is not changed.

Lineweaver-Burk plots for this type of inhibition are shown in fig. 20.

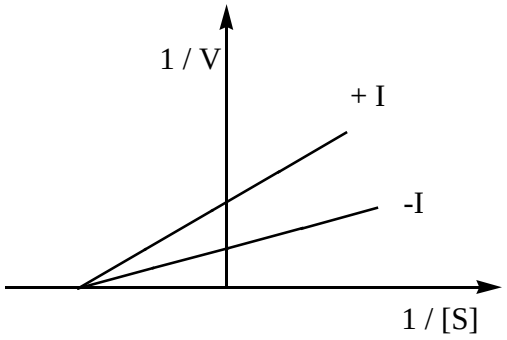


Figure 20. Lineweaver-Burk plots for non-competitive inhibition investigation.

For example: E – cytochrome C oxidase (heme-containing); I – cyanide ions CN^-

Heavy metal ions (lead, mercury), arsenic ions toxicity is explained from their influence on enzymes as non-competitive inhibitors to block SH-groups in active site of enzymes. Reversibility for this type of inhibition may be due to decrease of their concentration in the reaction medium due to dilution of solution where reaction occurs.

Allosteric inhibition features

It is usually the reversible inhibition. That is because ***the I makes linkage with allosteric centre by non-covalent bonds to change conformation of enzyme molecule (fig. 21).*** Allosterically regulated enzymes are key enzymes for metabolic processes. So, allosteric activation and inhibition are the most important regulative processes in promotion of homeostasis in a cell. ***Feed-back inhibition is discussed as the case of allosteric inhibition.*** Sometimes a product of enzymatic reaction (or terminal product of a process) may be as allosteric inhibitor at condition of its accumulation in a cell.

Allosteric inhibitor connects with allosteric centre

⇩

An enzyme conformation is changed at this moment

⇩

The conformation of active centre is changed, too (or there is the degradation of active centre)

⇩

Result: it is impossible to create the ES

Figure 21. All the steps for the influence of allosteric inhibitor on enzyme.

Example 1: NADH is produced due to isocitrate dehydrogenase reaction, under condition of its accumulation the enzyme activity is blocked. The terminal product of a process may be the feed-back inhibitor , too.

Example 2: Cholesterol synthesis from acetyl-SCoA is controlled so: the key enzyme – β hydroxy-β—methyl-glutaryl~SCoA-reductase is inactivated by cholesterol if its concentration is increased in a cell.

Example 3: Acetyl-CoA-carboxylase (the key enzyme in fatty acid synthesis) is regulated by feed-back influence of end-product – Palmityl-CoA.

Irreversible inhibition of enzyme activity

This type of inhibitors binds covalently or so tightly to the active centre of enzymes that they are inactivated irreversibly. There are those subtypes:

Affinity labels. There are substrate analogs that possess a highly reactive group that is not present on the natural substrate. The reactive group of ***I*** permanently blocks the active centre of the ***E*** from the ***S*** because the group reacts covalently with amino acid residue. The residue that is modified is not necessarily involved in catalysis.

Mechanism-based or suicide inhibitors. These are substrate analogs that are transformed by the catalytic action of the enzyme. Their structures are such that the product of this reaction is highly reactive and subsequently combines covalently with an amino acid residue in the active centre, thus inactivating the enzyme.

Transition – state analogs There are substrate-analogs which do not covalently modify the enzyme but bind the active centre so tightly that they irreversible inactivate the E.

Many highly toxic, naturally occurring and man-made compounds are irreversible enzyme inhibitors. Some organic compounds are poisons for humans (diisopropyl fluorophosphate, organophosphorus insecticides are among them). Phosphor-containing organic compounds inhibit acetyl choline transferase across blockage of OH-groups of serine residues in active sites of enzyme to cause CNS paralysis

Natural compounds used as drugs can also inhibit enzymes. For example:

1) Penicillin, which is a transition-state analog that inhibits the reaction with transpeptidase that is important in the development of bacterial membranes, thus destroying normal growth of the bacteria.

2) Allopurinol is the suicide inhibitor of xanthine oxidase and is used in the treatment of gout.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	The carbonic acid (H_2CO_3) is destructed up to carbon dioxide, that is released during breathing, and water with a help of one enzyme. Name the enzyme catalyzed this reaction: A. Carbonic Anhydrase B. Catalase C. Peroxidase D. Cytochrome C E. Cytochrome oxidase	
2.	The value of enzyme-substrate complex constant dissociation mathematically is not dependent from: A. Substrate concentration B. Time duration for the reaction C. Enzyme concentration D. ES complex concentration E. Affinity degree for enzyme to substrate	
3.	Some insecticides are poisons for humans because they block irreversibly the activity of very important enzyme in nervous tissue. It is: A. Cytochrome C oxidase B. ATP synthase C. Acetylcholine esterase D. Pyruvate kinase E. Lactate dehydogenase	
4.	Name a substance that competes with malonic acid to be attached to active site of Succinate dehydrogenase: A. Pyruvic acid B. Amber acid C. Malate D. Lactate E. α-Ketoglutarate	
5.	Name kinetic parameter of enzyme whose value is changed at the presence of competitive inhibitor: A. Vmax B. Km C. Optimal temperature D. Optimal pH E. Enzyme concentration	
6.	Name kinetic parameter of enzyme whose value is changed at the presence of non-competitive inhibitor: A. Vmax B. Km	

№	Test:	Explanation:
	C. Optimal temperature D. Optimal pH E. Enzyme concentration	
7.	The action of competitive inhibitor may be prevented by the: A. Increase of enzyme concentration B. The use of metal ion in the medium C. Increase of substrate concentration D. The use of allosteric activator E. The removal of the product from the medium	
8.	Continue the Phrase: “Small change in pH value for the reaction medium will change...": A. The level of enzyme molecule organization B. The degree of polarization for amino acid residues in active site C. The specificity of the enzyme D. Optical properties of the enzyme E. Biological function of the enzyme	
9.	Name factor of medium which can influence the charge of functional groups in active site of enzyme molecule: A. Temperature of medium B. Activator content in the medium C. pH of the medium D. Pressure E. Allosteric inhibitor content in the medium	
10.	The use of colors with high content of lead is limited in production of toys for kids. Lead ions toxicity is explained from their influence as inhibitors for enzymes. Name type of inhibition for lead ions: A. Non-competitive B. Competitive C. Uncompetitive D. Allosteric E. Irreversible	
11.	Treatment of methanol poisoning of a patient is the use of ethanol as preparation (per os or intravenously) in a quantity that can cause for healthy person toxicification. Find out explanation for effective ethanol use as drug in this case: A. The affinity of ethanol to active site of alcohol dehydrogenase is much higher then for methanol B. Ethanol is allosteric inhibitor of alcohol dehydrogenase C. Ethanol blocks the coenzyme of alcohol	

№	Test:	Explanation:
	dehydrogenase D. Ethanol is destroyed to form more toxic compounds as methanol E. Ethanol suppresses diffusion of methanol	
12.	Phosphor-containing organic compounds (mainly, they are poisons to cause CNS paralysis) inhibit acetyl choline transferase across blockage of OH-groups of serine residues in active sites of enzymes. Name type of inhibition for these compounds: A. Irreversible B. Reversible C. Competitive D. Non-competitive E. Feed-back inhibition	
13.	Acetyl-CoA-carboxylase (the key enzyme in fatty acid synthesis) is regulated by feed-back influence of end-product – Palmityl-CoA. Feed-back inhibition is subtype of: A. Allosteric inhibition B. Irreversible inhibition C. Competitive inhibition D. Covalent modification E. Uncompetitive inhibition	
14.	Structure feature for regulatory enzyme usually is the presence of allosteric center in their molecules. Find out its role: A. It attaches the regulator-substance B. It attaches the substrate C. It changes the structure of substrate D. It helps in dissociation of coenzyme E. It attaches the coenzyme	
15.	Heavy metal ions are very toxic. They block SH-groups that are placed in active centers of enzymes. Name the type of enzyme inhibition for heavy metal ions: A. Competitive B. Allosterical C. Non-competitive D. Uncompetitive E. Suicide	
16.	Some pharmaceutical preparations containing ions of arsenic and mercury are used in medical practice. Name type of inhibition of enzyme activity by these metal ions: A. Reversible B. Non-competitive C. Uncompetitive D. Competitive	

№	Test:	Explanation:
	E. Allosteric	
17.	Sulfonamide preparation was prescribed for the patient suffered from sore throat. Antimicrobial effect of this preparation is associated with the damage of folic acid synthesis. Name compound that competes with sulfonamide to be attach to active center of enzyme: A. Glutamic acid B. Citric acid C. Ubiquinone D. Succinate E. Para-amino benzoic acid	
18.	Proserin preparation is reversible inhibitor of acetylcholine esterase. Find out mechanism of proserin (P) action as inhibitor: A. Competition of P with acetylcholine to attach active site of enzyme B. Enzyme denaturation by P C. Covalent modification of active site of enzyme by P D. Fe^{2+} oxidation in active site of enzyme by P E. Covalent modification of allosteric site of enzyme by P	
19.	Proserin was used for the treatment of myasthenia and other disorders of muscular tissue. This preparation is competitive inhibitor of enzyme: A. Lactate dehydrogenase B. Citrate synthase C. Succinate dehydrogenase D. Arginase E. Acetylcholine esterase	

PRINCIPLES OF ENZYME ACTIVITY DETERMINATION. GENETIC DEFICIENCY OF ENZYMES. MEDICAL ENZYMOLOGY

(Krisanova N.V.)

INFORMATIONAL MATERIAL

A determination of enzyme activity in biological fluids

The determination of enzyme activity is of great importance for scientists. The enzyme activity is determined for tissue enzymes in homogenates of tissues or cellular fractions at research works. It is determined in whole blood, plasma or serum, in saliva, in gastric juice, in the urine, in cerebrospinal fluid for disease diagnostics in patients.

There are some methodic requirements for the enzyme activity determination in biological fluids:

- The substrate concentration must be more then substrate concentration for saturation of active centers of enzyme molecules found in investigated sample;
- The pH and temperature of the environment must be optimal;
- The activator for the enzyme in some cases must be added.

Total activity (T.A.) units:

- ***An International Unit (IU)*** is the amount of enzyme that catalyzes the transformation of 1 μmole of a substrate per minute under optimal conditions of measurement.
- ***Katal*** is the amount of enzyme that catalyzes the transformation of 1 mole of a substrate per second under optimal conditions of measurement.

These units are used for Total enzyme Activity (T.A.) determination.

Specific activity (S.A.) is the number of units of total activity per milligram of total protein [C] present in a sample: S.A. = $\frac{T.A}{[C]}$. This type of activity is used in researching works in biochemistry.

Turnover number (N) is the number of substrate molecules metabolized per one enzyme molecule per unit of time. For example, Carbonic unhydrase has

turnover number 36000000/min.

Clinical significance of some enzymes activity determination in biological fluids

The determination of enzyme activity in the blood plasma is of great importance for medicine. It helps to make diagnosis for some diseases, to find out the tissue damage, to differentiate the type of infringements for the same organ when other indexes of the blood plasma can't help.

Example 1: Aspartate aminotransferase (AsAT) activity in the blood plasma increases 10-100 times more then normal value at myocardium infarction in the first 3-4 hours of the damage development when cardiogram may be normal for patient.

Example 2 : The knowledge about tissue distribution of ***isozymes of Lactate Dehydrogenase (LDH):***

Myocardium	LDH_1 and LDH_2
Liver	$LDH_3 < LDH_4 < LDH_5$
Skeletal muscular tissue	$LDH_3 > LDH_4 > LDH_5$
Kidneys	LDH_3

It helps to find out the damage of some tissues if special type of isozymes is allocated from damaged tissue cell into the blood plasma. The determination of isozymes activity in the blood plasma is very important for diagnostic of heart, liver disease and many others (Fig. 22).

Example 3: Bilirubin indexes (conjugated and unconjugated) may be high in blood serum at various types of jaundice. The hepatic jaundice is accompanied with parenchyma damage of the liver. The liver parenchyma damage may be proved by the determination of ***alanine aminotransferase (AlAT) activity*** and ***choline esterase activity*** in the blood plasma of patients. Beside this the dynamic of choline esterase activity plays a valuable prognostic role at the treatment of patient: the decrease of the cholinesterase activity plays a role of a harbinger of the aggravation.

Figure 22. Enzymes with their respective substrates and inhibitors: the use in medicine.

Competitive inhibitors			
Enzyme	**Substrate**	**Inhibitor**	**Significance of inhibitor**
Monoamine oxidase	Epinephrine norepinephrine	Ephidrene, amphetamine	Useful for elevating catecholamine levels
Dihydrofolate reductase	Dihydrofolic acid	Aminopterin, amethopterin, methotrexate	Employed in the treatment of leukemia and other cancers
Acetylcholine esterase	Acetylcholine	Succinyl choline	Used in surgery for muscle relaxation, in anaesthetised patients
Dihydropteroate synthase	Para-Amino Benzoic Acid	Sulfonamide	Prevents bacterial synthesis of folic acid
Vitamin K epoxide reductase	Vitamin K	Dicumarol	Acts as an anticoagulant
Betta-Hydroxy-betta-Methyl-Glutaryl-CoA (HMG CoA)-reductase	HMG CoA	Lovastatin, compactin	Inhibits cholesterol biosynthesis
Irreversible inhibitor			
Enzyme	**Substrate**	**Inhibitor**	**Significance of inhibitor**
Aldehyde Dehydrogenase	Acetaldehyde	Disulfiram (antabuse)	Used in the treatment of alcoholism
Xanthine oxidase	Xanthine hypoxanthine	Allopurinol (suicide inhibitor)	Used in the control of gout to reduce excess production of uric acid from hypoxanthine
Cyclooxygenase	Arachidonic acid	Acetyl salicylic acid (aspirin) Phenyl butazone Indomethacin Ibuprofen	Anti-inflammatory drug : antipyretic (fever-reducing) and analgesic (pain relieving)
Aldehyde dehydrogenase	Acetyc aldehyde produced from ethanol	Teturam	Accumulation of acetic aldehyde in the blood of alcoholics will be, and it causes the aversion to alcohol under its use
Trypsin	Proteins of blood plasma	Trasilol and other protease inhibitors	To prevent spread proteolysis of proteins in pancreas and in the blood
Kallikrein	Proteins-regulators of blood vessels tonicity	Contrical	To control blood pressure in norm

Enzymes Use in Medicine

Basic sections	**Enzyme name**	**Examples of use**
Diagnostics a) serum	Lactate dehydrogenase [isozyme LDH_1]	Heart attack

enzyme The increased level	[isozyme LDH_5]	Liver diseases
	Aspartate aminotransferase [SGOT], AsAT	Heart attack (myocardial infarction)
	Alanine aminotransferase [SGPT], AlAT	Viral hepatitis, liver damage
	Creatine phosphokinase [CPK]: Isozyme MM (CPK_3) Isozyme MB (CPK_2)	 Muscle disorders Heart attack
	Acid phosphatase [ACP]	Prostate cancer
	Alcaline phosphatase [ALP]	Liver diseases, bone disorders
b) serum enzyme The lowered level	Choline esterase [ChE]	Liver parenchyma damage, hypothyroidism, nephritic syndrome, myocardial infarction
	γ-Glutamyl transpeptidase [GGT]	Alcoholism
	α-Amylase	Acute pancreatitis
	Lipase	Acute pancreatitis
	Aldolase	Muscular dystrophy
	5'-Nucleotidase	Hepatitis
	Glucose 6-phosphate dehydrogenase [G6PD]	Congenital deficiency with hemolytic anemia
	Ceruloplasmin	Wilson's disease
Treatment	Pepsin	Disordered digestion of proteins in stomach, deranged synthesis or secretion of pepsin
	Trypsin, chymotrypsin (immobilized forms)	Treatment of purulent wounds
	Streptokinase, urocaninase	Prevention of clots formation at transplantation of organs and other operations
	Hyaluronidase, Lidase	Resorption of a scar tissue, keloids due to the degradation of substrate - hyaluronic acid
	Asparaginase	Treatment of some malignant neoplasms, leucosis to prevent the accumulation of tumor growth factor -asparagine
	Nucleases (DNAase)	Viral conjunctivitis, rhinitis, purulent bronchitis
	Urease	Removal of urea from an organism in artificial kidney apparatus
	Streptodekase (immobilized enzyme)	To promote fibrinolysis at patients without normal duration of this pathway
Use of enzymes as analytical reagents	Glucose oxidase	The determination of glucose content in blood
	Cholesterol oxidase	The determination of cholesterol content in blood
	Lipase	The determination of triacylglycerols content in blood
	Urease	The determination of urea content in blood

Example 4: Amylase activity in blood plasma and in urine may be increased in 10-60 times or more at sharp pancreatitis in patients. This test is used also to check up the pancreatic gland function after treatment of patient with parotitis (mumps).

Genetic disorders of enzyme synthesis

A lot of genetic disorders are associated with the damage of enzymes synthesis or with the infringements of their regulation in tissues. These disorders are the most difficult in treatment, and the diagnosis is made at newborns or at prenatal state using the determination of some substrates or products concentration for enzymes that are in deficiency. DNA probes are available for prenatal diagnosis using amniotic liquid.

Example 1: Defects in the Phenylalanine 4-monooxygenase (hyperphenylalaninemia type I) or classic phenylketonuria. There is no transformation of phenylalanine into tyrosine in patient. Phenylalanine is accumulated in tissues and in the blood; the transformation of it may be to phenyl pyruvate, only. Phenyl pyruvate levels are also high in tissues and all the liquids. The major consequence of untreated type I hyperphenylalaninemia is mental retardation. Additional clinical signs include seizures, psychoses, eczema and a mould odour of urine. Screening of newborn infants for phenylketonuria now is compulsory (in a few days after born, 1-6 days).

	Normal Plasma / Urine	Phenylketonuric patient Plasma / Urine
Phenylalanine, mg/DL	1 - 2 / 30	15 - 63 / 300 - 1000
Phenylpyruvate, mg/DL	- / -	0,3 - 1,8 / 300 - 2000

Example 2: Alkaptonuria is caused by a defect in Homogentisate oxidase used for transformation of homogentesic acid to 4-maleylacetoacetate (tyrosine conversions). Homogentisate is accumulated in the blood and is excreted in large amounts in the urine causing the urine to darken after being exposed to air. Later in life, patients may develop pigmentation of connective tissue and suffer from arthritis.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	Twelve hours after an acute attack of retrosternal pain in the patient presented a jump of aspartate aminotransferase activity in his blood serum. What pathology is this deviation typical for? A Viral hepatitis B Myocardium infarction C Collagenosis D Diabetes mellitus E Diabetes insipidus	
2.	Marked increase of activity of MB-forms of CPK (creatine phosphokinase) and LDH-1 were revealed on the examination of the patient's blood. What is the most likely pathology? A Cholecystitis B Hepatitis C Rheumatism D Pancreatitis E Miocardial infarction	
3.	A patient presents high activity of LDH1, LDH2, aspartate aminotransferase, creatine phosphokinase. In what organ (organs) is the development of a pathological process the most probable? A In the heart muscle (initial stage of myocardium infarction) B In skeletal muscles (dystrophy, atrophy) C In kidneys and adrenals D In connective tissue E In liver and kidneys	
4.	6 hours after the myocardial infarction a patient was found to have elevated level of lactate dehydrogenase in blood. What isozyme should be expected in this case? A. LDH4 B. LDH1 C. LDH5 D. LDH3 E. LDH2	
5.	There is medicine named Teturam used in medical practice for the treatment and prophylaxis of alcoholism, and this preparation is inhibitor of aldehyde dehydrogenase. Accumulation of what compound in the blood of alcoholics will cause the aversion to alcohol under the use of this preparation?	

№	Test:	Explanation:
	A. Acetic aldehyde B. Ethanol C. Malonic dialdehyde D. Propionic aldehyde E. Methanol	
6.	A sick woman of 46 y.o. is suffered from progressive muscular dystrophy (Duchenne dystrophy). Name enzyme whose activity in the blood plasma is diagnostic test to prove this disorder: A. Creatine phosphokinase B. Lactate dehydrogenase C. Pyruvate dehydrogenase D. Glutamate dehydrogenase E. Adenylate kinase	
7.	The increase of activities for enzymes LDH1, LDH2, AsAT, creatine phosphate kinase was checked in the blood serum of diseased person. Name the probable organ (tissue) where pathology was developed: A. Heart muscle (beginning of myocardium infarction) B. Skeletal muscle (dystrophy or atrophy) C. Kidney and adrenal gland D. Connective tissue E. Liver and kidney	
8.	The previous diagnosis was made for the patient: myocardium infarction. Special feature of this pathology is the substantial increase in the blood serum of activity for: A. Catalase B. Glucose-6-phosphate dehydrogenase C. Alpha-amylase D. Arginase E. Creatine phosphate kinase	
9.	The investigation of diseased person's blood has revealed the excess increase of MB-isozyme of CPK (creatine phosphate kinase) and LDH1. Propose the probable pathology: A. Myocardium infarction B. Hepatitis C. Rheumatism D. Pancreatitis E. Cholecystitis	
10.	It was revealed acute panctreatitis in patient using biochemical investigation of his blood plasma. Find out biochemical test which can prove this diagnosis: A. Acidic phosphatase activity	

№	Test:	Explanation:
	B. Amino transferases activity C. Amylase activity D. Creatinine content E. Creatine phosphate kinase activity	
11.	The patient of 47y.o. with diagnosis myocardium infarction was admitted to reanimation department of hospital. What isozyme of LDH (lactate dehydrogenase) will be elevated first two days in this patient's blood plasma? A. LDH1 B. LDH2 C. LDH 3 D. LDH 4 E. LDH 5	
12.	The woman of 50y.o. with diagnosis myocardium infarction was admitted in the department of intensive therapy. Name the enzyme whose activity will be elevated two days in the blood plasma of this diseased person: A. Alanine aminotransferase B. Alanine peptidase C. Aspartate aminotransferase D. LDH4 E. LDH5	
13.	The young man of 18 y.o. with the damage of parenchyma of the liver has in his blood plasma probable elevated level of this enzyme activity: A. Alanine aminotransferase B. LDH1 C. Creatine kinase D. Acidic phosphatase E. Alpha-amylase	
14.	There was revealed acute increase of AsAT activity in the blood serum of patient for whom in 12 hours before acute attack of chest pain was checked. Find out the pathology whose development is associated with those change in the blood: A. Myocardium infarction B. Viral hepatitis C. Collagenosis D. Diabetes mellitus E. Diabetes insipidus	
15.	The determination of this enzyme activity in the urine is diagnostic test that is used for patients under acute pancreatitis to prove it. Name this enzyme:	

№	Test:	Explanation:
	A. Aldolase B. Amylase C. Lactate dehydrogenase D. Creatine phosphokinase E. Alanine aminopeptidase	
16.	The sick person was admitted to the hospital with previous diagnosis – acute pancreatitis. Name enzyme, whose activity must be determined in the blood serum and urine to prove this diagnosis: A. Alpha-amylase B. AlAT C. AsAT D. Lactate dehydrogenase E. Choline esterase	
17.	Name enzymes whose activity is in need to determine in the blood plasma to diagnise and to predict the development of disease for the patient with cardiac pathology: A. PDH, MDH, KGDH B. CPK (creatine phosphokinase), AlAT, AsAT C. Arginase, peptidase, acidic phosphatase D. Lysozyme, citrate synthase, aldolase E. Neuraminidase, hexokinase, pyruvate kinase	
18.	Name enzyme, whose activity determination in the blood serum is the most informative test at first hours of myocardium infarction development: A. Creatine phosphate kinase B. AsAT C. AlAT D. LDH E. Glutamate dehydrogenase	
19.	The patient has acute pancreatitis. Name preparation that will be recommended by doctor for the treatment to prevent autolysis of pancreas: A. Proteases inhibitors B. Proteases activators C. Trypsin D. Chymotrypsin E. Amylase	
20.	Hemorrhagic stroke is observed in patient. There is increase of kinins level in the blood of patient. Contrical preparation was prescribed for the patient to treat him. Name protease whose activity will be inhibited by this	

№	Test:	Explanation:
	preparation in patient's blood: A. Kallikrein B. Pepsin C. Trypsin D. Chymotrypsin E. Collagenase	
21.	Sick man 49 years old (a driver by profession) complains of unbearable retrosternal pain that was found in the cervical 2 hours ago. It is severe state, the patient has pale face, and, and his heart sounds are weakened. The laboratory investigation revealed high activity of Creatine phosphate kinase and LDH1. Name the disease which may by observed in patient: A. Acute myocardium infarction B. Acute pancreatitis C. Stenocardia D. Cholestasis E. Diabetes mellitus	
22.	The patient suffers from retrosternal pain from the left, the sweating and palpitation is observed for him, too. Choose enzymes whose activity is in need to determine in the blood to prove diagnosis: myocardium infarction: A. AsAT, CPK, LDH1 B. α-Fetoprotein, Aldolase, CPK C. Acidic phosphatase, LDH5, LDH4 D. Amylase, alkalic phosphatase, AlAT E. AlAT, Aldolase, LDH4	
23.	Keloids were remained on patient's body after burns. What enzyme-preparation may be used for their resorption? A. Asparaginase B. Nigedase C. Galactozidase D. Streptolidase E. Lidase	
24.	The preparation named "Lidase" is used in clinics for resorption of keloids and hematomas after burns and surgery operations. Name substrate for this enzyme preparation: A. Hyaluronic acid B. Dermatan sulfate C. Heparin D. Keratan sulfate E. Chondroitin-4-sulfate	
25.	The inhibitor for acetylcholine esterase was prescribed as preparation for the treatment of patient. Choose it from the list:	

№	Test:	Explanation:
	A. Aspirin B. Indometacin C. Allopurinol D. Sodium dichlorfenak E. Proserin	
26.	46 years old patient's blood serum creatine phosphate kinase activity was determined, and it was increased in values. Name the pathology that may be discussed for this patient: A. Chronic hepatitis B. Myocardium infarction C. Hemolytic anemia D. Acute pancreatitis E. Renal insufficiency	
27.	Pathogenic microorganisms contain aggression enzymes, that promote virulence for microorganisms. Find out those one: A. Lyase B. Carbounhydrase C. Hyaluronidase D. Oxidase E. Transferase	
28.	Fibrinolytic drugs can dissolve formed in human's blood thrombus. Choose those preparation: A. Riboflavin B. Streptokinase C. Isoniazide D. Phenobarbital E. Vicasol	
29.	Special growing factor is in need for tumor cell. Its destruction is made proposed enzyme used for leucosis treatment: A. Asparaginase B. Succinate dehydrogenase C. Aspartate aminotransferase D. Citrate synthase E. Glutaminase	
30.	This enzyme is prescribed as preparation for treatment of purulent wounds. Name it: A. Alkalic phosphatase B. Amylase C. Acidic phosphatase D. Trypsin E. Arginase	
31.	Pathogenic microorganisms during their entering and reproduction in human tissues produce different enzymes to help them in these actions. Find out those enzymes:	

№	Test:	Explanation:
	A. Oxidase, Catalase B. Lipase, Ligase C. Transferase, nuclease D. Esterase, protease E. Hyaluronidase, Lecithinase	
32.	Treatment of patients with purulent wounds is made using dressing with enzyme immobilized on them. Find out this enzyme: A. Trypsin B. Arginase C. Catalase D. Alkalic phosphatase E. Acidic phosphatase	
33.	The most informative test for early diagnosis of muscular dystrophies is the increase in the blood plasma of enzyme activity named: A. AsAT B. AlAT C. CPK-3 D. LDH1 E. Hexokinase	
34.	New antibiotics are produced from natural ones due to enzymatic reactions. Name synthetic form of enzymes frequently used in pharmaceutical industry: A. Immobilized enzyme B. Natural enzyme фермент C. Denaturated enzyme D. Enzyme complex with coenzyme E. Enzyme complex with activator	
35.	Increased total activity of LDH is found in the blood plasma of patient. It may be at disorders associated with diseases of heart, kidney and liver. Name additional clinical test to make differentiation in diagnosis of this patient: A. LDH isozymes content determination in blood plasma B. Glucose content determination in the blood C. Ketone bodies determination in the blood plasma D. Total cholesterol determination in blood plasma E. Amylase activity determination in blood plasma	
36.	The pharmaceutical preparation Asparaginase is used for the treatment of leucosis. Find out its mechanism of action: A. Protein synthesis stimulation B. Asparagine synthesis	

№	Test:	Explanation:
	C. Glutamine synthesis D. Glutamine desruction E. Asparagine degradation	
37.	The patient with myocardium infarction was prescribed fibrinolytic drug “Streptodekase” created using the linkage of enzyme with water-soluble polysaccharide template produced by method: A. Autolysis B. Ultracentrifugation C. Electrophoresis D. Enzyme mobilization E. Extraction	
38.	Five enzymes (LDH4, LDH5, AlAT, gamma-glutamyl transferase, carbamoyl ornithine transferase) activities are increased in blood plasma of patient. Name organ (tissue) whose damage is checked: A. Liver B. Heart C. Lungs D. Kidney E. Pancreas	
39.	Five enzymes (LDH4, LDH5, AlAT, gamma-glutamyl transferase, carbamoyl ornithine transferase) activities are increased in blood plasma of patient. Name organ (tissue) whose damage is checked: A. Liver B. Heart C. Lungs D. Kidney E. Pancreas	
40.	Trasilol is a preparation to treat acute pancreatitis, and it is found to block activity of special pancreatic enzyme. Choose it: A. Trypsin B. Amylase C. Lipase D. Elastase E. Phospholipase A2	
41.	Find out the enzyme whose activity is determined in the blood plasma of patient with bone tissue pathology: A. Pepsin B. Trypsin C. Amylase D. Acidic phosphatase E. Alkalic phosphatase	

№	Test:	Explanation:
42.	The degree of liver parenchyma damage may be estimated due to determination in the blood plasma of: A. Choline esterase activity B. Amylase activity C. LDH3 content D. Acidic phosphatase activity E. LDH1 and LDH2 content	

COMMON REGULARITIES OF METABOLISM. ANABOLIC AND CATABOLIC PROCESSES IN HUMANS. KREBS CYCLE

(Krisanova N.V.)

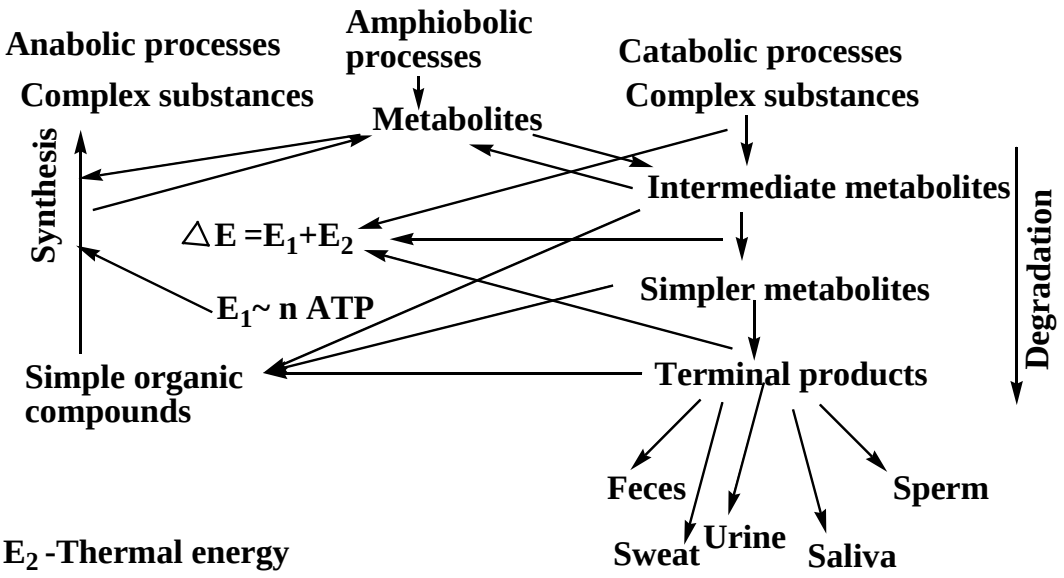
INFORMATIONAL MATERIAL

Any living system, if it is healthy, will be found in ***homeostasis state:*** it means that all the parameters for living system (physical indexes, levels of all the compounds that are useful for it) are in a region of normal average values. The promotion of this state is due to ***metabolism, that is sum total of all the metabolic pathways placed in the living system.***

Metabolic pathway is the sequence of chemical reactions catalyzed by enzymes to produce important terminal products for living system. Any metabolic pathway has initial substrates (they are placed in first reaction mainly) and terminal products. The rule to find out terminal products is to care for back-side products of intermediate reactions, which are produced but not involved in this pathway, and to care for terminal products of the last reaction. Intermediate products are named ***metabolites***.

Terminal products for human organism are those compounds which are produced but not involved in any reaction in human cells, they are: urea, uric acid, creatinine, indican, hippuric acid, 17-ketosteroids, salts of ammonia. The knowledge about them helps doctors to make diagnostics of diseases.

All the metabolic processes are divided in subtypes: ***anabolic, catabolic and amphybolic*** (Fig. 23). Anabolic pathways are synthetic one to produce complex molecules from simpler compounds with the use of energy, examples: replication, transcription, translation. The main class of enzymes to promote anabolic pathways is Ligases. Catabolic pathways are degradation processes to form simple molecules with the release of energy, examples: glycolysis, glycogenolysis, proteolysis, fatty acid oxidation, Krebs cycle.



E_1~ n ATP : Chemical energy of high energy-containing bonds in ATP molecule

△E - Total energy formed during degradation

Figure 23. Interrelations between anabolic, catabolic and amphybolic pathways in the living system.

Amphybolic process contains intermediate products which may be involved in both catabolic and anabolic pathways of living system. ***Anabolic process*** is the synthesis of complex compound from some simpler substances with the use of special energy sources such as ***nucleoside triphosphates. The main from them is ATP.*** Release of energy in a cell is due to ***catabolic pathways that are degradation pathways of some complex compounds into some simpler substances. Energy that is released during destruction of compound may both thermal and chemical placed in bonds of ATP (fig. 23).***

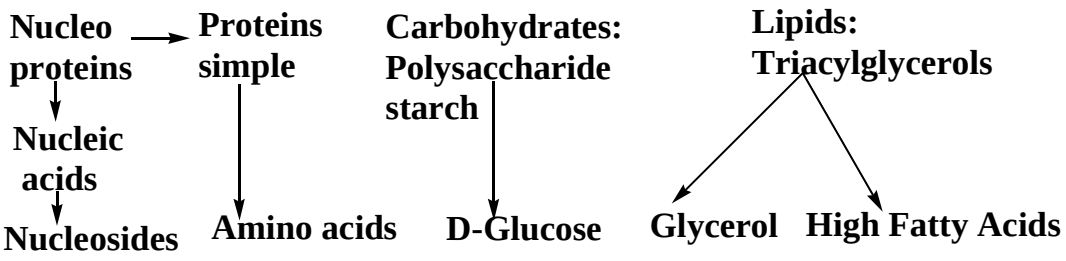
All the catabolic processes in the living system are divided in phases I, II, III.

Catabolic phase I reactions are catalyzed by single class of enzymes – hydrolases (Fig. 24).

The release of energy due to the function of this class of enzymes is impossible to keep in a form of ATP. This fact gives us understanding that only phases II and III are probable producers of ATP.

Catabolic phase I

It is proceed for *exogenous* substances in GIT, for *endogenous* substances it is in lysosomes and cytoplasm of cells



Products for catabolic phase I; any of them must be absorbed in the small intestine and trasfered across the bloodstream to the tissues

Figure 24. Initial substrates and terminal products for catabolic phase I.

Catabolic phase II (Fig. 25) is in cytoplasm and matrix of mitochondria (the latter place is associated with breakdown of pyruvate and high fatty acids (HFA beta-oxidation)):

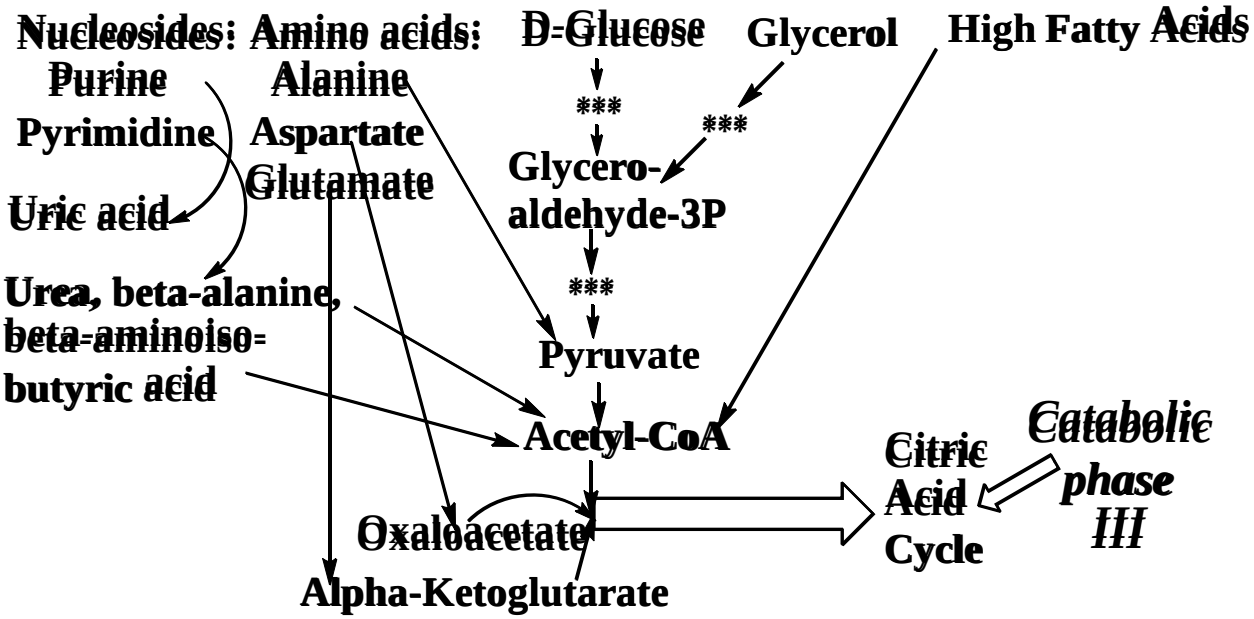


Figure 25. Catabolic phase II and its relations with Citric Acid Cycle (catabolic phase III).

Catabolic phase II for main molecules of a cell is finished by the formation of central key metabolite - acetyl-CoA. All the classes of enzymes except ligases may be used in catabolic phase II, but the class of oxido-reductases is the main promoter for the release of energy that may be utilized in production of ATP. Two types of oxidation may be found in cells of humans: aerobic (main) and anaerobic (in RBC, in cornea, lens, because mitochondria are absent there). Among all the pathways shown in the fig.3 glucose transformation up to pyruvate (glycolysis) and nucleoside degradation really found in cytoplasm of aerobic cells, other pathways may be both in cytoplasm and mitochondria or only in mitochondria as pyruvate transformation to acetyl-CoA, oxidation of fatty acids. Aerobic oxidation requires the presence of oxygen but the use of oxygen in a cell may be different (Fig. 26):

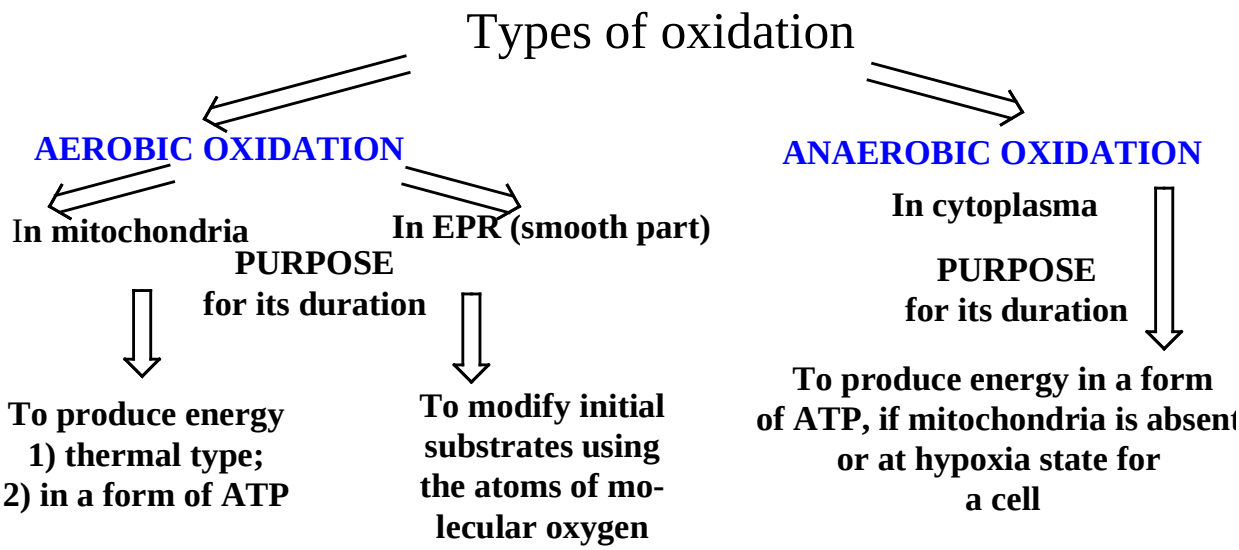


Figure 26. Features of aerobic and anaerobic oxidation in humans.

ATP may be synthesized using different ways in a cell:

1. Oxydative phosphorylation is the synthesis of ATP on the inner membrane of mitochondria due to energy produced in aerobic oxidation reactions (tissue respiration reactions)

2. Substrate phosphorylation (it is proceed in the cytoplasm, mainly):

$$ADP + X\text{-}H_2PO_3 \rightarrow ATP + X$$

Donor of phosphate $X\text{-}H_2PO_3$ may be high energy containing compound:

Creatine phosphate, Phosphoenolpyruvate, 1,3-Diphosphoglycerate.

The catabolic phase II is finished mainly by the production of active form of acetic acid named Acetyl-CoA. This molecule and oxaloacetate (may be produced in phase II from pyruvate, aspartic acid, etc.) are involved in Citric Acid Cycle (Fig. 27, CAC) – catabolic phase III, terminal.

Citric Acid Cycle may be named also as ***Tricarboxylic Acid Cycle (TAC)*** , because it contains some metabolites - tricarboxylic acids: citrate, cys-aconitate, isocitrate. The first name is associated with first product name: "citrate", that is produced from initial substrates acetyl-CoA and oxaloacetate. This process was studied in experiments in 1930th, it was finally identified in 1937 by Hans Adolf Krebs at the University of Sheffield, for which he received the Nobel Prize for Physiology or Medicine in 1953. So, the third name of this process is associated with surname, and it is Krebs cycle. All the reactions of this process are placed in the matrix of mitochondria, except Succinate dehydrogenase reaction (it is in the inner membrane).

Two oxidative decarboxylation reactions (isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase complex) prove, that this process is catabolic. Alpha-ketoglutarate dehydrogenase complex is composed from three enzymes and is related to multienzyme systems. Some intermediate metabolites are involved in anabolic pathways as synthesis of heme (succinyl-CoA), gluconeogenesis (oxaloacetate and all its precursors in CAC), in synthesis of non-essential amino acids (alpha-ketoglutarate → Glu, oxaloacetate → Asp). From this point of view, ***CAC is amphybolic pathway.***

Main products per one cycle of this process are:

- ***NADH (3 molecules), $FADH_2$*** linked to apoenzyme of succinate dehydrogenase (1 molecule)
- one molecule of high-energy bond containing substance - ***GTP*** (due to ***substrate phosphorylation*** catalyzed by succinyl-CoA thiokinase)
- ***two molecules of carbon dioxide*** (there is the utilization of two carbon atoms from oxaloacetate)

- ***oxaloacetate,*** that will be incorporated in the next round of the process.

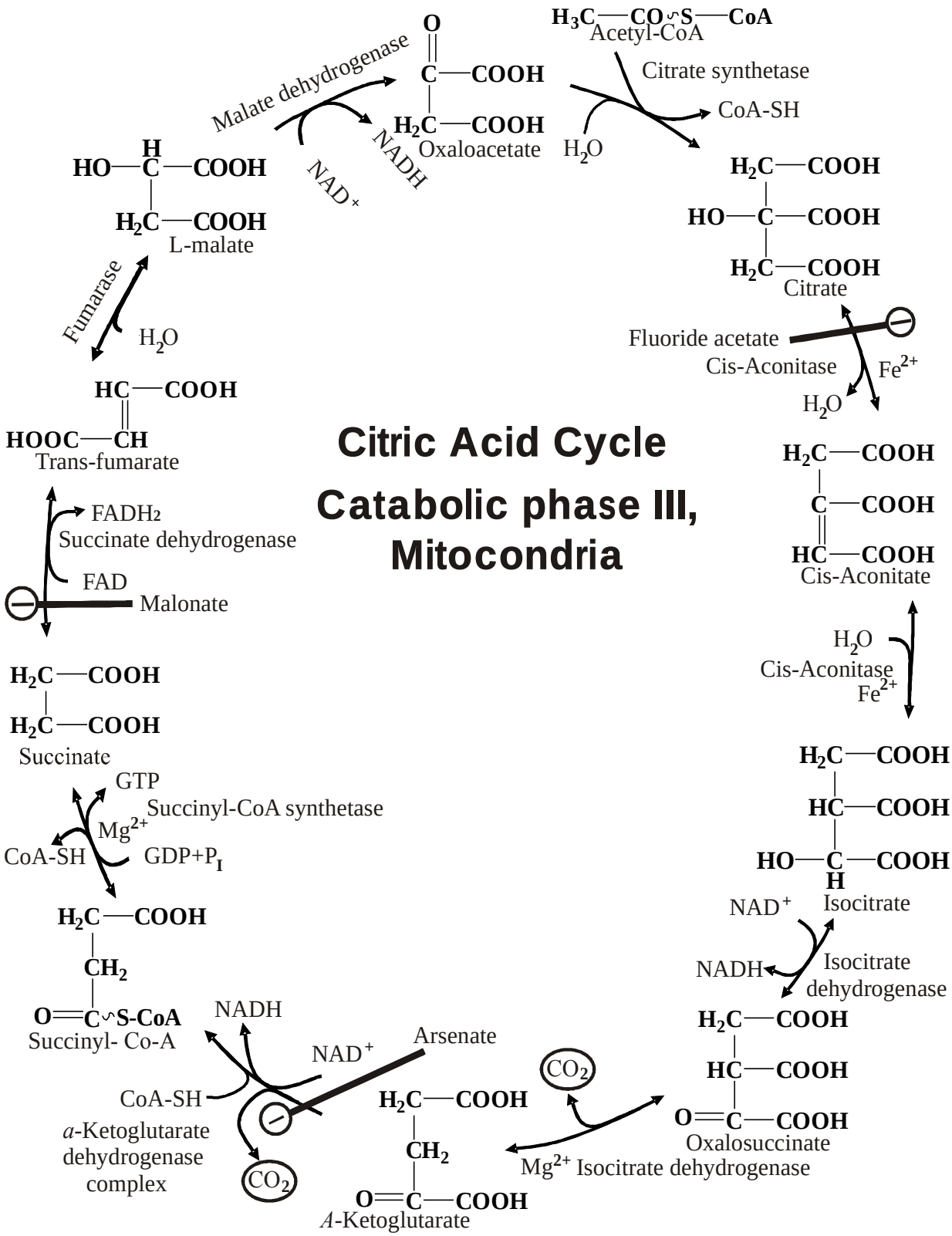


Figure 27. Citric Acid cycle reactions.

First products (3NADH and 1FADH$_2$) are found as initial donors of electrons for electron transport chain (ETC) located in the inner membrane of

mitochondria. Reduced forms of coenzyme and prosthetic group formed in catabolic phase II and III may be associated with probable energy releasein a form of ATP.

Factors needed for Citric acid cycle

duration normally with high rate

- *Ions : Mg^{2+} , Mn^{2+} , Fe^{2+}*
- *Vitamins : Thiamine, Riboflavin, Nicotinic acid or Nicotine amide, Pantothenic acid, Lipoic acid (all of them are in need for alpha-ketoglutarate dehydrogenase complex)*
- *ATP/ADP ratio in a cell is lower then 1*
- *NADH/NAD+ ratio in a cell is lower then 1*
- *High Fatty acyls-CoA are absent in the matrix of mitochondria*
- *Malonate concentration is low in the matrix*
- *Oxygen is present in a cell*

Activator for CAC:

Excess levels of ADP (for isocitrate dehydrogenase). This enzyme reaction is rate limited step for the whole process, look below inhibitors for this enzyme, too.

Inhibitors for CAC:

- Allosteric inhibitors with feed-back mechanism of action: ATP (for *isocitrate dehydrogenase, citrate synthase), NADH (for isocitrate dehydrogenase, citrate synthase), High Fatty acyls-CoA (for citrate synthase), Succinyl-CoA (for alpha-ketoglutarate dehydrogenase complex*
- Competitive inhibitors : malonate, oxaloacetate (*for succinate dehydrogenase)*
- Non-competitive inhibitor; fluoroacetate (*for cis-aconitase*)

The main role of CAC is to be a good producer of reduced forms of NADH and $FADH_2$ which are recognized as energy sources for ETC to produce energy used in oxidative phosphorylation. 12 ATP is sum total energy effect per one round of CAC, 11 ATP is produced due to oxidative phosphorylation.

CONCLUSIONS:

- Phases II, III of catabolic pathways prepare the cell to obtain intermediate metabolites named reduced forms of coenzymes (as NADH) or prosthetic groups (as FADH2) which are found in aerobic condition as donors of electrons for electron transport chain placed in the inner membrane of mitochondria.
- Any organic compound involved in catabolic pathway may be discussed as energy source for human cell except vitamins
- In aerobic condition the main way to produce ATP is oxidative phosphorylation
- In anaerobic condition for a cell energy sources (mainly ATP) are produced due to substrate phosphorylation using special compounds with high energy bond present in their structure
- The main producer of NADH is Citric Acid Cycle, and its blockage in any way of influence is dangerous for aerobic cell type: the cell will loose approximately 50% of total energy released due to catabolic pathways duration.
- Catabolic pathways produce some terminal products for humans, which are real terminal, because they are not used in humans in any reaction after their production, they are: uric acid, urea, creatinine, indican, hippuric acid.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	Examination of a patient revealed II grade obesity. It is known that he consumes a lot of sweets and fatty food, has sedentary way of life. That's why anabolic metabolism has the priority in his organism. Which of the following pathways is amphibolic? A. Glyconeogenesis B. Tricarboxylic acids Cycle C. Lipolysis D. Urea cycle E. Fatty acids oxidation	
2.	Name quantity of ATP synthesized after complete oxidation of 1 mole of acetyl-CoA in Tricarcarboxylic Acid Cycle:	

№	Test:	Explanation:
	A. 12 B. 1 C. 5 D. 8 E. 3	
3.	This compound is discussed as central (key) intermediate product for all the types of metabolisms (protein, lipid, carbohydrate, etc.). It is: A. Acetyl-CoA B. Succinyl-CoA C. Oxaloacetate D. Lactate E. Citrate	
4.	High energy containing compounds are in need to promote normal metabolism of substances.Find out those compound: A. Creatine phosphate B. Creatine C. Creatinine D. Glucose-6-phosphate E. Adenosine-5'-monophosphate.	
5.	Energy production is due to catabolic pathways, only. Name those one: A. Aerobic glycolysis B. Citric Acid Cycle C. Beta-oxidation of Fatty Acids D. Hexose Monophosphate Shunt E. Positions A, B, C are right	
6.	Propose the correct continuation of the phrase: "Citric Acid Cycle is…": A. The main producer of reduced forms of coenzymes B. Anabolic process C. Placed in cytoplasm of a cell D. The main producer of energy for erythrocytes E. Synthetic pathway	
7.	How many moles of high energy bond containing compound are produced due to substrate phosphorylation in one round of Citric Acid Cycle: A. Twelve B. Two C. One D. Three E. Four	
8.	Succinate conversion into fumarate is catalyzed by succinate dehydrogenase. Name competitive inhibitor for this enzyme:	

№	Test:	Explanation:
	A. Acetate B. Fumarate C. Malonate D. Malate E. Pyruvate	
9.	A boy of 2 years old has damaged energy exchange due to inhibition of oxidation processes and ATP synthesis. There is the decrease of Citric acid cycle metabolites content in his blood, too. It was proposed to think about probable inhibition of succinate dehydrogenase in boy's tissues as the reason of his state. Name the inhibitor for this enzyme: A. Aspartate B. Malonate C. Malate D. Glutamate E. Citrate	
10.	Citric Acid Cycle begins from condensation of oxaloacetate with acetyl-CoA to form citric acid. What is the role of oxaloacetate in this process? – It is: A. Inhibitor B. Repressor C. Reactivator D. Modificator E. Substrate	
11.	First substrate in first reaction of Krebs Cycle is: A. Acetyl CoA B. Glycine C. HCl D. Lipoprotein E. Glutamine	
12.	Krebs cycle does not occur in: A. Skeletal Muscle B. Heart C. RBC D. Liver E. All the above	
13.	Fluroacetate is found as non-competitive inhibitor for: A. Citrate synthetase B. Cis-Aconitase C. Succinate dehydrogenase D. Alpha-ketoglutarate dehydrogenase E. Malate dehydrogenase	
14.	The rate limited step for Citric Acid Cycle duration is the reaction catalyzed by: A. Citrate synthase	

№	Test:	Explanation:
	B. Cis-Aconitase C. Isocitrate dehydrogenase D. Alpa-ketoglutarate dehydrogenase E. Malate dehydrogenase	
15.	Specific inhibitor for succinate dehydrogenase is: A. Cyanide B. Malonate C. Arsenite D. Fluoride E. Malate	
16.	All these metabolic pathways take place inside the mitochondria except: A. Glycolysis B. Krebs cycle C. Urea cycle D. Oxidative phosphorylation E. Fatty acid β-oxidation	
17.	All these metabolic pathways are catabolic except: A. Glycolysis B. Krebs cycle C. Proteolysis D. Replication E. Fatty acid β-oxidation	
18.	Number of NADH molecules produced in Citric Acid Cycle per one round is: A. 2 B. 3 C. 4 D. 5 E. 6	
19.	Name substances which are really terminal products for catabolic pathways and for human organism: A. Uric acid and Urea B. Alanine and Pyruvate C. ATP and Carbon dioxide D. Amino acids and Keto acids E. Bilirubin and Urea	
20.	What class of enzymes is associated with duration of anabolic pathways, only: A. Isomerase B. Ligase C. Lyase D. Transferase E. Oxidoreductase	
21.	Exogenous substances may be involved in catabolic pathways to be used as energy sources for humans EXCEPT: A. Vitamins	

№	Test:	Explanation:
	B. Monosaccharides C. Amino acids D. Fatty acids E. Alcohols	
22.	The accumulation of NADH in the matrix of mitochondria is the signal to inhibit: A. Citrate lyase B. Cis-Aconitase C. Isocitrate dehydrogenase D. Fumarase E. Malate dehydrogenase	
23.	Amphybolic process must include intermediate metabolites which are involved in both anabolic and catabolic pathways of a cell. Choose those one: A. Proteolysis B. Hexose Monophosphate Shunt C. Citric Acid Cycle D. Malate-aspartate shuttle system E. All the above	
24.	Oxidative decarboxylation reactions occur two times in Citric Acid Cycle, but the mechanism of these reactions is not the same. Choose the conversion that may be named as oxidative decarboxylation and catalyzed by multienzyme system: A. Citrate is converted to Cis-Aconitate B. Isocitrate is converted to Alpa-ketoglutarate C. Alpa-ketoglutarate is converted to Succinyl-CoA D. Malate is converted to Oxaloacetate E. Succinate is converted to Fumarate	
25.	Name the regulatory enzyme from Citric Acid Cycle whose activity is stimulated by allosteric activator ADP at condition of its accumulation in the matrix of mitochondria: A. Citrate synthase B. Cis-Aconitase C. Isocitrate dehydrogenase D. Alpa-ketoglutarate dehydrogenase E. Succinate dehydrogenase	
26.	Urea is named as terminal product for humans, because: A. It is not produced in human tissues B. It is not involved in any transformation after its production C. It is removed across bile ducts only D. It is single molecule that is not utilized by human tissues	

№	Test:	Explanation:
	E. Positions C& D are right	
27.	Choose the value for ATP/ADP ratio in aerobic cell to stimulate the rate of Citric Acid Cycle duration: A. 0.3 B. 1.2 C. 1.5 D. 2.0 E. 2.5	
28.	Under the development of hypoxia state there is production of ATP in a cell mainly due to: A. Oxidative phosphorylation B. Protein kinase action C. Substrate phosphorylation D. Oxidative decarboxylation E. NADPH use	
29.	Catabolic phase I reactions for organic compounds in human organism may be placed in: A. Any place of human body B. Ribosome C. EPR rough part D. Gastrointestinal tract E. Cellular membrane	
30.	Vitamin B1 (thiamine) deficiency will cause the decrease of the rate of Citric Acid Cycle because one enzyme system in this process is in need of its derivative as coenzyme TPP. Name it: A. Isocitrate dehydrogenase B. Citrate synthase C. Pyruvate dehydrogenase complex D. Alpha-ketoglutarate dehydrogenase complex E. Malate dehydrogenase	

GENERAL BASES OF BIOENERGETICS

(Krisanova N.V.)

INFORMATIONAL MATERIAL

Tissue Respiration: stages, respiratory control

Tissue respiration is sum total of all the reactions which are producers of reduced forms of NADH, $FADH_2$, $FMNH_2$, NADPH – initial donors of electrons for electron transport chains (ETC), and these electrons are passed to molecular oxygen. If ETC is placed in the inner membrane of mitochondria and initial donors are NADH, $FADH_2$, $FMNH_2$, the release of energy may be utilized in a form of synthesized ATP molecules.

Tissue respiration is divided in three stages:

Stage I – it is catabolic phase II

Stage II – it is Krebs Cycle

Stage III – it is ETC reactions placed in the inner membrane of mitochondria and composed from complexes I, II, III, IV, ubiquinone (CoQ), cytochrome C. The moment of tissue respiration is the moment of electrons attraction by molecular oxygen.

All the factors, which control the rate of Krebs Cycle, are in control of tissue respiration and the main important is:

ATP/ADP ratio

- **It is very important to value the ratio ATP/ADP in a cell. It is named as *respiratory control*. It can be:**

$\frac{ATP}{ADP}$ **<1 , the rate of Krebs Cycle duration increases**

$\frac{ATP}{ADP}$ **>1, The rate of Krebs Cycle duration decreases**

$\frac{ATP}{ADP}$ **≠0; if it equals zero, it means the cell death**

ELECTRON TRANSPORT CHAIN (ETC) placed in the inner membrane is the sequence of enzymes and transporters of electrons (CoQ, Fe-S-Proteins) promoted the transfer of electrons from NADH, $FADH_2$ to molecular oxygen O_2. Due to experiments with inner membrane it was proved that:

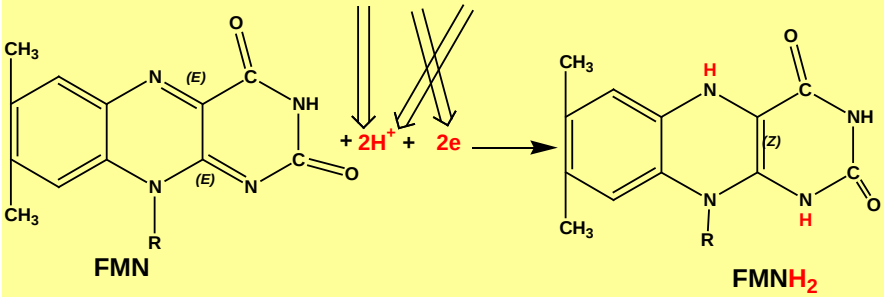
ETC contains four complexes:

	Composition of non-protein parts :
Complex I **NADH-coenzyme Q reductase (NADH-dehydrogenase)**	**FMN and Iron-Sulfur (Fe-S)- centers**
Complex II **Succinate dehydrogenase, Cytochrome b_{560}**	**FAD and Fe-S- centers**
Complex III **Cytochromes *b*, c_1** **Fe-S-proteins**	**Heme containing Fe $^{2+}$/ Fe $^{3+}$**
Complex IV **Cytochrome *C* oxidase (Cytochrome aa_3)**	**Heme containing Fe $^{2+}$ /Fe $^{3+}$** **Cu^{+}/Cu^{2+}**

Complex I is a transmembrane enzyme system which crosses the inner membrane in the direction from the matrix to the intramembrane space. It is shown below the

Complex I function at the moment of HADH oxidation

$NADH+H^+$



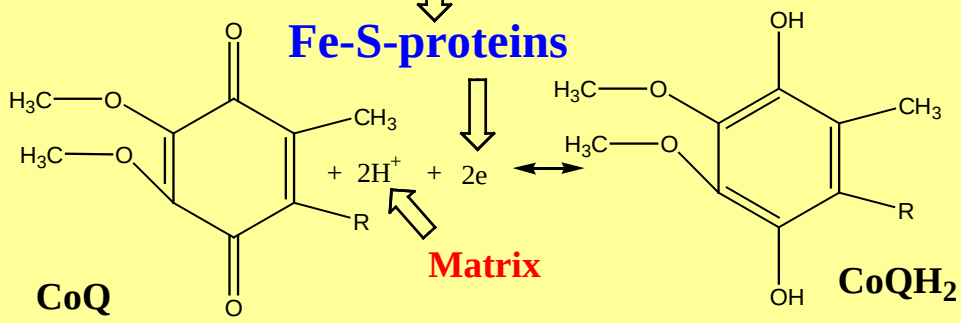
$NADH + H^+ + FMN{\bullet}E \rightarrow NAD^+ + FMNH_2{\bullet}E$ (1)

FMN• E – NADH-dehydrogenase (oxidized form)

Two electrons and two protons are accepted by FMN (the non-protein part of NADH-dehydrogenase) from $NADH+H^+$, and then across Fe-S-proteins of complex I electrons are passed to CoQ, but not protons – these particles from the matrix are accepted by CoQ:

Ubuquinone (CoQ) function at the moment of oxidation of complex I

$FMNH_2*E$



$$CoQ + FMNH_2{\bullet}E \rightarrow FMN{\bullet}E + CoQH_2 \quad (2)$$

The function of complex III

$$CoQH_2 + 2\ cyt\ b\ (Fe^{3+}) \rightarrow 2\ cyt\ b(Fe^{2+}) + + CoQ + 2H^+ \quad (3)$$

$$2\ cyt\ b(Fe^{2+}) + 2cyt\ c_1(Fe^{3+}) \rightarrow 2cyt\ b(Fe^{3+}) + +2\ cyt\ c_1(Fe^{2+}) \quad (4)$$

$$2\ cyt\ c(Fe^{3+}) + 2cyt\ c_1(Fe^{2+}) \rightarrow 2cyt\ c(Fe^{2+}) + + 2\ cyt\ c_1(Fe^{3+}) \quad (5)$$

Complex III also is transmembrane enzyme system, and it has ability to be a pump for protons. Complex IV is named cytochrome C oxidase (other name: cytochrome *aa*3), because it is acceptor of electrons from cytochrome C:

The function of complex IV

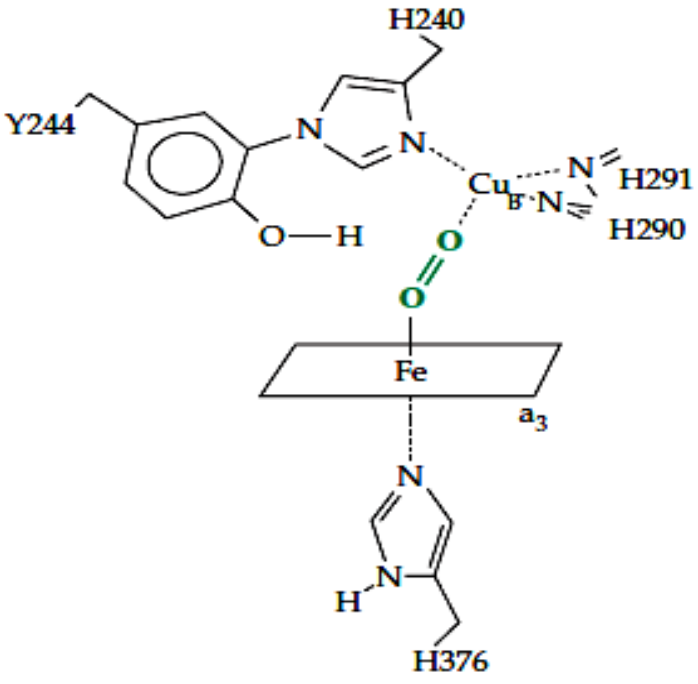
$$\text{cyt } aa_3(Fe^{3+}, Cu^{2+}) + 2 \text{ cyt c}(Fe^{2+}) \longrightarrow$$
$$\longrightarrow 2 \text{ cyt c}(Fe^{3+}) + \text{cyt } aa_3\ (Fe^{2+}, Cu^{+}) \quad (6)$$

$$\text{cyt } aa_3\ (Fe^{2+}, Cu^{+}) + \tfrac{1}{2}O_2 \longrightarrow$$
$$\longrightarrow \text{cyt } aa_3(Fe^{3+}, Cu^{2+}) + O^{2-}$$

or: (7)

$$4\,e^- + 8\,H^+_{IN} + O_2 \longrightarrow 2\,H_2O + 4\,H^+_{OUT}$$

After attraction of electrons complex IV has the ability to pass them to molecular oxygen which is also substrate for this complex (Fig. 28):



The $Cu_B \bullet A_3$ center of cytochrome oxidase

Figure 28. The mechanism of oxygen linkage by cytochrome *aa*3.

Oxygen becomes active particle oxide-anion (reaction 6), and then water is produced (reaction 7). Protons also may be pumped by this complex to intramembrane space of mitochondria.

Oxidative phosphorylation. Inhibitors of tissue respiration.

Uncouplers of oxidative phosphorylation and tissue respiration

The biophysical researches associated with determination of red-ox-potentials of each red-ox pair of ETC proved, that there is free energy formation thanks to each step of ETC. The change of red-ox-potential of each red-ox pair is estimated in volts due to polarography method. This change becomes more and more in the direction of electron transport from NADH to molecular oxygen (the biggest value is red-ox potential for pair cytochrome oxidase (Fe^{2+}/Cu^{+}) / O_2 , it equals +0,48 volt)

Some part of released energy is thermal energy, and another part may be transformed into electrochemical potential of the inner membrane of mitochondria and then used for ATP synthesis there. This process is named oxidative phosphorylation. The energy released for each electron pair passing through ETC is coupled with the formation of 3 ATP from ADP and inorganic phosphate, if one NADH is a donor of electrons to ETC.

Released energy is kept in two high-energy bonds of ATP molecule shown using sign **~ (Fig. 29).** There are three coupling sites in ETC, where complexes I, III, IV are placed. In each site energy is sufficient to support the formation of 1 mole of ATP. The change of red-ox potential in each site must be more then 0.22 volt.

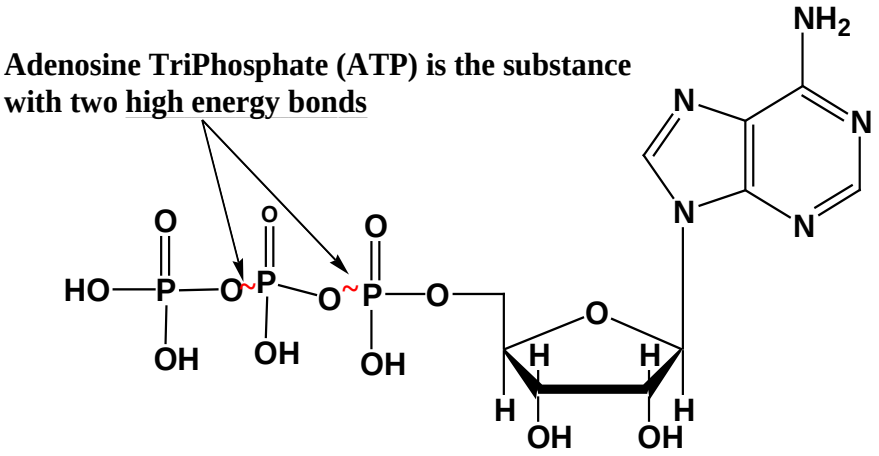


Figure 29. ATP structure.

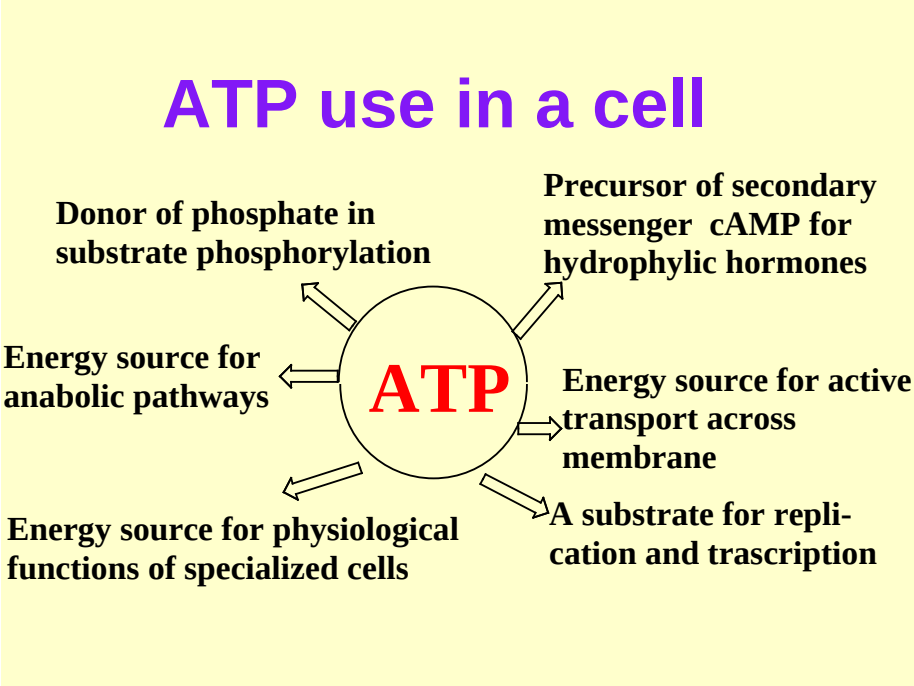


Figure 30. Production of ATP is mainly in mitochondria, but the use of ATP is very spread in a cell.

The chemiosmotic coupling hypothesis was probably the most widely accepted theory of oxidative phosphorylation. It was proposed by Peter Mitchell in 1961 to explain the mechanism of oxidative phosphorylation (Fig. 31).

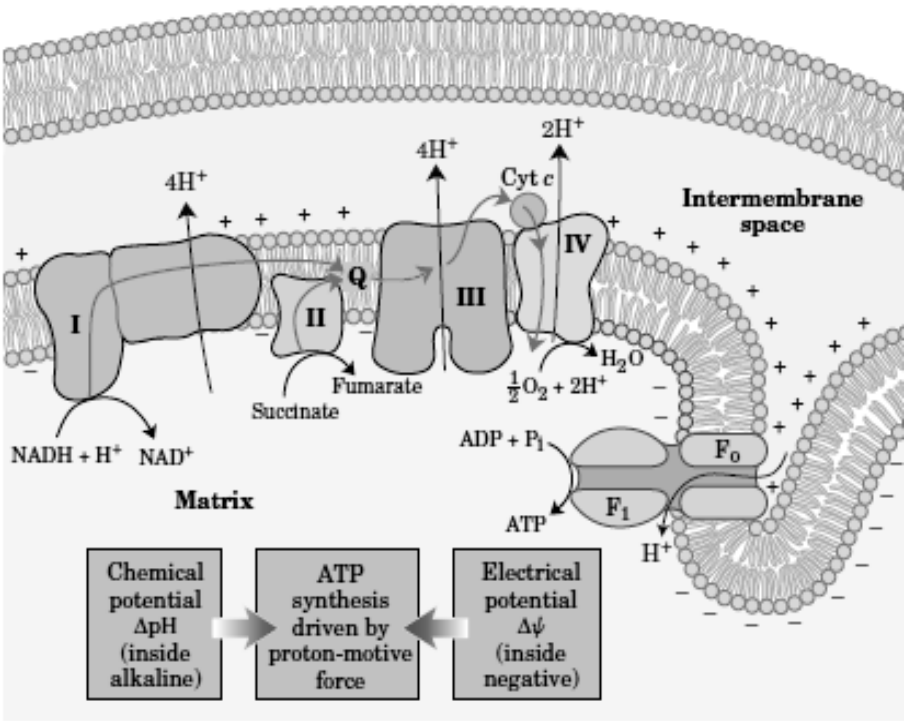


Figure 31. ETCs composition (long and short): pumps for protons are complexes I, III, IV. ATP-synthetase location and function.

The notions of chemiosmotic coupling hypothesis:

1. The inner membrane is not permeable for protons in any site of membrane. Some electron carriers are hypothesized to act as pumps, which cause directional pumping of protons across the inner membrane (Figure 2). As the electrons move down the chain, protons are expelled, penetrating from the matrix to the intramembrane space.

2. Because proton is a charged particle, the flow of free energy across the inner membrane is due to the combination of concentration gradient (ΔpH^+) and charge gradient $\Delta\Psi$. The sum of them is electrochemical potential of the inner membrane ($\Delta\mu H^+$).

3. It was proposed that chemical gradient of protons across the inner membrane of mitochondria serves as the means of coupling the energy flow of electron transport to the formation of ATP.

4. Protons pass back into the matrix at a special site or "pore" where ATP synthetase resides (figure 2). The dissipation of energy that occurs as the protons pass down the concentration gradient to the matrix allows the formation of ATP by synthetase. ATP synthetase is also known as H^+-ATPase or F_oF_1-ATPase. F_o spans the inner membrane and is composed of 4 types of subunits. The F_o forms a channel for protons from intramembrane space to the matrix. F_1 is tightly bound to F_o and sits on the matrix side of the inner membrane. F_1 is composed of 5 types subunits and it contains the catalytic site for ATP synthesis.

5. P. Mitchell suggested that ATP formation from ADP and phosphoric acid did not require energy. ATP is obtained in a linked form with F_1. Energy is required for ATP elimination from F_1. This action is due to the moment of protons moving through F_o as a channel for protons. This proton channel may be blocked by: Oligomycin and Dicyclohexylcarbodiimide (DCCD).

Coupling of oxidative phosphorylation to respiration act found in ETC is controlled

The rate of respiration may be controlled by the concentration of ADP because oxidation and phosphorylation are tightly coupled. The energy released from oxidation reactions is used to phosphorylate ADP to create ATP, which harnesses the energy for use throughout the body.

Five states of respiratory control have been defined in which the rate of respiration is limited by different factors:

State 1 is limited by availability of ADP and substrate (source of electrons).

State 2 is limited by the availability of substrate.

State 3 is limited by the capacity of the electron chain itself, when ADP, oxygen and substrate are saturating.

State 4 is limited by the availability of ADP.

State 5 is limited by availability of oxygen.

ADP/ATP transport is promoted by ATP/ADP translocase system placed in both two membranes of mitochondria, and the rate of this transport is the factor to control both oxidative phosphorylation and respiration rates. Inhibitors of this system are: Atractyloside and Bongkrekic acid.

The P/O ratio is a measure of how many moles of ATP are formed from ADP per gram atom of oxygen for given substrate.

Substrates that donate electrons to NAD yield P/O ratio of 3.

Substrates that donate electrons to FAD or FMN yield P/O ratio of 2.

This index value may be changed, if inhibitors of tissue respiration appear in a cell or substances – uncouplers are present there.

Inhibitors for tissue respiration

Inhibitors for complex I:

1) Rotenon, an insecticide;
2) Barbiturates (drugs for sleepless treatment);
3) Piericidin A, an antibiotic.

The activity of NADH-dehydrogenase equals zero and NADH accumulates in the matrix. P/O = 0 for substrates that donates electrons to NAD.

Inhibitors for complex II:

1) Malonic acid – the competitive inhibitor for succinate dehydrogenase;
2) Carboxin;
3) Thenoyl trifluoride acetone (TTFA).

Substances (2) and (3) block the electron transmission from $FADH_2$*SDHase to CoQ. P/O = 0 for succinate oxidation.

Inhibitors for complex III:

1) Dimercaprol;
2) Antimycin A, an antibiotic.

P/O may be lower then 3 or 2 (respectively oxidized substrate).

Inhibitors for complex IV:

1) Carbon monooxide which competes with oxygen for its binding site on CChOase;
2) Hydrogen sulphide;
3) Azides (an N_3 –containing organic compounds);
4) Cyanides block the heme-centers of cytochrome c oxidase, binding by covalent bonds.

In increased concentrations these substances block the tissue respiration completely. As the result the death will be for living system, and there is no any sense to determine the P/O ratio in this case.

Uncouplers of oxidative phosphorylation and tissue respiration

They are compounds that allow normal function of electron transport chain without production of ATP. ***Uncouplers allow leakage or transport of protons across the membrane, thus collapsing the proton gradient , because of their property to be attractors of protons and to be very lipophilic compounds. P/O equals 0 under their accumulation in a cell.*** Oxidative phosphorylation uncouplers are diverse group of compounds that include:

- ***2,4 - dinitrophenol (weight-loss drug in 1970s but was discontinued because of it toxicity);***
- ***dicumarol that is anticoagulant;***
- ***chlorcarbonylcyanide phenylhydrazone (CCCP);***
- ***thyroxin in high abnormal concentration;***
- ***valinomycin, an antibiotic.***

The release of energy is observed as thermal energy, only, in this case. There is the overheating of living system in this case. Under the hyperthyroidism development (T_3 and T_4 are in high concentration in human person) there is the infringement of thermal balance at diseased people, because T_4 becomes to be uncoupler.

Back-side products of ETC and their utilization in humans

The end-product of ETC is water that is produced from oxygen and protons due to complex IV function:

$$O_2 + 4\,H^+ + 4e \rightarrow 2H_2O$$

Some free radicals may be formed from oxygen during the ETC function as back-side products which are dangerous under their accumulation in a cell:

- ***Superoxide anion $Ó_2^-$,***
- ***Superoxide radical $Ó_2$,***
- ***Hydrogen peroxide radical HOÓ***
- ***Hydrogen peroxide H_2O_2***

All these particles are highly reactive, can react with and damage DNA, membrane phospholipids, modify proteins in a cell. There are some enzyme systems that can protect cells from free radicals damage:

- Superoxide dismutase: $2\,Ó_2^- + 2H^+ \rightarrow H_2O_2 + O_2$
- Peroxidase and Catalase: $2\,H_2O_2 \rightarrow 2H_2O + O_2$
- Glutathione peroxidase : $H_2O_2 + 2\,GSH \rightarrow 2H_2O + GS\text{-}SG$
- Glutathione reductase reduces the oxidative form GS-SG:

$$GS\text{-}SG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$$

Some vitamins are used as antioxidants: Retinol, L-ascorbic acid, vitamin E, vitamin K, $CoQH_2$; Melatonin and Selenium derivatives have ability to protect cells under the influence of free radicals and hydrogen peroxide, too.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	Cytochrome C preparation is used to improve tissue respiration and oxidation processes under the asphyxia state in newborns. Name the organic compounds class for cytochrome C: A. Hemoproteins B. Phosphoproteins C. Nucleoproteins D. Glycoproteins E. Lipoproteins	
2.	The overdose of hormone preparation thyroxin prescribed for patient caused the increase of body temperature. Hyperthermia was developed due to uncoupling of biological oxidation with: A. Beta-oxidation of fatty acids B. Oxidative phosphorylation C. Oxidative deamination of amino acids D. Oxidative decarboxylation of pyruvate E. Peroxidation of lipids	
3.	Anaerobic microorganisms die at the presence of oxygen because cytotoxic hydrogen peroxide is produced, and it is not destroyed under the absence in these microorganisms of enzyme named: A. Polymerase B. Protease C. Catalase D. Reductase E. Lactase	
4.	The patient got big dose of barbiturate derivative (hipnotic drug amital) that is inhibitor of NADH-dehydrogenase in electron transport chain of inner membrane of mitochondria. Choose process that is disturbed by this medicine in human organism: A. Melanin synthesis B. Ammonia utilization C. Lipid synthesis D. ATP synthesis E. Amino acid synthesis	

№	Test:	Explanation:
5.	During respiration the oxygen enters the organism. Name cellular compartment involved in oxidative phosphorylation at the presence of oxygen: A. Nucleus B. Mitochondria C. Endoplasmic reticulum D. Lysosome E. Ribosome	
6.	There is blockage of tissue respiration enzymes (cytochromes) in patient's organism. Name type of hypoxia that is developed in this patient: A. Hypoxic B. Tissue C. Respiratory D. Circulatory E. Hemic	
7.	Electron microscopy method fixed destruction of mitochondria. Name processes that were damaged in this case: A. Synthesis of ATP B. Lipolysis C. Glycolysis D. Lipogenesis E. Hexose monophosphate shunt	
8.	Production of energy used in ATP synthesis is due to many enzymes. Electron transport chain enzymes are placed in: A. Nucleus B. Lysosomes C. Ribosomes D. Mitochondria E. Peroxysome	
9.	Mitochondria is cellular organelle represented in all cells except erythrocytes, bacteria, blue-green algae. Name one from main functions of mitochondria in a cell: A. Secretion B. Construction of ribosome C. Hydrolysis of compounds D. Oxidative phosphorylation E. Lipolysis	
10.	Hydrogen peroxide is used in the treatment of bleeding wounds, and it is degraded during this operation by one enzyme from blood. Name this enzyme: A. Catalase B. Cytochrome oxidase C. Monoaminooxidase D. Lactate dehydrogenase	

№	Test:	Explanation:
	E. Aspartate aminotransferase	
11.	Antibiotic Olygomycin was prescribed for the treatment of patient suffered from tuberculosis. Name the process placed in mitochondria that is suppressed by this medicine: A. Oxidative decarboxylation B. Lipid peroxidation C. Oxidative phosphorylation D. Substrate phosphorylation E. Oxidation of NADH	
12.	It is known, that some compounds are uncouplers for tissue respiration and oxidative phosphorylation find out those one: A. Carbon monooxide B. Lactate C. 2.4-dinitrophenol D. Antimycin A E. Acetyl-CoA	
13.	Potassium cyanide is the poison that causes the human death immediately. Name mitochondrial enzymes which are blocked by cyanides: A. Cytochrome P-450 B. Flavoproteins - enzymes C. Cytochrome b_5 D. NAD^+ - dependent dehydrogenases E. Cytochrome c oxidase [cytochrome *aa*3]	
14.	Active forms of oxygen derivatives are produced during metabolic pathways duration (for example superoxide anion radical $^{\bullet}O_2^-$). This dangerous particle is destroyed by the enzyme: A. Superoxide dismutase B. Catalase C. Peroxidase D. Glutathione peroxidase E. Glutathione reductase	
15.	Potassium cyanide caused the death immediately with symptoms of total hypoxia. The most probable reason of cyanide toxic action is the inhibition of enzyme activity named: A. ATPase B. NADH-dehydrogenase C. ATP synthetase D. NADPH-dehydrogenase E. Cytochrome oxidase	
16.	Synthesis of ATP coupled with oxidation reactions and promoted by electron transport chain enzymes is named: A. Oxidative phosphorylation	

№	Test:	Explanation:
	B. Substrate phosphorylation C. Free oxidation D. Phosphorylation during photosynthesis E. Peroxide oxidation	
17.	Medical examiner has revealed that the death of 20 y.o. girl came due to poisoning by cyanides. Name the enzyme whose activity was blocked by cyanides: A. Malate dehydrogenase B. Cytochrome C oxidase C. Heme synthetase D. Aspartate aminotransferase E. Carbamoyl phosphate synthetase	
18.	Medical examiner has revealed that the death of 20 y.o. girl came due to poisoning by cyanides. Name the process whose damage caused the death of this girl: A. Tissue respiration B. Hemoglobin synthesis C. A transport of oxygen by hemoglobin D. Urea synthesis E. Malate-aspartate shuttle system	
19.	The patient in severe form of unconsciousness state was admitted to reanimation department. The overdose of barbiturates use was determined for him that caused symptoms of tissue hypoxia. Name part of ETC in mitochondrial inner membrane that is blocked by this compound: A. Ubiquinone B. Cytochrome b-cytochrom c1 C. Succinate-coenzyme Q-reductase D. Cytochrome C oxidase E. НАДН-coenzyme Q-reductase	
20.	Hyperthermia, bulimia, decrease of body mass is observed at patients with thyrotoxicosis. These clinical symptoms are developed due to infringements of : A. Lipid synthesis B. Oxidation of fatty acids C. Coupling of oxidation and oxidative phosphorylation D. Citric Acid Cycle E. ATP hydrolysis	
21.	Rotenone - insecticide poisoning was observed for the patient. Name part of ETC in mitochondrial inner membrane that is blocked by this compound: A. ATP-synthase B. Coenzyme Q – cytochrome C reductase C. Succinate-coenzyme Q-reductase	

№	Test:	Explanation:
	D. Cytochrome C oxidase E. NADH- coenzyme Q-reductase	
22.	Cyanides are powerful cell poisons that can cause the human death. Name enzyme of tissue respiration whose activity is blocked by cyanides: A. Cytochrome C oxidase B. Glucose-6-phosphate dehydrogenase C. Catalase D. Ferrochelatase E. Hemoglobin reductase	
23.	A woman 38 y.o. suffers from sweating, heart bit, the increase of body temperature in the evening. Basic metabolism rate is increased in 60%. Doctor made diagnosis: thyrotoxicosis. Name property of thyroxin (T4) that causes the increase of body heat production: A. T4 decreases deamination rate for amino acids B. T4 increases rate of coupling for oxidation and oxidative phosphorylation C. T4 causes the accumulation of acetyl-CoA D. T4 in excess levels is uncoupler for oxidation and oxidative phosphorylation E. T4 decreases the rate fatty acids oxidation	
24.	There is the reduction of molecular oxygen in mitochondrial electron transport chains up to hydrogen peroxide under the development of pathologies associated with hypoxia. Find out enzyme which promotes the degradation of cytotoxic compound – hydrogen peroxide: A. Catalase B. Cytochrome oxidase C. Succinate dehydrogenase D. Alpha-ketoglutarate dehydrogenase E. Aconitase	
25.	Increased production of thyroidal hormones T3 and T4, weight loss, tachycardia, psychic excitement and so on present in patient with thyrotoxicosis. How thyroidal hormones do effect on energy metabolism in the mitochondrion of cells? A. Disconnect oxidation and oxidative phosphorylation B. Activates phosphorylation of substance C. Stops phosphorylation of substance D. Stops respiratory chain E. Activates oxidative phosphorylation	
26.	A patient is followed up in an endocrinological dispensary on account of hyperthyroidism. Weight loss, tachycardia, finger tremor are accompanied with hypoxia similar symptoms – headache,	

№	Test:	Explanation:
	fatigue, eye flicker. Find out the result for the influence of high level of thyroid hormones on tissue respiration causing the development of hypoxia similar symptoms: A. Specific binding of active centers of respiratory enzymes B. Intensification of respiratory enzymes synthesis C. Competitive inhibition of respiratory enzymes D. Inhibition of respiratory enzymes synthesis E. Uncoupling of oxidation and phosphorylation	
27.	Profuse foam appeared when dentist put hydrogen peroxide on the mucous of the oral cavity. What enzyme caused such activity? A. Glucose-6-phosphatdehydrogenase B. Cholinesterase C. Acetyl transferase D. Catalase E. Methemoglobin reductase	
28.	During metabolic process active forms of the oxygen including superoxide anion radical are formed in human body. With help of what enzyme this anion is inactivated? A. Glutathione peroxidase B. Catalase C. Peroxidase D. Superoxide dismutase E. Glutathione reductase	
29.	Catabolic reaction is named as tissue respiration reaction if: A. It is producer of CO_2 B. It is the user of O_2 as a substrate C. It is producer of ATP D. It is producer of NADH or $FADH_2$ under aerobic condition E. Positions B & D are right	
30.	The energy of ATP may be used for the transport of some substances across membrane. Name those mechanism of the transport: A. Osmosis B. Filtration C. Simple diffusion D. Active transport E. Facilitated diffusion	
31.	Inherited disorders of glutathione peroxidase in RBC cause the hemolytic anemia in patients. Name process that is damaged under those condition in humans: A. Citric acid cycle B. Purine metabolism	

№	Test:	Explanation:
	C. Anaerobic glycolysis	
	D. Oxidation of fatty acids	
	E. Utilization of active forms of oxygen	

ANAEROBIC OXIDATION OF GLUCOSE – GLYCOLYSIS. SYNTHESIS OF GLUCOSE – GLUCONEOGENESIS

(Rudko N.P.)

INFORMATIONAL MATERIAL

Carbohydrates are aldehydes or ketones of high polyatomic alcohols or compounds that yield these derivatives in hydrolysis. They occur naturally in plants (where they are produced photosynthetically), animals and microorganisms and fulfil various structural and metabolic roles. Rice, potatoes, bread, corn, candy, and fruits are rich in carbohydrates.

Dietary carbohydrates are cleaved during digestion, forming monosaccharides (mainly glucose) that enter the blood. The major dietary carbohydrate is starch, the storage form of carbohydrate in plants. Monosaccharides (mainly glucose, some fructose and galactose) present in the diet or produced by the digestive process, are absorbed by the intestinal epithelial cells and passed into the blood.

The products of digestion enter cells from the blood.

There are various mechanisms of glucose transport across the cell membrane. Special proteins - glucose transporters (GLUTs) are a wide group of membrane proteins that facilitate the transport of glucose over a plasma membrane. Binding of glucose to one site provokes a conformational change associated with transport, and releases glucose to the other side of the membrane. Each glucose transporter isoform plays a specific role in glucose metabolism determined by its pattern of tissue expression, substrate specificity, transport kinetics, and regulated expression in different physiological conditions. 13 members of the GLUTs family have been identified. Greatest interest is GLUT4 as it is the insulin-regulated glucose transporter found primarily in adipose tissues and striated muscle (skeletal and cardiac).

When glucose enters cells, it is converted by hexokinase to glucose 6-phosphate, which is a pivotal compound in several metabolic pathways:

1) aerobic oxidation of glucose to CO_2 and H_2O;

2) anaerobic glycolysis, which produces lactate and generates ATP;

3) synthesis of glycogen or compounds such as glucose aminoglycans, proteoglycans;

4) pentose phosphate pathway (hexose monophosphate pathway HMP), which produces NADPH for reactions such as biosynthesis of fatty acids and ribose for nucleotide production.

The processes described above are stimulated by insulin to promote the decrease of blood glucose levels and the intracellular glucose level, too.

Ways of blood glucose pool use are submitted in Figure 32.

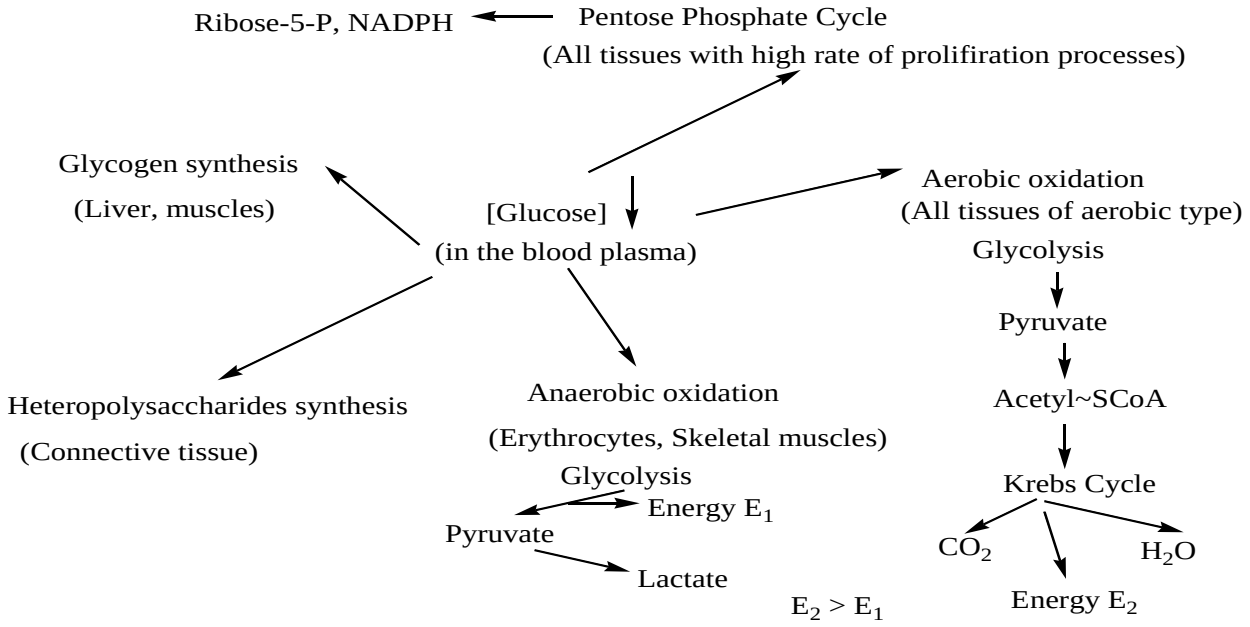


Figure 32. Glucose utilization processes in all types of tissues of human body.

Monosaccharides produced due to digestion leave the intestinal epithelial cells and enter the hepatic portal vein. Therefore, the liver is the first organ through which these products pass.

Glucose is the most acceptable form of carbohydrate to human tissues. All other carbohydrate in the body can be formed from glucose. Glucose is converted to other carbohydrate having specific functions.

The fate of glucose

in the liver

1. Glucose is oxidized to CO_2 and H_20 to meet the immediate energy needs of the liver.

2. Excess glucose is stored in the liver as glycogen, which is used during periods of fasting to maintain blood glucose.

3. Excess glucose may be converted (across acetyl-CoA) to fatty acids and a glycerol moiety (across dihydroxyacetone phosphate), which combine to form triacylglycerols (TG). TG are incorporated into VLDL to be released from the liver to the blood.

4. Glucose is involved into HMP

in the brain - which depends on glucose for its energy needs, glucose is oxidized to CO_2 and H_20, producing ATP; glucose is involved into HMP

in the red blood cells - they, lacking mitochondria, oxidize glucose to pyruvate and lactate, which are released into the blood; glucose is involved into HMP.

in the muscle cells

1. Glucose is oxidized to CO_2 and H_20 to generate ATP for muscle contraction.

2. Muscles store glucose as glycogen for use during contraction.

in the cells of adipose tissue – they take up glucose by a transport process that is stimulated by insulin and use it to produce energy and to form the glycerol moiety of its triacylglycerol stores.

in the cells of connective tissue – they take up glucose for synthesis of glucose aminoglycans

The processes that yield energy

Cells derive their energy from the chemical bonds of organic molecules. The metabolic processes that break down sugars to derive energy consist of two major types.

1. **Tissue respiration:** Break down 6C sugars in presence of oxygen to CO_2 and H_2O; most efficient source of energy.

2. **Fermentation:** In animals it usually named anaerobic glycolysis. It is break down 6C sugars to 3C or 2C compounds to derive some energy, occurs without oxygen.

Anaerobic glycolysis

It is the metabolic pathway in which any monosaccharide (mainly glucose) is split into two molecules of lactate to produce energy under anaerobic condition.

The catabolic pathway consists of eleven reactions each catalyzed by specific enzyme. In fact glycolysis is realized by means of the following developments:

1. To add phosphoryl groups to activate glucose.

Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated. Why phosphorylated intermediates are there? Phosphate groups are ionized at pH 7, giving each glycolytic intermediate a net negative charge. Because the plasma membrane is impermeable to charged molecules, the phosphorylated intermediates cannot disperse out of the cell. Energy used in the formation of the phosphate ester is partially conserved.

2. To convert the phosphorylated intermediates into high energy phosphate compounds and to couple the transfer of the phosphate to ADP to form ATP.

High energy phosphate compounds formed in glycolysis such as 1,3-bisphosphoglycerate and phosphoenolpyruvate donate phosphoryl groups to ADP to form ATP due to substrate phosphorylation. This reaction yields ATP when phosphoenolpyruvate is converted to pyruvate. This reaction could be the last, but in the process of glycolysis oxidized NAD^+ is used, which serves as an acceptor of electrons from the glyceraldehyde phosphate and thus it is converted to the reduced form NADH.

Thus, if glycolysis were to continue indefinitely, all of the NAD^+ would be used up, and glycolysis would stop. To allow glycolysis to continue, organisms must be able to oxidize NADH back to NAD^+.

There are three possible catabolic fates of the pyruvate & NADH formed in

glycolysis:

1. Under aerobic conditions, pyruvate is oxidized to CO_2 and NADH, $FADH_2$, which are oxidized to NAD^+, FAD due to oxidative phosphorylation yields ATPs.

2. Under anaerobic conditions NADH reduces pyruvate directly into lactate (anaerobic glycolysis (for example in muscle) or it is called homolactic fermentation in procaryotes).

3. Under anaerobic conditions in yeast, pyruvate is decarboxylated to yield CO_2 and acetaldehyde which is reduced by NADH to ethanol and NAD^+ is regenerated (alcoholic fermentation). Net reaction for the pathway named alcoholic fermentation of glucose is:

enzymes

$C_6H_{12}O_6 \rightarrow\text{----} \rightarrow 2C_2H_5OH + 2\ CO_2$

Biological significance of pyruvate conversion to lactate in anaerobic glycolysis (homolactic fermentation) and pyruvate to ethyl alcohol in alcoholic fermentation is replenishment of NAD^+.

Production of glucose by the liver

The liver has the major responsibility for maintaining blood glucose levels, initially by the process of glycogenolysis and subsequently by gluconeogenesis.

Glycogenolysis - About 2-3 hours after a meal, the liver begins to break down its glycogen stores by the process of glycogenolysis, and glucose is released into the blood. Glucose then can be taken up by various tissues and oxidized.

Glycogenolysis is stimulated by glucagons and epinephrine.

Gluconeogenesis is an anabolic pathway whereby non-carbohydrate precursors are converted to glucose. It is one of the two main mechanisms humans and many other animals use to keep blood glucose levels from hypoglycemia (dropping too low). This process occurs during periods of fasting, starvation, low-carbohydtrate diets, or intense exercise. After about 4-6 hours of fasting, the liver begins the process of gluconeogenesis. Within 30 hours, liver glycogen stores are

depleted, leaving gluconeogenesis as the major process responsible for maintaining blood glucose.

Gluconeogenesis occurs mainly in liver. It occurs to a more limited extent in kidney & small intestine under some conditions. Glucose is synthesized between almost nil and perhaps 200 g/day in adults in: liver (90%), kidney cortical layer (10%), small intestine (0,1%).

Location within the cell (if pyruvate is substrate):

- it is started in mitochondrion &
- is continued in cytoplasm &
- is finished in the lumen of the endoplasmic reticulum (Glucose-6-phosphatase is embedded in the endoplasmic reticulum membrane in liver cells)

Substrates:

lactate (produced in RBC, skeletal muscles due to anaerobic glycolysis);

glycerol (produced in adipocytes due to lipolysis);

glucogenic amino acids (all except Leu, Lys);

propionyl CoA (due to oxidation of odd carbon chain fatty acids taken up from vegetable foodstuff mainly)

Most precursors must enter the Krebs cycle at some point to be converted to oxaloacetate.

So, if lactate come from

- either erytrocytes (anaerobic glycolysis produced lactate is single pathway for ATP production there) or
- muscles (after exhausting muscular work, intensive physical trainings), or
- other organs at diseases of the respiratory system and circulatory disoders (hypoxia state).

It is converted to pyruvate by lactate dehydrogenase in cytosol, pyruvate is translocated to mitohondrion and then converted to oxaloacetate by pyruvate carboxylase. Oxaloacetate is involved in gluconeogenesis.

If amino acids such as alanine and other come from blood and muscle proteins they are converted in pyruvate (alanine) or other metabolites of Krebs

cycle by transaminases. In any case they are transformated in oxaloacetate for to be involved into gluconeogenesis.

If glycerol comes from adipocytes during fasting it turns into dihydroxyacetone phosphate and is involved in gluconeogenesis in the later stages, bypassing the reaction with oxaloacetate.

The major metabolic product produced under normal circumstances by erythrocytes and by muscle cells during intense exercise lactate is recycled to glucose through the liver in the Cori cycle (Fig. 33).

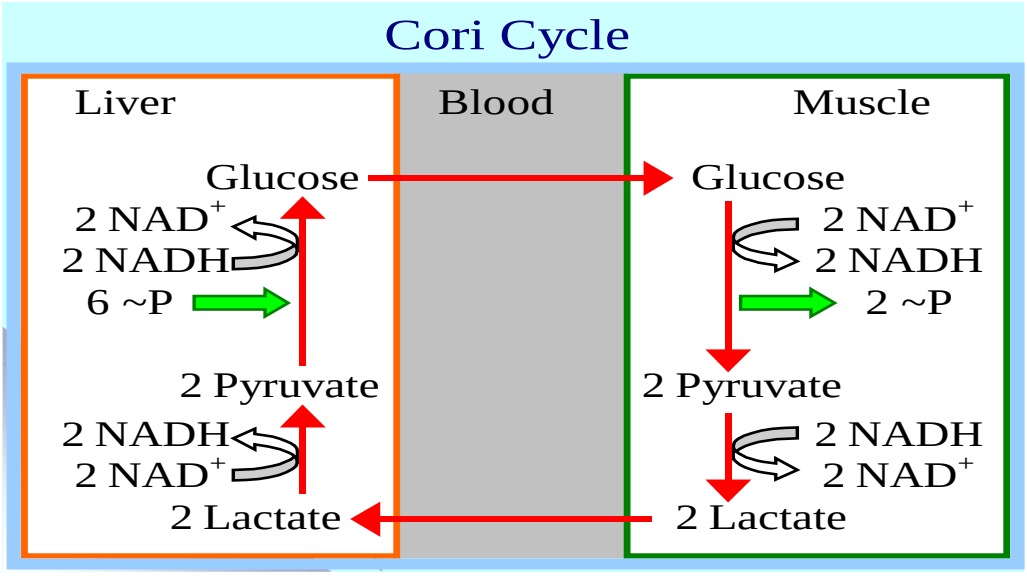


Figure 33. Cori cycle.

Characteristic of gluconeogenesis enzymes

Synthesis of glucose from pyruvate utilizes many of the same enzymes as glycolysis. Gluconeogenesis is not just the reverse of glycolysis. Three glycolysis reactions are essentially irreversible: hexokinase, phosphofructokinase1, pyruvate kinase. These steps must be bypassed in gluconeogenesis.

Two of the bypass reactions involve simple hydrolysis reactions:

Hexokinase or glucokinase (glycolysis) catalyzes: glucose + ATP → glucose-6-phosphate + ADP

- Glucose-6-Phosphatase (gluconeogenesis) catalyzes: glucose-6-phosphate + H_2O → glucose + P_i

Glucose-6-phosphatase is embedded in the endoplasmic reticulum (ER)

membrane in liver cells. Its catalytic site subunit is found to be exposed to the ER lumen, another subunit may function as a translocase, providing access of substrate to the active site.

Phosphofructokinase 1 (glycolysis) catalyzes: fructose-6-P + ATP → fructose-1,6-bisP + ADP

- Fructose-1,6-bisphosphatase (gluconeogenesis) catalyzes: fructose-1,6-bisP + H_2O→fructose-6-P + P_i

Bypass of pyruvate kinase

Pyruvate is converted to oxaloacetate before being changed to phosphoenolpyruvate:

1. **Pyruvate carboxylase** catalyses the ATP-driven formation of oxaloacetate from pyruvate and $CO_{2.}$ Pyruvate carboxylase requires vitamin biotin as a cofactor.

2. Then oxaloacetate (OA) must be converted into phosphoenolpyruvate (PEP). The oxaloacetate is decarboxylated and simultaneously phosphorylated, catalyzed by one of two isoforms of **phosphoenolpyruvate carboxykinase** (PEPCK) either in the cytosol or in the mitochondria to produce phosphoenolpyruvate. Under ordinary gluconeogenic condition, OA is converted into PEP by mitochondrial PEPCK; the resultant PEP is than transported out of the mitochondria, and converted into glucose by cytosolic gluconeogenic enzymes. However, during starvation when cytosolic NADH concentration is low and mitochrondrial NADH levels are high oxaloacetate may be used as a shuttle of reducing equivalents. As such OAA is converted into malate by mitochondrial malate dehydrogenase (MDH). After export into the cytosol, malate is converted back into OA, with contaminant reduction of NAD^+; OA is subsequently converted to PEP which is available for gluconeogenesis in the cytosol along with the transported reducing equivalent NADH. A metal ions such as Mn^{2+}, Mg^{2+} are required for the PEPCK reaction.

Energy balance for 1 mole of glucose synthesis from 2 moles of pyruvate:

Pyruvate carboxylase reaction – 2ATP;

Phosphoenolpyruvate carboxykinase reaction – 2 GTP;

1,3-diphosphoglycerate kinase reaction – 2 ATP;

In all : The use of 6 ATP for 1 mole of glucose synthesis from pyruvate or lactate.

Mitochondrial step in regulation of gluconeogenesis

Pyruvate carboxylase is allosterically activated by acetyl CoA.

Increases in concentrations of oxaloacetate and acetyl-CoA as initial substrates can increase the rate of Krebs cycle. However when ATP and NADH concentrations are high Krebs cycle is inhibited. As a result citrate is not formed, and pyruvate dehydrogenase complex is inhibited too. Accumulation of acetyl-CoA in the matrix enhances formation of oxaloacetate which is used in gluconeogenesis.

Reciprocal regulation of glycolysis and gluconeogenesis

If both pathways glycolysis & gluconeogenesis were simultaneously active in a cell, it would constitute a "futile cycle" that would waste energy.

Glycolysis: glucose + 2 ADP + 2 P_i $\rightarrow$ 2 lactate + 2 ATP

Gluconeogenesis: 2 lactate + 4 ATP + 2 GTP $\rightarrow$ glucose + 4 ADP + 2 GDP + 6 Pi

A futile cycle of both pathways would waste 4 ~P per cycle (glycolysis produces 2 ATP, but gluconeogenesis utilizes 6 ATP). To prevent the waste of a futile cycle, glycolysis & gluconeogenesis are **reciprocally regulated.**

Reciprocal hormonal regulation through fructose-2,6-bisphosphate

For understanding the reciprocal hormonal regulation through fructose-2,6-bisphosphate let’s consider some characteristics of key for duration and regulation of glycolysis enzyme phosphofructokinase:

There are two types of the enzyme:

Mammalian **PFK1:**

- catalyzes the irreversible transformation of fructose-6 phosphate to fructose-1,6 bisphosphate;

- is enzyme out of glycolysis;

- the main way of PFK1 activity regulation is allosteric;

- is a tetramer;

Mammalian **PFK2** or **fructose bisphosphatase 2** (**FBPase2**):

- catalyzes the reversible transformation of F6P to F2,6bisP;

- is enzyme for regulation of glycolysis in the liver;

- the main regulation of its activity is realized through phosphorylation-dephosphorylation (cAMP-dependent);

- PFK2 is a homodimer, half of PFK1 size;

- each polypeptide chain consisting of independent kinase and phosphatase domains.

So, fructose-2,6-bisphosphate stimulates glycolysis. It allosterically activates the glycolysis enzyme phosphofructokinase 1. It also activates transcription of the gene for glucokinase. Alimentary hyperglycemia after the use of high-carbohydrate meal induces glucokinase activity in hepatocytes too.

Fructose-2,6-bisphosphate allosterically inhibits the gluconeogenesis enzyme fructose-1,6-bisphosphatase.

Downstream effects of the cAMP cascade (due to glucagon):

Glycolysis slows because fructose-2,6-bisphosphate is not available to activate phosphofructokinase 1.

Gluconeogenesis increases because of the decreased concentration of fructose-2,6-bisphosphate, which would otherwise inhibit the gluconeogenesis enzyme fructose-1,6-bisphosphatase.

It is discussed two levels of regulation:

Local Control

It includes reciprocal allosteric regulation by adenine nucleotides:

Phosphofructokinase 1(glycolysis) is inhibited by ATP and activated by AMP, ADP

Fructose-1,6-bisphosphatase (gluconeogenesis) is inhibited by AMP

The opposite effects of adenine nucleotides on phosphofructokinase 1 and fructose-1,6-bisphosphatase insure that when cellular ATP is high (AMP would then be low), glucose is not degradated to make ATP.

When ATP is high it is more useful for a cell to store glucose as glycogen.

When ATP is low (AMP would then be high), the cell does not expend energy in synthesizing glucose.

Global Control in liver cells

It includes reciprocal effects of a cyclic AMP cascade, triggered by the hormone glucagon and epinephrine when blood glucose level is low.

Phosphorylation of enzymes & regulatory proteins in liver by protein kinase A (cAMP dependent protein kinase) results in: 1) inhibition of glycolysis; 2) stimulation of gluconeogenesis; 3) making glucose available for release to the blood.

Enzymes relevant to these pathways that are phosphorylated by protein kinase A include:

- pyruvate kinase, a glycolysis enzyme that is inhibited when phosphorylated;

- CREB (cAMP response element binding protein) which activates, through other factors, transcription of the gene for PEP Carboxykinase, leading to increased gluconeogenesis;

- bi-functional enzyme that makes and degradates an allosteric regulator, fructose-2,6-bisphosphate. The formation and degradation of fructose 2,6-bisphosphate as catalyzed by phosphofructokinase-2 (PFK2) and fructose bisphosphatase-2 (FBP2), two enzyme activities that occur on different domains of the same protein molecule. cAMP-dependent phosphorylation of the bi-functional enzyme activates FBPase2 domain and inhibits PFK2 domain**,** and vice versa. Concentration of fructose-2,6-bisphosphate thus decreases in liver cells in response to a cAMP signal cascade, activated by glucagon (epinephrine**)** when blood glucose concentration is low**.**

Gluconeogenesis regulation by glucocorticoids

Glucocorticoids (mainly cortisol) are required for the increase of gluconeogenesis rate in starvation and diabetes mellitus (subtype II). Their action is exerted directly on the liver and appears to involve modulation of PEPCK levels. Glucocorticoids increase the synthesis of this enzyme apparently through effects at the level of transcription. Glucocorticoids exert also permissive effects on the stimulation of gluconeogenesis in the liver by glucagon and epinephrine. The steroids are not required for cAMP generation or protein kinase A activation by these hormones, but appear to act by maintaining the responsiveness of certain enzymes to the effects of cAMP and alpha-adrenergic systems. It is proposed that this event involves the maintenance of a normal intracellular ionic environment.

Common problems associated with impaired glycolysis and gluconeogenesis

1. Lactic acidosis (lacticemia)

The increase of lactate levels in the blood causes an acidosis. Lactic acidosis can result from decreased utilization of lactate or increased formation of lactate. Anoxia or lack of oxygen may occur in condition that impair blood flow (at a shock), in respiratory disorders, and in severe anemia. Anoxia is a common cause of high blood lactate levels, because:

1) Phosphofructokinase-1 is activated in anaerobic condition;
2) Utilization of lactate is reduced without oxygen.

Lactic acidosis may be caused by hypoxia or by alcohol ingestion. Deficiency of oxygen results in increased NADH level, and more pyruvate than normal is converted to lactate. High NADH levels from alcohol metabolism cause pyruvate to be converted to lactate. Impossibility of lactate converting to pyruvate as the initial substrate for gluconeogenesis leads to inhibition of gluconeogenesis. That is why patients with acute alcohol poisoning have disturbed carbohydrate metabolism. Characteristic feature in this condition is the reduced rate of gluconeogenesis in the liver.

Normal blood level of lactate is less then 1,2 mmole/L; with lactic acidosis the blood lactate level may be 5 mmole/L or more. Lactate acidosis results in lowered blood pH and bicarbonate levels.

2. Pyruvate kinase deficiency

Deficiency of pyruvate kinase causes decreased production of ATP from anaerobic glycolysis. Erythrocytes have insufficient ATP for their sodium pumps; their membranes lyse, and a hemolytic anemia results.

3. Pyruvate carboxylase deficiency

The genetic defect of pyruvate carboxylase deficiency is the cause of delayed physical and mental development and early death in children. This defect is characterized by lacticemia, lactaciduria, disorders of a number of metabolic pathways. Pyruvate carboxylase deficiency is a rare condition, with an estimated incidence of 1 in 250,000 births worldwide.

Pyruvate carboxylase is a biotin-dependent mitochondrial enzyme that plays an important role in energy production and anaplerotic pathways. It catalyzes the conversion of pyruvate to oxaloacetate. Oxaloacetate is 1 of 2 essential substrates needed to produce citrate, the first metabolite in the first reaction of citric acid cycle. Oxaloacetate is also entry for most substrates of gluconeogenesis.

Thus pyruvate carboxylase deficiency results in malfunction of the citric acid cycle and of gluconeogenesis, thereby depriving the body of energy. Citric acid cycle derives energy from carbohydrates, while gluconeogenesis produces carbohydrate fuel for the body when carbohydrate intake is low. Loss of pyruvate carboxylase activity and citric acid cycle rate lead to depletion of glutamate production, important in the nervous system.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test tasks:	Explanations:
1.	A 7-year-old girl has signs of anemia. Laboratory examination revealed pyruvate kinase deficiency in the erythrocytes. What process disturbance plays the main role in	

№	Test tasks:	Explanations:
	anemia development? A. Tissue respiration B. Oxidative phosphorylation C. Peroxide decomposition D. Amino acids desamination E. Anaerobic glycolysis	
2.	The gluconeogenesis is activated in the liver after intensive physical trainings. What substance is utilized in gluconeogenesis first of all in this case: A. Glucose B. Glutamate C. Alanine D. Lactate E. Pyruvate	
3.	During starvation muscular proteins are involved into degradation to form free amino acids. These compounds will be the most probably used by the following process: A. Synthesis of higher fatty acids B. Oxidative decarboxylation C. Glycogenolysis D. Gluconeogenesis in muscles E. Gluconeogenesis in the liver	
4.	Cytoplasm of the myocytes contains a lot of dissolved metabolites resulting from glucose oxidation. Name the metabolite that turns directly into lactate: A. Oxaloacetate B. Glucose-6-phosphate C. Glycerophosphate D. Fructose-6-phosphate E. Pyruvate	
5.	A worker has decreased buffer capacity of blood due to exhausting muscular work. What acidic substance that came to blood caused this phenomenon? A. 3-Phosphoglycerate B. 1,3-Bisphosphoglycerate C. Lactate D. α-Ketoglutarate E. Pyruvate	
6.	The genetic defect of pyruvate carboxylase deficiency is the cause of delayed physical and mental development and early death in children. This defect is characterized by lacticemia, lactaciduria, disorders of a number of metabolic pathways. In	

№	Test tasks:	Explanations:
	particular, the following processes are inhibited: A. Citric acid cycle and gluconeogenesis B. Glycolysis and glycogenolysis C. Pentose phosphate pathway and glycolysis D. Lipolysis and lipogenesis E. Glycogenesis and glycogenolysis	
7.	Diseases of respiratory system and circulatory disorders impair the transport of oxygen, thus leading to hypoxia. Under these conditions the energy production is promoted by anaerobic glycolysis. As a result, the following substance is generated and accumulated in blood: A. Fumaric acid B. Pyruvic acid C. Glutamic acid D. Citric acid E. Lactic acid	
8.	Some students developed myokymia after continuous physical activity during physical exercises. The reason for such state was the accumulation of lactic acid in skeletal muscles. It was generated in the students' bodies after activation of the following process: A. Gluconeogenesis B. Lipolysis C. Glycolysis D. Pentose phosphate cycle E. Gluconeogenesis	
9.	Human red blood cells do not contain mitochondria. What is the main pathway for ATP production in these cells? A. Creatine kinase reaction B. Anaerobic glycolysis C. Cyclase reaction D. Aerobic glycolysis E. Oxidative phosphorylation	
10.	Prolonged fasting causes hypoglycemia which is amplified by alcohol consumption, as a result the following process is inhibited: A. Proteolysis B. Glycogenolysis C. Gluconeogenesis D. Lipolysis E. Glycolysis	

№	Test tasks:	Explanations:
11.	A patient is admitted to the hospital with signs of acute alcohol poisoning. What changes in carbohydrate metabolism are characteristic of this condition? A. There is reduced the rate of gluconeogenesis in the liver B. There is enhanced aerobic oxidation of glucose in muscles C. There is increased gluconeogenesis in liver D. Anaerobic breakdown of glucose prevails in muscles E. There is enhanced breakdown of glycogen in liver	
12.	Gluconeogenesis is activated in an athlete shortly after intense physical training. What is a substrate for gluconeogenesis: A. Leucine B. Oxaloacetate C. Lysine D. Glycogen E. Acetyl-CoA	
13.	Normal blood glucose level is maintained mainly through gluconeogenesis in a patient undergoing fasting therapy course. What amino acid is involved most actively in the synthesis of glucose in the human liver? A. Lysine B. Alanine C. Glutamic acid D. Leucine E. Valine	
14.	Intense pain occurs in the muscles of humans people after prolonged physical exertion. What changes in muscles are the most likely cause of this? A. Accumulation of lactic acid B. Accumulation of creatinine C. Increased degradation of proteins D. Increased excitability E. Elevated ADP	
15.	Some anaerobic bacteria converts generated due to glycolysis pyruvate to ethyl alcohol (alcoholic fermentation). What is the biological significance of this process? A. Replenishment of NAD^+ B. Production of lactate	

№	Test tasks:	Explanations:
	C. Production of ADP D. Replenishment of NADPH E. Production of ATP	
16.	It has been found in patient that after prolonged diets with sufficient amount of proteins, fats and with low carbohydrates the concentration of blood sugar is normal but liver glycogen is insignificantly reduced. Name process in patient's body that maintains mainly blood glucose level A. Lipogenesis B. Glycolysis C. Gluconeogenesis D. Glycogenolysis E. Glycogenesis	
17.	Gluconeogenesis playes an important role in maintaining of normal blood glucose level during fasting. Specify the main substrate of this process: A. Nucleic acid B. Acetone C. Cholesterol D. Amino acids E. Bile acids	
18.	Synthesis of ATP occurs due to substrate phosphorylation in anaerobic glycolysis. Spetial macroergic compounds are used in this process. Specify that one: A. Lactate B. Phosphoenolpyruvate C. Glucose 6-phosphate D. Pyruvate E. Glucose	
19.	It is known that normal blood glucose level is maintained during fasting through stimulation of gluconeogenesis. Which of the listed below substances may be used as a source for glucose synthesis? A. Ammonia B. Nicotinamide C. Alanine D. Urea E. Adenine	
20.	Find out name of the pathway proposed in common equation: enzymes $C_6H_{12}O_6 \rightarrow$---- $\rightarrow 2C_2H_5OH + 2\ CO_2$ A. Glucose oxidation B. Hydrolysis of glucose	

№	Test tasks:	Explanations:
	C. Lactic fermentation D. Glucose reduction E. Alcoholic fermentation of glucose	
21.	Erythrocytes for their livelihoods need energy in form of ATP. Specify the metabolic process, which provides the necessary amount ATP for erythrocyte: A. Pentose phosphate cycle B. Citric acid cycle C. Anaerobic glycolysis D. Beta-oxidation of fatty acids E. Gluconeogenesis	
22.	Fasting stimulates gluconeogenesis. Name vitamin involved in pyruvic acid carboxylation during gluconeogenesis: A. Nicotinamide B. Calciferol C. Folacin D. Biotin E. Retinol	
23.	There is alimentary hyperglycemia after the use of high-carbohydrate meal in human. What enzyme activity is induced in hepatocytes at these conditions? A. Glucokinase B. Glucose-6-Phosphatase C. Aldolase D. Isocitrate dehydrogenase E. Phosphorylase	
24.	Untrained people often have muscle pain after sprints as a result of lactate accumulation there. This might be caused by intensification of the following biochemical process: A Glycolysis B Gluconeogenesis C Pentose phosphate pathway D Lipogenesis E. Glycogenesis	
25.	Find out the cell compartment for glucose-6 phosphatase location in hepatocyte: A. Nucleous B. Mitochondrion C. Lysosome D. Plasma membrane E. Endoplasmic reticulum membrane	

№	Test tasks:	Explanations:
26.	How many macroergic molecules (ATP, GTP) are used for 1 molecule of glucose synthesis from pyruvate or lactate: A. 1 B. 2 C. 4 D. 6 E. 8	
27.	How glucocorticoids influence the carbohydrate metabolism? A. Activate gluconeogenesis B. Inhibit gluconeogenesis C. Activate glycolysis D. Inhibit glycolysis E. Inhibit alcoholic fermentation	
28.	Choose the terminal product of anaerobic glycolysis: A. Pyruvate B. Acetyl-SCoA C. Lactate D. CO_2, H_2O E. Oxaloacetate	
29.	Glucocorticoids increase the rate of gluconeogenesis across stimulation of transcription needed for some enzymes of this process. Name that enzyme: A. Glucokinase B. Hexokinase C. Phosphoenolpyruvate carboxykinase D. Pyruvate kinase E. Aldolase A	
30.	Choose a substance that **can not** be the use as the substrate for gluconeogenesis: A. Lactate B. Alanine C. Glycerol D. Lysine E. Aspartate	

AEROBIC OXIDATION OF MONOSACCHARIDES

(Rudko N.P.)

INFORMATIONAL MATERIAL

The complete aerobic oxidation of glucose up to CO_2 and H_2O is coupled to the synthesis of as many as 36 molecules of ATP:

$$C_6H_{12}O_6 + 6\ O_2 + 36\ P_i^{2-} + 36\ ADP^{3-} + 36\ H^+ \longrightarrow 6\ CO_2 + 36\ ATP^{4-} + 42\ H_2O$$

Aerobic oxidation of glucose consists of 3 main phases:

1) Glycolysis;

2) Oxidative decarboxylation of pyruvate;

3) Krebs cycle and oxidative phosphorylation

Each of these processes occurs in a specialized location in cells

Glycolysis occurs in the cytosol. This is glucose conversion to 2 molecules of pyruvate with 2 ATP and 2 NADH formation.

Oxidative decarboxylation of pyruvate occurs in the matrix of mitochondrion. This is 2 pyruvates conversion to 2 acetyl CoA with 2 NADH formation.

The Krebs cycle occurs in the matrix of mitochondrion. This is 2 acetyl CoA conversion to 4 CO_2 with 2 GTP and 6 NADH, and 2 $FADH_2$ formation. Oxidative phosphorylation occurs in the inner membrane of mitochondrion too. This is 10 NADH and 2 $FADH_2$ oxidation with H_2O and 34 ATP formation.

Glycolysis is the first of the three phases of aerobic oxidation of glucose.

Let’s come down to details of this stage. Glycolysis means “splitting of sugar”.

Functions of aerobic glycolysis:

a) to convert glucose to pyruvate which can be:

- burned for energy (due to pyruvate dehydrogenase and Krebs cycle) – most important for majority of tissues;

- or converted to fatty acid, cholesterol, amino acid synthesis etc.;

b) such intermediate as dihydroxyacetone phosphate can be reduced to glycerol phosphate either for use in the biosynthesis of lipids or for glycerol phosphate shuttle system which transfer reducing equivalents from cytoplasmic NADH into mitochondrion;

c) the reversible reactions of glycolysis in opposite direction of duration are used for gluconeogenesis.

Substrates for glycolysis:

- free (blood) glucose (nervous, blood cells, kidney medula and other tissues);
- glucose derived from digested diet starch, lactose, saccharose (liver and other tissues);
- glucose derived from glycogen (muscle tissue);
- galactose, fructose can be converted to intermediates of glycolysis and in this way enter the metabolic parthway (liver)

Product of the first stage pyruvate serves as a precursor in many metabolic reactions. The metabolic fate of pyruvate in aerobic conditions: pyruvate dehydrogenase complex (PDC) transforms it into acetyl CoA & thereby links the glycolysis to the citric acid cycle. PDC is organized in dodecahedral symmetry, and consists of a total of 96 subunits, organized into three functional proteins in the human enzyme (Fig. 34).

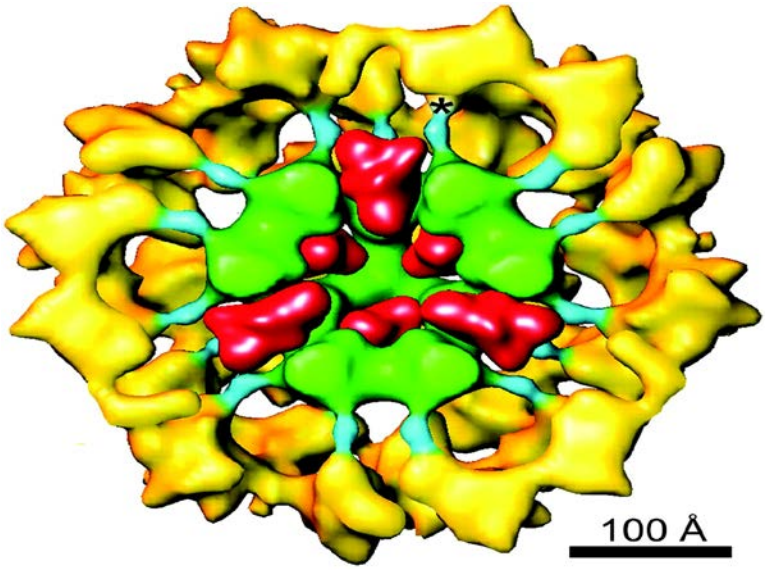


Figure 34. Cut-away model of the fully assembled PDC.

It conteins 5 kinds of coenzymes: TPP, FAD, NAD^+, CoA and lipoamide. These coenzymes are derivatives of vitamins: B_1, B_2, B_3 (PP), B_5 and lipoic acid respectively. So these vitamins deficiency can lead to reduced activity of pyruvate dehydrogenase complex. It is known that one of the possible reasons for accumulation of pyruvate in the blood plasma and its excretion with urine is hypovitaminosis B_1 because PDC activity is decreased.

Another example: it has been found out that one of pesticide components is sodium arsenate that blocks lipoic acid. As a result pyruvate dehydrogenase complex activity is impaired by this pesticide.

We have considered the metabolic fate for pyruvate, but as was shown earlier glycolysis produce 2 NADH per 1 glucose too. But the mitochondrial inner membrane is impermeable to NADH and a shuttle system is required for translocation of electrons produced during glycolysis across the inner membrane of the mitochondrion for oxidative phosphorylation in eukaryotes.

There are two shuttles for electrons transport from cytoplasm into mitochondrion.

The first is malate-aspartate shuttle. It consists of four protein parts:

- malate dehydrogenase in the mitochondrial matrix and intermembrane space;
- aspartate aminotransferase in the mitochondrial matrix and intermembrane space;
- malate-alpha-ketoglutarate antiporter in the inner membrane;
- glutamate-aspartate antiporter in the inner membrane.

Mechanism: The primary enzyme in the malate-aspartate shuttle is malate dehydrogenase. Malate dehydrogenase is present in two forms in the shuttle system: mitchondrial and cytosolic malate dehydrogenase. The two malate dehydrogenases catalyze their reaction in opposite directions in this process.

First, in the cytosol, malate dehydogenase reacts with oxaloacetate and NADH to produce malate and NAD^+. In this process two hydrogen atoms generated from NADH and an accompanying H^+ are attached to oxaloacetate to form malate.

Once malate is formed, the first antiporter (malate-alpha-ketoglutarate) imports the malate from the cytosol into the mitochondrial matrix and also exports alpha-ketoglutarate from the matrix into the cytosol simultaneously. After malate reaches the mitochrondial matrix it is converted by mitochrondrial malate dehydrogenase into oxaloacetate, during which NAD^+ is reduced with two electrons to form NADH and an H^+ is released. Since oxaloacetate cannot be transported into the cytosol Oxaloacetate is then transformed into aspartate by mitochrondrial aspartate aminotransferase. Since aspartate is an amino acid, an amino group needs to be added to the oxaloacetate. This is supplied by glutamate, which in the process is transformed into alpha-ketoglutarate by the same enzyme.

The second antiporter (the glutamate-aspartate antiporter) imports glutamate from the cytosol into the matrix and exports aspartate from the matrix to the cytosol. Once in the cytosol, aspartate is converted by cytosolic aspartate aminotransferase to oxaloacetate.

The net effect of the malate-aspartate shuttle is purely redox: NADH in the cytosol is oxidized to NAD^+, and NAD^+ in the matrix is reduced to NADH. The NAD^+ in the cytosol can then be reduced again by another round of glycolysis, and the NADH in the matrix can be used to pass electrons to the electron transport chain so that ATP can be synthesized.

Malate-aspartate shuttle is utilized in liver and heart. Most of other tissues use glycerol-3-phosphate shuttle.

The **glycerol-3-phosphate shuttle** is a mechanism that regenerates NAD^+ from NADH. Its importance in transporting reducing equivalents is secondary to the malate-aspartate shuttle.

In this shuttle, the enzyme called cytoplasmic glycerol-3-phosphate dehydrogenase (GPD) converts dihydroxyacetone phosphate to glycerol 3-phosphate by oxidizing one molecule of NADH to NAD^+ . Glycerol-3-phosphate gets converted back to dihydroxyacetone phosphate by a membrane-bound mitochondrial glycerophosphate dehydrogenase, this time reducing one molecule of enzyme-bound FAD to $FADH_2$. $FADH_2$ then reduces coenzyme Q (ubiquinone

to ubiquinol) which enters into oxidative phosphorylation. This reaction is irreversible.

Comparative characteristics of aerobic oxidation of glucose (to CO_2&H_2O) and anaerobic glycolysis energy balance

Aerobic oxidation of glucose (to CO_2&H_2O)

I. Glycolysis stage:

- 2 ATP (used for phosphorylation of glucose & fructose 6-P)

+ 4 ATP (produced by 1,3 bis P-glycerate and pyruvate kinases)

+ 6 ATP (if malate-aspartate shuttle translocates electrons from 2 NADH for oxidative phosphorylation (OP))

or + 4 ATP (if glycerol-3-phosphate shuttle translocates electrons from 2 NADH for OP)

= 8 (or 6)

II. Oxidative decarboxylation of pyruvate stage (2 pyruvates enter) :

+ 6 ATP (due to utilization of 2 NADH for OP)

III. Krebs cycle (2 acetyl CoA enter) stage:

+ 18 ATP (due to utilization of 6 NADH for OP)

+ 4 ATP (due to utilization of 2 FADH2 for OP)

+ 2 ATP (due to 2 GTP conversion)

= 24

In all = 38 (or 36) ATP

Anaerobic glycolysis:

- 2 ATP (used for phosphorylation of glucose & fructose 6-P)

+ 4 ATP (produced by 1,3 bis-P-glycerate kinase and pyruvate kinase)

2 NADH are not used for oxidative phosphorylation but are consumed in LDH reaction

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In all = 2 ATP

38 and 2 ATP per 1 molecule of glucose – it is very different. Anaerobic glycolysis give much less ATP than aerobic oxidation of glucose.

Skeletal muscles of trained person use glucose to produce ATP energy for muscle contraction for long-distance running due to aerobic oxidation (through aerobic glycolysis).

But aerobic glycolysis is normally faster than the Krebs cycle capacity, and lactate is the usual product of glycolysis even in resting muscle. The lactate/pyruvate ratio is about 10 in resting muscle, but in working muscle this ratio may hit 200.

Of crucial biomedical significance is the ability of anaerobic glycolysis to provide ATP in the absence of oxygen. For example, organ or tissue can produce ATP during hypoxia due to anaerobic glycolysis a short time in the case of thrombosis and other causes of circulatory disorders. When blood circulation in the damaged tissue is restored, then lactate accumulation comes to a stop and glucose consumption decelerated. These metabolic changes are caused by activation of the aerobic glycolysis as a more efficient process.

The fate of fructose and galactose

Although glucose is the most abundant monosaccharide derived from the diet, fructose and galactose are usually obtained in significant quantities. The major dietary source of fructose is the disaccharide sucrose (table sugar), but it is also present as the monosaccharide in fruit and in corn syrup, which is used as a sweetener.

After fructose and galactose enter cells, they are phosphorylated on carbon 1 and converted to intermediates in pathways of glucose metabolism. Fructose is metabolized mainly in the liver, where it is converted to fructose 1-phosphate by fructokinase and cleaved by aldolase B to produce dihydroxyacetone phosphate and glyceraldehyde, which may be phosphorylated to glyceraldehyde 3- phosphate.

These two triose phosphates are intermediates of glycolysis.

Fructose may be produced from sorbitol, which is generated from glucose.

Normal level of fructose in blood is 5.55-28.75 μmole/L (0.1-0.5 mg%); in urine – 0.17-0.36 mg/day (30-65 mg/day).

Galactose is phosphorylated by galactokinase to galactose 1-phosphate, which reacts with UDP-glucose. This reaction is catalized by galactose-1-phosphate uridylyltransferase with the concurrent formation of glucose-1-phosphate from UDP-glucose The second product is UDP-galactose, which is epimerized to UDP-glucose by UDP-glucose-4-epimerase . The net result is that galactose is converted to the glucose moieties of UDP-glucose and glucose 1-phosphate, intermediates in pathways of glucose metabolism. UDP-galactose may be used in the synthesis of glycoproteins, glycolipids, and proteoglycans. UDP-galactose may react with glucose in the mammary gland to form the milk sugar lactose. Galactose may be reduced to galactitol.

Pentose Phosphate Pathway (PPP)

The PPP (also called the hexose monophosphate shunt) is a process that primarily utilizes glucose 6-phosphate to generate NADPH and pentoses but allow also pentoses (ribose-5-phosphate) to enter glycolysis

Location in the body: everywhere but it is most active in:

- liver (for fatty acid (FA)&cholesterol(Ch)& nucleotide synthesis, detoxification (cytochrome P-450 system), bile acids formation;
- lactating mammary glands (for FA&Ch synthesis);
- adipose tissue (for FA synthesis);
- adrenal cortex, ovaries, testes (for steroid hormone synthesis);
- erythrocytes (for reducing of glutathione as an antioxidant);
- neutrophils (for generation of superoxide);
- rapidly proliferating cells (for nucleic acid&FA&Ch synthesis)

Location within the cell: cytosol

Regulation: the key enzyme is glucose 6-phosphate dehydrogenase:

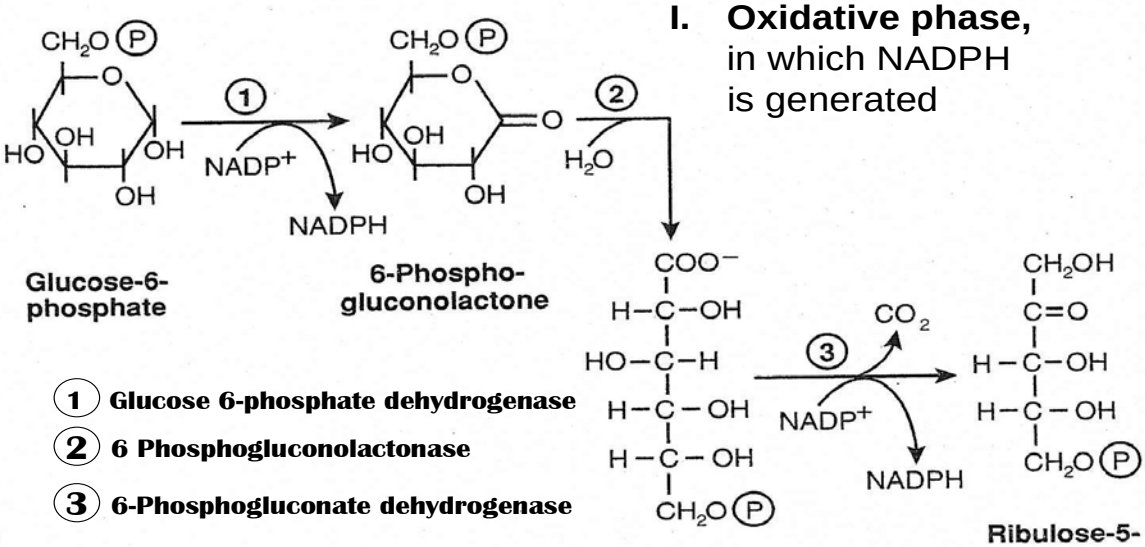
- NADPH, acyl CoA inhibit;
- $NADP^+$ activates;
- high glucose 6-phosphate activates;
- it is induced by insulin.

There are two phases in the PPP:

I. Oxidative phase

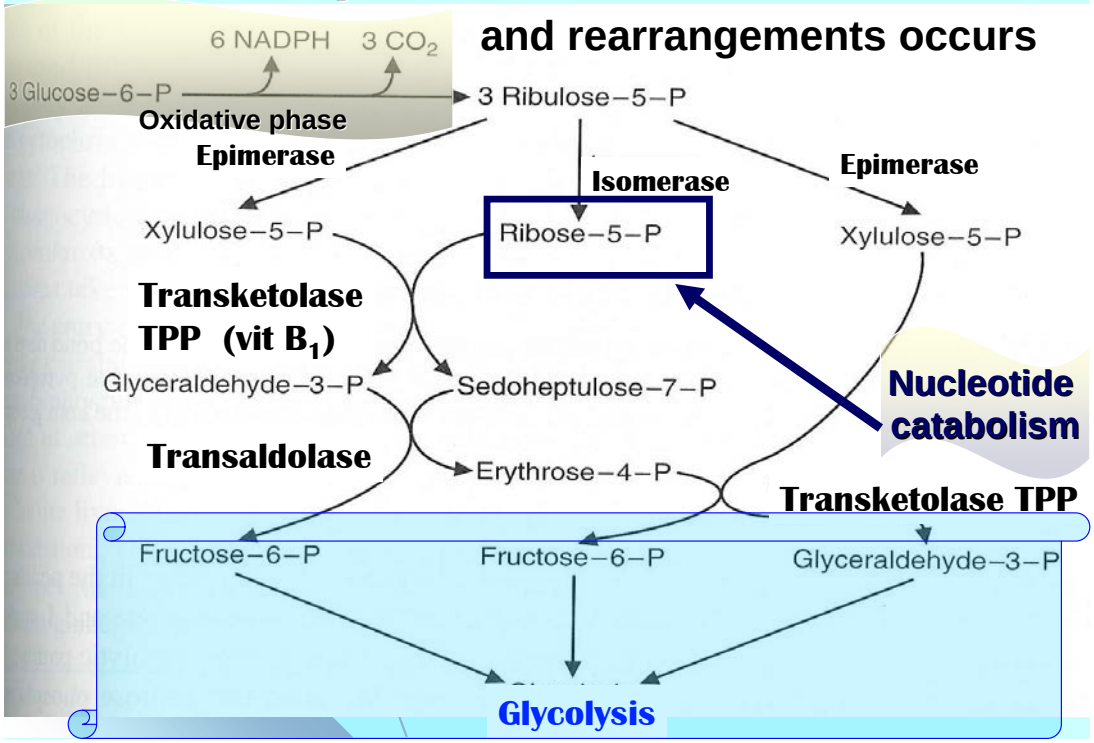
II. Non-oxidative phase

I. Oxidative phase, in which NADPH is generated



1. **Glucose 6-phosphate dehydrogenase**
2. **6 Phosphogluconolactonase**
3. **6-Phosphogluconate dehydrogenase**

Non-oxidative phase, in which ribose 5-P is formed and rearrangements occurs



Depending on relative needs of a cell for ribose-5-phosphate, NADPH, and ATP, the pentose phosphate pathway can operate in various modes, to maximize different products. There are three major scenarios:

1. Ribulose-5-phosphate may be converted to **ribose-5-phosphate**, a substrate for synthesis of nucleotides and nucleic acids. The pathway also produces some NADPH.
2. Glyceraldehyde-3-phosphate and fructose-6-phosphate, formed from the 5-carbon sugar phosphates, may be converted to glucose-6-phosphate for reentry into the linear portion of the Pentose phosphate pathway, maximizing formation of **NADPH**.
3. Any pentose may be formed as energy source to produce ATP, if transketolase will be reach with TPP (derived from vitamin B_1) and as a result glyceraldehyde-3-phosphate and fructose-6-phosphate, formed from the 5-carbon sugar phosphates, may enter glycolysis, for synthesis of **ATP**.

Ribose-1-phosphate generated during catabolism of nucleosides also enters the Glycolytic pathway in this way, after first being converted to ribose-5-phosphate. Thus the pentose phosphate pathway serves as an entry into glycolysis for both 5-carbon and 6-carbon sugars.

Common problems associated with galactose, fructose metabolism and pentose phosphate pathway

Galactosemia

Lactose in breast milk and infant formula is converted by intestinal lactase to glucose and galactose that are efficiently absorbed. In galactosemia, deficiency of galactose-1-phosphate uridyl transferase prevents the conversion of galactose into glucose-6-phosphate by the liver or erythrocytes. Most other organs do not metabolize galactose. The severe symptoms of galactosemia are caused by the reduction of galactose to galactitol (dulcitol) in the presence of the enzyme aldose reductase. High levels of galactitol cause cataracts, the accumulation of galactose-1-phosphate contributes to liver disease, and the accumulation of galactose

metabolites in urine can be measured as reducing substances. Infants with suspected galactosemia must be withdrawn from breast-feeding or lactose formulas and placed on nonlactose formulas.

There are 3 types of galactosemia or galactose deficiencies (Fig. 35).

Figure 35. Types of galactosemia.

Type	Name	Description of clinical symptoms
Type I	Classic galactosemia or galactose-1-phosphate uridyl transferase deficiency	Vomiting, diarrhea, general dystrophy, hepato- and splenomegaly, cataract, mental deficiency, adipose degeneration of liver, hypoglycemia are observed The symptoms are reduced upon termination of feeding milk. Is the most problematic, as galactose-free diets are not effective in treating neurocognitive deficiencies (in particular language disorders such as verbal dyspraxia) and ovarian failure. If a galactose-free diet is administered, cataracts and acute symptoms such as kidney and liver failure respond immediately.
Type II	Galactokinase deficiency	Cataracts, which are treatable by restricting galactose from the diet. Concentration of glucose in blood is not ussually changed
Type III	UDPgalactose-4-epimerase deficiency	Is extremely rare. It causes nerve deafness.

Classic galactosemia is more severe, causing elevation of galactose-1-phosphate, which inhibits phosphoglucomutase, interfering with glycogen synthesis and degradation.

Essential (familial) fructosuria

While most tissues cannot utilize fructose, the liver, kidneys, intestine, and adipose tissue can. Genetic fructokinase deficiency causes no symptoms. It can be detected by urine measurements of fructose that spills over into the urine. Unless care is taken, this could be misinterpreted as glucosuria, like that seen in diabetes, since both fructose and glucose are positive for a reducing-sugar test. Liver hexokinase rarely phosphorylates fructose to fructose-6-phosphate because the liver enzyme has a much greater affinity for glucose. However, adipose tissue hexokinase produces fructose-6-phosphate, which is involved into glycolysis. Fructokinase is an enzyme of the liver, intestine and kidney cortex. It produces fructose-1-phosphate which then can be acted upon by fructose-1-phosphate

aldolase (aldolase B), which splits it into dihydroxyacetone phosphate and glyceraldehyde. Glyceraldehyde and dihydroxyacetone phosphate proceed through glycolysis or gluconeogenesis through the action of triose kinase. Under normal circumstances, liver fructokinase phosphorylates fructose to fructose-1-phosphate, and fructose-1-phosphate aldolase acts upon it.

Fructose intolerance

The aldolase B that cleaves fructose phosphates is deficient. Fructose 1-phosphate accumulates and inhibits glucose production, causing severe hypoglycemia if fructose is ingested.

Glucose 6-phosphate dehydrogenase deficiency

One of the world's most common enzyme deficiencies is glucose-6-phosphate-dehydrogenase deficiency. This deficiency in erythrocytes is particularly prevalent among African and Mediterranean males. A deficiency in glucose-6-phosphate dehydrogenase blocks the pentose phosphate pathway and NADPH production. Without NADPH to maintain glutathione in its reduced form, erythrocytes have no protection from oxidizing agents. Red blood cells lyse and a hemolytic anemia may occur. This X-linked recessive deficiency is often diagnosed when patients develop hemolytic anemia after receiving oxidizing drugs such as antimalarial primaquine, pamaquine, antifever aspirin, sulfonamides or after eating oxidizing substances such as fava beans.

Just one enzyme of the pentose phosphate pathway is important in clinical diagnosis: transketolase activity is decreased in deficiency of vitamin B_1 (thiamine). Two diseases are associated with thiamine deficiency: Beri-beri and Wernicke-Korsakoff syndrome. Red cell transketolase activity determination may be used in the diagnosis of Wernicke's encephalopathy and other B_1-deficiency syndromes if the diagnosis is in doubt.

Essential pentosuria

L-Xylulose reductase (xylitol dehydrogenase) is deficient in essential pentosuria. L-Xylulose (a pentose) appears in the urine and gives a positive reducing-sugar test. The condition is benign.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test tasks:	Explanations:
1.	A child's blood presents high content of galactose, glucose concentration is low. There are such presentations as cataract, mental deficiency, adipose degeneration of liver. What disease is it? A. Diabetes mellitus B. Steroid diabetes C. Fructosemia D. Lactosemia E. Galactosemia	
2.	A child has got galactosemia. Concentration of glucose in blood has not considerably changed. Deficiency of what enzyme caused this illness? A. Phosphoglucomutase B. Amylo-l,6-glucosidase C. Galactokinase D. Galactose-1-phosphate uridyltransferase E. Hexokinase	
3.	When blood circulation in the damaged tissue is restored, then lactate accumulation comes to a stop and glucose consumption decelerated. These metabolic changes are caused by activation of the following process: A. Gluconeogenesis B. Aerobic glycolysis C. Anaerobic glycolysis D. Lipolysis E. Glycogen biosynthesis	
4.	It has been found out that one of pesticide components is sodium arsenate that blocks lipoic acid. What enzyme activity is impaired by this pesticide? A. Glutathione reductase B. Pyruvate dehydrogenase complex C. Glutathione peroxidase D. Methemoglobin reductase E. Microsomal oxidation	
5.	A baby has vomiting and diarrhea, general dystrophy, hepato- and splenomegaly are observed for him too. The symptoms are reduced upon termination of feeding milk. What is the basic hereditary defect is in the pathogenesis? A. Exocrine glands hypersecretion	

№	Test tasks:	Explanations:
	B. Phenylalanine metabolism disturbance C. Galactose metabolism disturbance D. Tyrosine metabolism disturbance E. Lack of glucose-6-phosphate dehydrogenase	
6.	Skeletal muscles of trained person use glucose to produce ATP energy for muscle contraction for long-distance running. Point out the main process of glucose utilization in these conditions: A. Anaerobic glycolysis B. Glycogenolysis C. Aerobic glycolysis D. Gluconeogenesis E. Glycogenesis	
7.	An increase in the size of the liver and spleen, and cataracts has been determined in a boy 2 years of age. In the blood sugar concentration is increased but glucose tolerance test is normal. Name substance whose metabolic genetic disorder is the cause of described condition? A. Sucrose B. Maltose C. Glucose D. Fructose E. Galactose	
8.	Enhanced hemolysis caused by deficiency of glucose-6-phosphate dehydrogenase is observed in 38 year old patient after taking aspirin and sulfonamides. This pathology is possibly due to disturbance of one from following coenzymes formation: A. $FADH_2$ B. NADPH C. Ubiquinone D. $FMNH_2$ E. Pyridoxal phosphate	
9.	Enhanced hemolysis is observed in three year old child with fever after taking aspirin. What congenital deficiency of the enzyme can cause hemolytic anemia in the child? A. Glucose-6-phosphate dehydrogenase B. Glucose-6-phosphatase C. γ-Glutamyl transferase D. Glycogen phosphorylase E. Glycerol phosphate dehydrogenase	
10.	In a sick child is found mental retardation,	

№	Test tasks:	Explanations:
	liver enlargement, loss of vision. The physician connects these symptoms with deficit of galactose-1-phosphate uridyltrasferase in the body. What pathological state the child has? A. Hypoglycemia B. Galactosemia C. Hyperlactatacidemia D. Hyperglycemia E. Fructosemia	
11.	Lack of glucose-6-phosphate dehydrogenase synthesis is observed in a child. What carbohydrate metabolic pathway is disturbed in the child? A. Glycogenesis B. Gluconeogenesis C. Glycogenolysis D. Pentose phosphate cycle E. Aerobic oxidation of glucose	
12.	How many phases are considered for aerobic oxidation of glucose A. 2 B. 3 C. 4 D. 5 E. 6	
13.	Glycolysis being amphiobolic process performs many functions. Which characteristic of glycolysis is correct: A. It converts glucose to pyruvate for energy production B. It converts glucose to ribose-5-phosphate for nucleotide synthesis C. It converts lactate to glucose preventing acidosis state D. It converts glucose to glycogen for storage E. It converts glycogen to glucose to maintain blood glucose levels	
14.	Such intermediate as dihydroxyacetone phosphate can be reduced to glycerol phosphate either for use in the biosynthesis of lipids or for reducing equivalents transfer from cytoplasmic NADH into mitochondrion. Choose the shuttle system used dihydroxyacetone phosphate as an acceptor of reducing equivalents from NADH: A. Malate-aspartate shuttle system	

№	Test tasks:	Explanations:
	B. Carnitine shuttle system C. Creatine shuttle system D. Glycerol phosphate shuttle system E. Glucose-alanine shuttle system	
15.	What compound can not be involved in glycolysis as a substrate: A. Glucose B. Galactose C. Fructose D. Glucose-6-phosphate E. Adenine	
16.	Product of the first stage of glucose aerobic oxidation pyruvate serves as a precursor in many metabolic reactions. The metabolic fate of pyruvate in aerobic conditions is: A. Transformation into lactic acid B. Transformation into acetyl CoA C. Transformation into ethanol D. Transformation into propanol E. Transformation into malonate	
17.	Providing the body with vitamins is essential for its normal energy state. One of the major enzyme systems is the pyruvate dehydrogenase complex. It conteins 5 kinds of coenzymes. Those coenzymes are derivatives of vitamins: A. B_1, B_2, B_3, B_4, B_5 B. B_1, B_2, B_3, B_5 and lipoic acid C. B_1, B_2, B_3, B_6 and pantothenic acid D. B_3, B_4, B_5, B_6, B_{12} E. A, D, K, E	
18.	What coenzymes are in structure of pyruvate dehydrogenase complex: A. NADH, $FADH_2$, pyridoxal phosphate B. NADH, biotin C. NADPH, biotin D. TPP, FAD, NAD^+, CoA, lipoamide E. TPP, methylcobalamin, lipoamide	
19.	There are 5 vitamins required for pyruvate dehydrogenase complex functioning. But it is known that the most common reason of decreased pyruvate dehydrogenase activity and consequently accumulation of pyruvate in the blood plasma and its excretion with urine is certain vitamin deficiency. Choose it: A. Vitamin B_1 B. Vitamin B_5 C. Vitamin B_6	

№	Test tasks:	Explanations:
	D. Vitamin D E. Vitamin A	
20.	A shuttle system is required for translocation of electrons produced during glycolysis across the inner membrane of mitochondrion for oxidative phosphorylation in eukaryotes. As an example is malate-aspartate shuttle. Which one of the following is not a part of this system: A. Malate dehydrogenase B. Aspartate aminotransferase C. Citrate-pyruvate antiporter D. Malate-alpha-ketoglutarate antiporter E. Glutamate-aspartate antiporter	
21.	The net effect of the malate-aspartate shuttle is purely redox: NADH in the cytosol is oxidized to NAD^+, and NAD^+ in the matrix is reduced to NADH. NADH in the mitochondria may be used for: A. Pentose phosphate pathway oxidative phase B. Pentose phosphate pathway non-oxidative phase C. Lactate formation D. Electron transport chain E. Acetyl-CoA formation	
22.	Glycerol-3-phosphate as a component of shuttle system gets converted back to dihydroxyacetone phosphate by enzyme glycerophosphate dehydrogenase. Where this enzyme is located? A. Cytosol B. Plasma membrane C. Endoplasmic reticulum D. Mitochondrial membrane E. Mitochondrial matrix	
23.	Mitochondrial glycerophosphate dehydrogenase is $FADH_2$ prosthetic group containing enzyme. $FADH_2$ must be turned to its oxidized form FAD for functioning of the shuttle system. What is reduced by $FADH_2$ of glycerophosphate dehydrogenase in the inner mitochondrial membrane: A. NAD^+ B. $NADP^+$ C. Coenzyme Q D. Coenzyme A	

№	Test tasks:	Explanations:
	E. Glutathione	
24.	Choose sum total of ATP for energy effect for aerobic oxidation of glucose in the liver: A. 2 ATP B. 6 ATP C. 12 ATP D. 36 ATP E. 38 ATP	
25.	Choose sum total of ATP for energy effect for anaerobic oxidation of glucose in the muscle: A. 2 ATP B. 6 ATP C. 12 ATP D. 36 ATP E. 38 ATP	
26.	Pentose phosphate pathway may be used for different purposes in various organs and tissues. Erythrocytes used it for: A. Steroid hormone synthesis B. Generation of superoxide C. Reducing of glutathione as an antioxidant D. Bile acids formation E. Nucleotide synthesis	
27.	Poin out all enzymes for pentose phosphate pathway location: A. Mitochondrial membrane B. Mitochondrial matrix C. Cytosol D. Plasma membrane E. Endoplasmic reticulum	
28.	Two phases are usually considered for pentose phosphate pathway. Which enzyme is related to non-oxidative phase: A. Glucose-6-phosphate dehydrogenase B. Glucose-6-phosphatase C. Transketolase D. Glucokinase E. Hexokinase	
29.	Two phases are usually considered for pentose phosphate pathway. Which enzyme is related to oxidative phase A. Glucose-6-phosphate dehydrogenase B. Glucose-6-phosphatase C. Transketolase D. Glucokinase E. Hexokinase	

№	Test tasks:	Explanations:
30.	One of the world's most common enzyme deficiencies is: A. Glucose-6-phosphate-dehydrogenase deficiency B. Galactose-1-phosphate uridyl transferase deficiency C. Galactokinase deficiency D. UDPgalactose-4-epimerase deficiency E. Fructokinase deficiency	

METABOLISM OF POLYSACCHARIDES. THE REGULATION AND DISORDERS OF CARBOHYDRATE METABOLISM

(Rudko N.P.)

INFORMATIONAL MATERIAL

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages and on hydrolysis give the constituent monosaccharides or oligosaccharides. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. Their function in living organisms is usually either structure- or storage-related. Polysaccharides are of two types: homopolysacharides (starch, glycogen, cellulose) and heteropolysaccharides (hyaluronic acid, heparin, chondroitin sulfates, keratan sulfate etc).

Glycosaminoglycans or mucopolysaccharides are long unbranched polysaccharides consisting of a repeating disaccharide unit. The repeating unit consists usually of an amino sugar (*N*-acetylglucosamine or *N*-acetylgalactosamine) along with a uronic sugar (glucuronic acid or iduronic acid) or galactose. Glycosaminoglycans being highly polar are useful to the body as a lubricant or as a shock absorber like hyaluronic acid. Chondroitin sulfate and dermatan sulfate help to maintain the structure and shapes of tissues; heparin used in clinical practice as an anticoagulant (it is appointed for a patient after surgery and is also the first line choice for thromboembolic diseases).

The most interesting polysaccharide in the sense of metabolism in human is glycogen. Glycogen is the storage form of glucose. These stores allow humans to eat intermittently by providing an immediate source of blood glucose for use as a metabolic fuel. Glycogen exists in the cytosol in granular form. It is a highly branched large polymer of glucose molecules linked by α-1,4-glycosidic bonds, branches arise by -1,6-glycosidic bonds. The major sites for glycogen storage are

the muscle and liver. The concentration of glycogen is higher in the liver. If muscle can only use its glycogen for its own energy needs, but liver mobilizes its glycogen for the release of glucose to the rest of the body.

Glycogen metabolism

Glycogenesis

It is activated in the liver first of all during essential hyperglycemia for the decrease of glucose level in the blood.

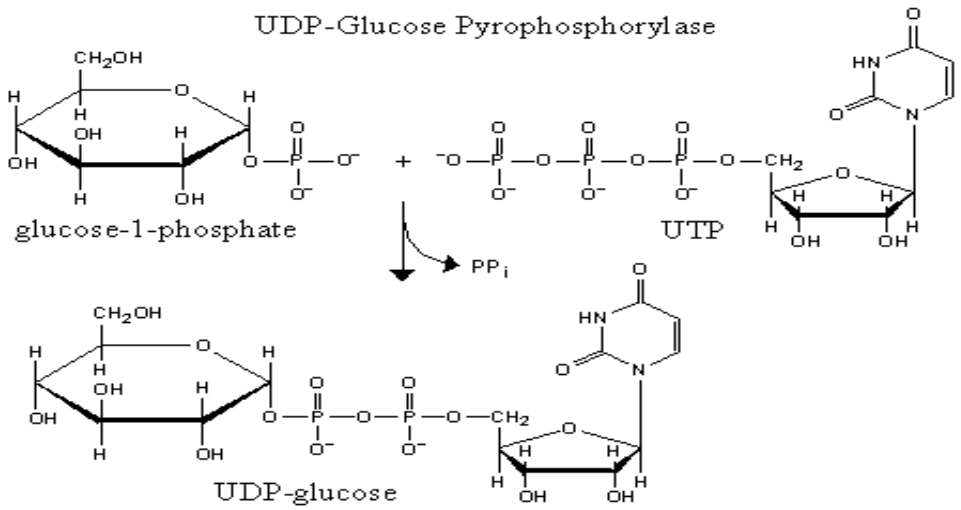
The overall reaction for glycogenesis (for the addition of each glucose residue):

$Glycogenin\text{-}(C_6H_{10}O_5)_n\ H + C_6H_{12}O_6 + ATP + UTP \rightarrow Glycogenin\text{-}(C_6H_{10}O_5)_{n+1}H + ADP + UDP + PPi$

Glycogenesis reaction sequence:

- Glucose is converted into glucose 6-phosphate by glucokinase or hexokinase
- Glucose 6-phosphate is converted into glucose 1-phosphate by the action of phosphoglucomutase
- Glucose 1-phosphate is converted into UDP-glucose by uridyl transferase (also named UDP-glucose pyrophosphorylase) and pyrophosphate is formed, which is hydrolyzed by pyrophosphatase into 2 molecules of Pi.

Glycogen (and all other polysaccharides) is synthesized from the active form of glucose. The immediate donor of glucose residues during the glycogenesis is uridine diphosphate glucose (UDP-glucose).



- Glycogenin (Fig. 35) initiates glycogen synthesis. It is an enzyme that catalyzes attachment of a glucose molecule from UDP-Glucose to one of its own tyrosine residues. This is repeated until a short seven more linear glucose polymer is built up on glycogenin (because glycogen synthase can only add to an existing chain of at least 8 glucose residues).

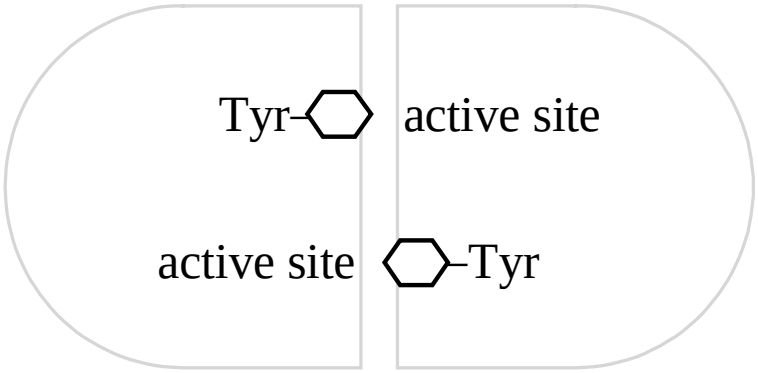


Figure 35. Glycogenin dimer structure.

- Glycogen synthase (specifically defined as UDP-glucose-glycogen glucosyl transferase) being the main enzyme then catalyzes elongation of glycogen chains:

UDP-glucose + Glycogen (n residues) → UDP + Glycogen (n+1 residues)

- Branches are made by branching enzyme (also known as amylo-α(1:4)-α(1:6)transglycosylase)

Glycogenolysis

It is the conversion of glycogen polymer to glucose monomers or its phosphate. In the liver it is used for the increase of glucose level in the blood during fasting (first 24 hours).

The overall reaction for glycogenolysis:

- for liver, kidney, small intestine

$Glycogenin\text{-}(C_6H_{10}O_5)_nH + Pi \rightarrow Glycogenin\text{-}(C_6H_{10}O_5)_{n-1}H + Glucose + Pi$

- for other tissues:

$Glycogenin\text{-}(C_6H_{10}O_5)_nH + Pi \rightarrow Glycogenin\text{-}(C_6H_{10}O_5)_{n-1}H + Glucose\ 6\text{-}P$

Glycogenolysis reaction sequence:

- Glycogen phosphorylase being the main enzyme then catalyzes cleavage of

the α(1→4) glycosidic linkages of glycogen, releasing glucose 1-phosphate as the reaction product. Pyridoxal phosphate (PLP), a derivative of vitamin B_6, serves as prosthetic group for glycogen phosphorylase.

- Debranching enzyme performs two functions:

1) Glucosyltransferase transfers three glucose residues from the four-residue glycogen branch to a nearby branch. This leaves on the donor branch a single glucose residue, connected to the main chain by an alpha-1,6 linkage

2) Glucosidase cleaves the remaining alpha-1,6 linkage, producing glucose and a linear chain of glycogen

- Phosphoglucomutase catalyzes the reversible reaction:

Glucose-1-phosphate → Glucose-6-phosphate

A scheme of Glycogen metabolism is submitted in Figure 36.

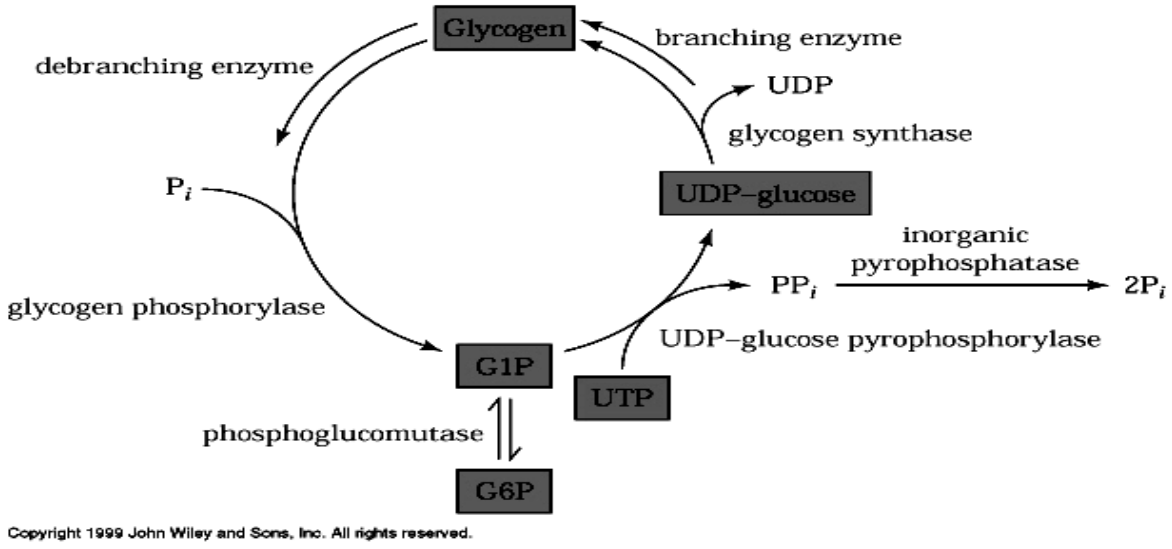


Figure 36. Glycogen metabolism: generally.

Regulation of glycogen metabolism

Glycogen synthase and glycogen phosphorylase are reciprocally regulated, both

1) by allosteric effectors and

2) by covalent modification (phosphorylation-dephosphorylation)

Reciprocal allosteric controls**:**

Glycogen phosphorylase in muscle is subject to allosteric regulation by:

- AMP activates;

- the released Ca^{2+} also activates phosphorylase kinase, which in muscle includes calmodulin as its δ-subunit. Phosphorylase kinase is partially activated by binding of Ca^{2+} to this subunit. Phosphorylation of the enzyme, via a cAMP cascade induced by epinephrine, results in further activation. These regulatory processes ensure release of phosphorylated glucose from glycogen, for entry into glycolysis to provide ATP needed for muscle contraction;

- ATP and glucose 6-phosphate inhibit.

An isozyme of glycogen phosphorylase expressed in liver is less sensitive to these allosteric controls.

Glycogen synthase *b* is activated by glucose 6-phosphate

Regulation by phosphorylation-dephosphorylation

- Both glucagon and epinephrine are produced in response to low blood glucose.
- The hormones activate receptors to trigger cAMP cascades (Fig. 37).
- Glucagon activates cAMP formation in a hepatocyte.
- Epinephrine activates cAMP formation in muscle cell.

Effect of insulin on glucose uptake and metabolism

- Insulin, produced in response to high blood glucose, triggers a separate signal cascade that leads to activation of phosphoprotein phosphatase. This phosphatase catalyzes removal of regulatory phosphate residues from glycogen phosphorylase, phosphorylase kinase, & glycogen synthase

- Insulin activates phosphodiesterase which cleaves cAMP. The opposite effect is caffeine which inhibits phosphodiesterase activity. Glycogen synthesis is reduced when caffeine poisoning occurs and as a result hyperglycemia may be observed in patients with diabetes.

- Thus insulin antagonizes effects of the cAMP cascade induced by glucagon & epinephrine. So, insulin suppresses glycogen phosphorylase (glycogen degradation) and stimulates glycogen synthase (glycogen synthesis).

Insulin binding its receptor starts a lot of other protein activation cascades.

These include: translocation of Glut-4 transporter to the plasma membrane and influx of glucose; glycolysis, fatty acid synthesis, etc.

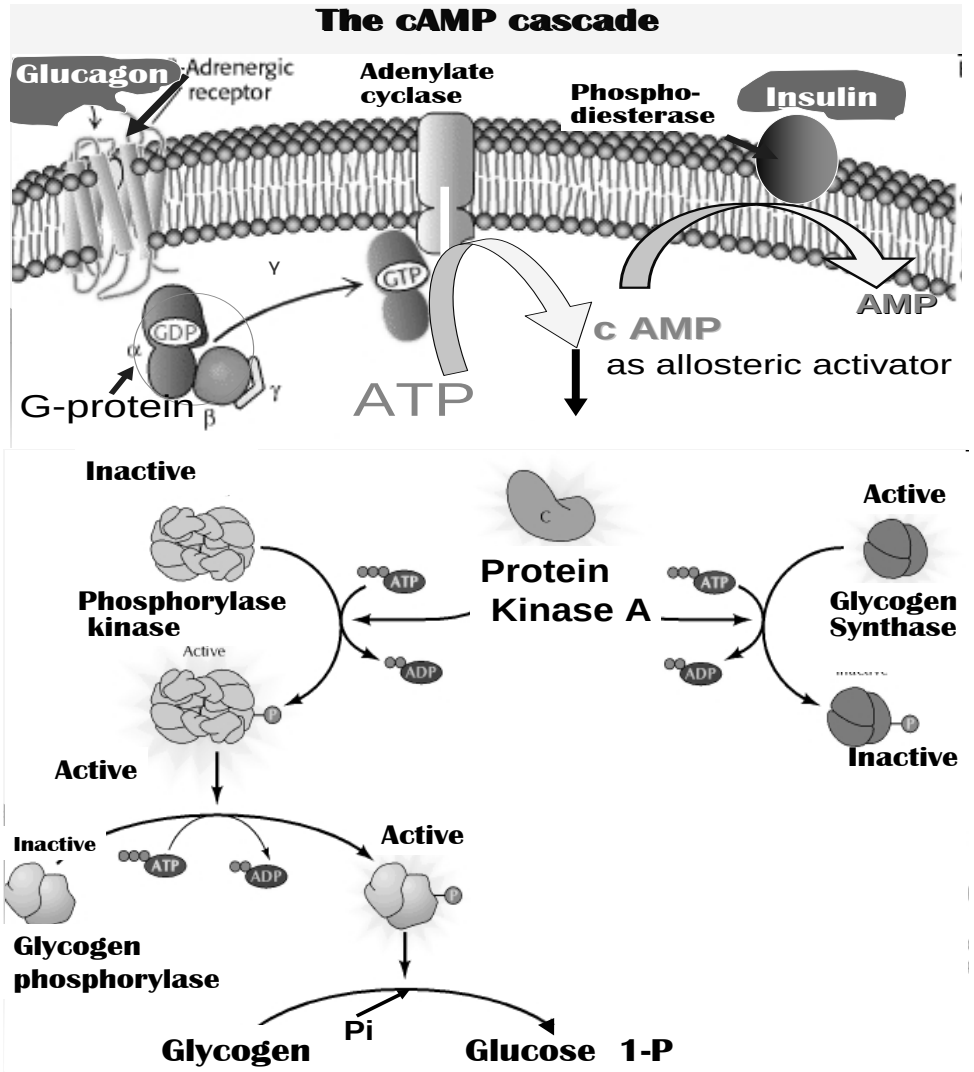


Figure 37. Reciprocal cAMP-dependent regulation of glycogen synthase and phosphorylase.

Common problems associated with carbohydrate metabolism

1. Diabetes mellitus

High blood glucose levels occur because of either a deficiency of insulin (insulin-dependent diabetes mellitus, IDDM) or the inability of tissues such as adipose and muscle to take up glucose in the presence of normal amounts of insulin (insulin resistance or noninsulin-dependent diabetes mellitus [NIDDM]). If insulin-deficiency diabetes mellitus is untreated, the body responds as if it is starving. Fuel stores are degraded in the face of high blood glucose, and

ketoacidosis may occur. Many metabolic pathways are affected.

Metabolic abnormalities in insulin-dependent diabetes mellitus:

- Glucose uptake by tissues is impaired, despite the fact that glucose is being released by the liver
- Glycolysis is depressed.
- Gluconeogenesis is stimulated.
- Glycogen stores are depleted.
- Lypolysis of triacylglycerols is depressed.
- Fatty acid oxidation is increased.
- Ketone bodies are synthesized in the liver and used as fuel by other tissues.
- Proteins are degraded, and amino acids are used as fuel.
- The levels of glucose, fatty acids, and ketone bodies in the blood become very high.
- Excess glucose leads to glycosylation of haemoglobin and possibly other proteins.

Haemoglobin A_1 reacts spontaneously with glucose to form a derivative known as glycosylated haemoglobin A_{1c} (Hb A_{1c}). Normally the concentration of HbA_{1c} in blood is very low, but in diabetes mellitus, where blood sugar levels may be high, the concentration of HbA_{1c} may reach 12% or more of the total haemoglobin. Because the average life of a red blood cell is 120 days, the amount of HbA_{1c} becomes a good indicator of blood glucose levels over 2-4 month period. Determination of the amount of glycosylated haemoglobin can estimate the hyperglycemia rate retrospectively (4-8 weeks before examination).

Cataract is considered a major cause of visual impairment in diabetic patients as the incidence and progression of cataract is elevated in patients with diabetes mellitus. The enzyme aldose reductase (AR) catalyzes the reduction of glucose to sorbitol through the polyol pathway, a process linked to the development of diabetic cataract. Extensive research has focused on the central role of the AR pathway as the initiating factor in diabetic cataract formation. It has been shown that the intracellular accumulation of sorbitol leads to osmotic changes

resulting in hydropic lens fibers that degenerate and form sugar cataracts. In the lens, sorbitol is produced faster than it is converted to fructose by the enzyme sorbitol dehydrogenase. In addition, the polar character of sorbitol prevents its intracellular removal through diffusion. The increased accumulation of sorbitol creates a hyperosmotic effect that results in an infusion of fluid to countervail the osmotic gradient. Another possible cause of cataracts is glycosylation of proteins and as a result disturbance of their structure and function.

The diagnosis of diabetes can be made on the basis of individual's response to the oral glucose load, commonly referred to as oral glucose tolerance test (OGTT) (Fig. 38).

Procedure:

1. A zero time (baseline) blood sample is drawn.
2. The patient is then given a measured dose (below) of glucose solution to drink within a 5 minute time frame.

Blood is drawn at intervals for measurement of glucose (blood sugar), and sometimes insulin levels. The intervals and number of samples vary according to the purpose of the test. For simple diabetes screening, the most important sample is the 2 hour sample and the 0 and 2 hour samples may be the only ones collected. A laboratory may continue to collect blood for up to 6 hours depending on the protocol requested by the physician.

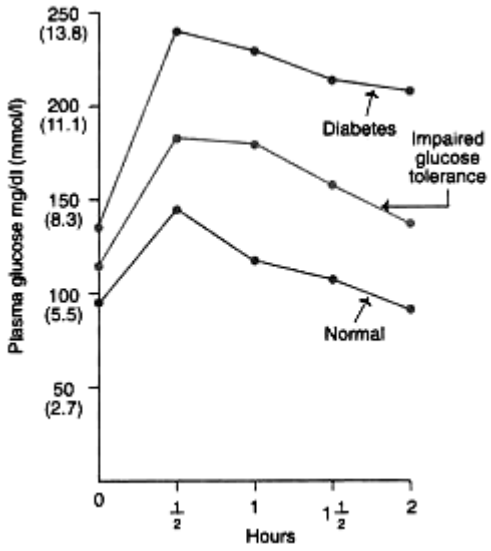


Figure 38. Results of oral glucose tolerance test:

Fasting plasma glucose (measured before the OGTT begins) should be below 6.1 mmol/L (110 mg/dL). Fasting levels between 6.1 and 7.0 mmol/L (110 and 125 mg/dL) are borderline ("impaired fasting glycaemia"), and fasting levels repeatedly at or above 7.0 mmol/L (126 mg/dL) are diagnostic of diabetes.

A 2 hour OGTT glucose level below 7.8 mmol/L (140 mg/dL) is normal, whereas higher glucose levels indicate hyperglycemia. Blood plasma glucose between 7.8 mmol/L (140 mg/dL) and 11.1 mmol/L (200 mg/dL) indicate "impaired glucose tolerance", and levels above 11.1 mmol/L (200 mg/dL) at 2 hours confirms a diagnosis of diabetes.

Clucose is not detected in any of the urine sample. If renal glycosuria (sugar excreted in the urine despite normal levels in the blood) is suspected, urine samples may also be collected for testing along with the fasting and 2 hour blood tests.

The most common cause of glucose excretion in urine (glycosuria) is diabetes mellitus. Therefore, glycosuria is the first line screening test for diabetes. Normally, glucose does not appear in urine until the plasma glucose concentration exceeds renal threshold 9-10 mmol/L (180 mg/dl).

A doctor normally can diagnose renal glycosuria when a routine urine test (Urinalysis) detects glucose in the urine, while a blood test indicates that the blood glucose level is normal.

Renal glycosuria, also known as renal glucosuria, is a rare condition in which the simple sugar glucose is excreted in the urine despite normal or low blood glucose levels. With normal kidney (renal) function, glucose is excreted in the urine only when there are abnormally elevated levels of glucose in the blood. However, in those with renal glycosuria, glucose is abnormally elevated in the urine due to improper functioning of the renal tubules, which are primary components of nephrons, the filtering units of the kidneys and as a result decreasing activity of glucose reabsorption enzymes.

2. Glycogen storage diseases

Glycogen accumulates primarily in the liver or muscle, or both. Enzyme deficiencies occur mainly in glycogen degradation or conversion to glucose. In the liver, glycogen storage diseases may produce conditions ranging from mild hypoglycemia to liver failure. In muscle, they may cause problems ranging from difficulty in performing strenuous exercise to cardiorespiratory failure (Fig. 39).

Glycogen Storage Diseases

Type	Enzyme deficiency	Tissue	Common name	Glycogen structure
I	Glucose-6-phosphatase	Liver	von Gierke's disease	Normal
II	α-1,4-Glucosidase	All lysosomes	Pompe's disease	Normal
III	Amylo-1,6-glucosidase (debranching enzyme)	All organs	Cori's disease	Outer chains missing or very short
IV	Amylo-(1,4→1,6)-transglycosylase (branching enzyme)	Liver, probably all organs	Andersen's disease	Very long unbranched chains
V	Glycogen phosphorylase	Muscle	McArdle's disease	Normal
VI	Glycogen phosphorylase	Liver	Hers' disease	Normal
VII	Phosphofructokinase	Muscle	Tarui's disease	Normal
VIII	Phosphorylase kinase	Liver	X-linked phosphorylase kinase defiency	Normal
IX	Phosphorylase kinase	All organs		Normal
0	Glycogen synthase	Liver		Normal, deficient in quantity

Figure 39. Glycogen storage diseases classification.

Glycogen storage disease type 0: a genetic defect in the isoform of the glycogen synthase enzyme expressed in liver causes a disease with symptoms that include:

- after eating a carbohydrate meal, elevated blood levels of glucose, lactate, and lipids are observed. Explanation: blood glucose is high because the liver cannot store it as glycogen. Some of the excess glucose is processed via glycolysis to produce lactate & fatty acid precursors;

- during fasting, hypoglycemia (low blood glucose) & high levels of circulating ketone bodies are observed. Explanation: blood glucose is low during

fasting because the liver lacks glycogen stores for generation of glucose. Ketone bodies are produced as an alternative fuel when glucose is inadequate.

- a deseased child behind in the mental development of their peers. There is vomiting, convulsions, loss of consciousness in the morning. In the blood - fasting hypoglycemia;

- it was found in the study of liver biopsy that glycogen synthesis does not occur in liver cells.

Treatment of this disease consists of frequent meals of complex carbohydrates (avoiding simple sugars that would lead to a rapid rise in blood glucose) and meals high in protein to provide substrates for gluconeogenesis.

3. Mucopolysaccharidoses and gangliosidoses (or sphingolipidoses)

A deficiency of lysosomal enzymes results in the inability to degrade the carbohydrate portions of proteoglycans or sphingolipids. Partially digested products accumulate in lysosomes. Tissues become engorged with these "residual bodies," and their function is impaired. These diseases are often fatal.

A mental and physical retardation, grave damage of internal connective tissue is observed in child. Urine analysis reveals excess levels of keratan sulfates in case if glucose aminoglycan metabolism is disturbed. Inherited disease, such as mucopolysaccharidosis, is manifested in metabolic disorders of connective tissue, bone and joint pathologies. The sign of this disease is the excessive urinary excretion of the glucose aminoglycans.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test tasks:	Explanations:
1.	A 45 y.o woman suffers from Cushing`s syndrome (steroid subtype of diabetes mellitus). Biochemical examination revealed hyperglycemia, hyperchloremia in this patient. Which of the processes below is the first to be stimulated? A. Gluconeogenesis B. Glucose reabsorption C. Glycogenolysis	

№	Test tasks:	Explanations:
	D. Glycolysis E. Glucose transport to the cell	
2.	Patient with diabetes mellitus experienced loss of consciousness and convulsions after an injection of insulin. What might be the result of biochemical blood analysis for concentration of glucose? A. 3.3 mmol/L B. 10.0 mmol/L C. 8.0 mmol/L D. 1.5 mmol/L E. 5.5 mmol/L	
3.	A child is languid, apathetic. Liver is enlarged, and liver biopsy revealed a significant amount of glycogen. Glucose concentration in the blood stream is below normal. What is the cause of low glucose levels: A. Low {absent} activity of hexokinase B. High activity of glycogen synthetase C. Deficit of gene that is responsible for the synthesis of glucose 1-phosphate uridine transferase D. High activity of glycogen phosphorylase in liver E. Low {absent} activity of glucose 6-phosphatase	
4.	A mental and physical retardation, grave damage of internal connective tissue is observed in child. Urine analysis reveals excess levels of keratan sulfates. Which of the following compounds metabolism is disturbed? A. Collagen B. Fibrinectin C. Glucose aminoglycan D. Ascorbic acid E. Elastin	
5.	Glycogen is synthesized from the active form of glucose. The immediate donor of glucose residues during the glycogenesis is: A. Glucose-3-phosphate B. Glucose-6-phosphate C. UDP-glucose D. Glucose-1-phosphate E. ADP-glucose	
6.	Inherited disease, such as mucopolysaccharidosis, is manifested in	

№	Test tasks:	Explanations:
	metabolic disorders of connective tissue, bone and joint pathologies. The sign of this disease is the excessive urinary excretion of the following substance: A. Lipids B. Amino acids C. Urea D. Glucose aminoglycans E. Glucose	
7.	A patient is ill with diabetes mellitus that is accompanied with hyperglycemia of over 7,2 millimole/L on an empty stomach. The level of what blood plasma protein can estimate the hyperglycemia rate retrospectively {4-8 weeks before examination}? A. C-reactive protein B. Ceruloplasmin C. Fibrinogen D. Albumin E. Glycosylated haemoglobin	
8.	According to results of glucose tolerance test, the patient has no disorder of carbohydrate tolerance. Despite that, glucose is detected in the patient's urine (5 mmole/l). The patient has been diagnosed with renal diabetes. What renal changes cause glucosuria in this case? A. Decreased activity of glucose reabsorption enzymes B. Exceeded glucose reabsorption threshold C. Increased activity of glucose reabsorption enzymes D. Increased glucose secretion E. Increased glucose filtration	
9.	A child has a history of hepatomegaly, hypoglycemia, seizures, especially on an empty stomach, and in stressful situations. The child is diagnosed with Gierke's disease. This disease is caused by the generic defect of the following enzyme: A. Glucokinase B. Glycogen phosphorulase C. Amyloid-1,6-glycosidase D. Phosphoglucomutase E. Glucose-6-phosphatase	
10.	Pancreas is known as a mixed secretion gland. Endocrine functions include	

№	Test tasks:	Explanations:
	production of insulin by beta cells. This hormone affects the metabolism of carbohydrates. What is its effect upon the activity of glycogen phosphorylase (GP) and glycogen synthase (GS)? A. It does not affect the activity of GP and GS B. It suppresses GP and stimulates GS C. It stimulates GP and suppresses GS D. It suppresses both GP and GS E. It stimulates both GP and GS	
11.	A year-old child behind in the mental development of their peers. There is vomiting, convulsions, loss of consciousness in the morning. In the blood - fasting hypoglycemia. What enzyme defect can be the reason for this state of the child? A. Sucrase B. Glycogen synthase C. Arginase D. Protein kinase E. Lactase	
12.	On the empty stomach in the patients blood glucose level was 5.65 mmol/L, in an hour after usage of sugar it was 8.55 mmol/L, in 2 hours – 4.95 mmol/L. Such indicators are typical for: A. Healthy person B. Patient with latent form of diabetes mellitus C. Patient with insulin-dependent diabetes mellitus D. Patient with non-insulin dependent diabetes mellitus E. Patient with thyrotoxicosis	
13.	Severe fasting hypoglycemia is identified in the study of patient blood. It was found in the study of liver biopsy that glycogen synthesis does not occur in the cells of the liver. What enzyme failure is the cause of the disease? A. Aldolase B. Fructose-1,6-bisphosphatase C. Glycogen synthase D.Phosphorylase E. Pyruvate carboxylase	
14.	Mucopolysaccharidosis refers to storage diseases because of the lack of enzymes	

№	Test tasks:	Explanations:
	breaks splitting of polysaccharides. Patients observed increase their release with urine and accumulation in certain cell organelles. What organelles accumulate mucopolysaccharides? A. Lysosomes B. Golgi apparatus C. Nucleus D. Endoplasmic reticulum E. Mitochondria	
15.	A 62-year-old female patient has developed a cataract (lenticular opacity) secondary to the diabetes mellitus. What type of protein modification is observed in case of diabetic cataract? A. Glycosylation B. Phosphorylation C. ADP-ribosylation D. Methylation E. Limited proteolysis	
16.	Blood glucose concentration is 15 mmol/L (renal reabsorption threshold is 10 mmol/L) in a patient. The consequence of this would be: A. Decrease in urine output B. Reducing the secretion of vasopressin C. Reducing the secretion of aldosterone D. Reducing reabsorption of glucose E. Glycosuria	
17.	On the empty stomach in the patients blood glucose level was 5.6 mmol/L, in an hour after usage of sugar it was 13.8 mmol/L, in 3 hours – 9.2 mmol/L. Such indicators are typical for: A. Healthy person B. Patient with latent form of diabetes mellitus C. Patient with insulin-dependent diabetes mellitus D. Patient with non-insulin dependent diabetes mellitus E. Patient with thyrotoxicosis	
18.	Andersen's disease belongs to a group of hereditary diseases, developing as a result of congenital deficiency of the synthesis of certain enzymes of glycogenolysis. What enzyme failure is the molecular basis for glycogen storage disease? A. Glycogen synthase	

№	Test tasks:	Explanations:
	B. Amilo (1.4-1.6) transglycosidase C. Lysosomal glycosidases D. Phosphofructokinase E. Glucose-6-phosphatase	
19.	Caffeine inhibits phosphodiesterase activity, which converts cAMP to AMP. What process velocity is reduced when caffeine poisoning occurs: A. Glycogen synthesis B. Pentose phosphate pathway C. Phosphorylation of proteins D. Lipolysis E. Glycolysis	
20.	The main reserve of carbohydrates in the body is glycogen. What organ deposits the greatest part of glycogen? A. Pancreas B. Spleen C. Kidney D. Liver E. Heart	
21.	A glycosaminoglycan having anticoagulant effect is appointed for a patient after surgery. Name of the substance: A. Chondroitin-6-sulfate B. Heparin C. Keratan sulfate D. Hyaluronic acid E. Chondroitin-4-sulfate	
22.	Proteoglycans in the body perform a variety of functions. What heteropolysaccharide used in clinical practice as an anticoagulant? A. Heparin B. Dermatan sulfate C. Chondroitin sulfate D. Keratan sulfate E. Hyaluronic acid	
23.	Blood glucose level is first increased and then reduced after consumption of food rich in carbohydrates by the action of insulin. What process is activated by the hormone? A. Glycogen synthesis B. Breakdown of proteins C. Breakdown of glycogen D. Gluconeogenesis E. Breakdown of lipids	
24.	A woman of 45 years old, a long time	

№	Test tasks:	Explanations:
	suffering from diabetes, after insulin administration appeared weakness, pallor, palpitations, anxiety, double vision, numbness of the lips and tongue tip. Blood glucose levels is 2.5 mmol/L. What complication is developed in the patient? A.Hyperglycemic coma B. Hyperosmolar coma C. Uremic coma D. Hypoglycemic coma E. Hyperketonemic coma	
25.	Hyperglycemia, glycosuria, high density of urine, increased the amount of glucocorticoids in the blood are observed, concentration of 17-ketosteroids in the blood and urine is increased? What disease is in the patient? A. Steroid diabetes B. Diabetes mellitus C. Diabetes insipidus D. Hepatic diabetes E. Renal diabetes	
26.	Transmission of information from peptide hormones into the cell occurs via intracellular secondary messengers, with the participation of adenylate cyclase. What reaction is catalyzed by adenylate cyclase? A. Synthesis of ATP from AMP and pyrophosphate B. Synthesis of cAMP C. Cleavage of ADP to form inorganic phosphate and AMP D. The splitting of ATP to ADP and inorganic phosphate E. The splitting of ATP to cAMP and pyrophosphate	
27.	Which one of the following enzymes is associated with glycogen synthesis? A. Branching enzyme B. Glycogen phosphorylase C. Phosphorylase kinase D. Debranching enzyme E. Glucose-6-phosphatase	
28.	Point out the main process maintaining the blood glucose level during fasting: A. Glycolysis B. Hexose monophosphate shunt C. Glycogenolysis in the muscles	

№	Test tasks:	Explanations:
	D. Glycogenolysis in the liver E. Gluconeogenesis in the muscles	
29.	Insulin stimulates all the processes listed below except: A. Glycogenesis B. Hexose monophosphate shunt C. Transport of glucose through cell membrane D. Glycolysis E. Glycogenolysis	
30.	A child has been diagnized for hypoglycemia, enlarged liver, and excess fat deposition in the cheeks. A liver biopsy reveals excess glycogen in hepatocytes. There is hypothetically Pompe's desease. Deficiency of which of the following enzymes might explain this phenotype? A. Glucose 6-phosphatase B. Glucosyl -4:6 transferase C. Lysosomal α-1,4-glucosidase D. α-1,4-galactosidase E. Amylo α -1,6 glucosidase	

LIPOPROTEINS OF BLOOD PLASMA. METABOLISM OF TRIACYLGLYCEROLS AND OF GLYCEROPHOSPHOLIPIDS

(Ivanchenko D.G.)

INFORMATIONAL MATERIAL

Lipids are some organic compounds non-soluble in the water but soluble in organic solvents represented in human tissues.

Among them are naturally-occurring molecule, such as fats, oils, waxes, cholesterol, steroids, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, triacylglycerols phospholipids, and others.

Although the term “lipids” is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides and should not be confused with the term fatty acid. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol (Fig. 40).

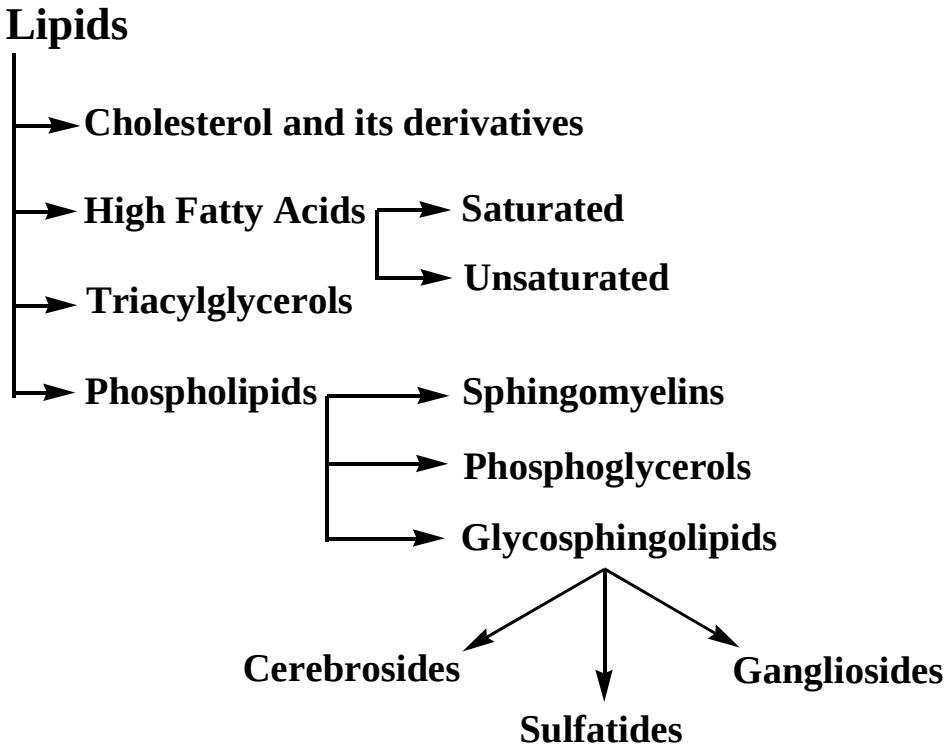


Figure 40. The classification of lipids.

The main biological functions of lipids include energy storage, acting as

structural components of cell membranes, and participating as important signaling molecules.

Triacylglycerols

The simplest lipids constructed from fatty acids are the triacylglycerols (TG), also referred to as triglycerides or neutral fats. Triacylglycerols are composed of three fatty acid residues each in ester linkage with a single glycerol (Fig. 41). Those containing the same kind of fatty acid in all three positions are called simple triacylglycerols and are named after the fatty acid they contain. Simple triacylglycerols of 16:0, 18:0, and 18:1, for example, are tripalmitin, tristearin, and triolein, respectively (Fig. 41). Most naturally occurring triacylglycerols are mixed; they contain two or three different fatty acids. To name these compounds unambiguously, the name and position of each fatty acid acyl must be specified.

Triacylglycerols are nonpolar, hydrophobic molecules, essentially insoluble in water. Lipids have lower specific gravities than water, which explains why mixtures of oil and water (oil-and-vinegar salad dressing, for example) have two phases: oil, with the lower specific gravity, floats on the aqueous phase.

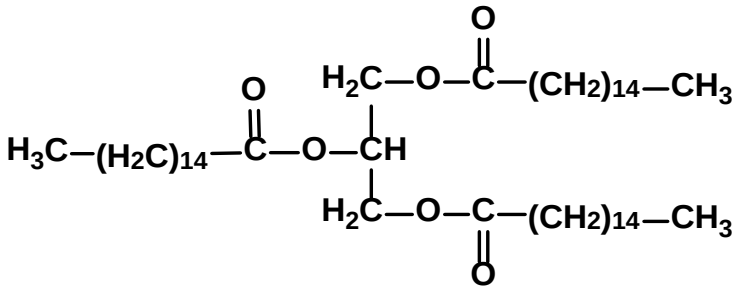


Figure 41. Tripalmitoyl glycerol structure.

In vertebrates, specialized cells named adipocytes, or fat cells, store large amounts of triacylglycerols as fat droplets that nearly fill the cell. There are two significant advantages to use triacylglycerols as stored fuels, rather than polysaccharides such as glycogen and starch. First, the carbon atoms of fatty acids are more reduced than those of sugars, and oxidation of triacylglycerols yields more than twice as much energy, gram for gram, as the oxidation of carbohydrates.

Second, because triacylglycerols are hydrophobic and therefore unhydrated, the organism that carries fat as fuel does not have to carry the extra weight of water of hydration that is associated with stored polysaccharides (2 g per gram of polysaccharide).

Phospholipids

A 1,2-diacylglycerol that has a phosphate group esterified at carbon atom 3 of the glycerol backbone is a **glycerophospholipid,** also known as a *phosphoglyceride* or a *phosphatidic acid* (Fig. 42). These lipids form one of the largest class of natural lipids and one of the most important. They are components of cell membranes and are found in small concentrations in other parts of cells, too. It should be noted that all glycerophospholipids are members of the broader class of lipids known as **phospholipids.**

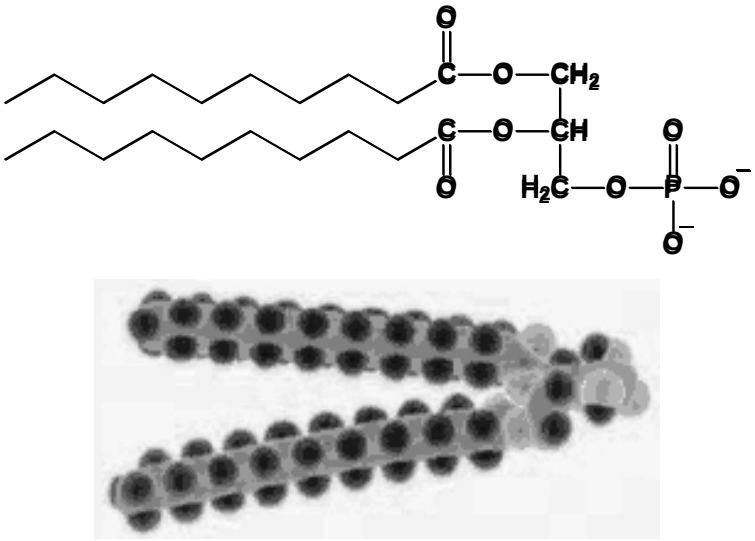


Figure 42. Phosphatidic acid, the parent compound for glycerophospholipids.

Phosphatidic acid, the parent compound for the glycerol-based phospholipids, consists of glycerol-3-phosphate, with fatty acids esterified at the 1- and 2-positions. Phosphatidic acid is found in small amounts in most natural systems and is an important intermediate in biosynthesis of more common glycerophospholipids (Fig. 43). In these compounds, a variety of polar groups are

esterified to the phosphoric acid moiety of the molecule. The phosphate, together with such esterified entities, is referred to as a “head” group. Phosphatides with choline or ethanolamine are referred to as **phosphatidylcholine** (known commonly as **lecithin**) or **phosphatidylethanolamine,** respectively. These phosphatides are two of the most common constituents of biological membranes. Other common *head groups* found in phosphatides include glycerol, serine, and inositol. Another kind of glycerol phosphatide found in many tissues is **diphosphatidylglycerol.** First observed in heart tissue, it is also named **cardiolipin.** In cardiolipin, a phosphatidylglycerol is esterified through the C-1 hydroxyl group of the glycerol moiety of the head group to the phosphoryl group of another phosphatidic acid molecule.

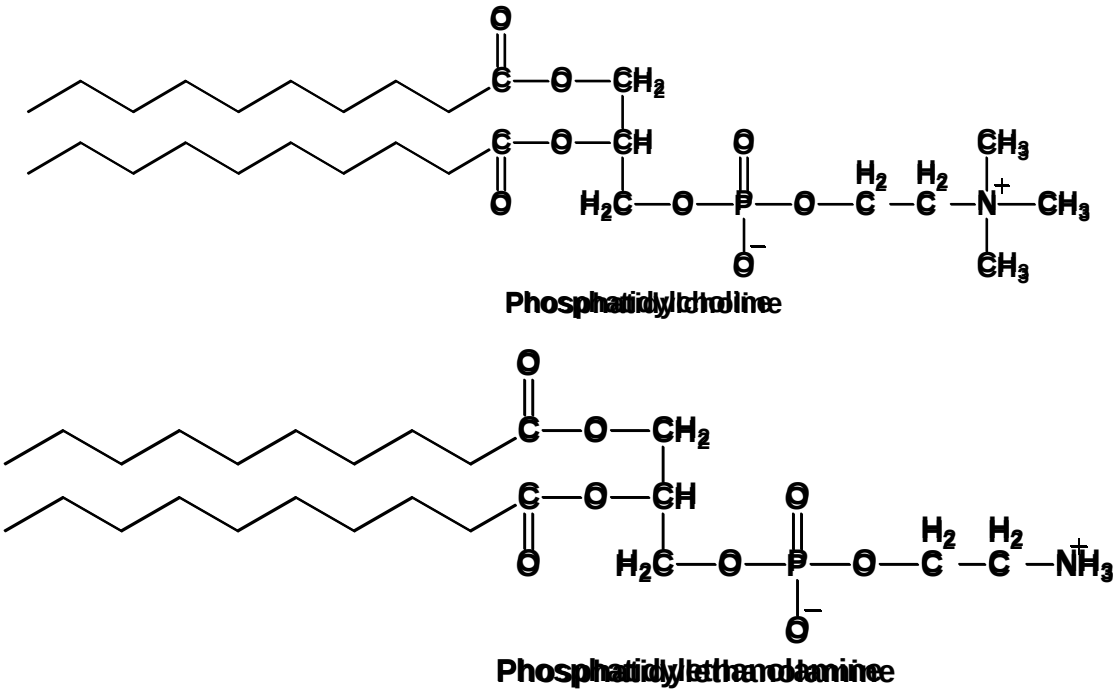


Figure 43. Structures of several glycerophospholipids.

Phosphatides exist in many different varieties, depending on the fatty acids esterified to the glycerol group. As we shall see, the nature of fatty acids can greatly affect the chemical and physical properties of phosphatides and the membranes that contain them. In most cases, glycerol phosphatides have a saturated fatty acid at position 1 and an unsaturated fatty acid at position 2 of glycerol. Thus, **1-stearoyl-2-oleoyl-phosphatidylcholine** is a common constituent

in natural membranes, but **1-linoleoyl-2-palmitoylphosphatidylcholine** is not.

Sphingolipids

These lipids are found in the cellular membranes of all eukaryotic cells, although the concentration is highest in cells of central nervous system. Sphingolipids do not have a glycerol backbone; they are formed from aminoalcohol sphingosine. (Fig. 44). Shingosine is derived from serine and a specific fatty acid, palmitate. Ceramides are amides formed from sphingosine by attaching fatty acid to its amino group. Various sphingolipids are then formed by attaching different groups to hydroxyl group on ceramide. As reflected in the names for cerebrosides and gangliosides, these sphingolipids contain sugars attached to hydroxyl group of ceramide through glycosidic bonds. They are glycolipids (more specifically, glycosphingolipids). Sphingomyelin, which contains a phophorylcholine group attached to ceramide, is a component of cell membranes and the myelin sheath around neurons.

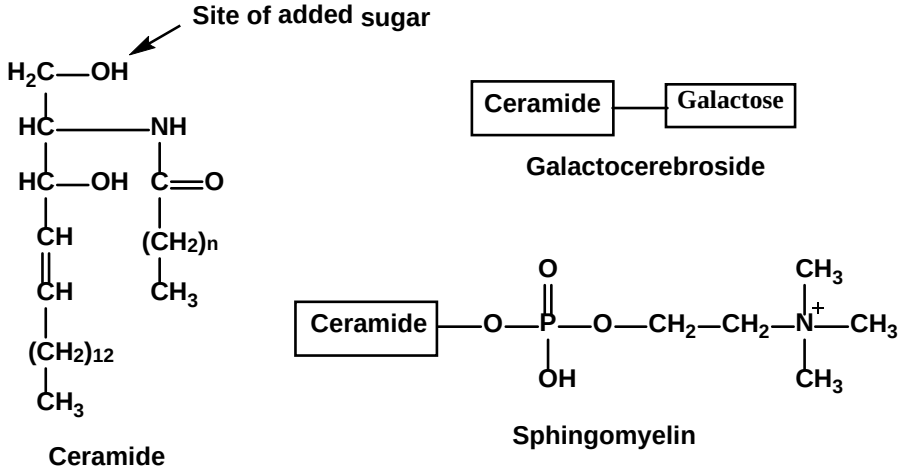


Figure 44. Sphingolipids, derivatives of ceramide.

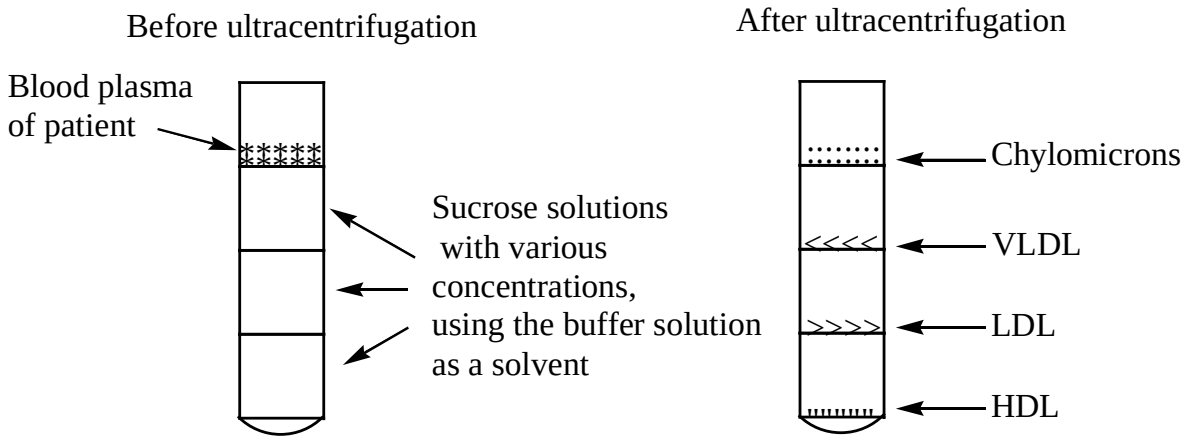
The ivestigation methods for lipids and lipoproteins

Two main methods for separation of lipids and lipoproteins are used: ultracentrifugation and electrophoresis.

Ultracentrifugation

Sucrose solutions with various concentrations are added step by step to the

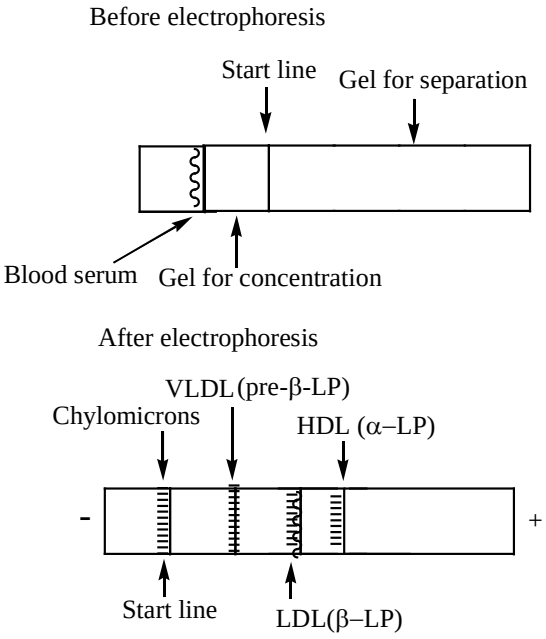
test centrifugal tube and the blood plasma of patient is plotted on the surface of this mixture. Then there is the centrifugation using special centrifuge (~ 24 hours) that gives 50000g.



Then there is the ability to take any fraction of lipoproteins for further study.

Electrophoresis

Poly Acryl Amide Gel (PAAG) may be used as a carrier for plotting of blood serum (0,2 mL). pH of buffer solution must be about 7,4-7,6 and after electrophoresis and painting of fractions we can see the result:



Plasma lipoproteins: classification, structure, composition and function

The term lipoprotein can describe any protein that is covalently linked to

lipid groups (e.g., fatty acids or prenyl groups), it is most often used for a group of molecular complexes found in the blood plasma of mammals (especially humans). Plasma lipoproteins transport lipid molecules (triacylglycerols, phospholipids, and cholesterol) through the bloodstream from one organ to another. Lipoproteins also contain several types of lipid-soluble molecules (fat-soluble vitamins A, E, D, K and several carotenoids) (Fig. 45). The protein components of lipoproteins are named **apolipoproteins** or **apoproteins**.

The most hydrophobic TG and cholesterol esters are placed in site of those lipoprotein particles named **micella**. Phospholipids create monolayer turned to around medium. Proteins are placed mainly on the surface of those micella to be involved in receptor-dependent endocytosis.

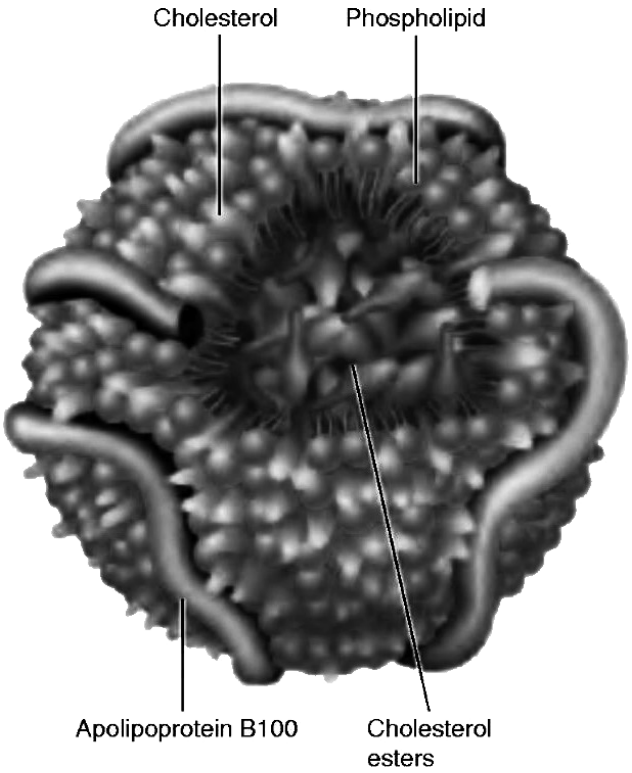


Figure 45. Plasma Lipoprotein micella structure.

The small particles of plasma lipoprotein, which carry triacylglycerols, may be separated according to their densities by centrifugation. They have been classified into five groups of increasing density: **chylomicrons (Chm),** very low density lipoproteins **(VLDL)**, intermediate density lipoproteins (**IDL**), low density **(LDL),** and high density lipoproteins **(HDL)** (Fig. 46). Each lipoprotein particle

contains one or more apolipoproteins, whose sizes vary from the enormous 4536-residue apoB-100 to apoC-II and apoC-III, each of which contains just 79 residues, and the 57-residue apoC-I (Fig. 47).

Figure 46. Classes of Lipoprotein Particles

Class	Diametr (nm)	Density (g/ml)	Composition (weight %)*				
			Surface components			Core lipids	
			Protein	Phospho-lipid	Cholesterol	Cholesteryl esters	Triacyl-glycerol
Chylo-microns	75-1200	0.930	2	7	2	3	86
VLDL	30-80	0.930-1.006	8	18	7	12	55
IDL	25-35	1.006-1.019	19	19	9	29	23
LDL	18-25	1.019-1.063	22	22	8	42	6
HDL2	9-12	1.063-1.125	40	33	5	17	5
HDL3	5-9	1.125-1.210	45	35	4	13	3
Lp(a)	25-30	1.040-1.090					

*Data from Havel, R. J., and Kane, J. P. (1995) in *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II (Scriver C. R., Beaudet A.L., Sly W. S., and Valle D., eds), pp. 1841 – 1852, McGraw-Hill, New York.

Chylomicrons (Chm), which are assembled by the intestinal mucosa, function to keep exogenous triacylglycerols and cholesterol absorbed from the gastrointestinal tract. Precursors (**nascent** forms – newly released, synthesized, immature) involved into different interactions during their circulation in the blood stream to be converted to **remnant** (parts left over after the loss of components) forms which are ready for the degradation. These lipoproteins are released into the intestinal lymph (known as chyle), which are transported through the lymphatic vessels before draining into the large body veins via the thoracic duct. After a fatty meal, the otherwise clear chyle takes on a milky appearance in the blood.

Chm reach the liver across vena porta and adhere to binding sites on the inner surface (endothelium) of the capillaries in skeletal muscle and adipose tissue. There, within minutes after entering the bloodstream, the chylomicron triacylglycerols are hydrolyzed through the action of lipoprotein lipase (LPL), an

extracellular enzyme that is activated by apoC-II. Then tissues take up the liberated monoacylglycerol and fatty acid hydrolysis products. The chylomicrons shrink as their triacylglycerols are progressively hydrolyzed until they are reduced to cholesterol-enriched chylomicron remnants. The chylomicron remnants reenter the circulation by dissociating from the capillary endothelium and are subsequently taken up by the liver. Utilization of Chm is due to apoE-receptor depended endocytosis. It should be noted that any lipoprotein class is degraded due to the same mechanism. Chm therefore function to deliver dietary triacylglycerols and cholesterol to the liver, muscle and adipose tissue (Fig. 48).

Figure 47. Properties of Major Plasma Apolipoproteins

Designation	№ residues	Mass (kDa)	Source	Function
A-I	243	29		Major HDL protein, cofactor of LCAT
A-II	–	17.4	Liver and intestine	Unknown
A-IV	376	44.5	Intestine	Unknown
B-100	4536	513	Liver	VLDL formation; ligand for LDL receptor
B-48	2152	241	Intestine	Chylomicron formation, ligand for liver Chm receptor
C-I	57	6.6	Liver	Inhibition of Cholesteryl ester transfer protein
C-II	79	8.9	Liver	Cofactor for lipoprotein lipase
C-III	79	8.8	Liver	Inhibits lipoprotein lipase and hepatic lipase
D	–	31	Many tissues	Structural component of lipocalins (are a family of proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids)
E	299	34	Liver, VLDL	Ligand for Chm receptor
(a)	Variable			Ligand for liver chylomicron receptor

VLDL, which are synthesized in the liver as lipid transport vehicles, are also degraded by lipoprotein lipase. The VLDL remnants appear in the circulation, first as IDL and then as LDL. In the transformation of VLDL to LDL, all their proteins but apoB-100 are removed and much of their cholesterol is esterified by the HDL-associated enzyme lecithin–cholesterol acyltransferase (LCAT), which is activated by apoA-I and catalyzes those reaction: esterification of cholesterol by LCAT is made at the moment of a contact of HDL with these lipoprotein or with outer surface of cellular membrane of any peripheral cell where excess cholesterol occurs.

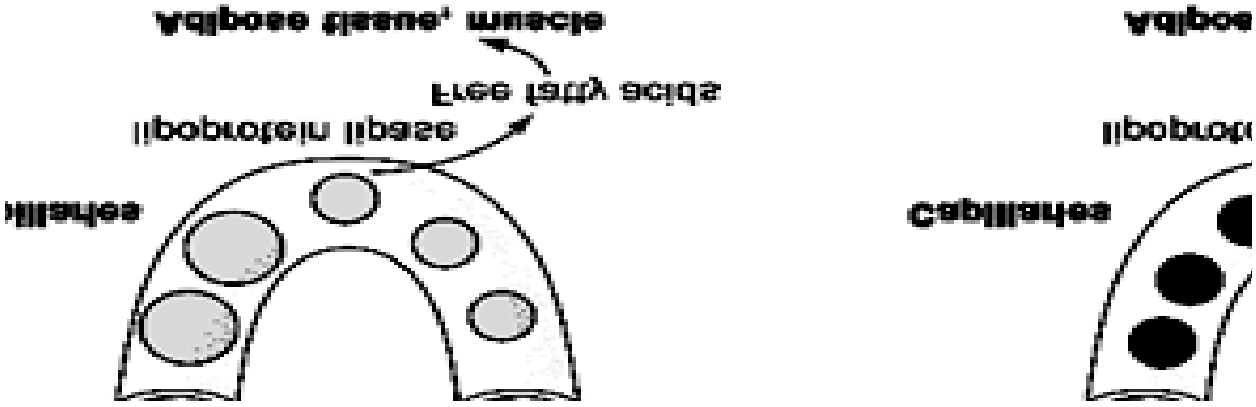


Figure 48. Model for plasma triacylglycerol and cholesterol transport in humans.

ApoB-100, a 4536-residue monomeric glycoprotein (and thus one of the largest monomeric proteins known), has a hydrophobicity approaching that of integral proteins and contains relatively few amphipathic helices. Hence, in contrast to the other, less hydrophobic plasma apolipoproteins, apoB-100 is neither water-soluble nor transferred between lipoprotein particles. Each LDL particle contains but one molecule of apoB-100, which immunoelectron microscopy indicates has an extended form that covers at least half of the particle surface. Chylomicrons, however, contain apoB-48, a 2152-residue protein that is identical in sequence to the N-terminal 48% of apoB-100. Indeed, both proteins are encoded by the same gene.

Cholesterol is an essential component of animal cell membranes. The

cholesterol may be externally supplied or, if this source is insufficient, internally synthesized. Michael Brown and Joseph Goldstein have demonstrated that cells obtain exogenous cholesterol mainly through the endocytosis (engulfment) of LDL in complex with LDL receptor (LDLR), a cell-surface transmembrane glycoprotein that specifically binds apoB-100. LDLR also binds chylomicron remnants via their apoE components. Such receptor-mediated endocytosis (Fig. 10) is a general mechanism whereby cells take up large molecules, each through a corresponding specific receptor. LDL specifically binds to LDL receptors (LDLRs) on clathrin-coated pits (**1**). These bud into the cell (**2**) to form coated vesicles (**3**), whose clathrin coats depolymerize as triskelions, resulting in the formation of uncoated vesicles (**4**). These vesicles then fuse with vesicles called endosomes (**5**), which have an internal pH of ~ 5.0. The acidity induces LDL to dissociate from LDLR. LDL accumulates in the vesicular portion of the endosome, whereas LDLR concentrates in the membrane of an attached tubular structure, which then separates from the endosome (**6**) and subsequently recycles LDLR to the plasma membrane (**7**). The vesicular portion of the endosome (**8**) fuses with a lysosome (**9**), yielding a secondary lysosome (**10**), wherein the apoB-100 component of LDL is degraded to its component amino acids and the cholesteryl esters are hydrolyzed by a lysosomal lipase to yield cholesterol and fatty acids. An LDLR molecule cycles in and out of the cell every 10 to 20 minutes during its ~ 20-hour lifetime.

Any excess intracellular cholesterol is reesterified for storage within the cell through the action of acyl-CoA:cholesterol acyltransferase (ACAT). The overaccumulation of cellular cholesteryl esters is prevented by two feedback mechanisms:

1. High intracellular levels of cholesterol suppress the synthesis of LDLR, thus decreasing the rate of LDL accumulation by endocytosis.

2. Excess intracellular cholesterol inhibits the biosynthesis of cholesterol.

HDL has essentially the opposite function of LDL: It removes cholesterol from the tissues. Nascent forms of HDL are produced in the liver, but remnant HDL are assembled in the plasma from components obtained largely through the

degradation of other lipoproteins. Circulating HDL acquires its cholesterol by extracting it from cell-surface membranes and converts it to cholesteryl esters through the action of LCAT, an enzyme that is activated by apoA-I. HDL therefore functions as a cholesterol scavenger.

The liver is the only organ capable of disposing of significant quantities of cholesterol (by its conversion to bile acids).This occurs through the mediation of both LDLR and a specific HDL receptor named SR-BI (for scavenger receptor class B type I). About half of the VLDL, after its degradation to IDL and LDL, is taken up by the liver via LDLR-mediated endocytosis (Fig. 49). However, hepatocytes (liver cells) take up cholesteryl esters from HDL by an entirely different mechanism: rather than being engulfed and degraded, the SR-BI–bound HDL selectively transfers its component cholesteryl esters to the cell. The lipid-depleted HDL then dissociates from the cell and reenters the blood circulation.

Reasons of Hyperlipoproteinemies development in human

Hyperlipoproteinemia is a metabolic disorder characterized by abnormally elevated concentrations of specific lipoprotein particles in the plasma.

According the classification (Fridrickson E., et all) hyperlipoproteinemies are divided in five types:

Type I (Hyperchylomicronemia). Defect in human organism: decreased lipoprotein lipase, altered ApoC-II. Diagnostic results: very high level of chylomicrons in the blood serum on empty stomach. Slight higher levels for VLDL and triacylglycerols. Xanthomatosis in patients is associated with this type of hyperlipoproteinemia.

Type II (Hyper-β-lipoproteinemia). Subtype IIa. Defect in human organism: LDL receptor deficiency. Diagnostic results: high levels of LDL and total cholesterol in patients. Subtype IIb. Defect in human organism: decreased LDL receptor and increased ApoB. Diagnostic results: high levels of LDL, VLDL, cholesterol and triacylglycerols. Ischemic heart disease and hypertension is observed at patients.

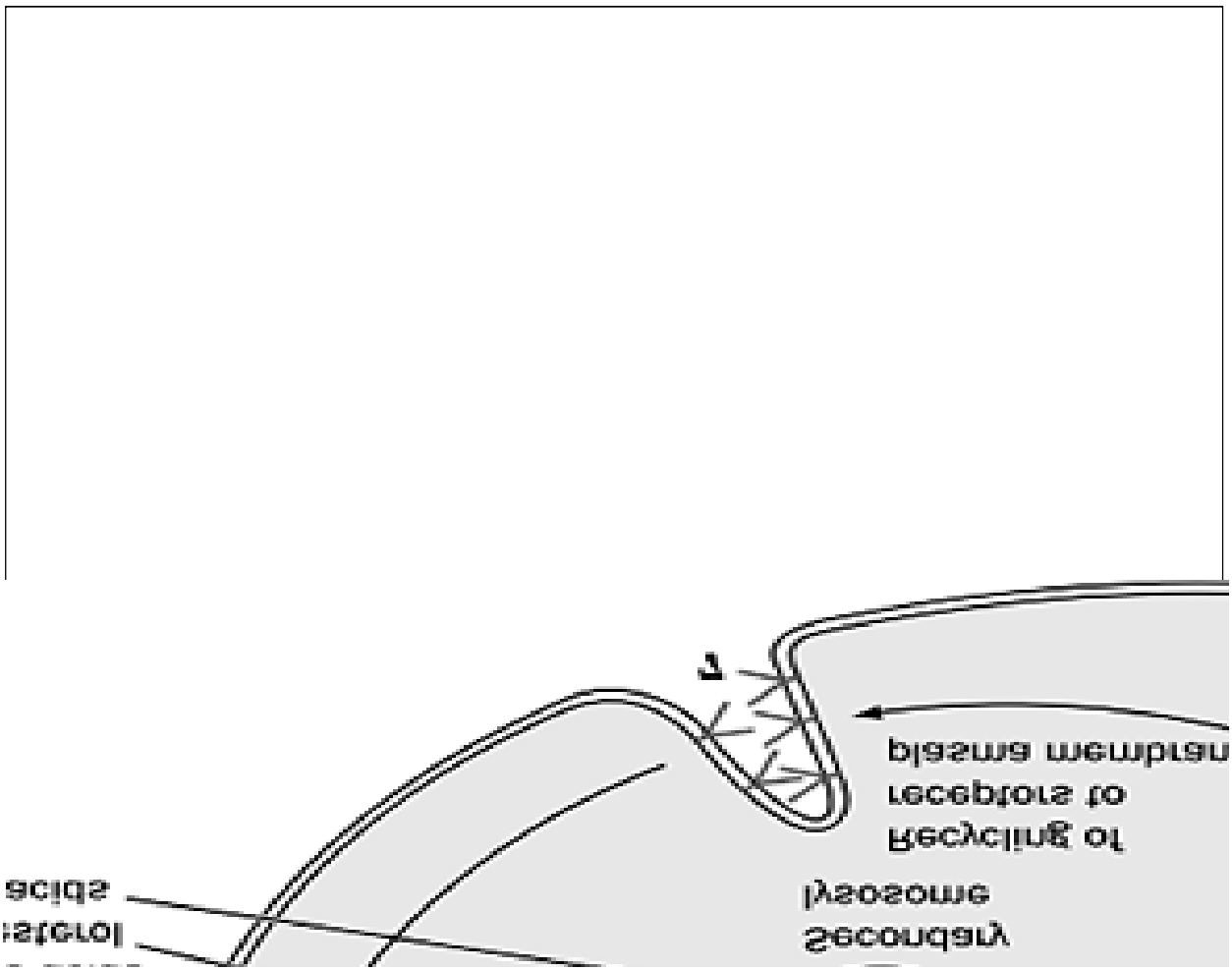


Figure 49. Sequence of events in the receptor-mediated endocytosis of LDL.

Type III (Dis-β-lipoproteinemia). Defect in human organism: ApoE-II synthesis. Diagnostic results: high levels of IDL in the blood plasm that are absent at healthy adults, high levels of cholesterol. These changes associated with problems in heart system: atherosclerosis of blood vessels, thrombosis may be at patient too.

Type IV (Hyper-pre-β-lipoproteinemia). Diagnostic results: high levels of VLDL, but LDL are slight higher or normal. Chylomicrons are absent. Diabetes mellitus with obesity and ischemic heart disease are associated with this type of hyperlipoproteinemia.

Type V (Hyper-pre-β-lipoproteinemia accompanied with Hyperchylomicronemia). Diagnostic results: high levels of chylomicrons and VLDL. Xanthomatosis is represented, also. This state may be in patients with

latent form of insulin-independent diabetes mellitus, but ischemic heart disease is not observed at patient, in this case.

Triacylglycerols degradation in adipose tissue

The mobilization of fatty acids from triacylglycerol stores depends upon hormone-sensitive lipase (Fig. 50). This enzyme is activated by cAMP-dependent phosphorylation (catalyzed by protein kinase A) and moves from the cytoplasm to surfaces of lipid droplets in response to catecholamines and other lipolytic hormones (glucagon, STH, ACTH) (Fig. 51). The increased level of cyclic AMP then stimulates protein kinase A, which phosphorylates two key proteins: perilipin A, a fat-droplet-associated protein, and hormone-sensitive lipase. Fatty acids are a major fuel for aerobic cells (except neurons).

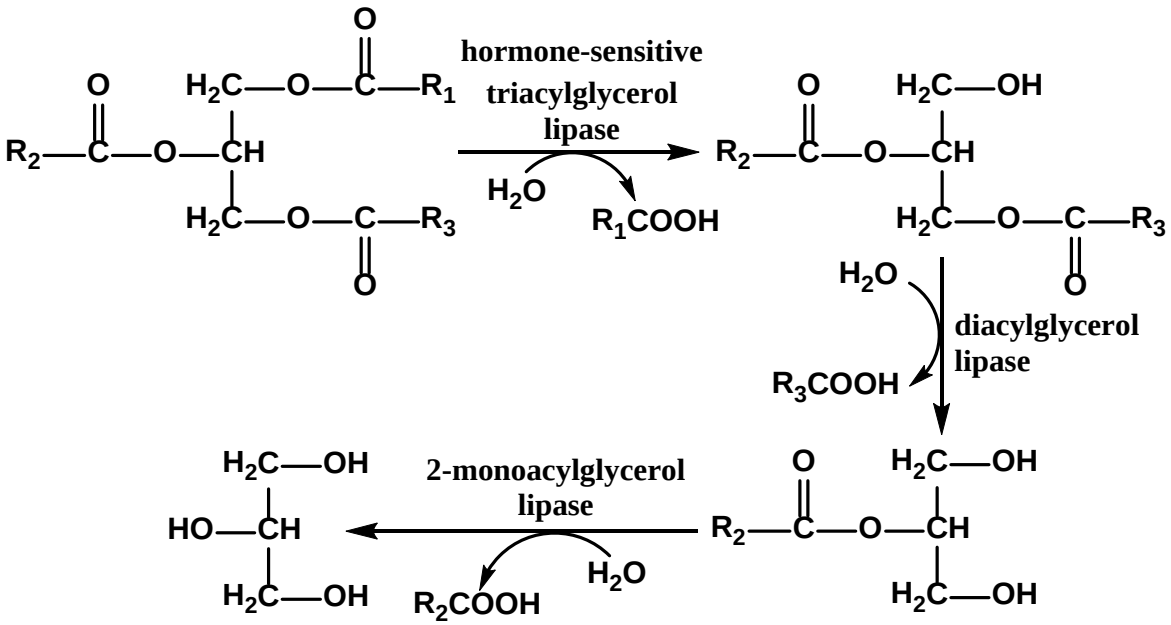


Figure 50. Hydrolysis of triacylglycerols.

Both products of lipolysis (i.e., fatty acids and glycerol) are released into the blood. Fatty acids are carried to tissues for use in synthesis of triacylglycerols, phospholipids, and other membrane lipids. Glycerol is transported to the liver where it can be used in lipid or glucose synthesis (Fig. 52). After their transport across the adipocyte plasma membrane, fatty acids become bound to serum albumin. The albumin-bound fatty acids are carried to tissues throughout the body,

where they are oxidized to generate energy. Fatty acids are transported into cells by a protein in the plasma membrane. This process is linked to the active transport of sodium. The amount of fatty acid that is transported depends on its concentration in blood and the relative activity of the fatty acid transport mechanism.

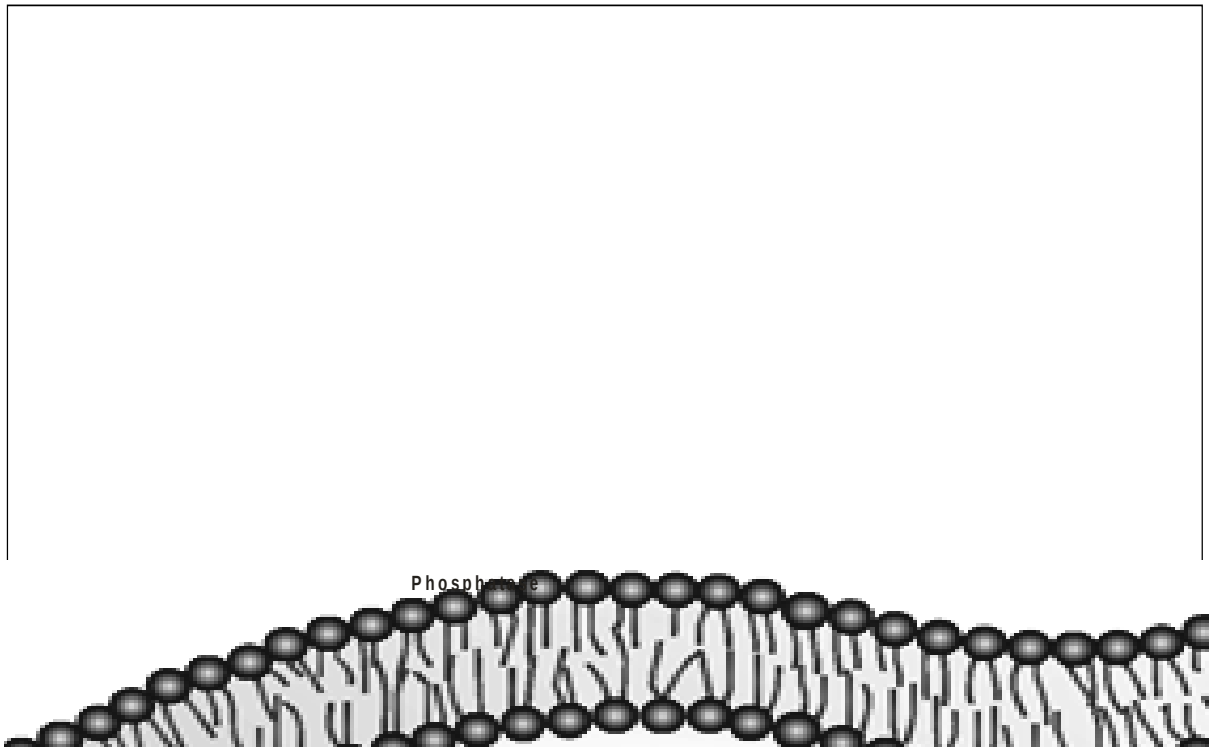


Figure 51. Diagrammatic view of lipolysis.

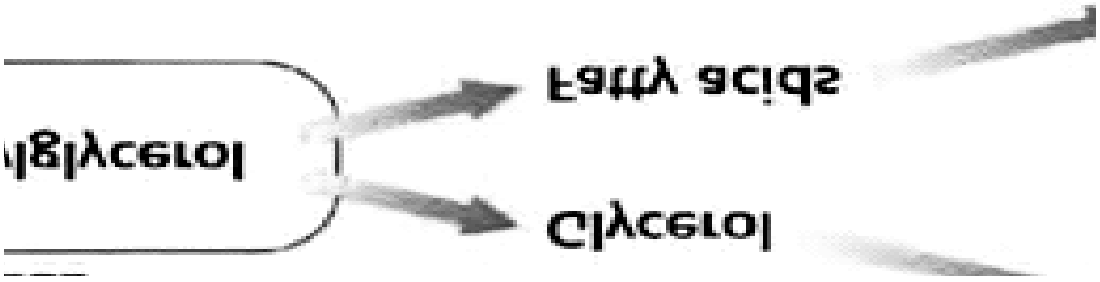


Figure 52. Lipolysis products and their metabolism in tissues:

Starvation → Glucagon↑→ Triacylglycerol lipase activity ↑;

Physical loading → Epinephrine, STH↑ → Triacylglycerol lipase activity ↑;
Emotional stress → ACTH, Epinephrine → Triacylglycerol lipase activity ↑;
Extensive growing → STH → Triacylglycerol lipase activity ↑.

Synthesis of Triacylglycerols

Triacylglycerols are synthesized from fatty acyl-CoA esters and glycerol-3-phosphate or dihydroxyacetone phosphate (Fig. 53). The initial step in this process is catalyzed either by glycerol-3-phosphate acyltransferase in mitochondria and the ER, or by dihydroxyacetone phosphate acyltransferase in the ER or peroxisomes. In the liver glycerol-3-phosphate is formed from glycerol under glycerolkinase action. In the latter case, the product acyl-dihydroxyacetone phosphate is reduced to the corresponding lysophosphatidic acid by an NADPH-dependent reductase. The lysophosphatidic acid is converted to a triacylglycerol by the successive actions of 1-acylglycerol-3-phosphate acyltransferase, phosphatidic acid phosphatase, and diacylglycerol acyltransferase. The intermediate phosphatidic acid and 1,2-diacylglycerol (DAG) can also be converted to phospholipids. The acyltransferases are not completely specific for particular fatty acyl-CoAs, either in chain length or in degree of unsaturation, but in triacylglycerols of human adipose tissue, palmitate tends to be concentrated at position 1 and oleate at position 2 of glycerol fragment.

The dihydroxyacetone phosphate used to make glycerol-3-phosphate for triacylglycerol synthesis comes either from glucose via the glycolytic pathway or from oxaloacetate via an abbreviated version of gluconeogenesis termed glyceroneogenesis. Glyceroneogenesis is necessary in times of starvation, since approximately 30% of the fatty acids that enter the liver during a fast are reesterified to triacylglycerol and exported as VLDL. Adipocytes also carry out glyceroneogenesis in times of starvation. They do not carry out gluconeogenesis but contain the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK), which is upregulated when glucose concentration is low, and participates in the glyceroneogenesis required for triacylglycerol biosynthesis.

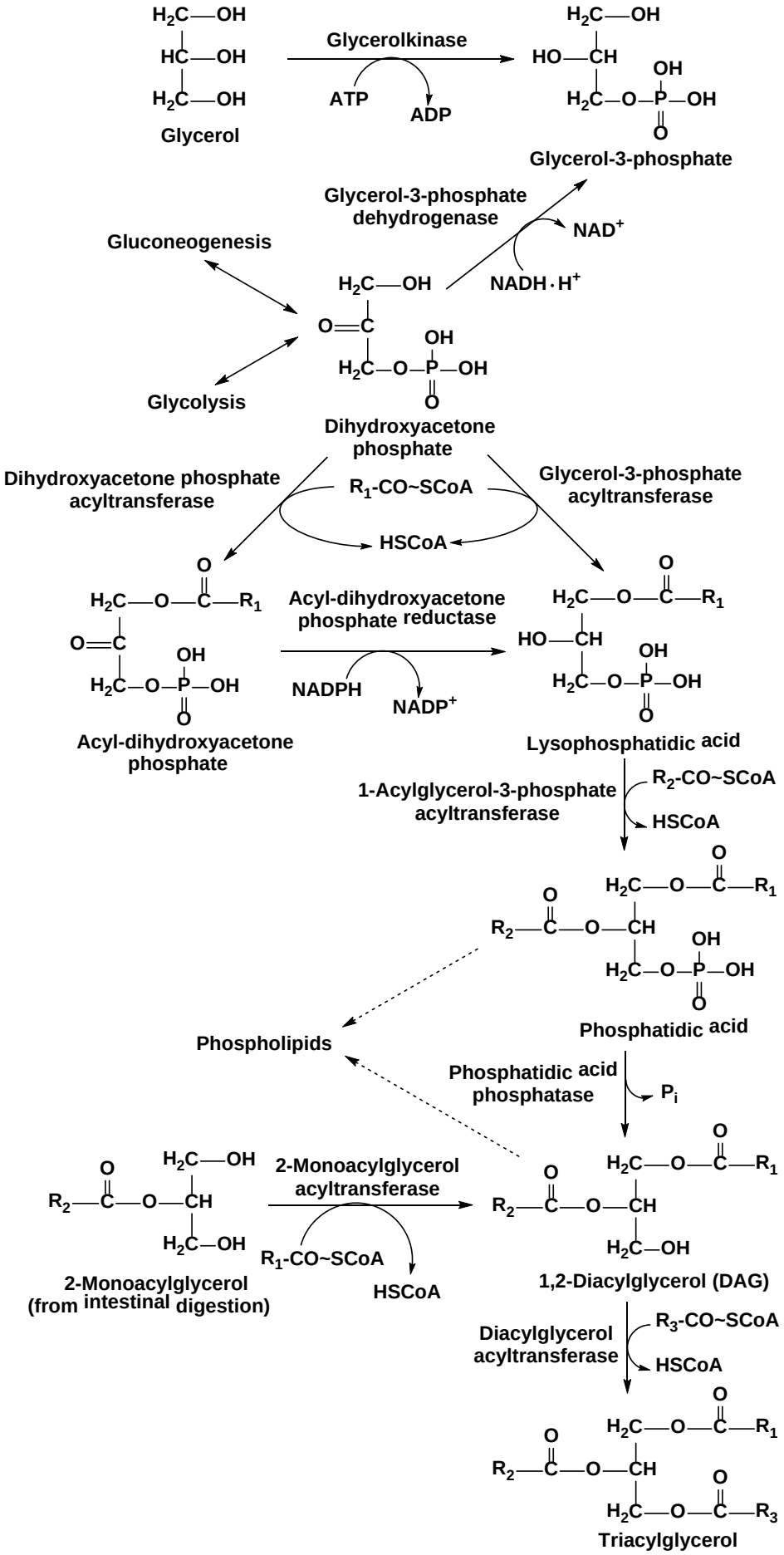


Figure 53. The reactions of triacylglycerol biosynthesis.

Lipid biosynthesis is controlled by long-term regulation, with insulin stimulating and starvation inhibiting the synthesis of acetyl-CoA carboxylase and fatty acid synthase. The presence in the diet of polyunsaturated fatty acids also decreases the concentrations of these enzymes. The amount of adipose tissue lipoprotein lipase, the enzyme that initiates the entry of lipoprotein-packaged fatty acids into adipose tissue for storage, is also increased by insulin and decreased by starvation. In contrast, the concentration of heart lipoprotein lipase, which controls the entry of fatty acids from lipoproteins into heart tissue for oxidation rather than storage, is decreased by insulin and increased by starvation. Starvation and/or regular exercise, by decreasing the glucose concentration in the blood, change the body's hormone balance. This situation results in long-term changes in gene expression that increase the levels of fatty acid oxidation enzymes and decrease those of lipid biosynthesis.

If balanced diet is created by human person ability for TG synthesis is considered mainly in the liver after portion of carbohydrates intake with food products or due to appearence of 2-monoacylglycerols after their absorption or resynthesis in the small intestine wall and transport with Chm across vena portae to the liver.

Main signal to stimulate enzymes of TG synthesis is accumulation of HFA acyl-CoAs inside the hepatocyte.

Beginning of TG synthesis in adipose tissue is observed in humans under excess intake of exogenous carbohydrates and TG. To like eating cakes with butter cream – direct way to obesity whose development is promoted by described metabolic pathway duration.

Phospholipid metabolism

The "complex lipids" are dual-tailed amphipathic molecules composed of either 1,2-diacylglycerol or N-acylsphingosine (ceramide) linked to a polar head group that is either a phosphate ester (Fig. 43). Note also that these substances are synthesized in membranes, mostly on the cytosolic face of the endoplasmic

reticulum, and from there are transported to their final cellular destinations.

The triacyglycerol precursors 1,2-diacyl glycerol and phosphatidic acid are also the precursors of certain glycerophospholipids (Figs. 54). Activated phosphate esters of the polar head groups react with the C3 OH group of 1,2-diacylglycerol to form the phospholipid's phosphodiester bond. In some cases the phosphoryl group of phosphatidic acid is activated and reacts with the unactivated polar head group.

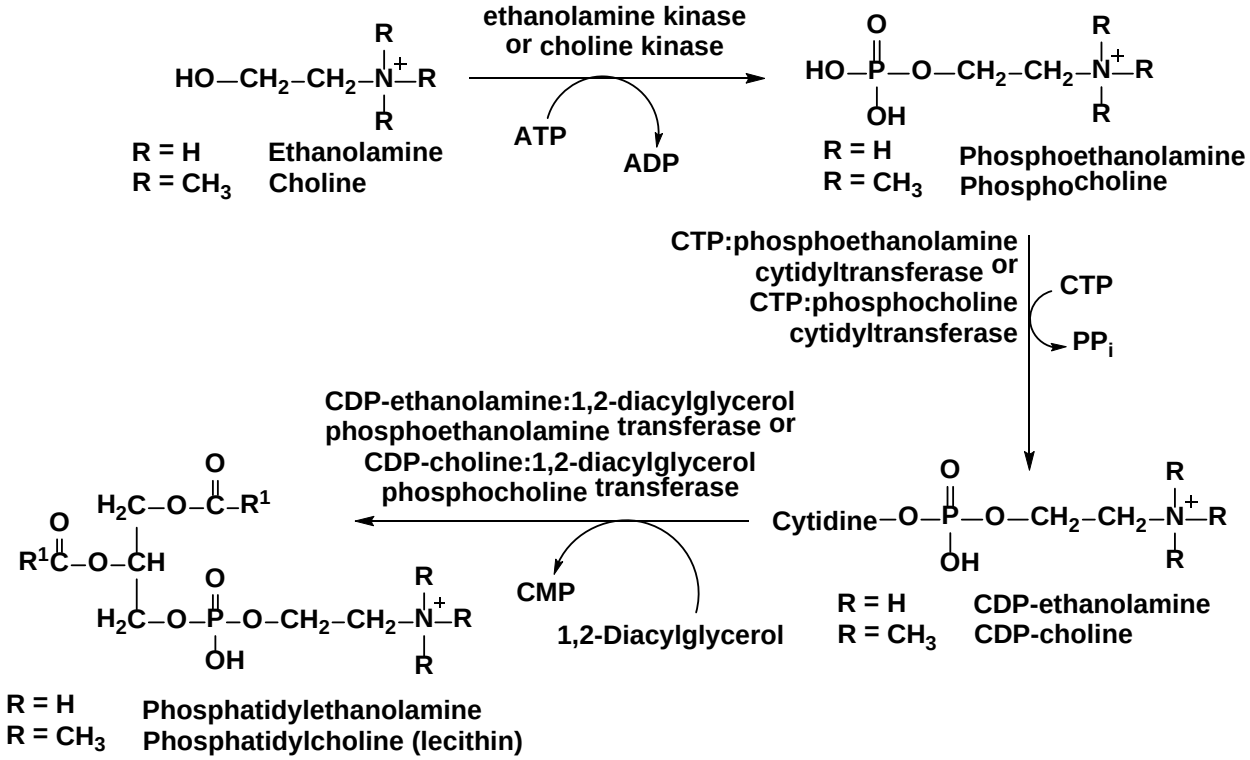


Figure 54. The biosynthesis of phosphatidylethanolamine and phosphatidylcholine.

The mechanism of activated phosphate ester formation is the same for both the polar head groups ethanolamine and choline (Fig. 55):

1. ATP first phosphorylates the OH group of choline or ethanolamine.

2. The phosphoryl group of the resulting phosphoethanolamine or phosphocholine then attacks CTP, displacing PP_i , to form the corresponding CDP derivatives, which are activated phosphate esters of the polar head group.

3. The C3 OH group of 1,2-diacylglycerol attacks the phosphoryl group of the activated CDP-ethanolamine or CDP–choline, displacing CMP to yield the corresponding glycerophospholipid.

The liver also converts phosphatidylethanolamine to phosphatidylcholine by trimethylating its amino group, using S-adenosylmethionine as the methyl donor.

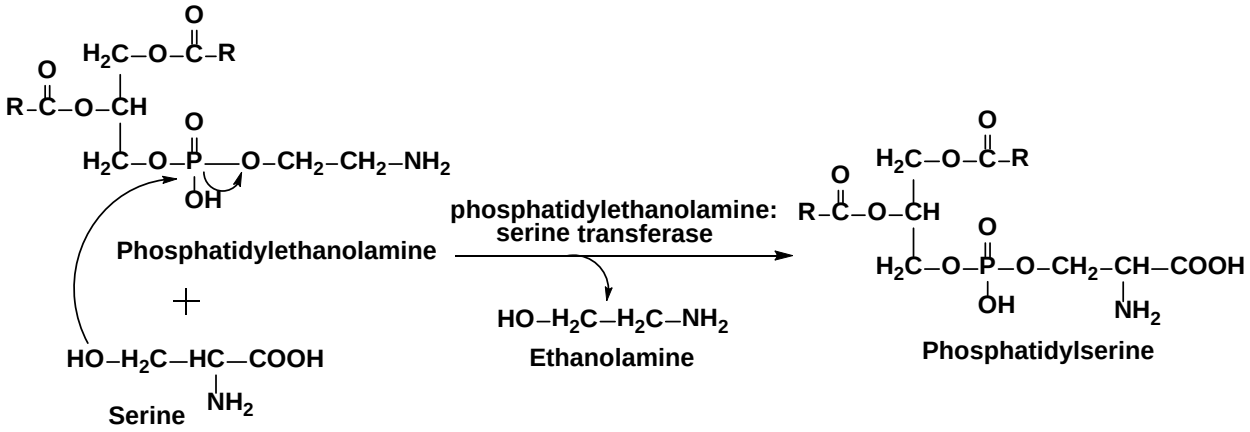


Figure 55. Phosphatidylserine synthesis.

Phosphatidylserine is synthesized from phosphatidylethanolamine by a head group exchange reaction catalyzed by phosphatidylethanolamine:serine transferase in which serine's OH group attacks the donor's phosphoryl group (Fig. 55). The original head group is then eliminated, forming phosphatidylserine.

In the synthesis of phosphatidylinositol and phosphatidylglycerol, the hydrophobic tail is activated rather than the polar head group. Phosphatidic acid, the precursor of 1,2-diacylglycerol, attacks the α-phosphoryl group of CTP to form the activated CDP-diacylglycerol and PP_i. Phosphatidylinositol results from the attack of inositol on CDP-diacylglycerol. Phosphatidylglycerol is formed in two reactions: attack of the C1-OH group of glycerol-3-phosphate on CDP-diacylglycerol, yielding phosphatidylglycerol phosphate; and hydrolysis of the phosphoryl group to form phosphatidylglycerol.

It should be noted that phospholipids and TG synthesis in the liver have common steps in the beginning of their duration. After phosphatidic acid formation their transportations are differ. What pathway will be predominated? The answer for this question is depended situation in hepatocytes which mostly realized due to reaction Glycerol:HFA:Lipotropic factors. Lipotropic factors are Choline, Methionine, Vitamins B_6, B_{12}, CTP. The lower content of lipotropic factors – the higher rate for TG synthesis.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	Synthesis of phospholipids is disordered under the liver fat infiltration. Indicate which of the following substances can enhance the process of methylation during phospholipids synthesis? A. Glucose B. Citrate C. Methionine D. Glycerin E. Ascorbic acid	
2.	Fatty liver infiltration was developed in experimental animal that was kept on protein-free diet. Particularly it is the result of methylating agents deficiency. This is caused by disturbed generation of the following metabolite: A. Choline B. DOPA C. Cholesterol D. Acetoacetate E. Linoleic acid	
3.	A 12-year old patient was found to have blood serum cholesterol at a rate of 25 mmol/l. The boy has a history of hereditary familial hypercholesterolemia, which is caused by the impaired synthesis of protein receptors for: A. Low density lipoproteins B. High density lipoproteins C. Intermediate density lipoproteins D. Very low density lipoproteins E. Chylomicrons	
4.	Disruption of nerve fiber myelinogenesis causes neurological disorders and mental retardation. These symptoms are typical for hereditary and acquired alterations in the metabolism of: A. Phosphatidic acid B. Cholesterol C. Sphingolipids D. Neutral fats E. Higher fatty acids	
5.	Glycerol metabolism is closely associated with tissue glycolysis. Name the compound of intermediary glycerol metabolism that is directly involved in glycolysis:	

№	Test:	Explanation:
	A. Diacylglycerol B. Triacylglycerol C. Phosphoenolpyruvate D. Dihydroxyacetone phosphate E. Glycerate	
6.	Biological importance of glycolysis is estimated as to be a source of energy for tissue cell or to be the source of compounds that are used for synthesis of lipids. Name those compound: A. Pyruvate B. Dihydroxyacetone phosphate C. Gluconic acid D. Phosphoenolpyruvate E. Lactic acid	
7.	Lipotropic factors should be prescribed to the patient after viral hepatitis to prevent fatty liver infiltration. Name one of them: A. Tryptophan B. Choline C. Vicasol D. Contrycal E. Allopurinol	
8.	Patient with diabetes mellitus, who takes long time insulin preparation, complains from weight gain. Select a possible mechanism for the side effect of insulin: A. Proteins degradation is activated B. Promotes the conversion of proteins to fats C. Inhibits the glycolysis D. Inhibits the absorption of lipids E. Inhibits the fat mobilization from the depot	
9.	It was the block of bile flow into duodenum of experimental animal under the ligation of his common bile duct. Name substances which hydrolysis will be violated in this case: A. Lipids B. Carbohydrates C. Proteins and carbohydrates D. Lipids and carbohydrates E. Proteins	
10.	Fat in adipose tissue is reduced at regular intense physical loads. It is utilized in cells to release to the bloodstream compounds: A. Lipoproteins	

№	Test:	Explanation:
	B. Glucose C. Chylomicrons D. Free fatty acids and glycerol E. Ketone bodies	
11.	Lipoproteins are transport form of lipids in the blood. Point out the transport form of cholesterol to the liver: A. HDH B. LDL C. VLDL D. Interferons E. Albumins	
12.	Lipids are transported by lipoproteins in the blood. Specify lipoprotein class that is formed in the small intestine wall after excess lipids intake from foods: A. HDL B. Chylomicrons C. LDL D. VLDL E. IDL	
13.	Liver requires a mechanism for phosphatidylcholine (PC) producing because it exports significant amounts of PC to the bile and to the blood to be as component of serum lipoproteins. This mechanism includes three methylation steps to produce PC from phosphatidylethanolamine. What is the methyl group donor for methylation: A. S-adenosylmethionine B. N-guanosylmethionine C. Cytidine diphosphate-choline D. Uridine diphosphate-methionine E. Homosycteine	
14.	There are two sources of glycerol 3-phosphate for triacylglycerol synthesis. Adipose tissue is strictly dependent on glucose uptake to produce dihydroxyacetone phosphate for glycerol 3-phosphate formation. But liver can use diverse (glucose independent) way for glycerol 3-phosphate synthesis. Point out the liver specific enzyme for this transformation: A. Glycerol 3-phosphate dehydrogenase B. Glycerol kinase C. Enolase D. Aldolase	

№	Test:	Explanation:
	E. Acetyl CoA carboxylase	
15.	Point out the terminal product of triacylglycerols lipolysis in adipose tissue: A. Bile acids B. Mineral acids C. Glycerol D. 2-Monoacylglycerol E. Diacylglycerol	
16.	Glycerol as product of lipolysis may be transported to the liver where it is phosphorylated to glycerol 3-phosphate and may enter glycolysis due to glycerol 3-phosphate dehydrogenase to form: A. 3-Phosphoglycerate B. Dihydroxyacetone phosphate C. Glyceraldehyde 3 phosphate D. 1,3-Biphosphoglycerate E. Phosphoenolpyruvate	
17.	Point out the lipoproteins of the blood plasma containing the highest mass of triacylglycerols: A. HDL B. LDL C. IDL D. Chylomicrons E. VLDL	
18.	Blood plasma of patient with hyperlipoproteinemia type I remains milky even after a long fast due to markedly elevated and persistent chylomicrons. What abnormality is possible in these patients? A. Obstruction of the bile duct B. Deficient pancreatic lipase C. Defective synthesis of apoB-48 D. Deficient lecithin cholesterol acyltransferase (LCAT) E. Deficient lipoprotein lipase	
19.	Hormone sensitive triacylglycerol lipase is not activated by one hormone from following list. Point out it. A. Insulin B. Glucagon C. Epinephrine D. Norepinephrine E. None of above	
20.	Choose the incorrect statement about the glycerol use as a substrate for gluconeogenesis:	

№	Test:	Explanation:
	A. Glycerol is released from adipose stores of triacylglycerol B. The liver takes up the glycerol and phosphorylates it C. Glycerol is released from adipocyte being phosphorylated before D. Glycerol phosphorylation is supplied with 1 ATP E. Conversion of glycerol to glycerol-3-phosphate is catalyzed by glycerol kinase	
21.	Which of the following apoprotein is an activator of LCAT (Lecithin-cholesterol acyltransferase) A. Apo B 100 B. Apo B 48 C. Apo D D. Apo AI E. Apo CII	
22.	Name a class of lipoproteins that will be least in migration from start-line in electrophoresis: A. Chylomicrons B. VLDL C. LDL D. IDL E. HDL	
23.	Endogenous triglyceride carrier in blood plasma is mainly: A. Chylomicron B. VLDL C. LDL D. HDL E. IDL	
24.	Find out main lipoprotein class that is produced in mucosa cells of the small intestine wall for the transport of resynthesized lipids: A. Chylomicrons B. VLDL C. HDL D. IDL E. LDL	
25.	Classification of lipids is based on their ability to be dissolved in organic solvents and their inability to give solutions in water. Find out lipids in this list: A. Lecithin B. Phosphatidyl ethanol amine C. 1.2-dioleyl glycerol	

№	Test:	Explanation:
	D. All these compounds E. Palmitic acid	
26.	Tissue lipolysis is associated with cleavage of one type of bond, only. It is: A. Hydrogen bond B. Ester bond C. Peptide bond D. Double bond E. Disulfide bond	
27.	Hormone-sensitive triacylglycerol lipase is stimulated by all these hormones except: A. Insulin B. Epinephrine C. ACTH D. Growth hormone E. Glucagon	
28.	Choose name of enzyme that is not involved in tissue lipolysis: A. Diacylglycerol lipase B. Monoacylglycerol lipase C. Pancreatic lipase D. Triacylglycerol lipase E. Sphyngomyelinase	
29.	The liver is tissue type where triacylglycerol and glycerophospholipid synthesis are the most extensive. First steps in these pathways (from glycerol free and acyl-CoAs) are the same but they become differ at conversion of: A. Monoacylglycerol phosphate B. Glycerol-3-phosphate C. CDP-glycerol D. Diacylglycerol E. Phosphatidic acid	
30.	Steatosis is caused by the accumulation of triacylglycerols in hepatocytes. One of the mechanisms of this disease development is a decrease in the utilization of VLDL neutral fat. What lipotropic factors intake with food prevent the development of steatosis? A. Valine, B_3, B_2 B. Arginine, B_2, B_3 C. Alanine B_1, PP D. Methionine, B_6, B_{12} E. Isoleucine, B_1, B_2	

HIGH FATTY ACIDS AND KETONE BODIES METABOLISM

(Ivanchenko D.G.)

INFORMATIONAL MATERIAL

Common information about Fatty acids

Fatty acids (FA) are usually straight aliphatic chains with a methyl group at one end (named the ω-carbon) and a carboxyl group at the other end (Fig. 56). Most FA in humans have an even number of carbon atoms, usually between 14 and 24 (Fig. 57). Saturated fatty acids have single bonds between the carbons in the chain, and unsaturated fatty acids contain one or more double bonds. The most common saturated fatty acids present in a cell are palmitic acid (C_{16}) and stearic acid (C_{18}). Although these two fatty acids are generally named by their trivial names, shorter fatty acids are often named by the Latin word for the number of carbons, such as octanoic acid (C_8) and decanoic acid (C_{10}). Term high fatty acid is proposed for all FA which contain more then C_{10}.

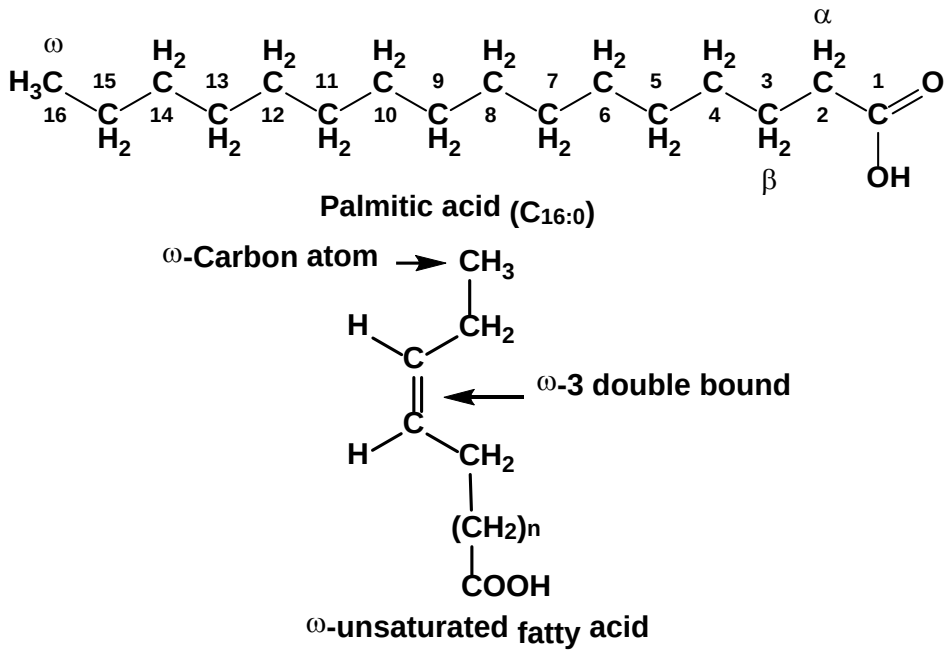


Figure 56. Examples of fatty acids structure.

Fatty acid carbon atoms are numbered starting at the carboxyl terminus. Carbon atoms 2 and 3 are often referred to as α and β, respectively. The position of a double bond is represented by the symbol Δ followed by a superscript number.

For example, *cis*-Δ^9 means that there is a *cis* double bond between carbon atoms 9 and 10; *trans*-Δ^2 means that there is a *trans* double bond between carbon atoms 2 and 3. Alternatively, the position of a double bond can be denoted by counting from the distal end, with the ω-carbon atom (the methyl carbon) as number 1. An ω-3 fatty acid, for example, has the structure shown in Fig. 56.

Figure 57. Common biological fatty acids.

Number of carbon	Common name	Symbol	Structure
Saturated fatty acids			
12	Lauric acid	12:0	$CH_3(CH_2)_{10}COOH$
14	Myristic acid	14:0	$CH_3(CH_2)_{12}COOH$
16	Palmitic acid	16:0	$CH_3(CH_2)_{14}COOH$
18	Stearic acid	18:0	$CH_3(CH_2)_{16}COOH$
20	Arachidic acid	20:0	$CH_3(CH_2)_{18}COOH$
22	Behenic acid	22:0	$CH_3(CH_2)_{20}COOH$
24	Lignoceric acid	24:0	$CH_3(CH_2)_{22}COOH$
Unsaturated fatty acids (all double bonds are cis)			
16	Palmitoleic acid	16:1	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
18	Oleic acid	18:1	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
18	Linoleic acid	18:2	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COOH$
18	α-Linolenic acid	18:3	$CH_3CH_2(CH=CHCH_2)_3(CH_2)_6COOH$
18	γ-Linolenic acid	18:3	$CH_3(CH_2)_4(CH=CHCH_2)_3(CH_2)_3COOH$
20	Arachidonic acid	20:4	$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$
24	Nervonic acid	24:1	$CH_3(CH_2)_7CH=CH(CH_2)_{13}COOH$

Saturated fatty acid chains can pack closely together to form ordered, rigid arrays under certain conditions, but unsaturated fatty acids prevent such close packing and produce flexible, fluid aggregates. Some fatty acids are not synthesized by mammals and yet are necessary for normal growth and life. These ***essential fatty acids*** include *linoleic* and *γ-linolenic acids*. These must be obtained by mammals in their diet (specifically from plant sources). *Arachidonic acid* may be synthesized in mammals from linoleic acid. At least one function of the essential fatty acids is to serve as a precursor for the synthesis of *eicosanoids*, such as *prostaglandins*, a class of compounds that exert hormone-like effects in many physiological processes.

β-Oxidation of high fatty acids

After FA transport across the adipocyte plasma membrane, fatty acids become bound to serum albumin. Appearance of free FA in the blood plasma mainly associated with previous tissue lipolysis duration in a ratio 1 Alb: 4 FA. The albumin-bound fatty acids are carried to tissues throughout the body, where they may be oxidized to generate energy. Fatty acids are a major fuel for aerobic cells (except neurons). Fatty acids are transported into cells by a protein in the plasma membrane. This process is linked to the active transport of sodium. The amount of fatty acid that is transported depends on its concentration in blood and the relative activity of the fatty acid transport mechanism.

Most fatty acids are degraded by the sequential removal of two-carbon fragments from the carboxyl end of fatty acids. During this process, referred to as β-oxidation, acetyl-CoA is formed as the bond between the α- and β-carbon atoms is broken.

β-Oxidation occurs primarily within mitochondria. Before β-oxidation begins, each fatty acid is activated in a reaction with ATP and CoASH (derivative of pantothenic acid, vitamin B_5). The enzyme that catalyzes this reaction, acyl-CoA synthetase, is found in the outer mitochondrial membrane. Because the mitochondrial inner membrane is impermeable to most acyl-CoA molecules, a special carrier named carnitine is used to transport acyl groups into the mitochondria (Fig. 58).

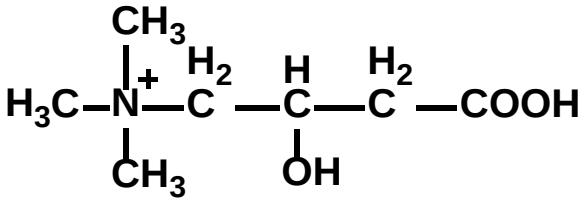


Figure 58. Structure of Carnitine.

Carnitine-mediated transport of acyl groups into the mitochondrial matrix is accomplished through the following mechanism (Fig. 59):

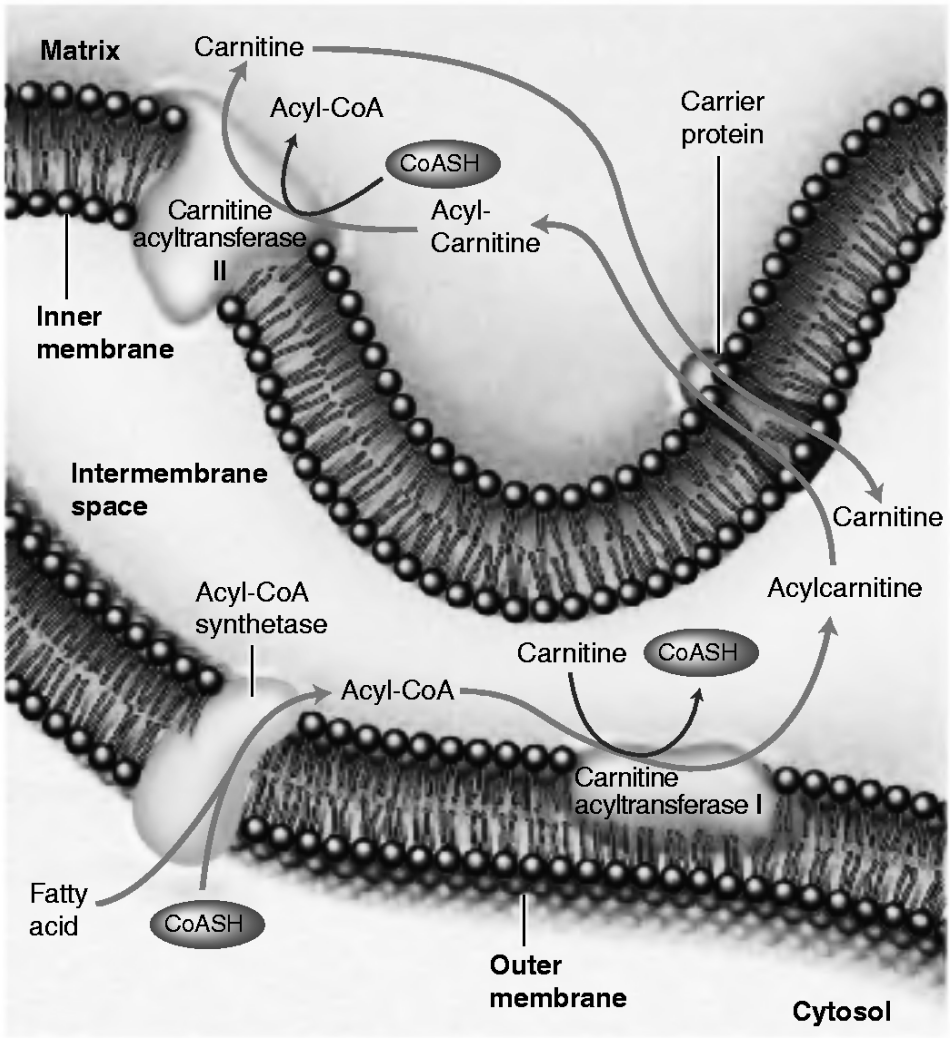


Figure 59. Fatty Acid Transport into the Mitochondria.

1. Each acyl-CoA molecule is converted to an acylcarnitine derivative (Fig. 60):

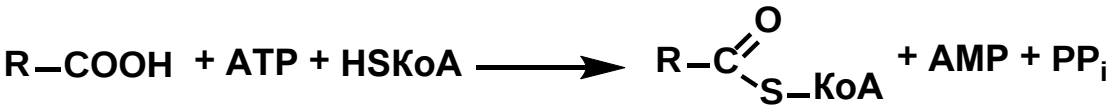


Figure 60. Activation of fatty acids.

This reaction is catalyzed by carnitine acyltransferase I.

2. A carrier protein (carnitine-acylcarnitine translocase) within the mitochondrial inner membrane transfers acylcarnitine into the mitochondrial matrix.

3. Acyl-CoA is regenerated by carnitine acyltransferase II.

4. Camitine is transported back into the intermembrane space by the carrier protein. It then reacts with another acyl-CoA.

A summary of the reactions of the β-oxidation of saturated fatty acids is shown in Figure 61. The pathway begins with an oxidation-reduction reaction, catalyzed by acyl-CoA dehydrogenase (an inner mitochondrial membrane flavoprotein), in which one hydrogen atom each is removed from the α- and β-carbons and transferred to the enzyme-bound FAD. The $FADH_2$ produced in this reaction then donates 2 electrons to the mitochondrial electron transport chain. There are several isozymes of acyl-CoA dehydrogenase, each specific to a different fatty acid chain length. The product of this reaction is trans-α,β-enoyl-CoA.

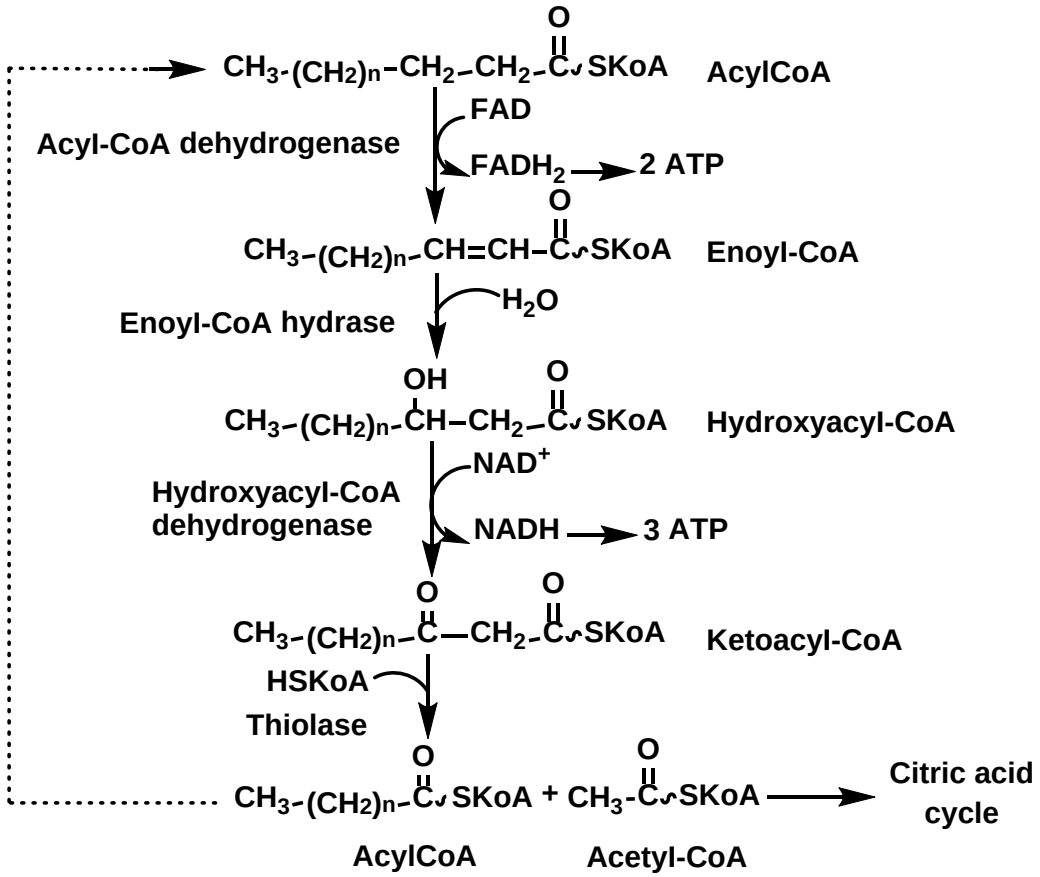


Figure 61. β-Oxidation of saturated Acyl-CoA.

The second reaction, catalyzed by enoyl-CoA hydrase, involves a hydration of the double bond between the α- and β-carbons. The β-carbon is now hydroxylated. In the third reaction this hydroxyl group is oxidized. The production of a β-ketoacyl-CoA is catalyzed by β-hydroxyacyl-CoA dehydrogenase. The

electrons transferred to NAD^+ are later donated to Complex I of the ETC. Finally, thiolase (sometimes referred to as β-ketoacyl-CoA thiolase) catalyzes a C_α-C_β cleavage. In this reaction, sometimes named a *thiolyric cleavage*, an acetyl-CoA molecule is released. The other product, an acyl-CoA, now contains two fewer C atoms. The four steps constitute one cycle of β-oxidation. During each later cycle, a two-carbon fragment is removed. This process, sometimes called the β-oxidation spiral, continues until, in the last cycle, a four-carbon acyl-CoA is cleaved to form two molecules of acetyl-CoA.

β-Oxidation of unsaturated and odd-chain fatty acids

The oxidation of unsaturated fatty acids presents some difficulties, yet many such FA are available in the diet. Most of these reactions are the same as those for saturated fatty acids. In fact, only two additional enzymes – an isomerase and a reductase – are needed to degrade a wide range of unsaturated FA.

Consider the oxidation of palmitoleate. This C_{16} unsaturated fatty acid, which has one double bond between C-9 and C-10, is activated and transported across the inner mitochondrial membrane in the same way as saturated fatty acids. Palmitoleoyl-CoA then undergoes three cycles of degradation, which are carried out by the same enzymes as those in the oxidation of saturated fatty acids. However, the cis-Δ^3-enoyl-CoA formed in the third round is not a substrate for acyl-CoA dehydrogenase. The presence of a double bond between C-3 and C-4 prevents the formation of another double bond between C-2 and C-3. This impasse is resolved by a new reaction that shifts the position and configuration of the cis-Δ^3 double bond. Cis-Δ^3 enoyl-CoA isomerase converts this double bond into a trans-Δ^2 double bond. The subsequent reactions are those of the saturated fatty acid oxidation pathway, in which the trans-Δ^2-enoyl-CoA is a regular substrate.

Another problem arises with the oxidation of polyunsaturated fatty acids. Consider linoleate, a C_{18} polyunsaturated fatty acid with cis-Δ^9 and cis-Δ^{12} double bonds. The cis-Δ^3 double bond formed after three rounds of β-oxidation is converted into a trans-Δ^2 double bond by the aforementioned isomerase. The acyl-CoA produced by another round of oxidation contains a cis-Δ^4 double bond.

Dehydrogenation of this species by acyl-CoA dehydrogenase yields a 2,4-dienoyl intermediate, which is not a substrate for the next enzyme in the β-oxidation pathway. This impasse is circumvented by 2,4-dienoyl-CoA reductase, an enzyme that uses NADPH to reduce the 2,4-dienoyl intermediate to trans-Δ^3-enoyl-CoA. Cis-Δ^3-enoyl-CoA isomerase then converts trans-Δ^3-enoyl-CoA into the trans-Δ^2 form, a customary intermediate in the β-oxidation pathway. Only two extra enzymes are needed for the oxidation of any polyunsaturated fatty acid. Odd-numbered double bonds are handled by the isomerase, and even-numbered ones by the reductase and the isomerase.

Most fatty acids have even numbers of carbon atoms and are therefore completely converted to acetyl-CoA. Some plants and marine organisms, however, synthesize fatty acids with an odd number of carbon atoms. The final round of β-oxidation of these fatty acids forms propionyl-CoA, which is converted to succinyl-CoA for entry into the citric acid cycle.

The conversion of propionyl-CoA to succinyl-CoA involves three enzymes (Fig. 62). The first reaction is that of propionyl-CoA carboxylase, a biotin-dependent enzyme with subunit composition $\alpha_6\beta_6$.

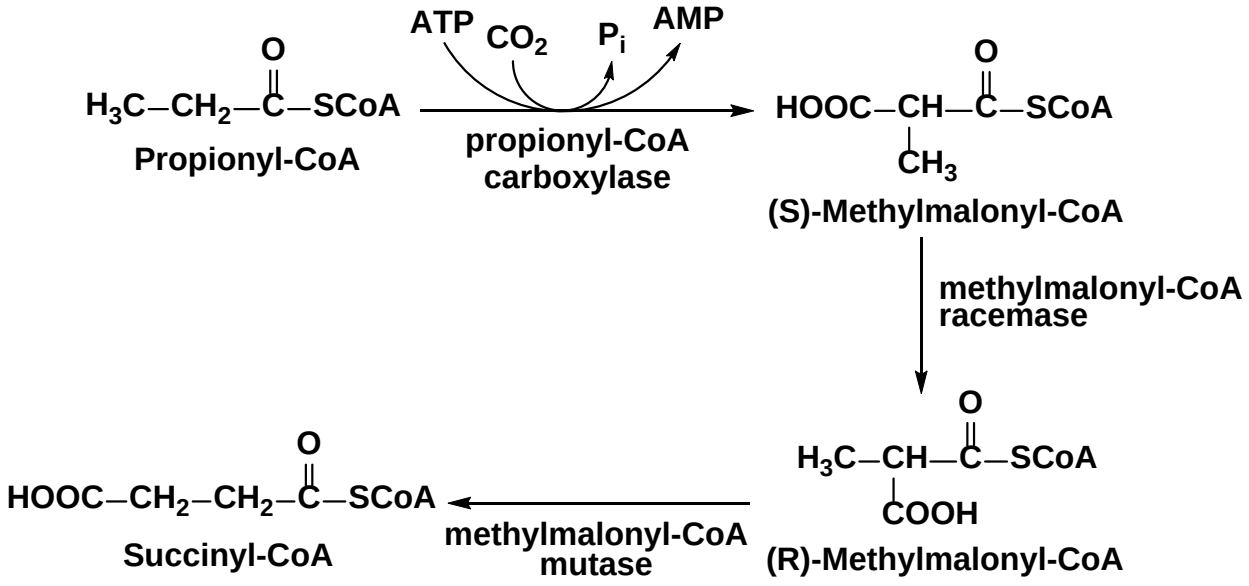


Figure 62. Conversion of propionyl-CoA to succinyl-CoA.

Methylmalonyl-CoA mutase, which catalyzes the third reaction of the propionyl-CoA to succinyl-CoA conversion (Fig. 62), is specific for (R)-

methylmalonyl-CoA even though propionyl-CoA carboxylase stereospecifically synthesizes (S)-methylmalonyl-CoA. This diversion is rectified by methylmalonyl-CoA racemase, which interconverts the (R) and (S) configurations of methylmalonyl-CoA. Methylmalonyl-CoA mutase catalyzes the conversion of a metabolite to a succinyl-CoA (C_4) citric acid cycle intermediate.

Fatty acids biosynthesis

FA biosynthesis occurs through condensation of C_2 units, the reverse of the β-oxidation process. The pathway of fatty acid synthesis differs from that of fatty acid oxidation. Figure 63 outlines fatty acid oxidation and synthesis with emphasis on the differences between these pathways. Whereas fatty acid oxidation occurs in the mitochondrion and utilizes fatty acyl-CoA esters, fatty acid biosynthesis occurs in the cytosol with the growing fatty acids esterified to acyl-carrier protein (ACP). ACP, like CoA, contains a phosphopantetheine group that forms thioesters with acyl groups. The phosphopantetheine phosphoryl group is esterified to a Ser OH group of ACP, whereas in CoA it is esterified to AMP. In animals, ACP is part of a large multifunctional protein (Type I ACP), whereas in E. coli it is a 125-residue polypeptide (Type II ACP). The phosphopantetheine group is transferred from CoA to apo-ACP to form the active holo-ACP by phosphopantetheine transferase (alternatively, ACP synthase).

The redox coenzymes of the animal fatty acid oxidative and biosynthetic pathways differ (NAD^+ and FAD for oxidation; NADPH for biosynthesis) as does the stereochemistry of their intermediate steps, but their main difference is the manner in which C_2 units are removed from or added to the fatty acyl thioester chain. In the oxidative pathway, β-ketothiolase catalyzes the cleavage of the C_α–C_β bond of β-ketoacyl-CoA so as to produce acetyl-CoA and a new fatty acyl-CoA, which is shorter by a C_2 unit. In the biosynthetic pathway, the condensation reaction is coupled to the hydrolysis of ATP, thereby driving the reaction to completion. This process involves two steps: (1) the ATP-dependent carboxylation of acetyl-CoA by acetyl-CoA carboxylase (ACC) to form malonyl-CoA, and (2)

the exergonic decarboxylation of the malonyl group in the condensation reaction catalyzed by fatty acid synthase.

Figure 63. Comparison of fatty acid β-oxidation and fatty acid biosynthesis.

The synthesis of FA from acetyl-CoA and malonyl-CoA involves seven enzymatic reactions that yield mainly palmitic acid, which takes place in the cytoplasm. Acetyl-CoA must be transferred from mitochondria to the cytoplasm (Fig. 64). Mitochondria, however, are not readily permeable to acetyl-CoA. Recall that carnitine carries only long-chain fatty acids. The barrier to acetyl-CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane. Citrate is formed in the mitochondrial matrix by the condensation of acetyl CoA with oxaloacetate. When present at high levels, citrate is transported to the cytoplasm, where it is cleaved by citrate lyase.

Differences occur in (1) cellular location, (2) acyl group carrier, (3) electron acceptor/donor, (4) stereochemistry of the hydration/dehydration reaction, and (5) the form in which C_2 units are produced/donated.

Oxaloacetate formed in the transfer of acetyl groups to the cytoplasm must be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Hence, a series of bypass reactions are needed. Most important, these reactions generate much of the NADPH needed for fatty acid

synthesis. First, oxaloacetate is reduced to malate by NADH. This reaction is catalyzed by a malate dehydrogenase in the cytoplasm.

$$\text{Oxaloacetate} + \text{NADH} + H^+ \leftrightarrow \text{Malate} + NAD^+$$

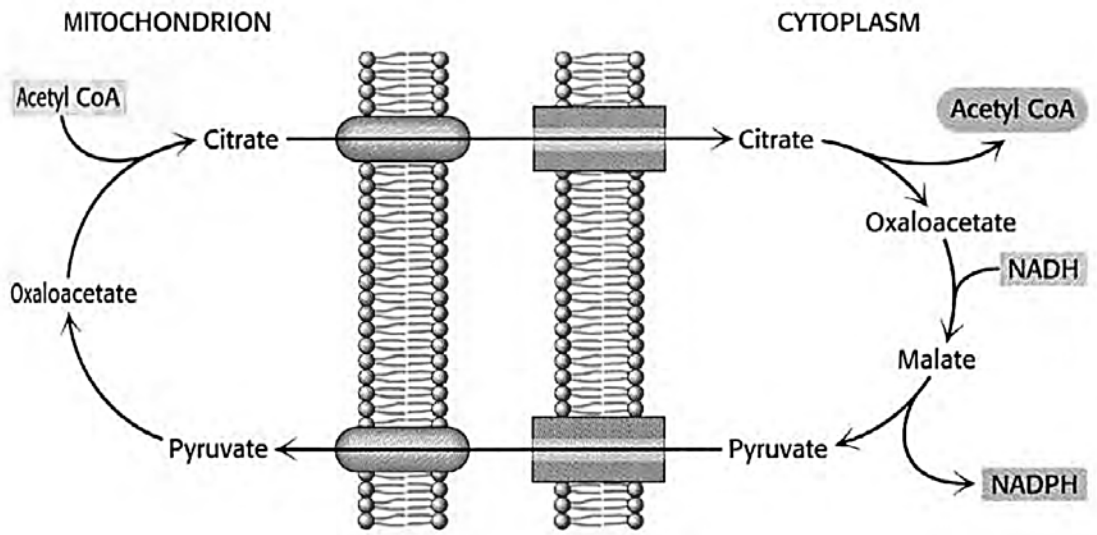


Figure 64. Transfer of acetyl-CoA to the cytoplasm.

Second, malate is oxidatively decarboxylated by an $NADP^+$-linked malate enzyme (also named malic enzyme).

$$\text{Malate} + NADP^+ \rightarrow \text{Pyruvate} + CO_2 + \text{NADPH} + H^+$$

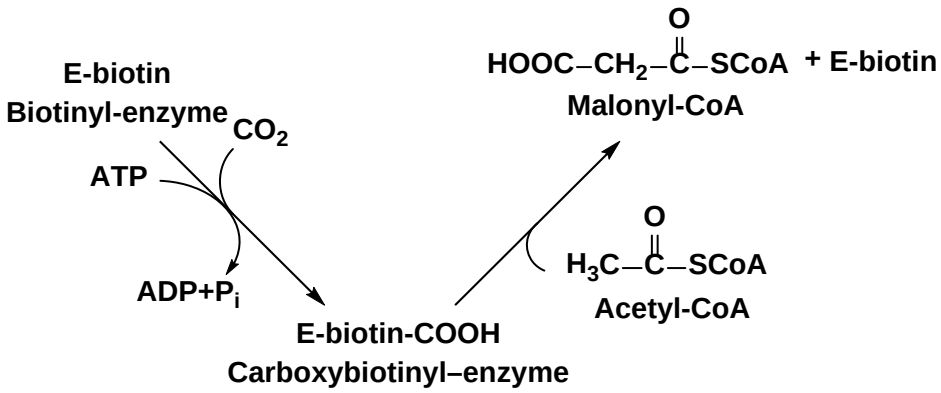
The pyruvate formed in this reaction readily enters mitochondria, where it is carboxylated to oxaloacetate by pyruvate carboxylase.

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{Oxaloacetate} + \text{ADP} + P_i$$

Thus, one molecule of NADPH is generated for each molecule of acetyl-CoA that is transferred from mitochondria to the cytoplasm. Hence, eight molecules of NADPH are formed when eight molecules of acetyl-CoA are transferred to the cytoplasm for palmitate synthesis. The additional six molecules of NADPH required for this process come from the pentose phosphate pathway.

ACC (acetyl-CoA carboxylase) is a biotin-dependent enzyme that catalyzes the first committed step of fatty acid biosynthesis and one of its rate-controlling steps. It is a member of a family of biotin-dependent carboxylases that, in humans, has only three members besides ACC: propionyl-CoA carboxylase, pyruvate carboxylase, and β-methylcrotonyl-CoA carboxylase. The ACC reaction, like those of other biotin-dependent carboxylases, occurs in two steps, a CO_2 activation and a

carboxylation:



Acetyl-CoA carboxylase plays an essential role in regulating fatty acid synthesis and degradation. Recall that this enzyme catalyzes the committed step in fatty acid synthesis: the production of malonyl-CoA (the activated two-carbon donor). This important enzyme is subject to both local and hormonal regulation.

Acetyl-CoA carboxylase is switched off by phosphorylation and activated by dephosphorylation (Fig. 65). cAMP-dependent protein kinase (cAMPK) converts the carboxylase into an inactive form by modifying a single serine residue. cAMPK is essentially a fuel gauge; it is activated by cAMP. Thus, the carboxylase is inactivated when the energy charge is low. Fats are not synthesized when energy is required.

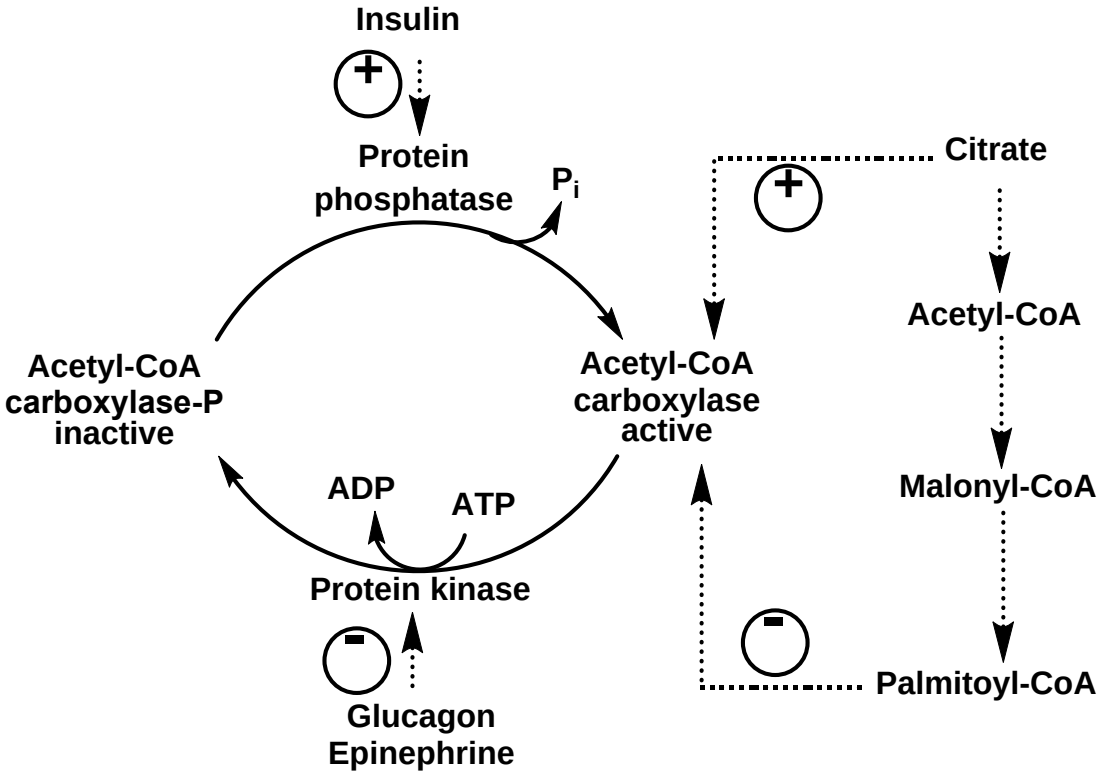


Figure 65. Control of acetyl-CoA carboxylase.

The carboxylase is also allosterically stimulated by citrate. Citrate acts in an unusual manner on inactive acetyl-CoA carboxylase, which exists as isolated dimers. Citrate facilitates the polymerization of the inactive dimers into active filaments (Fig. 66). Citrate-induced polymerization can partly reverse the inhibition produced by phosphorylation. The level of citrate is high when both acetyl-CoA and ATP are abundant, signifying that raw materials and energy are available for fatty acid synthesis. The stimulatory effect of citrate on the carboxylase is counteracted by palmitoyl-CoA, which is abundant when there is an excess of fatty acids. Palmitoyl-CoA causes the filaments to disassemble into the inactive subunits. Palmitoyl-CoA also inhibits the translocase that transports citrate from mitochondria to the cytoplasm, as well as glucose 6-phosphate dehydrogenase, which generates NADPH in the pentose phosphate pathway.

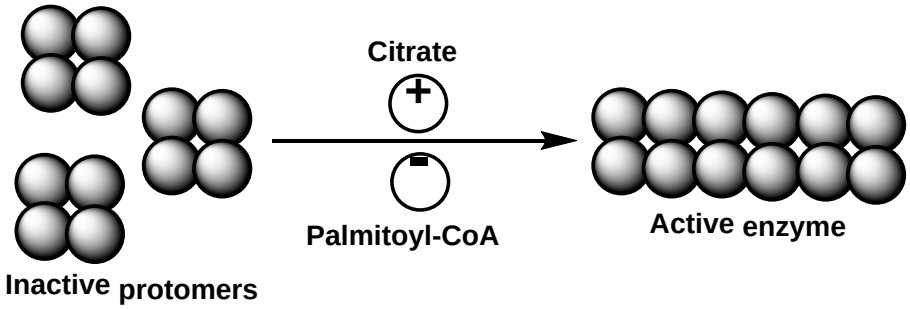


Figure 66. Activation of acetyl-CoA carboxylase by polymerization.

Acetyl-CoA carboxylase also plays a role in the regulation of fatty acid degradation. Malonyl-CoA, the product of this reaction, is present at a high level when fuel molecules are abundant. Malonyl-CoA inhibits carnitine acyltransferase I, preventing the entry of fatty acyl-CoAs into mitochondrial matrix in times of plenty. Malonyl-CoA is an especially effective inhibitor of carnitine acyltransferase I in heart and muscle, tissues that have little fatty acid synthesis capacity of their own. In these tissues, acetyl-CoA carboxylase may be a purely regulatory enzyme.

Carboxylase is controlled by hormones glucagon, epinephrine, and insulin, which reflect the overall energy status of the organism. *Insulin stimulates fatty acid synthesis by activating the carboxylase, whereas glucagon and epinephrine have*

the reverse effect.

Regulation by Glucagon and Epinephrine. Consider, again, a person who has just awakened from a night's sleep and begins a bout of exercise. As mentioned, glycogen stores will be low, but lipids are readily available for mobilization.

As stated earlier, the hormones glucagon and epinephrine, present under conditions of fasting and exercise, will stimulate the release of fatty acids from triacylglycerols in fat cells, which will be released into the blood, and probably from muscle cells, where they will be used immediately as fuel. These same hormones will inhibit fatty acid synthesis by inhibiting acetyl-CoA carboxylase. Although the exact mechanism by which these hormones exert their effects is not known, the net result is to augment the inhibition by the cAMP-dependent kinase. This result makes sound physiological sense: when the energy level of the cell is low, as signified by high concentration of AMP, and the energy level of the organism is low, as signaled by glucagon, fats should not be synthesized. Epinephrine, which signals the need for immediate energy, enhances this effect. Hence, these hormones switch off fatty acid synthesis by keeping the carboxylase in inactive phosphorylated state.

Regulation by Insulin. Now consider the situation after the exercise has ended and the runner has had a meal. In this case, the hormone insulin inhibits the mobilization of FA from adipocytes and stimulates their accumulation in a form of triacylglycerols in adipose tissue. Insulin also stimulates fatty acid synthesis by activating acetyl-CoA carboxylase. Insulin stimulates the carboxylase by stimulating the activity of a protein phosphatase that dephosphorylates and activates acetyl-CoA carboxylase. Thus, the signal molecules glucagon, epinephrine, and insulin act in concert on triacylglycerol metabolism and acetyl-CoA carboxylase to carefully regulate the utilization and storage of fatty acids.

Reactions of fatty acid synthesis are catalysed by a multifunctional enzyme known as fatty acid synthase (FAS) complex. In eukaryotic cells, including man, FAS exists as a dimer with two identical units (Fig. 67). Each monomer possesses

the activities of seven different enzymes and an acyl carrier protein (ACP) bound to 4'-phosphopantetheine. FAS functions as a single unit catalysing all the seven reactions to produce at the same time two palmityl-CoA. Dissociation of the synthase complex in two monomers results in loss of the enzyme activities.

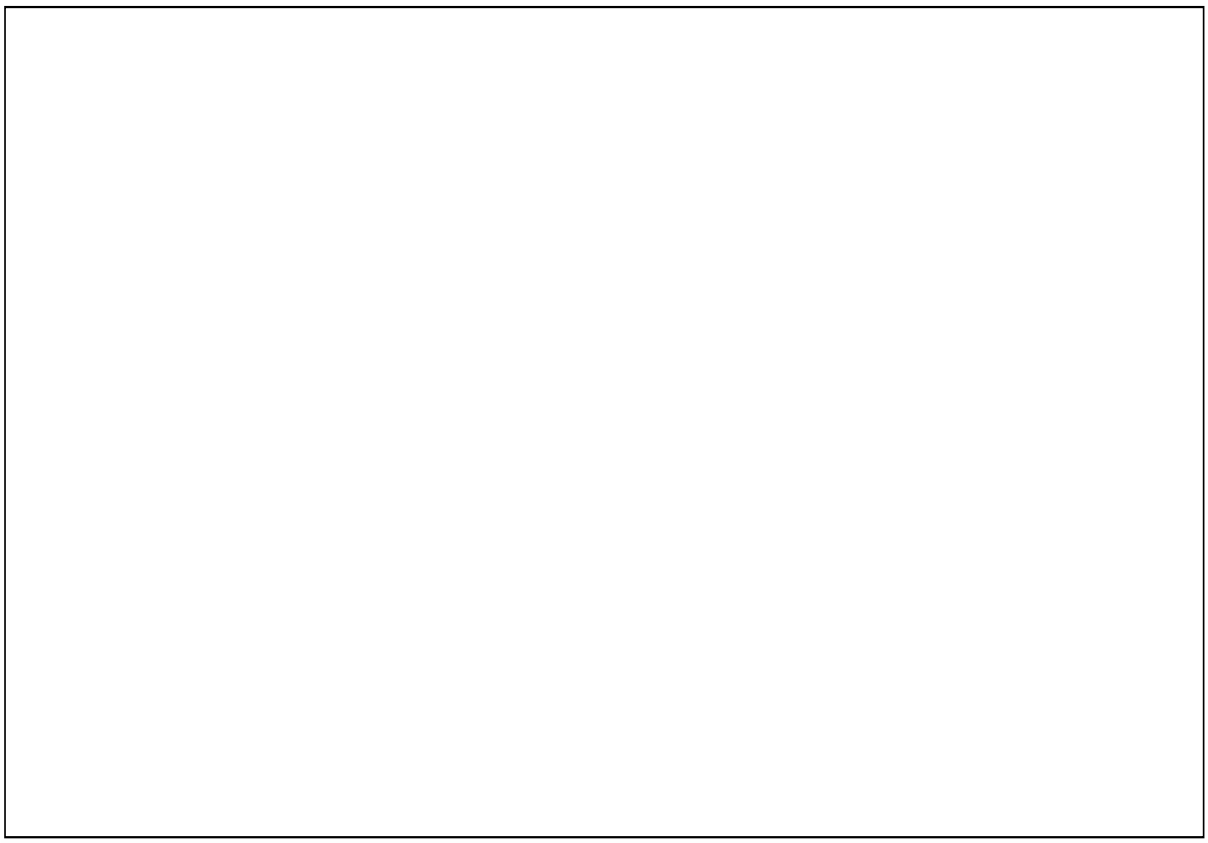


Figure 67. Fatty acid synthase complex.

The sequence of reactions of the extramitochondrial synthesis of fatty acids (palmitate) is depicted in Fig. 68-70.

1. The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalysed by the enzyme, acetyl CoA-ACP transacylase. The acetyl unit is then transferred from ACP to cysteine residue of the enzyme. Thus ACP site falls vacant.

2. The enzyme malonyl-CoA-ACP transacylase transfers malonate from malonyl-CoA to bind to ACP.

3. The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP). The malonyl moiety loses CO_2 which was added by acetyl-CoA carboxylase. Thus, CO_2 is never incorporated into fatty acid carbon chain. The

decarboxylation is accompanied by loss of free energy which allows the reaction to proceed forward. This reaction is catalyzed by β-ketoacyl ACP synthase.

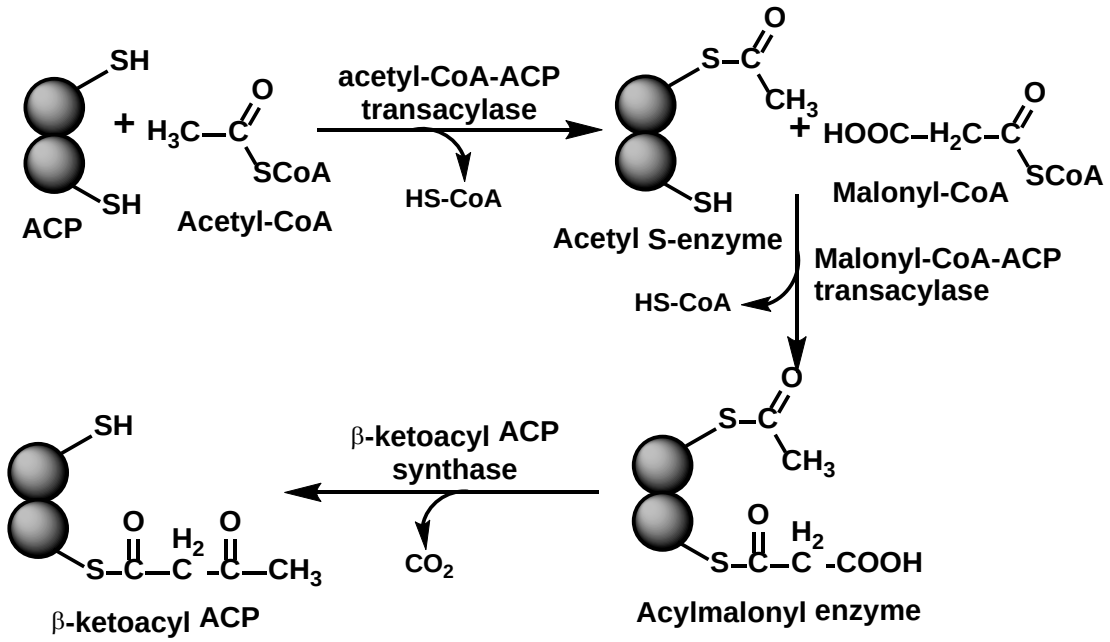


Figure 68. β-Ketoacyl ACP formation.

4. β-Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group. The reducing equivalents are supplied by NADPH.

5. β-Hydroxyacyl ACP undergoes dehydration. A molecule of water is eliminated and a double bond is introduced between α and β carbons.

6. A second NADPH-dependent reduction, catafysed by enoyl-ACP reductase occurs to produce acyl-ACP. The four-carbon unit attached to ACP is butyryl group. The carbon chain attached to ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl-CoA). At the end of 7 cycles, the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid-namely palmitate-bound to ACP is produced.

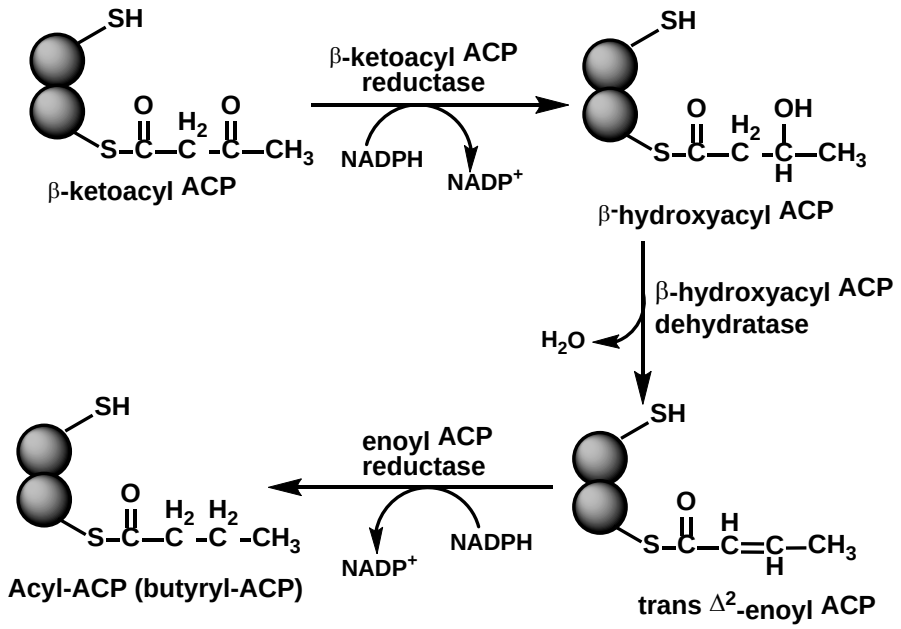


Figure 69. Butyryl ACP synthesis.

7. The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase. This completes the synthesis of palmitate.

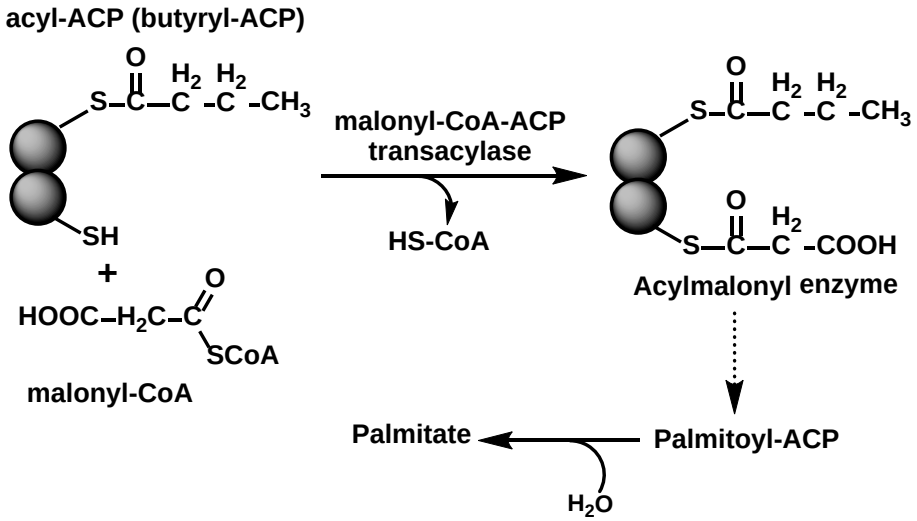


Figure 70. Palmitate formation.

Palmitate (16:0), the normal product of the animal fatty acid synthase pathway, is the precursor of longer chain saturated and unsaturated fatty acids through the actions of elongases and desaturases. Elongases are present in both the mitochondrion and the ER (endoplasmic reticulum) but the mechanisms of elongation at the two sites differ. Mitochondrial elongation occurs by successive addition and reduction of acetyl units; the only chemical difference between these two pathways occurs in the final reduction step in which NADPH takes the place

of $FADH_2$ as the terminal redox coenzyme (Fig. 71). Elongation in the ER involves the successive condensations of malonyl-CoA with acyl-CoA. These reactions are each followed by NADPH-associated reductions similar to those catalyzed by FAS, the only difference being that the fatty acid is elongated as its CoA derivative rather than as its ACP derivative.

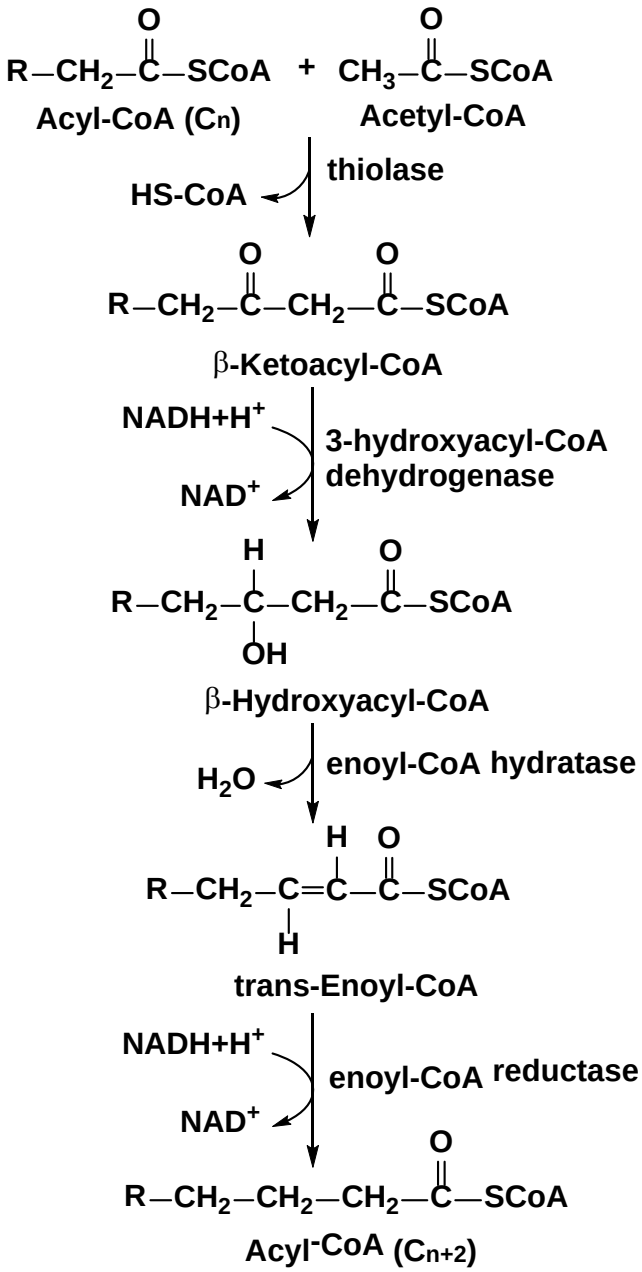
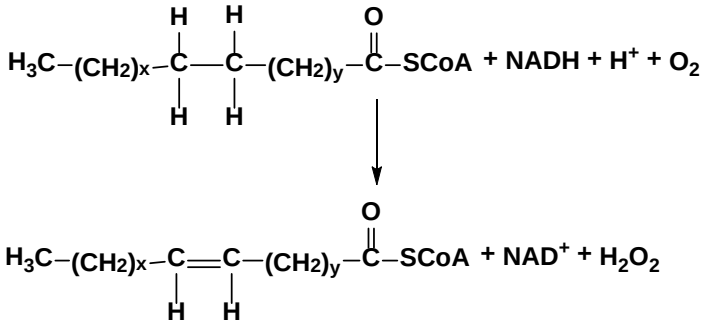


Figure 71. Mitochondrial fatty acid elongation.

Unsaturated fatty acids are produced by terminal desaturases. Mammalian systems contain four terminal desaturases of broad chain-length specificities designated Δ^9-, Δ^6-, Δ^5-, and Δ^4-fatty acyl-CoA desaturases. These membrane-bound, nonheme iron-containing enzymes catalyze the general reaction



where x is at least 5 and where $(CH_2)_x$ can contain one or more double bonds. The $(CH_2)_y$ portion of the substrate is always saturated. Double bonds are inserted between existing double bonds in the $(CH_2)_x$ portion of the substrate and the CoA group such that the new double bond is three carbon atoms closer to the CoA group than the next double bond (not conjugated to an existing double bond) and, in animals, never at positions beyond C9. Mammalian terminal desaturases are components of mini-electron-transport systems that contain two other proteins: cytochrome b_5 and NADH-cytochrome b_5 reductase. The electron-transfer reactions mediated by these complexes occur at the inner surface of the ER membrane (Fig. 72) and are therefore not associated with oxidative phosphorylation.

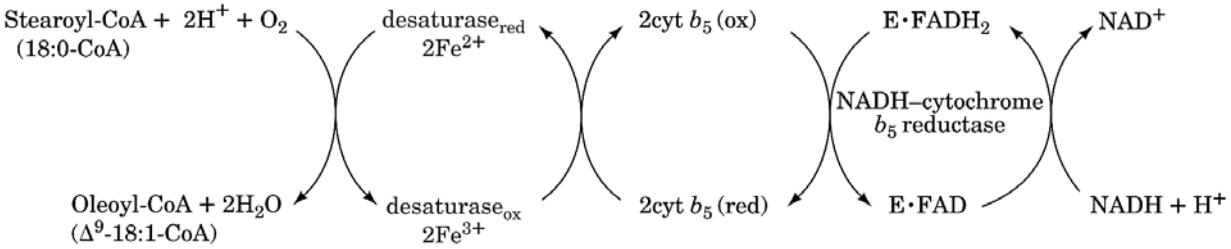


Figure 72. The electron-transfer reactions mediated by the Δ^9-fatty acyl-CoA desaturase complex.

Ketone bodies

Acetate, Acetone and β-Hydroxybutyrate are named as Ketone bodies. They are preferred as energy source during their conversions in the heart, skeletal muscle and kidney. The brain, under normal circumstances, uses only glucose as its energy source (fatty acids are unable to pass the blood–brain barrier), but during starvation, ketone bodies become the brain's major fuel source. Ketone bodies are water-soluble equivalents of fatty acids except acetone. The end product for their

catabolic pathway is acetyl-CoA that is involved in Krebs Cycle to be utilized there. Acetone cannot be utilized in humans, it is very inert molecule.

Ketone bodies synthesis is activated in the case of acetyl-CoA accumulation in the cytoplasm of hepatocytes. It may be during extended β-oxidation of HFA and tissue lipolysis.

Acetoacetate formation occurs in three reactions (Fig. 73):

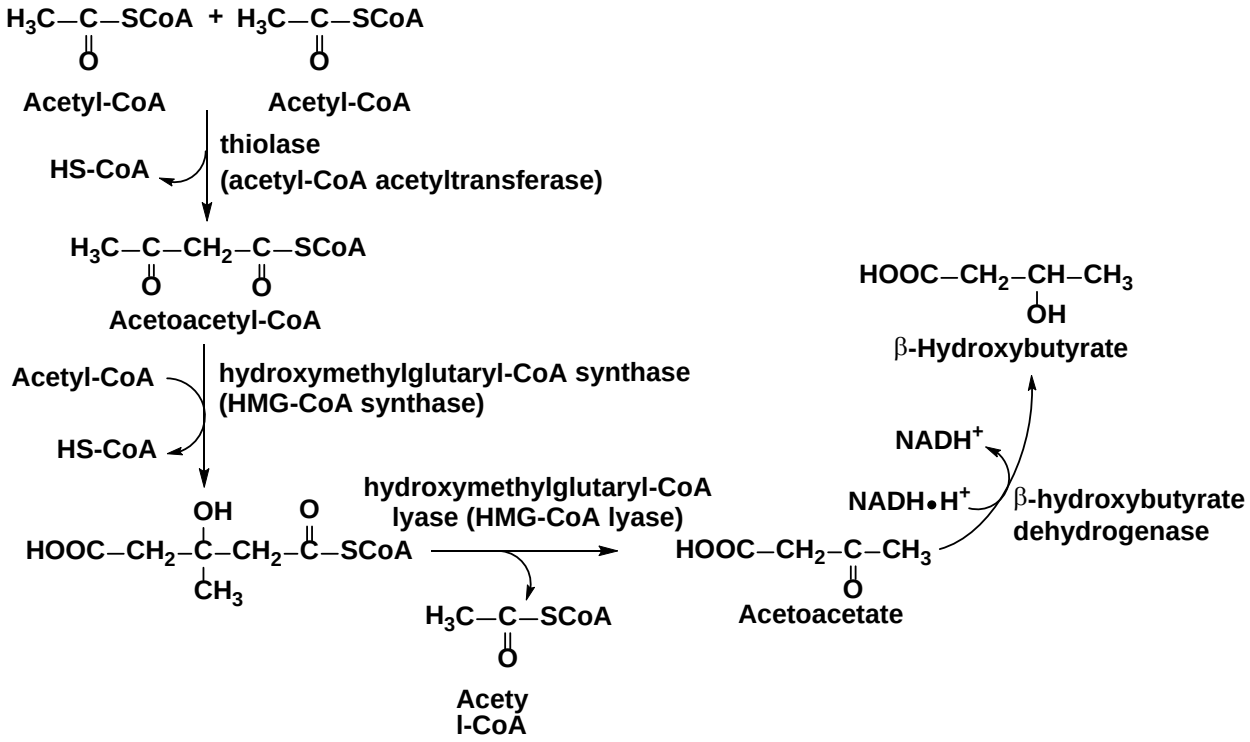


Figure 73. Ketogenesis.

1. Two molecules of acetyl-CoA are condensed to acetoacetyl-CoA by thiolase (also called acetyl-CoA acetyltransferase) working in the reverse direction from the way it does in the final step of β-oxidation.

2. Condensation of the acetoacetyl-CoA with a third acetyl-CoA by HMG-CoA synthase forms β-hydroxy-β-methylglutaryl-CoA (HMG-CoA).

3. Degradation of HMG-CoA to acetoacetate and acetyl-CoA is catalyzed by HMG-CoA lyase.

Acetoacetate may be reduced to β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase.

The liver releases acetoacetate and β-hydroxybutyrate, which are carried by the bloodstream to the peripheral tissues for use as alternative fuels. There, these products are converted to acetyl-CoA as is diagrammed in Fig. 74. Succinyl-CoA, which acts as the CoA donor, can also be converted to succinate with the coupled synthesis of GTP in the succinyl-CoA synthetase reaction of the citric acid cycle. The "activation" of acetoacetate bypasses this step and therefore "costs" the free energy of GTP hydrolysis. The liver lacks 3-ketoacyl-CoA transferase, which permits it to supply ketone bodies to other tissues.

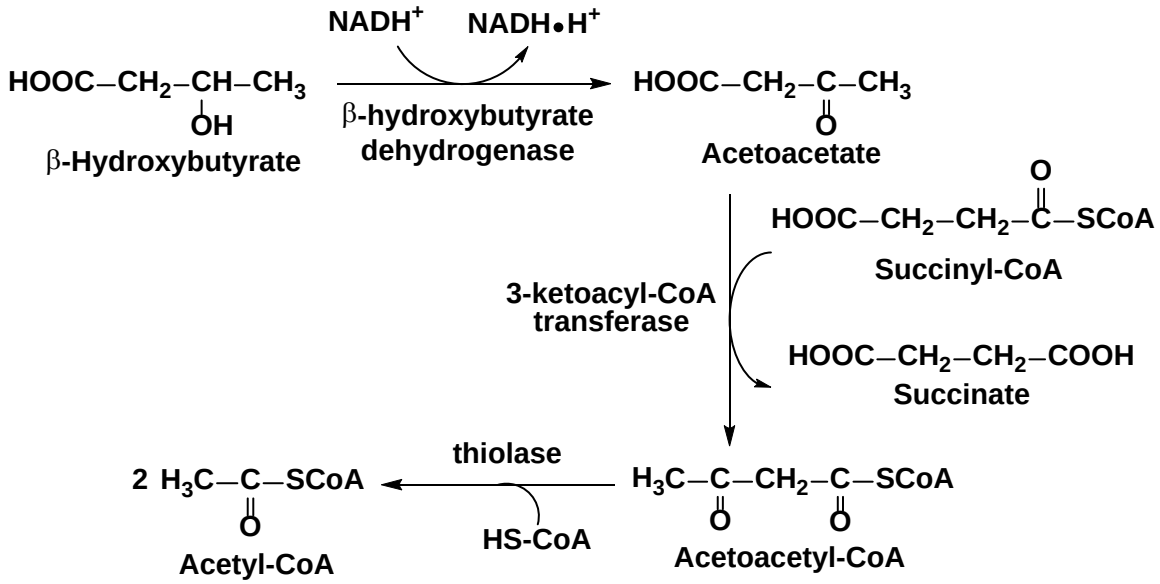


Figure 74. The metabolic conversion of ketone bodies to acetyl-CoA.

The ketone bodies level of the blood serum of healthy humans must be in the region 0.034-0.43 mmol/L. In the blood ketone bodies levels increase sufficiently, as they do after about 20 days of starvation, they are a valuable energy substrates for the brain and may account for up to 75% of brain oxidation. Ketone bodies concentration is elevated in patients with diabetes mellitus, too.

The most common of these conditions is diabetic ketosis in patients with insulin-dependent diabetes mellitus. It curtails fatty acid mobilization by adipose tissue. The absence of insulin has two major biochemical consequences (Fig. 75).

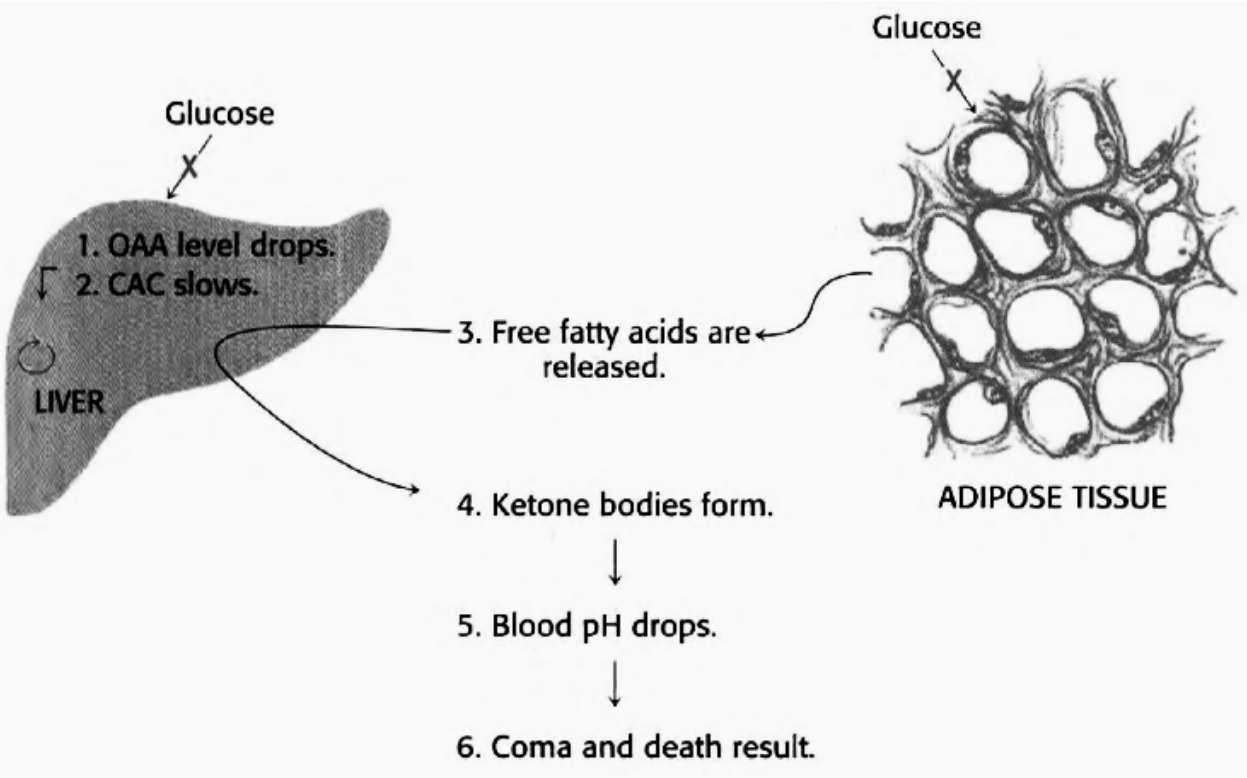


Figure 75. Diabetic ketosis results when insulin is absent.

OAA **–** glucose-derived oxaloacetate; CAC – citric acid cycle.

First, the liver cannot use glucose and consequently cannot provide oxaloacetate to process fatty acid-derived acetyl- CoA. Second, adipose cells continue to release fatty acids into the bloodstream, which are taken up by the liver to be involved in β-oxidation with the production of excess Acetyl-CoA which is converted into ketone bodies. The liver thus produces large amounts of ketone bodies, which are moderately strong acids, except acetone. As the result for patient is severe metabolic acidosis. The decrease in pH impairs tissue function, most importantly in the central nervous system.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	An experimental animal has been given excessive amount of carbon labeled glucose for a week. What compound this label will be found in? A. Phenylalanine	

№	Test:	Explanation:
	B. Methionine C. Palmitic acid D. Vitamin A E. Arachidonic acid	
2.	A 1 y.o. child with symptoms of muscle affection was admitted to the hospital. Examination revealed carnitine deficiency in his muscles. Biochemical base of this pathology is disturbed process of: A. Regulation of Ca^{2+} level in mitochondria B. Transporting of fatty acids to the matrix of mitochondria C. Actin and myosin synthesis D. Lactic acid utilization E. Substrate phosphorylation	
3.	A sportsman was recommended to take a medication that contains carnitine in order to improve his sport results. What process is promoted by carnitine? A. Synthesis of steroid hormones B. Fatty acids transport to mitochondrions C. Tissue respiration D. Synthesis of ketone bodies E. Synthesis of proteins	
4.	The patient with diabetes mellitus has been delivered in the hospital in a state of unconsciousness. His arterial pressure is low. The patient has acidosis. Point out substances, whose accumulation in the blood results in these manifestations: A. Ketone bodies B. Monosaccharides C. Cholesterol esters D. High fatty acids E. Amines	
5.	Patients who suffer from severe diabetes mellitus and don't receive insulin have metabolic acidosis. This is caused by increased concentration of the following metabolites: A. Ketone bodies B. Unsaturated fatty acids C. Cholesterol D. Triacylglycerols E. Fatty acids	
6.	A patient with high rate of obesity was advised to use carnitine as a additive in order to enhance "fat burning". What is the	

№	Test:	Explanation:
	role of carnitine in the process of fat oxidation? A. Transport of FFA (free fatty acids) from cytosol to the mitochondria B. Activation of intracellular lipolysis C. It takes part in one of reactions of FFA beta-oxidation D. FFA activation E. Transport of FFA from fat depots to other tissues	
7.	Examination revealed carnitine deficiency in patient's muscles. What process disturbance is the biochemical basis of this pathology? A. Transporting of fatty acids to mitochondria B. Regulation of Ca^{2+} level in mitochondria C. Substrate phosphorylation D. Lactic acid utilization E. Actin and myosin synthesis	
8.	A 39-year-old female patient with a history of diabetes mellitus was hospitalized in a comatose state with diabetic ketoacidosis. This condition had been caused by an increase of the following metabolite level: A. Alpha-ketoglutarate B. Citrate C. Acetoacetate D. Malonate E. Aspartate	
9.	Lipids are the most valuable energetic material for the body. Point out the main way of fatty acids conversion in the mitochondria of cells to produce energy: A. Decarboxylation B. α-Oxidation C. γ-Oxidation D. β-Oxidation E. Reduction	
10.	Vitamins and vitamin-like compounds are needed for activation and transfer of HFA through the mitochondrial membrane. Select one of them: A. Ubiquinone B. Biotin C. Carnitine D. Riboflavin E. Thiamine	

№	Test:	Explanation:
11.	The patient was prescribed L-carnitine. Name substances whose transmembrane transport is provided by this drug: A. Amino Acids B. Glucose C. Purine nucleotides D. Pyrimidine nucleotides E. High fatty acid	
12.	The patient with atherosclerosis was prescribed Linetol that contains essential fatty acids. Point out the essential component of this drug: A. Crotonic acid B. Stearic acid C. Palmitic acid D. Oleic acid E. Linoleic acid	
13.	The patient eats daily a few raw eggs, which contain antivitamin biotin – avidin. Point out the stage of lipid metabolism that is disrupted: A. Oxidation of glycerol B. Biosynthesis of fatty acids C. The biosynthesis of cholesterol D. Lipids absorption E. Transport of lipids in the blood	
14.	Increased amount of free fatty acids is observed in the blood of patients with diabetes mellitus. It may be caused by: A. Increased activity of triglyceride lipase of adipocytes B. Storage of palmitoyl-CoA C. Activation of ketone bodies utilization D. Activation of apolipoproteins synthesis E. Decreased activity of phosphatidylcholine-cholesterol-acyltransferase blood plasma	
15.	Choose the allosteric activator of acetyl-CoA-carboxylase (the key enzyme of HFA synthesis): A. Malate B. Oxaloacetate C. Citrate D. Succinate E. Fumarate	
16.	The removal of two-carbon units from fatty acyl-CoA involves four sequential reactions. Which of the following reaction sequences is correct for the pathway of	

№	Test:	Explanation:
	beta-oxidation: A. Oxidation, dehydration, oxidation, cleavage B. Hydrogenation, dehydration, hydrogenation, cleavage C. Dehydrogenation, hydration, dehydrogenation, cleavage D. Reduction, hydration, dehydrogenation, cleavage E. Reduction, dehydration, reduction, cleavage	
17.	Choose the products for the first round of stearic acid beta-oxidation: A. 129 ATP B. 1 Oleyl-CoA, 12 ATP C. 2 Acetyl-CoA, 2 $FADH_2$, 1 ATP D. 1 Palmitoyl-CoA, 1 acetyl-CoA, 1 $FADH_2$, 1 NADH E. 1 Stearyl-CoA, 1 acetyl-CoA, 1 $FADH_2$, 1 NADH	
18.	Fatty acids are activated in catabolic processes so: they... A. Are phosphorylated by ATP B. Don't change the structure C. Are converted to acyl-SCoA due to ATP energy D. Are linked to HS-CoA without any energy use E. Interact with carnitine	
19.	Acetyl CoA is the source of carbon atoms while NADPH provides the reducing equivalents for fatty acid synthesis. Which of the following enzymes take part in the formation of both mentioned substrates (Acetyl-CoA and NADPH): A. Citrate synthase, pyruvate dehydrogenase (PDH), Glucose-6-phospate dehydrogenase (G-6-P DH) B. Citrate lyase, G-6-P DH, malic enzyme C. Citrate lyase, PDH, pyruvate kinase D. G-6-P DH, gluconolactone hydrolase, phosphogluconate dehydrogenase E. Pyruvate carboxylase, PDH, acyl-CoA dehydrogenase	
20.	Which of the following substances is immediate precursor of acetoacetate in ketogenesis pathway? A. Beta-hydroxybutyrate B. Acetoacetyl CoA	

№	Test:	Explanation:
	C. Beta-hydroxybutyryl CoA D. Acetyl CoA E. Beta-hydroxy-beta-methylglutaryl CoA	
21.	There are two end products in beta-oxidation of odd chain fatty acids. They are: A. Acetyl-CoA and malonyl-CoA B. Acetyl-CoA and oxaloacetate C. Acetyl-CoA and propionyl-CoA D. Acetyl-CoA and butiryl-CoA E. Methylmalonyl-CoA and malonyl-CoA	
22.	What sentence is reasonable in regard to fatty acid synthesis: A. It occurs in the mitochondrial matrix B. It requires NADPH and ATP C. It is catalysed by fatty acid synthase, an enzyme complex that requires biotin as a coenzyme D. It is activated by glucagons E. It requires specific enzyme propionyl-CoA carboxylase	
23.	The acetyl-CoA required for fatty acid synthesis is formed in the cytoplasm as a result of one enzyme activity. Point out this enzyme: A. Malic enzyme B. Isocitrate dehydrogenase C. Citrate lyase D. Thiolase E. Pyruvate decarboxylase	
24.	Acetyl CoA carboxylase is key enzyme in fatty acid synthesis. Point out the coenzyme of this enzyme: A. NADH B. $FADH_2$ C. Biotin D. Phosphopantetheine E. CoA-SH	
25.	A microsomal enzyme system is responsible for the formation of some unsaturated fatty acids. Point out the enzyme of this system: A. NADH-cytochrome b_5 reductase B. NADH coenzyme Q reductase C. Succinate coenzyme Q reductase D. Cytochrome oxidase E. Coenzyme Q-cytochrome c reductase	
26.	Point out the possible substrate for acyl-CoA-dehydrogenase (beta-oxidation of	

№	Test:	Explanation:
	HFA): A. Acetyl-SCoA B. Enoyl-SCoA C. Butyryl-SCoA D. Beta-hydroxyacyl-SCoA E. Beta-ketoacyl-SCoA	
27.	A microsomal enzyme system named fatty acyl-CoA desaturase is responsible for the formation of monounsaturated fatty acids: oleic and palmitoleic acids. Name the coenzyme which is an electron donor for this enzyme system: A. NADH B. TPP C. Biotin D. Phosphopantetheine E. CoA-SH	
28.	Point out the process or reaction where acetone is formed as end- product: A. Beta-oxidation of HFA B. Decarboxylation of acetoacetic acid C. Condensation of two acetyl-CoA molecules D. Synthesis of HFA E. Decarboxylation of beta-hydroxybutyric acid	
29.	Name ketone bodies which are excreted with urine in diabetic ketoacidosis: A. Acetoacetic and pyruvic acids B. Acetoacetic and oxaloacetic acids C. Acetoacetic and pyruvic acids D. Acetoacetic and alpha-ketoglutaric acids E. Acetoacetic and beta-hydroxybutiric acids	

CHOLESTEROL METABOLISM. THE REGULATION AND DISORDERS OF LIPIDS METABOLISM: OBESITY, ATHEROSCLEROSIS

(**Ivanchenko D.G.**)

INFORMATIONAL MATERIAL

Steroids

Steroids contain a four-ring structure named the steroid nucleus (Fig. 76). Cholesterol is the steroid precursor in human cells from which all of the steroid hormones are synthesized by modifications to the ring or C_{20} side chain. Although cholesterol is not water soluble, it is converted to amphipathic water-soluble bile salts such as cholic acid. Bile salts line the surfaces of lipid droplets called micelles in the lumen of the intestine, where they keep the droplets emulsified in the aqueous environment.

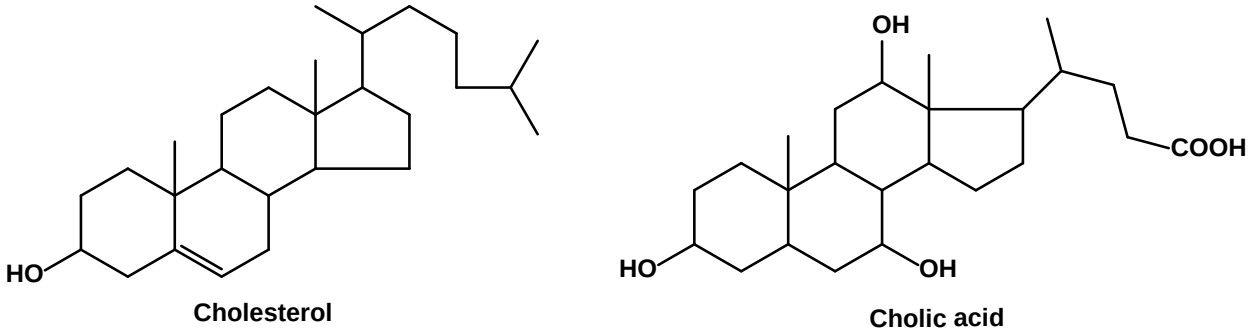


Figure 76. Cholesterol and cholic acid.

Cholesterol metabolism in humans

All ways of the cholesterol metabolism and its utilization are represented in the figure 77.

Synthesis of cholesterol

Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by liver (50 %), intestine (15 %), skin, adrenal cortex, reproductive tissue etc. The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell.

For the production of one mole of cholesterol, 18 moles of acetyl-CoA, 36

moles of ATP and 16 moles of NADPH are required.

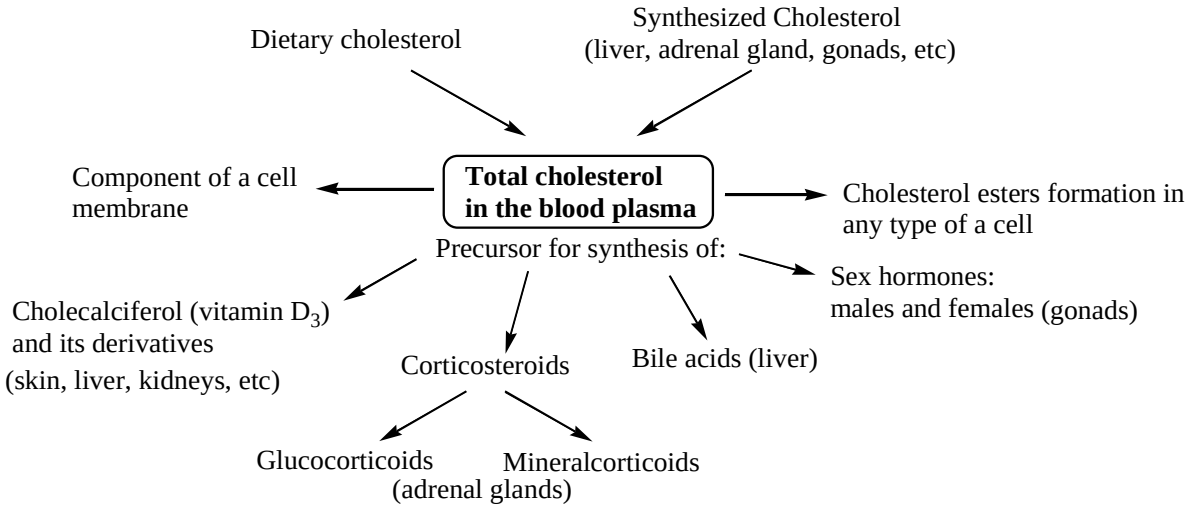


Figure 77. All the ways for the formation of cholesterol pool in the blood plasma.

All 27 carbon atoms of cholesterol are derived from acetyl-CoA in a five-stage synthetic process:

1. Synthesis of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA)
2. Synthesis of mevalonate
3. Synthesis of isoprene units
4. Synthesis of squalene
5. Squalene cyclization and cholesterol formation.

1. Synthesis of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA).

The first stage in the synthesis of cholesterol is the formation of β-hydroxy-β-methylglutaryl-CoA from acetyl-CoA (Fig. 78). Two moles of acetyl-CoA condense to form acetoacetyl-CoA. Another molecule of acetyl-CoA is then added to produce HMG-CoA. HMG-CoA synthesis requires the participation of two enzymes: thiolase and HMG-CoA synthase. The enzymes forming the HMG-CoA leading to ketone bodies occur in the mitochondria, whereas those responsible for the synthesis of the HMG-CoA that is destined for cholesterol biosynthesis are located in the cytosol. Their catalytic mechanisms, however, are identical.

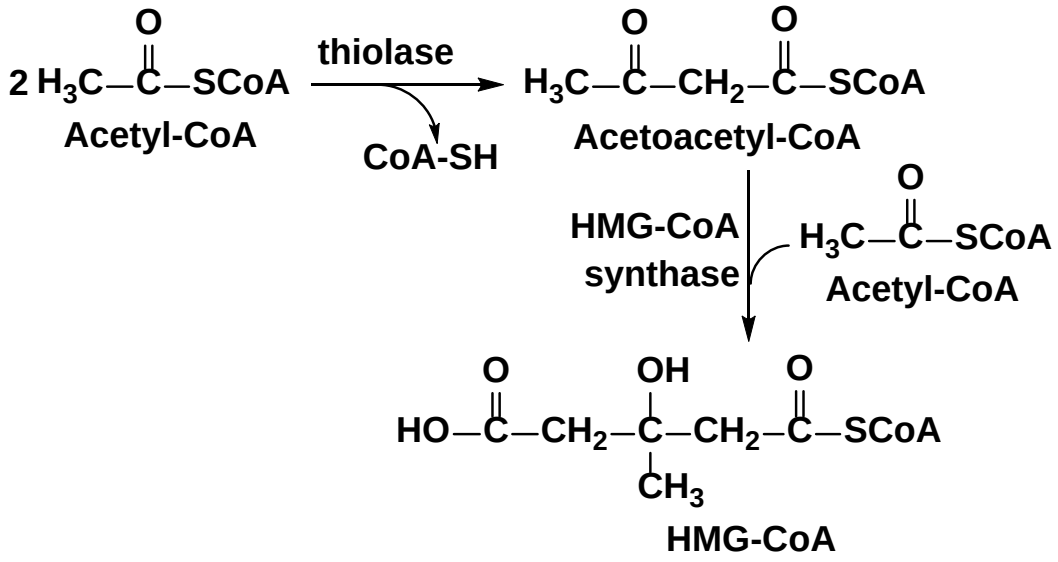


Figure 78. Synthesis of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA).

2. Synthesis of mevalonate.

The CoA thioester group of HMG-CoA is reduced to an alcohol in an NADPH-dependent four-electron reduction catalyzed by HMG-CoA reductase, yielding mevalonate (Fig. 79).

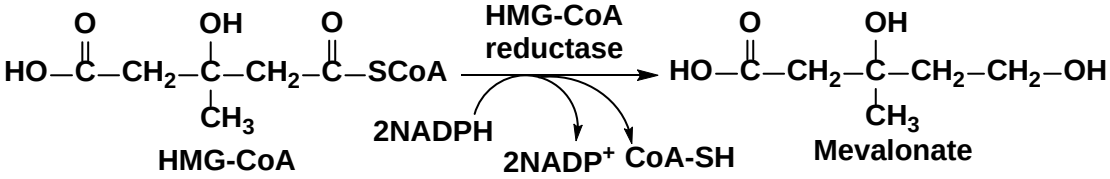


Figure 39. Synthesis of mevalonate.

HMG-CoA reductase mediates the rate-limiting step of cholesterol biosynthesis and is the most elaborately regulated enzyme of this pathway. This 888-residue endoplasmic reticulum membrane-bound enzyme is regulated by competitive and allosteric mechanisms, phosphorylation/dephosphorylation, and long-term regulation. Cholesterol itself is an important feedback regulator of the enzyme.

3. Synthesis of isoprene units.

Mevalonate is converted into 3-isopentenyl pyrophosphate in three consecutive reactions requiring ATP (Fig. 80). In the last step, the release of CO_2 yields isopentenyl pyrophosphate, an activated isoprene unit that is a key building

block for many important biomolecules throughout the kingdoms of life. The latter isomerizes to dimethylallyl pyrophosphate. Both isopentenyl pyrophosphate and dimethylallyl pyrophosphate are 5-carbon isoprenoid units.

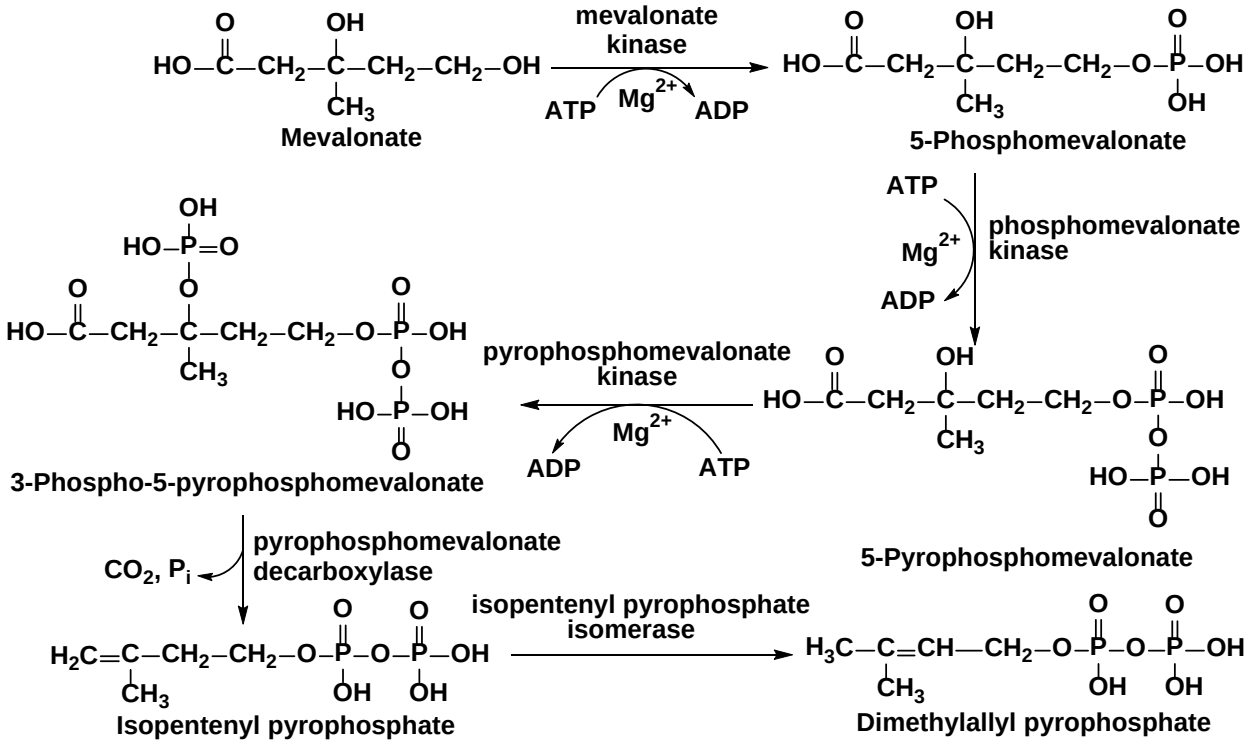


Figure 80. Synthesis of isoprene units.

4. Synthesis of squalene.

Four isopentenyl pyrophosphates and two dimethylallyl pyrophosphates condense to form the C_{30} cholesterol precursor squalene in three reactions catalyzed by two enzymes (Fig. 81):

1. Prenyltransferase (farnesyl pyrophosphate synthase) catalyzes the head-to-tail (1'-4) condensation of dimethylallyl pyrophosphate and isopentenyl pyrophosphate to yield geranyl pyrophosphate.

2. Prenyltransferase catalyzes a second head-to-tail condensation of geranyl pyrophosphate and isopentenyl pyrophosphate to yield farnesyl pyrophosphate.

3. The endoplasmic reticulum enzyme squalene synthase then catalyzes the head-to-head (1-1') condensation of two farnesyl pyrophosphate molecules to form squalene. Farnesyl pyrophosphate is also a precursor of dolichol, farnesylated and geranylgeranylated proteins, and ubiquinone.

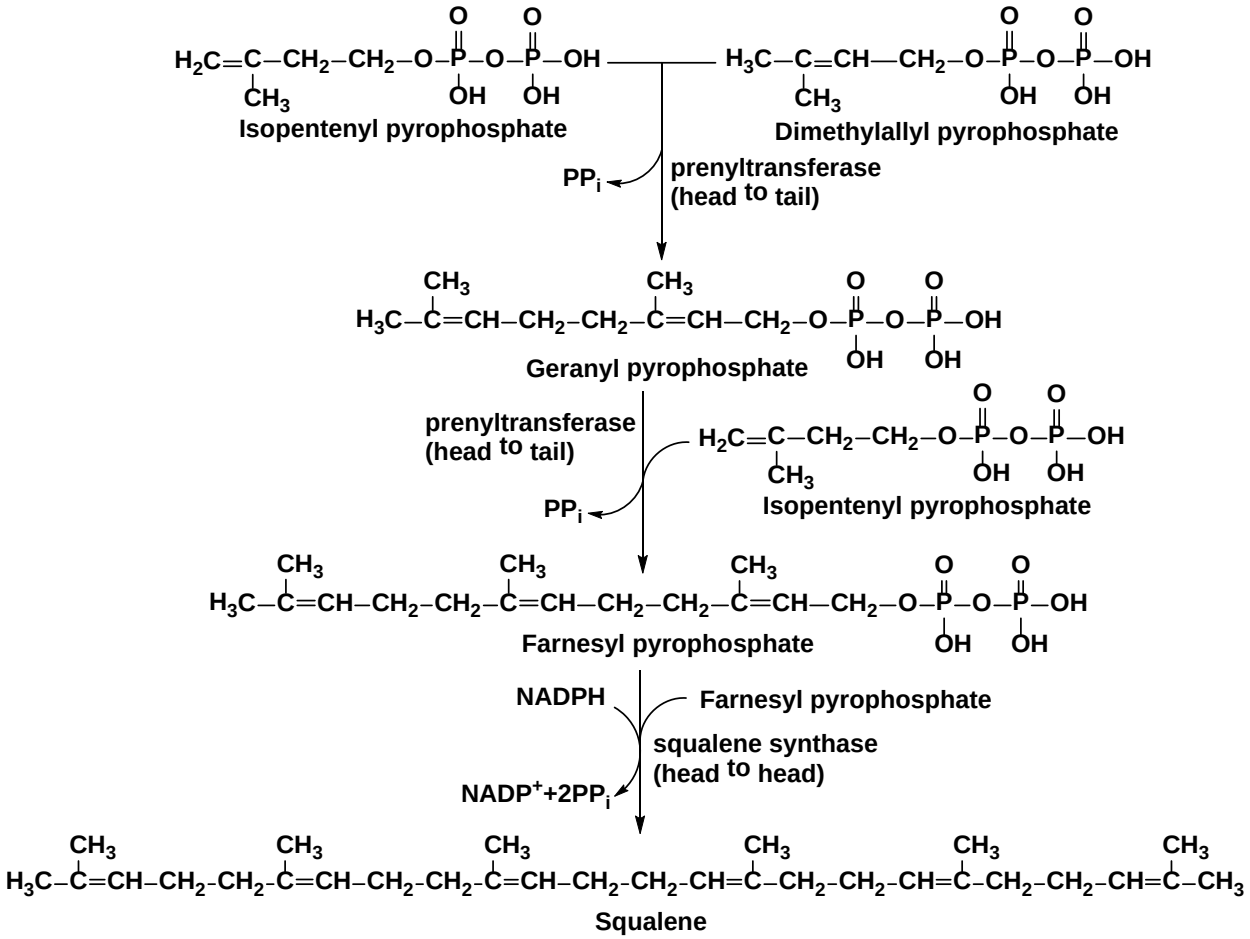


Figure 81. Synthesis of squalene.

5. Squalene cyclization and cholesterol formation.

Squalene undergoes hydroxylation and cyclization utilizing O_2 and NADPH to form lanosterol. The formation of cholesterol from lanosterol is a multistep process with a series of about 19 enzymatic reactions (Fig. 82).

The following are the most important reactions:

- Elemination the carbon atoms from 30 to 27.
- Removal of two methyl groups from C_4 and one methyl group from C_{14}.
- Shift of double bond from C_8 to C_5.
- Reduction in the double bond present between C_{24} and C_{25}.

Control of Cholesterol Biosynthesis

Cholesterol may be obtained from the diet or it may be synthesized de novo. An adult person on a low-cholesterol diet typically synthesizes about 800 mg of

cholesterol per day. The rate of cholesterol formation by these organs is highly responsive to the cellular level of cholesterol. This feedback regulation is mediated primarily by changes in the amount and activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase).

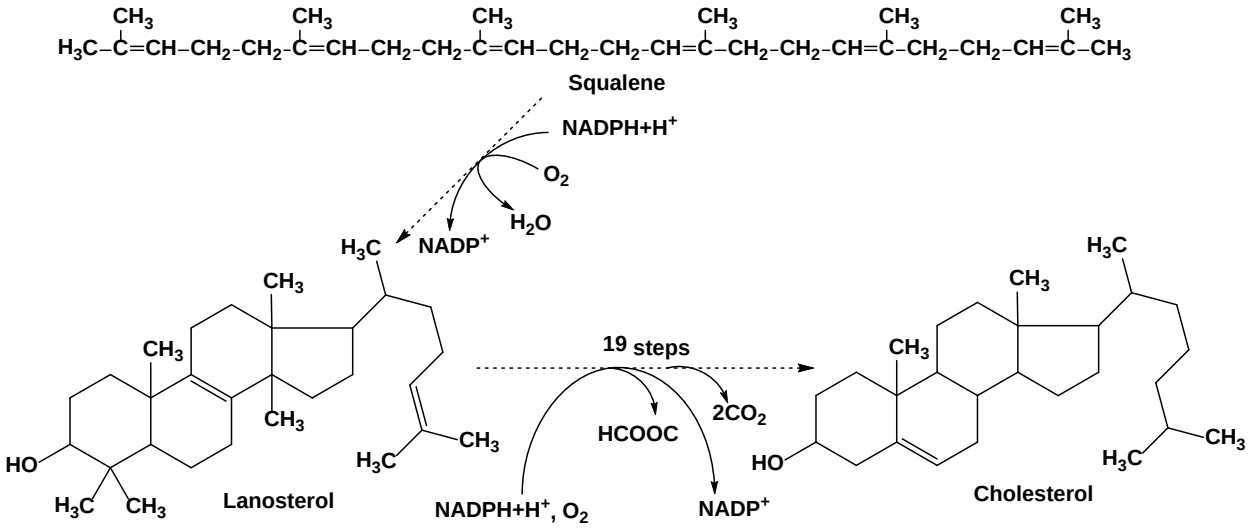


Figure 82. Squalene cyclization and cholesterol formation

The main way in which HMG-CoA reductase is controlled is by long-term feedback control of the amount of enzyme present in a cell. When either LDL-cholesterol or mevalonate levels fall, the amount of HMG-CoA reductase present in the cell can rise as much as 200-fold, due to an increase in enzyme synthesis combined with a decrease in its degradation. When LDL-cholesterol or mevalonolactone (an internal ester of mevalonate that is hydrolyzed to mevalonate and metabolized in the cell) are added back to a cell, these effects are reversed.

The mechanism by which cholesterol serves to control the expression of the >20 genes involved in its biosynthesis and uptake, such as those encoding HMG-CoA reductase and the LDL receptor, has been elucidated by Michael Brown and Joseph Goldstein.

The rate of mRNA synthesis of HMG-CoA reductase is controlled by the sterol regulatory element binding protein (SREBP). This transcription factor binds to a short DNA sequence named the sterol regulatory element (SRE) on the 5' side

of the reductase gene. It binds to the SRE when cholesterol levels are low and enhances transcription. In its inactive state, the SREBP resides in the endoplasmic reticulum membrane, where it is associated with the SREBP cleavage activating protein (SCAP), an integral membrane protein. SCAP is the cholesterol sensor. When cholesterol levels fall, SCAP escorts SREBP in small membrane vesicles to the Golgi complex, where it is released from the membrane by two specific proteolytic cleavages (Fig. 83). The released protein migrates to the nucleus and binds the SRE of the HMG-CoA reductase gene, as well as several other genes in the cholesterol biosynthetic pathway, to enhance transcription. When cholesterol levels rise, the proteolytic release of the SREBP is blocked, and the SREBP in the nucleus is rapidly degraded. These two events halt the transcription of genes of the cholesterol biosynthetic pathways.

The degradation of the reductase is stringently controlled. The enzyme is bipartite: its cytoplasmic domain carries out catalysis and its membrane domain senses signals that lead to its degradation. The membrane domain may undergo structural changes in response to increasing concentrations of sterols, and it makes the enzyme more susceptible to proteolysis. The reductase may be further degraded by ubiquitination and targeting to the 26S proteasome under some conditions.

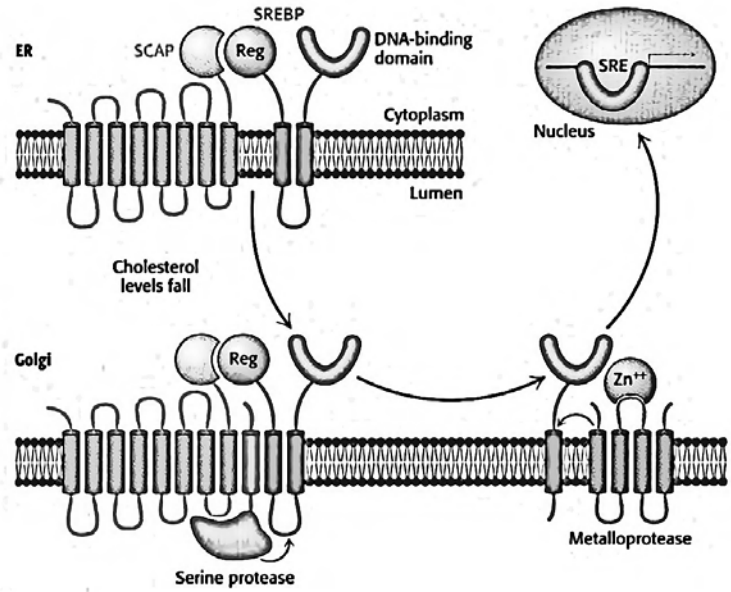


Figure 83. The cholesterol-mediated proteolytic activation of SREBP (Goldstein, J., Rawson, R.B., and Brown, M., Arch. Biochem. Biophys. 397, 139 (2002)).

HMG-CoA reductase exists in interconvertible more active and less active forms, as do glycogen phosphorylase, glycogen synthase, pyruvate dehydrogenase, and acetyl-CoA carboxylase, among others. The unmodified form of HMG-CoA reductase is more active and the phosphorylated form is less active. HMG-CoA reductase is phosphorylated (inactivated) at its Ser 871 in a bicyclic cascade system by the covalently modifiable enzyme AMP-dependent protein kinase (AMPK), which also acts on acetyl-CoA carboxylase. It appears that this control is exerted to conserve energy when ATP levels fall and AMP levels rise, by inhibiting biosynthetic pathways. This hypothesis was tested by Brown and Goldstein, who used genetic engineering techniques to produce hamster cells containing a mutant HMG-CoA reductase with Ala replacing Ser 871 and therefore incapable of phosphorylation control. These cells respond normally to feedback regulation of cholesterol biosynthesis by LDL-cholesterol and mevalonate but, unlike normal cells, do not decrease their synthesis of cholesterol on ATP depletion, supporting the idea that control of HMG-CoA reductase by phosphorylation is involved in energy conservation.

In this way glucagon and glucocorticoids favour the formation of inactive HMG-CoA reductase (phosphorylated form) and, thus, decrease cholesterol synthesis. On the other hand, insulin and thyroxine increase cholesterol production by enhancing the formation of active HMG-CoA reductase (dephosphorylated form).

The drugs lovastatin (also called mevinolin and sold as Mevacor), pravastatin (Pravachol), and simvastatin (Zocor) are fungal products, which are used to decrease the serum cholesterol level in patients with hypercholesterolemia. The synthetic inhibitor atorvastatin (Lipitor) is presently one of the most widely prescribed drugs in USA. These drugs, collectively known as statins, are competitive inhibitors of HMG-CoA reductase and, therefore, reduce cholesterol synthesis. About 50 to 60 % decrease in serum cholesterol level has been reported by a combined use of these two drugs.

Cholesterol Degradation Pathways

The steroid nucleus (ring structure) of the cholesterol cannot be degraded to CO_2 and H_2O. Cholesterol (50 %) is converted to bile acids (excreted in feces), serves as a precursor for the synthesis of steroid hormones, vitamin D_3, coprostanol and cholestanol. The latter two are the fecal sterols, besides cholesterol.

Bile Salts Formation. Bile salts are polar derivatives of cholesterol. These compounds are highly effective detergents because they contain both polar and nonpolar regions. Bile salts are synthesized in the liver, stored and concentrated in the gall bladder, and then released into the small intestine. Bile salts, the major constituent of bile, solubilize dietary lipids. Solubilization increases the effective surface area of lipids with two consequences: (1) more surface area is exposed to the digestive action of lipases and (2) lipids are more readily absorbed by the intestine. Bile salts are also the major breakdown products of cholesterol.

Bile acid synthesis, which occurs in the liver, is outlined in Figure 84.

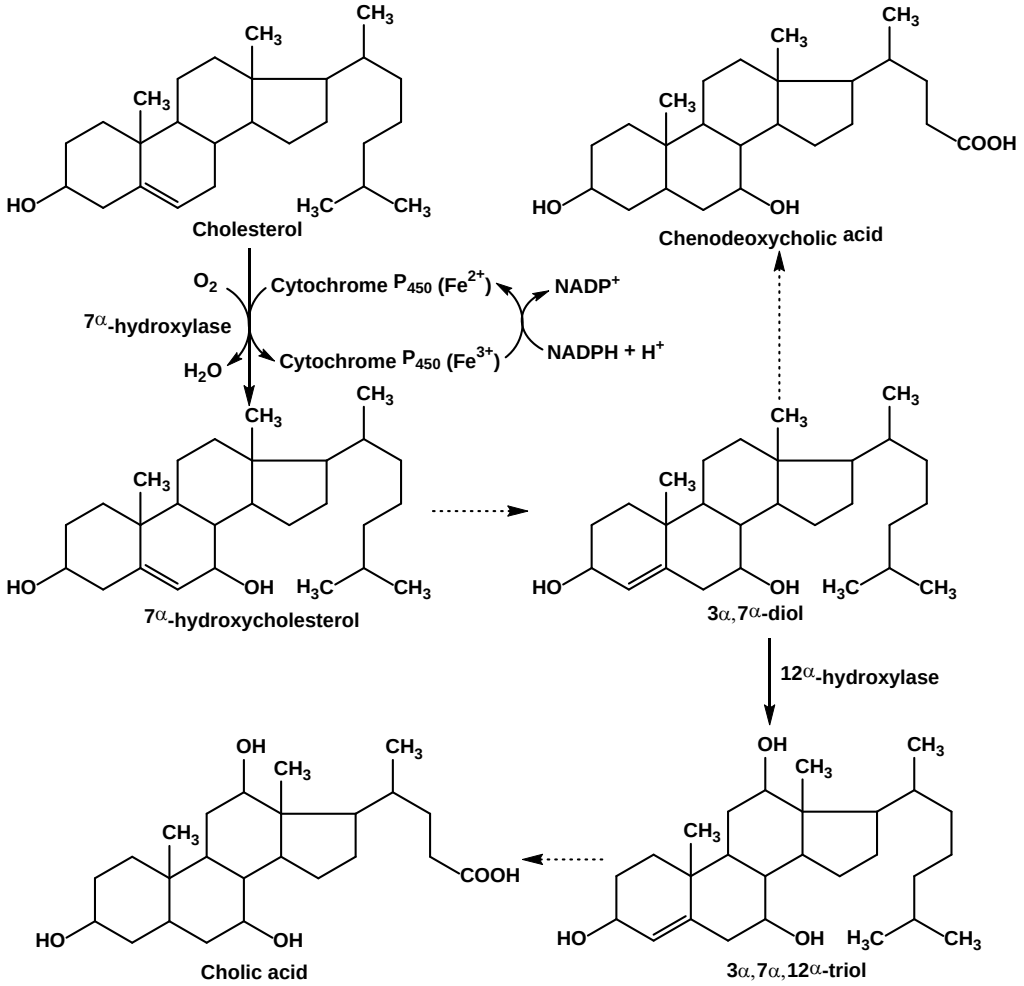


Figure 84. Synthesis of bile acids.

The conversion of cholesterol to 7α-hydrocholesterol, catalyzed by cholesterol-7α-hydroxylase (a microsomal enzyme), is the rate-limiting reaction in bile acid synthesis. In later reactions, the double bond at C-5 is rearranged and reduced, and an additional hydroxyl group is introduced. Two different sets of compounds are produced. One set has α-hydroxyl groups at positions 3, 7, and 12, and produces the cholic acid series of bile salts. The other set has α-hydroxyl groups only at positions 3 and 7 and produces the chenodeoxycholic acid series. Three carbons are removed from the side chain by an oxidation reaction. The remaining 5-carbon fragment attached to the ring structure contains a carboxyl group. The products of this process, cholic acid and chenodeoxycholic acid, are converted to bile salts by microsomal enzymes that catalyze conjugation reactions. These conversion steps help to increase solubility of intermediate metabolites. Most bile acids are conjugated with glycine or taurine (Fig. 85).

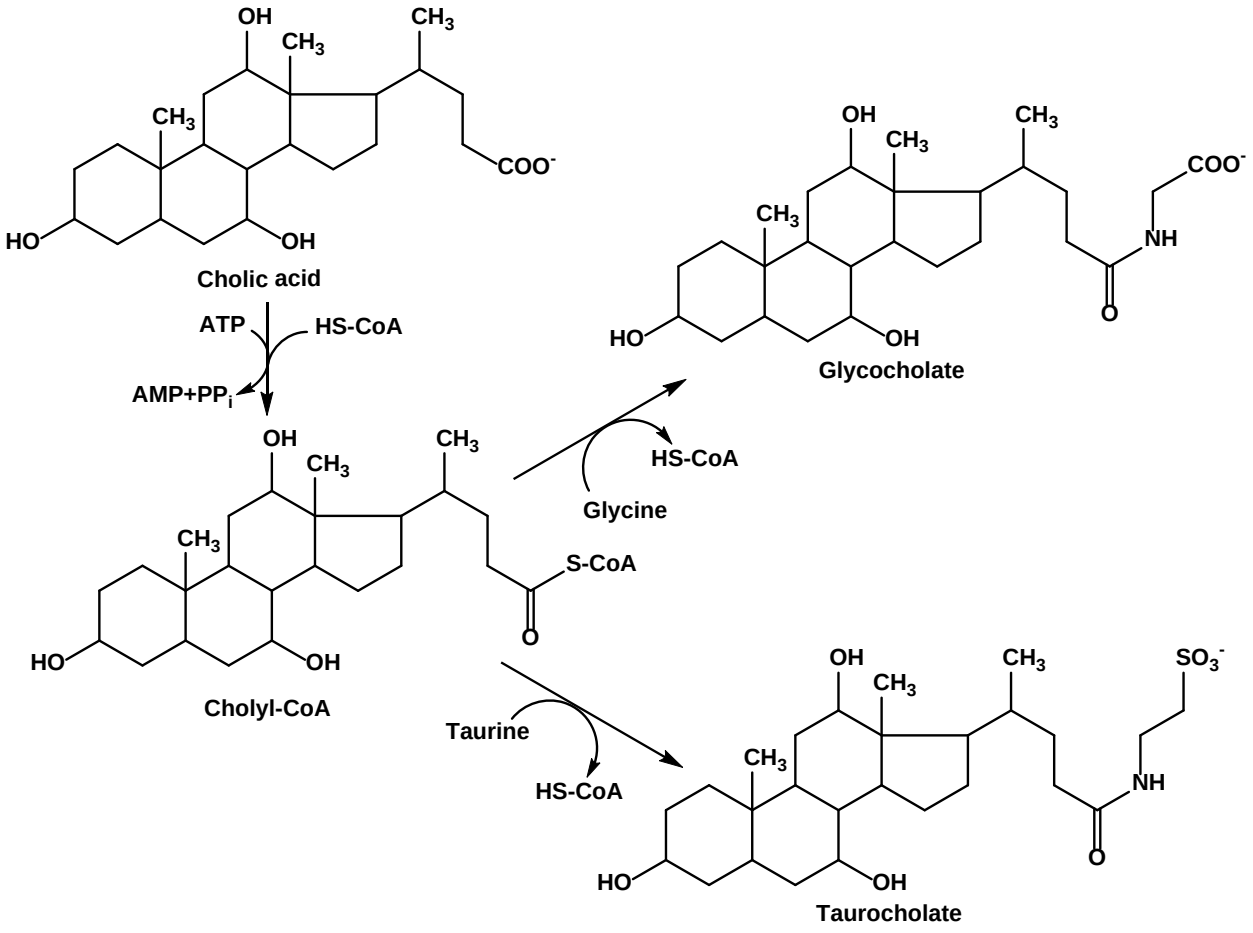


Figure 85. Synthesis of glycine and taurine conjugates.

In the bile, the conjugated bile acids exist as sodium and potassium salts which are known as bile salts. In the intestine, a portion of primary bile acids undergoes deconjugation and dehydroxylation to form secondary bile acids (deoxycholic acid and lithocholic acid). These reactions are catalyzed by bacterial enzymes in the intestine.

Enterohepatic circulation. The conjugated bile salts synthesized in the liver accumulate in gall bladder. From there they are secreted into the small intestine where they serve as emulsifying agents for the digestion and absorption of fats and fat soluble vitamins. A large portion of bile salts (primary and secondary) are reabsorbed and returned to the liver through portal vein. Thus the bile salts are recycled and reused several times in a day. This is known as enterohepatic circulation. About 12-32 g of bile salts are secreted into the intestine each day and reabsorbed. However, a small portion of about 0.2-0.6 g/day is lost in the feces. An equal amount (0.2-0.6 g/day) is synthesized in liver to replace the lost bile salts. The fecal excretion of bile salts is the only route for the removal of cholesterol from the body (Fig. 86).

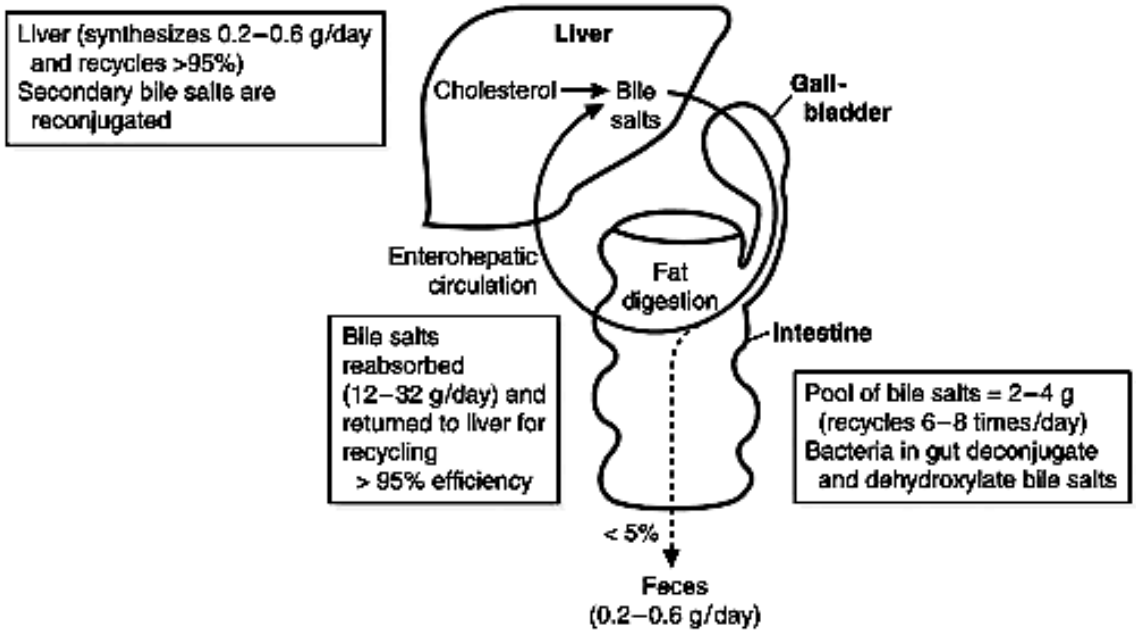


Figure 86. Overview of bile salt metabolism.

Steroid Hormones Formation. Cholesterol is the precursor of the five major classes of steroid hormones: progestagens, glucocorticoids,

mineralocorticoids, androgens, and estrogens. These hormones are powerful signal molecules that regulate a host of organismal functions.

Progesterone, a progestagen, prepares the lining of the uterus for the implantation of an ovum. Progesterone is also essential for the maintenance of pregnancy. The major site of synthesis of this class of hormones is the corpus luteum.

Androgens (such as testosterone) are responsible for the development of male secondary sex characteristics, whereas estrogens (such as estrone) are required for the development of female secondary sex characteristics. The major sites of synthesis of this class of hormones are the testes.

Estrogens, along with progesterone, also participate in the ovarian cycle. Glucocorticoids (such as cortisol) promote gluconeogenesis and the formation of glycogen, enhance the degradation of fat and protein, and inhibit the inflammatory response. They enable animals to respond to stress; indeed, the absence of glucocorticoids can be fatal. The major sites of synthesis of this class of hormones are the ovaries.

Mineralocorticoids (primarily aldosterone) act on the distal tubules of the kidney to increase the reabsorption of Na^+ and the secretion of K^+ and H^+, which leads to an increase in blood volume and blood pressure. The major site of synthesis of this class of hormones is the adrenal cortex.

Steroid hormones bind to and activate receptor molecules that serve as transcription factors to regulate gene expression. These small similar molecules are able to have greatly differing effects because the slight structural differences among them allow interactions with specific receptor molecules.

Cholesterol is converted to progesterone in the first two steps of synthesis of all steroid hormones. Cytochrome $P450_{SCC}$ (side-chain cleavage enzyme system or cholesterol desmolase) is located in the mitochondrial inner membrane and removes six carbons from the side chain of cholesterol, forming pregnenolone, which has 21 carbons (Fig. 87). The next step, the conversion of pregnenolone to progesterone, is catalyzed by 3β-hydroxysteroid dehydrogenase, an enzyme that is

not a member of the cytochrome P450 family. Other steroid hormones are produced from progesterone by reactions that involve members of the P450 family. As the synthesis of the steroid hormones is discussed, notice how certain enzymes are used in more than one pathway. Defects in such enzymes will lead to multiple abnormalities in steroid synthesis, which, in turn, results in a variety of abnormal phenotypes.

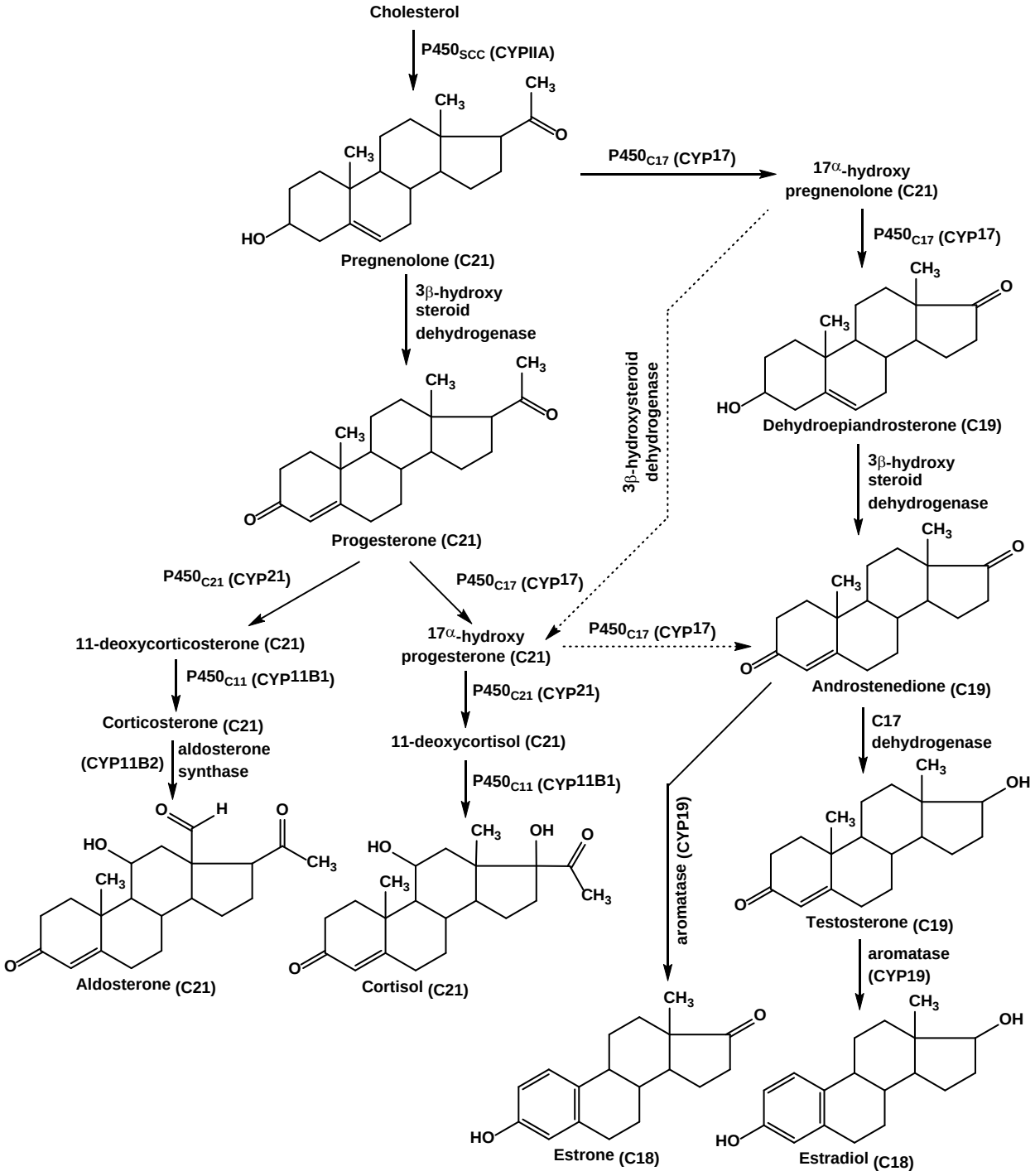


Figure 87. Synthesis of steroid hormones.

Synthesis of cortisol. The adrenocortical biosynthetic pathway that leads to cortisol synthesis occurs in the middle layer of the adrenal cortex known as the zona fasciculata. Free cholesterol is transported by an intracellular carrier protein to the inner mitochondrial membrane of cells (Fig. 88), where the side chain is cleaved to form pregnenolone. Pregnenolone returns to the cytosol, where it forms progesterone.

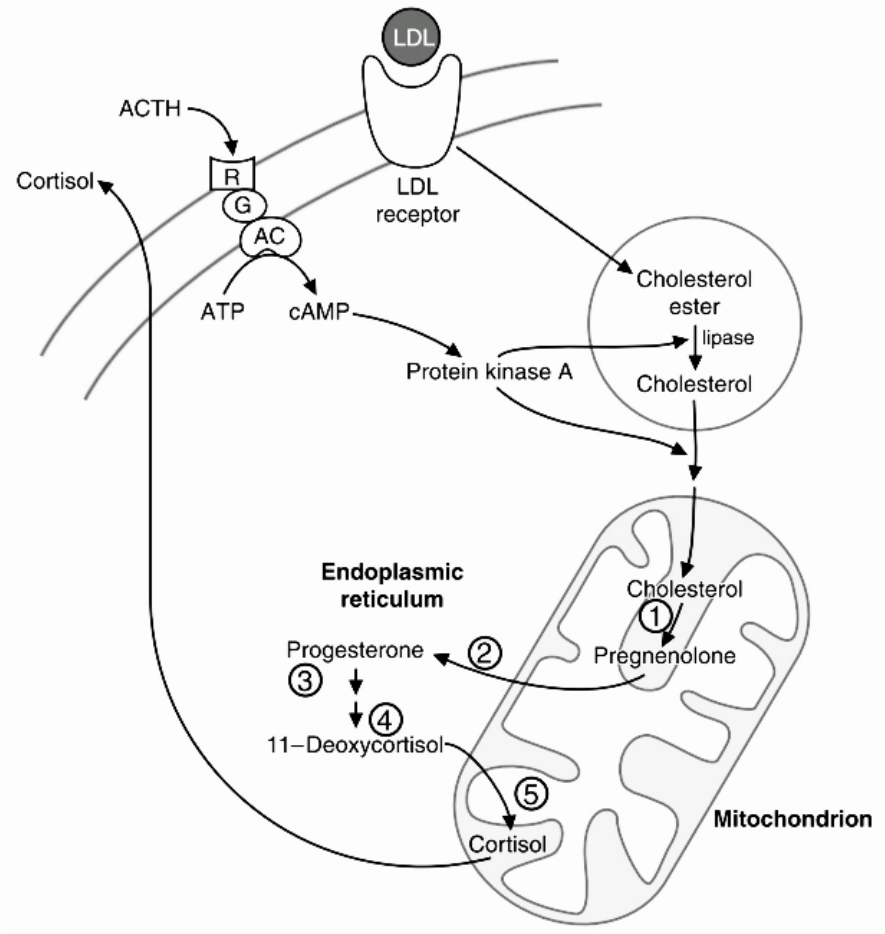


Figure 88. Cellular route for cortisol synthesis.

1 – Cholesterol desmolase (involved in side chain cleavage); 2 – 3β-hydroxysteroid dehydrogenase; 3 – 17α-hydroxylase; 4 – 21-hydroxylase; 5 – 11β-hydroxylase.

In the membranes of the endoplasmic reticulum, the enzyme $P450_{C17}$ catalyzes the hydroxylation of C17 of progesterone or pregnenolone and can also catalyze the cleavage of the 2-carbon side chain of these compounds at C17 (a C17-C20 lyase activity). These two separate functions of the same enzyme allow

further steroid synthesis to proceed along two separate pathways: the 17-hydroxylated steroids that retain their side chains are precursors of cortisol (C21), whereas those from which the side chain was cleaved (C19 steroids) are precursors of androgens (male sex hormones) and estrogens (female sex hormones).

In the pathway of cortisol synthesis, the 17-hydroxylation of progesterone yields 17-α-hydroxyprogesterone, which, along with progesterone, is transported to the smooth endoplasmic reticulum. The membrane-bound $P450_{C21}$ (21-α-hydroxylase) enzyme catalyzes the hydroxylation of C21 of 17-α-hydroxyprogesterone there to form 11-deoxycortisol (and of progesterone to form deoxycorticosterone, a precursor of the mineralocorticoid, aldosterone) (Fig. 47).

The final step in cortisol synthesis requires transport of 11-deoxycortisol back to the inner membrane of the mitochondria, where $P450_{C11}$ (11-β-hydroxylase) receives electrons from electron transport protein intermediates (adrenodoxin, which when oxidized is reduced by adrenodoxin reductase). This enzyme transfers these reducing equivalents by way of oxygen to 11-deoxycortisol for hydroxylation at C11 to form cortisol. The rate of biosynthesis of cortisol and other adrenal steroids is dependent on stimulation of the adrenal cortical cells by adrenocorticotropic hormone (ACTH).

Synthesis of aldosterone. The synthesis of the potent mineralocorticoid aldosterone in the zona glomerulosa of the adrenal cortex also begins with the conversion of cholesterol to progesterone (Figs. 87, 88). Progesterone is then hydroxylated at C21, a reaction catalyzed by $P450_{C21}$, to yield deoxycorticosterone. The $P450_{C11}$ enzyme system then catalyzes the reactions that convert deoxycorticosterone to corticosterone. The terminal steps in aldosterone synthesis, catalyzed by the P450 aldosterone system, involve the oxidation of corticosterone to 18-hydroxycorticosterone, which is oxidized to aldosterone.

The primary stimulus for aldosterone production is the octapeptide angiotensin II, although hypernatremia (greater than normal levels of sodium in the blood) may directly stimulate aldosterone synthesis as well. ACTH has a permissive action in aldosterone production. It allows cells to respond optimally to

their primary stimulus, angiotensin II.

Synthesis of the adrenal androgens. Adrenal androgen biosynthesis proceeds from cleavage of the 2-carbon side chain of 17-hydroxypregnenolone at C17 to form the 19-carbon adrenal androgen dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEAS) in the zona reticulosum of the adrenal cortex (Fig. 87). These compounds, which are weak androgens, represent a significant percentage of the total steroid production by the normal adrenal cortex, and are the major androgens synthesized in the adrenal gland.

Androstenedione, another weak adrenal androgen, is produced when the 2-carbon side chain is cleaved from 17α-hydroxyprogesterone by the C17-C20 lyase activity of $P450_{C17}$. This androgen is converted to testosterone primarily in extraadrenal tissues. Although the adrenal cortex makes very little estrogen, the weak adrenal androgens may be converted to estrogens in the peripheral tissues, particularly in adipose tissue.

Synthesis of testosterone. Luteinizing hormone (LH) from the anterior pituitary stimulates the synthesis of testosterone and other androgens in Leydig cells of human testicle. In many ways, the pathways leading to androgen synthesis in the testicle are similar to those described for the adrenal cortex. In the human testicle, the predominant pathway leading to testosterone synthesis is through pregnenolone to 17α-hydroxypregnenolone to DHEA (the Δ^5 pathway), and then from DHEA to androstenedione, and from androstenedione to testosterone (Fig. 87). As for all steroids, the rate-limiting step in testosterone production is the conversion of cholesterol to pregnenolone. LH controls the rate of side-chain cleavage from cholesterol at carbon 21 to form pregnenolone, and thus regulates the rate of testosterone synthesis. In its target cells, the double bond in ring A of testosterone is reduced through the action of 5α-reductase, forming the active hormone dihydrotestosterone (DHT).

Synthesis of estrogens and progesterone. Ovarian production of estrogens, progestins (compounds related to progesterone), and androgens requires the activity of the cytochrome P450 family of oxidative enzymes used for the synthesis

of other steroid hormones. Ovarian estrogens are C18 steroids with a phenolic hydroxyl group at C3 and either a hydroxyl group (estradiol) or a ketone group (estrone) at C17. Although the major steroid-producing compartments of the ovary (the granulosa cell, the theca cell, the stromal cell, and the cells of the corpus luteum) have all of the enzyme systems required for the synthesis of multiple steroids, the granulosa cells secrete primarily estrogens, the thecal and stromal cells secrete primarily androgens, and the cells of the corpus luteum secrete primarily progesterone.

The ovarian granulosa cell, in response to stimulation by follicle-stimulating hormone (FSH) from the anterior pituitary gland and through the catalytic activity of P450 aromatase, converts testosterone to estradiol, the predominant and most potent of the ovarian estrogens (Fig. 87). Similarly, androstenedione is converted to estrone in the ovary, although the major site of estrone production from androstenedione occurs in extraovarian tissues, principally skeletal muscle and adipose tissue.

Vitamin D_3. The D_3 vitamin is sterol derivative in which the steroid B ring is disrupted at its 9,10 position. The natural form of the vitamin, vitamin D_3 (cholecalciferol), is nonenzymatically formed in the skin of animals through the photolytic action of UV light on 7-dehydrocholesterol (is an intermediate in cholesterol synthesis) (Fig. 89). On the basis of its mechanism of action in the body, cholecalciferol should be called a prohormone, a hormone precursor. Dietary forms of vitamin D are absorbed through the aid of bile salts in the small intestine. Whether absorbed in the intestine or photosynthesized in the skin, cholecalciferol is then transported to the liver by a specific vitamin D-binding protein (DBP), also known as transcalciferin. Vitamin D_3 gains biological activity through further metabolic processing, first in the liver and then in the kidney:

1. In human liver, vitamin D_3 is hydroxylated to form 25-hydroxycholecalciferol in an O_2-requiring reaction catalyzed by either of two cytochrome P450's: CYP27A1 and CYP2R1 (or 25-hydroxylase).

2. The 25-hydroxycholecalciferol is transported to the kidney, where it is

further hydroxylated by CYP27B1 (or 1α-hydroxylase) to yield the active hormone 1,25-dihydroxycholecalciferol [1,25$(OH)_2D_3$]. CYP27B1 is activated by PTH, so this reaction is an important control point in Ca^{2+} homeostasis.

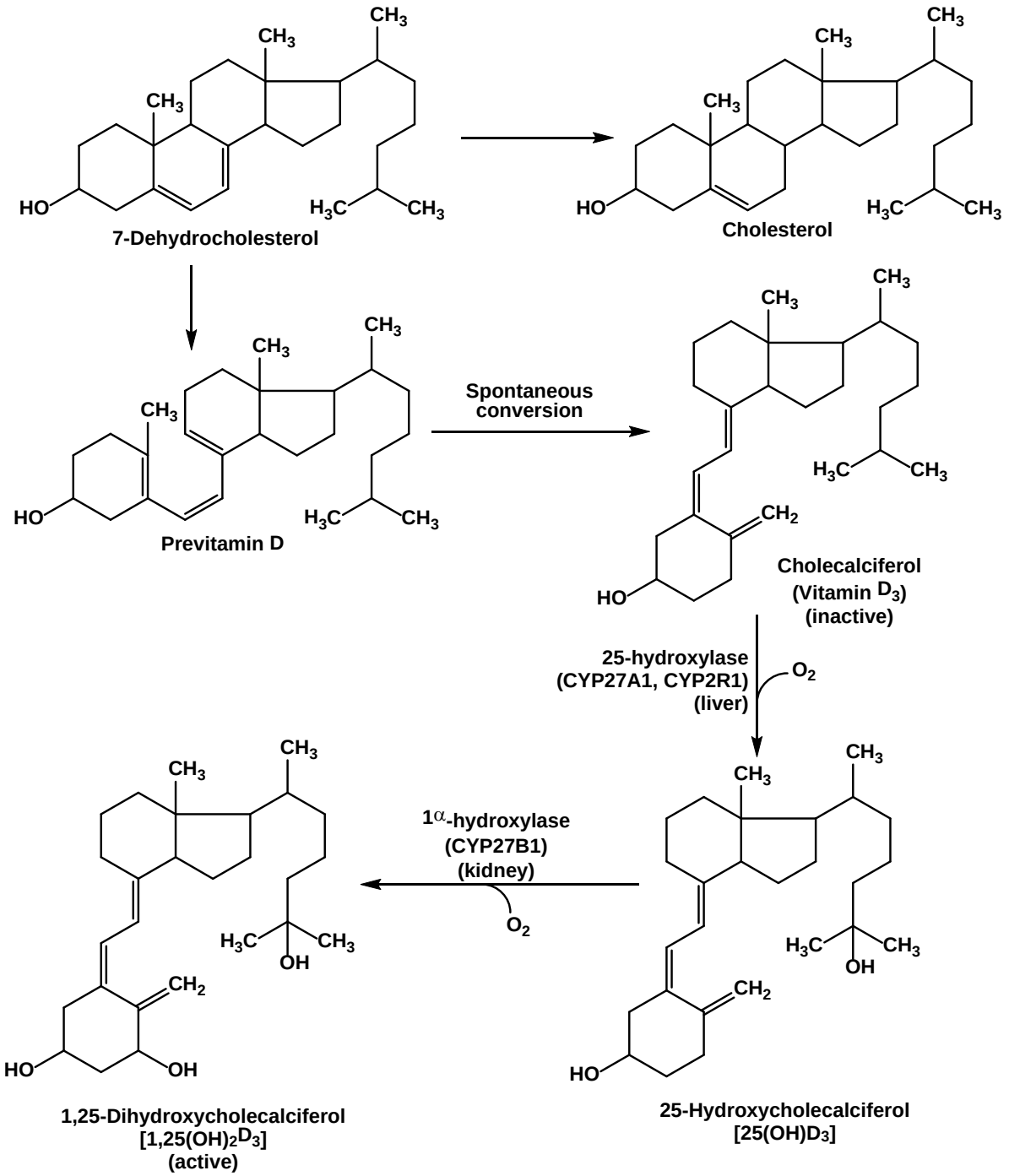


Figure 89. Synthesis and activation of vitamin D_3.

$1,25(OH)_2D_3$ acts to increase serum [Ca^{2+}] by promoting the intestinal absorption of dietary Ca^{2+} and by stimulating Ca^{2+} and PO_4^{3-} reabsorption in kidneys. Intestinal Ca^{2+} absorption is stimulated through increased synthesis of a

Ca^{2+}-binding protein, which functions to transport Ca^{2+} across the intestinal mucosa. $1,25(OH)_2D_3$ binds to cytoplasmic receptors in intestinal epithelial cells that, on transport to the nucleus, function as transcription factors for the Ca^{2+}-binding protein. The maintenance of electroneutrality requires that Ca^{2+} transport be accompanied by that of counterions, mostly P_i (H_3PO_4), so that $1,25(OH)_2D_3$ also stimulates the intestinal absorption of Pi. The observation that $1,25(OH)_2D_3$, like PTH, stimulates the release of Ca^{2+} and P_i from bone seems paradoxical in view of the fact that low levels of $1,25(OH)_2D_3$ result in subnormal bone mineralization. Presumably the increased serum [Ca^{2+}] resulting from $1,25(OH)_2D_3$-stimulated intestinal uptake of Ca^{2+} causes bone to take up more Ca^{2+} than it loses through direct hormonal stimulation. In addition, vitamin D_3 has been shown to modulate the immune response, provide protection against certain types of cancers, and has been implicated in preventing/reversing heart disease.

Vitamin D_3, unlike the water-soluble vitamins, is retained by the body, so that excessive intake of vitamin D_3 over long periods causes vitamin D_3 intoxication (although note that most individuals, particularly the elderly and those with limited sun exposure, have less than the recommended levels of vitamin D_3 in their blood). The consequent high serum [Ca^{2+}] results in aberrant calcification of a wide variety of soft tissues. The kidneys are particularly prone to calcification, a process that can lead to the formation of kidney stones and ultimately kidney failure. In addition, vitamin D_3 intoxication promotes bone demineralization to the extent that bones are easily fractured. The observation that the level of skin pigmentation in indigenous human populations tends to increase with their proximity to the equator is explained by the hypothesis that skin pigmentation functions to prevent vitamin D intoxication by filtering out excessive solar radiation.

Lipid metabolism disorders

Obesity

Obesity is an abnormal increase in the body weight due to excessive fat

deposition. Men and women are considered as obese if their weight due to fat (in adipose tissue), respectively, exceeds more than 20 % and 25 % of body weight.

There is strong evidence to suggest that obesity has genetic basis. Thus, a child born to two obese people has about 25% chances of being obese. One gene namely *ob gene*, expressed in adipocytes (of white adipose tissue) producing a protein named *leptin*, is closely associated with obesity.

Leptin is regarded as a body weight regulatory hormone. It binds to a specific receptor in the brain and functions as a lipostat. When the fat stores in the adipose tissue are adequate, leptin levels are high. This signals to restrict the feeding behaviour and limit fat deposition. Further, leptin stimulates lipolysis and inhibits lipogenesis. Any genetic defect in leptin or its receptor will lead to extreme overeating and massive obesity. Treatment of such obese individuals with leptin has been shown to reverse obesity.

During starvation, leptin levels fall which promote feeding, and fat production and its deposition.

Obesity is a major problem in developed and increasingly in underdeveloped countries. There is particular concern about the marked increase in obesity in children, which can lead to major health problems in later life and will result in a massive increase in financial expenditure on health provision in the future: some obese children are now developing type 2 diabetes as young as 12.

There are at least three concerns about the diet of children in developed countries since they may lead to disease in adulthood or even earlier. The concerns are related to:

• the fat content;

• the trans-fatty acid content;

• the sugar (sucrose) content.

Fat content. The large amount of fat (as a high percentage of energy intake) is one factor that can lead to obesity, which increases the risk of developing type 2 diabetes. The increased availability of “fast food” and snacks that contain a high percentage of fat are temptations to children, in whom appetite is large, to

accommodate sufficient intake of food to support growth. If the energy intake is higher than expenditure, obesity can result.

Trans-fatty acids. The phospholipids in the plasma and in membranes of all cells contain long-chain polyunsaturated fatty acids (PUFA). During periods of growth and development of organs, PUFAs are required for phospholipid synthesis. The PUFAs are, of course, obtained from dietary triacylglycerol and phospholipids. The double bonds in most natural fatty acids are cis not trans. Nonetheless trans-fatty acids do occur in dietary fats. If the diet contains trans-fatty acids, they might be incorporated into the phospholipids along with the cis-fatty acids and hence into membranes. The presence of these abnormal fatty acids will modify the structure of the phospholipids which could impair the function of the membrane. There are two main sources of trans-fatty acids in the diet: foods produced from ruminants contain trans-fatty acids due to the activity of bacteria in the rumen; commercial hydrogenation of oils results in conversion of some cis into trans bonds. These artificially hydrogenated fats are used in the preparation of children's favourite foods such as potato crisps (chips), biscuits and pastries, and fast food such as beefburgers. Cells particularly exposed to such fatty acids are the endothelial cells. Damage to membranes of endothelial cells can lead to local inflammation and predispose to atherosclerosis.

Sugar. The hydrolysis of sucrose in the intestine produces both glucose and fructose, which are transported across the epithelial cells by specific carrier proteins. The fructose is taken up solely by the liver. Fructose is metabolised in the liver to the triose phosphates, dihydroxyacetone and glyceraldehyde phosphates. These can be converted either to glucose or to acetyl-CoA for lipid synthesis. In addition, they can be converted to glycerol 3-phosphate which is required for, and stimulates, esterification of fatty acids. The resulting triacylglycerol is incorporated into the VLDL which is then secreted. In this way, fructose increases the blood level of VLDL.

The metabolism of VLDL by lipoprotein lipase in the capillaries in many tissues results in the formation of low density lipoprotein (LDL), which is

atherogenic, so that diets high in sucrose are a risk factor for development of atherosclerosis. Many children in developed countries now consume large quantities of soft drinks containing sucrose or fructose. According to the above discussion, this could lead to atherosclerosis in later life.

Atherosclerosis of blood vessels in humans

Atherosclerosis of blood vessels in humans usually associated with the state of Hypercholesterolemia. It is determined in patients with cholesterol level more then 5.72 mmole/L (normal is 3,9-5,72 mmol/L) in the blood plasma. This state is correlated with one of the following abnormalities:

1) elevated concentrations of VLDL with normal concentrations of LDL;
2) elevated LDL with normal VLDL;
3) elevation of both lipoprotein fractions;
4) the inverse relationship between HDL and LDL may be also. The most predictive relationship is the LDL:HDL cholesterol ratio, and it must be not higher then 3.5 for healthy people.

The accumulation of LDL in the blood may be in patients with genetic defects associated with:

a) a defect of synthesis of receptors to LDL – apoB-100 receptors. It results in high levels of cholesterol (about 18 mmol/L) and LDL;

b) a decrease of synthesis of triacylglycerol lipase linked with VLDL. This defect results the increase of VLDL, then LDL (cholesterol and TG concentrations are increased);

c) the low activity of Endothelial triacylglycerol lipase (the same results);

d) a decrease of synthesis of Lecithin Cholesterol Acyltransferase (LChATase) linked with HDL. In this case the surplus of cholesterol can’t be transformed into cholesterol ester, and transported from peripheral cell to the liver by HDL.

These changes can cause the atherosclerosis and are considered as primary reasons of this pathology. The surplus of cholesterol accumulates in the arterial walls. LDL and VLDL (in high levels) can penetrate the vascular wall from the

blood plasma to act subsequently as a primary substrate causing the atherosclerotic lesion of arteries.

The secondary reasons of atherosclerosis may be the diseases such as diabetes mellitus, lipid nephrosis, hypothyroidism, and other conditions of hyperlipidemia.

Coronary heart disease is caused by atherosclerosis of arteries and can be finished by myocardium infarction in patient.

The treatment of hypercholesterolemia state depends upon its reason. It may include:

1) ***Cholesterol free diet*** (for all reasons of this state);

2) ***Unsaturated High fatty acids*** must be in high concentration in food products (for all reasons of this state)

Fish oils. These contain high levels of omega-3 fatty acids, which have a number of properties that could explain why fish oils or a diet high in oily fi sh have a protective effect:

• They lower the blood level of VLDL and therefore they lower plasma level of LDL.

• They decrease the formation of blood clots.

• They decrease the formation of thromboxane A_2 and prostacyclin I_2 in favour of thromboxane A_3 and prostacyclin I_3, changes which protect against thrombosis.

• They have a hypotensive effect.

The major omega-3 fatty acid in fish oil is eicosapentaenoic acid, which contains five double bonds compared with only four present in the omega-6 fatty acid, arachidonic acid. When eicosapentaenoic acid is substrate for eicosanoid production, it gives rise to prostacyclins and thromboxanes of the three series whereas when arachidonic acid is substrate, it gives rise to the two series, thromboxane A_2 and prostacyclin I_2. Thromboxane A_3 has much less of a thrombolytic effect than thromboxane A_2 whereas prostacyclin I_3 has more of an antithrombotic activity than prostacyclin I_2. Hence, the risk of formation of a thrombus is decreased when omega-3 fatty acids are the substrate for the

cyclooxygenase. There is considerable epidemiological evidence that fish oils are protective against atherosclerosis;

3) ***Antioxidants.*** These are naturally occurring compounds that have the ability to lower the levels of free radicals: they include vitamins C and E, the carotenoids and the flavonoids. Vitamin E and the carotenoids are particularly important in preventing oxidation of the unsaturated fatty acids within the LDL particle and within membranes of cells;

4) ***Physical activity.*** Evidence for the beneficial effects of physical activity on the development of atherosclerosis first arose from a series of epidemiological studies. This activity is now known to cause several changes, all of which are benefi cial in decreasing the risk of development of atherosclerosis. These are:

- a fall in the total serum level of cholesterol;
- an increase in the serum HDL-cholesterol level;
- a fall in the serum LDL-cholesterol level;
- a fall in the plasma triacylglycerol level;
- loss of weight;
- reduction of blood pressure.

It also increases the sensitivity of tissues to insulin, which may provide better control of the blood glucose level to minimise the risk of damage to LDL by glycosylation;

5) ***The use of drugs – inhibitors for β-hydroxy-β-methylglutaryl-CoA reductase***: Lovastatin, Mevastatin;

6) ***The use of drug – Cholestyramine resin*** to block the reabsorption of bile acids in the small intestine. In this case the cholesterol is utilized in higher quantity up to bile acids.

7) ***The use of drugs Clofibrate an gemfibrozil*** that divert the hepatic inflow of free fatty acids into oxidation, thus decreasing the secretion of VLDL by the liver. This drugs stimulate the hydrolysis of VLDL triacylglycerols by lipoprotein lipase;

8) ***The use of drug Probucol.*** It increases the rate of LDL catabolism via receptor-independent pathways, but its antioxidant properties may be more important in preventing accumulation of oxidized LDL in arterial walls;

9) ***The use of Nicotinic acid*** to reduce the flux of fatty acids by inhibiting of adipose tissue lipolysis thereby inhibiting VLDL production by the liver.

Genetic disorders of phospholipid metabolism

Hexosaminidase A deficiency results in a group of neurodegenerative disorders caused by intralysosomal storage of the specific glycosphingolipid, GM2 ganglioside. The prototype hexosaminidase A deficiency is ***Tay-Sachs disease***, also known as the acute infantile variant. Tay-Sachs disease is characterized by progressive weakness, loss of motor skills, decreased attentiveness, and increased startle response beginning between ages three and six months with progressive evidence of neurodegeneration including: seizures, blindness, spasticity, eventual total incapacitation, and death, usually before age four years. The juvenile (subacute), chronic, and adult-onset variants of hexosaminidase A deficiency have later onsets, slower progression, and more variable neurologic findings, including: progressive dystonia, spinocerebellar degeneration, motor neuron disease, and, in some individuals with adult-onset disease, a bipolar form of psychosis.

Acute infantile hexosaminidase A deficiency (Tay-Sachs disease, TSD). Affected infants generally appear to be completely normal at birth. Mild motor weakness begins between age three and six months, along with myoclonic jerks and an exaggerated startle reaction to sharp noise.

By age six to ten months, the infant fails to achieve new motor skills or even loses previously demonstrated skills. Decreasing visual attentiveness and unusual eye movements are associated with pallor of the perifoveal macula of the retina with prominence of the fovea centralis, the so-called cherry-red spot, which is seen in virtually all affected children.

After age eight to ten months, progression of the disease is rapid. Spontaneous or purposeful voluntary movements diminish, and the infant becomes

progressively less responsive. Vision deteriorates rapidly. Seizures are common by age 12 months. Subtle partial complex seizures or absence attacks typically become more frequent and more severe.

Progressive enlargement of the head typically begins by age 18 months; it results from reactive cerebral gliosis, not hydrocephalus.

Further deterioration in the second year of life results in: decerebrate posturing, difficulties in swallowing, worsening seizures, and finally an unresponsive, vegetative state. Death from bronchopneumonia usually occurs between age two and four years.

Juvenile (subacute) hexosaminidase A deficiency. Juvenile hexosaminidase A deficiency often begins with ataxia and incoordination between age two and ten years. Speech, life skills, and cognition decline. Spasticity and seizures are present by the end of the first decade of life. Loss of vision occurs much later than in the acute infantile form of the disease, and a cherry-red spot is not consistently observed. Instead, optic atrophy and retinitis pigmentosa may be seen late in the course. A vegetative state with decerebrate rigidity develops by age ten to 15 years, followed within a few years by death, usually from infection. In some cases, the disease pursues a particularly aggressive course, culminating in death in two to four years.

Chronic and adult-onset hexosaminidase A deficiency. These conditions represent a spectrum of later-onset, more slowly progressive neurodegenerative disorders, associated with low levels of residual HEX A enzyme activity. Early symptoms may range from muscle weakness to extrapyramidal findings to altered cerebellar manifestations.

In the chronic form, central nervous system involvement is widespread, although certain neurologic findings may predominate over others. Psychomotor regression may be less prominent. The age of onset ranges from early childhood to the end of the first decade. In some individuals, extrapyramidal signs of dystonia, choreoathetosis, and ataxia may be evident. In others, cerebellar signs of dysarthria, ataxia, incoordination, and abnormalities of posture develop between

age two and ten years; mentation and verbal skills tend to be involved later in the course. The clinical presentation of the chronic form of hexosaminidase A deficiency may suggest possible diagnosis of spinocerebellar degeneration, Friedreich ataxia, or amyotrophic lateral sclerosis (ALS).

Individuals with adult-onset disease tend to show progressive muscle wasting, weakness, fasciculations, and dysarthria, indistinguishable from progressive adolescent-onset spinal muscular atrophy (Kugelberg-Welander disease) or early-onset ALS. Upper motor neuron signs, nonspecific cerebellar atrophy, and abnormalities of saccades may be present.

Cognitive dysfunction and dementia can be observed. As many as 40% of individuals have psychiatric manifestations (without dementia) including: recurrent psychotic depression, bipolar symptoms, and acute hebephrenic schizophrenia with disorganization of thought, agitation, delusions, hallucinations, and paranoia. Impairment of executive functioning and memory has also been observed.

EXERCISES FOR INDEPENDENT WORK. In the table with test tasks emphasize keywords, choose the correct answer and justify it:

№	Test:	Explanation:
1.	After intake of food rich in fats a patient feels nausea and sluggishness; with time there appeared signs of steatorrhea. Blood cholesterol concentration is 9,2 micromole/L. This condition was caused in the intestine by lack of: A. Phospholipids B. Chylomicrons C. Fatty acids D. Bile acids E. Amino acids	
2.	A patient has disturbed absorption of fat digestion products. It might have been caused by a deficit of those components in the small intestine cavity: A. Bile acids B. Bile pigments C. Lipolytic enzymes D. Sodium ions E. Lipid soluble vitamins	
3.	Examination of cell culture got from a	

№	Test:	Explanation:
	patient with lysosomal pathology revealed accumulation of great quantity of lipids in lysosomes. What of the following diseases this disturbance is typical for? A. Gout B. Phenylketonuria C. Wilson disease D. Tay-Sachs disease E. Galactosemia	
4.	A caprological survey revealed light-colored feces containing drops of neutral fat. The most likely reason for this condition of the disorder is: A. Pancreatic juice secretion B. Intestinal juice secretion C. Gastric juice acidity D. Intestinal absorption E. Bile inflow into the bowel	
5.	The patient with diabetes mellitus has been delivered in the hospital in the state of unconsciousness. Kussmaul breathing, blood pressure 80/50 mm Hg, acetone breath. Which substances accumulation in the body can be explained by the occurrence of these disorders? A. Ketone bodies B. Monosaccharides C. Cholesterol esters D. High fatty acids E. Amines	
6.	A 6 year old child was delivered to the hospital. Examination revealed that the child couldn't fix his eyes, didn't keep his eyes on toys, eye ground had the cherry-red spot sign. Laboratory analyses showed that brain, liver and spleen had high content of ganglioside glycometide. What congenital disease is found in kid? A. Tay-Sachs disease B. Wilson's syndrome C. Turner's syndrome D. Niemann-Pick disease E. MacArdle disease	
7.	A 67-year-old male patient consumes eggs, pork fat, butter, milk and meat. Blood tests results: cholesterol – 12.3 mmol/l, total lipids – 8.2 g/l, increased low-density lipoprotein fraction (LDL). What type of hyperlipoproteinemia is observed in the	

№	Test:	Explanation:
	patient? A. Hyporlipoproteinemia type I. B. Hyperlipoproteinemia type IV C. Cholesterol, hyperlipoproteinemia D. Hyperlipoproteinemia type IIa E. Hyperlipoproteinemia type IIb	
8.	Feces of the patient contain high amount of undigested fats and have grayish-white color. Specify the cause of this phenomenon: A. Hypovitaminosis B. Obturation of bile duct C. Irritation of intestinal epithelium D. Enteritis E. Hypoactivation of pepsin by hydrochloric acid	
9.	A drycleaner's worker has been found to have hepatic steatosis. This pathology may be caused by the disruption of synthesis of the following substance: A. Phosphatidylcholine B. Cholic acid C. Tristearateglycerol D. Urea E. Phosphatidic acid	
10.	Increased HDL levels decrease the risk of atherosclerosis. What is the mechanism of HDL anti-atherogenic action? A. They activate the conversion of cholesterol to bile acids B. They are involved in the breakdown of cholesterol C. They promote absorption of cholesterol in the intestine D. They supply tissues with cholesterol E. They remove cholesterol from tissues	
11.	Point out the disorder of fat metabolism which occurs in adipose tissue: A. Ketosis B. Retention hyperlipidemia C. Fatty liver D. Steatorrhea E. Obesity	
12.	The patient has elevation of LDL and VLDL levels in blood plasma. Name the pathology which is characterized by these changes: A. Osteoarthritis	

№	Test:	Explanation:
	B. Gout C. Gastritis D. Atherosclerosis E. Leukemia	
13.	The patient has elevation of chylomicrons level in blood plasma, especially after eating fatty meals. Hyperproteinemia type I is observed, which is associated with deficiency of the enzyme: A. Lipoprotein lipase B. Prostaglandin-endoperoxide synthase C. Adenylate cyclase D. Protein kinase E. Phospholipase C	
14.	Examination of the patient has revealed elevation levels of LDL in the blood serum. Name the disease which is characterized by these changes: A. Glomerulonephritis B. Acute pancreatitis C. Gastritis D. Atherosclerosis E. Pneumonia	
15.	Colloidal properties of bile are violated in the gallbladder during inflammation process. This can lead to the formation of gallstones. Name the compound which crystallization leads to the formation of gallstones: A. Cholesterol B. Oxalates C. Hemoglobin D. Albumin E. Urates	
16.	Drugs of different groups are used for the treatment and prevention of atherosclerosis. The cholesterol-lowering drugs of lipid nature include: A. Polyunsaturated fatty acids B. Pravastatin (Pravachol), Rosuvastatin (Crestor) C. Allopurinol D. Aspirin E. Heparin	
17.	Examination of the patient has revealed fat malabsorption. Point out what substance deficiency in the intestine may cause this: A. Bile acids B. Cholesterol	

№	Test:	Explanation:
	C. Bicarbonates D. Bile pigments E. Lecithins	
18.	A man has symptoms of atherosclerotic lesions of the cardiovascular system. Select the index of the biochemical analysis of blood which is increased: A. Concentration of LDL B. Concentration of chylomicrons C. The activity of pancreatic lipase D. The activity of LDH5 E. Concentration of HDL	
19.	The patient suffers from cerebral atherosclerosis. A blood test revealed hyperlipoproteinemia. Name the class of lipoproteins which concentration is most probable increased: A. Complexes of globulins with steroid hormones B. Complexes of fatty acids with albumins C. HDL D. LDL E. Chylomicrons	
20.	The healthy adult person has to care Cholesterol levels in the blood plasma in some regions to prevent the development of atherosclerosis of blood vessels. Find out these regions: A. 2.97-5.5 mmole/l B. 1.55-2.97 mmole/l C. 5.46-8.96 mmole/l D. 2.4-5.5 mmole/l E. 4.22-6.11 mmole/l	
21.	Some metabolic pathways for cholesterol in human organism are related to catabolic one. Find out them: A. Bile acids production B. Glucocorticoids production C. Mineral corticoids production D. Vitamin D_3 and calcitriol formation E. Any one represented in the list	
22.	The dietary cholesterol level is a factor to block cholesterol synthesis in the liver. It is possible to prevent this blockage if Cholesterol percentage in total food lipids will be not more then: A. 0.05 % B. 2 % C. 5 %	

№	Test:	Explanation:
	D. 10 % E. 20 %	
23.	HMG-reductase becomes active in liver cell, if: A. cAMP levels are increased B. Ratio Insulin/Glucagon<1 C. It is dephosphorylated D. Reductase kinase is active E. Mevalonic acid is accumulated	
24.	The most unsaturated and the longest intermediate metabolite of cholesterol synthesis is: A. Farnesyl pyrophosphate B. Mevalonate C. Isopentenyl pyrophosphate D. Squalene E. Geranyl pyrophosphate	
25.	Steroidogenous tissues are those one where cholesterol synthesis and its transformation occur mostly. Find out those tissues: A. Aorta endothelial tissue B. Liver C. Adrenal cortex D. Gonads E. Any one represented in the list	
26.	The primary reason of atherosclerosis development in human person may be associated with: A. Any one represented in the list B. Deficiency of apoprotein B100-receptors in liver cell C. Genetic decrease of endothelial Lipoprotein lipase activity D. Deficiency of lipoprotein lipase linked with VLDL E. Deficiency of Lecithin Cholesterol Acyltransferase	
27.	The secondary reason of atherosclerosis development in human person may be associated with: A. Arthritis B. Diabetes mellitus subtype II C. Scurvy D. Viral hepatitis E. Gout	
28.	This coenzyme is the most frequently used in transformations of cholesterol and its synthesis, too. Name it: A. FAD	

№	Test:	Explanation:
	B. TPP C. NADPH D. NAD^+ E. Carboxybiotin	
29.	Name non-protein compounds produced from free cholesterol in the liver, only: A. VLDL B. Cholecalciferol & Cholesterol esters C. Estradiol & Testosterone D. Bile acids & Cholesterol esters E. LDL	

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ANSWERS TO TESTS TASKS:

Classification, physicochemical properties and functions of simple proteins in humans. The methods for indication, separation and release of proteins from biological fluids

1	2	3	4	5	6	7	8	9	10
C	C	D	B	D	A	C	A	A	A
11	12	13	14	15	16	17	18		
A	A	D	C	E	A	E	E		

Conjugated proteins. the methods of allocation and quantitative determination of proteins in biological fluids

1	2	3	4	5	6	7	8	9	10
D	E	E	A	A	D	A	B	C	E
11	12	13	14	15	16	17	18	19	
E	A	C	A	A	D	D	E	C	

Enzymes: Structure and physicochemical properties. Classification and nomenclature of enzymes.

1	2	3	4	5	6	7	8	9	10
A	B	B	E	A	A	A	B	C	C
11	12	13	14	15	16	17	18	19	20
C	D	E	A	D	A	E	A	B	A
21	22	23	24	25	26	27	28	29	30/31
A	C	A	E	A	A	B	B	D	A/A

The mechanism of action and kinetic properties of enzymes. The regulation of enzymatic activity.

1	2	3	4	5	6	7	8	9	10
A	B	C	B	B	A	C	B	C	A
11	12	13	14	15	16	17	18	19	
A	A	A	A	C	B	E	A	E	

Principles of enzyme activity determination. Genetic deficiency of enzymes. Medical enzymology.

1	2	3	4	5	6	7	8	9	10
B	E	A	B	A	A	A	E	A	C
11	12	13	14	15	16	17	18	19	20
A	C	A	A	B	A	B	A	E	A
21	22	23	24	25	26	27	28	29	30
A	A	E	A	E	B	C	B	A	D
31	32	33	34	35	36	37	38	39/40	41/42
E	A	A	A	A	E	D	A	A/A	E/A

Common regularities of metabolism. Anabolic and catabolic processes in humans. Krebs Cycle.

1	2	3	4	5	6	7	8	9	10
B	A	A	A	E	A	C	C	B	E
11	12	13	14	15	16	17	18	19	20
A	C	B	C	B	A	D	B	A	B
21	22	23	24	25	26	27	28	29	30
A	C	E	C	C	B	A	C	D	D

General bases of bioenergetics

1	2	3	4	5	6	7	8	9	10
A	B	C	D	B	B	A	E	D	A
11	12	13	14	15	16	17	18	19	20
C	C	E	A	E	A	B	A	E	C
21	22	23	24	25	26	27	28	29	30/31
E	A	D	A	A	E	D	D	E	D/E

Anaerobic oxidation of glucose – glycolysis. Synthesis of glucose – gluconeogenesis

1	2	3	4	5	6	7	8	9	10
A	D	E	E	C	A	E	C	B	C
11	12	13	14	15	16	17	18	19	20
A	B	B	A	A	C	D	B	C	E
21	22	23	24	25	26	27	28	29	30
C	D	A	A	E	D	A	C	C	D

Aerobic oxidation of monosaccharides

1	2	3	4	5	6	7	8	9	10
E	C	B	B	C	C	E	B	A	B
11	12	13	14	15	16	17	18	19	20
D	B	A	D	E	B	B	D	A	C
21	22	23	24	25	26	27	28	29	30
D	D	C	E	A	C	C	C	A	A

Metabolism of polysaccharides. The regulation and disorders of carbohydrate metabolism

1	2	3	4	5	6	7	8	9	10
A	D	E	C	C	D	E	A	E	B
11	12	13	14	15	16	17	18	19	20
B	A	C	A	A	E	B	B	A	D
21	22	23	24	25	26	27	28	29	30
B	A	A	D	A	E	A	D	E	C

Lipoproteins of blood plasma. Metabolism of triacylglycerols and of glycerophospholipids

1	2	3	4	5	6	7	8	9	10
C	A	A	C	D	B	B	E	A	D
11	12	13	14	15	16	17	18	19	20
A	B	A	B	C	B	D	E	A	C
21	22	23	24	25	26	27	28	29	30
D	A	B	A	D	B	A	C	E	D

High fatty acids and ketone bodies metabolism

1	2	3	4	5	6	7	8	9	10
C	B	B	A	A	A	A	C	D	C
11	12	13	14	15	16	17	18	19	20
E	E	B	A	C	C	D	C	B	E
21	22	23	24	25	26	27	28	29	
C	B	C	C	A	C	A	B	E	

Cholesterol metabolism. The regulation and disorders of lipids metabolism: obesity, atherosclerosis

1	2	3	4	5	6	7	8	9	10
D	A	D	E	A	A	E	B	A	E
11	12	13	14	15	16	17	18	19	20
E	D	A	D	A	A	A	A	D	A
21	22	23	24	25	26	27	28	29	
E	A	C	D	A	A	B	C	B	