MINISTRY OF HEALTH OF UKRAINE
ODESA NATIONAL MEDICAL UNIVERSITY
Department of Medical Biology and Chemistry

CONFIRMED by
Vice-rector for scientific and pedagogical work
Eduard BURIACHKIVSKYI
"___" _____ 2024

METHODOLOGICAL DEVELOPMENT TO THE LECTURES ON THE EDUCATIONAL DISCIPLINE

Faculty, course	**International faculty, 1st, 2d year**
Specialty	**222 "Medicine"**
Academic discipline	**Biological and bioorganic chemistry**

The program was approved:
Meeting of the Department of Medical Biology and Chemistry
Odesa National Medical University
Protocol № 1 dated 26 august 2024
Head of Department _____ Hennadii STEPANOV
(signature)

Developers:
Associate Professor, Head of the Department, Ph.D., Stepanov H. F.
Associate professor of the department, Ph.D., Burdina Y.F.
Associate Professor, Ph.D., Storchylo O.V.
Associate professor, Ph.D., Assoc. Shirykalova A.O.
Associate professor, Ph.D. Grekova A.V.
Senior lecturer Kostina A.A.
Senior lecturer, Ph.D. Vasylieva A.G.
assistant, Gridina I.R.

Lecture № 1

"Lipids, phospholipids, steroids: properties and biological role. Characteristics of phospholipids as functional components of biomembranes"

Actuality of theme: Lipids, along with other organic compounds, are the structural basis of all living organisms and play an important role in life processes. Lipids have plastic, energetic, regulatory and protective functions. Fats are an obligatory component of food. Knowledge of the structure and chemical properties of lipids is necessary for further study of their biological functions in the courses of biological chemistry, physiology. Violation of lipid metabolism affects the general state of the body. Knowledge of the general properties of lipids (saponifiable and unsaponifiable) will allow future doctors to understand the pathogenesis of diseases, competently diagnose it and solve the issues of medical therapy.

Aims: to form a systematic knowledge of the structure, chemical properties and biological role of simple saponifiable lipids - triacylglycerols and their structural components as a chemical basis for studying the structure of biological membranes and lipid exchange processes.

Basic concepts: lipids, triacylglycerols, structure of biological membrane, lipid exchange processes, saponifiable and unsaponifiable lipids.

Plan and organizational structure of the lecture:

1. The main structural components of lipids.
2. Classification of lipids and their biological functions.
3. Features of the structure of the structural components of saponifiable lipids.
4. Chemical properties of lipids.
5. Fats, oils, waxes. Their consistency and chemical properties.
6. Analytical characteristics of fats.
7. Surface-active properties of lipids, diphilic structure of their molecules.

Content of lecture material (lecture text)

Lipids are fat-like substances that are part of living organisms, poorly soluble in water and well soluble in non-polar organic solvents. Under this name, substances that are extracted from plant and animal tissues by extraction with non-polar organic solvents are combined different in chemical structure and biological functions.

Lipid classification

LIPIDS subdivided into

- simple (triglycerides, waxes)
- complex (phospholipids, sphingolipids, lipoproteins, glycolipids)
- steroids (and their derivatives)
- isoprenoids (vitamin A, carotene, lycopene, terpenes)
- a group of others (diacylglycerols, higher carboxylic acids, etc.)

Depending on the ability to hydrolysis with the formation of salts of higher fatty acids (soaps), lipids are divided into saponifiable and unsaponifiable ones.

Saponifiable Lipids

Saponifiable lipids consist of two or more structural components into which they break down upon hydrolysis by acids, alkalis, or lipase enzymes.

Classification and basic structural components.

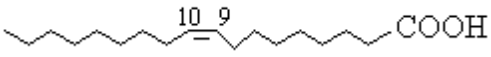
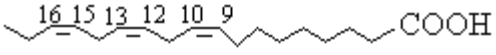
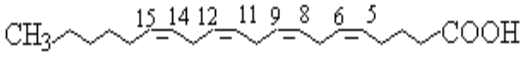
The main structural components of saponifiable lipids are alcohols and higher fatty acids. Saponifiable lipids of a more complex structure may contain residues of phosphoric acid, amino alcohols, as well as residues of mono- and oligosaccharides.

Higher fatty acids are saturated or unsaturated carboxylic acids isolated from fats by hydrolysis. Their structure is characterized by the following main features:

- have an unbranched structure with an even number of carbon atoms from C_4 to C_{80}, but most often there are acids of the composition C_{16}, C_{18} and C_{20};
- unsaturated acids, as a rule, contain a double bond at position 9;
- if there are several double bonds, then they are separated by a CH_2 group;
- double bonds in unsaturated acids have a cis configuration.

Table 1

Essential fatty acids in lipids

Title	The number of C atoms	Formula	Structure
Saturated			
Oil	C_4	C_3H_7COOH	$CH_3(CH_2)_2COOH$
Caproic	C_6	$C_5H_{11}COOH$	$CH_3(CH_2)_4COOH$
Caprylic	C_8	$C_7H_{15}COOH$	$CH_3(CH_2)_6COOH$
Capric	C_{10}	$C_9H_{19}COOH$	$CH_3(CH_2)_8COOH$
Lauric	C_{12}	$C_{11}H_{23}COOH$	$CH_3(CH_2)_{10}COOH$
Myristic	C_{14}	$C_{13}H_{27}COOH$	$CH_3(CH_2)_{12}COOH$
Palmitic	C_{16}	$C_{15}H_{31}COOH$	$CH_3(CH_2)_{14}COOH$
Stearic	C_{18}	$C_{17}H_{35}COOH$	$CH_3(CH_2)_{16}COOH$
Arachinic	C_{20}	$C_{19}H_{39}COOH$	$CH_3(CH_2)_{18}COOH$
Unsaturated			
Oleic	C_{18}	$C_{17}H_{33}COOH$	
Linoleic	C_{18}	$C_{17}H_{31}COOH$	
Linolenic	C_{18}	$C_{17}H_{29}COOH$	
Arachidonic	C_{20}	$C_{19}H_{31}COOH$	

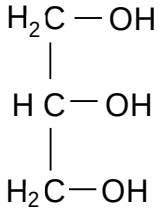
According to the position of the double bond relative to the last carbon atom, polyunsaturated fatty acids are divided by

• ω-6 fatty acids – linoleic (C18:2; 9,12), γ-linolenic (C18:3; 6,9,12), arachidonic (C20:4; 5,8,11,14). These acids form vitamin F, and are found in vegetable oils.

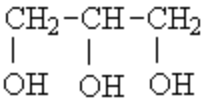
• ω-3-fatty acids – α-linolenic (C18:3; 9,12,15), timnodonic (eicosopentaenoic, C20:5; 5,8,11,14,17), clupanodonic (decosopentaenoic, C22:5; 7, 10,13,16,19), cervonic (decosohexaenoic, C22:6; 4,7,10,13,16,19). The most significant source of acids of this group is the fat of fish from the cold seas. An exception is α-linolenic acid, which is found in hemp, linseed, and corn oils.

Unsaturated fatty acids (linoleic, linolenic, arachidonic) are indispensable and enter the human body mainly with vegetable oils. Saturated fatty acids are synthesized in the body from acetic acid enzymatically.

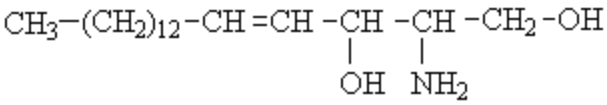
In the composition of lipids, higher fatty acids are linked by ester or amide bonds with alcohols, the most important of which are the trihydric alcohol glycerol and the amino alcohol sphingosine.



Glycerol

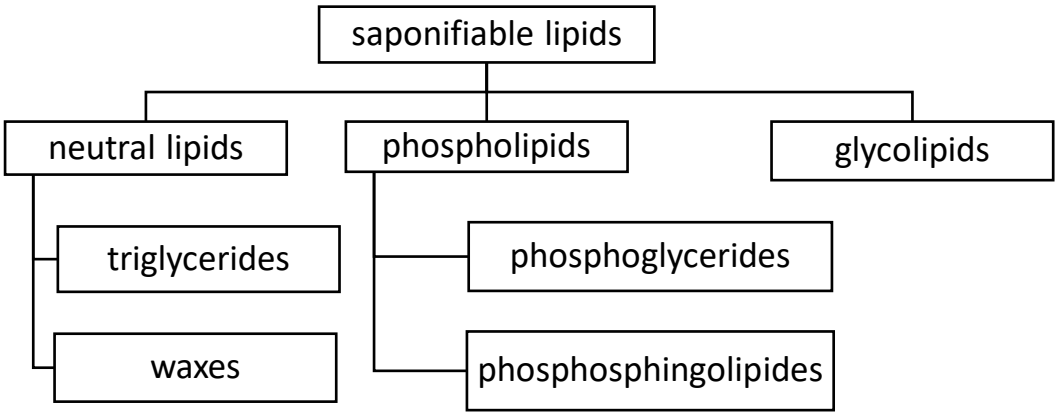


glycerol



sphingosine

In accordance with their chemical structure and biological functions, three main groups of saponifiable lipids are distinguished: neutral lipids, phospholipids and glycolipids.



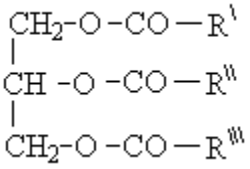
1.2. Neutral lipids

Neutral lipids are esters of higher fatty acids and alcohols (higher monatomic, glycerol, cholesterol, etc.). The most important of these are triacylglycerides and waxes.

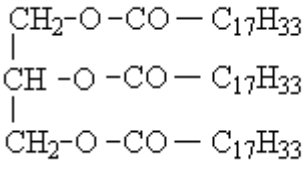
Triacylglycerides

Triacylglycerides are esters of glycerol and higher fatty acids.

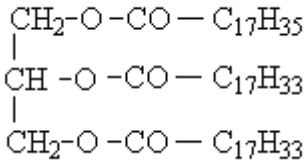
General formula:



Simple triacylglycerides contain identical, mixed residues of different fatty acids. The names of triacylglycerides are based on the names of the acyl residues that make up their fatty acids.

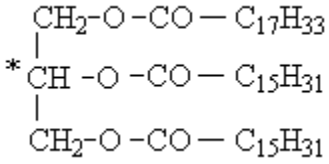


trioleinoglycerol



distearoyl-2-oleinoglycerol

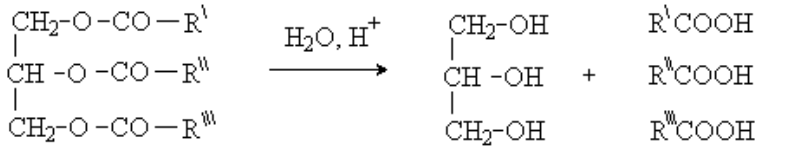
Mixed triacylglycerides may contain a chiral carbon atom in position 2 and have enantiomers, for example:

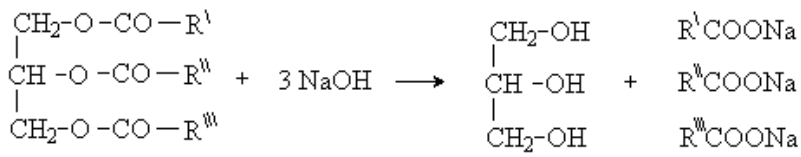


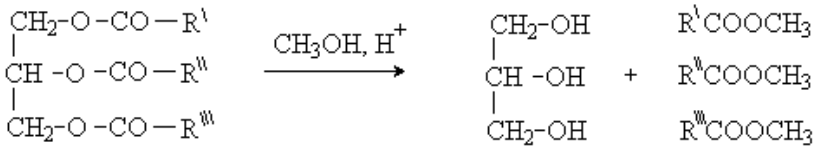
Triacylglycerides are low-polar, water-insoluble substances, since their molecules do not contain highly polar or charged groups. Triacylglycerides, containing predominantly unsaturated acid residues, are, under ordinary conditions, liquids, triglycerides of saturated acids are solids. They are part of animal fats and vegetable oils, which are mixtures of triacylglycerides. Animal fats contain mainly triacylglycerides with saturated acid residues and therefore have a solid consistence. Vegetable oils comprise mainly unsaturated acid residues and are liquids.

Solid fats, when the temperature rises, become liquid within a certain temperature range, as they consist of a mixture of various triglycerides.

The chemical properties of triacylglycerides are determined by the presence of an ester bond and unsaturation. As esters, triacylglycerides are hydrolyzed by acids and alkalis, and also enter into a transesterification reaction.

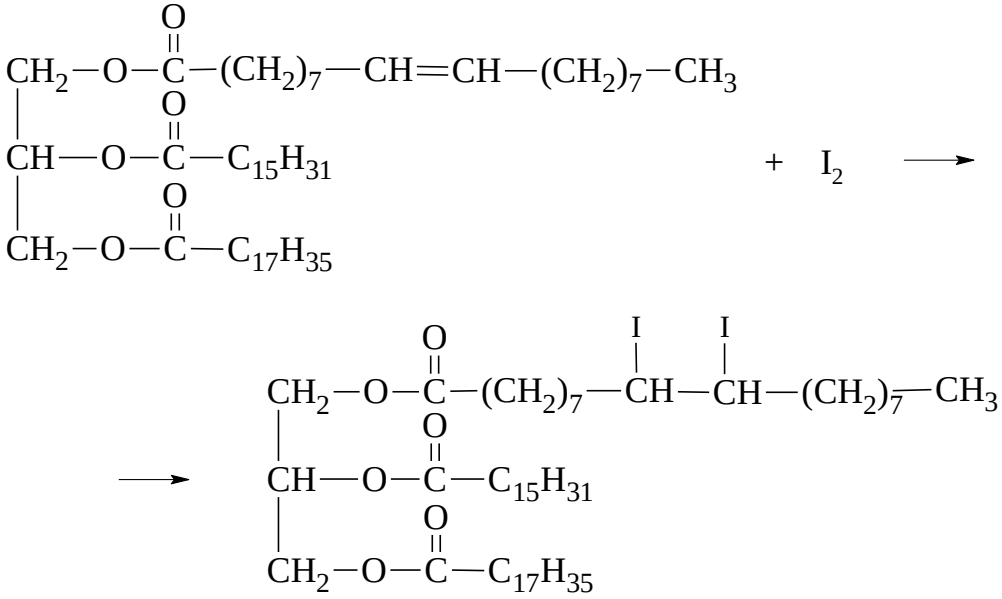






With alkaline hydrolysis (saponification) of fats, salts of fatty acids (soaps) are formed. Their molecules are diphilic (they contain a polar “head” and a non-polar “tail”), which causes their surface-active properties and a washing effect.

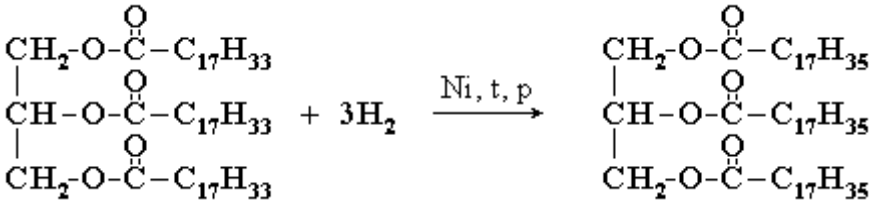
Triacylglycerides containing unsaturated fatty acid residues enter into double bond addition reactions.



The halogen addition reaction is used to determine the content of unsaturated acid residues in fats. A quantitative characteristic of the degree of fat unsaturation is the iodine number — the amount of iodine (in g) that 100 g of fat can absorb.

The higher the iodine number, the more unsaturated acids are part of the fat (oil). For solid fats, the iodine number is 35 - 85, for oils - 150 - 200. So, the iodine number of butter is 36, pork fat is 59, human fat is 64, corn oil is 121, sunflower oil is 145, linseed oil is 179 .

An important industrial process is the hydrogenation of fats – the catalytic hydrogenation of vegetable oils, as a result of which hydrogen saturates double bonds, and liquid oils turn into solid fats (margarine).



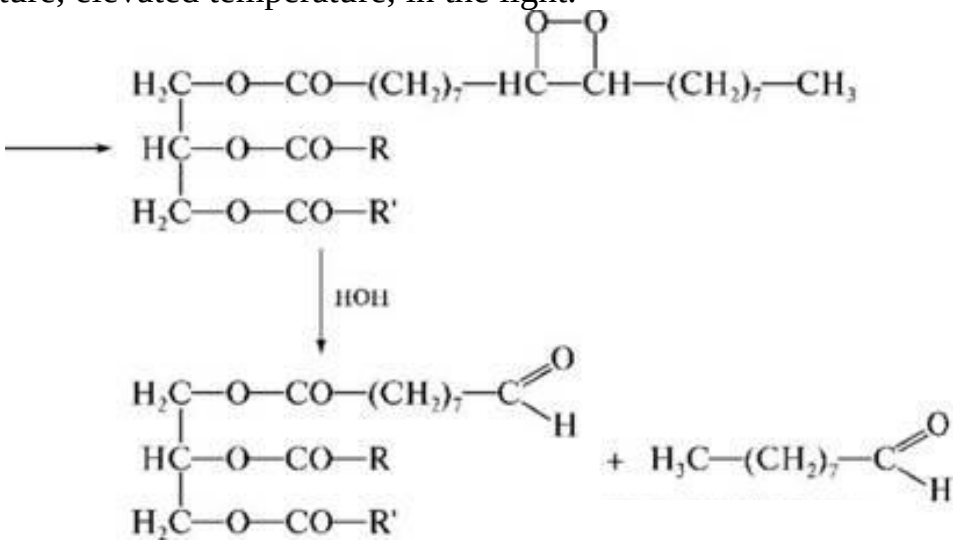
glycerol trioleate glycerol triostearate

Vegetable oils have the highest nutritional value, which, along with essential fatty acids, contain the necessary phospholipids, vitamins, useful phytosterols (vitamin D precursors) for the body and practically do not contain cholesterol.

Most fats in the air rancid – they get an unpleasant taste and smell. Rancidity occurs due to the oxidation of unsaturated higher fatty acids and is accompanied by hydrolysis.

There are two types of rancidity – hydrolytic and oxidative. Free fatty acids, such as butyric, aldehydes and short-chain ketones, which also have an unpleasant odor

and taste, can also form. The rancidity process is accelerated in the presence of moisture, elevated temperature, in the light.



pelargonic aldehyde

In the human body, lipid peroxidation is initiated by the H-O • and H-O-O • radicals or hydrogen peroxide H_2O_2. Their number increases sharply with the absorption of a dose of ionizing radiation. The methylene group located in the vicinity of the double bond of the hydrocarbon fragment of the acid residue is subjected to free radical attack.

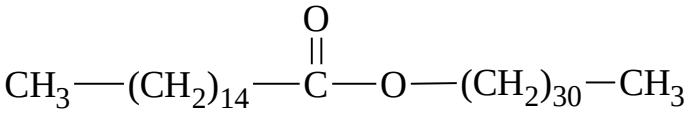
The regioselectivity of this process is explained by the formation of an allyl type radical, which is stabilized by the conjugation of an unpaired electron with double-bond electrons. The final oxidation products are short chain organic acids. They have an unpleasant smell of sweat or rancid oil.

Lipid peroxidation is one of the most important oxidative processes in the human body and is the main cause of damage to cell membranes.

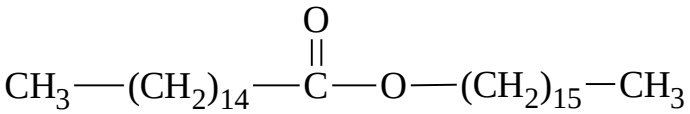
In cells under normal conditions, the self-oxidation of unsaturated fats is completely inhibited due to the presence of vitamin E, various enzymes, as well as ascorbic acid.

Waxes are esters of fatty acids and higher monohydric alcohols (C12 - C46). Waxes are part of the protective coating of plant leaves and the skin of humans and animals. They give the surface a characteristic shine and water-repellent properties, which is important for maintaining water inside the body and creating a barrier between the body and the environment.

Beeswax



myricyl palmitate



cetyl palmitate (Spermaceti)

1.3. Phospholipids

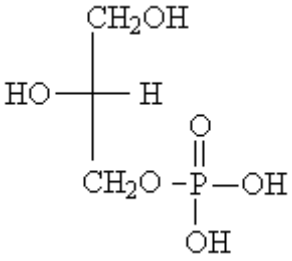
Phospholipids are complex lipids and are the main components of cell membranes in which lipid bilayers form. Alcohols are present in the PL composition: aminoethanol, choline, amino acids serine, threonine, polyhydric cyclic alcohol inositol.

The polar structure (hydrophilic) and nonpolar (hydrophobic) are distinguished in the PL structure. The presence of two sites with opposite physicochemical properties is called amphiphilic. The residue of phosphoric acid is always ionized.

PL possess surfactant properties, form film layers well at the separation of two phases, are active emulsifiers, and easily form complexes with proteins. These complexes are called lipoproteins (LP). If arachidonic acid is present in the PL, then such a phospholipid, being in the cell membrane, takes part in the formation of intracellular regulators: prostaglandins, thromboxanes, which are formed from arachidonic acid.

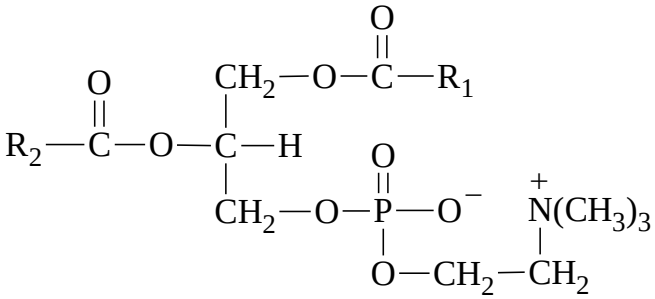
Phosphoglycerides

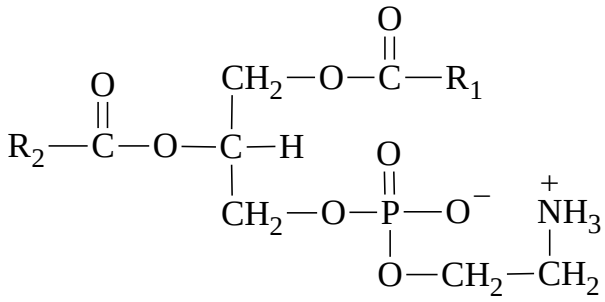
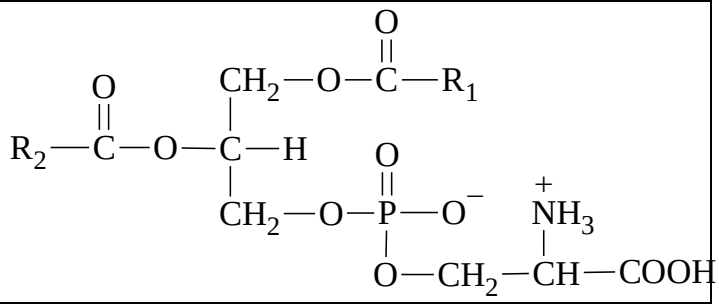
The main structural components that make up the phosphoglyceride molecule are glycerin, fatty acids, phosphoric acid, amino alcohols (ethanolamine or choline) or the amino acid serine. They are considered as derivatives of L-glycero-3-phosphate



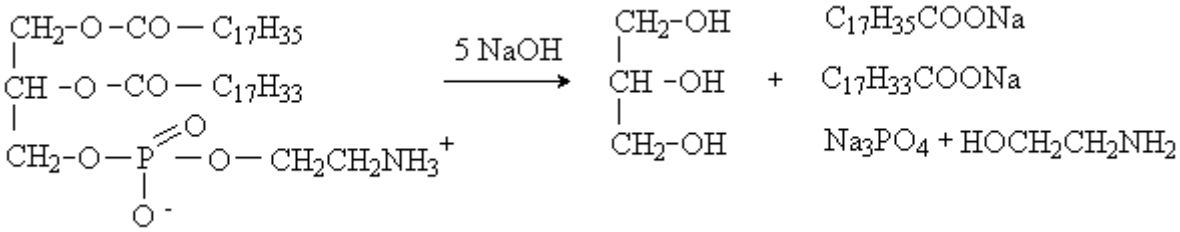
in which the alcohol groups are esterified with fatty acids and the residue of the phosphoric acid forms an ester bond with the amino alcohol. The general formula of phosphoglycerides:

Depending on the structure of the amino alcohol, phosphatides are divided into **phosphatidylcholines, phosphatidylethanolamines and phosphatidylserines.**

Phosphatide	Amino alcohol	Formula
Phosphatidyl choline (Lecithins)	Choline	

Phosphatidyl Ethanolamines	Ethanolamine	
Phosphatidyl serine	Serine	

When heated in acidic and alkaline medium, phosphoglycerides are hydrolyzed, breaking up into the main structural components.

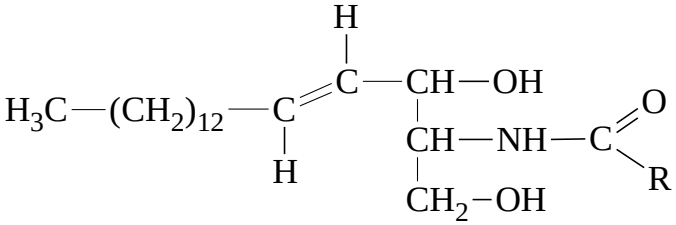


Sphingolipids

Sphingolipids are structural analogues of glycerophospholipids, in which instead of glycerol an unsaturated diatomic amino alcohol is present.

Ceramides

Ceramides are N-acylated derivatives of sphingosine. The general structure of ceramides is as follows:

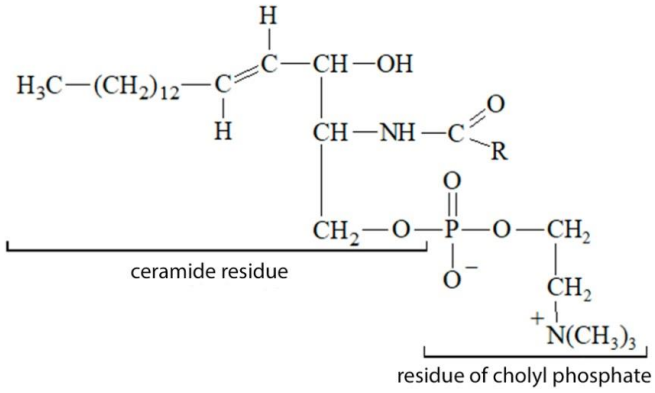


Ceramides are found in nature both in free form (in lipids of the liver, spleen, red blood cells), and as part of sphingolipids.

Sphingomyelins

Other sphingolipids can also be considered as derivatives of ceramides. The main group of sphingolipids consists of **sphingomyelins** ceramide derivatives in which the primary hydroxyl group of ceramide is esterified with phosphoric acid containing a choline residue.

The general structure of sphingomyelins can be represented as follows:



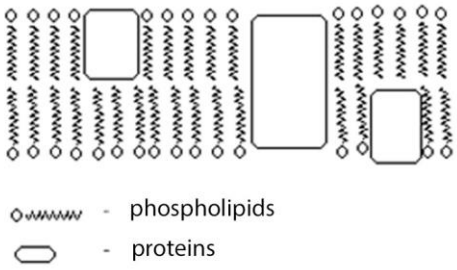
where R is the CO- residue of palmitic, stearic, lignoceric or nervonic acid.

Sphingolipids are found in cell membranes of microorganisms, plants, viruses, insects, fish and higher animals.

Phospholipid molecules are diphilic. They contain a polar hydrophilic “head” and a non-polar hydrophobic “tail”. In the aquatic environment, they are able to form spherical micelles - liposomes, which can be considered as a model of cell membranes.

Phospholipids are the main structural components of cell membranes. According to the liquid-mosaic model, cell membranes are considered as lipid bilayers. In such a bilayer, the hydrocarbon radicals of phospholipids due to hydrophobic interactions are inside, and the polar groups of lipids are located on the outer surface of the bilayer. Protein molecules are embedded in the liquid lipid bilayer.

liposome structure

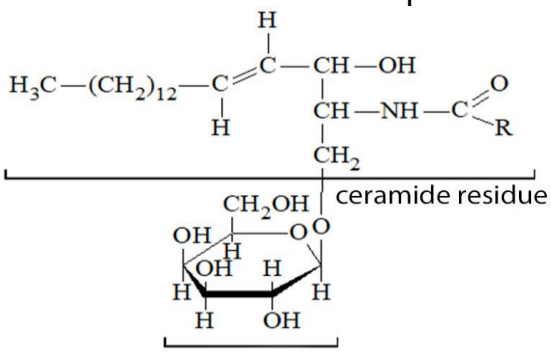


cell membrane structure

1.4. Glycolipids

Among glycosphingolipids, **cerebrosides and gangliosides** are most common.

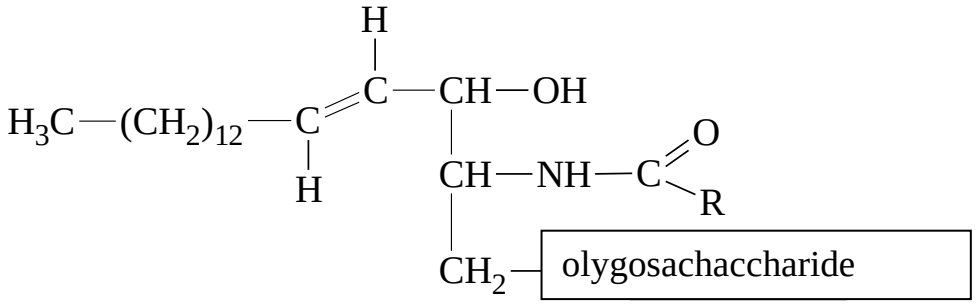
The general structure of cerebrosides can be represented as follows:



β-D galactopyranose residue

Cerebrosides are found mainly in myelin sheaths and membranes of brain nerve cells.–In addition to sphingosine, they contain higher acids (cerebric, nerve, lignoceric), as well as residues of hexoses glucose or galactose.

The structure of gangliosides is similar to cerebrosides, but at the same time they have a more complex and diverse composition. In addition to these components, they contain neuraminic acid and heteropolysaccharides:



The gray matter of the brain is richest in gangliosides.

Lipoproteins are lipid molecules bound to proteins. There are a lot of them in the membranes, proteins can penetrate the membrane through, located under or above the membrane, can be immersed in a lipid bilayer at various depths.

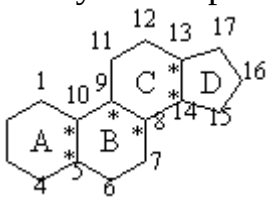
Lipid function

Function	Examples and explanations
Energy	The main function of triglycerides. When splitting 1 g of lipids, 38.9 kJ are released
Structural	Phospholipids, glycolipids and lipoproteins are involved in the formation of cell membranes.
Reserve	Fats and oils are a reserve nutrient in animals and plants. It is important for animals hibernating during the cold season or making long transitions through the area where there are no power sources. Plant seed oils are essential for providing energy for seedling.
Protective	The layers of fat and fatty capsules provide amortization of the internal organs. Layers of wax are used as a water-repellent coating in plants and animals.
Heat insulating	Subcutaneous fatty tissue prevents the outflow of heat into the surrounding space. Important for aquatic mammals or mammals living in cold climates.
Metabolic water source	When 1 kg of fat is oxidized, 1.1 kg of water is released. Important for desert dwellers.

Catalytic	Fat-soluble vitamins A, D, E, K are cofactors of enzymes, i.e., these vitamins themselves do not have catalytic activity, but enzymes cannot perform their functions without them.

2.2 Steroids

Steroids are natural biologically active compounds whose structure is based on the hydrocarbon steran. Like terpenes, steroids belong to isoprenoids and are associated with them by common biosynthesis pathways.

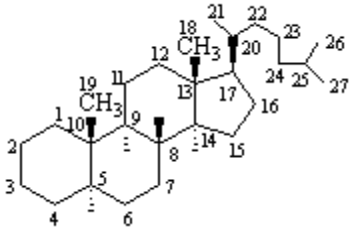


steran

Three main groups of steroids are distinguished depending on the substituents: sterols, bile acids and steroid hormones.

Sterols

Sterols are natural alcohols of a number of steroids, the basis of the carbon skeleton of which is cholestane hydrocarbon.



cholestan

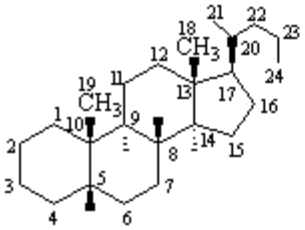
Sterols are present in all tissues of animals and plants. They are intermediate products in the biosynthesis of bile acids and steroid hormones. Examples of animal steroids are cholestanol and cholesterol.

Cholesterol is the most common sterol in animals and humans. It is present in all animal lipids, blood and bile. The brain contains 7% cholesterol per dry weight. Violation of the exchange of cholesterol leads to its deposition on the walls of arteries and atherosclerosis, as well as to the formation of gallstones.

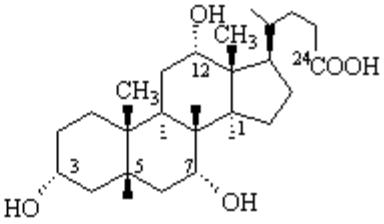
* Contrary to popular belief, cholesterol is not an “enemy of the human race,” but serves as the basis for the synthesis of steroid hormones. In general, cholesterol is a rather important and moderately harmless metabolite, which plays an important role in ensuring the barrier function of cell membranes. In addition, it protects cell membranes from electrical breakdown and prevents the autooxidation of membrane lipids. A lack of cholesterol (hypocholesterolemia) can result in an increased risk of tumor and viral diseases. However, an excess of cholesterol (hypercholesterolemia) makes it difficult to transfer calcium ions and its concentration in the cytoplasm increases. This leads to increased cell division and the development of atherosclerosis.

Bile acids

Bile acids are hydroxycarboxylic acids of a number of steroids. The basis of the structure of bile acids is cholane hydrocarbon.



cholan

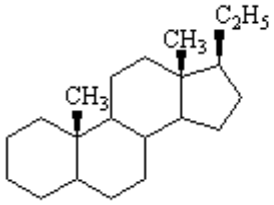


cholic acid

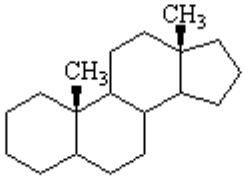
Bile acids are formed in the liver from cholesterol. Sodium and potassium salts of bile acids are surfactants. Emulsifying fats, they contribute to their absorption and digestion.

Steroid hormones

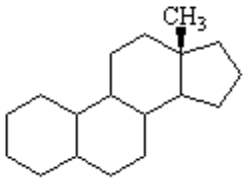
Steroid hormones are physiologically active substances of a number of steroids produced by the endocrine glands. According to the chemical structure and biological effect, hormones of the adrenal cortex (corticosteroids), male sex hormones (androgens) and female sex hormones (gestagens and estrogens) are distinguished. Each type of steroid hormone corresponds to a hydrocarbon, which forms the basis of their carbon skeleton. For corticosteroids and progestogens, this is pregnan, androgen - androstane, estrogen - estran.



Pregnan

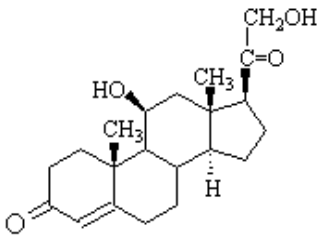


androstane

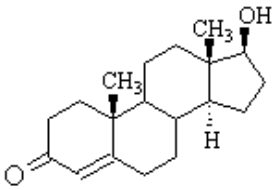


estran

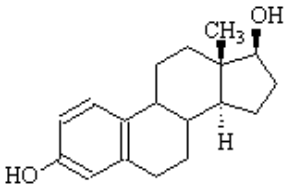
The figure shows examples of some steroid hormones produced by different endocrine glands.



corticosterone



testosterone



estradiol

Corticosterone is a hormone of the adrenal cortex, regulates carbohydrate metabolism, acts as an insulin antagonist, increasing blood sugar. Testosterone is a male sex hormone that stimulates the development of secondary sexual characteristics. Estradiol is a female sex hormone that controls the menstrual cycle.

General material and educational and methodological support of the lecture:

- Working program of the discipline
- Silabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational tasks

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.

2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.

3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.

4. Construction features, chemical properties and the biological role of carbohydrates. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova, K.V. Bevziuk. Teaching aid. Odesa, 2017. – 44 p.

5. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

6. Lipids: classification, structural features, properties and biological role. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova. Teaching aid. Odesa, 2017. – p. 32.

Additional:

7. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.

8. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.

9. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.

10. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.

11. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.

12. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 2

“Structure and chemical properties of carbohydrates”

Actuality of theme: Carbohydrates are part of the cells and tissues of all plant and animal organisms. By mass, carbohydrates make up the bulk of organic matter on Earth. In nature, they are of great importance as a source of energy in metabolic processes (in plants - starch, in animal organisms - glycogen), are structural components of plant cell walls (cellulose), bacteria (muramin) and constituents of vital substances (nucleic acids, coenzymes, vitamins). Some carbohydrates and their derivatives are used as medicines.

Polysaccharides play an important role in human life. For example, glycogen, in animal organisms, is a structural analog of starch. Strong branching from the main chain contributes to their energetic function, since only with large end residues is it

possible to ensure rapid detachment of the required number of glucose molecules. Heteropolysaccharides in the body are bound to proteins and form complex supramolecular complexes. The most important class of organic compounds found in nature. The best known are glucose, starch, cellulose, glycogen, heparin, etc., which play an important role in human and animal life processes.

Aims: To consolidate and expand knowledge of the principles of stereoisomerism, tautomeric equilibrium, and chemical properties of the most important monosaccharides involved in life processes, as well as to acquire practical skills in identifying carbohydrates. Study the most important representatives of reducing and non-reducing saccharides, their cyclo-oxo-tautomeric forms, bond type and properties.

Basic concepts: carbohydrates, classification of carbohydrates, aldoses, ketoses, monosaccharides, glucose, galactose, mannose, fructose, disaccharides, bioses, polysaccharides, heteropolysaccharides, glycogen, starch, cellulose, heparin, classification of biogenic elements.

Plan and organizational structure of the lecture:

1. Classification of monosaccharides .
2. Nomenclature of carbohydrates.
3. The phenomenon of optical isomerism, chirality. 4.
4. D- and L- stereochemical series of carbohydrates.
5. Cyclo-oxo-tautomerism of sugars.
6. Anomerism in monose series. Mutarotation phenomenon.
7. Classification of disaccharides according to their ability to redox reactions.

Types of glycosidic bonds between monosaccharide residues.

8. Structure, properties and role in structure formation of polysaccharides, their tautomeric forms.
9. Structure and properties of lactose and sucrose. Inversion of sucrose by hydrolysis.
10. Homopolysaccharides as polyglycosides.
11. Structure, biological role and application of starch, structure of amylose and amylopectin.
12. Structure and biological role of glycogen and cellulose.
13. Heteropolysaccharides.

Content of lecture material (lecture text)

Carbohydrates include heterofunctional compounds of the polyhydroxycarbonyl series and their derivatives.

Carbohydrates are part of the cells and tissues of all plant and animal organisms and, respectively, comprise 80% and 2% of the dry matter mass.

Biological functions of carbohydrates

1. Energy – carbohydrates are the main type of cell fuel. When 1 mole of glucose is burned, 3060 J of energy is released, which is consumed in endothermic biological processes, turning into heat and partially accumulating in ATP.

2. Plastic – carbohydrates are an essential component of intracellular structures and membranes of plant and animal origin. The main substance of the intercellular matrix of connective tissue is proteoglycans – high molecular weight carbohydrate-protein components.

3. Synthetic – carbohydrates participate in the synthesis of nucleic acids, are part of coenzymes, glycolipids, glycopeptides, glycoproteins.

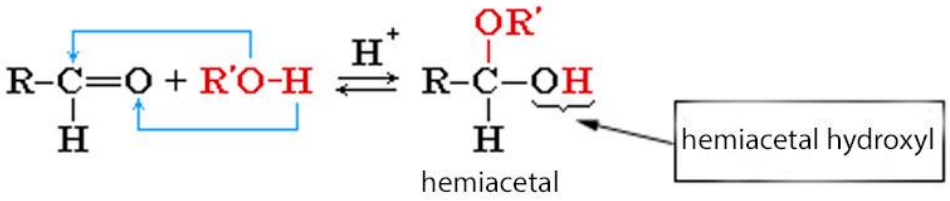
4. Protective – carbohydrates participate in maintaining the body's immunity. Thyroid-stimulating hormone controls the function and development of the thyroid gland, being a glycoprotein, i.e. a complex of carbohydrates with proteins.

5. Specific – individual carbohydrates are involved in conducting nerve impulses, the formation of antibodies, and ensuring the specificity of the blood group.

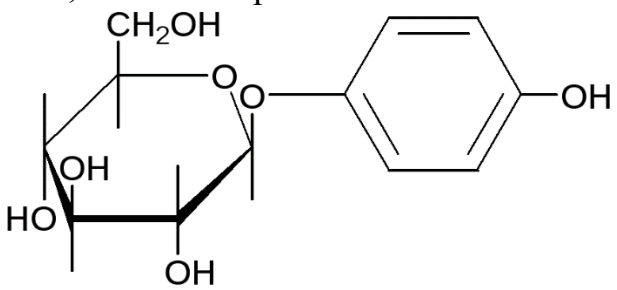
6. Regulatory – plant foods contain polysaccharide - cellulose, which improves bowel function and increases secretion in the stomach.

All of the above emphasizes the need for optimal provision of the body with carbohydrates. On average, normal – 450 g per day.

The chemical transformations of biologically active substances that are contained in the body are based on the chemical reactions of various functional groups, as well as transformations that are due to their mutual influence. The structure of natural carbohydrates, one of the main sources of energy in our body, is based on the reactions of the aldehyde group with the hydroxyl group (the formation of hemiacetals).



In the form of acetals, “side” compounds are excreted from the body:



The aldehyde react with ammonia or with substituted amines underlies the synthesis of amino acids from carbohydrate metabolism products. A similar mechanism for the interaction of acetaldehyde (the product of ethanol oxidation) with the amines of the body (biogenic amines) is the basis of the toxic effect of ethanol on the human body. The interaction of aldehydes (aldol condensation) underlies the production of polysaccharides in plant and animal cells.

Classification and nomenclature of carbohydrates

Carbohydrates are a class of organic compounds, representatives of which are in all living organisms. It was previously revealed that many compounds of this class have a molecular formula of the type Cx ($H_2$0) y. The name was proposed by the Russian chemist K. Schmidt (1844). However, further studies have proved that this definition does not cover many compounds, for example, deoxy derivatives of hexoses, etc.

The source of carbohydrates for all living organisms is photosynthesis, which is carried out by plants. Animal organisms receive monosaccharides from plant sources, and then use them, including for the synthesis of polysaccharides. The process can be shown in the form of such scheme:

$$xCO_2 + yH_2O \xrightarrow{h\nu} C_x(H_2O)_y + xO_2$$

Thus, carbohydrates are a kind of chemical depot of energy storage. This energy is released in animal organisms as a result of the metabolism of carbohydrates, which is reduced, from a chemical point of view, to their oxidation.

A small part of the released energy is converted into heat, and a large part is deposited during the synthesis of adenosine triphosphate (ATP), and then it is used in vital processes (muscle contraction, transmission of a nerve impulse, etc.).

Carbohydrates are also structural components of a number of vital substances - nucleic acids, vitamins, coenzymes.

The well-known representative of carbohydrates - glucose - is an essential component of blood and human tissues. Carbohydrates can be divided into two main groups:

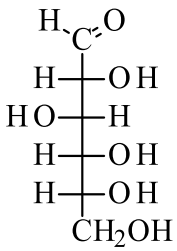
Simple carbohydrates, or simple sugars – monosaccharides, or monoses that are not able to hydrolysis.

Complex carbohydrates, or complex sugars are polysaccharides, or polyoses that can hydrolyze to simple carbohydrates. Among them, a group of relatively low molecular weight compounds (oligosaccharides) is distinguished, which during hydrolysis form from 2 to 10 monosaccharide molecules.

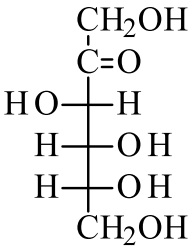
Monosaccharides are polyhydroxycarbonyl compounds. They are classified according to two characteristics: the length of the carbon chain and the nature of the oxo group.

Monosaccharides, depending on the length of the carbon chain (3-10 atoms) are divided into trioses, tetrose, pentoses, hexoses, heptoses, etc. In nature, pentoses and hexoses are most common.

Monosaccharides that contain an aldehyde group are called aldoses, and with ketone group are called ketoses (for example, hexoses).



glucose



fructose

According to the IUPAC nomenclature, any aldopentose has the name 2, 3, 4, 5-tetrahydroxypentanal, and aldohexose - 2, 3, 4, 5, 6-pentahydroxyhexanal. However, the international nomenclature in the chemistry of carbohydrates is practically not used, and they use trivial names.

Stereoisomerism of carbohydrates. Cyclo-oxo-tautomerism.

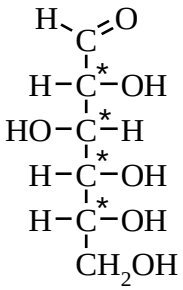
Monosaccharide molecules contain several chiral centers; therefore, several stereoisomers correspond to the same structural formula. The number of isomers is calculated by the formula: $N = 2^n$,

where N – number of isomers; n – number of chiral centers.

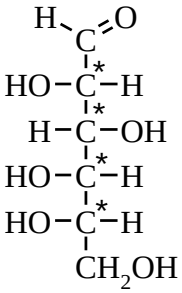
There are four chiral carbon atoms in the molecules of aldohexoses, the total number of stereoisomers according to the Fisher formula

$$N = 2^4 = 16$$

Thus, for each of the optical isomers there is one of its optical antipodes - the enantiomer, the rest - diastereomers. So, 16 aldoghexoses make up 8 pairs of antipodes that belong to the D and L rows.

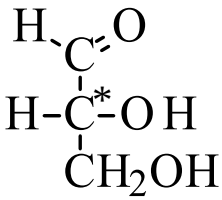


D - glucose

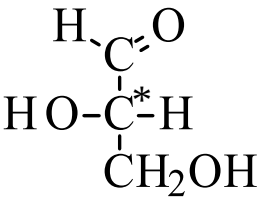


L - idose

The relative configuration of monosaccharides is determined by the configuration standard (D-glycerol aldehyde). The configuration of the chiral center farthest from the oxo group is compared with it.



D (+) - Glycerin aldehyde
R (lat. Rectus - right)



L (-) - Glycerin aldehyde
S (lat. Sinister - left)

According to stoichiometric nomenclature, the D-, L-system in most cases is replaced by the R-, S-system, which is the main one when considering the spatial model of the molecule, in which seniority of substituents near the chiral center is taken into account. The seniority of the substituents is determined by the atomic number of the element in the TEC associated with the chiral center. The model is positioned so that the youngest deputy, as a rule, was the most distant from the observer. If the precedence of the other three substituents decreases clockwise, then this configuration is denoted by R, counterclockwise - S.

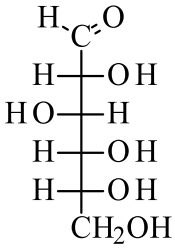
The presence of chiral centers in the monosaccharide molecule indicates that they are optically active, i.e. able to deflect the plane of polarization of light by a certain

angle a. The sign in parentheses in the name of the carbohydrate is not associated with their belonging to the D- or L-series. It is determined experimentally and depends on the contribution of all chiral centers in the molecule.

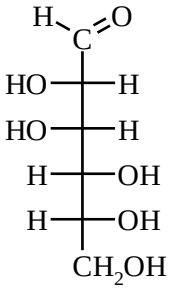
The optical activity of carbohydrates is indicated by a (+) sign for dextrorotatory compounds and a (-) sign for levorotatory compounds.

Among aldohexoses and ketohexoses of the D-stereochemical series, there are both levorotatory and dextrorotatory compounds.

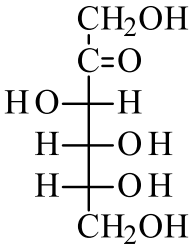
Fischer projection formulas are used to represent stereoisomers in monosaccharides.



D (+) – glucose



D (+) - mannose



D (-) – fructose

The predominant majority of natural monosaccharides belongs to the D-series. Living organisms do not "recognize" and do not know how to process L-glucose. L-glucose is not amenable to alcoholic fermentation by yeast cells.

During the study of the chemical properties of monosaccharides, it was determined that, although they are aldehyde or keto alcohols in structure, they do not exhibit all characteristic reactions to the -C = O group:

- do not form bisulfite derivatives;

-do not give staining with fuchsulfuric acid.

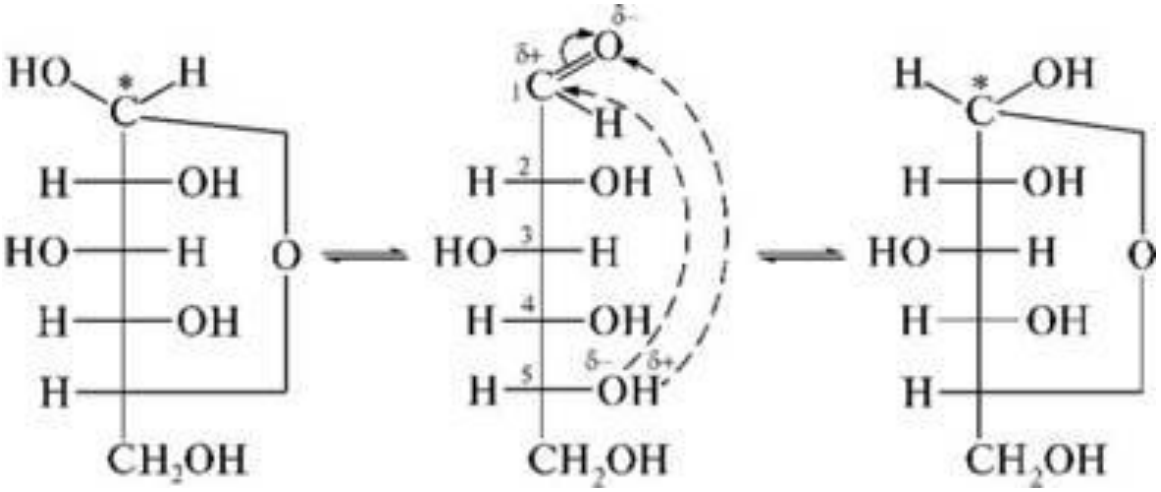
There are reactions that cannot be explained on the basis of the above structure of sugars, for example, the phenomenon of mutarotation and the formation of glycosides. The number of isolated isomers was twice as large as would be expected according to the formula $N = 2^n$. 32 isomers of aldoghexoses are known instead of 16. For all aldoghexoses isolated from living organisms or synthesized, the relative configurations of substituents near asymmetric atoms are established.

For the first time, the assumption of the cyclic structure of glucose was put forward by our compatriot A.A. Collie (1870), and soon by the German scientist B. Tollens (1883).

In five- and six-carboxylic chains, two functional groups may come closer together — the aldehyde (ketone) and hydroxyl groups near the C-4 or C-5 carbon atom. Due to this intramolecular interaction, a cyclic hemi-acetal is formed.

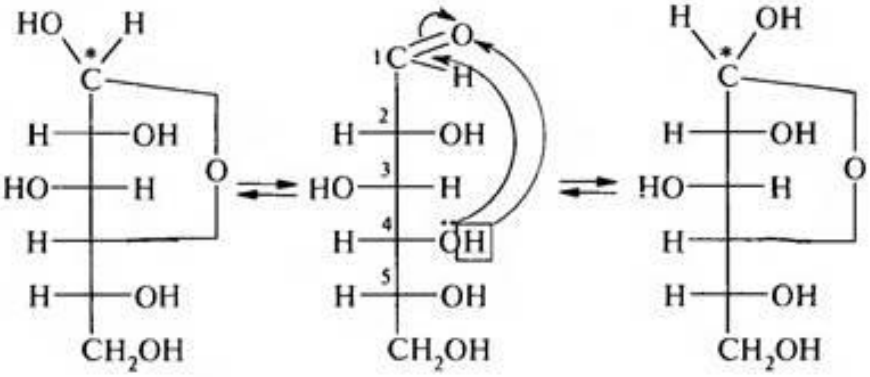
If a five-membered cycle is formed, closed to an oxygen atom, then such a cycle is called furanose, and if it is six-membered, it is called pyranose. The OH group that formed is called hemi-acetal, or glycoside. For example, glucose exists in five forms, four of them cyclic:

Collie Tollens Formulas
hemi-acetal hydroxyl



β-D – glucopyranose (cyclic form) *D – glucose (oxoform)* *α-D - glucopyranose (cyclic form)*

In a cyclic form, an additional center of chirality arises, which is called anomer, and the two stereoisomers that formed are α- and β- anomers.

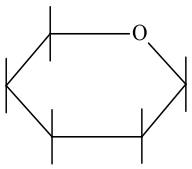


β-D – glucofuranose *D – glucose* *α-D - glucofuranose*

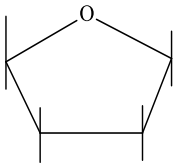
In the α-anomer, the configuration of the anomeric center is the same as the configuration of the "terminal" chiral center in the monosaccharide molecule, and in the β-anomer it is the opposite.

In general, the α- and β-anomers due to the presence of several more centers of chirality in the molecule are not enantiomers, but diastereomers. They have different physical and chemical properties.

Anomers is a special case of epimers. It is convenient to use Haworth formulas rather than Collie-Tollens formulas to depict oxygen-containing cycles. They are depicted as flat polygons that are perpendicular to the plane of the picture. The oxygen atom is located in the pyranose cycle in the far right corner, and in the furanose cycle - beyond the plane of the figure

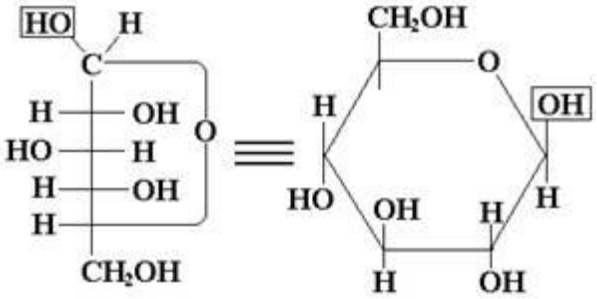


Pyranose cycle



furanose cycle

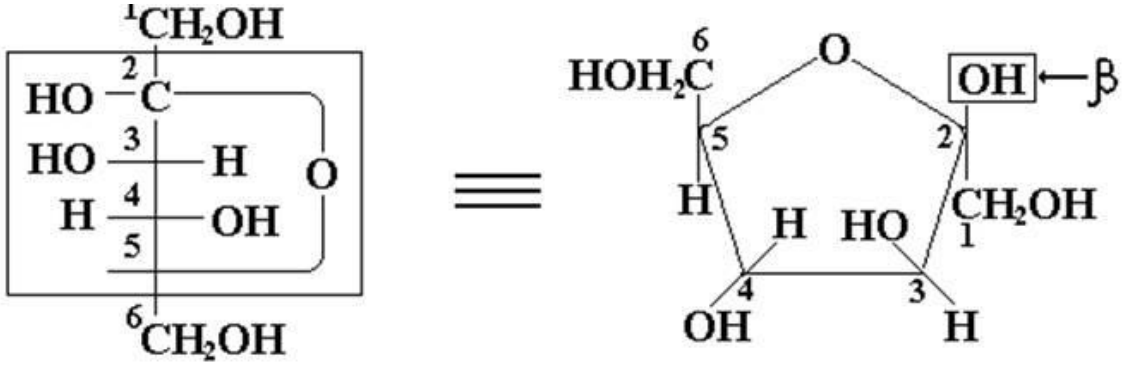
The transition from projection formulas to Haworth projection formulas is carried out in this way. All substituents located to the left of the carbon chain are located above the plane of the oxygen-containing cycle, and those located to the right are located below the plane. In aldhexoses of the D series, the CH_2OH group is always located above the plane.



β-D-glucopyranose

In the solid state, monosaccharides have a cyclic structure. Depending on which solvent D-glucose was recrystallized, it can be obtained either as an α- or β-anomer. They differ in the value of specific rotation: for the α-anomer - + 112 °, for the β-anomer - + 19 °. After a while, a freshly prepared solution from each glucose anomer gradually changes the rotation angle to + 52.5 °. The change in time of the angle of rotation of the plane of polarization of light by sugar solutions is called mutarotation. The chemical basis of mutarotation is the ability of sugars to cyclo-oxo-tautomerism, or ring-chain tautomerism.

Thus, in an aqueous solution, D-glucose exists in the form of five tautomers. Similar tautomeric transformations occur in ketohexoses with the predominant majority of furanose forms.



Collie-Tollens formula
D-fructose

Haworth formula
D-fructofuranose

Reactivity of Monosaccharides

Monosaccharides enter into most of the reactions that are characteristic of alcohols and oxo compounds.

The biological effect of monosaccharides is due to their chemical structure. The structure of sugars determines the mechanism of reactions that underlie biochemical transformations. Thus, the formation of cyclic structures led to the appearance of the most reactive hemi-acetal hydroxyl.

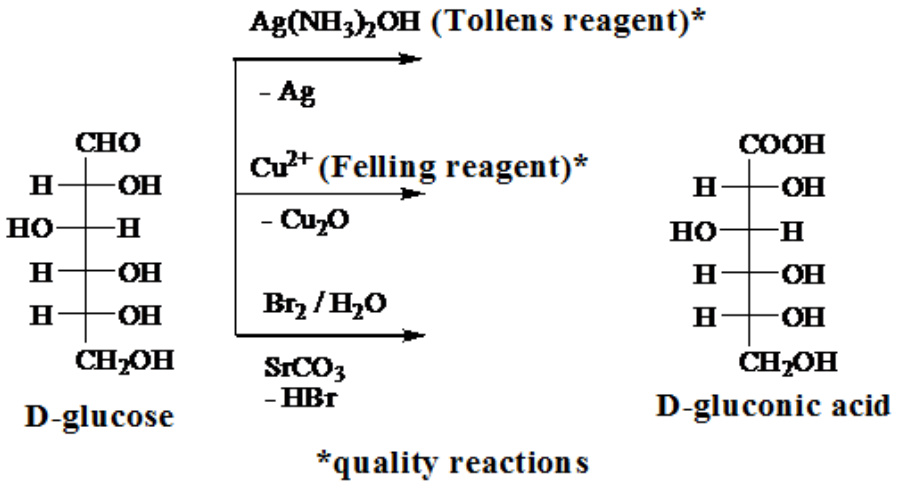
Due to the high reactivity of hemi-acetal hydroxyl, important metabolites are formed.

The chemical properties of monosaccharides are due to the presence of three types of functional groups in the molecule (carbonyl, alcohol hydroxyls and glycosidic (hemi-acetal) hydroxyl). The chemical properties of monosis are characterized by two groups of reactions – according to the oxo form (those associated with carbonyl transformations) and cyclic forms (those associated with reactions of hydroxyl groups).

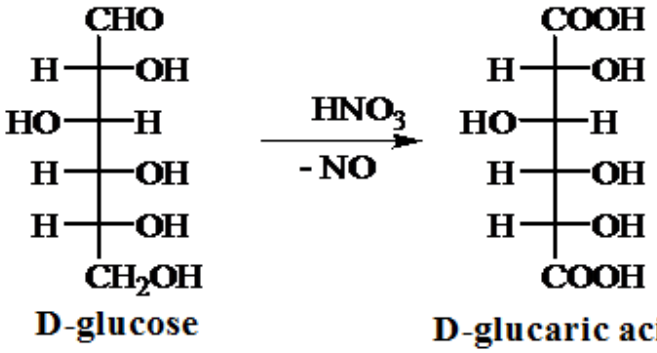
1. Oxo-reactions (open chain form) monoses

These reactions take place in polar solvents (often in aqueous solutions) with the obligatory participation of the carbonyl group.

a) Oxidation



b) Concentrated Nitric Acid Oxidation.

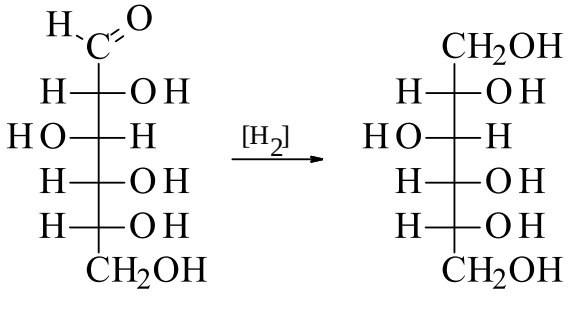


When concentrated HNO_3 is used, in addition to the carbonyl group, the terminal CH_2-OH group is oxidized and a number of glycaric (araic, aldaric, sugar) acids are formed.

D-mannaric acid → from D-mannose

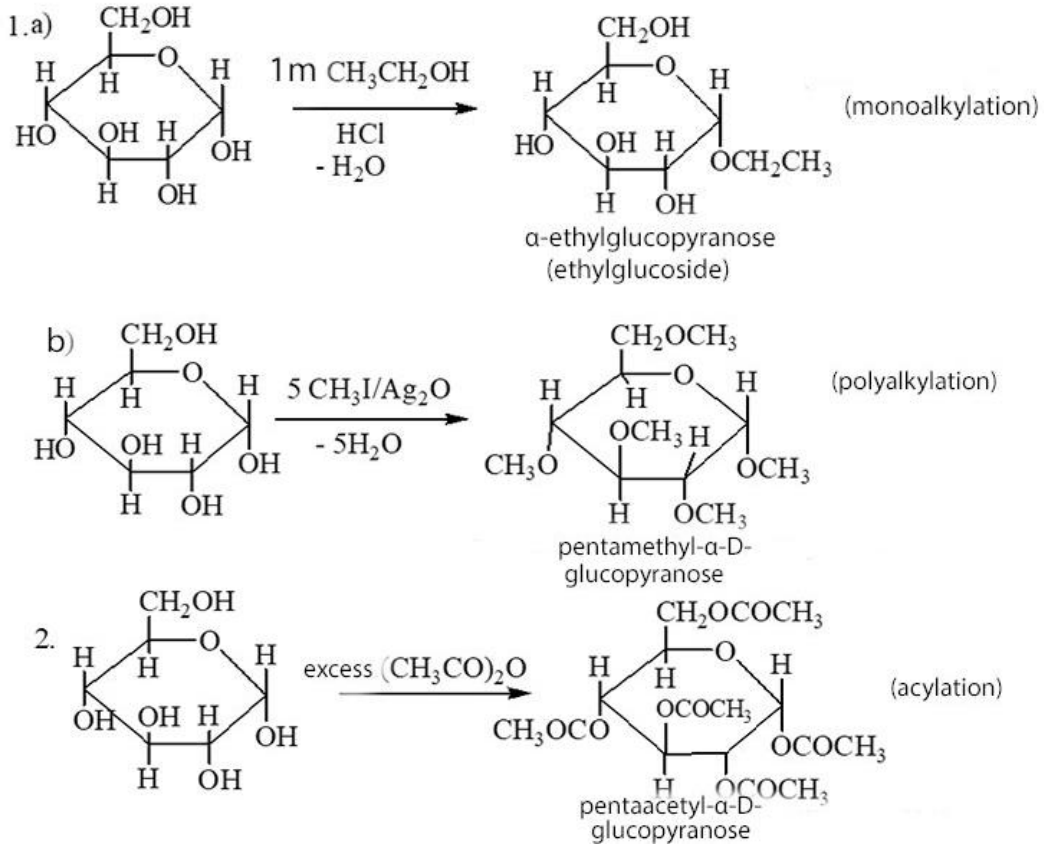
D-galactaric acid →f rom D-galactose

2. Reduction



D-mannitol → from D-mannose , D-galactite → from D-galactose

Cyclic reactions



3. The most important property of monosaccharides is their enzymatic fermentation, i.e. decay of molecules into fragments under the influence of various enzymes. Hexoses are mainly fermented in the presence of enzymes secreted by yeast, bacteria or molds. Depending on the nature of the active enzyme, reactions of the following types are distinguished:

a) $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ (alcoholic fermentation);

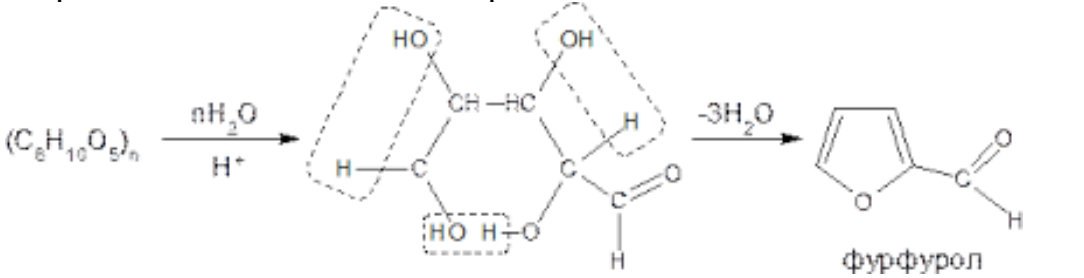
b) $C_6H_{12}O_6 \rightarrow 2CH_3\text{-}CH(OH)\text{-}COOH$ (lactic acid fermentation);

c) $C_6H_{12}O_6 \rightarrow C_3H_7COOH + 2CO_2 + 2H_2O$ (butyric acid fermentation);

d) $C_6H_{12}O_6 + O_2 \rightarrow HOOC\text{-}CH_2\text{-}C(OH)(COOH)\text{-}CH_2\text{-}COOH + 2H_2O$ (citric acid fermentation);

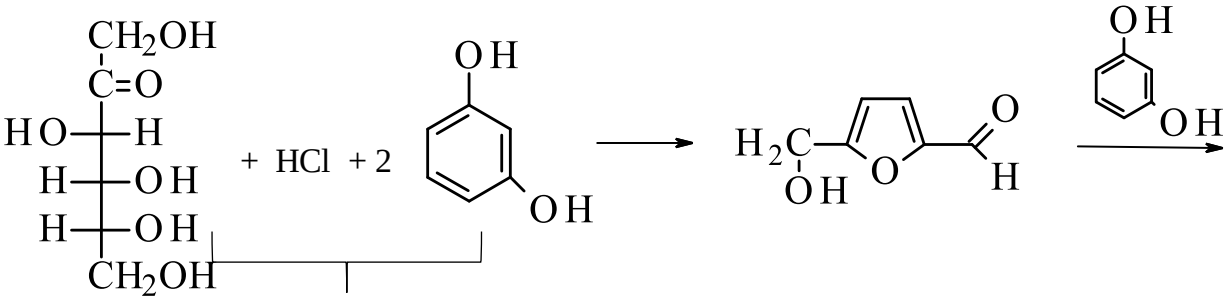
f) $2C_6H_{12}O_6 \rightarrow C_4H_9OH + CH_3\text{-}CO\text{-}CH_3 + 5CO_2 + 4H_2$ (acetone-butanol fermentation).

4. A characteristic reaction to pentoses is the formation of furfural. This reaction proceeds when heated in the presence of mineral acids.

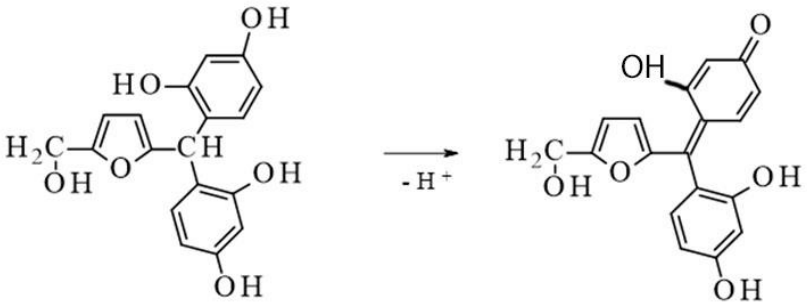


Aldopentose *furfural*

Furfural gives a red color with aniline (a qualitative reaction to pentoses). 5-Hydrosimethylfurfural gives a red color with resorcinol (Selivanov's reaction to fructose, which previously isomerizes into glucose).



D-fructose *Selivanov's Reagent*

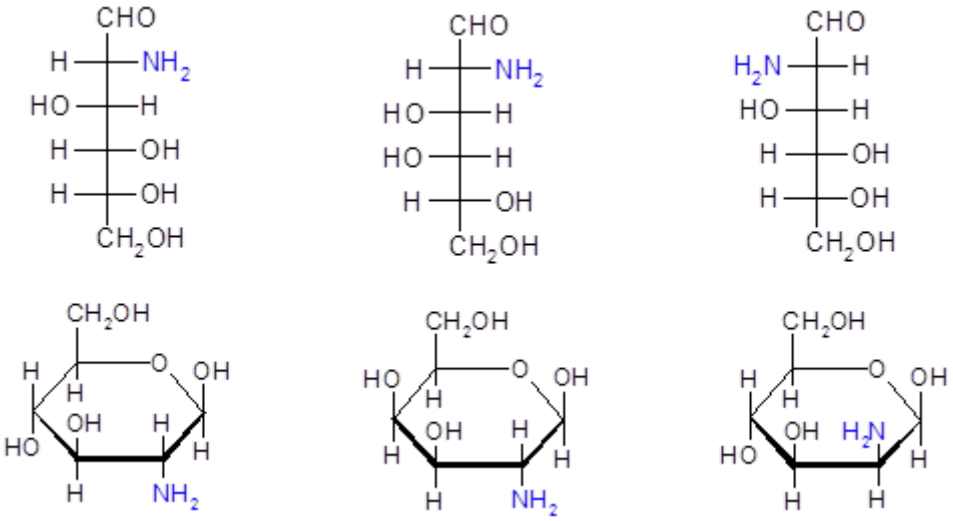


Amino sugars

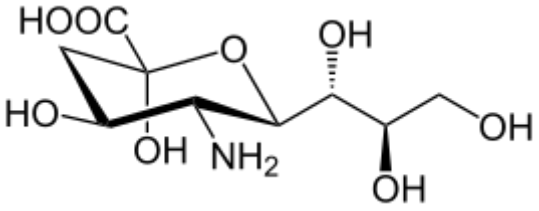
Amino sugars are derivatives of carbohydrates formed by the substitution of one or more hydroxyl groups for an amino group.

In the case of direct attachment of an amino group to the glycosidic carbon atom, such compounds are called glycosylamines or N-glycosides.

The most important representatives of amino sugars are glucosamine, or chitosamine (2-deoxy-2-amino-D-glucose) and galactosamine, or chondrosamine (2-deoxy-2-amino-D-galactose). They are part of various heteropolysaccharides that play an important physiological role in animals and humans.



Neuraminic acid, or nonulosamic acid, is formed from the aminosaccharide mannosamine by its condensation with pyruvic acid. It is very reactive due to a large number of functional groups, so its content in the body in free form is small. It is a part of blood glycoproteins, brain glycolipids and other connective tissue substances. Its N-acetyl derivatives are called sialic acids and are contained in salivary gland secretion, mucus, in mitochondria membranes, where they take part in membrane permeability processes.

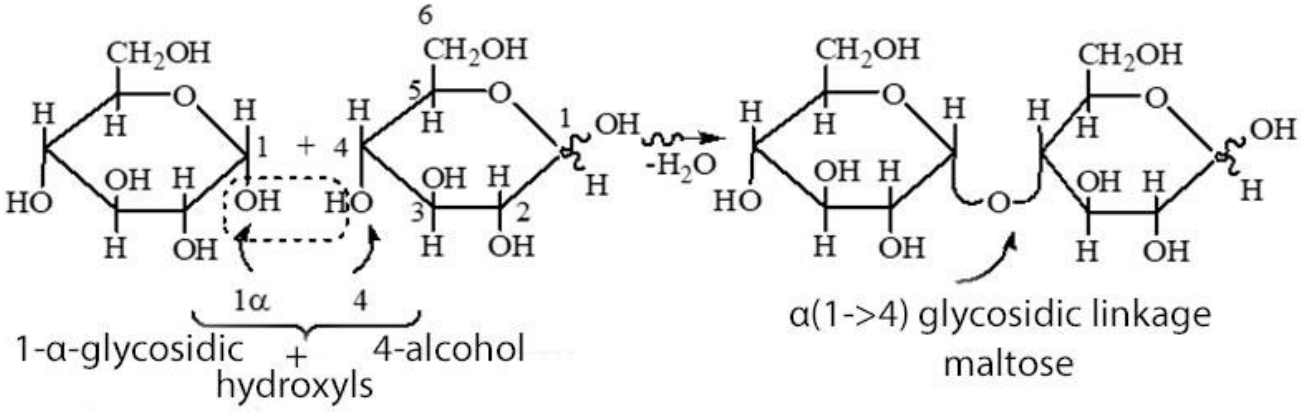


Neuraminic acid

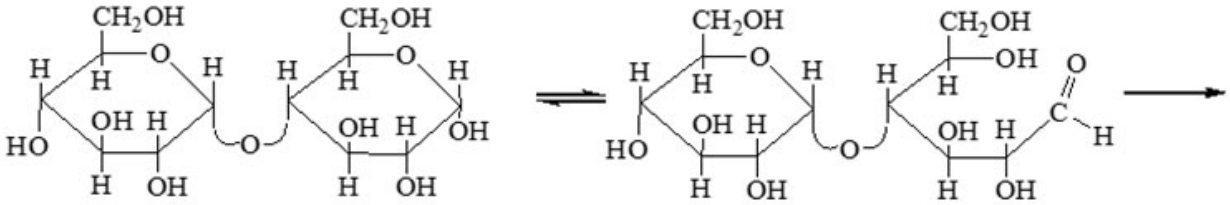
Amino sugars and their methyl derivatives are part of the molecules of a number of antibiotics: D-gulosamine (2-amino-2-deoxy-D-gulose) - streptomycin, deosamine (3-dimethylamino-3,4,6-trideoxy-D-glucose) - erythromycin, mycaminose (3-dimethylamino-3,6-dideoxy-D-glucose - carbomycin, etc.). N-acetylfucosamine was isolated from glycolipids of microorganisms.

Disaccharides

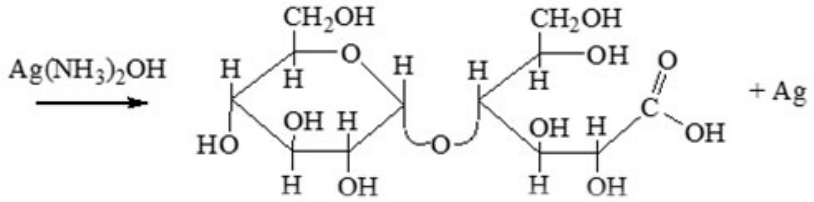
Disaccharides are carbohydrates, the molecules of which consist of two monosaccharide residues joined together by the interaction of hydroxyl groups (two hemi-acetal or one hemi-acetal and one alcohol). The absence or presence of glycosidic (hemi-acetal) hydroxyl is reflected in the properties of disaccharides. Bioses are divided into two groups: reducing and non-reducing bioses. Reducing bios they are capable of exhibiting the properties of reducing agents and, when interacting with an ammonia silver solution, are oxidized to the corresponding acids, they contain glycosidic hydroxyl in their structure, and the connection between monoses is glycoside-glycosic. Scheme of the formation of reducing biosis, for example, maltose:



The reduction properties of maltose are manifested in the oxidation of Br_2 / H_2O, Tollens and Felling reagents, and maltobionic acid (a series of bionic acids) is formed.

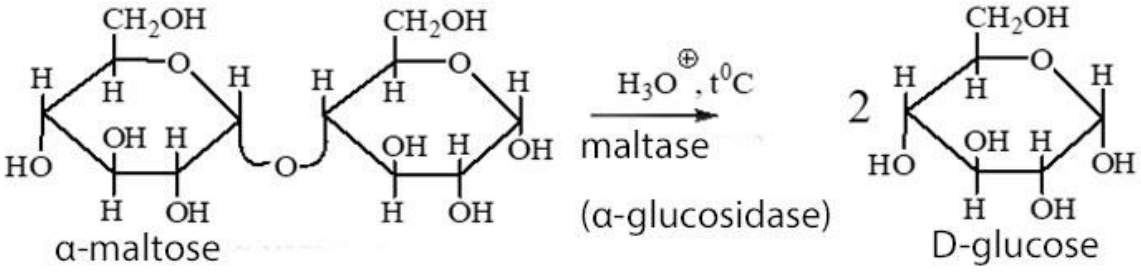


α-maltose



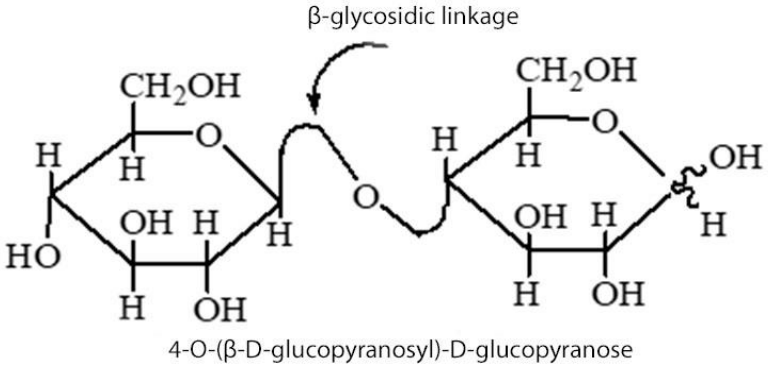
D-maltobionic acid

For disaccharides, a hydrolysis reaction is characteristic, as a result of which two monosaccharide molecules are formed:



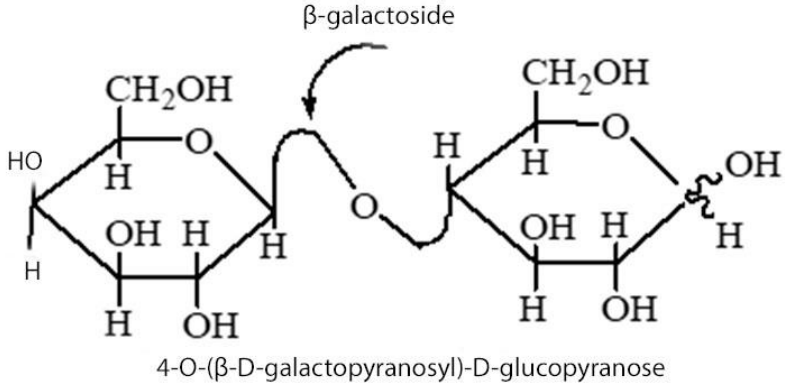
α-maltose

Cellobiose D-glucose 1β-4 D-glucose



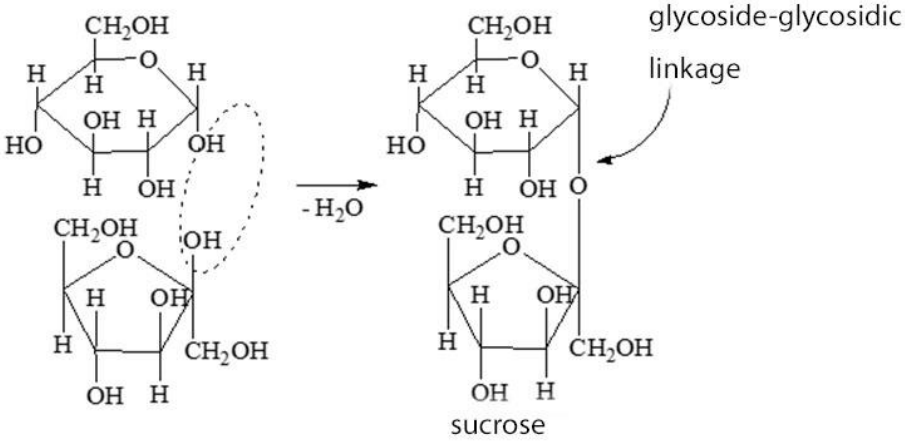
4-O-(β-D-glucopyranosyl)-D-glucopyranose

Lactose D-galactose 1β-4D-glucose

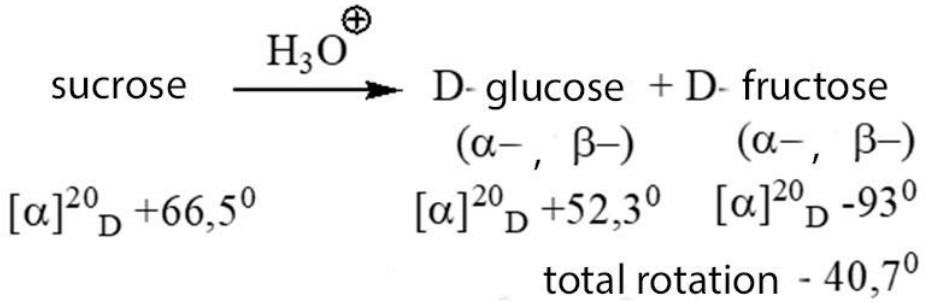


4-O-(β-D-galactopyranosyl)-D-glucopyranose

An example of the most common disaccharides in nature is sucrose (beet or cane sugar). The sucrose molecule consists of α-D-glucopyranose and β-D-fructofuranose, connected to each other due to the interaction of hemiacetal (glycosidic) hydroxyls. Bioses of this type do not exhibit reducing properties, since they do not contain a glycosidic hydroxyl in their structure; the connection between monoses is glycoside-glycosidic. Such disaccharides are called non-reducing, i.e. not able to oxidize. β-D-glucopyranose and α-residues of Scheme of sucrose formation:



Sucrose inversion. With acid hydrolysis of D (+) sucrose or with invertase, equal amounts of D (+) glucose and D (-) fructose are formed. Hydrolysis is accompanied by a change in the sign of the specific rotation angle [α] from positive to negative, which is why the process is called inversion, and a mixture of D (+) glucose and D (-) fructose is called invert sugar.

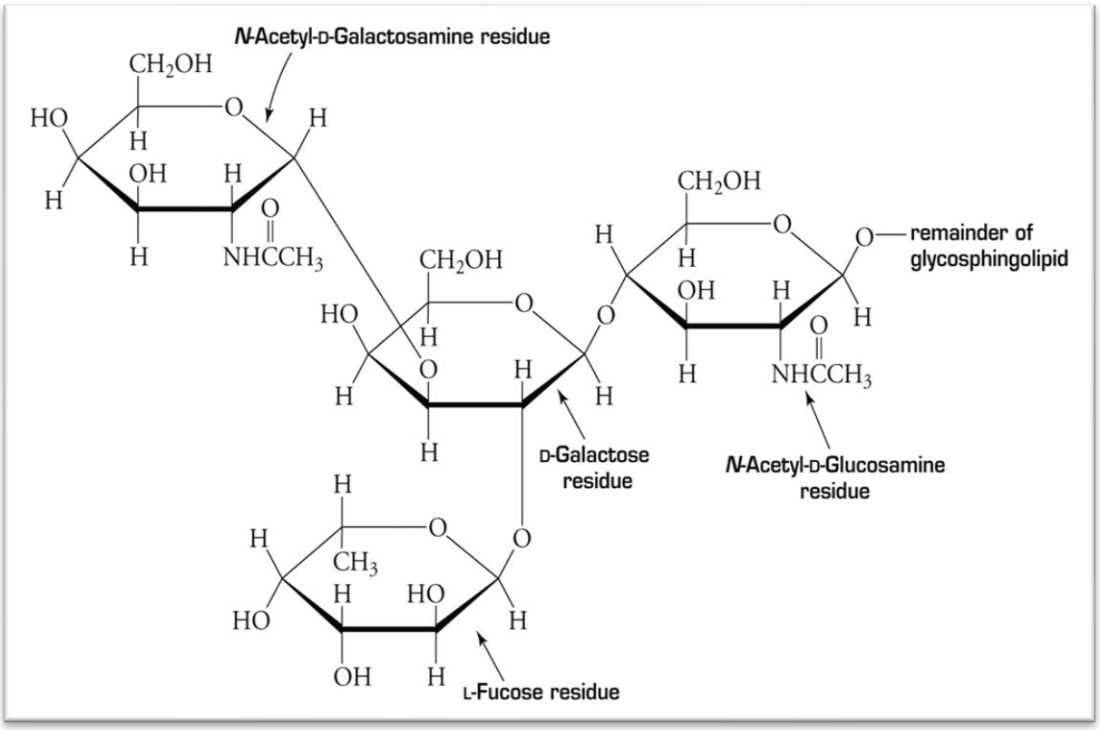


Oligosaccharides with 3 or more monosaccharides **N-Acetylgalactosamine (GalNAc)**, is an amino sugar derivative of galactose. ***The blood group antigens are oligosaccharides that are attached to lipids and proteins found on cell surfaces.***

It is typically the first monosaccharide that connects serine or threonine in particular forms of protein *O*-glycosylation.

N-Acetylgalactosamine is necessary for intercellular communication, and is concentrated in sensory nerve structures of both humans and animals.

GalNAc is also used as a targeting ligand in investigational antisense oligonucleotides and siRNA therapies targeted to the liver, where it binds to the asialoglycoprotein receptors on hepatocytes.



Polysaccharides (polyoses)

Polysaccharides are polymers of 10 or more monosaccharide units

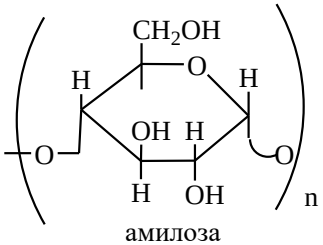
Homopolysaccharides contain a single type of monosaccharide unit.

Heteropolysaccharides contain more than one typee of monosaccharide unit.

Polysaccharides are natural high-molecular carbohydrates whose macromolecules are composed of monosaccharide residues. The main representatives: starch and cellulose, which are built from the remains of one monosaccharide - D - glucose. Starch and cellulose have the same molecular formula: $(C_6H_{10}O_5)_n$, but different properties. This is due to the peculiarities of their spatial structure. Starch consists of residues α - D - glucose, and cellulose - from β - D - glucose.

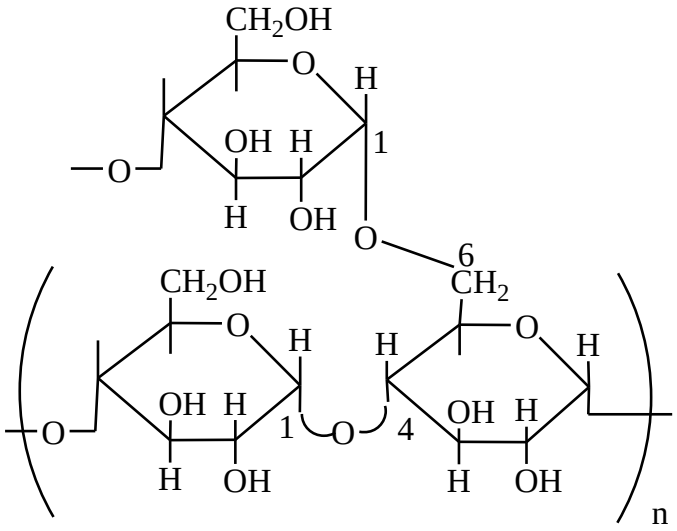
Starch is a reserve polysaccharide of plants, accumulates in the form of grains in the cells of seeds, bulbs, leaves, stems, is a white amorphous substance, insoluble in cold water. Starch is a mixture of amylose and amylopectin, which are built from α-D-glucopyranose residues.

Amylose is a linear polysaccharide, the link between the residues of D-glucose is 1 α - 4. The shape of the chain is spiral, one coil of the spiral contains 6 residues of D-glucose. The amylose content in starch is 15 - 25%.



амилоза amylose

Amylopectin is a branched polysaccharide; the bonds between D-glucose residues are 1α-4 and 1α-6. The amylopectin content in starch is 75-85%.



амилопектин

amylopectin

Chemical characterization

1. Eter and ester formation (similar to bios).

2. Qualitative reaction – staining with the addition of iodine: for amylose - in blue, for amylopectin – in red. During the interaction of amylose with iodine in an aqueous solution, iodine molecules enter the internal channel of the spiral, forming the so-called inclusion – clathrate compound.

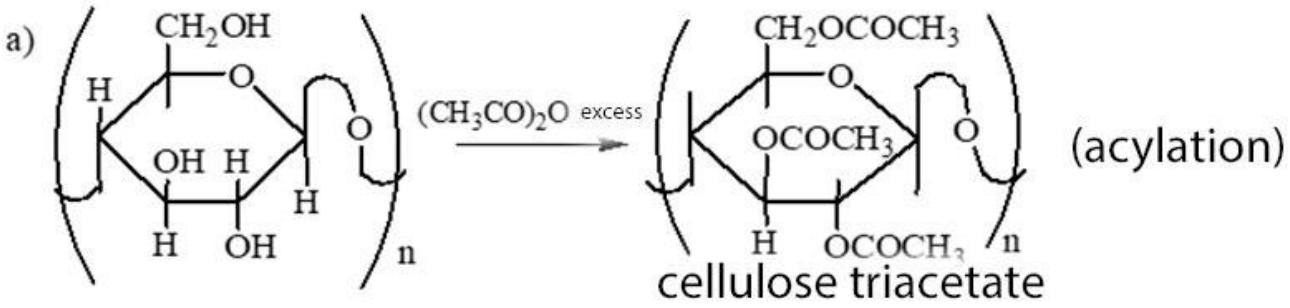
3. Acid hydrolysis of starch: starch → dextrins → maltose → α - D - glucose.

Cellulose. The structural polysaccharide of plants is constructed from β-D-glucopyranose residues, the nature of the compound is 1β-4. The cellulose content, for example, in cotton is 90-99%, in hardwood - 40-50%. This biopolymer has great mechanical strength and acts as a supporting material for plants, forming the walls of plant cells. Used in the manufacture of fibers and paper. Cellulose polysaccharide chains are stretched and bundled and held by hydrogen bonds. Cellulose chains are linear in structure.

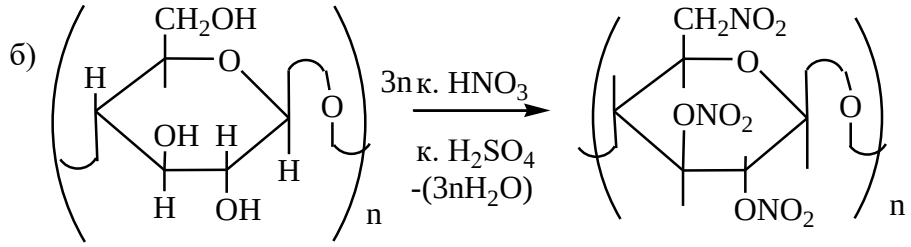
Chemical properties

1. Acid hydrolysis (saccharification): cellulose → cellobiose → β - D - glucose.

2.Ester formation



Acetate fiber is made from cellulose acetate solutions in acetone. Plasticized cellulose acetate, as well as cellulose acetate-butyrate (mixed cellulose ester with acetic and butyric acids) are used in the production of plastics.



cellulose trinitrate (nitrocellulose)

Nitrocellulose is explosive, constitutes the basis of smokeless powder. Pyroxylin is a mixture of cellulose di- and trinitrates, used for the manufacture of celluloid, collodion, photographic films, varnishes.

Heteropolysaccharides

A polysaccharide that contains different types of monosaccharides is known as a heteropolysaccharide.

Heteropolysaccharides are polymers built from a large number of different monosaccharide units and their derivatives. In biochemistry and physiology of humans and animals the most important heteropolysaccharides are glycosaminoglycans.

Glycosaminoglycans are heteropolysaccharides built from repeating disaccharide residues. The monosaccharide components of the disaccharide residues of glycosamine glycosphingolipids are most often hexuronic acids (glucuronic or sometimes iduronic, etc.) and N-acetyl derivatives of hexosamines (glucosamine, galactosamine).

Glycosaminoglycans include numerous animal biopolymers that make up the intercellular matrix of connective tissue, which fills the space between individual cells. The outdated name of these compounds - mucopolysaccharides - indicates that the compounds of this class were first isolated from mucin, a component of mucus, which is a lubricating substance that acts as a physiological lubricant. The most studied glycosamine glycans are hyaluronic acid, chondroitin sulfates, dermatan sulfates, keratan sulfates, heparan sulfates, which are part of the skin, tendons, cartilage of joints, providing mechanical strength and elasticity of organs, elasticity of their joints. Glycosaminoglycan heparin is a natural anticoagulant.

Glycosaminoglycans are polyanionic molecules. At least one of the monosaccharide components in the molecules of glycosamine glycans carries an acid group - carboxyl or sulfate group, which provides their high hydrophilicity, i.e. the ability to retain a significant amount of water in biological tissues.

All glycosaminoglycans perform their biochemical and physiological functions being bound to proteins. Covalent complexes of connective tissue glycosaminglycans (hyaluronic acid, chondroitin sulfates, etc.) with proteins are called proteoglycans, which are representatives of mixed biopolymers (glycoconjugates).

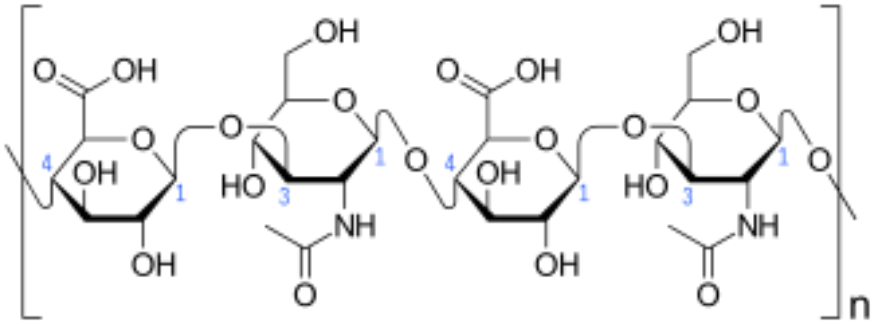
Some of the important heteropolysaccharides are:

1. Hyaluronic Acid:

Hyaluronic acid is a polymer of disaccharides, which are composed of D-glucuronic acid and N-acetyl-D-glucosamine, linked via alternating β-(1→4) and β-(1→3) glycosidic bonds. Hyaluronic acid can be 25,000 disaccharide repeats in length.

Polymers of hyaluronic acid can range in size from 5,000 to 20,000,000 Da in vivo. The average molecular weight in human synovial fluid is 3–4 million Da, and hyaluronic acid purified from human umbilical cord is 3,140,000 Da.

Hyaluronic acid is energetically stable, in part because of the stereochemistry of its component disaccharides.[citation needed] Bulky groups on each sugar molecule are in sterically favored positions, whereas the smaller hydrogens assume the less-favorable axial positions.

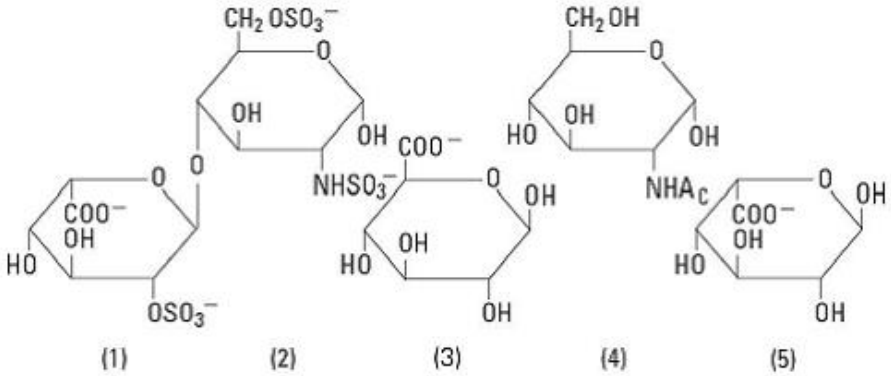


Hyaluronic acid has been used in various formulations to create artificial tears to treat dry eye. It is a common ingredient in skin care products, it is used as a dermal filler in cosmetic surgery. It is typically injected using either a classic sharp hypodermic needle or a micro-cannula. Some studies have suggested that the use of micro-cannulas can significantly reduce vessel embolisms during injections. Currently, hyaluronic acid is used frequently as a soft tissue filler due to its bio-compatibility and reversibility. Complications include the severing of nerves and microvessels, pain, and bruising. Some side effects can also appear by way of erythema, itching, and vascular occlusion; vascular occlusion is the most worrisome side effect due to the possibility of skin necrosis, or even blindness in a patient. In some cases, hyaluronic acid fillers can result in a granulomatous foreign body reaction.

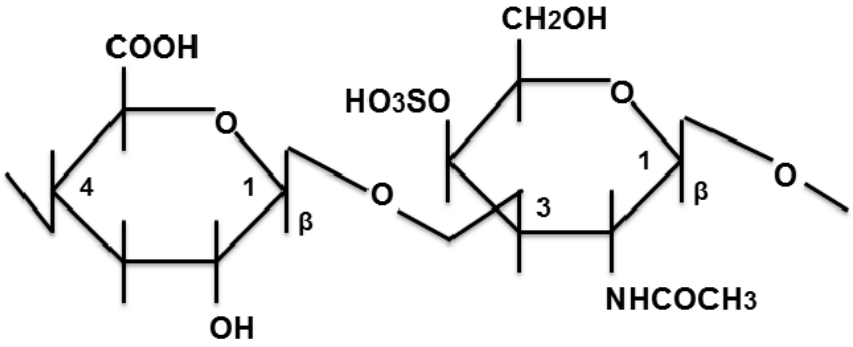
2. **Heparin:** It is made up of D-glucuronic acid, L-iduronic acid, N-sulfo-D-glucosamine and is largely distributed in mast cells and blood.

Native heparin is a polymer with a molecular weight ranging from 3 to 30 kDa, although the average molecular weight of most commercial heparin preparations is in the range of 12 to 15 kDa.

Heparin is an injectable anticoagulant that is used to prevent the formation of blood clots in the vessels. It is a highly-sulfated glycosaminoglycan and the most highly negatively charged biological molecule known to mankind. It is also used to create an anti-clotting surface inside various medical devices such as renal dialysis machines and test tubes.



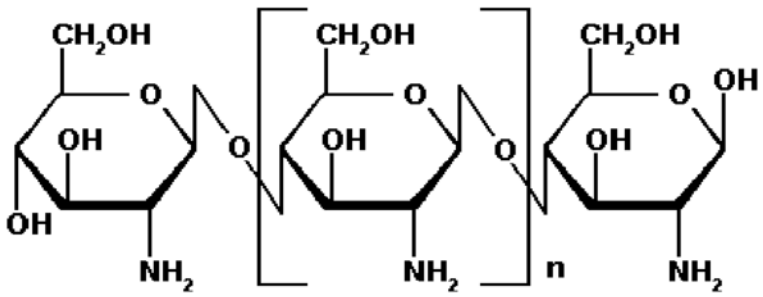
3. **Chondroitin-4-sulfate:** Its component sugars are D-glucuronic acid and N-acetyl-D-galactosamine-4-O-sulfate. It is present in the cartilages.



Chondroitin sulfate is a natural substance used for the treatment of osteoarthritic conditions. It is an essential component of cartilage and plays an important role in the elasticity and function of articular cartilage where it is mainly attached covalently to core proteins in the form of proteoglycans. It is a polymer with a wide molecular weight range composed of an alternating sequence of sulfated and/or unsulfated d-glucuronic acid (GlcA) and N-acetyl- d-galactosamine (GalNAc) residues linked through alternating β-(1 → 3) and β-(1 → 4) bonds. The presence of sulfate groups makes it extremely hydrophilic. Predominant sources of chondroitin sulfate raw materials are bovine trachea, porcine skin and rib cartilage, and shark cartilage.

4. **Chitosan** is a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide.

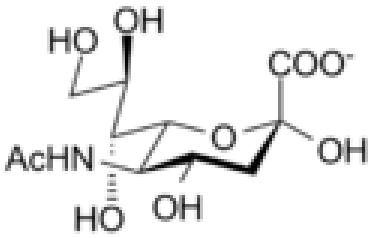
Chitosan has a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In winemaking, it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it is useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.



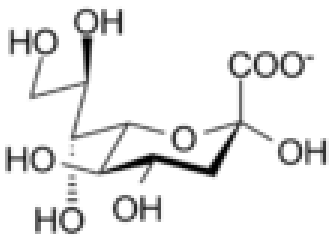
5. **Gamma globulin:** N-acetyl-hexosamine, D-mannose, D-galactose are the component sugars of this polysaccharide. It is found in the blood.

6. **Sialic acids** - are a group of glucose aminglicans that contain neuramyl acids and carbohydrate derivatives. They are a class of alpha-keto acid sugars with a nine-carbon backbone Compounds of neuramilic acid with acetic acid are sialic acids. They are found in cell membranes, saliva and other biological fluids. For the diagnosis

of a number of inflammatory diseases (rheumatism, tuberculosis), in which their level in the blood is elevated, their determination is carried out.



N-Acetylneuraminic acid
Neu5Ac



2-Keto-3-deoxynonic acid
Kdn

General material and educational and methodological support of the lecture:

- Working program of the discipline
- Silabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational tasks

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Construction features, chemical properties and the biological role of carbohydrates. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova, K.V. Bevziuk. Teaching aid. Odesa, 2017. – 44 p.
5. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
6. Lipids: classification, structural features, properties and biological role. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova. Teaching aid. Odesa, 2017. – p. 32.

Additional:

7. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
8. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
9. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
10. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.

11. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
12. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 3

«Proteinogenic amino acids, peptides, proteins: structure, properties, biological role. Classification»

Actuality of theme: Protein chemistry has always united the ideas and methods of biology, medicine, chemistry, and physics. Proteins provide the material basis for the chemical activity of the cell. It is conventionally considered that peptides contain up to 100 amino acids in a molecule and have a molecular weight of about 1000, while proteins have more than 100 amino acid residues. The biological activity of proteins is closely related to the high level of organization of the molecule. Therefore, living organisms synthesize proteins of the necessary conformation.

Aims: Сформировать знания структуры и химических свойств наиболее важных α-аминокислот *in vivo* и *in vitro*.

Basic concepts: protein, proteins, amino acids, essential amino acids, amphoteric properties of amino acids.

Plan and organizational structure of the lecture:

1. Nomenclature of amino acids.
2. Structure of the 20 most important α-amino acids.
3. Classification of amino acids.
4. Isomerization of amino acids. D- and L-genetic series.
5. Methods of production of amino acids.
6. Physical and chemical properties of amino acids.
7. General chemical properties of amino acids (reactions of deamination, overamination, decarboxylation, specific properties of α-, β-, γ-amino acids).
8. Reactions of polycondensation of amino acids to form peptides and proteins.

Content of lecture material (lecture text)

Proteins (proteins) are high molecular organic substances built from amino acid residues. Proteins are the basis of the structure and function of all living organisms. Proteins can perform a variety of functions.

The main functions of proteins:

1. Catalytic function. Today, most enzymes, or biological catalysts, are proteins. The rate of chemical reactions in biological systems depends on this function of proteins.

2. Transport function. Blood oxygen is transported by hemoglobin molecules, which are a protein of red blood cells. Serum albumin takes part in the transport of lipids, forms complexes with organic and inorganic substances and ensures their delivery to target organs.

3. Protective function. In response to the entry into the body of substances bearing the imprint of genetic foreignness, specific protective antibody proteins are synthesized. The protective function of proteins is also manifested in their ability to coagulate (fibrinogen), which protects the body from blood loss during wounds.

4. Contractile function. Specific muscle tissue proteins (actin and myosin) play a major role in the act of muscle contraction and relaxation. Proteins of the cytoskeleton, which ensure chromosome divergence during mitosis, also have a contractile ability.

5. Structural function. Structural proteins (collagen, keratin, elastin, etc.) occupy the first place in terms of the number of proteins in the human body. Proteins are involved in the formation of cell membranes, the interstitial substance of connective tissue, and in combination with carbohydrates are part of a number of secrets (mucin, mucoids, etc.).

6. Hormonal function. Hormonal regulation occupies an important place in the regulation of metabolism, and a number of hormones are represented by proteins, polypeptides or amino acid derivatives.

7. Nutritional (reserve) function. There are special reserve proteins that feed the fetus (ovalbumins) and the baby (albumin and casein).

In addition, proteins are involved in the expression of genetic information, the transmission of nerve impulses, support the oncotic pressure of blood and cells, provide homeostasis pH of the internal medium of the body.

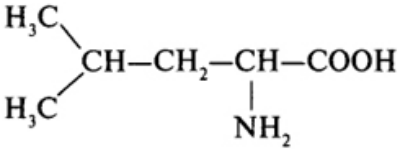
The organs and tissues of animals contain a large amount of proteins. Proteins in the human body account for 45% of the dry weight. The most rich in protein are striated muscles, lungs, spleen, and kidneys (72–84%). Organs with a moderate protein content include skin, brain, and nerve tissue, heart, and digestive system organs (47–63%). In the hard tissues of bones, teeth and adipose tissue, proteins are contained in a small amount (14-20%).

Nomenclature, features of the spatial and structural structure of natural amino acids

Amino acids are a large class of organic compounds, a characteristic feature of which is the presence of two functional groups — a carboxyl and an amino group — in the composition of the molecule. A special group is made up of natural amino acids. They can be conditionally divided into 2 groups:

- ✓ amino acids that are involved in the formation of peptides and proteins. They are characterized only by the α-structure and all belong to the L-stereo series.

✓ amino acids that have biological activity, but are not monomers of natural polymers of proteins and peptides.

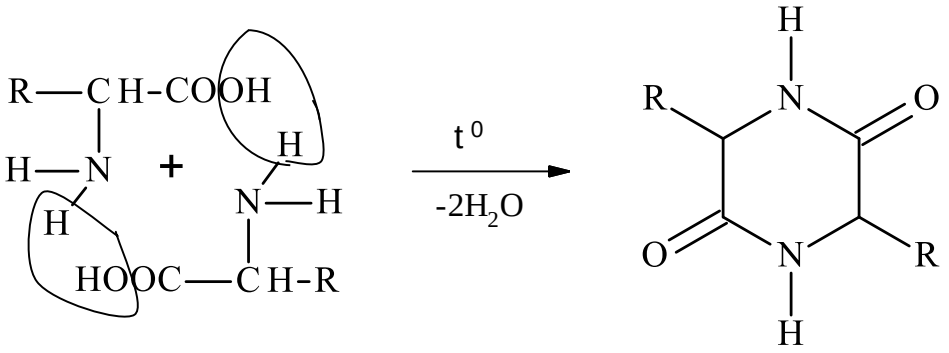


Leucine

2-amino-4-methylpentanoic acid, α-amino-γ-methylvaleric acid

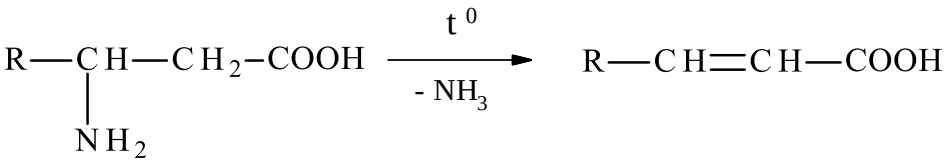
The specific properties of α, β, γ, δ are amino acids.

- **α-aminoacids**



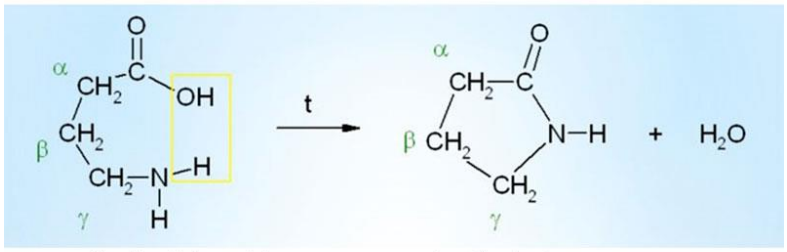
Diketopiperazine
(lactam modification)

- **β-aminoacids**



- **γ, δ - aminoacids**

γ and δ amino acids eliminate water after heating and form cyclic amide - lactam



γ-aminobutiric acid γ-butirolactam

Natural α-L - amino acids are monomers of polypeptides and proteins.

Usually about 20 natural amino acids are secreted, from which the whole set of natural proteins of plant and animal origin is formed.

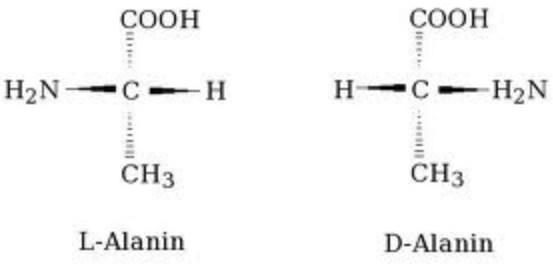
A single genetic code of nature determines the unity of the amino acid composition of proteins.

Nomenclature of natural amino acids: trivial names apply.

Structural features and stereochemistry

Natural amino acids belong to the L - stereo series and have α-structure. This means that both functional groups, amino and carboxyl, are linked to a common carbon

atom, which is always optically active (with the exception of glycine, aminoacetic acid). The fundamental works of E. Fisher, P. Carrer are devoted to investigation of the spatial structure of natural amino acids.



L-Alanin D-Alanin

Bitter sweet

Most natural amino acids have only one asymmetric carbon atom, but two amino acids – threonine and isoleucine – contain two chiral centers.

Classification of Natural Amino Acids

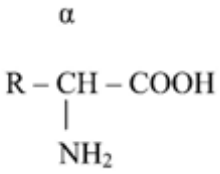
Natural amino acids are classified according to several characteristics:

1) biological: in relation to metabolism in the human body, two types of amino acids are distinguished.

a) Interchangeable: (synthesized in human cells): alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, proline, serine, tyrosine, cysteine.

b) Irreplaceable (not synthesized in human cells, must be supplied with food): valine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine. For children, arginine and histidine are additionally irreplacable.

2) The basic structural units of proteins are amino acids. Natural proteins are built from 20α amino acids. α-amino acids are derivatives of carboxylic acids in which the hydrogen atom of the α-carbon is substituted by an amino group:



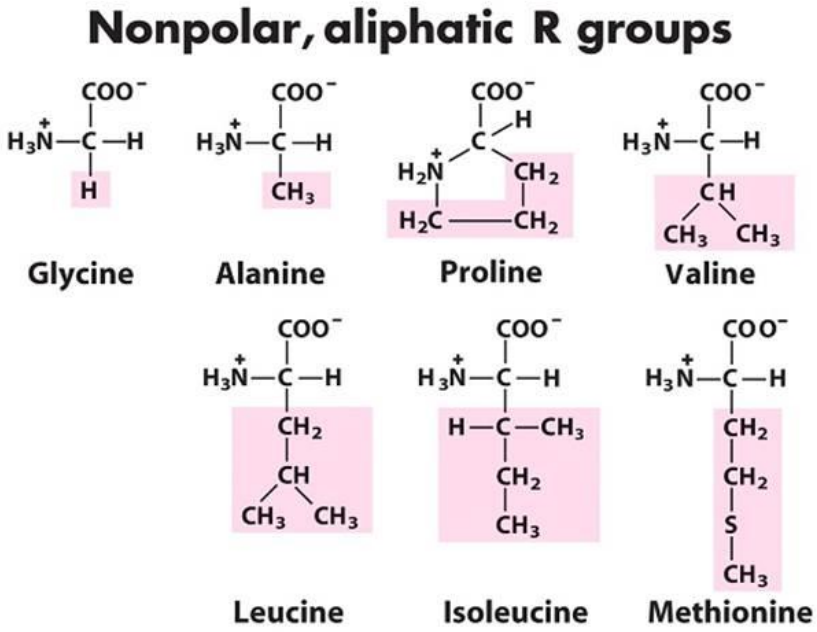
The radical R is a group of atoms that are not involved in the formation of a peptide bond. All structural features and functions of protein molecules are determined by the chemical nature of the radical R.

The classification of amino acids is based on the chemical structure of amino acid radicals.

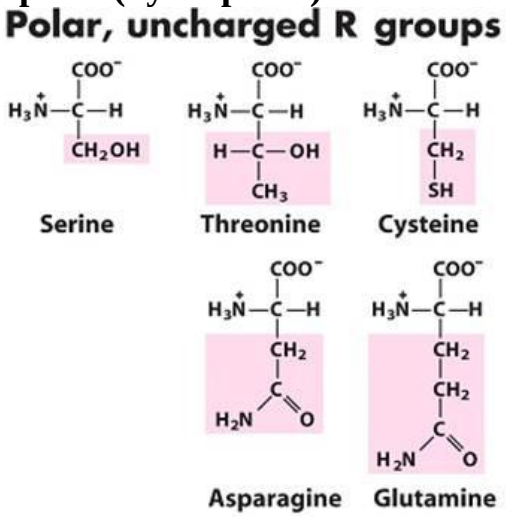
Modern rational classification is based on the polarity of radicals.

There are five classes of amino acids.

1. **Amino acids with non-polar (hydrophobic) radicals:**



2. Amino acids with polar (hydrophilic) radicals:

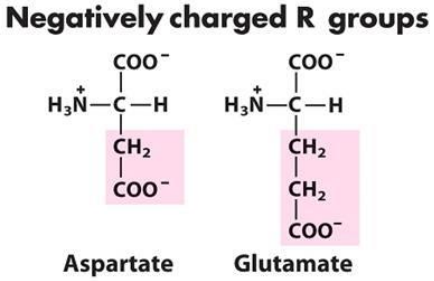


3. Amino acids with negatively charged radicals:

-COOH $\rightarrow$ -COO $^{-}$ + H^+ (the side chain acquires a negative charge);

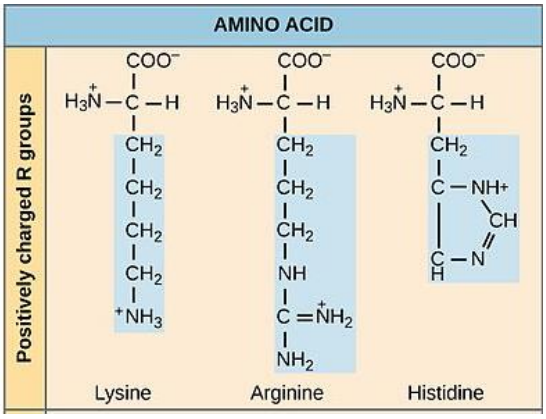
-SH$\rightarrow$ -S^-+H^+ (the side chain acquires a negative charge);

Ar-OH$\rightarrow$Ar-O^{-}+H^+ (the side chain acquires a negative charge)



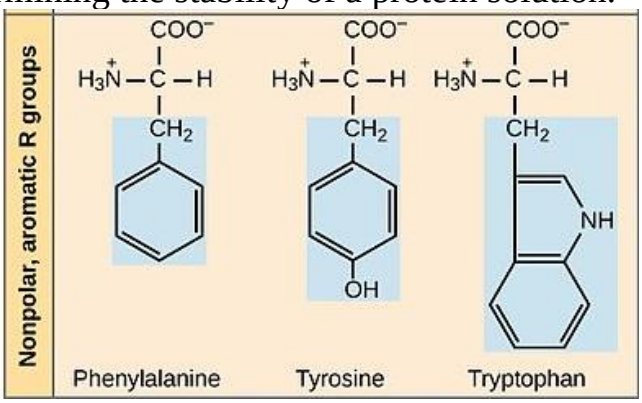
4. Amino acids with positively charged radicals:

-NH_2 + H^+ $\rightarrow$ -NH_3^+



5. Amino acids with aromatic radicals:

In an aqueous solution, molecules of amino acids and proteins are usually charged, and the presence of a charge in combination with a stable hydration shell is an important factor determining the stability of a protein solution.

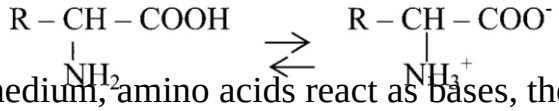


Acid-base properties of amino acids

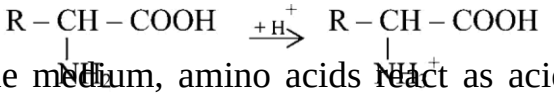
According to the protolytic theory of acids and bases, amino acids belong to ampholytes, because contain acid and base centers in the composition of the molecule. In an aqueous solution, the amino acid molecule exists in the form of a bipolar ion (zwitter-ions).

Depending on the pH of the medium, this or that charge may prevail.

Amino acids are amphoteric substances, readily soluble in water, dissociate in aqueous solutions with the formation of bipolar ions (zwitterions):



In an acidic medium, amino acids react as bases, the amino group is a proton acceptor:



In an alkaline medium, amino acids react as acids, the carboxyl group is a proton donor.

In strongly acidic medium: (pH = 1-2), a cationic form of amino acid is formed. In a strongly alkaline medium: (pH = 13-14), the anionic form of amino acid predominates. There are pH values specific for each amino acid in which the number of anionic forms in solution is equal to the number of cationic forms. In this case, it is necessary to take into account the presence of ionogenic side in branch chain.

The pH value at which the total charge of the amino acid molecule is 0 is called the isoelectric point of the amino acid (pI).

If the pH of the solution corresponds to the isoelectric point of amino acid, then during electrophoresis there is no movement of the molecule in the solution. If the pH of the solution is less than pI, then the cationic form of amino acid moves to the cathode. If the pH of the solution is more than pI, then the anionic form of amino acid moves to the anode. This is the basis for the separation of amino acids by electrophoresis method.

Aqueous solutions of amino acids have buffering properties.

Obtaining α-amino acids

❖ Protein hydrolysis

The disadvantage is the destruction of certain amino acids (serine, threonine, tryptophan).

❖ Biotechnological production method - based on the ability of special microorganisms to produce a specific amino acid in a nutrient medium.

❖ 3. Chemical syntheses

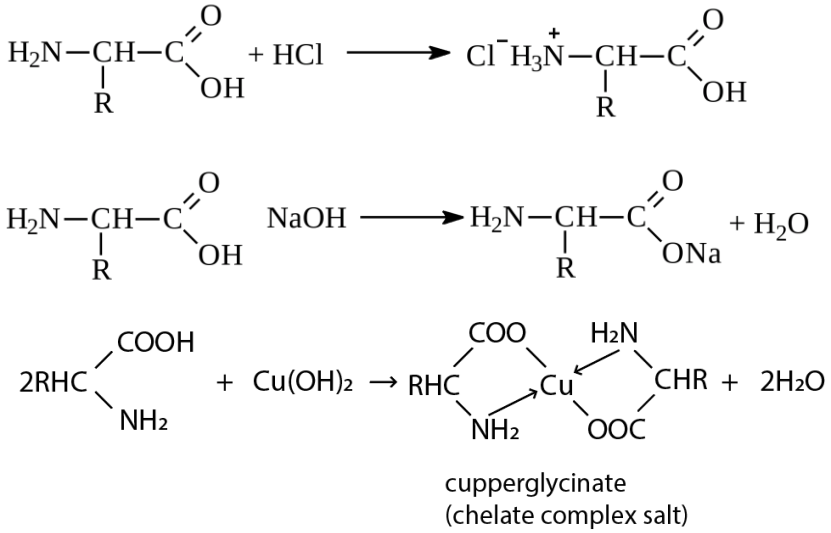
Ammonolysis of halogen acids (Emil Fischer) - the action of ammonia excess on α-halogen acids.

The Strecker-Zelinsky synthesis is the preparation of α-amino acids from aldehydes or ketones by the action of NH_3 and HCN followed by hydrolysis of the resulting α-aminonitriles.

Chemical properties of amino acids

Amino acids are heterofunctional organic compounds that enter into reactions characteristic of carboxyl groups, amino groups, and exhibit a number of specific biochemical properties.

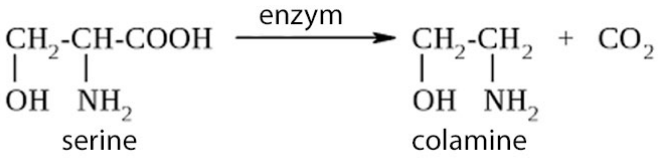
- **As ampholytes amino acids form salts when interacting with acids and bases.**



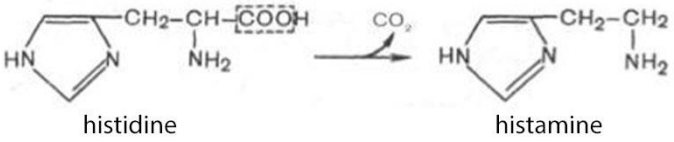
- **Decarboxylation**

In vivo:

*r*efers to the general reaction of all natural α-amino acids, in which decarboxylase enzymes are involved with the participation of vitamin B6 in two active forms of pyridoxalphosphate and pyridoxaminophosphate. Substances with pronounced biological activity are formed - biogenic amines.

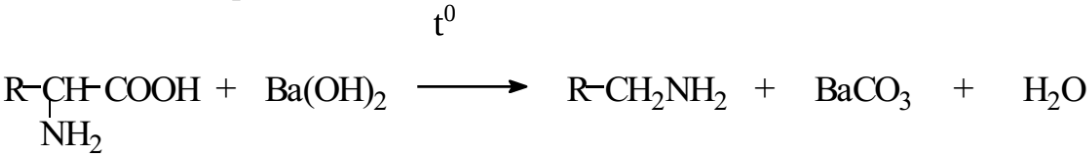


Ethanolamine is involved in the synthesis of phospholipids.



Histamine is a mediator of the allergic reaction of the body. When decarboxylation of glutamic acid takes place GABA (gamma-aminobutyric acid) is formed, which is a mediator of inhibition of the nervous system.

In vitro: In the presence of barium water:



- **Deamination**

  In vivo:

  There are two types of deamination: non-oxidative and oxidative.

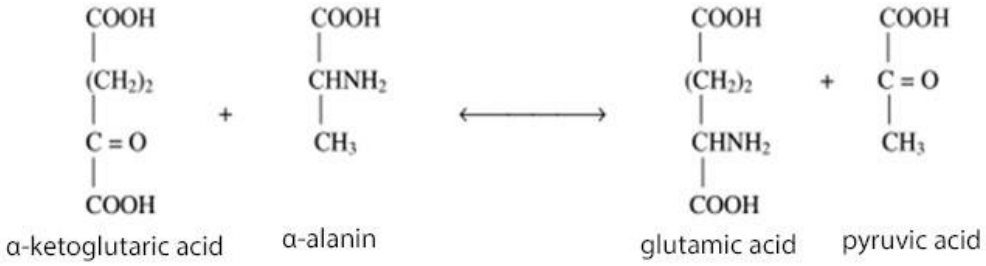
- **Non-oxidative deamination**

  There are several types of non-oxidative deamination:

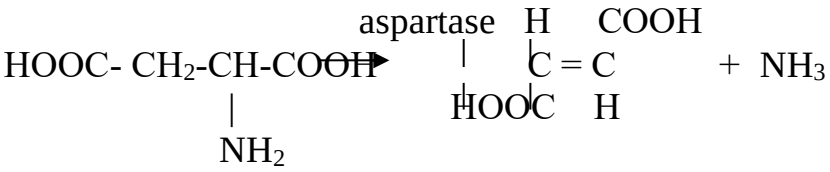
- elimination of the amino group - with unsaturated acid formation
- hydrolytic deamination - the amino acid is converted to hydroxy acid
- reducing deamination - a saturated amino acid is formed
- transamination. It represents the main direction of amino acid metabolism in the human body.

- **Transamination**

  This extremely important reaction, which takes place in all tissues of the human body (especially active in the liver, kidney, and myocardium), is reduced to the interconversion of two different amino acids and keto acids - a new amino acid and a new keto acid are formed. As a result of transamination, amino acids entering the composition of proteins enter the metabolism, and replaceable acids are synthesized.

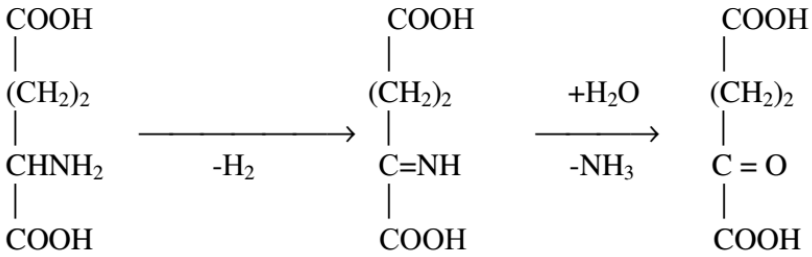


Elimination of the amino group



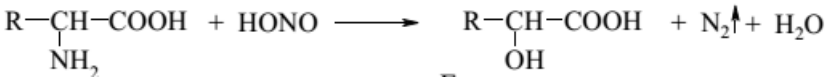
Aspartic acid fumaric acid (trans isomer)

Oxidizing deamination



In vitro:

Van Slyke`s Reaction is Amino Group Quantification Method



hydroxyacid

Polycondensation reactions are the method of peptide obtaining.

Peptide synthesis

The following transformations of the starting amino acids are necessary for the peptide synthesis:

1) Preliminary temporary protection of all (or some) functional groups that do not participate in the formation of the peptide bond.

There are two types of protecting groups:

a) N-protective (temporary protection of the NH_2 group);

b) C-protection (temporary protection -COOH group).

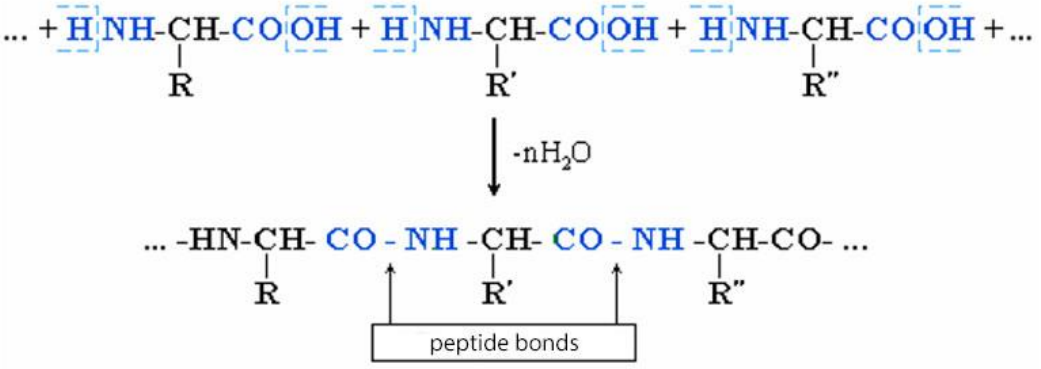
2. Additional activation of those groups that directly form a peptide bond.

3. Removal of protective groups after the formation of the peptide bond.

Polymers consisting of α-amino acids are called polypeptides, or peptides. The amide bond (CO-NH) in such compounds is called the peptide bond (peptide group).

In biological systems, the synthesis of polypeptides occurs on ribosomes, each amino acid is associated with transport RNA, different acyl-tRNAs sequentially lengthen the polypeptide chain in accordance with the triplet code of mRNA localized on the ribosome. The beginning of the polypeptide chain is an amino acid, containing a free α-amino group and end-containing a free α-carboxyl group.

The consecutive connection of amino acids is called the primary structure of the protein.



Peptide nomenclature

Polypeptides are called derivatives of the C-terminal amino acid, the name begins with the N-terminal amino acid, lists everything in order (changing the ending to yl), ends with the C-terminal amino acid. Full names can be recorded, abbreviated in Latin transcription.

Example: glycylalanyl glutamyl valine (gly-ala-gly-val)

The use of amino acids. Medical - biological significance of amino acids

In addition to participating in the biosynthesis of proteins, amino acids perform many other independent functions.

1. Amino acids participate in the biosynthesis of neurotransmitters and hormones:

- Acetylcholine mediator of the parasympathetic nervous system is formed from the amino acid serine

- from phenylalanine or tyrosine, a mediator of the sympathetic nervous system noradrenaline is formed, norepinephrine and hormones adrenaline, thyroxine

- GABA is synthesized from glutamic acid

2. Amino acids glycine, glutamic acid have neurotransmitter functions

3. Aspartic acid is necessary in the synthesis of nitrogenous nucleic acid bases (adenine, guanine, uracil, thymine, cytosine)

4. Glutamic and aspartic acids are involved in the neutralization of ammonia

5. Amino acid methionine transfers its active methyl group to form thymine, choline, adrenaline.

6. Under conditions of carbohydrate starvation, glucose is synthesized from amino acids in the human body.

Therefore, amino acids are used as medical preparation: glutamine, methionine, glycine, cysteine, tryptophan.

Physico-chemical properties of proteins. The concept of structural organization of proteins

The characteristic physical properties of proteins are the high viscosity of solutions, limited ability to diffuse, the ability to significantly swell, optical activity,

mobility in an electric field. Proteins have a high hydrophilicity, that is caused by the high oncotic pressure of proteins. Protein solutions have low osmotic pressure.

Proteins are capable of absorbing ultraviolet radiation with a wavelength of less than 280 nm.

Protein molecules have a large molecular weight (6,000-1,000,000) and are not able to penetrate semipermeable artificial membranes and biomembranes of healthy living organisms.

The form of protein molecules

In nature, there are two types of protein molecules: filiform (fibrillar) and spherical (globular). The physicochemical and biological properties of proteins in a free or bound state are determined by their spatial structure.

Protein denaturation is a process of the protein, olecule spartial structure disorder, leading to the loss of its characteristic properties under the influence of various physical and chemical factors. Externally, denaturation is manifested by a loss of solubility, an increase in viscosity, and a sharp decrease in the biological activity of the protein.

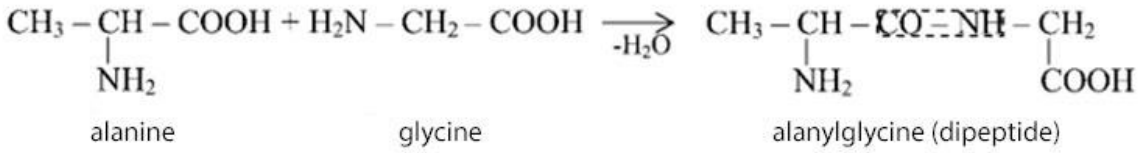
Protein renaturation is the reverse process with complete restoration of the structure and function of the protein molecule. It is possible with a short action of the denaturing agent. Denaturation is irreversible in disturbance of the quaternary, tertiary and secondary structures of the protein.

Isoelectric point of proteins

The pH of the solution, at which the total charge of the protein molecules is zero, is the isoelectric point of the protein (pI). It is determined by the amino acid composition of the protein. At the isoelectric point, proteins are the least stable in solution, easily precipitated.

The concept of the structural organization of protein

Proteins are complex polypeptides, the amino acids in which are linked by peptide bonds resulting from the interaction of the α-carboxyl and α-amino groups of amino acids.



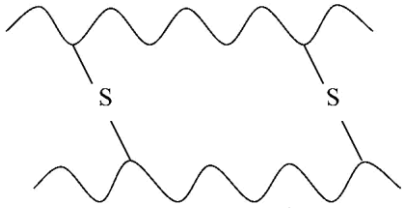
Other amino acids can attach to the dipeptide, forming a tri-, tetra-, pentapeptide, and so on up to the formation of a large polypeptide. The sequence of amino acids in the polypeptide is the primary structure of the protein.

In total, there are four levels of structural organization of the protein - primary, secondary, tertiary and quaternary structure. The structure of most proteins consists of three levels.

Primary and secondary protein structure

The primary structure of the protein is the sequence of amino acid residues in the polypeptide.

Some proteins are represented not by one, but by several polypeptide chains linked together by disulfide bonds.



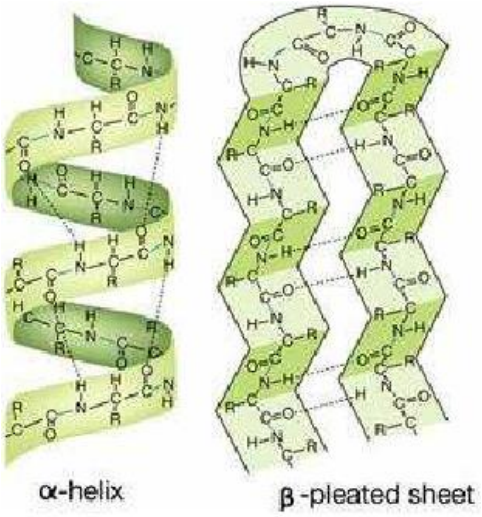
To determine the primary structure of the protein by hydrolysis, the quantitative ratio of amino acids in the protein molecule is determined. Then, the chemical nature of the terminal amino acids of the polypeptide chain, which contains one free NH_2 and COOH group, is determined. The Sanger method and the phenylhydantoin method are used to determine the N-terminal amino acid; the Akabori method is used to determine the C-terminal amino acid.

The primary structure of the protein is characterized by several features:

- The primary structure of the protein is genetically determined and unique, the replacement of amino acids within the polypeptide chain leads to a change in the structure and function of the protein.
- The primary structure of the protein is stable, which is ensured by dipeptide and to a lesser extent disulfide bonds.
- The number of amino acid combinations in the polypeptide is very large, repeating amino acid sequences are rare. Identical amino acid sequences can occur in the region of active centers of enzymes.
- The primary structure of the protein determines the secondary, tertiary and quaternary structure of the protein molecule.

The secondary structure of the protein is the configuration of the polypeptide chain, its more compact packaging in a spiral or some other conformation. The configuration process is in accordance with the program embedded in the primary structure of the protein. There are two main configurations of the polypeptide chain: the α-helix and the β-folded layer.

The active principle in the formation of the α and β structures is the ability of amino acids to form hydrogen bonds. The stability of the secondary structure is ensured mainly by hydrogen bonds and to a lesser extent peptide and disulfide bonds. A hydrogen bond is a weak electrostatic interaction between an electronegative atom (O or N atom) and a hydrogen atom covalently bonded to another electro-negative atom. The main types of hydrogen bonds:



The structure of the α-helix has a number of patterns. For each step of the helix there are 3.6 amino acid residues, the step of the helix is 0.54 nm per turn and 0.15 nm per one amino acid residue. The helix angle of 26 °, after every five turns, the structural conformation of the polypeptide is repeated. When a β-structure is formed, two or more linear polypeptide chains arranged parallel or antiparallel are linked by hydrogen bonds between peptide bonds. In nature, there are proteins whose secondary structure is neither an α- nor β-structure (for example, collagen). Now, the existence of two intermediate levels of the organization of a protein molecule between the secondary and tertiary structure has been proved so-called - sub-secondary structures and structural domains.

Tertiary and quaternary protein structure

The tertiary structure of the protein is the spatial orientation of the polypeptide helix, which follows the secondary structure of the compaction method of the protein molecule. The process of packing the polypeptide chain is folding.

The spatial structure of proteins depends on the ionic strength and pH of the solution, temperature and other factors. In the stabilization of the spatial structure of proteins, along with covalent bonds (peptide and disulfide), the so-called non-covalent bonds electrostatic interactions of charged groups, van der Waals forces, interactions of non-polar lateral amino acid radicals, hydrophilic-hydrophobic interactions, etc. take part, which include hydrogen bonds. Non-covalent bonds play a major role. The tertiary structure of the protein is formed spontaneously and is completely determined by the primary structure of the protein. The main driving force behind the emergence of the three-dimensional structure is the interaction of amino acid radicals with water molecules, consisting in the fact that hydrophobic radicals of amino acids are oriented inside the protein molecule, while hydrophilic radicals are oriented outward. As a result, the thermodynamically most favorable conformation of the protein molecule is formed, which is characterized by the least free energy. Conformations of proteins are stable, the main forms of conformations are the T-form (tensed) and the R-form (relaxed). The three-dimensional structure of the protein contains functional information that determines all the biological properties of proteins. Violation of the tertiary structure of the protein entails the loss of its biological properties.

The quaternary structure of a protein is the spatial orientation of several polypeptide chains with their own primary, secondary and tertiary structure, with the formation of a macromolecule.

Individual polypeptide chains – protomers (monomers, subunits) - do not have biological activity and acquire it with a certain mode of spatial association. The resulting molecule is an oligomer (multimer).

The quaternary structure is stabilized due to non-covalent bonds between the contact pads of the protomers complementary to each other. The quaternary structure is found in several hundred proteins. The hemoglobin molecule is a tetramer – consists of two α and two β chains. The phosphorylase enzyme consists of two identical subunits of two peptide chains. The lactate dehydrogenase enzyme contains two types of polypeptide chains - M (muscle) and B (brain) and can exist in five forms, called isoenzymes, or multiple forms of enzymes.

Classification of proteins

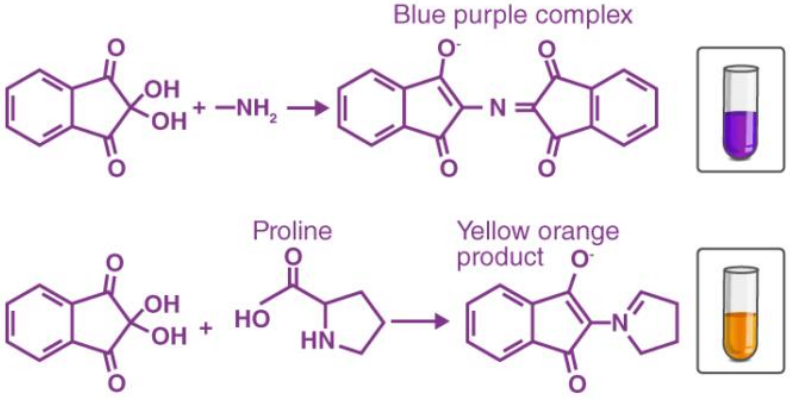
In accordance with the functions performed, 12 main classes of proteins are distinguished:

1) catalytically active proteins (enzymes);
2) proteins - enzyme inhibitors;
3) proteins - regulators of genome activity;
4) protective proteins: proteins of the immune and coagulation system;
5) toxic proteins;
6) transport proteins;
7) membrane proteins;
8) contractile proteins;
9) receptor proteins;
10) hormone proteins;
11) proteins - envelopes of viruses;
12) proteins with other functions.

Qualitative reactions to amino acids, peptides, proteins

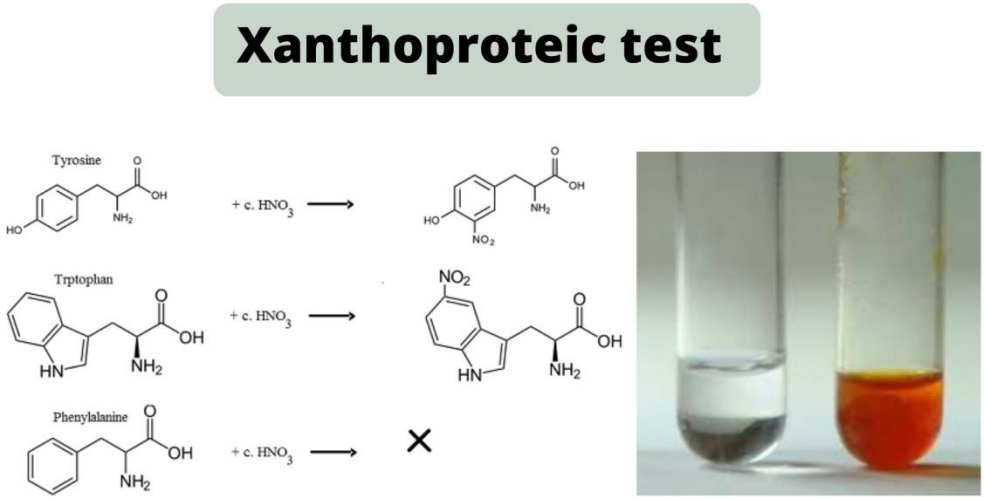
Amino acids can be detected using color reactions: ninhydrin, xanthoprotein, Fole, Milon, biuret test, etc. These reactions are nonspecific, because based on the detection of individual fragments in the structure of amino acids, which can occur in other compounds.

Ninhydrin reaction, a color reaction used for the qualitative and quantitative determination of α-amino acids, amino acid amines. When ninhydrin (triketohydrin dehydrate, $C_9H_6O_4$) is heated in an alkaline medium with substances having primary amino groups ($-NH_2$), a product is formed that has a stable intense blue-violet color with maximum absorption about 570 nm. Since absorption at this wavelength linearly depends on the number of free amino groups, the ninhydrin reaction served as the basis for their quantitative determination by colorimetry or spectrophotometry. This reaction is also used to determine secondary amino groups (> NH) in the amino acids proline and hydroxyproline; in this case a bright yellow product forms colors. Sensitivity - up to 0.01%. Modern automatic amino acid analysis is carried out by combining the ion-exchange separation of amino acids and their quantitative determination using the ninhydrin reaction. When separating mixtures of amino acids by paper chromatography, it is possible to determine each amino acid in an amount of at least 2-5 micrograms.



The amount of amino acids can be judged by the color intensity. This reaction is positive not only with free amino acids, but also with peptides, proteins, etc.

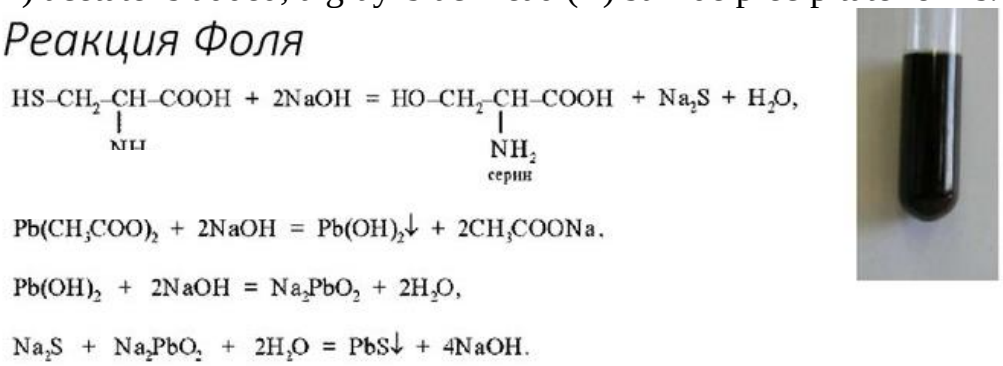
The xanthoprotein reaction allows you to detect aromatic amino acids (phenylalanine, tyrosine, histidine, tryptophan), based on the electrophilic substitution reaction in the aromatic nucleus (nitration).



When concentrated nitrate acid acts on tyrosine, for example, a yellow colored product forms.

Foley's reaction

This is a reaction to cysteine and cystine. During alkaline hydrolysis, "weakly bound sulfur" in cysteine and cystine is quite easily cleaved, resulting in the formation of hydrogen sulfide, which, reacting with alkali, gives sodium or potassium sulfides. When lead (II) acetate is added, a gray-black lead (II) sulfide precipitate forms.



Biuret test

Biuret reaction may be used as a color reaction to proteins. In an alkaline medium in the presence of cuprum (II) salts, they give a violet color. The color is due to the formation of the complex compound cuprum (II), with peptide group —CO — NH—, which is characteristic of proteins. This reaction got its name from a urea derivative - biuret, which is formed by heating of urea with the elimination of ammonia:

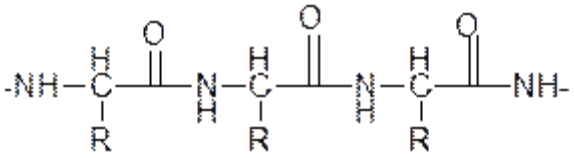
$$H_2N\text{-}CO\text{-}NH_2 + H_2N\text{-}CO\text{-}NH_2 \rightarrow H_2N\text{-}CO\text{-}NH\text{-}CO\text{-}NH_2 + H_2O$$

biuret

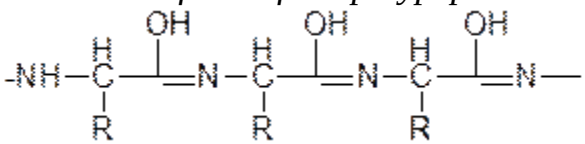
In addition to proteins and biuret, other compounds containing this group also give the same coloration: amides, carboxylic acid imides, as well as compounds

containing -CO-NH- or -CO-NH-CO- in the molecule. Proteins, some amino acids, peptides, biuret and middle peptones also give a reaction.

The color of the complex obtained by the biuret reaction with various peptides is slightly different and depends on the length of the peptide chain. Peptides with a chain length of four amino acid residues and above form a red complex, tripeptides form a violet, and dipeptides form a blue.

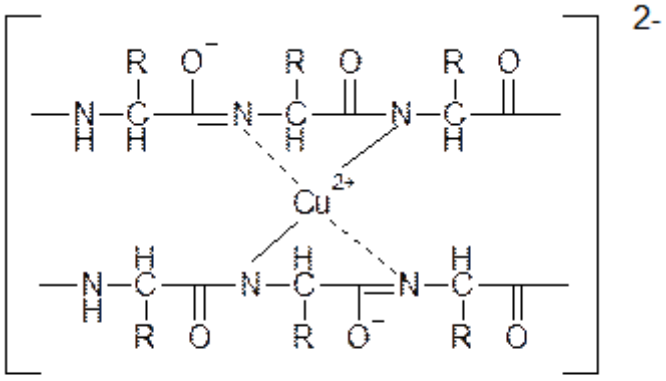


ketone form of the polypeptide



enol form of the polypeptide

When the polypeptide interacts with $Cu(OH)_2$, a complex forms, the structure of which can be shown as follows:



General material and educational and methodological support of the lecture:

- Working program of the discipline
- Silabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational tasks

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.

4. Construction features, chemical properties and the biological role of carbohydrates. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova, K.V. Bevziuk. Teaching aid. Odesa, 2017. – 44 p.
5. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
6. Lipids: classification, structural features, properties and biological role. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova. Teaching aid. Odesa, 2017. – p. 32.

Additional:

7. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
8. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
9. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
10. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
11. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
12. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 4

«Heterocyclic compounds as structural components of nucleic acids. The structure of nucleic acids.»

Actuality of theme: The great importance of heterocyclic compounds lies in the fact that they are the basis of many natural biologically active substances and medicines. The most well-known and widely used drugs of natural and synthetic origin more than 62% are heterocyclic compounds. Alkylated pyrrole nuclei are the basis of important biologically active compounds: hemin, chlorophyll, vitamin B12; β-lactam antibiotics - penicillin and cephalosporin - have saved millions of human lives. Nucleic acids are natural biopolymers whose monomers are mononucleotides. Nucleic acids play a major role in the transmission of genetic information and in controlling the process of protein synthesis.

Aims: To form knowledge about the structure and features of the chemical behavior of five-membered heterocyclic compounds with biological activity; to know the principles of structure and chemical properties of nucleic acids and their monomers - nucleotides for understanding their biosynthesis and biological role in the body.

Basic concepts: five-membered heterocyclic compounds, six-membered heterocyclic compounds, natural biologically active substances, nucleosides, nucleotides, ATP, DNA, structure of DNA.

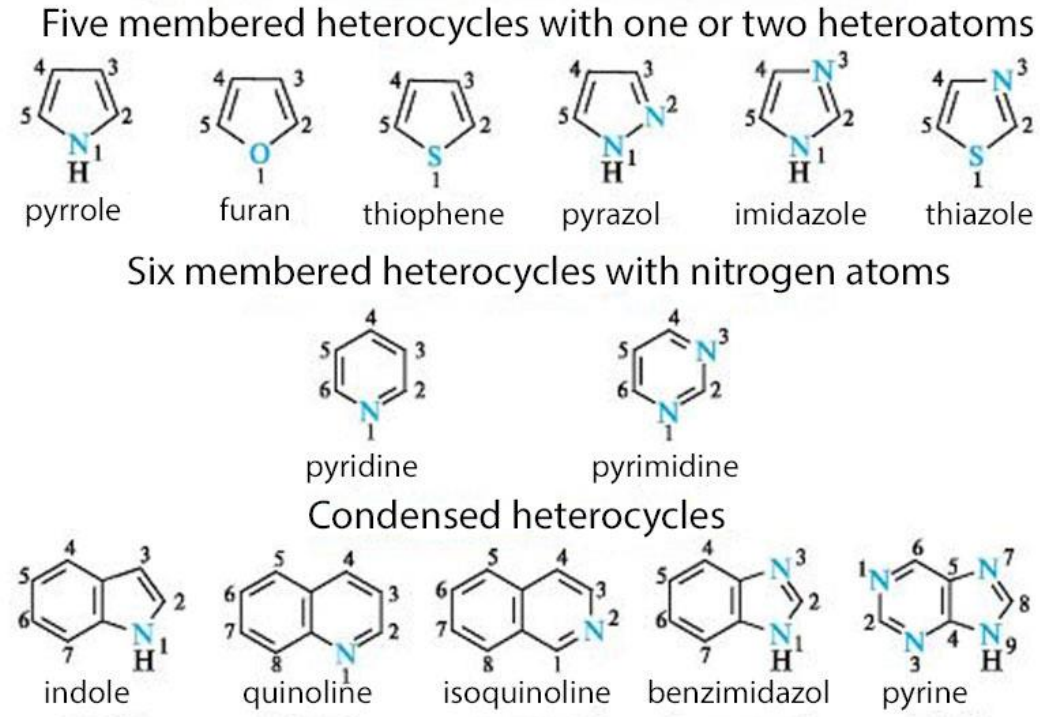
Plan and organizational structure of the lecture:

1. Classification of heterocycles by cycle size, number and type of heteroatoms.
2. Five-membered heterocycles with one and two heteroatoms.
3. six-membered heterocyclic compounds.
4. Pyrimidine and purine derivatives.
5. Structure of nucleotides - components of nucleic acids
6. Structure and significance of 3',5' - c-AMP, its role in the action of hormones on cells.
7. Phosphorylated nucleotide derivatives, biological significance of ADP and ATP.
8. Nucleic acids.
9. The structure and biochemical functions of DNA.

Content of lecture material (lecture text)

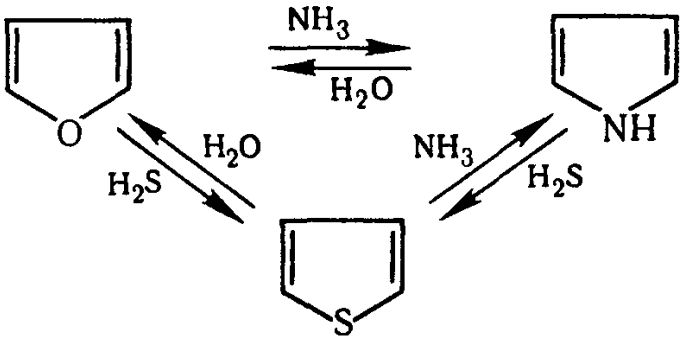
Cyclic organic compounds, which include, in addition to carbon atoms, one or more atoms of other elements (heteroatoms) - O, N, S are called **heterocyclic compounds**.

Classification of heterocyclic compounds



Five-membered heterocycles with one heteroatom

This group includes five-membered aromatic heterocycles: **pyrrole, furan and thiophene.** All three heterocycles are connected with each other by mutual transitions (t ° = 400°, Al_2O_3)



furan thiophene pyrrole

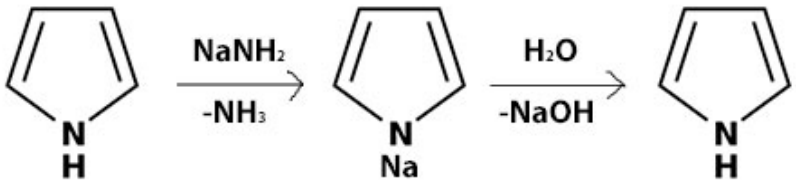
Pyrrole, furan and thiophene belong to the so-called **π-excess** heterocycles, i.e. to compounds with increased electron density inside the ring, since the six-electron π cloud is delocalized in them at 5 atoms of the cycle. These heterocycles have **aromaticity (Hückel rule):**

a) the cyclic system is **flat**;

b) has a **continuous** conjugated chain;

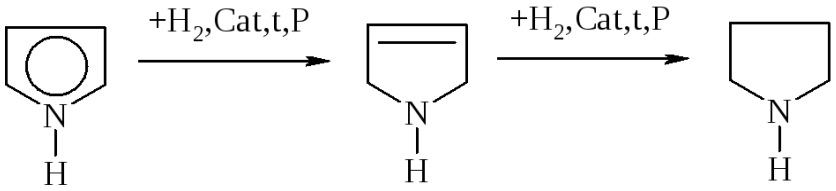
c) contains **(4n + 2)** π-generalized electrons, where n = 1, 2, 3, etc. which is determined by number of cycles.

So, in **pyrrole** from the nitrogen atom, an unshared electron pair located on a non-hybridized p-orbital is included in an aromatic sextet. Three electrons in sp^2 hybrid orbitals participate in the formation of three σ bonds. The atom of the Nitrogen in this electronic state is called **pyrrole nitrogen** and has acidic properties:



sodium pyrrol

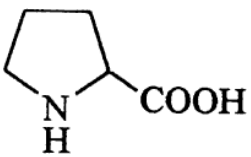
Pyrrole is able to **recover** in the presence of HI, the process is stepwise:



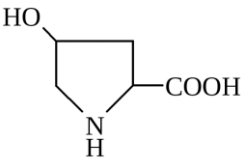
pyrroline pyrrolidine

Pyrrolidine (a cyclic secondary amine) has strongly basic properties. Its core is part of a number of organic compounds.

pyrrolidine

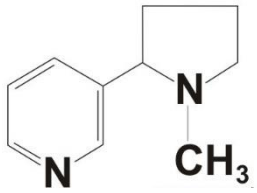


imino acid proline

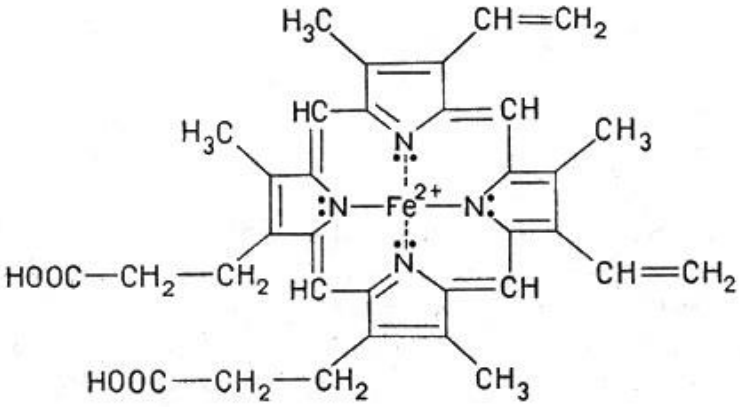


imino acid hydroxyproline

Nicotine alkaloid (up to 8% found in tobacco)



Tetrapyrrole compounds are an important group of nitrogen-containing natural substances, which include four pyrrole rings Pyrrole → Porfin → Protoporphyrin → Gem



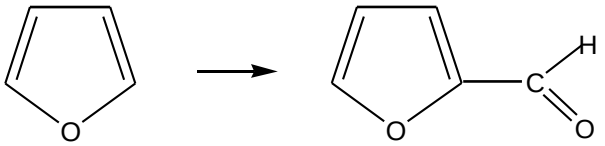
Ferroprotoporphyrin (heme)

The heme contains an atom of a divalent ferrum bound to porphyrin - this is the **prosthetic** group of the complex hemoglobin protein (oxygen-carrying).

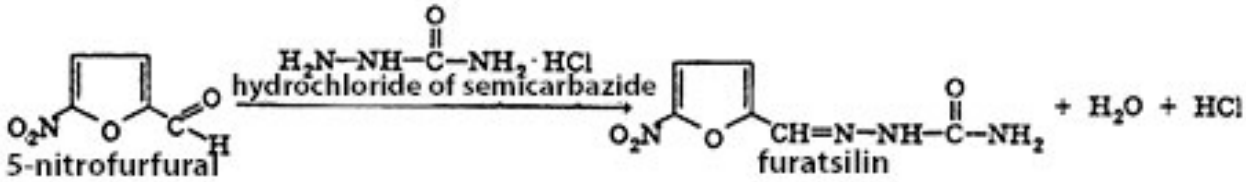
The complex of porphyrin with ferrum is part of a number of enzyme systems: cytochromes, catalase, peroxidase. The complex of porphyrin with magnesium is the basis of the chlorophyll molecule. Pyrrole nuclei connected with cobalt are part of vitamin B_{12} (cyancobalamine), which is necessary for normal hematopoiesis.

Furan and its derivatives resemble pyrrole compounds, they easily enter into electrophilic substitution reactions - they are nitrated, sulfonated. The substituent enters the α-position. Furan is capable of reduction, oxidation reactions.

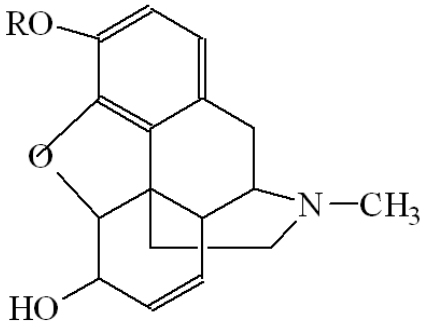
furan furfural



Furan nitro derivatives are medicines - furatsilin, furazomedon, which are effective in purulent-inflammatory processes caused by microorganisms (dysentery, typhoid fever).

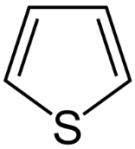


Furan is a part of narcotic substances - morphine, heroin, codeine, etc.



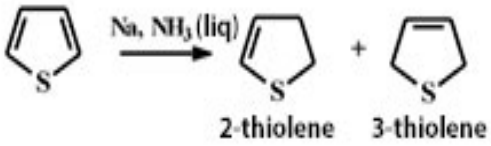
R=H – morphine
R=CH_3 - codeine

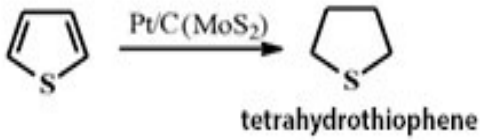
Morphine has a strong analgesic effect. It includes phenanthrene structure. The occurrence of addiction to morphine is known, which leads to the development of addiction.



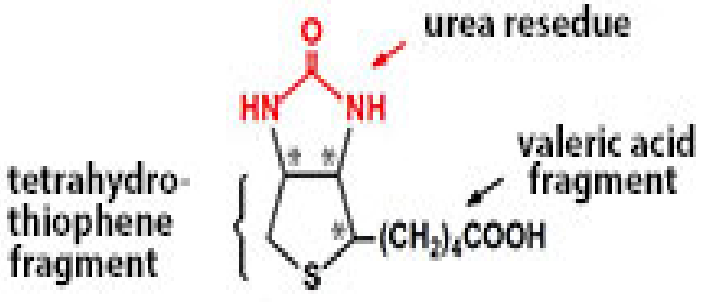
Thiophene – of the five-membered heterocyclic compounds under consideration with one heteroatom, it is closest to benzene in chemical and physical properties (boiling point of thiophene is 84 ° C, benzene 80 ° C). Thiophene is found in coal tar. Thiophene derivatives thereof are part of the ichthyol ointment, which has anti-inflammatory, antiseptic and local analgesic effects.

The reduction of thiophene in the presence of a palladium catalyst leads to the formation of tetrahydrothiophene.

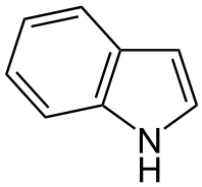




Biotin (vitamin H) is derivative of tetrahydrothiophene. Biotin consists of imidazoline ring that is cis-fused to a tetrahydrothiophene ring bearing a valerate side chain. The chirality at each of its three asimetric centers is indicated (*). Biotin is essential human nutrient. For lack of vitamin H in food the protein and fat metabolism breaks that causes dermatic diseases.



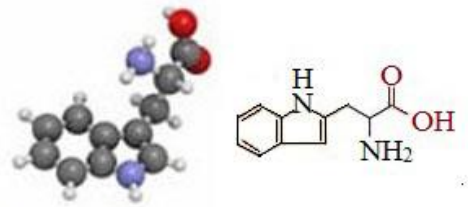
biotin (vitamin H)



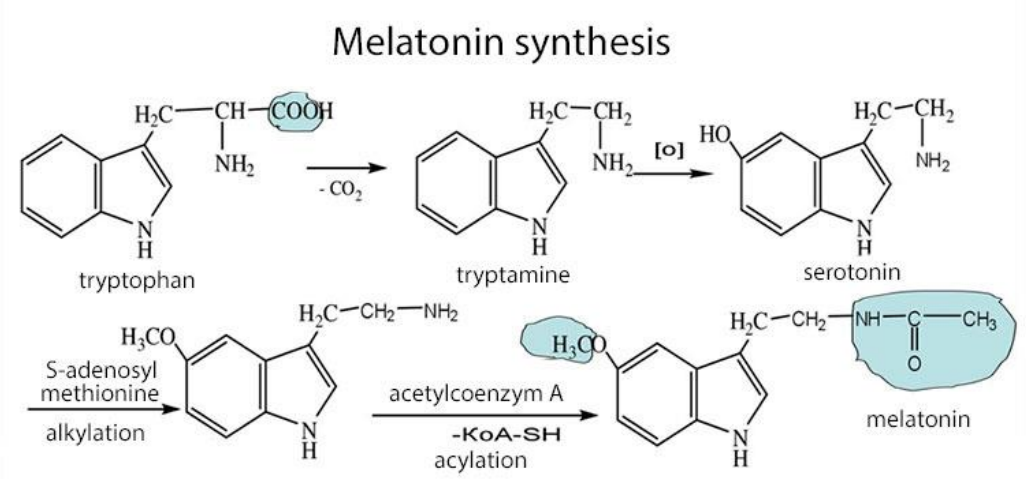
Indole (benzpyrrole)

Indole (benzpyrrole) is a condensed heterocyclic compound composed of benzene and pyrrole nuclei having a common joint. Indole is aromatic. Like naphthalene, its socialized π-system contains 10 electrons (4n + 2, for n = 2). By its properties, indole resembles pyrrole. It practically does not possess the basic properties, in some reactions it behaves like weak NH-acid, it quickly darkens in air due to oxidation. Indole enters into electrophilic substitution reactions actively, the β-position of the pyrrole core of indole being the most reactive.

Many indole derivatives are found in nature and have diverse biological activity.



Tryptophan (α-amino-β-indolylpropionic acid) is an amino acid that is part of proteins. In the process of metabolism, tryptophan is capable of hydroxylation, decarboxylation.

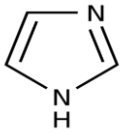


Serotonin is one of the mediators of the brain. Violation of its normal metabolism in the body leads to the development of schizophrenia. Serotonin is part of some biologically active substances that dramatically disrupt mental activity. So, psilocybin, lysergic acid diethylamide (LSD), which cause visual hallucinations, contains serotonin.

Melatonin is the main epiphysis hormone, the regulator of daily rhythms.

Five-membered heterocycles with two heteroatoms

Five-membered heterocycles with two heteroatoms are more stable, and they are characterized by lower activity in electrophilic substitution reactions compared to five-membered heterocycles with one heteroatom. They are prone to tautomeric transformations and to the formation of intermolecular hydrogen bonds.

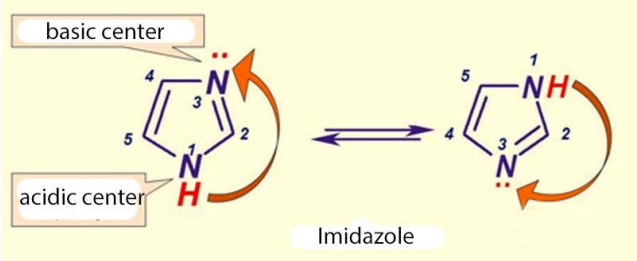


Imidazole is a five-membered heterocycle with two nitrogen atoms located in the first and third positions of the ring. One of these atoms is similar to the nitrogen atom in pyrrole and is responsible for the weakly acidic properties of imidazole, the other is similar to the "pyridine" nitrogen atom and determines the weakly basic properties of imidazole. Thus, imidazole is an amphoteric compound, it forms salts with strong acids and with alkali metals.

Tautomerism is an equilibrium dynamic isomerism. Its essence lies in the mutual conversion of isomers with the transfer of any mobile group and the corresponding redistribution of electron density.

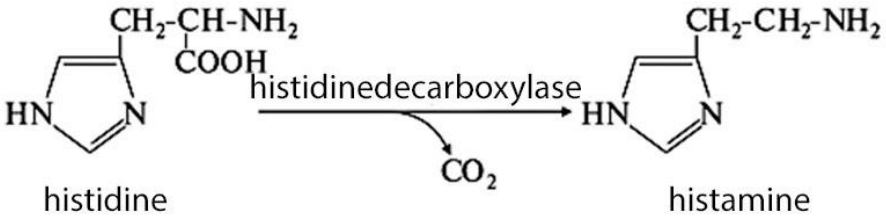
Prototropic (azole) tautomorism of heterocycles

Tautomerism is dynamic equilibrium isomerism. Tautomers convert to each other spontaneously and exist in dynamic equilibrium due to carrying over some mobile group.

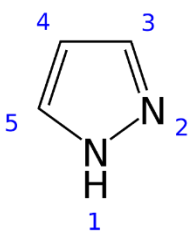


Hydrogen transfer is carried out so quickly (approximately 10 times per second) that it is not possible to isolate individual 4- or 5-monosubstituted imidazoles (“fast” tautomerism). Many imidazole derivatives are found in nature and have great biological significance. The most important are the amino acid histidine and the product of its decarboxylation histamine.

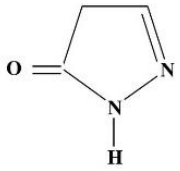
Histidine (α-amino-β-imidazolylpropionic acid) is part of many proteins, including globin. In hemoglobin, due to the "pyridine" nitrogen atom of the imidazole fragment of this acid, the globin protein binds to the heme ferrum atom.



Pyrazole is an isomer of imidazole. Nitrogen atoms in the pyrazole cycle are located nearby. The chemical behavior of these two isomers has much in common.



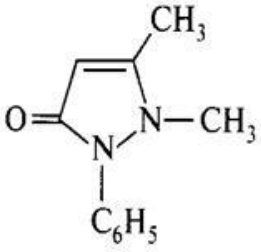
Pyrazole derivatives were not found in nature, but important drugs were created on its basis. Most of them are derivatives of pyrazolone-5



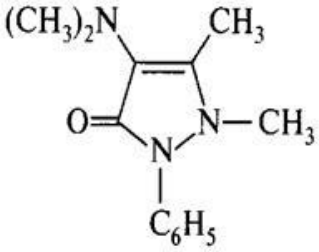
Pyrazolone-5

Pyrazolone-5 is the basic structure of analgetic drugs.

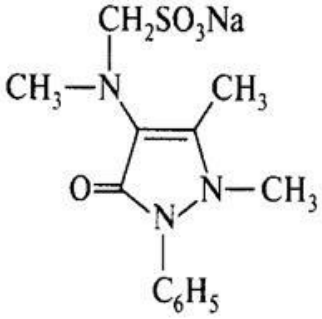
Pyrazolone medicines



Antipyrine (1-phenylpyrazolone-5)



amidopyrile (2,3-dimethyl-1-phenyl pyrazolone-5 -(2,3-dimetyl- -4-dimethylamino-pyrazolone-5)

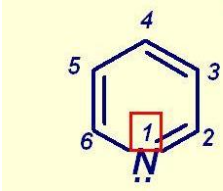


analgin

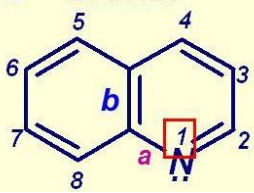
Antipyrine and amidopyrine are widely used in medicine as antipyretic, analgesics and sedatives. Analgin - is a sulfo derivative of amidopyrine. It may be used in liquid form. It is superior in activity and speed of reaction than amidopyrine and antipyrine.

Six membered heterocycles with one heteroatom

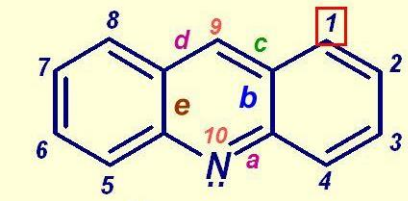
This group includes aromatic heterocyclic compounds containing a six-membered ring with one nitrogen heteroatom: pyridine, quinoline, isoquinoline, acridine – azines.



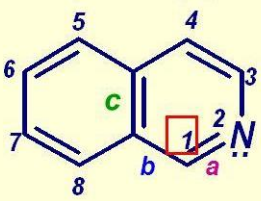
Pyridine azine



Quinolone, benzo[b]-pyridine



Acridine dibezo[b,e] pyridine



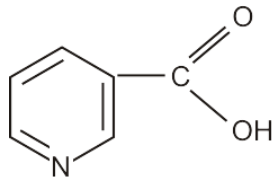
Isoquinoline benzo[c]-pyridine

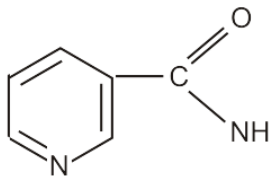
Pyridine C_5H_5N is the most important of the natural six-membered heterocycles with one heteroatom. It has a characteristic unpleasant odor, poisonous, inhalation of its vapor can lead to severe damage to the nervous system. Pyridine is an aromatic heterocyclic compound having basic properties. The presence of a heteroatom leads to

an uneven distribution of electron density. Thus, in pyridine, the nitrogen atom is in a state of sp_2 hybridization (two of the three sp_2 hybrid orbitals form σ bonds). It supplies one p-electron to an aromatic sextet.

Nicotinic acid and nicotinamide

Nicotinic acid and its amide, nicotinamide, have gained fame as two forms of vitamin PP, used in medicine to treat pellagra (antipellagric vitamin).

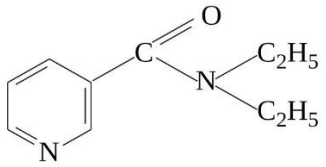




2

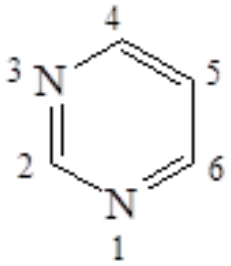
Nicotinic acid Nicotinamide

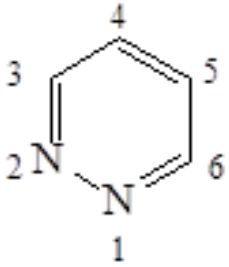
Nicotinamide is an integral part of the enzyme systems of dehydrogenases responsible for redox processes in the body (NAD-nicotinamide adenine dinucleotide), and nicotinic acid diethylamide (cordiamine) serves as an effective stimulator of the central nervous system. They can be obtained from nicotinic acid by common methods:

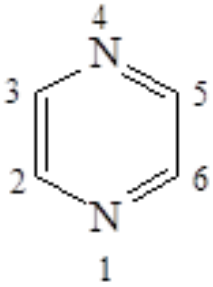


Six-membered heterocycles with two nitrogen atoms

This group includes three isomeric six-membered heterocycles - pyridazine, pyrimidine, pyrazine.



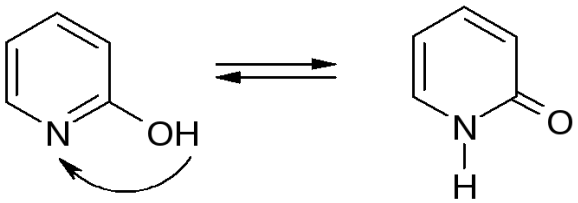




Pyrimidine Pyridazine Pyrazine

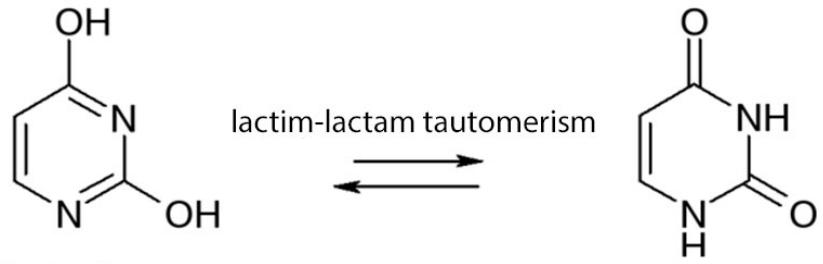
The introduction of the second nitrogen atom into the six-membered ring further reduces the activity of the heterocyclic nucleus (compared to pyridine) in electrophilic substitution reactions. The basicity of diazines also decreases. The introduction of electron-donating — OH and NH_2 groups into the molecule significantly increases their reactivity.

Especially important are the hydroxy and amino derivatives of pyrimidine - uracil, thymine and cytosine - the components of nucleic acids. They are characterized by lactim-lactam tautomerism, which occurs due to the transfer of hydrogen between nitrogen and oxygen.



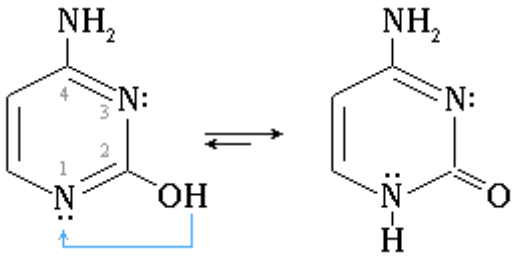
lactim form lactam form

2-hydroxypyridine



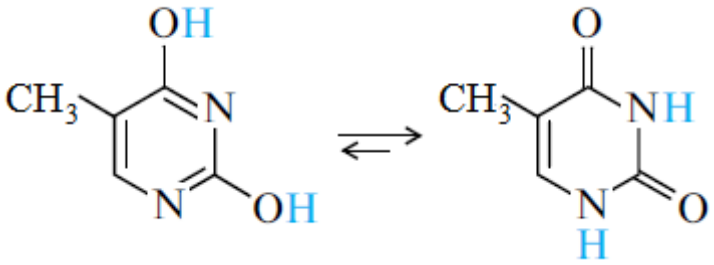
lactim form lactam form

uracil (2,4-dihydroxypyrimidine)



lactim form lactam form

cytosine (4-amino-2-hydroxypyrimidine)

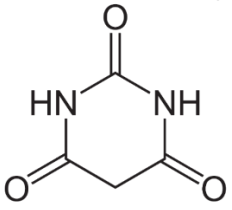


lactim form lactam form

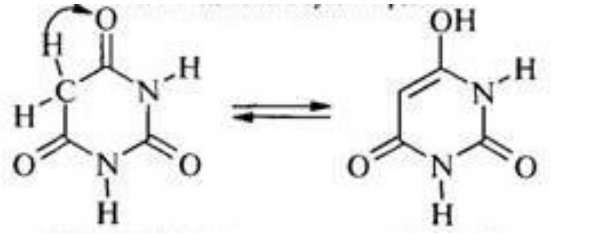
thymine (5-methyluracil)

Barbituric acid and its derivatives

They contain a pyrimidine ring and form two types of tautomeric forms:

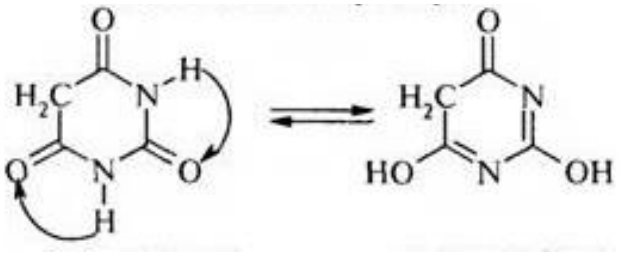


Keto-enol tautomerism



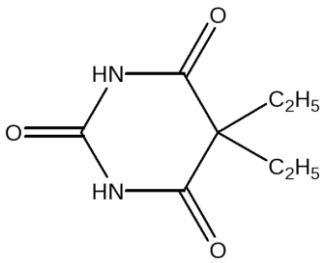
keto form enol form

lactim-lactam tautomerism

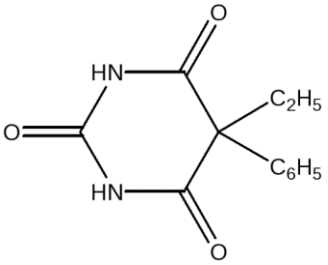


lactam form lactim form

Derivatives of barbituric acid belong to narcotic hypnotics.



Barbital (Veronal, 5.5-diethyl-barbituric acid)

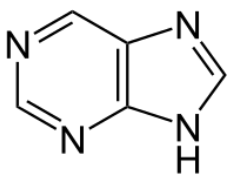


Phenobarbital (luminal, 5-ethyl-5-phenyl-barbituric acid)

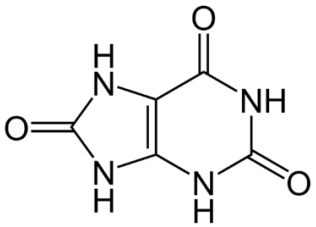
The continuous use of barbiturates for a long period leads to the development of addiction and can be the cause of drug dependence (mental and physical). Drug withdrawal in the presence of drug dependence is accompanied by severe mental and somatic disorders (withdrawal syndrome). There is anxiety, irritability, fear, vomiting, visual impairment, convulsions, etc. In severe cases, death may occur.

Purin and its derivatives

Purine is a bicyclic heterocyclic compound formed by the condensed nuclei of pyrimidine and imidazole.



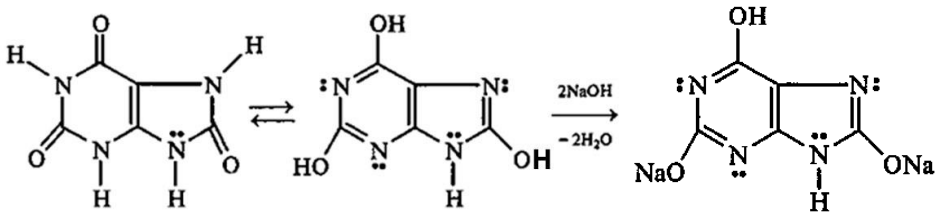
Purine



Uric acid

The purine system is aromatic and submits to the Hückel rule. Purine is resistant to oxidizing agents, soluble in water, forms salts with both strong acids and alkali metals.

Uric acid is the end product of the metabolism of purine compounds in the body. Uric acid is dibasic, poorly soluble in water, but easily soluble in alkalis. Uric acid salts are called urates. With some disorders in the body, they are deposited in the joints, for example, with gout, as well as in the form of kidney stones, causing severe pain.



Uric acid Sodium urate

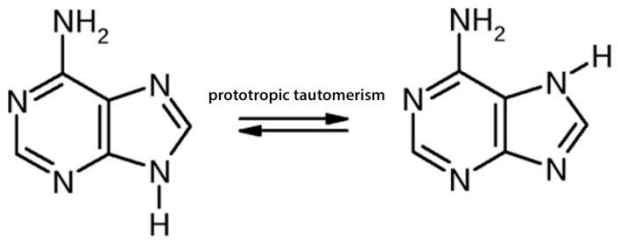
Aminopurines - components of nucleic acids

Of the aminopurines, the most important are 6-aminopurine or adenine, 2-amino-6-hydroxypurin or guanine, which are essential components of nucleic acids.

Adenine Guanine

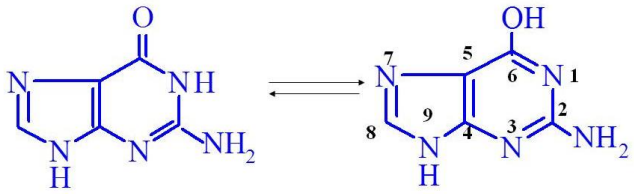
For adenine, prototropic tautomerism is possible due to the migration of hydrogen between N_7 and N_9 in the imidazole ring:

tautomeric forms of 6-aminopurine (adenine)



For guanine, in addition to prototropic tautomerism, lactim-lactam tautomerism is possible.

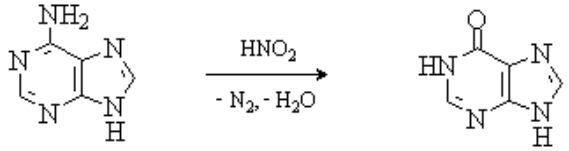
Lactim-lactam tautomerism



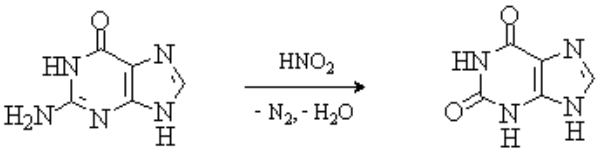
lactam form lactim form

Guanine (2-amino-6-hydroxypurine)

In the process of metabolism in the body (in vivo) and outside the body (invitro), under the influence of HNO_2 deamination of adenine and guanine occurs with the formation of hypoxanthine and xanthine, respectively, which are the precursors of uric acid during the breakdown of aminopurines.

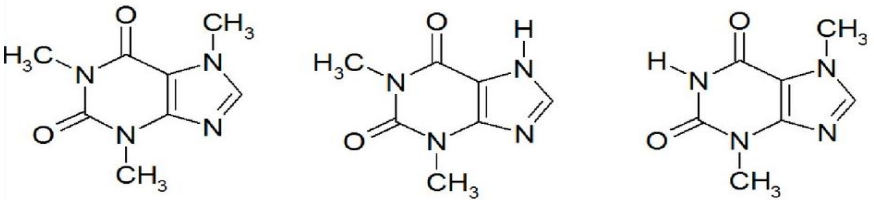


Adenine Hypoxanthine



Guanine Xanthine

Methylated xanthines (alkaloids) are caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine).



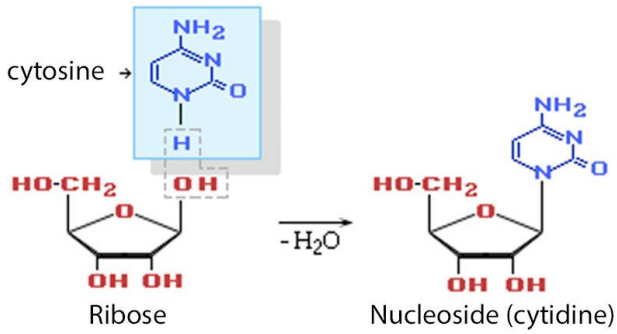
Caffeine Theophylline Theobromine

found in tea, coffee, mate leaves, guarana paste, cola nuts — *principal alkaloid of cacao bean, also in cola nuts and tea* — *small amounts in tea*

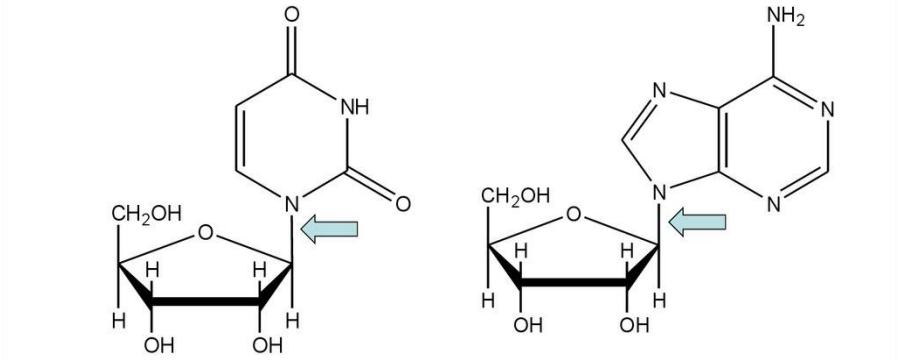
Nucleosides

Nucleoside is called N-β-glycoside, in which the aglycon (non-carbohydrate part) is a nitrogenous base derived from pyrimidine or purine. Depending on which monosaccharide is part of the nucleoside, they are divided into two types - ribosides and deoxyribosides.

Nucleoside components and structure



Nucleosides are N-glycosides of ribose and deoxyribose with pyrimidine and purine bases. N-glycosidic linkage may be hydrolyzed in acidic medium.



Uridine Adenosine

Nucleosides are intermediate compounds in the synthesis of nucleotides, and are not involved in other metabolic processes in the cell, but synthetic nucleosides are used as drugs. They have less toxicity than the aglycon (derivative of pyrimidine or purine) included in their composition, nucleosides are better absorbed compared to free nitrogen bases.

Nomenclature

Nucleosides containing **pyrimidine** in their name have an ending **idine.**

Nucleosides containing **purine** in their name have an ending – **ozine.**

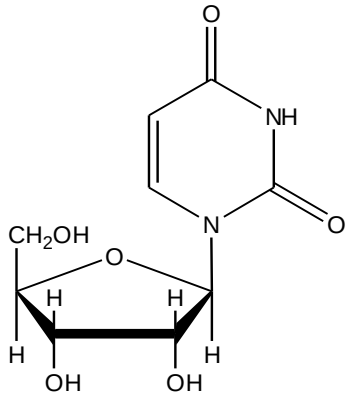
Pay attention to the nomenclature of nucleosides containing thymine.

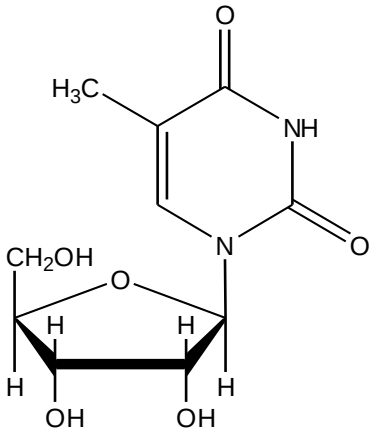
Thymine is the base of DNA, and if the nucleoside contains deoxyribose, then the name of the nucleoside (**thymidine**) does not need to emphasize the chemical nature of the carbohydrate. If thymine is associated with ribose, which is an atypical biological situation, then the name indicates the name of the carbohydrate (**thymidine riboside**)

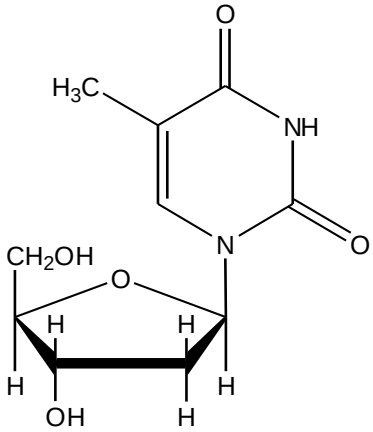
The most common nucleosides

Type of bond - N-β-glycosidic linkage

Nitrogen base	Nucleoside carbohydrate ribose	Nucleoside carbohydrate deoxyribose
uracil	uridine	deoxyuridine
cytosine	cytidine	deoxycytodine
thymine	ribothymidine	thymidine
adenine	adenosine	deoxyadenosine
guanine	guanosine	deoxyguanosine
hypoxanthine	inosine	deoxyinosine

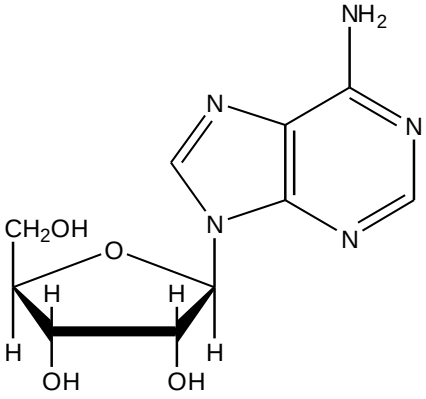




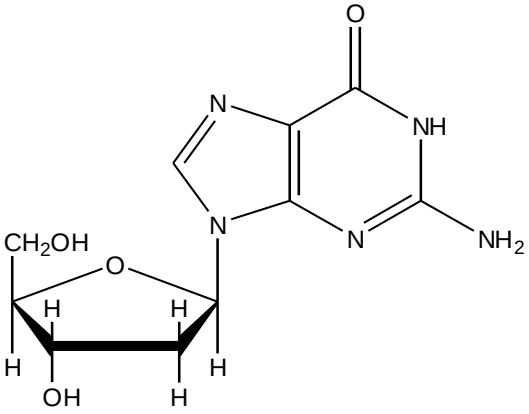


Uridine thymidine riboside thymidine

In natural nucleosides containing adenine, guanine, hypoxanthine and other purine compounds, an N_9-β-glycosidic bond is always formed



Adenosine



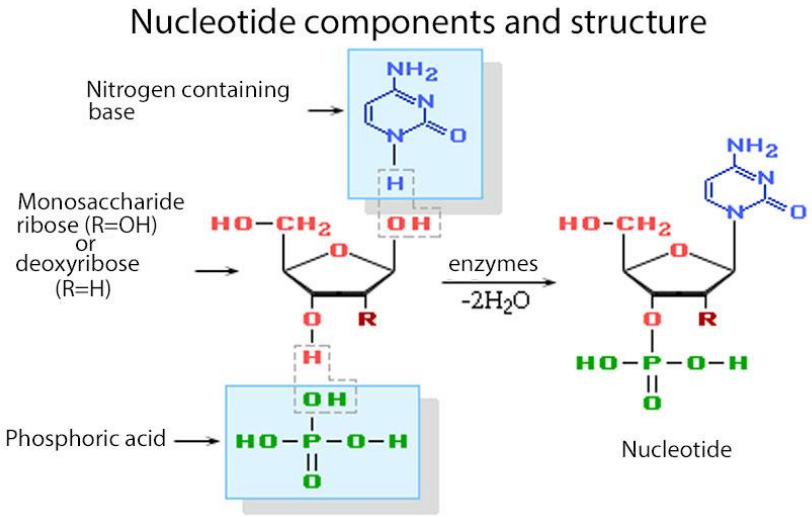
Deoxyguanosine

All the above figures show the actual spatial relative position of the carbohydrate and nitrogen base:

- rotation around the glycosidic bond is difficult;
- the location of the pentose cycle to the left of the bond corresponds to the only correct image of the **β-glycosidic** bond;
- carbonyl and amino groups of the cycles are turned in the opposite direction from pentose (so these groups will be able to participate in the creation of complementary pairs).

Nucleotides

Nucleotides are phosphoric esters of nucleosides. Their chemical composition: nitrogen base + pentose + phosphoric acid.



Phosphorus esters are formed with the participation of the hydroxyl groups of pentoses. The position of the phosphorus-ester groups is usually designated using the notation ('), for example: 5', 3'. Nucleotides play an extremely important role in the life of the cell.

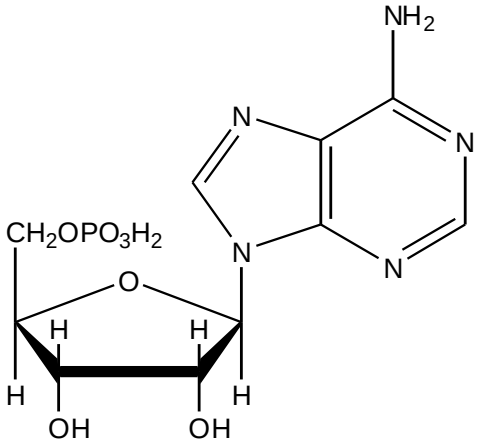
Nucleotide classification

Nucleotides consisting of one nitrogen base molecule, pentose, phosphoric acid are called mononucleotides. Mononucleotides may contain one molecule of phosphoric acid, two or three molecules of phosphoric acid, connected to each other. The combination of two mononucleotides is called a dinucleotide. In the composition of the dinucleotide, various nitrogenous bases or one other cyclic compound are usually present. A special role in biochemical processes is played by cyclic mononucleotides.

The nomenclature of mononucleotides.

Depending on the amount of phosphate residues, “monophosphate”, “diphosphate”, “triphosphate” is added to the name of the nucleoside, indicating their position in the pentose cycle — a digital designation of the place with a badge (').

The position of the phosphate group in position (5 ') is the most common and typical, therefore, it can be omitted (AMP, GTP, UTP, dAMP, etc.) The remaining provisions are indicated necessarily (3'-AMP, 2'-AMP, 3'-dAMP)



5'-adenosine monophosphate (5'- AMP or AMP)

Names of the most common nucleotides

nucleoside	nucleoside monophosphate	nucleoside diphosphate	nucleoside triphosphate
adenosine	5'-Adenosine Monophosphate (5'-AMP or AMP) 5' adenylic acid	5'-adenosine diphosphate (5'-ADP or ADP)	5'-adenosine triphosphate (5'-ATP or ATP)
adenosine	3'-adenosine monophosphate (3'-AMP) 3 'adenylic acid	not found in vivo	not found in vivo
guanosine	5'-guanosine monophosphate (5'- GMF or GMF)	5'-guanosine diphosphate (5'- GDF or GDF)	5'-guanosine triphosphate (5'-GTF or GTF)
guanosine	3'-guanosine monophosphate (3'-GMF) 3'-guanylic acid	not found in vivo	not found in vivo
deoxy adenosine	5'-deoxyadenosine monophosphate (5'-dAMP or dAMP)	5'-deoxyadenosine diphosphate (5'-dADP or dADP)	5'-deoxyadenosine triphosphate (5'-DATP or DATP)
uridine	5'-uridine monophosphate (5'-UMF or UMF)	5'-uridine diphosphate (5'-UDP or UDP)	5'-uridine triphosphate (5'- UTF or UTF)
cytidine	5'-cytidine monophosphate (5'-CMF or CMF)	5'-cytidine diphosphate (5'- CDF or CDF)	5'-cytidine triphosphate (5'- CTF or CTF)

Nucleotides formed with the participation of ribose can contain phosphoric acid residues in three positions (5 ', 3', 2 '), and with the participation of deoxyribose - only in two positions (5', 3 '), there is no hydroxy group in position 2'. This fact is very important for the structure of DNA.

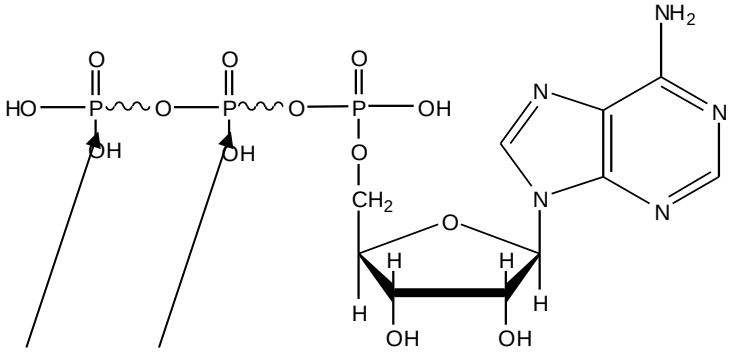
The absence of a hydroxy group in the second position has two important consequences: - the polarization of the glycosidic bond in DNA decreases and it becomes more resistant to hydrolysis. - 2-O-deoxyribose cannot undergo either epimerization or conversion to ketosis.

In the cell, the nucleoside monophosphate is sequentially converted to diphosphate and then to triphosphate. For example: AMP → ADP → ATP

The biological role of nucleotides

All nucleoside diphosphates and nucleoside triphosphates are high-energy (macroergic) compounds.

Nucleoside triphosphates participate in the synthesis of nucleic acids, provide activation of bioorganic compounds and biochemical processes that take place with the expenditure of energy. Adenosine triphosphate (ATP) is the most common macroergic compound in the human body. The ATP content in skeletal muscle of mammals is up to 4 g / kg, the total content is about 125 g. In humans, the ATP metabolism rate reaches 50 kg / day. The hydrolysis of ATP produces adenosine diphosphate (ADP)



Macroergic bonds

ATP contains various types of chemical bonds:

1. N-β-glycosidic
2. Ester
3. Two anhydride (biologically macroergic)

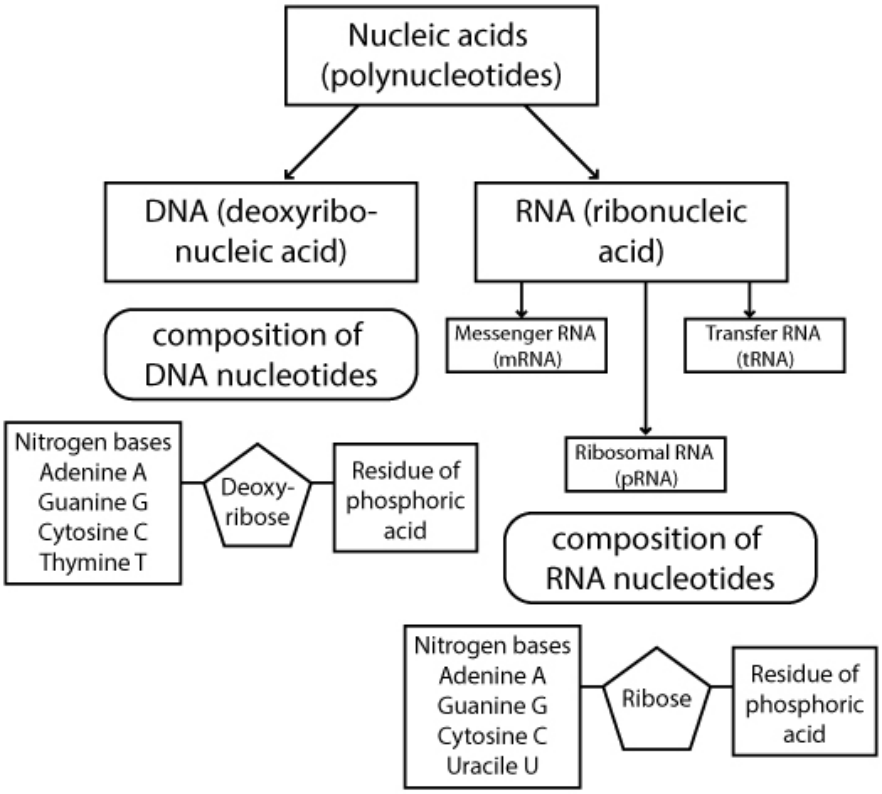
Under in vivo conditions, the hydrolysis of the ATP macroergic bond is accompanied by the release of energy (about 35 kJ / mol), which provides other energy-dependent biochemical processes.

$$ATP + H_2O \xrightarrow{\text{enzyme ATP hydrolase}} ADP + H_3PO_4$$

In aqueous solutions, **ADP and ATP** are unstable. At 0^oC ATP is stable in water for only a few hours, and when boiled for 10 minutes. Under the action of alkali, two terminal phosphates (anhydride bonds) are hydrolyzed easily, and the latter (ester bond) is hydrolized difficultly. With acid hydrolysis, the N-glycosidic bond breaks easily. ATP was first isolated from muscles in 1929 by K. Loman. Chemical synthesis was carried out in 1948 by A. Todd.

The structure of nucleic acids

The primary structure of RNA and DNA is the serial connection of nucleotides in the polynucleotide chain. The skeleton of the polynucleotide chain consists of carbohydrate and phosphate residues, heterocyclic nitrogenous bases are connected to carbohydrates through an N-β-glycosidic bond. From a biological point of view, triplets — blocks of nucleotides from three nitrogen bases, each of which encodes an amino acid or has a specific signaling function — are of utmost importance.



The structure of the nucleic acid can be represented schematically:

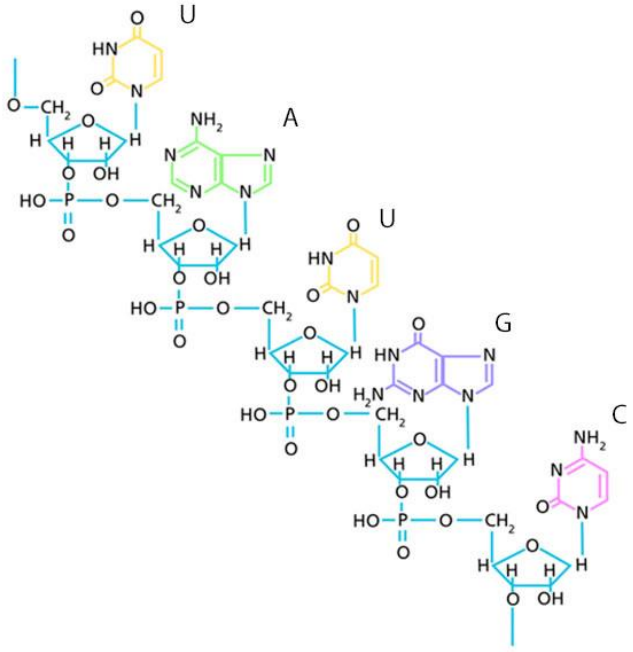
5', 3' 5', 3' 5', 3'

phosphate — pentose — phosphate — pentose — phosphate — pentose —

OH | | |

| nitrogen base nitrogen base nitrogen base

In the primary structure of DNA, the beginning of the chain is determined by pentose containing phosphate at position 5 '. The pentoses in the polynucleotide chain are joined via 3 ′ → 5 ′ phosphate bonds. At the end of the chain in the 3'-pentose position, the OH-group remains free.



Higher Order DNA Structure - Double Helix

The scientific description of the secondary structure of DNA refers to the greatest discoveries of mankind in the twentieth century. Biochemist D. Watson and physicist F. Crick in 1953 proposed a model of the structure of DNA and the mechanism of the replication process. In 1962 they were awarded the Nobel Prize.

In a popular form, the story is described in James Watson's book “The Double Helix”. The book very interestingly describes the story of a joint work, with humor and easy irony of the author for such a significant event, the happy “culprits” of which were two young scientists. Since the discovery of the DNA structure, mankind has received an instrument for the development of a new direction - biotechnology, protein synthesis by recombination of genes.

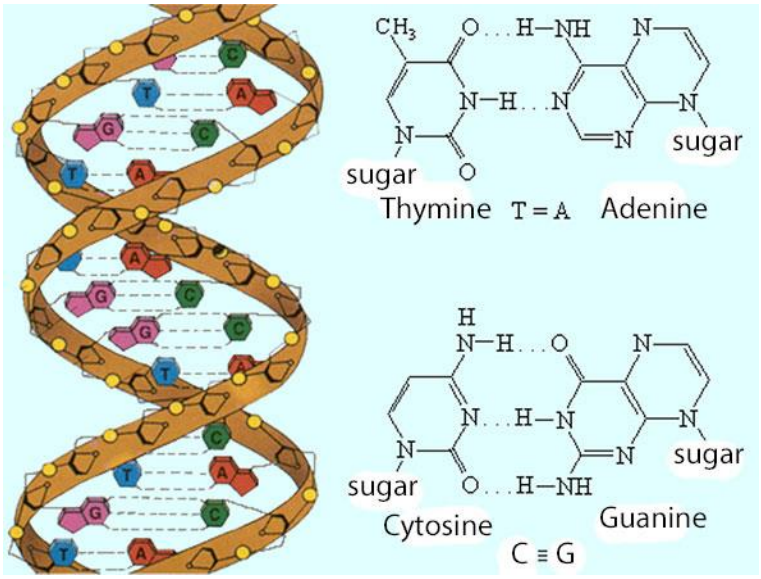
The discovery of the structure of DNA was facilitated by the research of E. Chargaff regarding the chemical composition of DNA. He found out:

- the number of pyrimidine bases is equal to the number of purine bases
- the amount of thymine is equal to the amount of adenine, and the amount of cytosine is equal to the amount of guanine.

$$A = T \; G = C$$
$$A + G = T + C$$
$$A + C = T + G$$

These relationships are called the Chargaff`s rule.

The DNA molecule is two twisted spirals. The skeleton of each spiral is a chain of alternating residues of deoxyribose and phosphoric acid. The spirals are oriented in such a way that they form two unequal spiral grooves that run parallel to the main axis. These grooves are filled with histone proteins. Nitrogenous bases are located inside the spiral, almost perpendicular to the main axis and form complementary pairs A ... T and G ... C between the chains.



The total length of DNA molecules in each cell reaches 3 cm. The diameter of the cell is on average 10^{-5} m, the diameter of DNA is only $2 \cdot 10^{-9}$ m. The main parameters of the double helix:

- Diameter is 1.8 - 2nm
- 10 nucleotides on one turn
- coil pitch is ~ 3.4 nm
- the distance between two nucleotides is 0.34 nm.

The bases are perpendicular to the axis of the chain.

- antiparallel directions of polynucleotide chains
- The connection between the furanose cycles of deoxyribose through phosphoric acid is carried out from position 3` to position 5` in each of the chains.
- The beginning of the chain - the pentose hydroxyl group is phosphorylated at position - 5`, the end of the chain is the free pentose hydroxyl group at position 3`.

- In the composition of DNA and RNA, nucleoside fragments are in the anti-conformation of the purine pyrimidine cycle located to the right of the glycosidic bond. Only this position allows the formation of a complementary pair (see nucleotide formulas)

- Between nitrogenous bases there are three types of interactions:

- "Transverse", complementary pairs of two chains are involved. There is a "cyclic" electron transfer between two nitrogenous bases (T - A, Y - C), an additional π - electronic system is formed, which provides additional interaction and protects the nitrogenous bases from undesirable chemical influences. Two hydrogen bonds are established between adenine and thymine, and three hydrogen bonds between guanine and cytosine.

- "Vertical" (stacking), due to stacking, the nitrogenous bases of one chain are involved. "Stacking interaction" is even more important in the stabilization of the structure than the interaction in complementary pairs

- Interaction with water plays a significant role in maintaining the spatial structure of the double helix, which takes the most compact structure to reduce the contact surface with water and directs hydrophobic heterocyclic bases into the spiral.

General material and educational and methodological support of the lecture:

- Working program of the discipline
- Silabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational tasks

Literature

Basic:

1. Biological and Bioorganic Chemistry: Bioorganic Chemistry: textbook / B.S. Zimenkovsky, V.A. Muzychenko, I.V. Nizhenkovska, G.O. Syrova. — 3rd edition – 2020. – 288 p.
2. Biological and Bioorganic Chemistry. Biological Chemistry: textbook / Yu.I. Gubsky, I.V. Nizhenkovska, M.M. Korda et al. — 2nd edition – 2021 – 544 p.
3. Bioorganic Chemistry. Rineyskaya O.N. textbook. – 2018. – 174 p.
4. Construction features, chemical properties and the biological role of carbohydrates. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova, K.V. Bevziuk. Teaching aid. Odesa, 2017. – 44 p.
5. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.
6. Lipids: classification, structural features, properties and biological role. Ia.F. Burdina, A.V. Grekova, S.V. Shcherbakov, T.A. Sidelnikova. Teaching aid. Odesa, 2017. – p. 32.

Additional:

7. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
8. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
9. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
10. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
11. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
12. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 5

Topic: Biochemistry as a science: biomolecules; metabolic pathways. Enzymes: structure, properties, classification and nomenclature. Kinetics and regulation of enzymatic reactions. Regulatory enzymes. Cofactors and coenzymes. Medical enzymology.

Relevance of the topic: An organism can live and develop only under the conditions of the course of reactions in it, related to the energy supply of the processes of synthesis and decomposition of substances. All processes that take place in the body differ in speed and coordination. Digestion, energy supply, synthesis of structural components of cells and tissues, growth, reproduction, muscle contraction, blood clotting and other processes are related to the work of enzymes. Therefore, it is important to reveal the functioning of enzymatic processes occurring in membranes and organelles for the integration of metabolism in individual cells.

Purpose: study of the general patterns of enzymatic catalysis and use of enzyme activity research in the diagnosis of various pathological conditions.

Basic concepts:

1. What are enzymes?
2. Code of enzymes.
3. Active and allosteric centers of enzymes.
4. Kinetics, energetics of the enzymatic reaction.
5. Enzyme activators, inhibitors.
6. Isoenzymes, polyenzyme systems.
7. Enzymopathology, enzyme diagnostics, enzyme therapy.

Plan and organizational structure of the lecture:

1. Structure of enzymes.
2. Nomenclature, classification, properties of enzymes
3. Mechanism of enzyme action, enzyme centers,
4. Kinetics, energetics of the enzymatic reaction
5. Enzyme activators, inhibitors
6. Isoenzymes, polyenzyme systems
7. Regulation of enzymatic activity
8. Basics of medical enzymology

Content of the lecture material

CONTENTS

- Chemistry
- Classification
- Mechanism of Enzyme Action
- Enzyme Kinetics
- Inhibition
- Activation
- Specificity

Introduction

- Enzymes are *biological catalysts* that speed up the rate of the biochemical reaction.
- Most enzymes are three dimensional *globular proteins* (tertiary and quaternary structure).
- Some special RNA species also act as enzymes and are called *Ribozymes* e.g. hammerhead ribozyme.

Hammerhead enzyme

Structure of Enzymes

- The *active site* of an enzyme is the region that binds substrates, co-factors and prosthetic groups and contains residue that helps to hold the substrate.
- Active sites generally occupy less than 5% of the total surface area of enzyme.
- Active site has a *specific shape* due to tertiary structure of protein.
- A change in the shape of protein affects the shape of active site and function of the enzyme.

Active Site

- Active site can be further divided into:

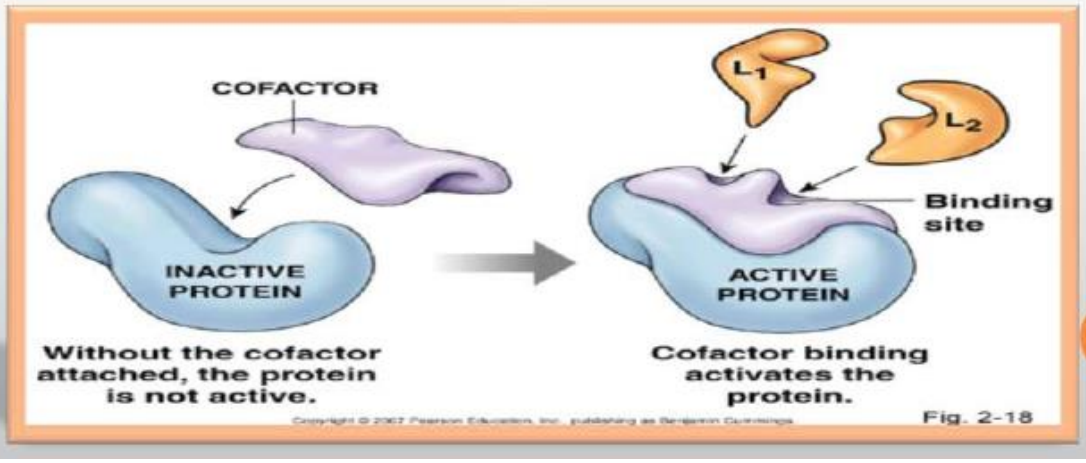
It chooses the substrate and binds it to active site.

It performs the catalytic action of enzyme.

CO-FACTORS

- Co-factor is the non protein molecule which carries out chemical reactions that can not be performed by standard 20 amino acids.

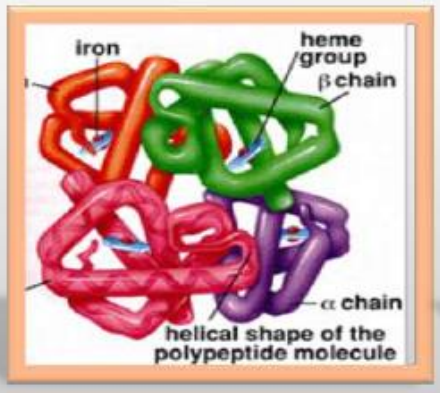
- Co-factors are of two types:
  - Organic co-factors
  - Inorganic cofactors



TYPES OF ORGANIC CO-FACTORS

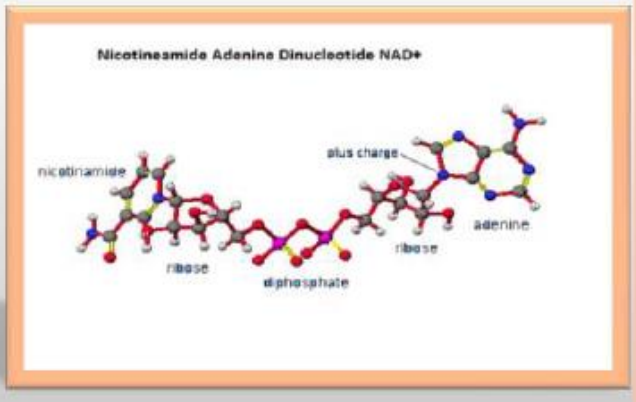
Prosthetic Group

- A prosthetic group is a tightly bound organic co-factor e.g. Flavins, heme groups and biotin.



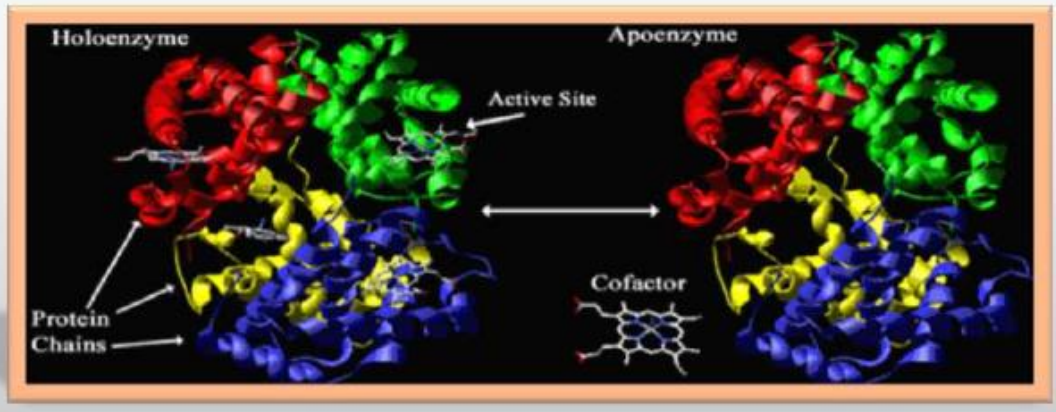
Coenzyme

- A coenzyme is loosely bound organic co-factor. E.g. NAD^+



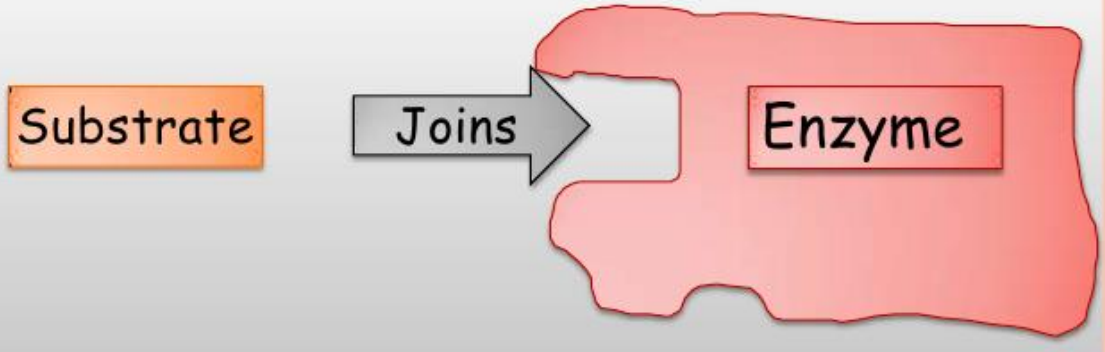
Types of co-factors Continued…

- An enzyme with it's co-factor removed is designated as *apoenzyme*.
- The complete complex of a protein with all necessary small organic molecules, metal ions and other components is termed as *holoenzyme* of *holoprotein*.



SUBSTRATE

- The reactant in biochemical reaction is termed as substrate.
- When a substrate binds to an enzyme it forms an enzyme-substrate complex.



CHARACTERISTICS

- Enzymes *speed up* the reaction by lowering the activation energy of the reaction.
- Their presence *does not effect* the nature and properties of *end product*.
- They are *highly specific* in their action that is each enzyme can catalyze one kind of substrate.
- Small amount of enzymes can accelerate chemical reactions.
- Enzymes are *sensitive* to change in pH, temperature and substrate concentration.
- *Turnover number* is defined as the number of substrate molecules transformed per minute by one enzyme molecule.

Catalase turnover number = 6 x106/min

NOMENCLATURE OF ENZYMES

- An enzyme is named according to the name of the substrate it catalyses.
- Some enzymes were named before a systematic way of naming enzyme was formed.

  Example: pepsin, trypsin and rennin
- By adding suffix **-ase** at the end of the name of the substrate, enzymes are named.
- Enzyme for catalyzing the hydrolysis is termed as hydrolase.

  Example :

maltose + water $\xrightarrow{\text{maltase}}$ glucose + glucose

EXAMPLES

substrate	enzymes	products
lactose	lact**ase**	glucose + galactose
maltose	malt**ase**	Glucose
cellulose	cellul**ase**	Glucose
lipid	lip**ase**	Glycerol + fatty acid
starch	amyl**ase**	Maltose
protein	prote**ase**	Peptides + polypeptide

CLASSIFICATION OF ENZYMES

- A systematic classification of enzymes has been developed by *International Enzyme Commission*.
- This classification is based on the type of reactions catalyzed by enzymes.
- There are *six* major classes.
- Each class is further divided into sub classes, sub sub-classes and so on, to describe the huge number of different enzyme-catalyzed reactions.

Classification of enzymes

Continued……..

ENZYME CLASS	REACTION TYPE	EXAMPLES
Oxidoreductases	Reduction-oxidation (redox)	Lactate dehydrogenase
Transferases	Move chemical group	Hexokinase
Hydrolases	Hydrolysis; bond cleavage with transfer of functional group of water	Lysozyme
Lysases	Non-hydrolytic bond cleavage	Fumarase
Isomerases	Intramolecular group transfer (isomerization)	Triose phosphate isomerase
Ligases	Synthesis of new covalent bond between substrates, using ATP hydrolysis	RNA polymerase

MECHANISM OF ENZYME ACTION

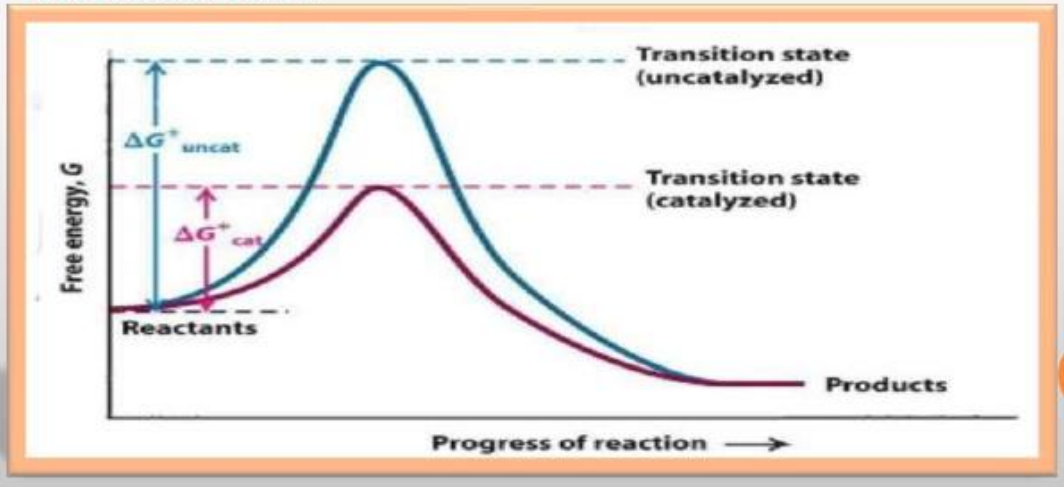
- The catalytic efficiency of enzymes is explained by two perspectives:

Thermodynamic changes

Processes at the active site

THERMODYNAMIC CHANGES

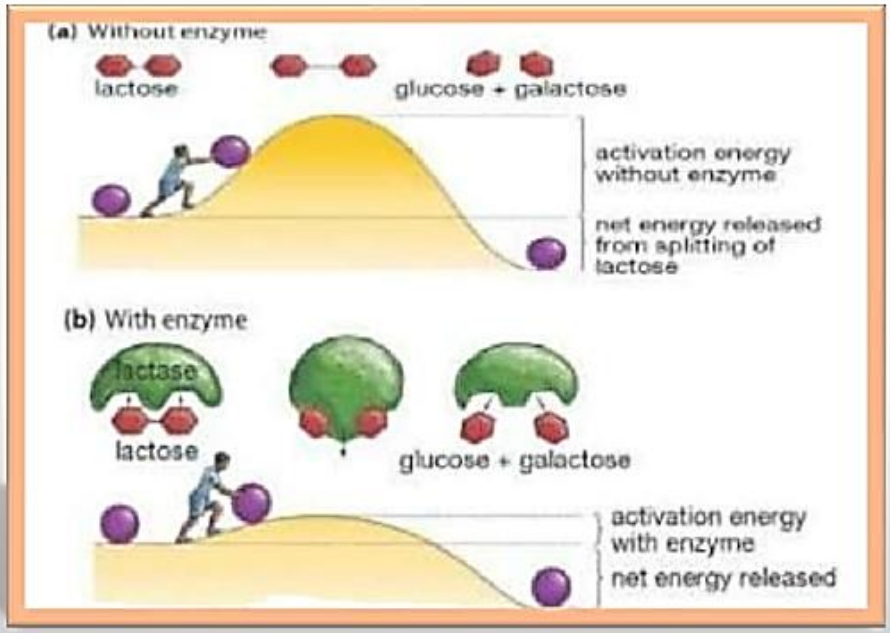
- All chemical reactions have energy barriers between reactants and products.
- The difference in transitional state and substrate is called *activational barrier*.



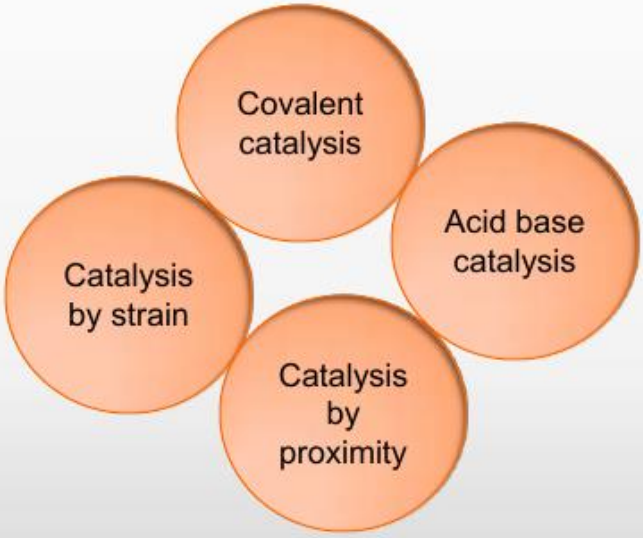
THERMODYNAMIC CHANGES

- Only a few substances cross the activation barrier and change into products.
- That is why rate of uncatalyzed reactions is much slow.
- Enzymes provide an alternate pathway for conversion of substrate into products.
- Enzymes accelerate reaction rates by forming transitional state having low activational energy.
- Hence, the reaction rate is increased many folds in the presence of enzymes.
- The total energy of the system remains the same and equilibrium state is not disturbed.

THERMO-DYNAMIC CHANGES OVERVIEW

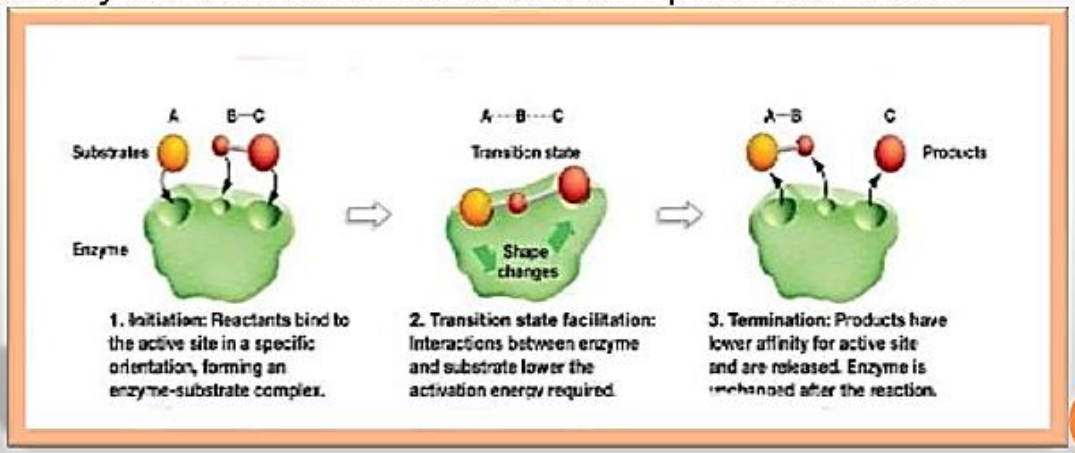


PROCESSES AT THE ACTIVE SITE



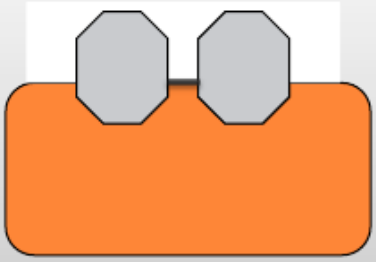
COVALENT CATALYSIS

- Enzymes form covalent linkages with substrate forming transient enzyme-substrate complex with very low activation energy.
- Enzyme is released unaltered after completion of reaction.



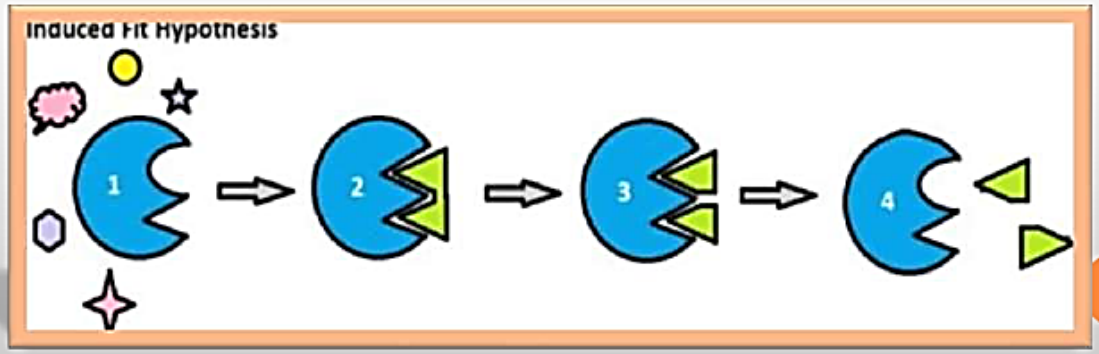
LOCK AND KEY MODEL

- Proposed by EMIL FISCHER in 1894.
- Lock and key hypothesis assumes the active site of an enzymes are rigid in its shape.
- There is no change in the active site before and after a chemical reaction.



INDUCED FIT MODEL

- More recent studies have revealed that the process is much more likely to involve an induced fit model(proposed by DANIAL KOSH LAND in 1958).
- According to this exposure of an enzyme to substrate cause a change in enzyme, which causes the active site to change it's shape to allow enzyme and substrate to bind.



INTRODUCTION

"It is a branch of biochemistry in which we study *the rate of enzyme catalyzed reactions*."

- Kinetic analysis reveals the number and order of the individual steps by which enzymes transform substrate into products

- Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of that enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme

RATES OF REACTION AND THEIR DEPENDENCE ON ACTIVATION ENERGY

- Activation Energy (Ea):

"The least amount of energy needed for a chemical reaction to take place."

- Enzyme (as a catalyst) acts on substrate in such a way that they lower the activation energy by changing the route of the reaction.
- The reduction of activation energy (Ea) increases the amount of reactant molecules that achieve a sufficient level of energy, so that they reach the activation energy and form the product.

Example:

- *Carbonic anhydrase* catalyses the hydration of 10^6 CO_2 molecules per second which is 10^7x faster than spontaneous hydration.

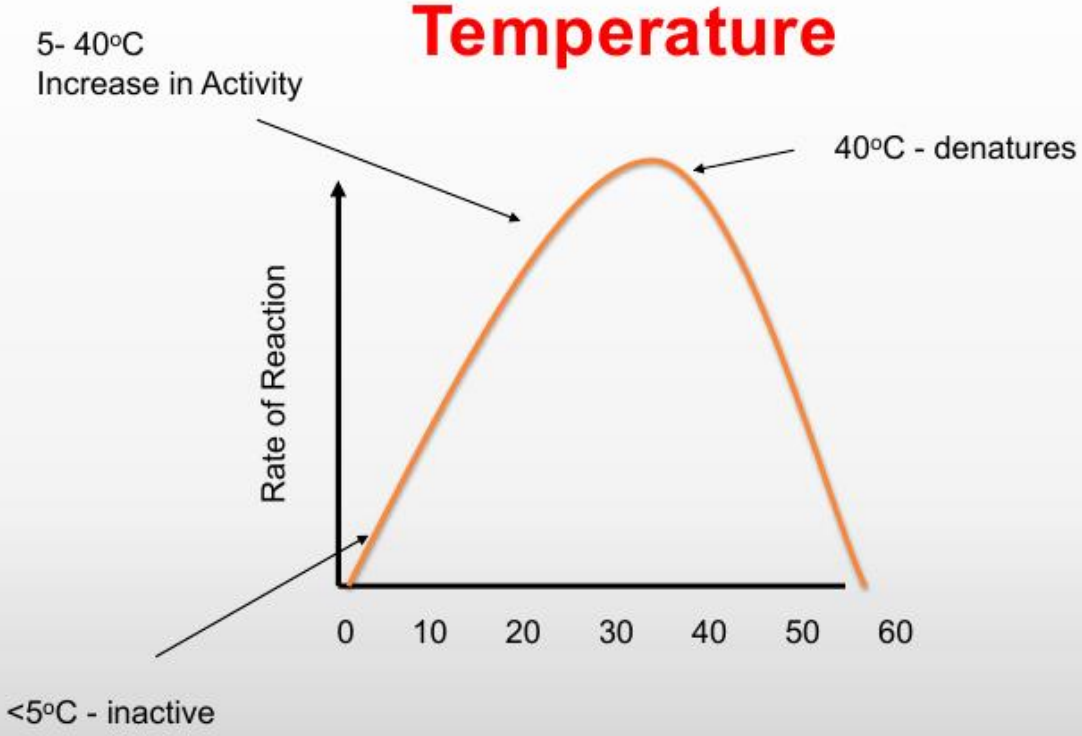
KINETICS OF ENZYMES CATALYSIS

- Enzymes catalysis:

" It is an increase in the rate of reaction with the help of enzyme(as catalyst)."

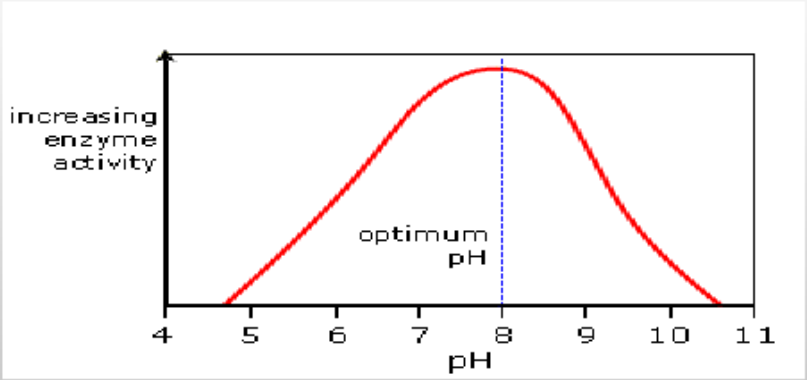
- Catalysis by enzymes that proceed via unique reaction mechanism, typically occurs when the transition state intermediate forms a covalent bond with the enzyme(covalent catalysis).
- During the process of catalysis enzymes always emerge unchanged at the completion of the reaction.

Temperature

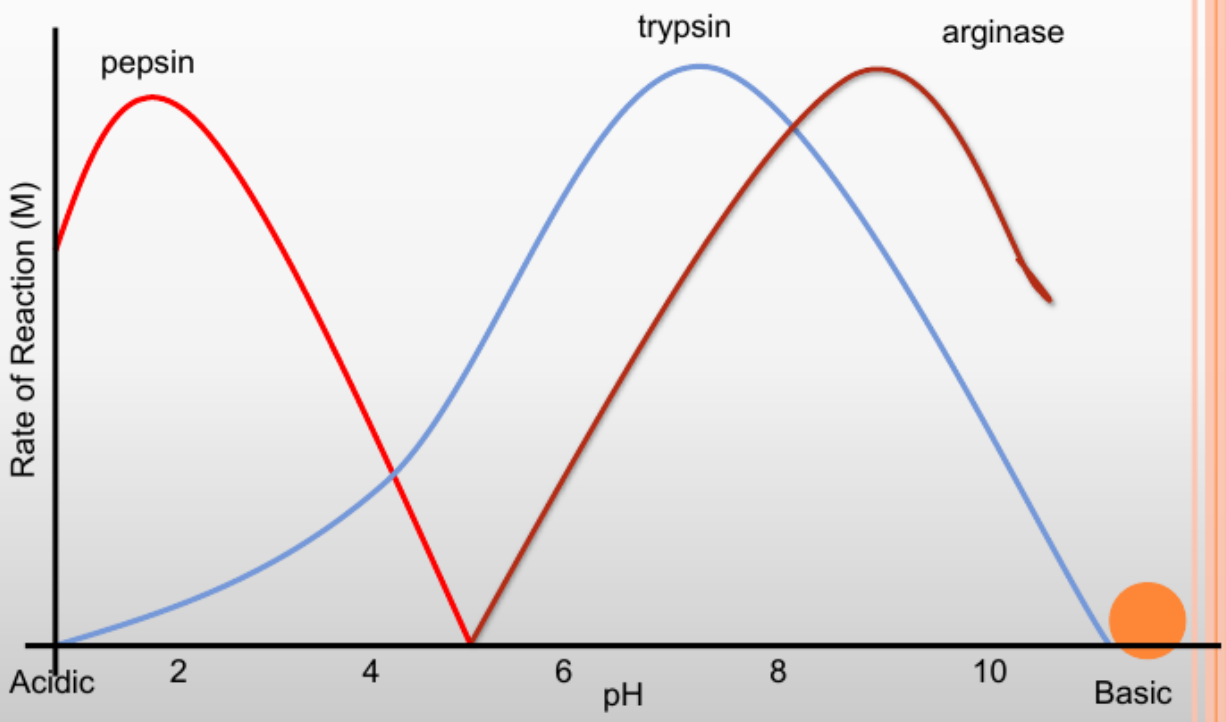


EFFECT OF pH

- Rate of almost all enzymes catalyzed reactions depends on pH
- Most enzymes exhibit optimal activity at pH value between *5 and 9*
- High or low pH value than optimum value will cause ionization of enzyme which result in denaturation of enzyme



pH AFFECTS THE FORMATION OF HYDROGEN BONDS AND SULPHUR BRIDGES IN PROTEINS AND SO AFFECTS SHAPE.



MICHAELIS-MENTEN EQUATION

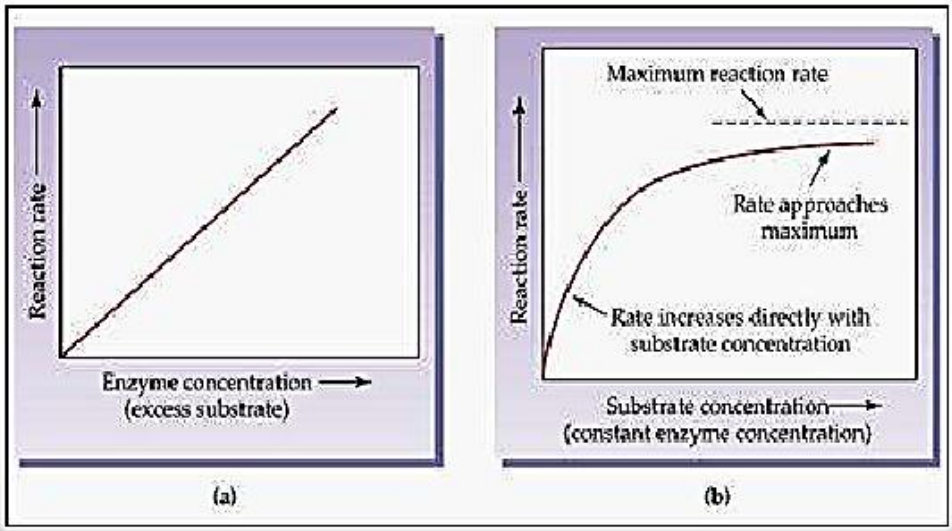
- Michaelis-Menten Equation:

"It is an equation which describes how reaction velocity varies with substrate concentration."

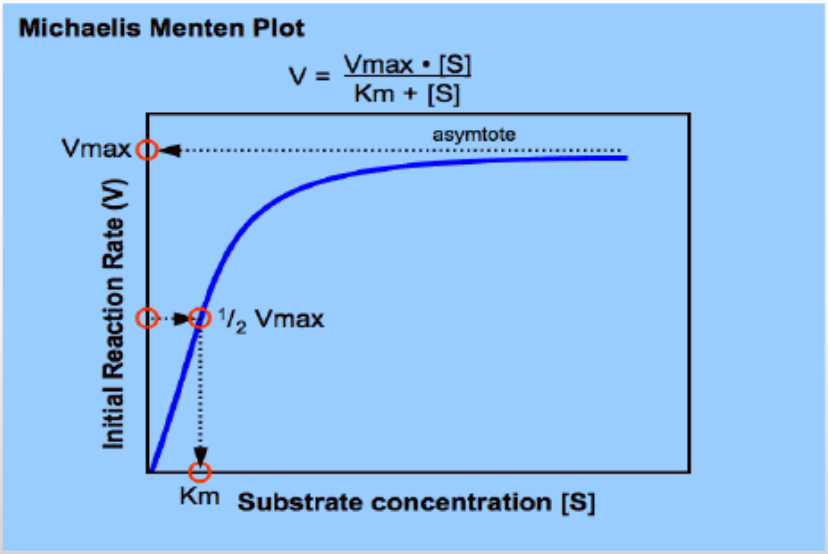
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

- Where
  - V_o is the initial reaction velocity.
  - V_{max} is the maximum velocity.
  - K_m is the Michaelis constant = $(k_{-1}+k_2)/k_1$.
  - [S] is the substrate concentration.

SUBSTRATE CONCENTRATION



SUBSTRATE CONCENTRATION



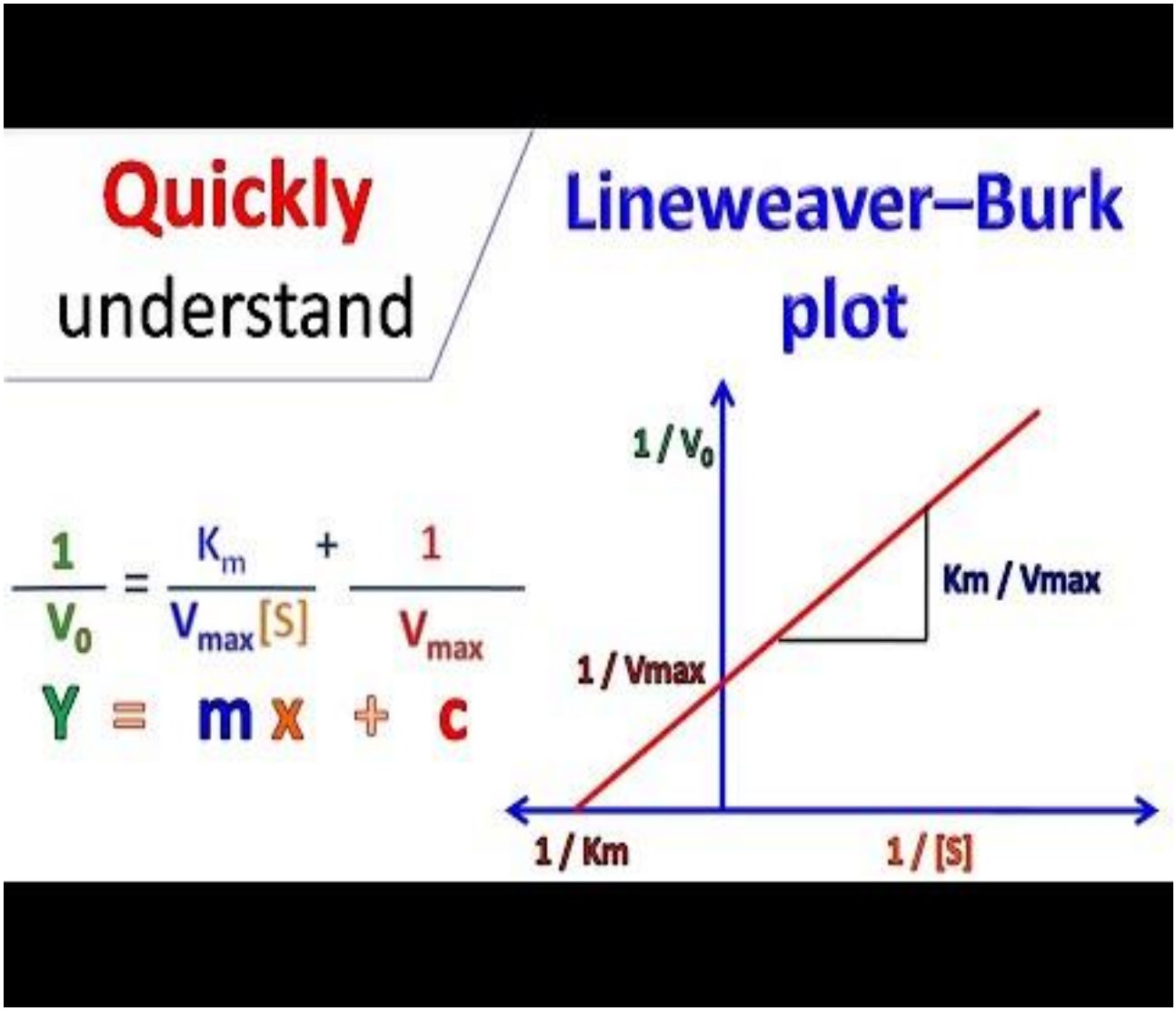
Lineweaver–Burk plot

- The Lineweaver–Burk plot or double reciprocal plot is a common way of illustrating kinetic data. This is produced by taking the reciprocal of both sides of the Michaelis–Menten equation. This is a linear form of the Michaelis–Menten equation and produces a straight line with the equation:
- $y = mx + c$ with a y-intercept equivalent to $1/V_{max}$ and an x-intercept of the graph representing $-1/K_M$

Double reciprocal form of the Michaelis-Menten equation:

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[s]} + \frac{1}{V_{max}}$$

$$\frac{1}{v} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}}$$



Pharmaceutical Importance

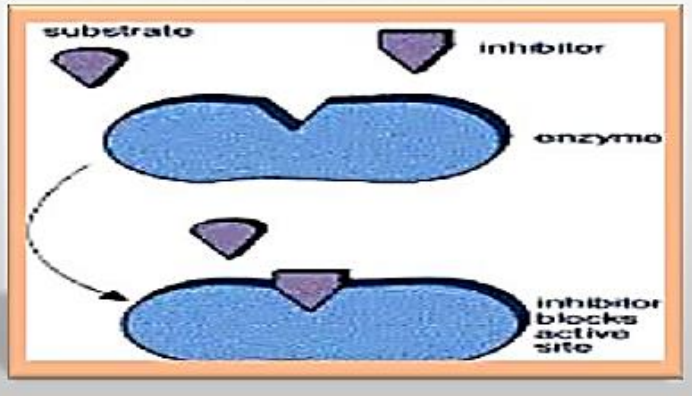
- Enzymes are virtually involved in all physiological processes which makes them the *targets of choice for drugs* that cure or ameliorate human disease.
- Applied enzyme kinetics represents the *principal tool* by which scientist identify and characterize therapeutic agents that selectively inhibit the rates of specific enzymes catalyzed processes.
- Enzymes kinetics thus play a critical role in drug discovery as well as elaborating the mode of action of drugs.

Inhibition

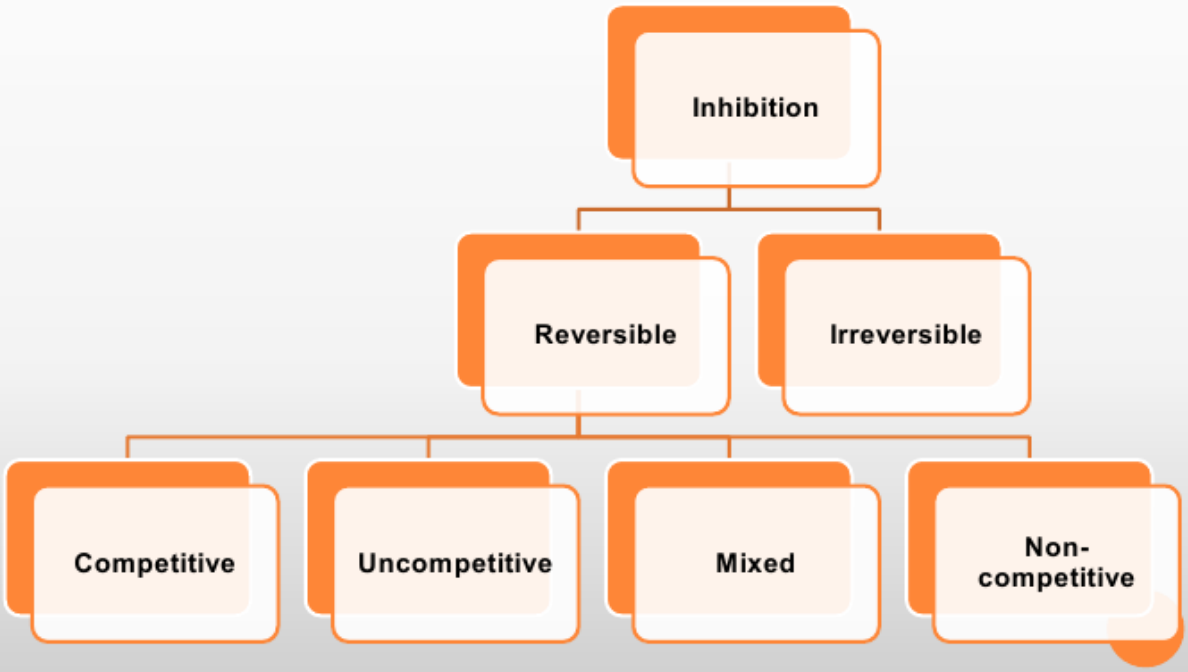
- The prevention of an enzyme process as a result of interaction of inhibitors with the enzyme.

➢ INHIBITORS:

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called an inhibitor.



TYPES OF INHIBITION



REVERSIBLE INHIBITION

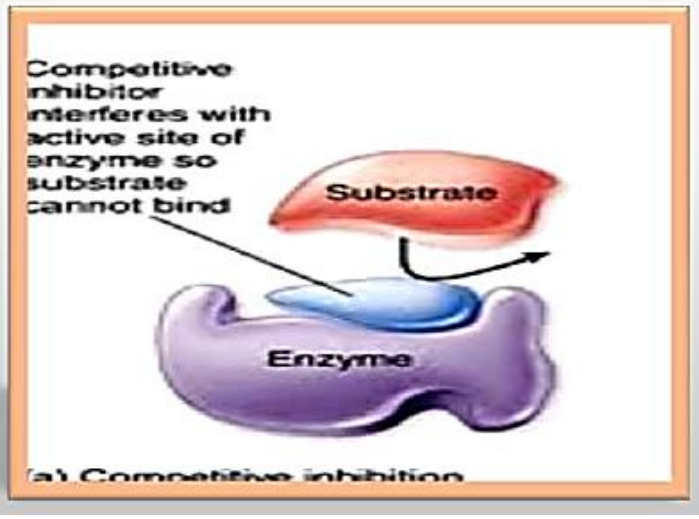
- It is an inhibition of enzyme activity in which the inhibiting molecular entity can associate and dissociate from the protein's binding site.

TYPES OF REVERSIBLE INHIBITION

- There are four types:
  - Competitive inhibition.
  - Uncompetitive inhibition.
  - Mixed inhibition.
  - Non-competitive inhibition.

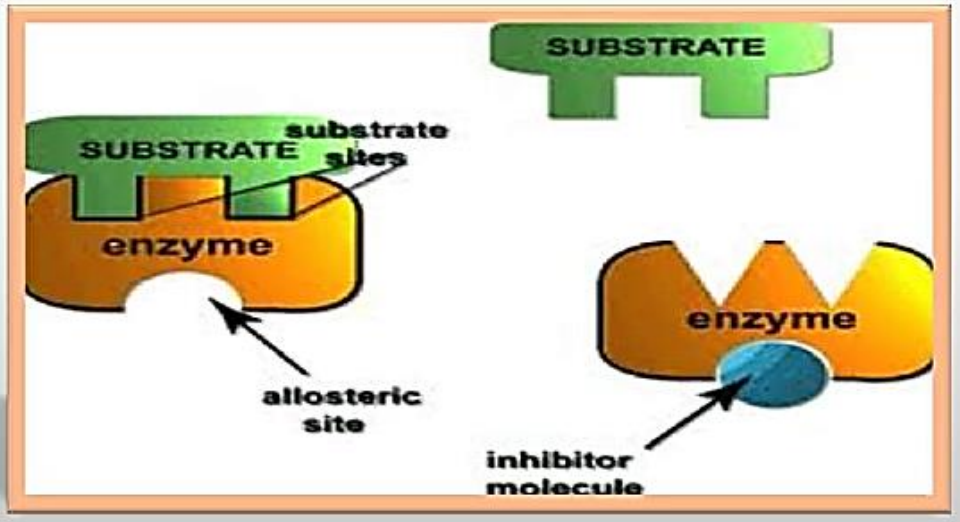
COMPETITIVE INHIBITION

- In this type of inhibition, the inhibitors compete with the substrate for the active site. Formation of E.S complex is reduced while a new E.I complex is formed.



UNCOMPETITIVE INHIBITION

- In this type of inhibition, inhibitor does not compete with the substrate for the active site of enzyme instead it binds to another site known as *allosteric* site.



MIXED INHIBITION

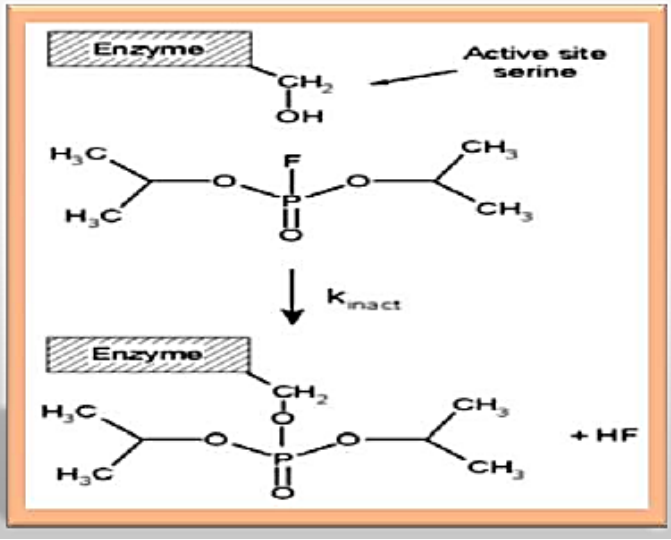
- In this type of inhibition both E.I and E.S.I complexes are formed.
- Both complexes are catalytically inactive.

NON COMPETITIVE INHIBITION

- It is a special case of inhibition.
- In this inhibitor has the same affinity for either enzyme E or the E.S complex.

IRREVERSIBLE INHIBITION

- This type of inhibition involves the *covalent attachment* of the inhibitor to the enzyme.
- The *catalytic activity* of enzyme is completely lost.
- It can only be restored only by synthesizing molecules.



ACTIVATION

- Activation is defined as the conversion of an inactive form of an enzyme to active form which processes the metabolic activity.

TYPES OF ACTIVATION

- Activation by co-factors.
- Conversion of an enzyme precursor.

ACTIVATION BY CO FACTORS

- Many enzymes are activated by co-factors.

Examples:

- *DNA polymerase* is a holoenzyme that catalyzes the polymerization of de -oxyribonucleotide into a DNA strand. It uses Mg- ion for catalytic activity.
- *Horse liver dehydrogenase* uses Zn- ion for it's activation.

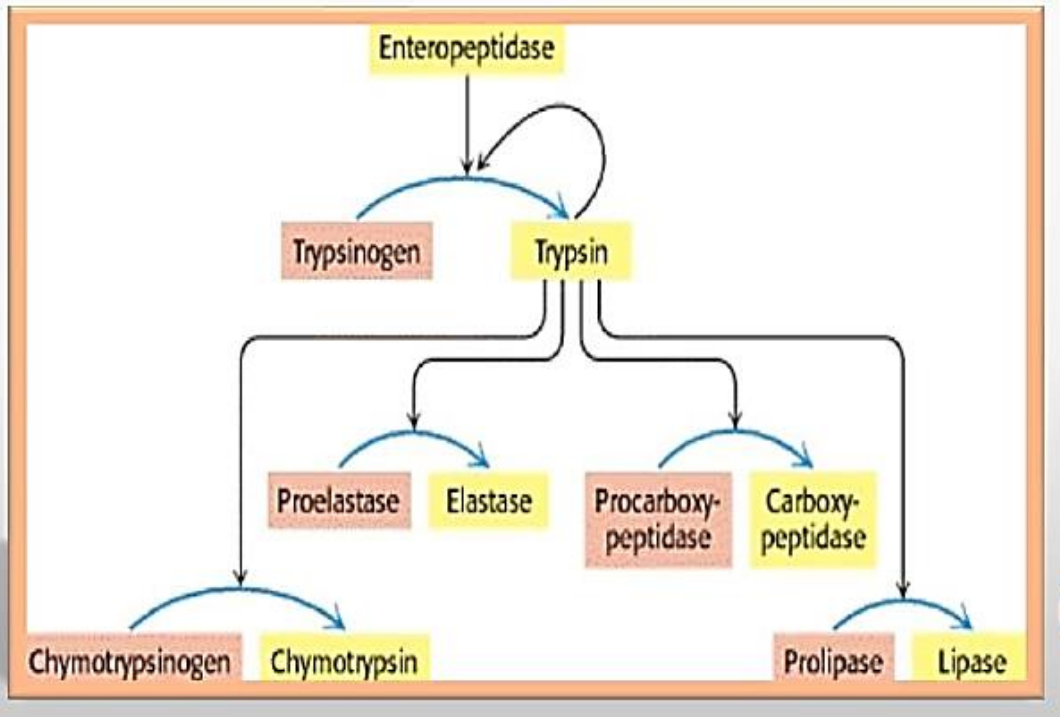
CONVERSION OF AN ENZYME PRECURSOR

- Specific proteolysis is a common method of activating enzymes and other proteins in biological system.

Example:

- The generation of trypsin from trypsinogen leads to the activation of other zymogens.

ZYMOGEN ACTIVATION BY PROTEOLYTIC CLEAVAGE

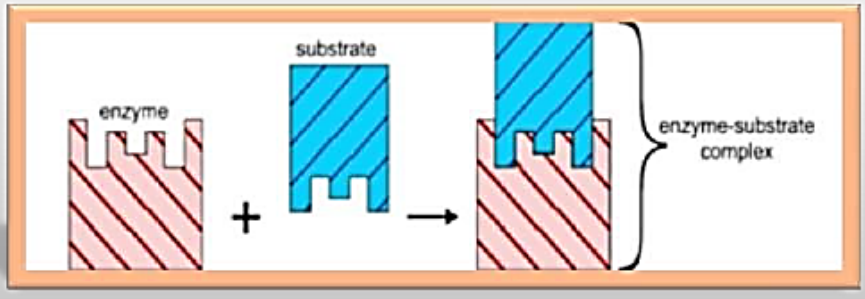


ENZYME SPECIFICITY

- Enzymes are highly specific in nature, interacting with one or few substrates and catalyzing only one type of chemical reaction.
- Substrate specificity is due to complete fitting of active site and substrate .

Example:

- Oxydoreductase do not catalyze hydrolase reactions and hydrolase do not catalyze reaction involving oxidation and reduction.



TYPES OF ENZYME SPECIFICITY

- Enzymes show different degrees of specificity:

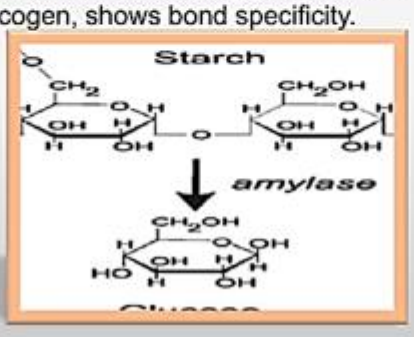
- Bond specificity.
- Group specificity.
- Absolute specificity.
- Optical or stereo-specificity.
- Dual specificity.

BOND SPECIFICITY

- In this type, enzyme acts on substrates that are similar in structure and contain the same type of bond.

Example :

- *Amylase* which acts on α-1-4 glycosidic ,bond in starch dextrin and glycogen, shows bond specificity.

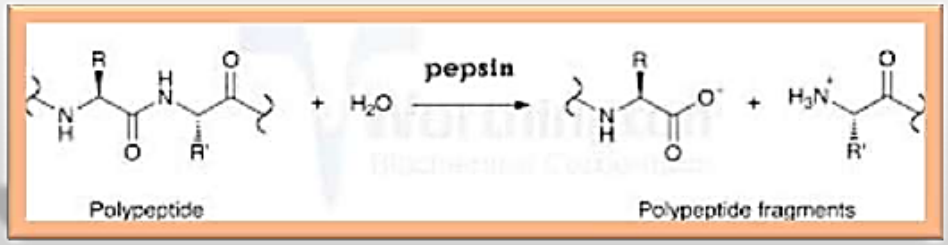


GROUP SPECIFICITY

- In this type of specificity, the enzyme is specific not only to the type of bond but also to the structure surrounding it.

Example:

- *Pepsin* is an endopeptidase enzyme, that hydrolyzes central peptide bonds in which the amino group belongs to aromatic amino acids e. g phenyl alanine, tyrosine and tryptophan.

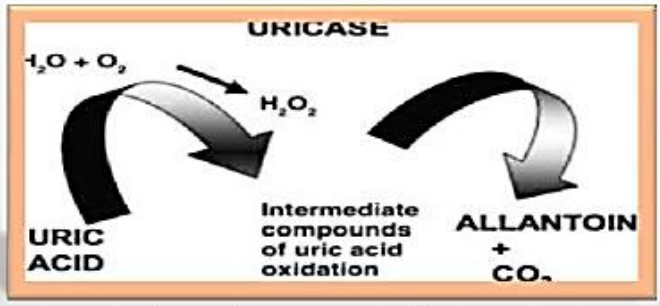


SUBSTRATE SPECIFICITY

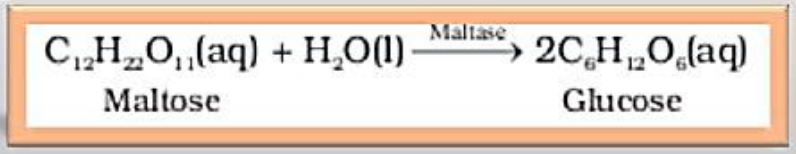
- In this type of specificity ,the enzymes acts only on one substrate

  Example :

- *Uricase* ,which acts only on uric acid, shows substrate specificity.



- *Maltase* , which acts only on maltose, shows substrate specificity.

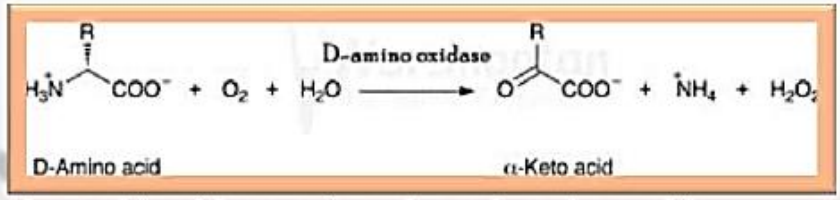


OPTICAL / STEREO-SPECIFICITY

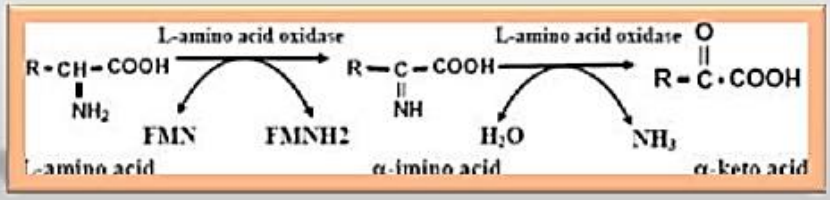
- In this type of specificity , the enzyme is not specific to substrate but also to its optical configuration

  Example:

- D amino acid oxidase acts only on D amino acids.



- L amino acid oxidase acts only on L amino acids.

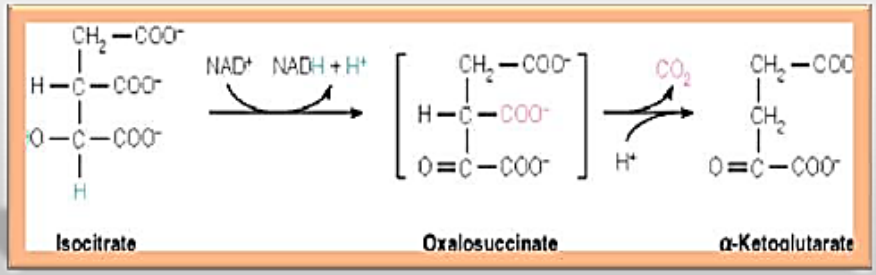


DUAL SPECIFICITY

- There are two types of dual specificity.
- The enzyme may act on one substrate by two different reaction types.

Example:

- *Isocitrate dehydrogenase* enzyme acts on isocitrate (one substrate) by oxidation followed by decarboxylation(two different reaction types) .



General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

Introduction to biochemistry. Biochemical components of cells

1. Biological chemistry (biochemistry) as a science. The place of biochemistry among other medical and biological disciplines.
2. Objects of study and tasks of biochemistry. The leading role of biochemistry in establishing the molecular mechanisms of the pathogenesis of human diseases.
3. Connection of biochemistry with other biomedical sciences. Medical biochemistry. Clinical biochemistry. Biochemical laboratory diagnostics.
4. History of biochemistry; development of biochemical research in Ukraine.
5. Biochemical components of the cell, their biochemical functions. Classes of biomolecules. Hierarchy of biomolecules, their origin.

Enzymes and coenzymes. Regulation of metabolism.

1. Enzymes: definition; properties of enzymes as biological catalysts.

2. Classification and nomenclature of enzymes, characteristics of individual classes of enzymes.
3. Structure and mechanisms of action of enzymes. Active and allosteric (regulatory) centers.
4. Cofactors and coenzymes. The structure and properties of coenzymes, vitamins as precursors in the biosynthesis of coenzymes.
5. Coenzymes: types of reactions catalyzed by individual classes of coenzymes.
6. Isoenzymes, peculiarities of structure and functioning, importance in diagnosis of diseases.
7. Mechanisms of action and kinetics of enzymatic reactions: dependence of reaction speed on substrate concentration, pH and temperature.
8. Enzyme activators and inhibitors: examples and mechanisms of action.
9. Types of enzyme inhibition: reverse (competitive, non-competitive) and irreversible inhibition.
10. Regulation of enzymatic processes. Ways and mechanisms of regulation: allosteric enzymes; covalent modification of enzymes.
11. Cyclic nucleotides (cAMP, cGMP) as regulators of enzymatic reactions and biological functions of the cell.
12. Enzymopathies are congenital (hereditary) defects in the metabolism of carbohydrates, amino acids, porphyrins, and purines.
13. Enzyme diagnosis of pathological processes and diseases.
14. Enzymotherapy – use of enzymes, their activators and inhibitors in medicine.
15. Principles and methods of detecting enzymes in biological objects. Units of activity and amount of enzymes.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 6

Topic: Bioenergetics: general ways of catabolism of carbohydrates, lipids, amino acids. Cycle of tricarboxylic acids. Biological oxidation and oxidative phosphorylation. Electron transport chain in mitochondria.

Relevance of the topic: The energy balance of energy intake and expenditure must be maintained in the body. Living organisms receive energy in the form of potential energy accumulated in the chemical bonds of fat, protein and carbohydrate molecules. In the process of biological oxidation, this energy is released. Part of it is used for the synthesis of ATP, the other part of this energy is converted into heat.

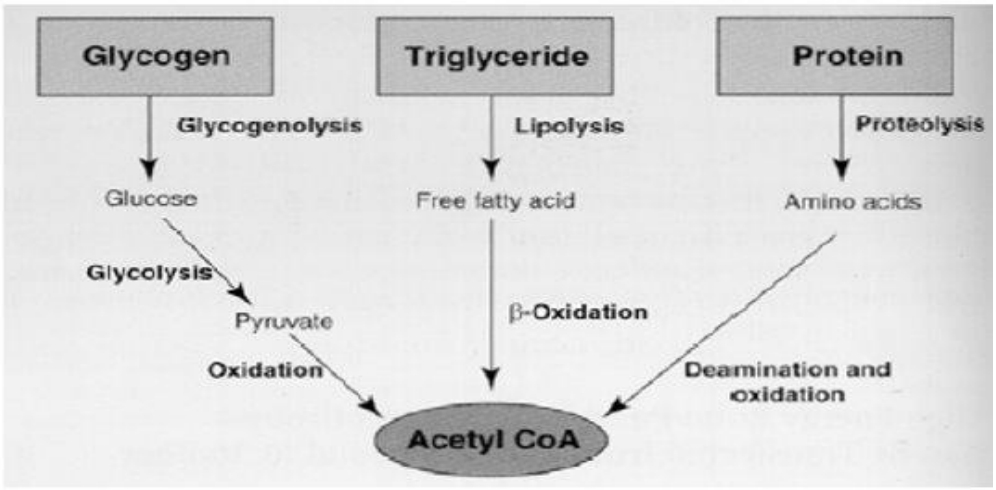
The integration of metabolic transformations of proteins, fats and carbohydrates is manifested in the existence of common precursors and common intermediate products of metabolism. This is a common carbon fund, a common intermediate product of metabolism - acetyl-CoA and other substances. Final transformation pathways, such as the tricarboxylic acid cycle and the respiratory chain reactions that occur in mitochondria, also link metabolic processes at different stages.

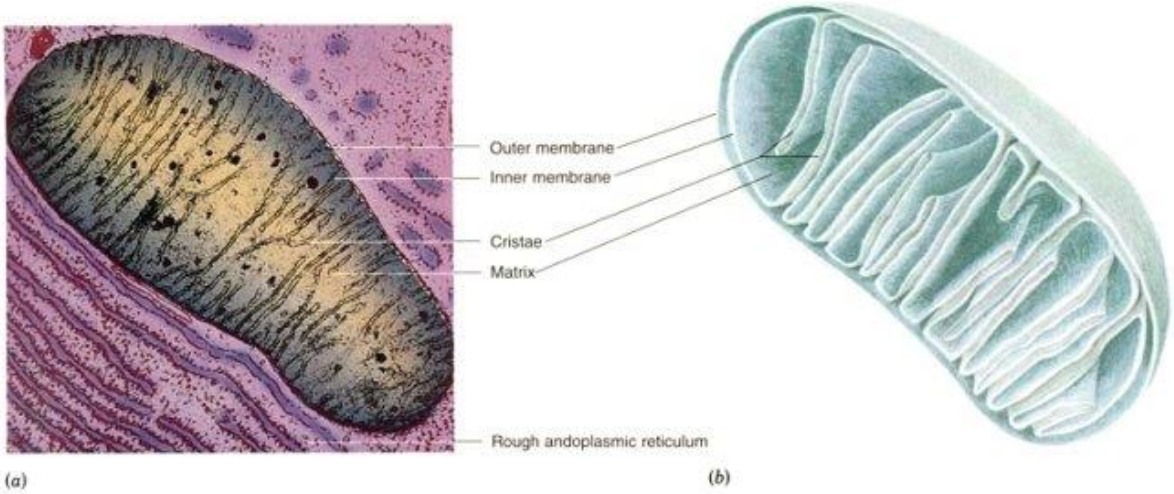
Purpose: to study the general pathways of catabolism of the main classes of organic compounds and the generation of energy in the body.

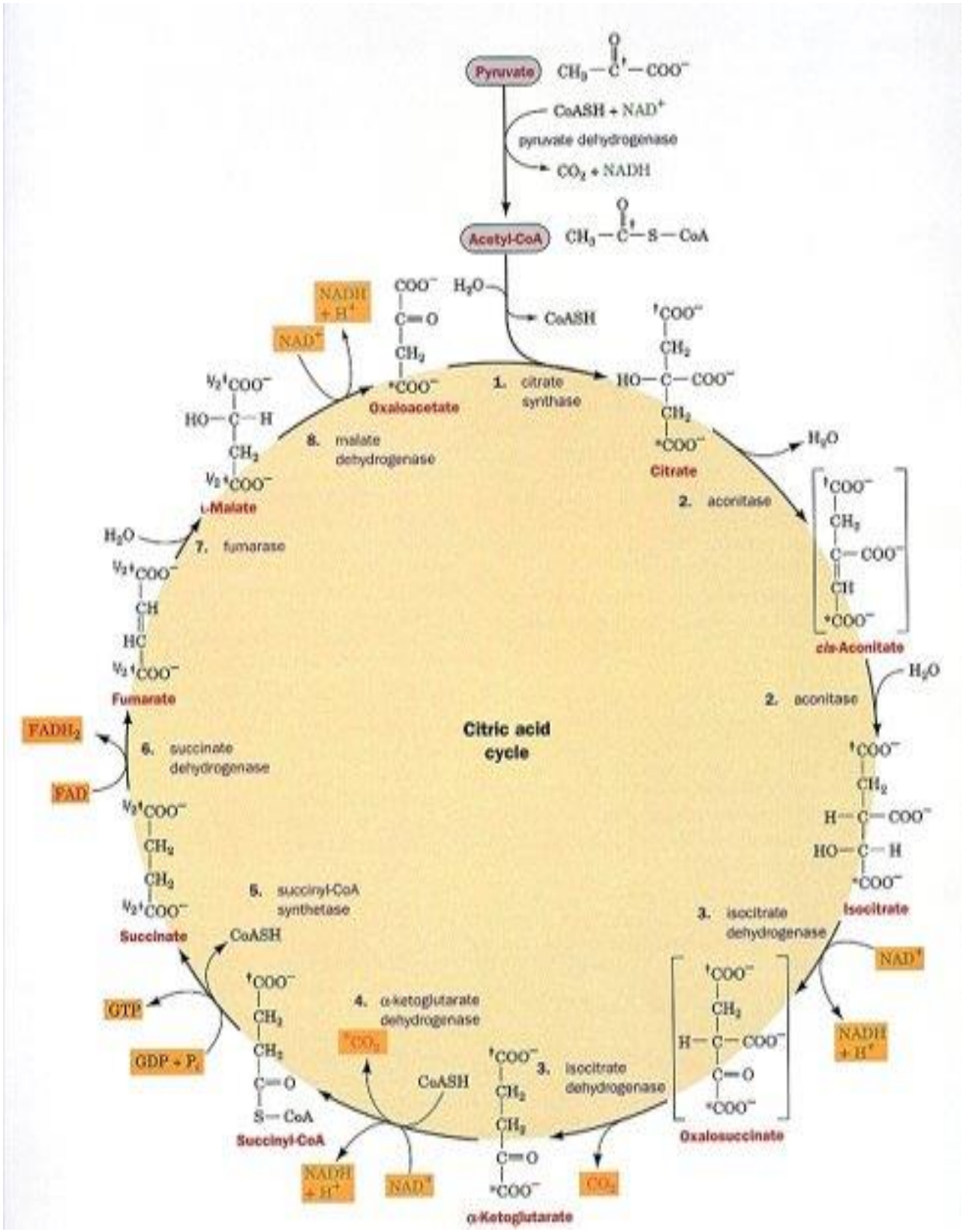
Basic concepts:
1. Anabolism, catabolism, metabolism.
2. Energy balance.
3. Anaplerotic reactions.
4. Amphibolic reactions.
5. Cytochrome P450.
6. Free radicals.
7. Antioxidant systems.

Plan and organizational structure of the lecture:
1. General ways of catabolism.
2. Krebs cycle. Sequence of reactions.
3. Energy balance of the Krebs cycle.
4. Anaplerotic reactions.
5. Amphibolic reactions.
6. Microsomal oxidation.
7. Free radical oxidation.
8. Antioxidant systems.

Content of the lecture material


Glycogen
Triglyceride
Protein
Glycogenolysis
Lipolysis
Proteolysis
Glucose
Free fatty acid
Amino acids
Glycolysis
Pyruvate
β-Oxidation
Oxidation
Deamination and oxidation
Acetyl CoA


Outer membrane
Inner membrane
Cristae
Matrix
Rough andoplasmic reticulum
(a)
(b)



IN GENERAL THE TCA CYCLE IS INHIBITED BY A HIGH ENERGY CHARGE AND STIMULATED BY LOW ENERGY CHARGE

AMPHIBOLIC NATURE

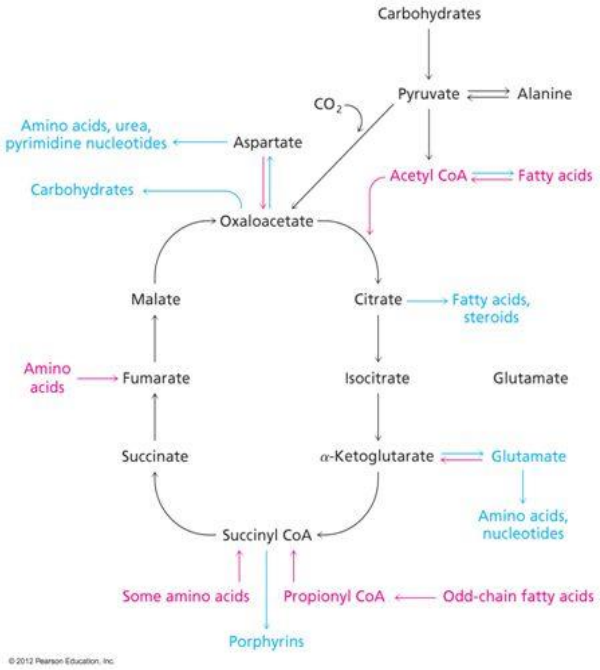
- Kreb cycle is both catabolic and anabolic in nature. Hence regarded as Amphibolic.
- Involved in gluconeogenesis , transamination and deamination.
- Also provide precursors for many biosynthetic pathway. For ex.

  1. alfa-ketoglutarate and oxaloacetate : serve as precursor of amino acids, aspartate and glutamate by simple transamination and required for synthesis of non-essential amino acids ,purines and pyrimidines.

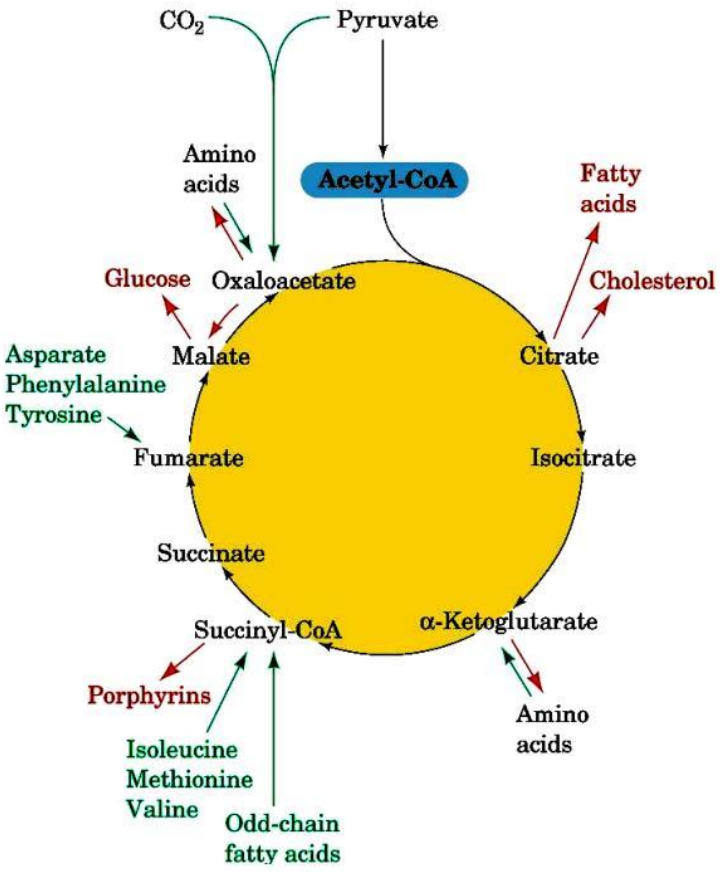
  2. succinyl CoA : used for synthesis of heme.

Anaplerotic Reactions

- "Filling up" reactions
- Formation of oxaloacetate by pyruvate carboxylase
- No net carbohydrates from Acetyl CoA in mammals



Amphibolic Nature of Citric Acid Cycle



Bioenergetic processes: biologic oxidation, oxidative phosphorylation, ATP synthesis

Biological oxidation of organic compounds can occur in several ways:

a) by splitting off the hydrogen atoms from the substrates - dehydrogenation of substrates;

b) by splitting off electrons from the substrates;

c) by addition of oxygen, if this is the transfer of electrons from a substance which is oxidized to the oxygen molecule (microsomal oxidation).

An important step in the aerobic oxidation is a cellular respiration - the multi-enzymatic process of transfer of protons and electrons in the chain of respiratory enzymes to the oxygen.

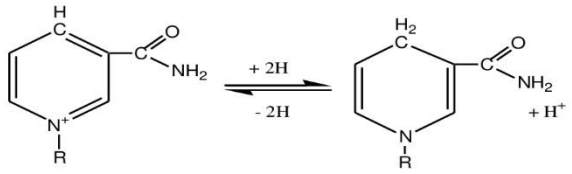
Biochemical mechanisms of cellular respiration.

The first stage of cellular respiration

It starts with the dehydrogenation of substrates - the action of nicotinamide enzymes

$$\text{Sub } H_2 + NAD(P) \xrightarrow{2H^+ + 2e^-} \text{Sub} + NAD(P)H + H^+$$

As a result of this reaction substrate is oxidized and recovers NAD(P) is redused.



The second phase - the action of flavin enzymes

Flavin enzymes can take hydrogen from nicotinamide enzymes or directly from the substrates. Hydrogen acceptor of vitamin B_2 is a component.

$$NE\ (NADH + H^+) + FE \longrightarrow (NAD^+) + FEH_2$$

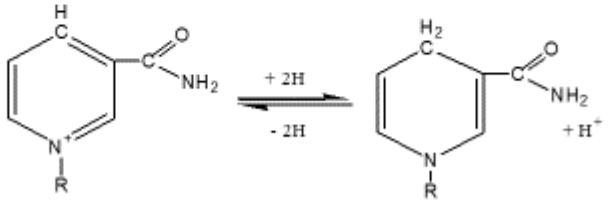
Biochemical mechanisms of cellular respiration.

The first stage

It starts with the dehydrogenation of substrates - the action of nicotinamide enzymes

$$Sub\ H_2 + NAD(P)^+ \xrightarrow{2H + 2\ e^-} Sub + NAD\ (P)\ H + H^+$$

As a result of this reaction is oxidized substrate and reduced NAD $(P)^+$.

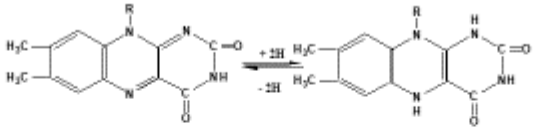


The second phase

- the action of flavin enzymes

Flavin enzymes can make hydrogen from nicotinamide enzymes or directly from the substrates. Hydrogen acceptor is a component of vitamin B_2.

$$NE\ (NADH + H^+) + FE \longrightarrow (NAD^+) + FEH_2$$



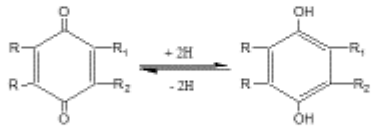
Stage III –

- effect of ubiquinone (coenzyme Q).

–

$$FEH_2 + CoQ \xrightarrow{2H^+ +2\ e^-} CoQH_2 + FE$$

Formed reduced form of ubiquinone and the oxidized form of flavin enzymes.



Oxidized form of coenzyme Q Reduced form of coenzyme Q

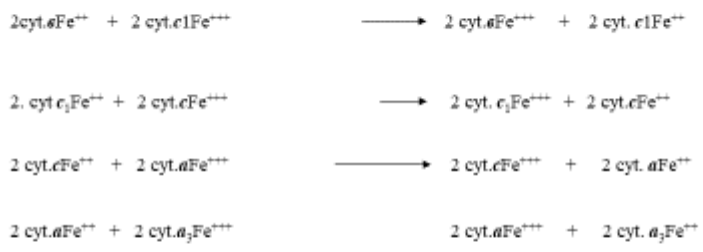
Stage IV - the system of cytochromes.

Non-protein part of cytochrome - ferrum-porphirine complexes, which contain iron.

Iron in the cytochrome tends to change the valence, which is associated with the accession of electrons to iron, or their impact

Ubiquinone, oxidized, transfers electrons to 2 of cytochrome c, and protons temporarily remain in solution. After ubiquinone path of protons and electrons apart.

$2H^+ \quad 2\,e^-$



Phase V

- Transfer of electrons from reduced cytochrome oxidase (cyt. a_3) on oxygen Formed negatively charged, active oxygen ion.

$$2 \text{ cyt. } a_3Fe^{++} + \tfrac{1}{2} O_2 \xrightarrow{-2e^-} 2 \text{ cyt. } aFe^{+++} + O^{--}$$

Phase VI

- combination of highly ionized oxygen and protons, which remained in the matrix after ubiquinone, with formation of water:

$$O^{--} + 2H^+ \rightarrow H_2O$$

The whole system of tissue respiration can be represented as the general pattern of enzyme complexes:

I - NADH-KoQH$_2$-reductase (inhibitor rotenone)

II - succinate- KoQH$_2$ reductase (inhibitor - carboksin)

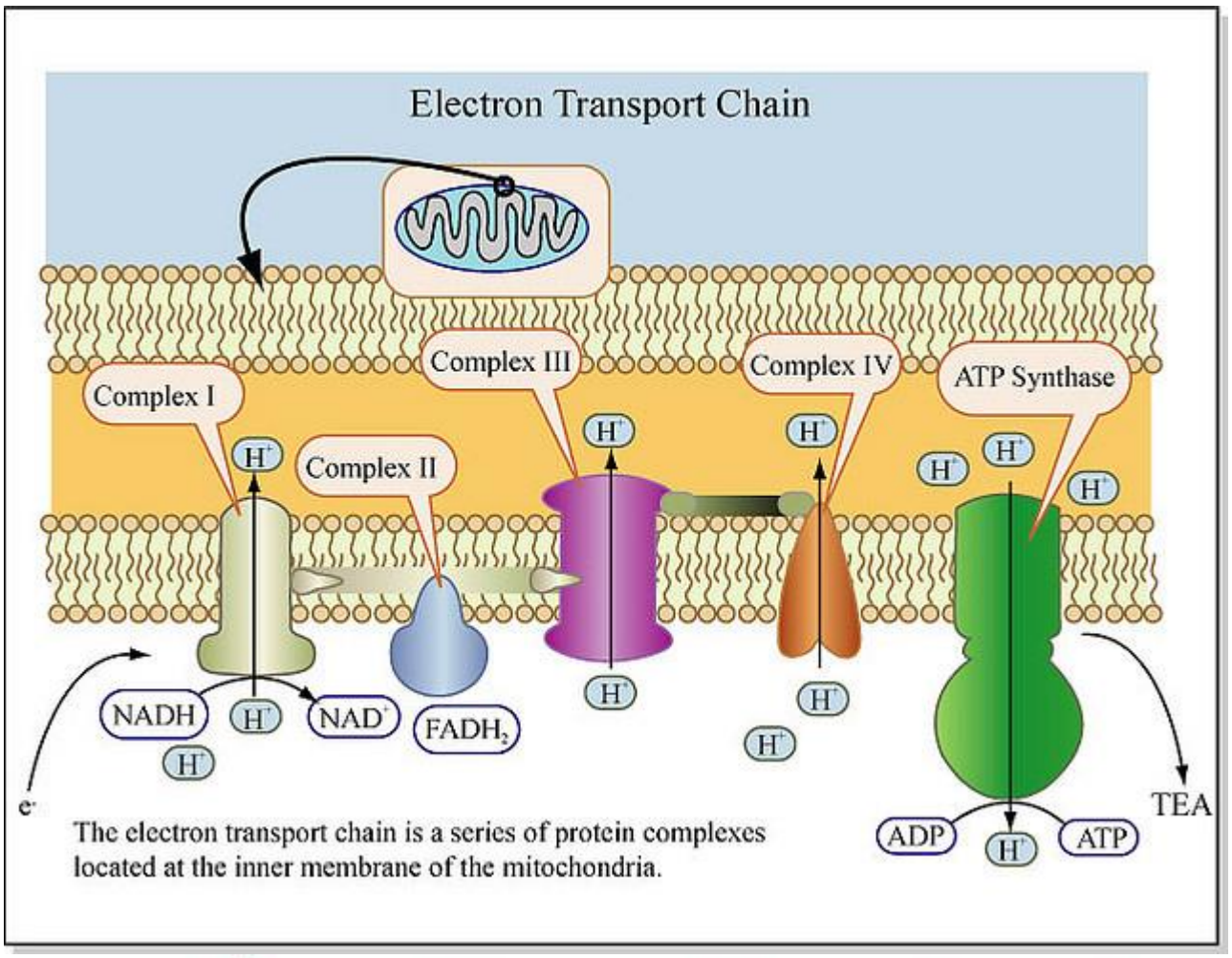
III - KoQN2-cytochrome c reductase (inhibitor - antimycin A)

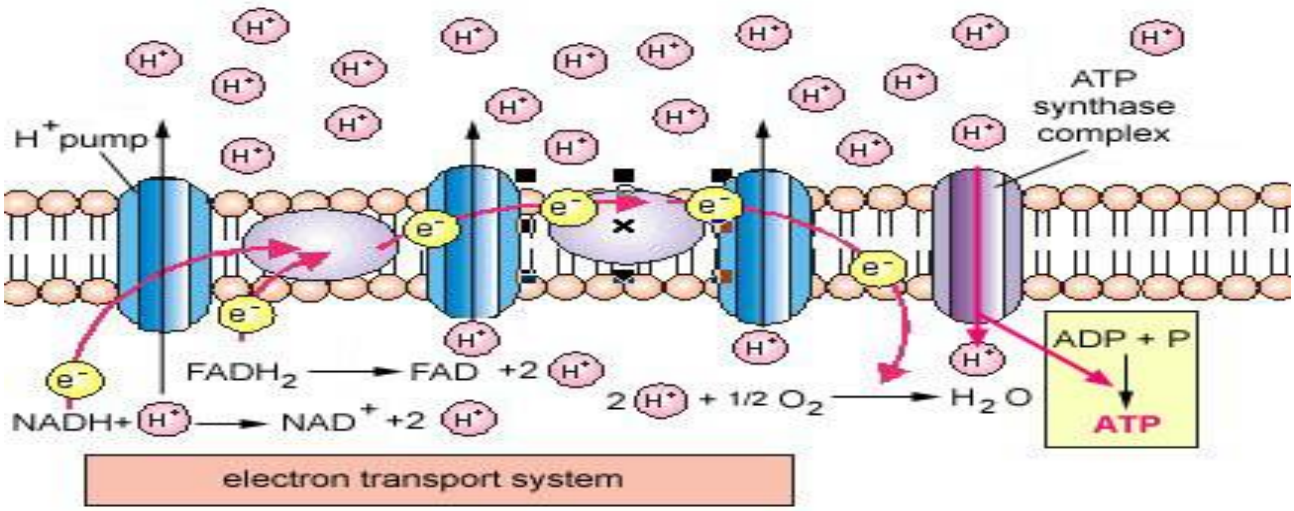
IV – cytochrome a-cytochrome oxidase (inhibitors: carbon monoxide, cyanide)

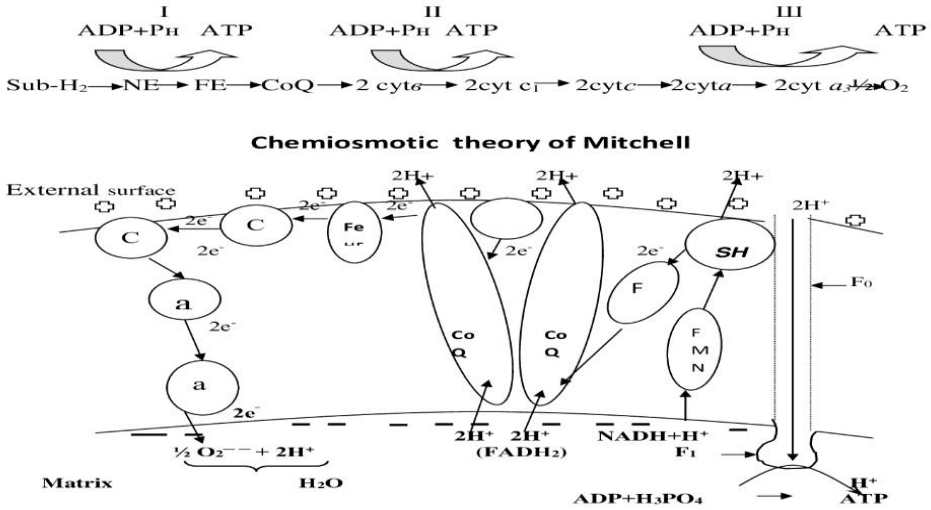
The sequence and location of these or other enzymes in the process of tissue respiration is determined by:

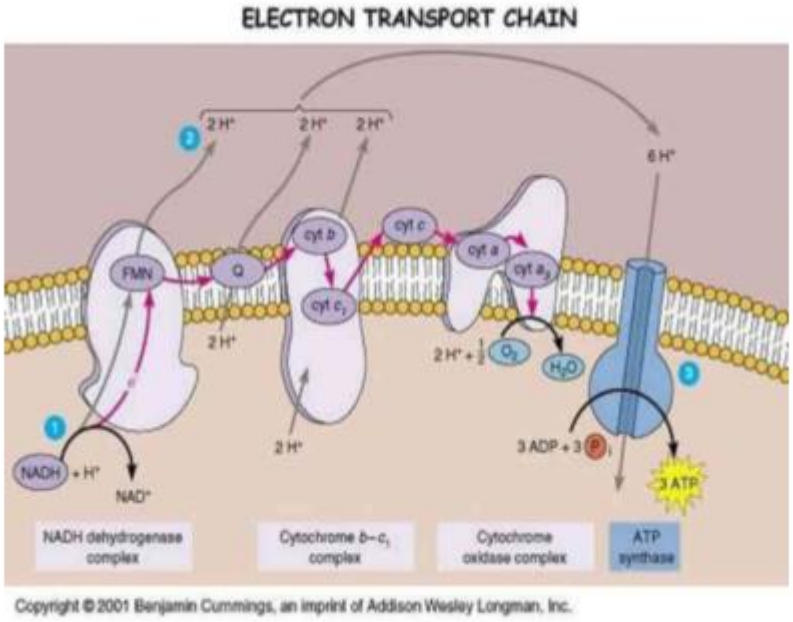
1) rate of oxidation and reduction components of the respiratory chain enzymes;

2) the redox potential of the components of the respiratory chain enzymes.


Electron Transport Chain
Complex I
Complex II
Complex III
Complex IV
ATP Synthase
NADH
NAD+
FADH2
TEA
ADP
ATP
The electron transport chain is a series of protein complexes located at the inner membrane of the mitochondria.


H+ pump
ATP synthase complex
FADH2 → FAD +2
NADH+ → NAD+ +2
2 + 1/2 O2 → H2O
ADP + P
ATP
electron transport system





The formation of free radicals, peroxides and antioxidants.

In the chain of cellular respiration at the level of ubiquinone, which is a division of the flow of protons and electrons, some electrons are "lost" and a molecule of oxygen does not receive four electrons in the outer orbit, but only one.

Free radical - a molecule or part thereof, which has an unpaired electron on the molecular or atomic outer orbit. The presence of such an electron gives the system a very high reactivity in chemical transformations and, therefore, the possibility of damage to biologically important molecules.

Superoxide anion oxygen can also act as an oxidant and (take electrons from the substrates), and as a reluctant (electrons give the substrate). In both cases, the substrate, which interacts with superoxide anion, oxygen turns into a free radical.

Formation of free radicals, peroxides and antioxidants.

In the chain of tissue respiration at the level of ubiquinone, which is a division of the flow of protons and electrons, some electrons "lost" and a molecule of oxygen does not receive four electrons in the outer orbit, but only one. Free radical - a molecule or part thereof, which has an unpaired electron on the molecular or atomic outer orbit. The presence of such an electron gives the system a very high reactivity in chemical transformations and, therefore, the possibility of damage to biologically important molecules. Superoxide anion oxygen can also act as an oxidant and (take electrons from the substrates), and as a reluctant (electrons give the substrate). **In both cases, the substrate, which interacts with superoxide anion, oxygen turns into a free radical.**

$$O_2 \uparrow -e^- \qquad O_2 + e^- \rightarrow O_2^- + e^- + 2H^+ \rightarrow H_2O_2$$

At accumulation in the tissues of hydrogen peroxide superoxide anion oxygen can interact with it to form a free hydroxyl radical (Haber-Weiss reaction):

$$O_2^- + H_2O_2 \longrightarrow OH^- + OH^\bullet + O_2$$

Superoxide anion oxygen can interact with the free hydroxyl radicals to form hydroxyl and singlet oxygen $^1O_2$, which has two electrons in the outer orbit are multidirectional spin.

$$O_2^- + OH^\bullet \rightarrow OH^- + {}^1O_2$$

Formation of superoxide increases sharply with active phagocytosis by leucocytes. Free radicals are formed by enzymatic splitting of ATP, with the appearance and conduct excitation along the nerve, the work of the sodium pump in plasma membranes of cells, the oxidation of polyunsaturated fatty acids, which are part of the plasma membrane. Formations of at the same hydrogen peroxide fatty acids, in contrast to the hydrophobic fatty acids, which reduce the permeability of cell membranes, are hydrophilic properties and "washed out" from the membrane surface. This is probably one of the mechanisms for restoring the structure of the plasma membrane and at the same time it leads to an increase in their permeability.

$$\begin{matrix} R \\ | \\ CH_2 \\ | \\ R_1 \end{matrix} + O_2^- \longrightarrow \begin{matrix} R \\ | \\ CH\text{-}O\text{-}O^- \\ | \\ R_1 \end{matrix} + \begin{matrix} R_2 \\ | \\ CH_2 \\ | \\ R_3 \end{matrix} \longrightarrow \begin{matrix} R \\ | \\ CH\text{-}O\text{-}OH \\ | \\ R_1 \end{matrix} + \begin{matrix} R_2 \\ | \\ CH^* \\ | \\ R_3 \end{matrix}$$

Toxic effects of free radicals and hydrogen peroxide is determined by the fact that they can dramatically change the structure of virtually all biological macromolecules - nucleic acids, proteins, fats,

carbohydrates, and disrupt their function.

The first line of defense cells against free radicals by a group of enzymes superoxide dismutase, which trap superoxide radicals O2, which are generated in the cell, and connecting them with two protons is converted into hydrogen peroxide and molecular oxygen

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Two other groups of enzymes - catalase and peroxidase - are designed to capture hydrogen peroxide. They catalyze the last two-electron reduction in water:

a) either using hydrogen peroxide as an electron donator (in the case of catalase)

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$$

a) either involving different reluctant (in the case of peroxidase)

$$H_2O_2 + 2DH_2 \rightarrow 2H_2O + DH_2 + D$$, here **D-** regenerator

Glutathione peroxidase catalyzes the oxidation of glutathione by hydrogen peroxide:

$$H_2O_2 + 2GluSH \rightarrow 2H_2O + Glu\text{-}S\text{-}S\text{-}Glu$$

or organic peroxides:

$$2ROOH + 2\ GluSH \rightarrow R\text{-}OH + H_2O + Glu\text{-}S\text{-}S\text{-}\ Glu$$

In the structure of glutathione peroxidase is selenium (Se) instead of sulfur (S) of cysteine, so selenium is necessary for the functioning of antioxidant system.

$$E\text{-}SeH + R - O\text{-}OH \longrightarrow E - SeOH + R - OH$$

Reduced glutathione peroxidase	Lipids hydro peroxide	Oxidated glutathione peroxidase	Hydroxycompounds

GP oxidized with the participation of reduced glutathione into a restored glutathione, and reduced glutathione oxidized:

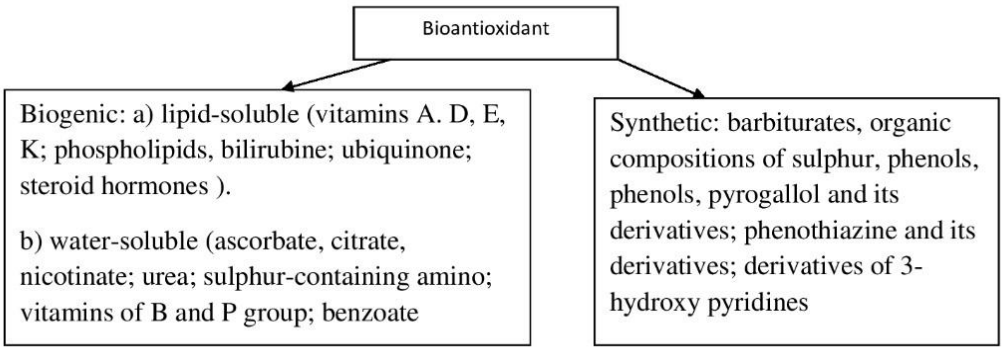
E-SeOH + 2 **GluSH** ⟶ E –SeH + **Glu-S-S- Glu** + H_2O

Oxidized GP Reduced glutathione Reduced GP Oxidized glutathione

Glutathione with the participation of glutathione reductase (coenzyme NADPH + H^+) becomes a reduced form

Glu-S-S- Glu + NADP+H^+ → 2 **GluSH**+ NADP

The second line of defense - is available in any cell of a group of substances called antioxidants. Antioxidants inhibit free-radical, non-enzymatic oxidation of energy substrates, especially unsaturated fatty acids, carbohydrates, hydrocarbons, and some amino acids, reducing output toxic oxidation products. Bioantioxidant system (BAO) of an organism or cell consists of exogenous antioxidants, which are delivered with the food, and endogenous synthesized in itself. For the convenience of study they are classified on the nutrient antioxidants and synthetic.



Microsomes:

- Microsomal enzyme system→ mixed function oxidase → mono-oxygenases

- Its components include

  Cytochrome P450

  Flavinoprotein (co-enzymes in redox reaction) NADPH

  Molecular oxygen,

Drug + enzyme +oxygen molecule +NADPH +Flavoprotein

4

Oxidation Reactions

Microsomal oxidation

- occurs in microsomes
- e.g. cytochrome P450 enzymes, NADPH and oxygen

Non microsomal oxidation

- occurs in cytosol or mitochondria
- e.g. oxidases and dehydrogenases.
  - Alcohol – Dehydrogenase
  - Adrenaline – MAO
  - Xanthine – Xanthine oxidase

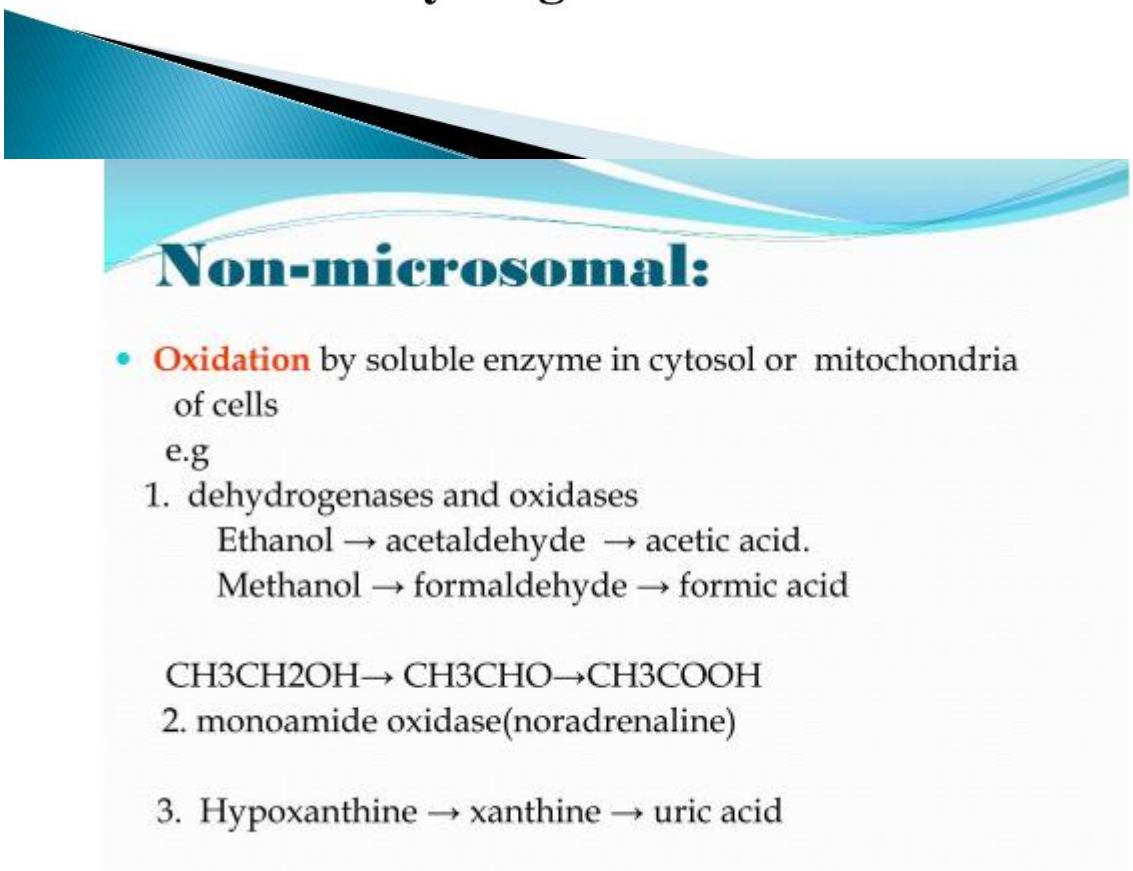
Oxidation Reactions

Microsomal oxidation (CYT-P450).

Oxidation by cytochrome P450 enzymes

Non-microsomal oxidation.

Oxidation by soluble enzymes in *cytosol* or *mitochondria* of cells (as oxidases and dehydrogenases) e.g. monoamine oxidase (MAO) and alcohol dehydrogenase.



General material and educational and methodological support of the lecture:
- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks

- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Exchange of substances (metabolism) - general patterns of catabolic and anabolic processes.
2. Joint stages of intracellular catabolism of biomolecules: proteins, carbohydrates, lipids.
3. Cycle of tricarboxylic acids. Localization, sequence of enzymatic reactions, significance in metabolism.
4. Energy balance of the cycle of tricarboxylic acids. Physiological significance of CTC reactions.
5. Reactions of biological oxidation; types of reactions (dehydrogenase, oxidase, oxygenase) and their biological significance. Tissue respiration.
6. Enzymes of biological oxidation in mitochondria: pyridine-, flavin-dependent dehydrogenases, cytochromes.
7. The sequence of the components of the mitochondrial respiratory chain. Molecular complexes of the inner membranes of mitochondria.
8. Oxidative phosphorylation: coupling points of electron transport and phosphorylation, coefficient of oxidative phosphorylation
9. Chemiosmotic theory of oxidative phosphorylation, mitochondrial ATP synthetase.
10. Inhibitors of electron transport and uncouplers of oxidative phosphorylation.
11. Microsomal oxidation: cytochrome P-450; molecular organization of the electron transport chain.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 7

Topic: Carbohydrate metabolism: glycolysis, glycogenolysis, oxidative decarboxylation of pyruvate, interconversion of monosaccharides, metabolism of fructose, galactose. Carbohydrate metabolism: Glycogen biosynthesis, pentose phosphate pathway, gluconeogenesis. Enzymopathies of carbohydrate metabolism (glycogen storage diseases). Diabetes mellitus.
Relevance of the topic: Carbohydrates are the main component of biomass on our planet, they make up the main structural material of plants, and are also one of the important food products for mammals, including humans. At the molecular level, relative to humans, they play the role of the main source of energy, take part in the structure and maintenance of the functions of cell membranes, are part of the intercellular substance and provide the adhesive properties of cells. Oxidation of carbohydrates is the basis of bioenergetics of nervous tissue. Violation of carbohydrate metabolism is the cause of a number of diseases: such as diabetes, galactosemia, rheumatism and others.

Purpose: to study the general patterns of carbohydrate metabolism as the main source of energy in the human body; to study the main mechanisms of carbohydrate metabolism disorders, which are the cause of a number of diseases: such as diabetes, galactosemia, rheumatism, and others.

Basic concepts:
1. Glycolysis, glycogenolysis. Dichotomous way.
2. Shuttle mechanisms.
3. Multienzyme complex.
4. PPP. Apotomic way.
5. Corey cycle.
6. Galactosemia.
7. Diabetes.
8. Glycogen storage diseases.

Plan and organizational structure of the lecture:
1. Sequence of reactions of glycolysis and glycogenolysis.
2. Biological significance of glycolysis.
3. Oxidative decarboxylation of pyruvate.
4. Metabolism of fructose, galactose. Interconversion of monosaccharides
5. Pentose phosphate pathway. Sequence of reactions and characteristics of enzymes. Biological role of PPP.
6. Biosynthesis of glycogen.
7. Gluconeogenesis. Glucose-lactate and glucose-alanine cycles.
8. Violation of carbohydrate metabolism.
9. Hereditary enzymopathies of carbohydrate metabolism.
10. Diabetes mellitus.

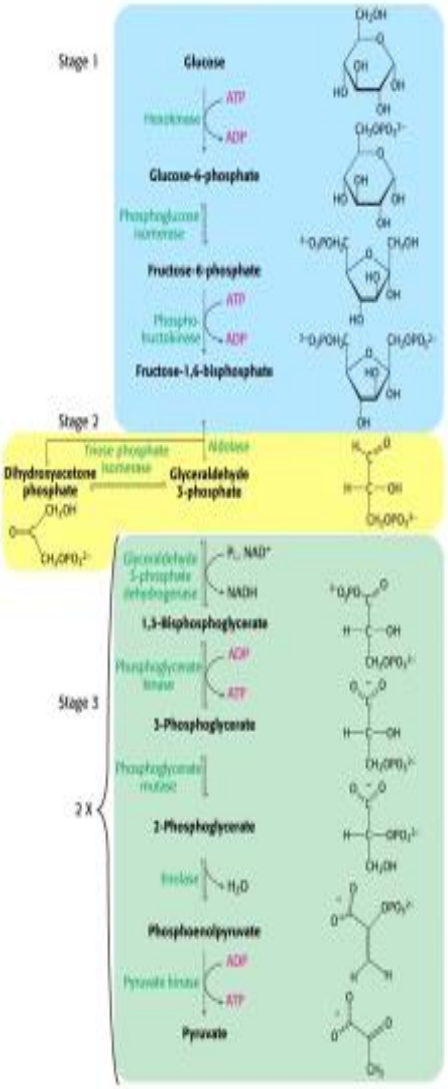
Content of the lecture material

- Greek word - “glycos” - meaning sweet/sugar and “lysis” - meaning dissolution
- Glycolysis is the first step in the breakdown of glucose to extract energy for cellular metabolism. Glycolysis consists of an energy-requiring phase followed by an energy-releasing phase.
- Glycolysis is a series of reactions that extract energy from glucose by splitting it into two three-carbon molecules called pyruvates.
- Sequence of reactions converting glucose to lactate with production of ATP.
- Embden-Meyerhof pathway

Stage 1 - Investment of ATP. Glucose is phosphorylated. The negative charge concent glucose in the cell and glucose becomes less stable.

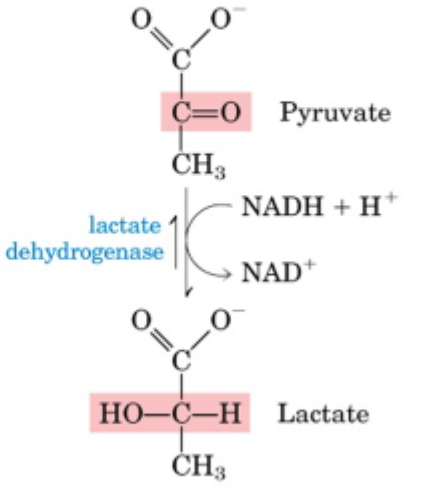
Stage 2 – The 6 carbon sugar is split to two 3-carbon fragments.

Stage 3 – Energy yielding phase. The oxidation of the 3-carbon fragments yields ATP



Under anaerobic conditions pyruvate is converted to lactate.
(exercising muscle is an example)

The NAD^+ that is consumed in the glyceraldehyde 3-phosphate reaction is produced in the lactate DH reaction. The redox balance is maintained. The activities of glyceraldehyde 3-phosphate DH and lactate DH are linked metabolically.



$\Delta G'^{\circ} = -25.1$ kJ/mol

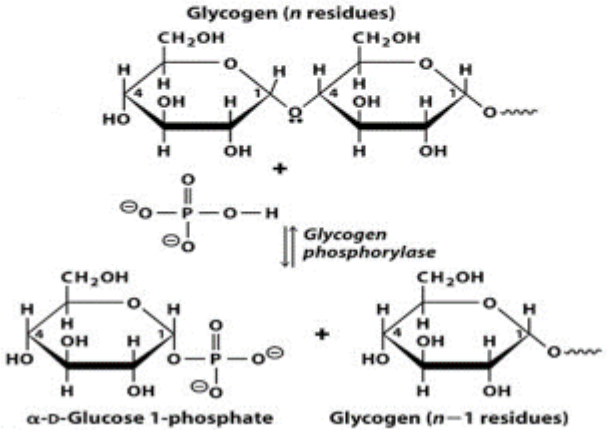
Glycolytic oxidoreduction

The NAD^+ that is consumed in the glyceraldehyde 3-phosphate reaction is produced in the lactate DH reaction. Thus, redox balance is maintained.

The $NADH \cdot H^+$ that is produced in the glyceraldehyde 3-phosphate reaction is consumed in the lactate DH reaction. Thus, redox balance is maintained.

Glucose + 2 P_i +2 ADP → 2 lactate + 2 ATP + 2 H_2O

Glycogenolysis refers to the breakdown of glycogen, a branched polymer of glucose,into monomers to continue glycolysis or to maintain blood glucose level. It usually occurs in liver and muscles.



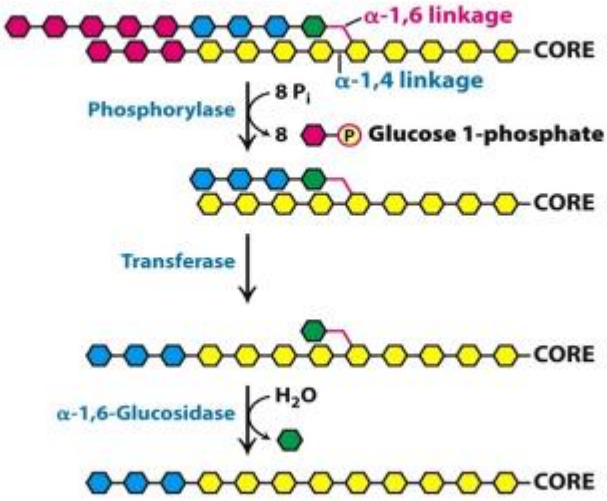
1. Action of Glycogen phosphorylase:

- This enzyme catalyses the reaction in which an (α1→4) glycosidic linkage between two glucose residues at a nonreducing end of glycogen undergoes attack by inorganic phosphate(P_i), removing the terminal glucose residue as α-D-glucose 1-phosphate
- It is a phosphorolysis reaction

2. Debranching enzyme:

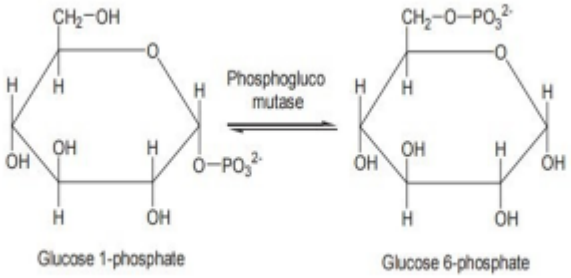
- It consists of two enzymes that catalyze twosuccessive reactions
- Oligo(α1→6) to (α1→4) glucotransferase shifts a block of three glucosyl residues from one outer branch to the other. This transfer exposes a single glucose residue joined by anα-1,6-glycosidic linkage
- α-1,6-glucosidase hydrolyses the α-1,6- glycosidic bond, resulting in the release of a free glucose molecule

PROCESS OF GLYCOGENOLYSIS



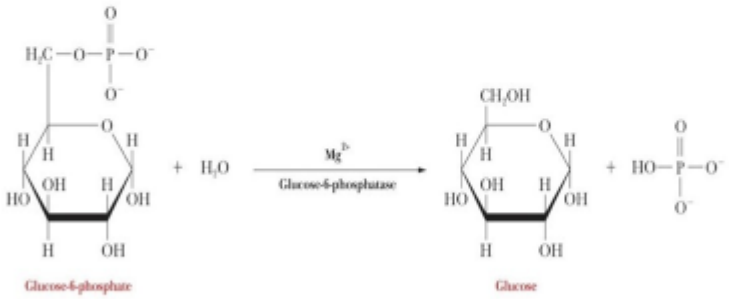
3. Conversion of Glucose 1-phosphate to Glucose 6-phosphate:

- Enzyme – Phosphoglucomutase
- Reversible reaction
- Initially phosphorylated at a ser residue, the enzyme donates a phosphoryl group to C-6 of the substrate, then accepts a phosphoryl group for C-1
- The glucose 6-phosphate formed from glycogen in skeletal muscle can enter glycolysis and serve as an energy source to support muscle contraction



4. Conversion of Glucose 6-phosphate to Glucose:

- In liver, glycogen breakdown serves a different purpose – to release glucose into the blood when the blood glucose level drops, as it does between meals
- Enzyme – glucose 6-phosphatase
- The enzyme is present in liver and kidney



Oxidative decarboxylation of Pyruvate

•In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are degraded to the Acetyl group of Acetyl-CoA, the form in which the citric acid cycle accepts most of its fuel input.

•The oxidative decarboxylation of Pyruvate to form Acetyl-CoA is the link between Glycolysis and the Citric acid cycle.

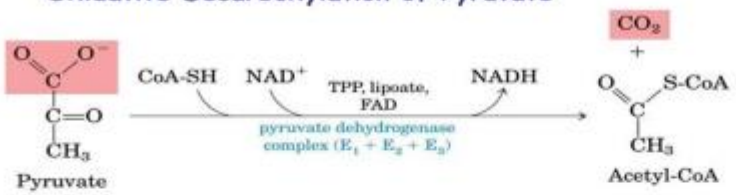
•The reaction occurs in the mitochondrial matrix.

•The pyruvate derived from glucose by glycolysis is dehydrogenated to yield acetyl CoA and CO_2 by the enzyme pyruvate dehydrogenase complex (PDC)

•It is an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO_2 and the two remaining carbons become the acetyl group of Acetyl-CoA.

•High activities of PDC are found in cardiac muscle and kidney.

Oxidative Decarboxylation of Pyruvate

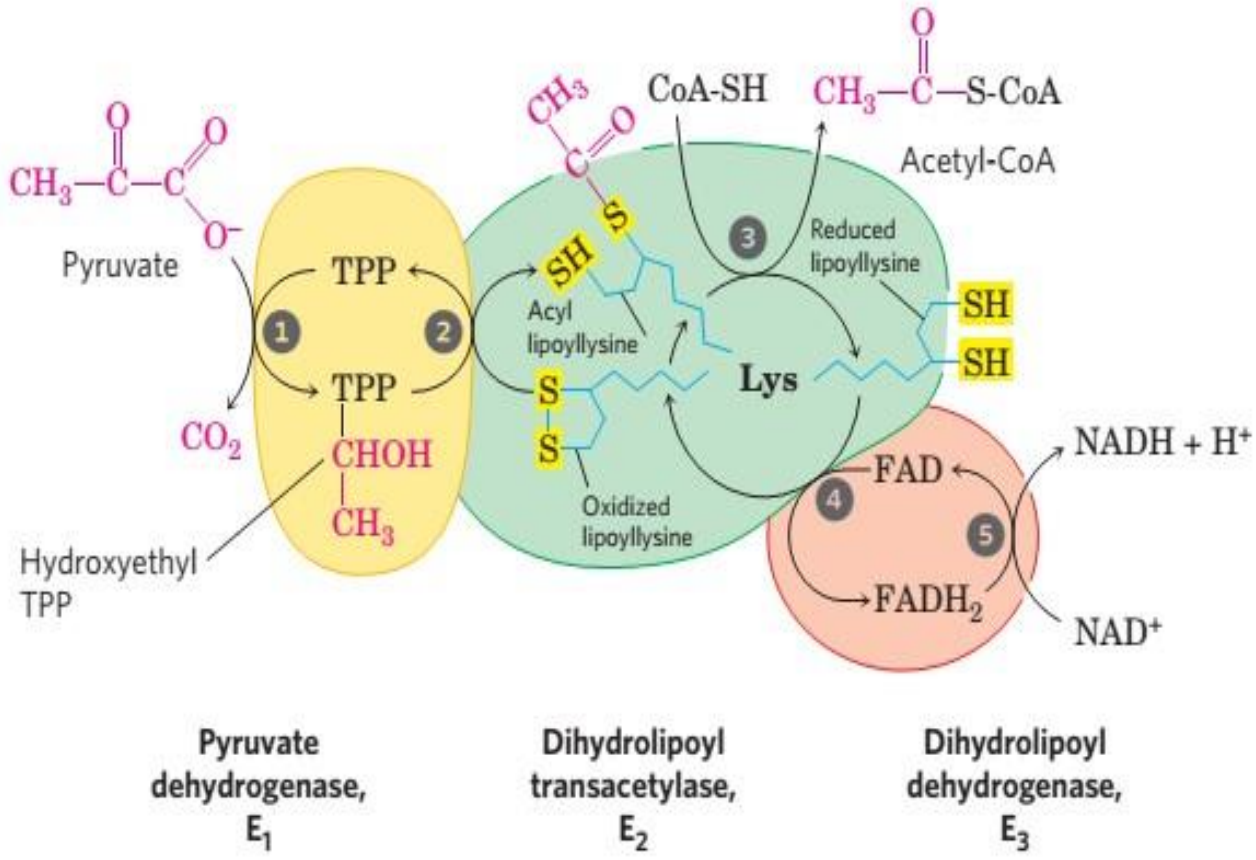


- Pyruvate Dehydrogenase complex is a large multi-subunit complex located in the mitochondria
- Irreversible reaction; Acetyl CoA cannot be converted into pyruvate
- Pyruvate dehydrogenase is not a part of citric acid cycle but it a major source of fuel for citric acid cycle which is Acetyl CoA
- Pyruvate Dehydrogenase complex is aggregate of three enzymes:
  1- Pyruvate dehydrogenase component called (pyruvate decarboxylase)
  2- Dihydrolipoyl transacetylase
  3- Dihydrolipoyl dehydrogenase

Each subunit of this large complex catalyzes a part of the overall reactions.

PDC contains 5 coenzymes:

Thiamine pyrophosphate (TPP), Lipoic acid (LA), Coenzyme A (CoA), Flavin adenine dinucleotide (FAD) and Nicotinamide adenine dinucleotide (NAD^+).



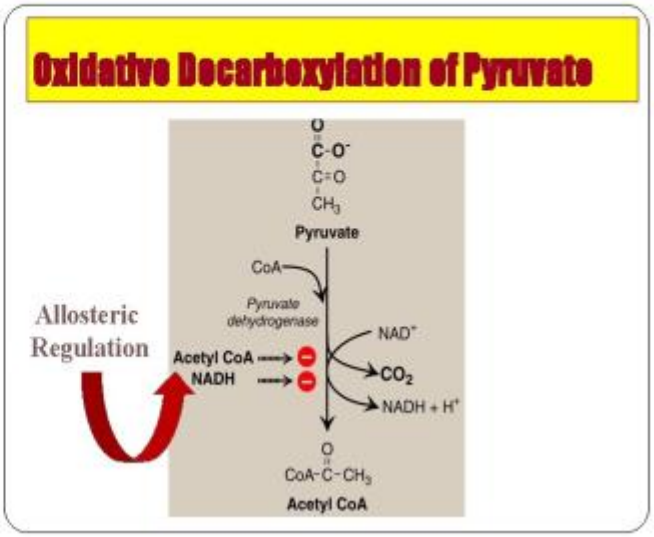
Regulation of Oxidative Decarboxylation of Pyruvate:

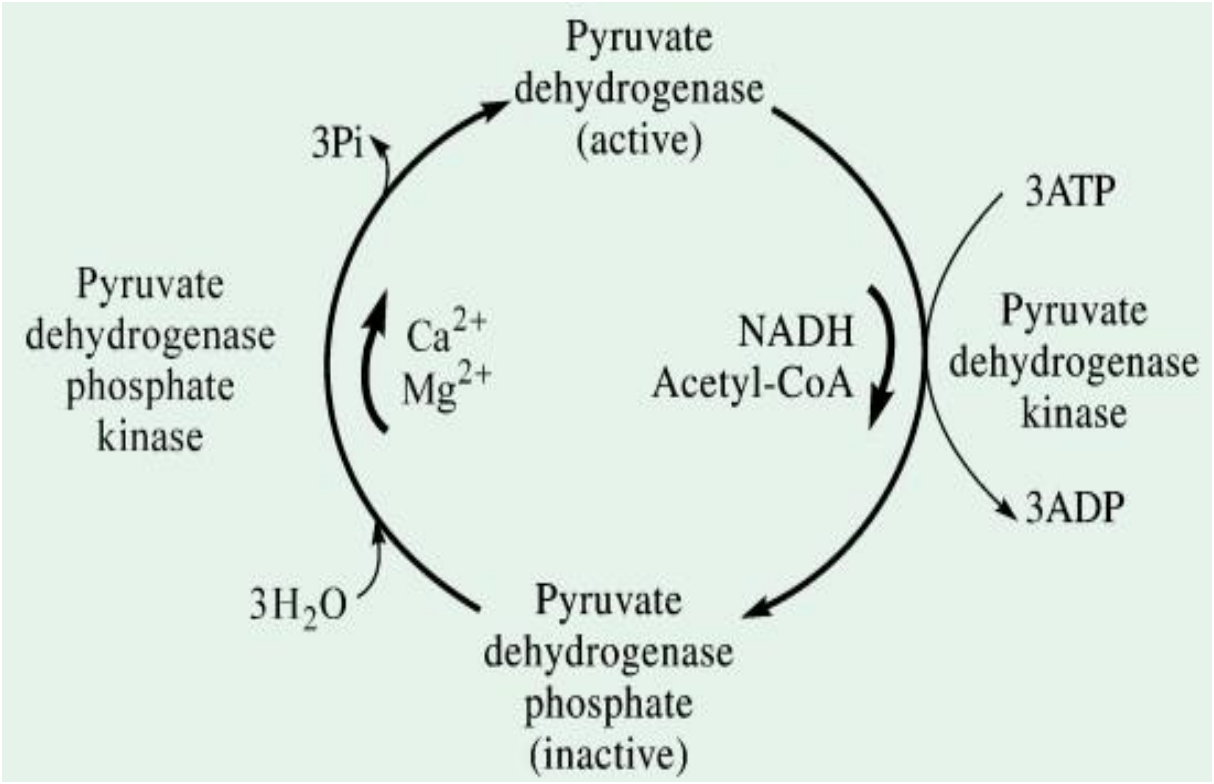
PDC which catalyzes the oxidative decarboxylation of pyruvate is regulated in 3 ways:

•End-product inhibition:
Acetyl-CoA and NADH-H⁺, both end products of the pyruvate dehydrogenase reaction, are potent allosteric inhibitors of the enzyme. The inhibitory effects are reversed on the addition of coenzyme A and NAD^+ respectively.

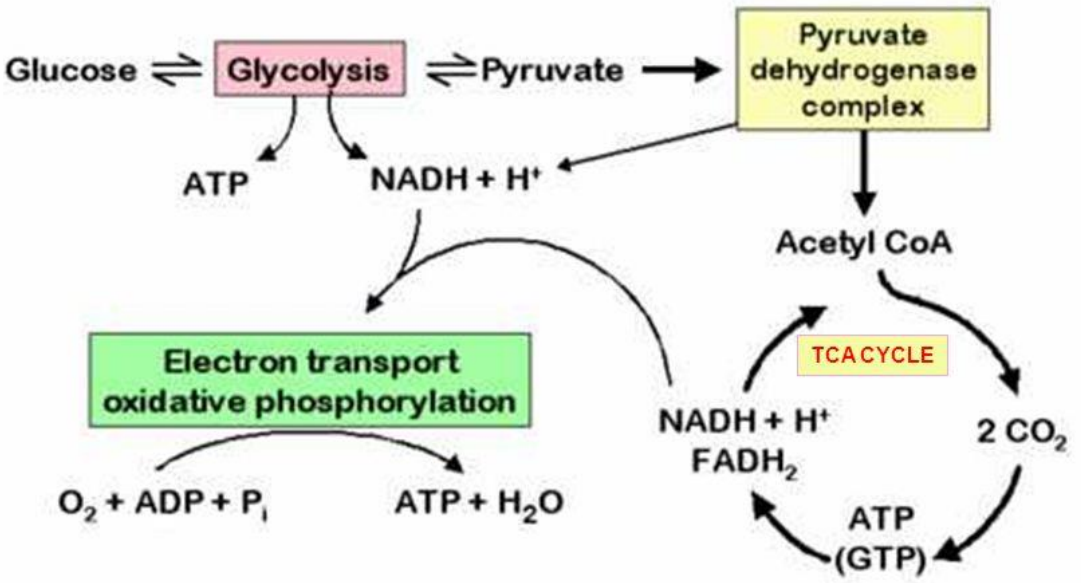
•Feedback regulation:
The activity of PDC is controlled by the energy charge. The pyruvate dehydrogenase component is specifically inhibited by GTP and activated by AMP.

•Covalent modification:
Under conditions of high concentrations of ATP, acetyl-CoA and those of the intermediates of TCA cycle, further formation of acetyl-CoA is slowed down. This is accomplished by covalent modification.





Aerobic oxidation



3

ATP from Glucose

One glucose molecule undergoing complete oxidation provides:

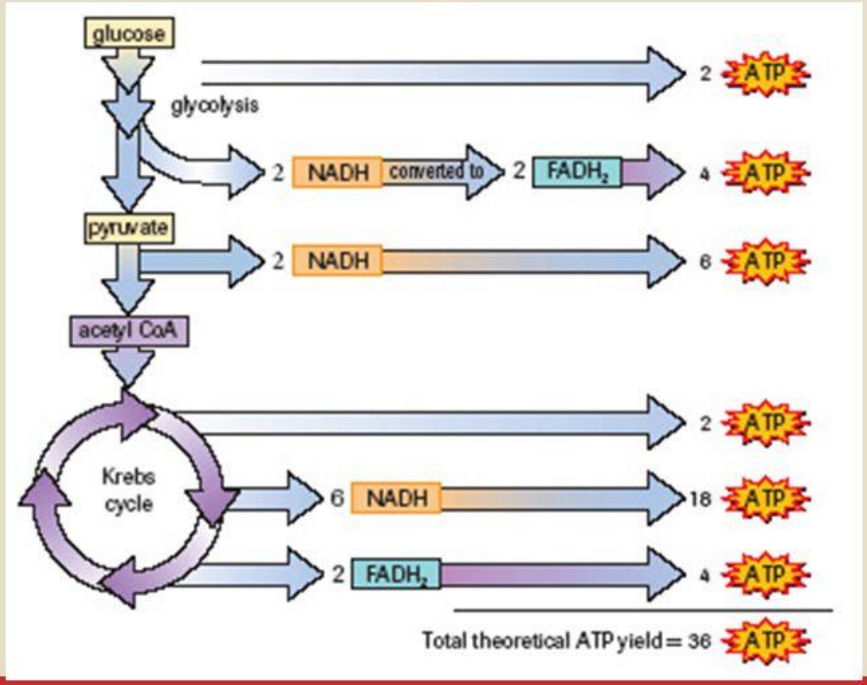
From glycolysis	6 – 8 ATP
From 2 pyruvate	6 ATP
From 2 acetyl CoA	24 ATP
	36-38 ATP

Overall ATP Production for one glucose

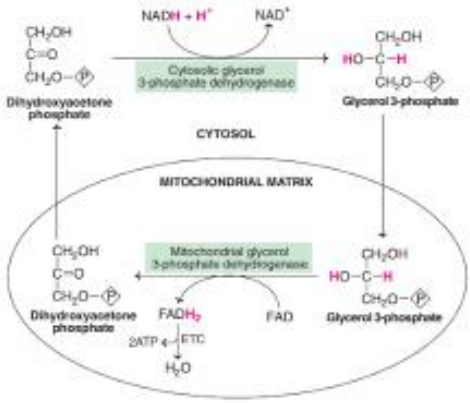
$$C_6H_{12}O_6 + 6O_2 + (36-38)ADP + (36-38)P_i \longrightarrow 6CO_2 + 6H_2O + (36-38)ATP$$

glucose

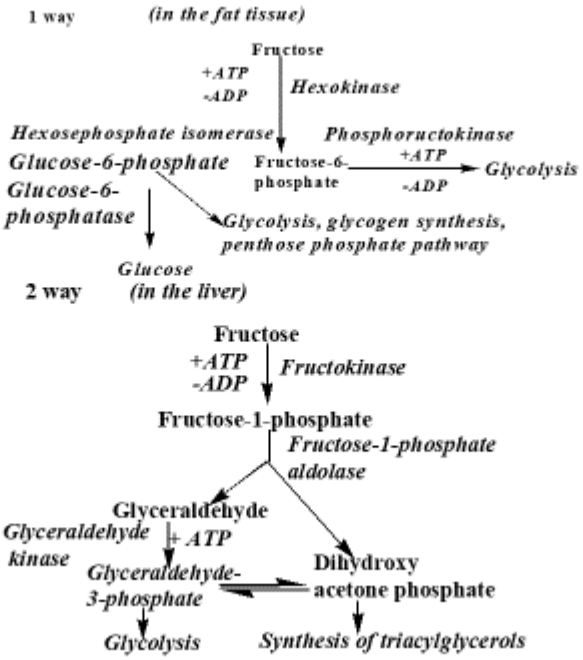
The Aerobic Respiration Energy Balance Sheet



Shuttle mechanisms

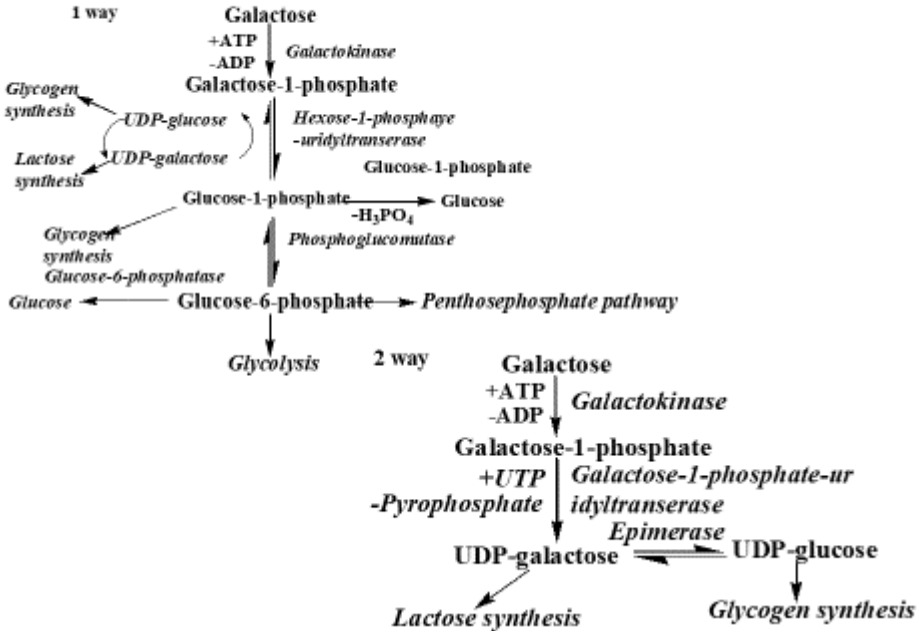


Metabolism of fructose



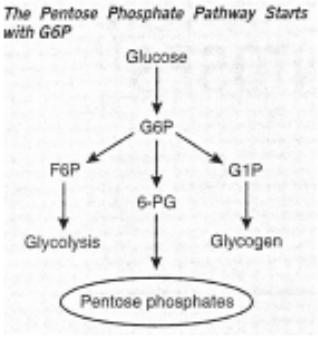
Metabolism of galactose.

Galactose is produced by the hydrolysis of disaccharide - lactose in the small intestine.



•The Pentose Phosphate Pathway

•The pentose phosphate pathway takes place entirely within the cytoplasm and is also known as the hexose monophosphate shunt or phosphogluconate pathway.
•The most important function of the pentose phosphate pathway is to reduce 2 molecules of $NADP^+$ to $NADPH{\cdot}H^+$ or each glucose-6-phosphate that is oxidatively decarboxylated to ribulose-5-phosphate.
•$NADPH{\cdot}H^+$ is critical to maintaining reduced glutathione levels in cells which is required to minimized damage from reactive oxygen species.
•The pentose phosphate pathway is also responsible for producing ribose-5-phosphate which provides the ribose sugar backbone that anchors the nucleotide base to DNA and RNA polymers.



The pentose phosphate pathway can be divided into 2 phases:

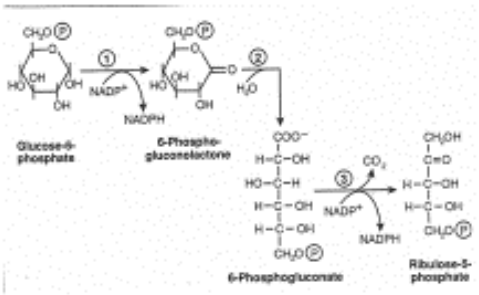
- the oxidative phase, which generates $NADPH{\cdot}H^+$ and pentose phosphates
- the nonoxidative phase, which interconverts C3, C4, C5, C6 and C7 sugar phosphates.

•3 enzymatic reactions in the oxidative phase:

•1. Oxidation of glucose-6P by the enzyme glucose-6-P dehydrogenase (G6PD) to 6-phosphogluconolactone is coupled to the reduction of $NADP^+$ resulting in the formation of 1 molecule of $NADPH{\cdot}H^+$. This is the commitment step in the pathway.

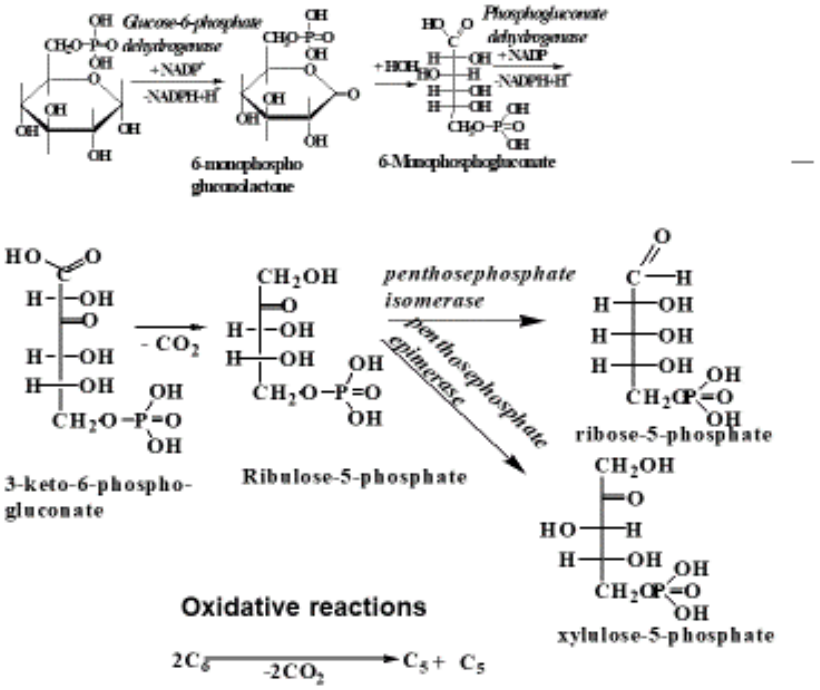
•2. 6-phosphogluconolactone is hydrolyzed by lactonase to produce the open chain monosaccharide-6-phosphogluconate.

•3. 6-phosphogluconate is then oxidized and decarboxylated by 6-phosphogluconate dehydrogenase to generate ribulose-5-P, CO2 and the second molecule of $NADPH{\cdot}H^+$.



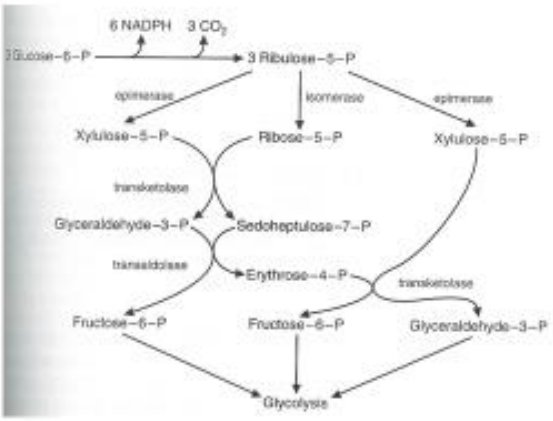
Pentose phosphate pathway of carbohydrate metabolism.

Aerobic step

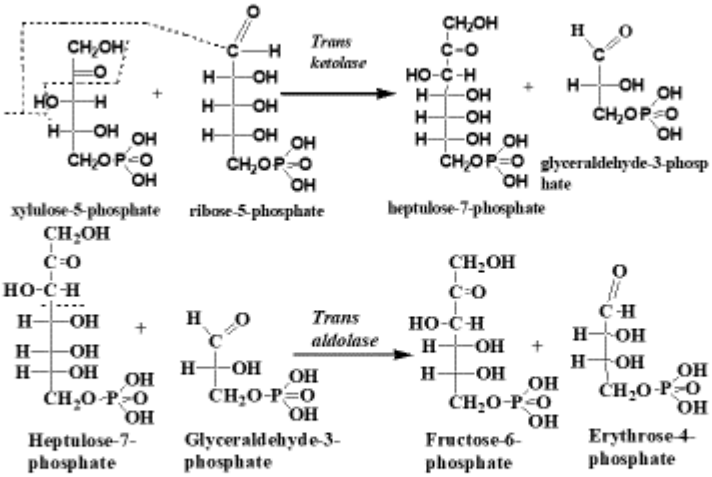


The non-oxidative phase of the PPP

In cells that require high levels of NADPH·H$^+$ for biosynthetic reactions, the ribulose-5-P produced in the oxidative phase needs to be converted back into glucose-6-P to maintain flux through the glucose-6-P dehydrogenase reaction.



Anaerobic step



Non-oxidative reactions

•***Under deficiency of erythrose-4-phosphate*** **the accumulation of pentose phosphates takes place.**

$$C_6 \longrightarrow 2C_3$$

$$C_6 + C_3 \xrightarrow{\text{transketolase}} C_4 + C_5$$

$$C_3 + C_4 \xrightarrow{\text{transaldolase}} C_7$$

$$C_7 + C_3 \xrightarrow{\text{transketolase}} C_5 + C_5$$

• ***In excess of erythrose-4-phosphate*** **there is involvement of pentose phosphates, which were formed by the degradation of nucleic acids or nucleotides in the formation of metabolites of glycolysis.**

$$C_5 + C_5 \xrightarrow{\text{transketolase}} C_7 + C_3$$

$$C_7 + C_3 \xrightarrow{\text{transaldolase}} C_4 + C_6$$

$$C_4 + C_5 \xrightarrow{\text{transketolase}} C_6 + C_3$$

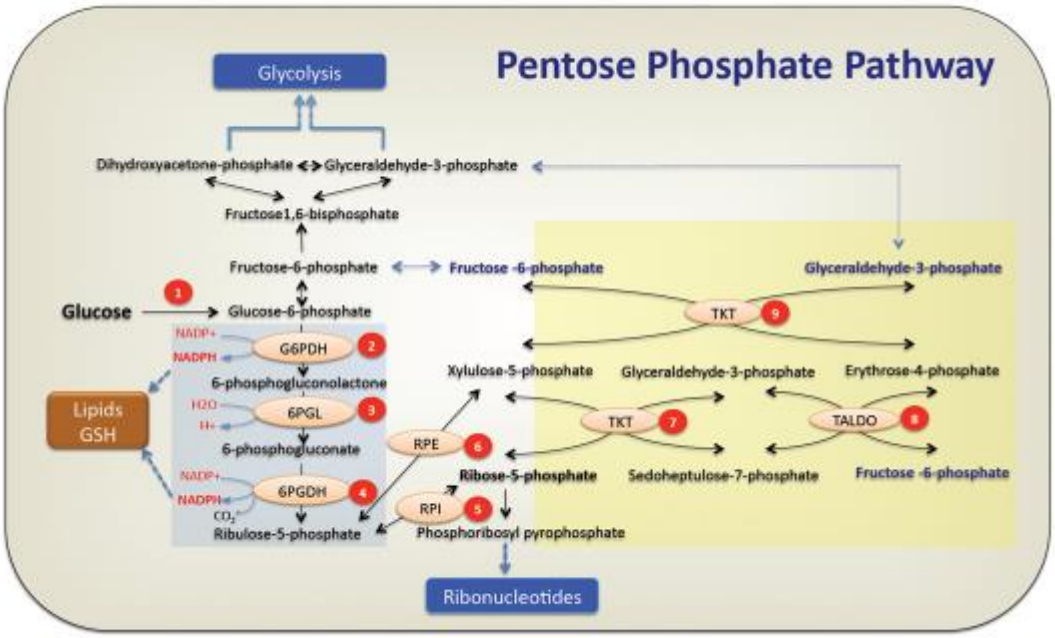
$$C_4 + C_5 \xrightarrow{\text{transketolase}} C_6 + C_3$$

•Metabolic flux through the Pentose Phosphate Pathway is tightly-regulated:

•1. If increased NADPH·H^+ is required form biosynthetic pathways, or to provide reducing power for detoxification, then fructose-6P and glyceraldehyde-3P are used to resynthesize glucose-6P and thereby maintain flux through the oxidative phase of the pathway.

•2. If cells need to replenish nucleotide pools due to high rates of DNA and RNA synthesis, then the bulk of ribulose-5P is converted to ribose-5P to stimulate nucleotide biosynthesis.

•3. If ATP levels in the cell are low, and NADPH·H^+ levels are not limiting, then glucose-6-P-dehydrogenase is inhibited and the pentose phosphate pathway is bypassed so that glucose-6P can be metabolized directly by the glycolytic pathway.



Regulation of pentose phosphate pathway

•The entry of glucose 6-phosphate into the pentose phosphate pathway is controlled by the cellular concentration of $NADPH{\cdot}H^+$

•$NADPH{\cdot}H^+$ is a strong inhibitor of glucose-6-phosphate dehydrogenase

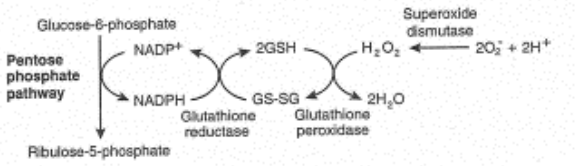
•As $NADPH{\cdot}H^+$ is used in various pathways, inhibition is relieved, and the enzyme is accelerated to produce more $NADPH{\cdot}H^+$

•The synthesis of glucose 6-phosphate dehydrogenase is induced by the increased insulin/glucagon ratio after a high carbohydrate meal.

•Pentose phosphate pathway protects cells against reactive oxygen species (ROS), molecular oxygen and partially reduced, reactive forms of oxygen. Reduction of molecular O2 in a series of one-electron steps yields superoxide, hydrogen peroxide, hydroxyl radical, and water.
The intermediate, activated forms of oxygen are known as reactive oxygen species (ROS)

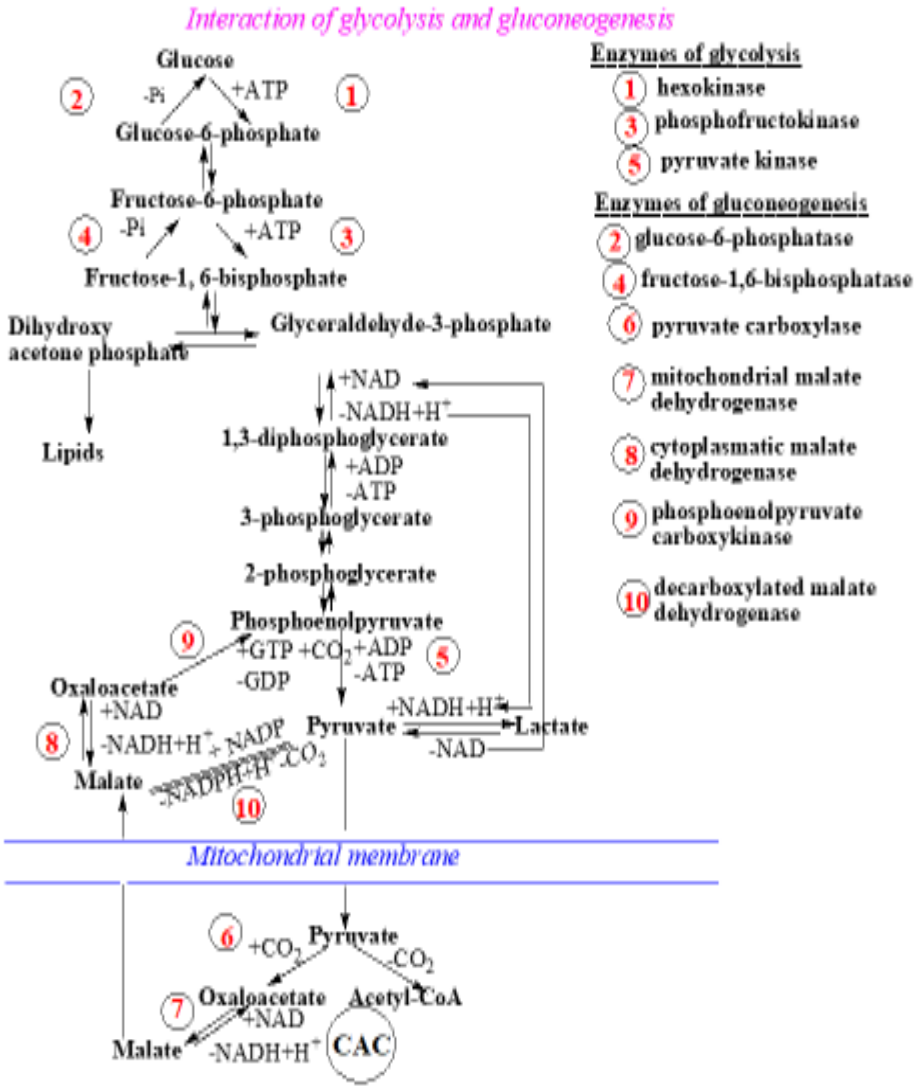
•Role of $NADPH{\cdot}H^+$ and glutathione in protecting cells against ROS

•Reduced glutathione (GSH) protects the cell by destroying hydrogen peroxide and hydroxyl free radicals. Regeneration of GSH from it oxidized form (GS-SG) requires the $NADPH{\cdot}H^+$ produced in the glucose 6-phosphate dehydrogenase reaction.

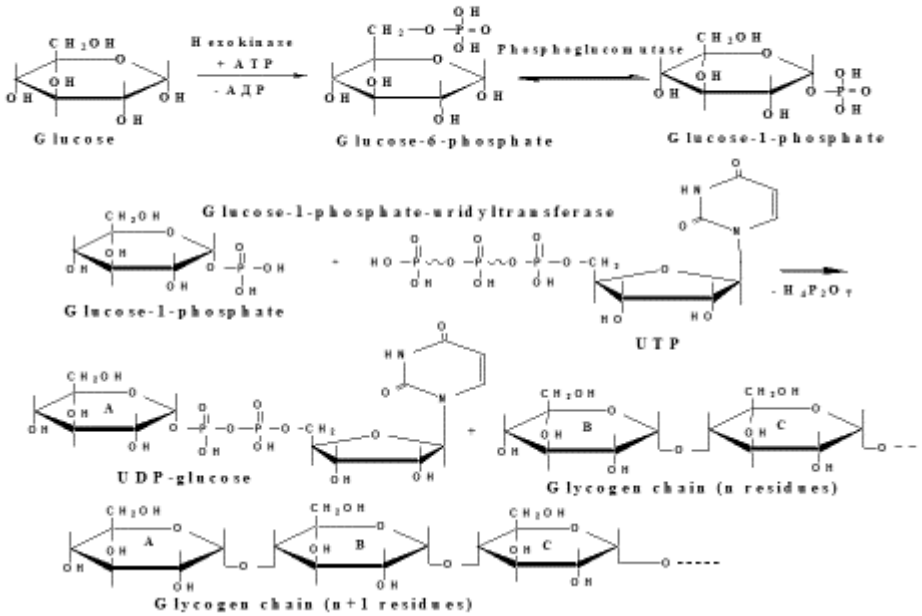


Clinical aspects.

•Glucose-6-phosphate dehydrogenase deficiency causes hemolytic anemia
•Mutations present in some populations causes a deficiency in glucose 6-phosphate dehydrogenase, with consequent impairment of $NADPH{\cdot}H^+$ production.
•Detoxification of H2O2 is inhibited, and cellular damage results - lipid peroxidation leads to erythrocyte membrane breakdown and hemolytic anemia.
•Most G6PD-deficient individuals are asymptomatic - only in combination with certain environmental factors (sulfa antibiotics, herbicides, aspirin, antimalarials) do clinical manifestations occur.
•toxic ingredient of fava beans



Biosynthesis of glycogen (glycogenesis).

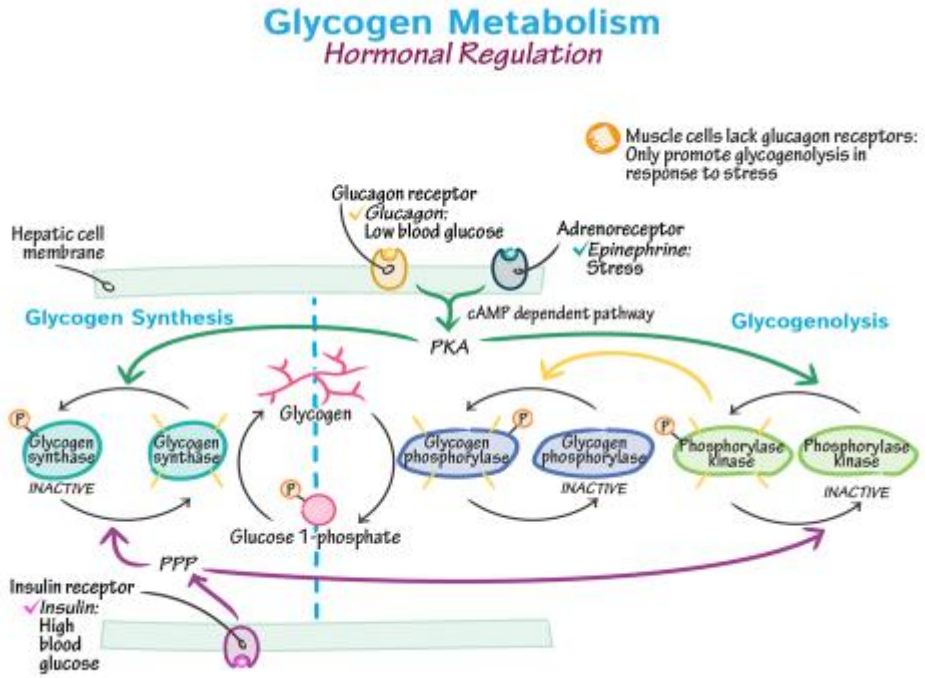


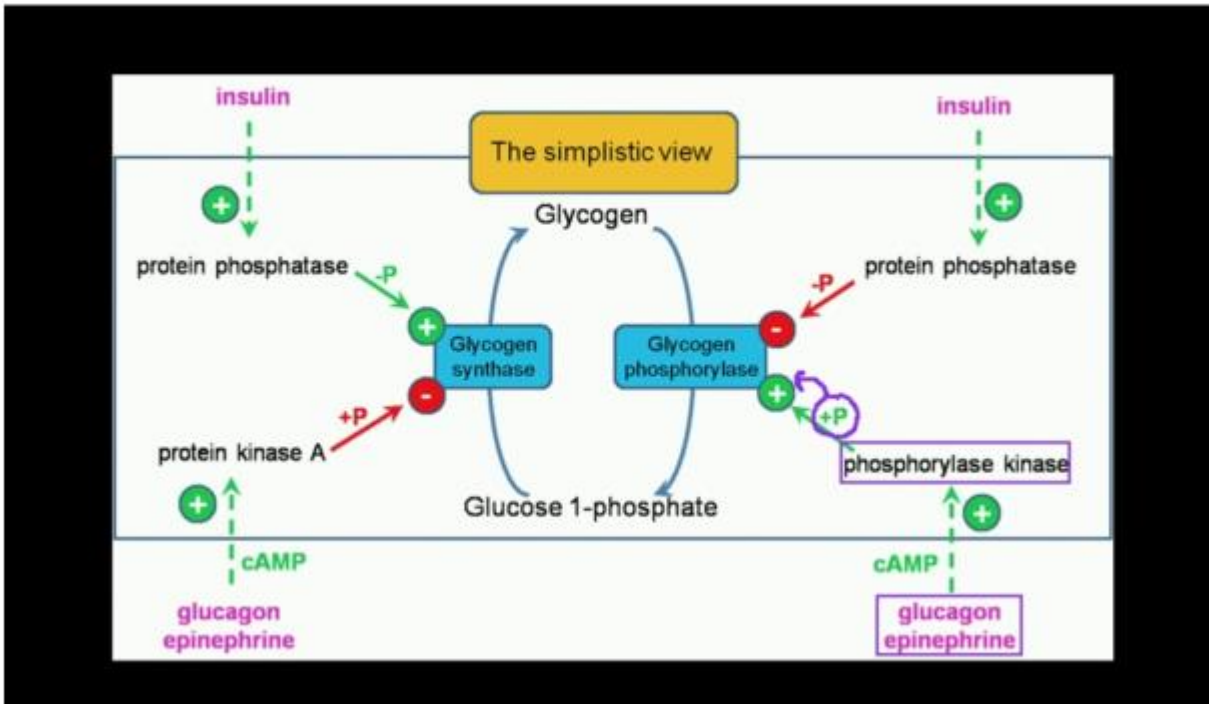
The biological role of the pentose phosphate pathway of carbohydrate metabolism

1. It provides pentose phosphates to organism that are necessary for the biosynthesis of nucleotides, nucleic acids - DNA and RNA.

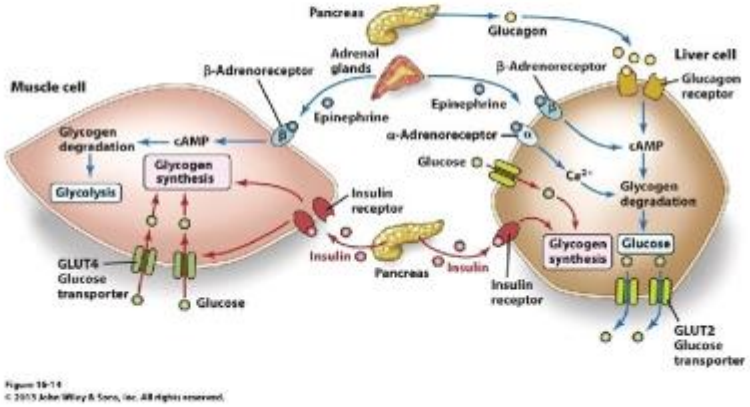
2. It delivers the reduced forms of $NADP^+$ ($NADPH{\cdot}H^+$), which are the donors of hydrogen for the biosynthesis of fatty acids, cholesterol, bile acids, steroid hormones, purine bases, etc. Also there are used in the microsomal oxidation in the detoxication of the drugs and toxins in the liver. It satisfies the body's requirement for $NADPH+H^+$ approximately in 50%.

3. In the anaerobic phase of pentose phosphate pathway the fructose-6-phosphate, glyceraldehyde-3-monophosphate and dihydroxy acetone phosphate are formed, which provide the relationship of glycolysis and pentose phosphate pathway.





Hormonal Control of Glycogen Metabolism



Type	Disease Name	Defective enzyme	Glycogen levels	Glycogen structure	Principal tissue affected
I	Von Gierke's disease	Glucose-6-phosphatase (G6pase)	High	Normal	Liver, kidney
II	Pompe's disease	α-1,4 Glucosidase	Very high	Normal	All organs
III	Cori's Forbes' disease	Debranching enzyme	High	Short outer branches	Liver, Heart, Muscle
IV	Andersen's disease	Branching enzyme	Normal	Long outer branches	Liver, Spleen, Muscle
V	McArdle's disease	Muscle Phosphorylase	High	Normal	Muscle
VI	Hers' disease	Liver Phosphorylase	High	Normal	Liver
VII	Tarui's disease	Phosphofructokinase	High	Normal	Muscle
VIII	Hepatic phosphorylase kinase deficiency	Phosphorylase kinase	High	Normal	Liver

All glycogen storage disease are characterized by hepatomegaly, abnormal liver function.

Diagnostics is:

1) **determining the content of glucose and lactate in the blood,**
2) **load with adrenaline,**
3) **biopsy of tissue,**
4) **determining the structure of glycogen and its location in the cell,**
5) **enzyme indicators.**

General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Aerobic and anaerobic oxidation of glucose, general characteristics of the processes.
2. Anaerobic oxidation of glucose. Sequence of reactions and enzymes of glycolysis.
3. Aerobic oxidation of glucose. Stages of conversion of glucose to CO2 and H2O.
4. Oxidative decarboxylation of pyruvate. Enzymes, coenzymes and the sequence of reactions in a multienzyme complex.
5. Glycolytic oxidoreduction: substrate phosphorylation and shuttle mechanisms of glycolytic NADH oxidation.
6. Comparative characteristics of bioenergetics of aerobic and anaerobic glucose oxidation, Pasteur effect.
7. The phosphorolytic pathway of glycogen cleavage in the liver and muscles. Regulation of glycogen phosphorylase activity.
8. Mechanisms of reciprocal regulation of glycogenolysis and glycogenesis due to cascading cAMP-dependent phosphorylation of enzyme proteins.
9. Metabolic ways of transformation of fructose and galactose; hereditary enzymopathies of their metabolism.
10. Biosynthesis of glycogen: enzymatic reactions, physiological significance. Regulation of glycogen synthase activity.

11. The role of adrenaline, glucagon and insulin in hormonal regulation of glycogen metabolism in muscles and liver.
12. Genetic disorders of glycogen metabolism (glycogenosis, aglycogenosis).
13. Gluconeogenesis: substrates, enzymes and physiological significance of the process.
14. Glucose-lactate (Cori cycle) and glucose-alanine cycles.
15. Blood glucose (glucosemia): normoglycemia, hypo- and hyperglycemia, glucosuria. Diabetes mellitus is a pathology of glucose metabolism.
16. Hormonal regulation of blood glucose concentration and metabolism.
17. Pentose phosphate pathway of glucose oxidation: scheme of the process and biological significance.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 8

Topic: Lipid metabolism. Catabolism of triacylglycerols: oxidation of fatty acids and glycerol; ketogenesis. Lipid metabolism. Lipogenesis. Cholesterol metabolism. Regulation and pathology of lipid metabolism: obesity, atherosclerosis.

Relevance of the topic: Lipids play an important role in vital processes. These are the main components of biomembranes that affect their permeability, participate in the transmission of nerve impulses, create intercellular contacts, form the body's energy reserve, create waterproof and heat-insulating coatings in animals and plants, protect organs and tissues from mechanical impact. Assimilation of knowledge on the specified topic is certainly an integral part of the formation of the scientific worldview of the future doctor and a prerequisite for the development of methods and means of pharmacological correction of disorders of lipid metabolism, including obesity, atherosclerosis.

Purpose: to study the general patterns of lipid metabolism and their use as energy in the human body; to study the main mechanisms of carbohydrate metabolism disorders, which are the cause of a number of diseases: such as obesity, atherosclerosis, and others.

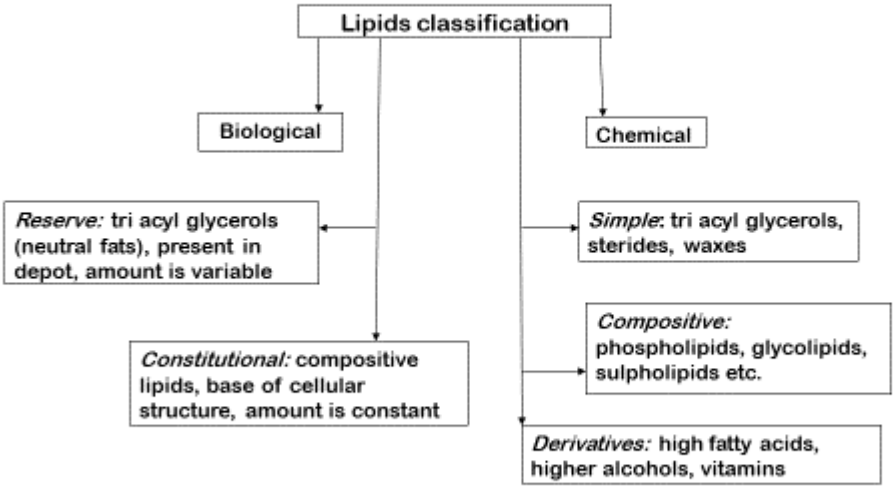
Basic concepts:

1. Monoacyl glycerol.
2. Triacyl glycerol.
3. Carnitine.
4. Ketone bodies.
5. Sphingolipidosis.
6. Lipoproteins.
7. Obesity.
8. Atherosclerosis.
9. Gallstone disease.

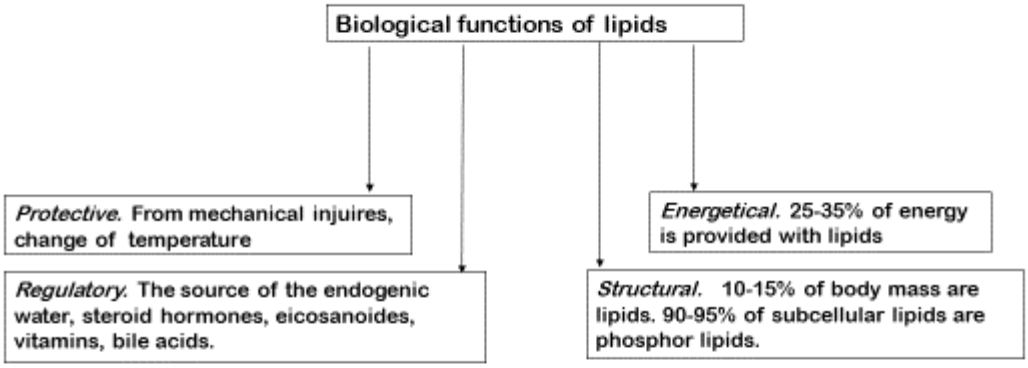
Plan and organizational structure of the lecture:

1. Biological role of lipids.
2. Oxidation of triacylglycerols.
3. Oxidation of HFA. Biological role.
4. Oxidation of glycerol.
5. The energy balance of the oxidation of HFA and glycerol.
6. Metabolism of acetoacetic acid.
7. Formation of ketone bodies, their biological role.
8. Biosynthesis of HFA. Sequence of reactions and characteristics of enzymes.
9. Glycerol biosynthesis.
10. Biosynthesis of triacylglycerols.
11. Formation of complex lipids
12. Biosynthesis and biotransformation of cholesterol.
13. Disorders of lipid metabolism.

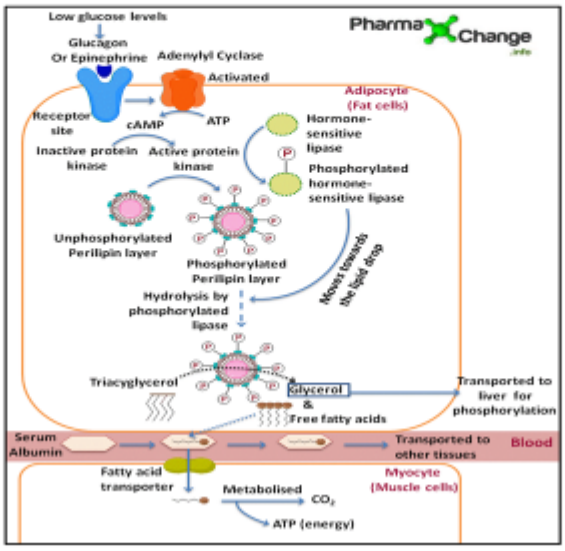
Content of lecture material



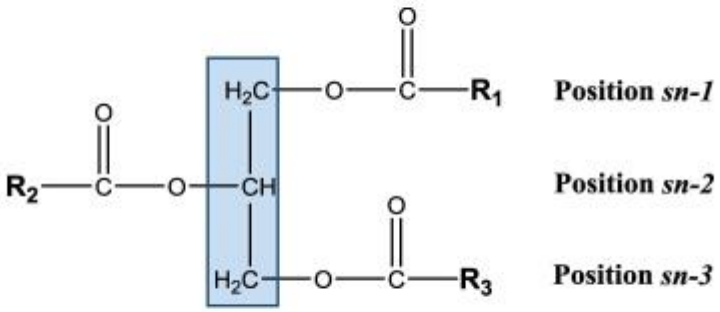
Lipids are organic compounds, are nonpolar molecules, which are soluble only in nonpolar solvents and insoluble in water.



Lipolysis is the metabolic pathway through which triacylglycerols are hydrolyzed into a glycerol and 3 fatty acids. Occurs in adipocytes. Lipolysis is induced by glucagon, epinephrine, norepinephrine, growth hormone, cortisol. Lipolysis is activated when: under normal physiological stressful situations - emotional stress, muscle work, fasting, in pathological conditions - type I diabetes mellitus, other hormonal diseases (hypercortisolism, hyperthyroidism).



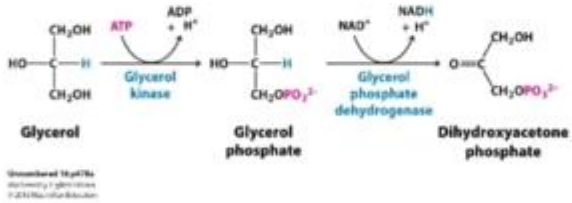
Structure of triacylglycerol



Oxidation of glycerol

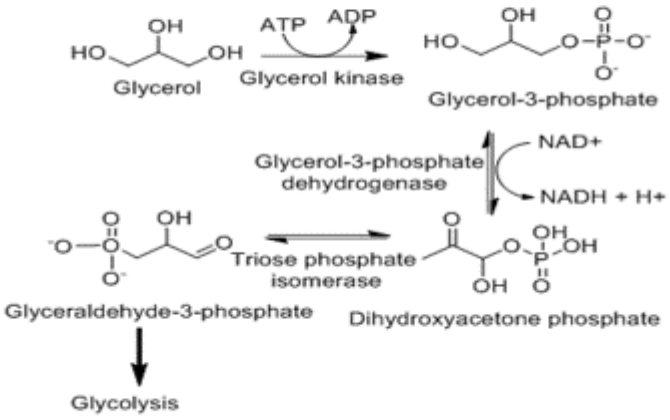
1) Phosphorylation of glycerol in the cytosol of cells to glycerol phosphate.

2) Oxidation of glycerol phosphate in the mitochondria to dihydroxy acetone phosphate.



Dihydroxy acetone phosphate diffuses from the mitochondria into the cytosol.

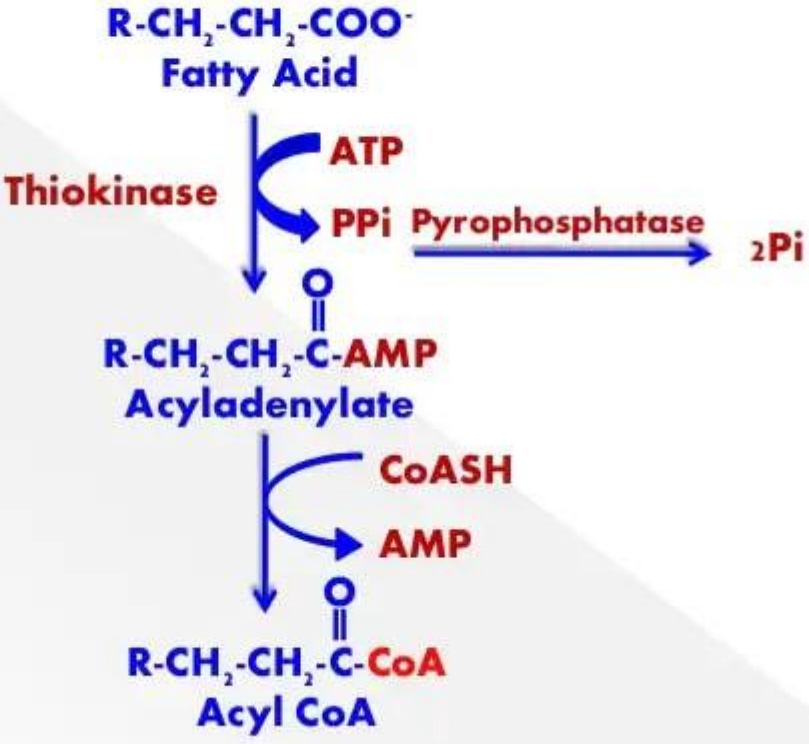
3) Isomerization of dihydroxy acetone phosphate in the cytosol in glycerol phosphate, and its conversion into pyruvate and acetyl-CoA.



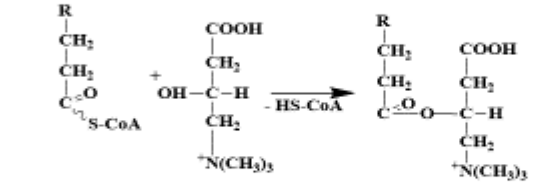
Bological role of oxidation of glycerol

1. Oxidation of glycerol releases energy, which is reserved in the form of ATP.
2. Glycerol phosphate is used for the biosynthesis of tri acyl glycerides and phospholipids.
3. Derived from glycerol dihydroxy acetone phosphate and glyceraldehyde phosphate can be used for the biosynthesis of carbohydrates (glucose).

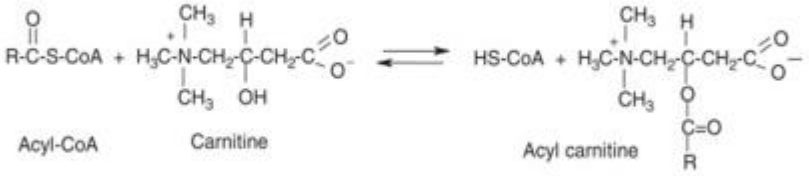
Activation of fatty acid to Acyl CoA



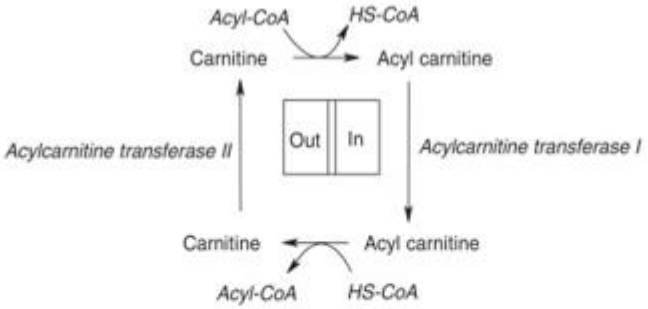
Since acyl-CoA is formed in the cytoplasm, and the oxidation of fatty acids occurs in mitochondria, acyl by of the using carnitine transporter (Carnitine shuttle) is transferred from the cytoplasm to the mitochondria.

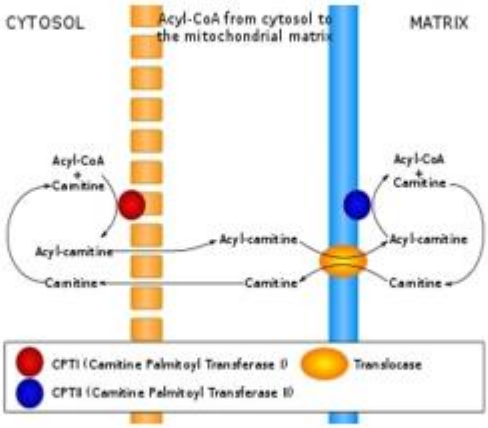


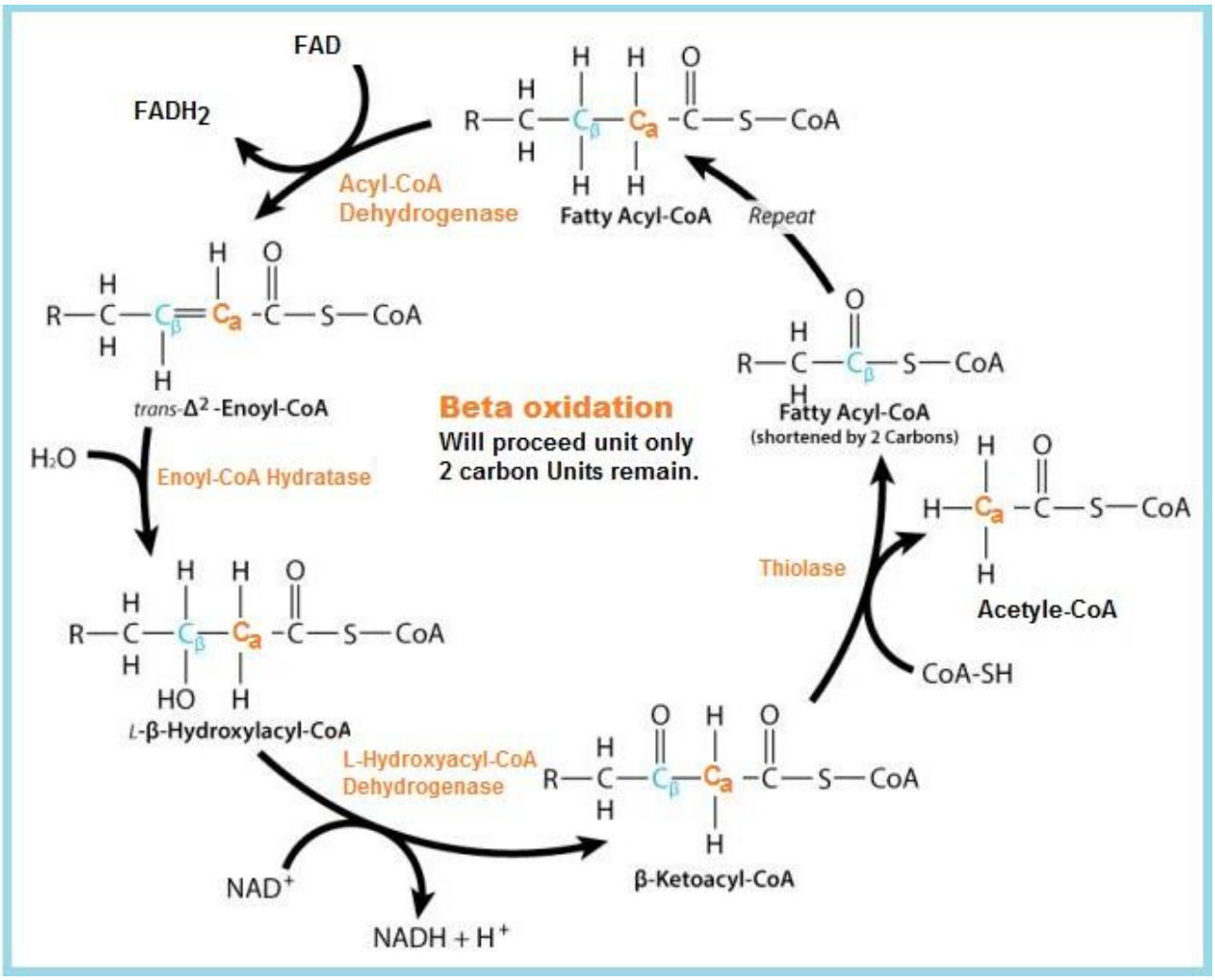
Acyl-CoA **Carnitine (γ-trimethylamino β--hydroxybutyrate)** **Acyl-carnitine**



Acyl-CoA Carnitine Acyl carnitine




CYTOSOL
Acyl-CoA from cytosol to the mitochondrial matrix
MATRIX
Acyl-CoA + Carnitine
Acyl-carnitine
Carnitine
Translocase
CPTI (Carnitine Palmitoyl Transferase I)
CPTII (Carnitine Palmitoyl Transferase II)


FAD
FADH2
Acyl-CoA Dehydrogenase
Fatty Acyl-CoA
Repeat
trans-Δ2-Enoyl-CoA
H2O
Enoyl-CoA Hydratase
Beta oxidation
Will proceed unit only
2 carbon Units remain.
Fatty Acyl-CoA
(shortened by 2 Carbons)
Thiolase
Acetyle-CoA
CoA-SH
L-β-Hydroxylacyl-CoA
L-Hydroxyacyl-CoA Dehydrogenase
β-Ketoacyl-CoA
NAD+
NADH + H+

Calculations

Carbons in Fatty Acid	Acetyl CoA C/2	β-oxidation cycles (C/2) -1
12	6	5
14	7	6
16	8	7
18	9	8

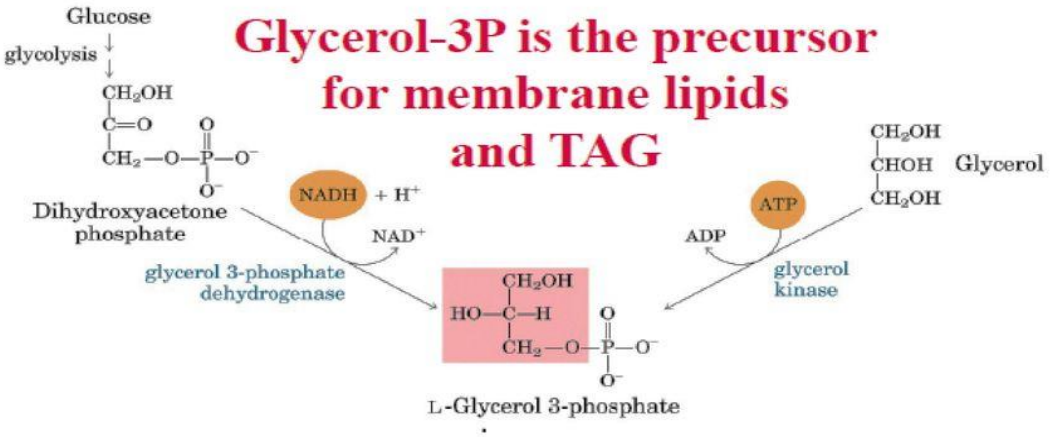
Note: In each round of β-oxidation *one molecule* of $FADH_2$ and *one molecule* of $NADH+H^+$ are produced which generates 2 and 3 ATP molecules, respectively

Biosynthesis of glycerol

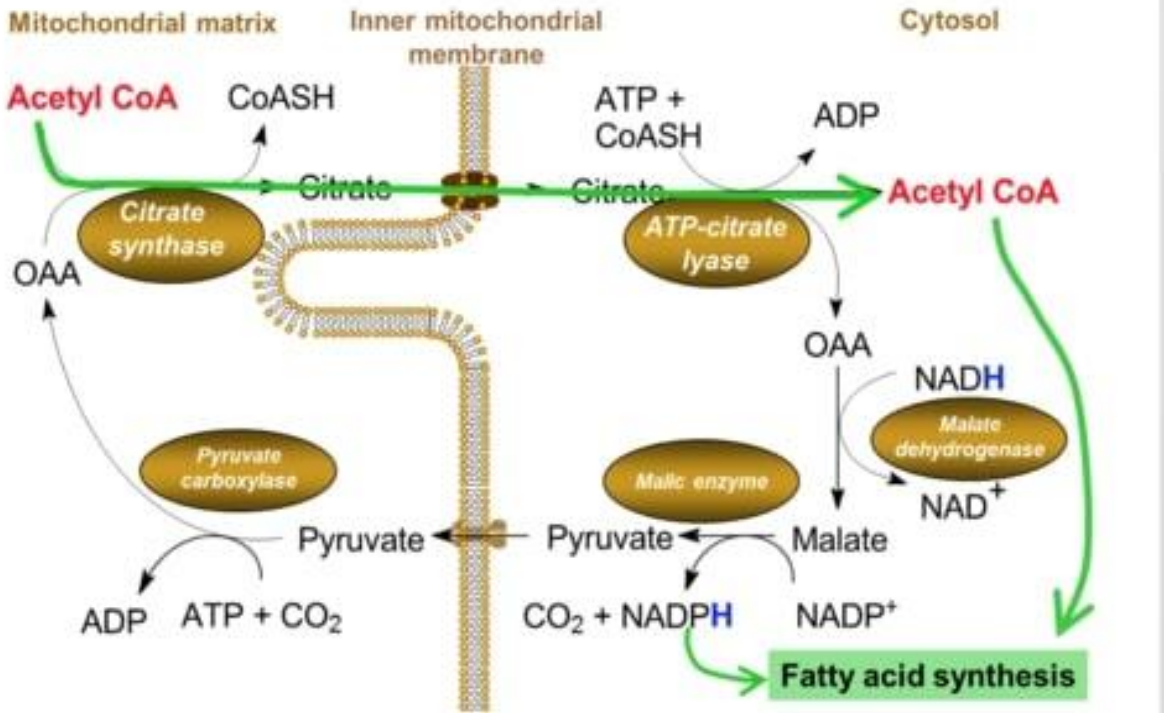
- Glucose is oxidized via glycolysis to **dihydroxy acetone phosphate**

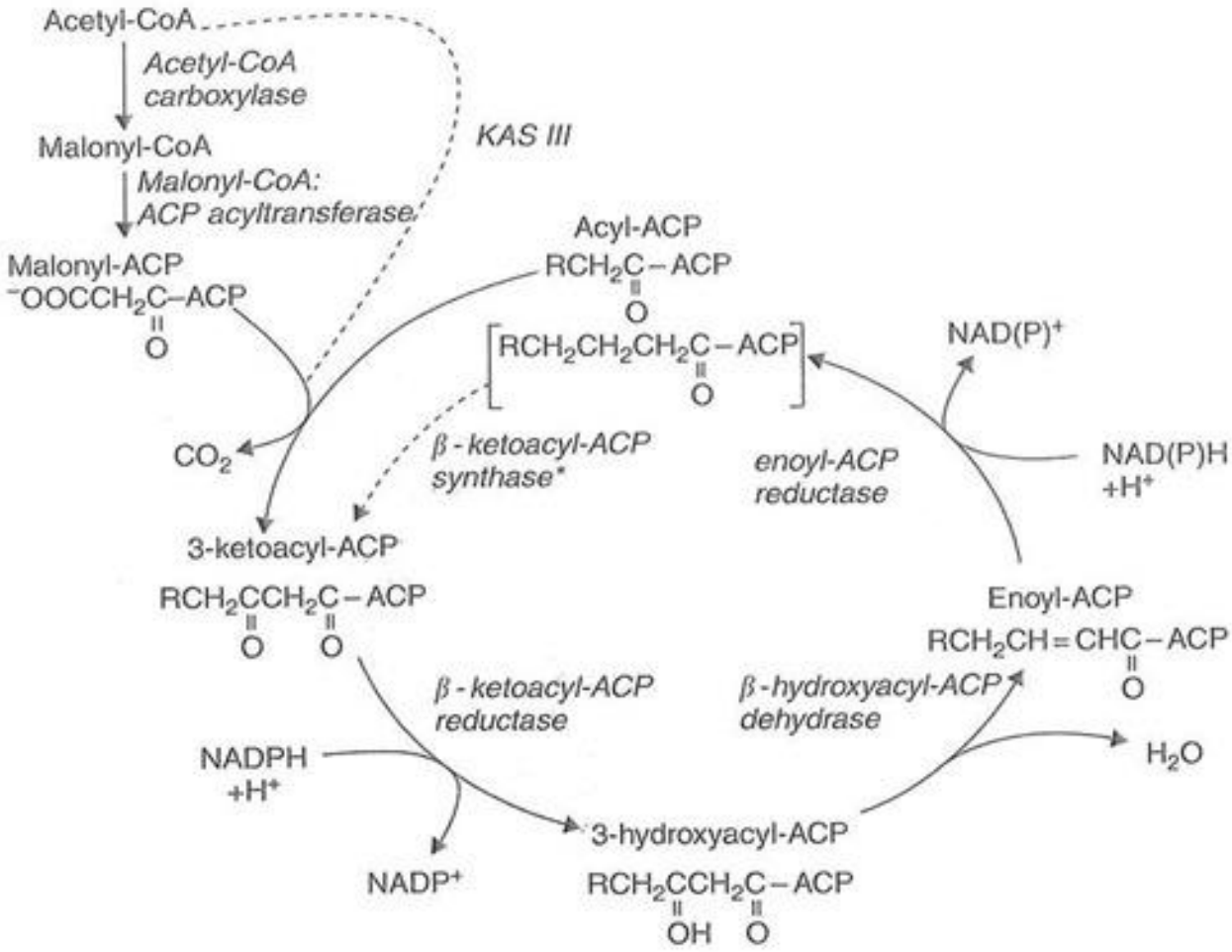
  ⬇

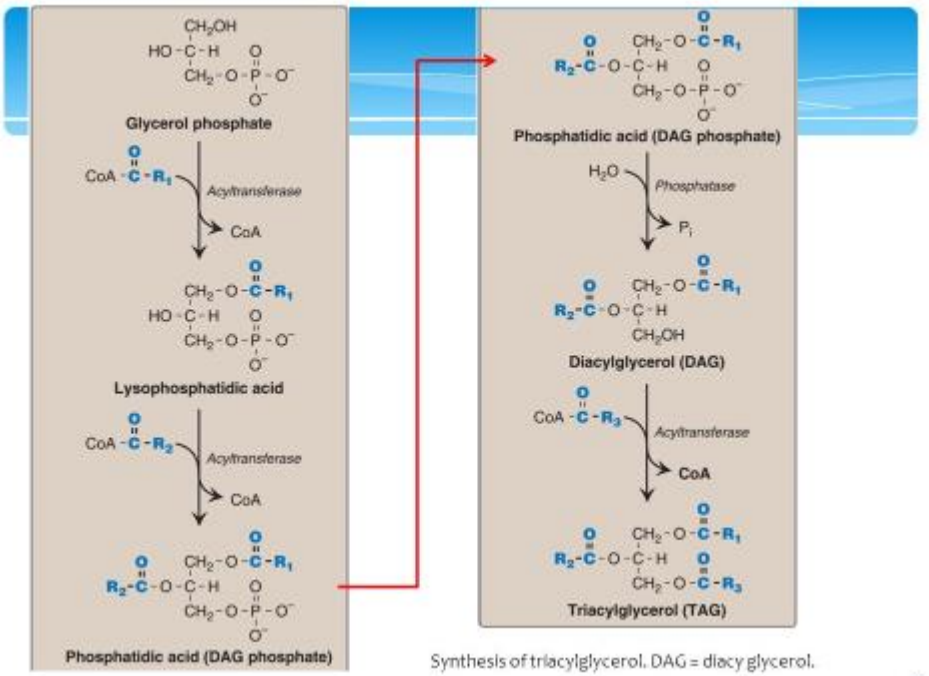
  reduced to **glycerol-3 phosphate** by the enzyme glycerol-3 phosphate dehydrogenase.



The citrate shuttle transfers acetyl CoA from mitochondria to cytosol

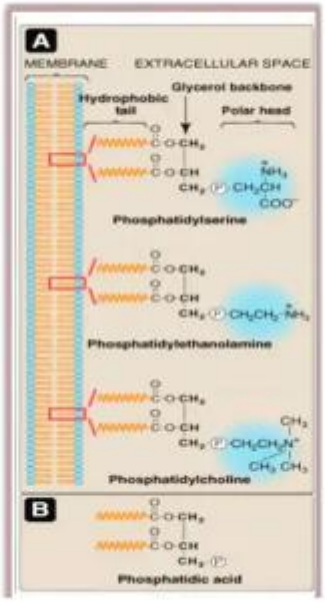


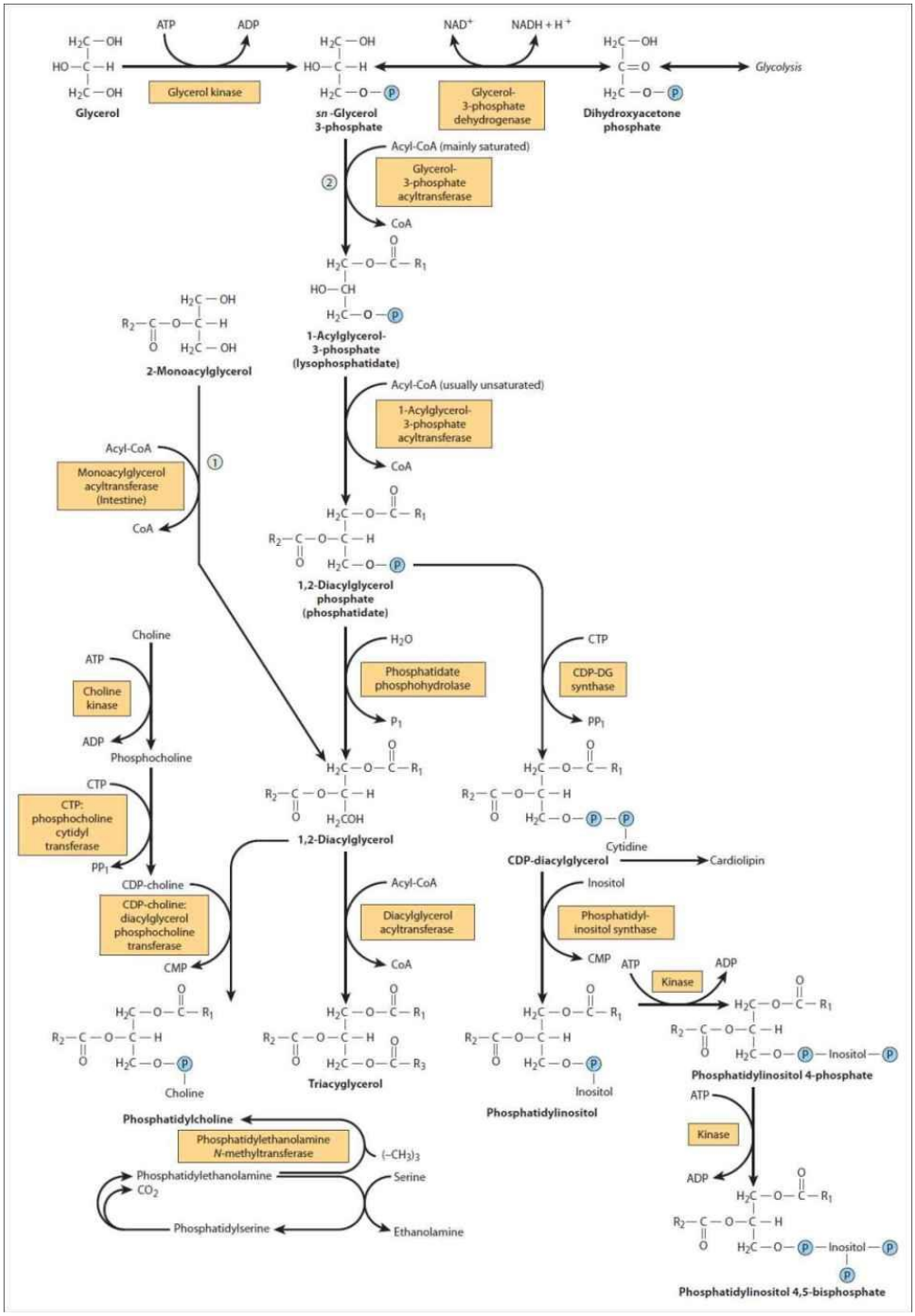




Synthesis of triacylglycerol. DAG = diacy glycerol.

Phosphilipids



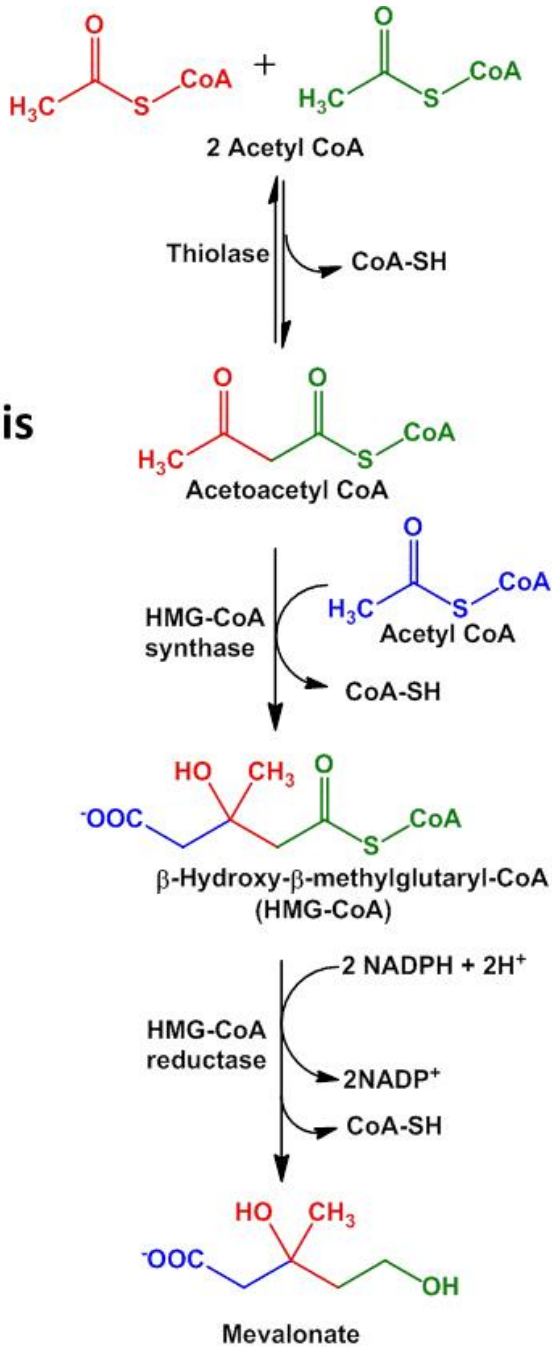

ATP
ADP
Glycerol kinase
Glycerol
NAD+
NADH + H+
Glycerol-3-phosphate dehydrogenase
sn-Glycerol 3-phosphate
Dihydroxyacetone phosphate
Glycolysis
Acyl-CoA (mainly saturated)
Glycerol-3-phosphate acyltransferase
CoA
1-Acylglycerol-3-phosphate (lysophosphatidate)
2-Monoacylglycerol
Acyl-CoA (usually unsaturated)
1-Acylglycerol-3-phosphate acyltransferase
Acyl-CoA
Monoacylglycerol acyltransferase (Intestine)
1,2-Diacylglycerol phosphate (phosphatidate)
Choline
Choline kinase
Phosphocholine
H2O
Phosphatidate phosphohydrolase
P1
CTP
CDP-DG synthase
PP1
CTP: phosphocholine cytidyl transferase
1,2-Diacylglycerol
CDP-diacylglycerol
Cytidine
Cardiolipin
CDP-choline
CDP-choline: diacylglycerol phosphocholine transferase
CMP
Acyl-CoA
Diacylglycerol acyltransferase
Inositol
Phosphatidyl-inositol synthase
Kinase
Phosphatidylinositol 4-phosphate
Choline
Triacylglycerol
Phosphatidylinositol
Phosphatidylcholine
Phosphatidylethanolamine N-methyltransferase
(–CH3)3
Phosphatidylethanolamine
CO2
Serine
Phosphatidylserine
Ethanolamine
Phosphatidylinositol 4,5-bisphosphate

FUNCTIONS OF CHOLESTEROL

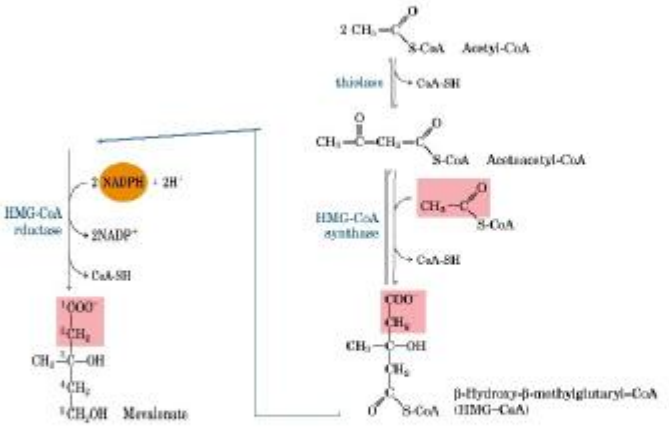
Cholesterol is the most abundant sterol in humans and performs a number of essential functions. For example-

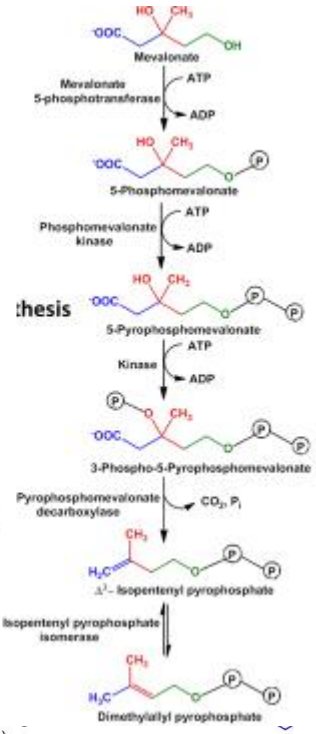
- It is a major constituent of the plasma membrane and of plasma lipoproteins.
- It is a precursor of bile salts,
- It is a precursor of steroid hormones that include adrenocortical hormones, sex hormones, placental hormones etc
- Also a precursor of vitamin D, cardiac glycosides, Sitosterol of the plant kingdom, and some alkaloids.
- It is required for the nerve transmission. Cholesterol is widely distributed in all cells of the body but particularly abundant in nervous tissue.

Cholesterol Biosynthesis
Stage 1

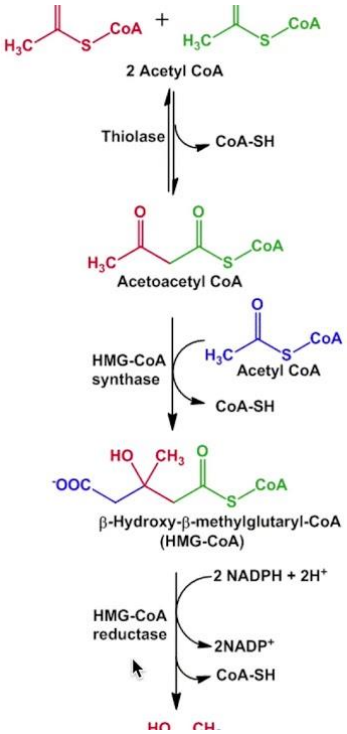


- Mevalonate formation is the first stage of cholesterol synthesis (summary so far)



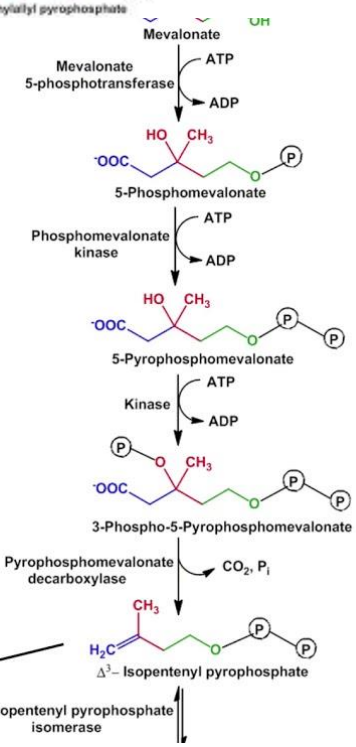


Pharma Change

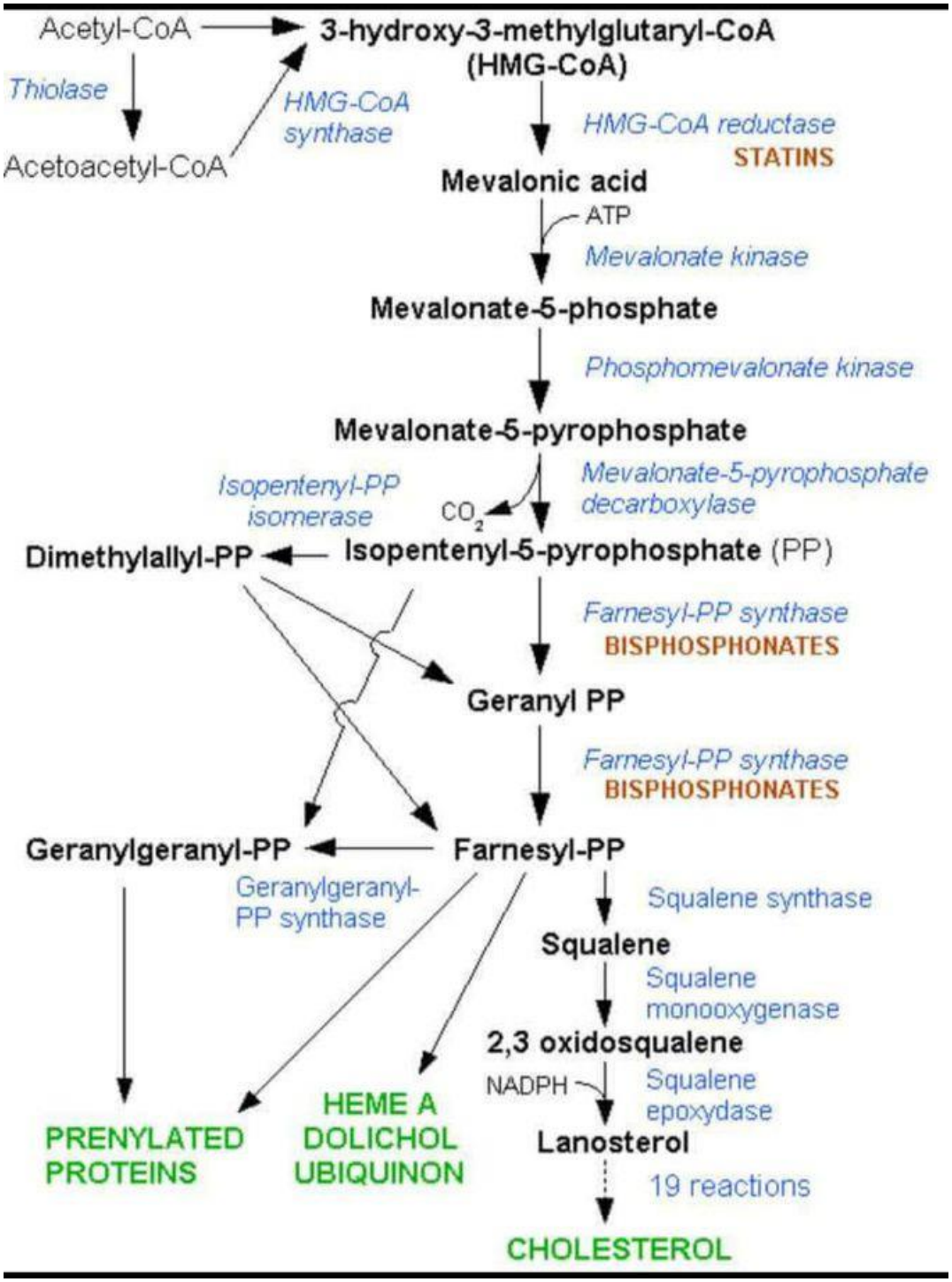


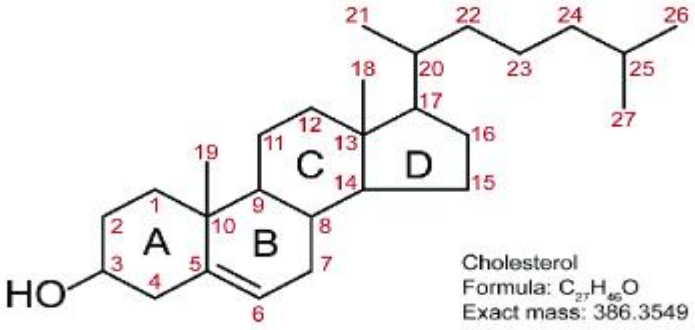
mevalonate (3 acetyl-CoA's per mevalonate).

- Notice that mevalonate synthesis requires 2 molecules of NADPH.
- HMG-CoA is a branch point in metabolism.
  - When body is in catabolic state (fasting), the liver generates ketone bodies from HMG-CoA.
  - During energy excess, **HMG-CoA reductase** converts HMG-CoA to mevalonate.
- **HMG-CoA reductase** is the committed and heavily regulated enzyme of cholesterol and isoprenoid synthesis.
- Phase 2 of **cholesterol synthesis** involves conversion of mevalonate to the basic **isoprenoid units** (IPP and DMAP).
- IPP and DMAP are the basic units of all **isoprenes** and their derivatives (cholesterol, steroids, bile acids, vitamin D, CoQ).



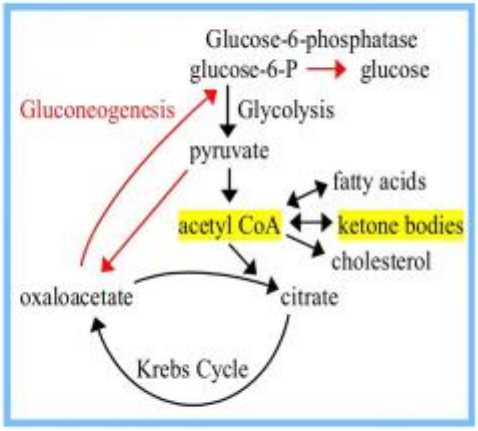
Larger Isoprenes
Squalene
(Next Video)

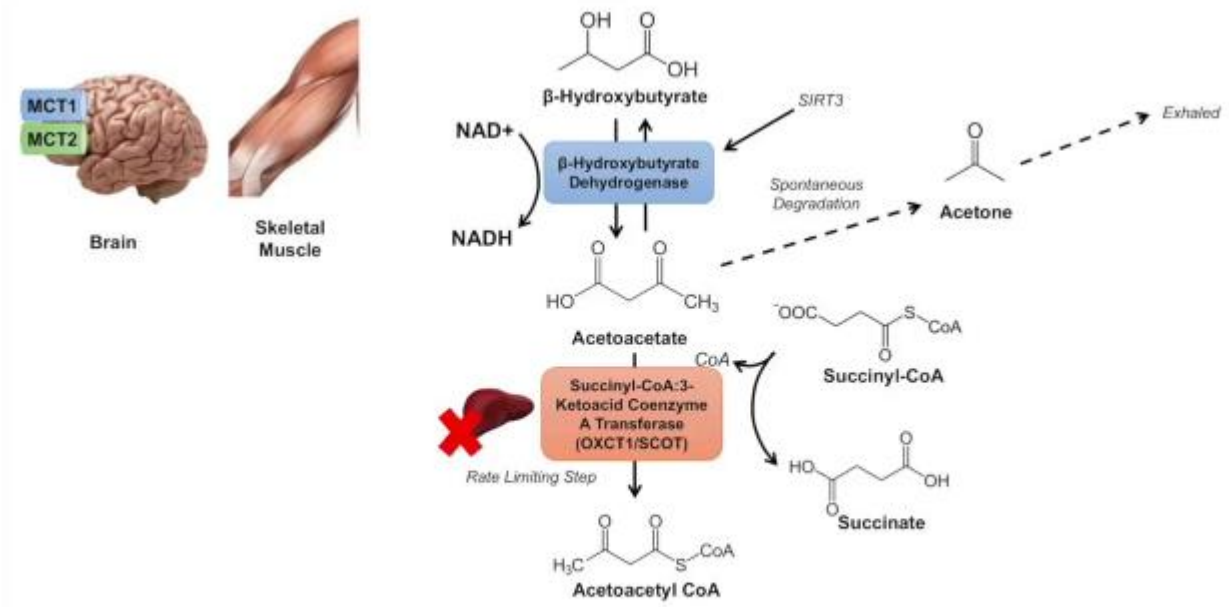

Acetyl-CoA
3-hydroxy-3-methylglutaryl-CoA
(HMG-CoA)
Thiolase
HMG-CoA
synthase
HMG-CoA reductase
STATINS
Acetoacetyl-CoA
Mevalonic acid
ATP
Mevalonate kinase
Mevalonate-5-phosphate
Phosphomevalonate kinase
Mevalonate-5-pyrophosphate
Isopentenyl-PP
isomerase
CO_2
Mevalonate-5-pyrophosphate
decarboxylase
Dimethylallyl-PP
Isopentenyl-5-pyrophosphate (PP)
Farnesyl-PP synthase
BISPHOSPHONATES
Geranyl PP
Farnesyl-PP synthase
BISPHOSPHONATES
Geranylgeranyl-PP
Farnesyl-PP
Geranylgeranyl-
PP synthase
Squalene synthase
Squalene
Squalene
monooxygenase
2,3 oxidosqualene
NADPH
Squalene
epoxydase
HEME A
DOLICHOL
UBIQUINON
PRENYLATED
PROTEINS
Lanosterol
19 reactions
CHOLESTEROL



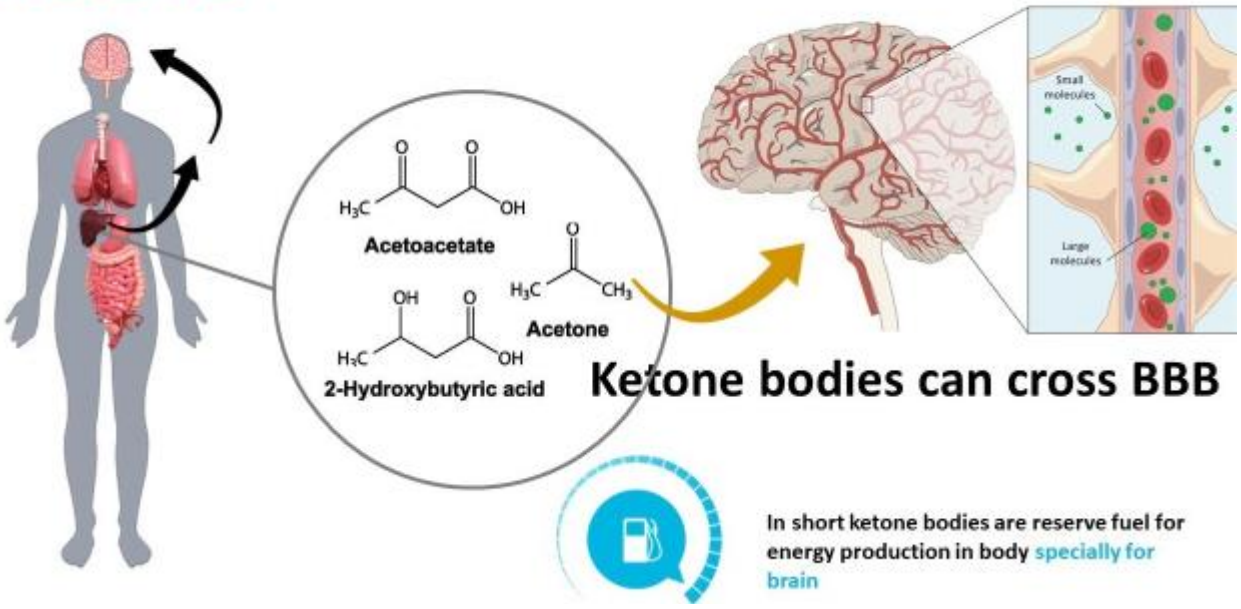
Ketone Bodies Metabolism

- Ketone synthesis occurs in the Liver - Mitochondria
- During prolonged starvation, fasting (and in diabetes) oxaloacetate is depleted in liver due to gluconeogenesis
- This impedes entry of acetyl-CoA into Krebs cycle.
- Acetyl-CoA in liver mitochondria is converted then to ketone bodies - Acetone, Acetoacetate & β-hydroxybutyrate.



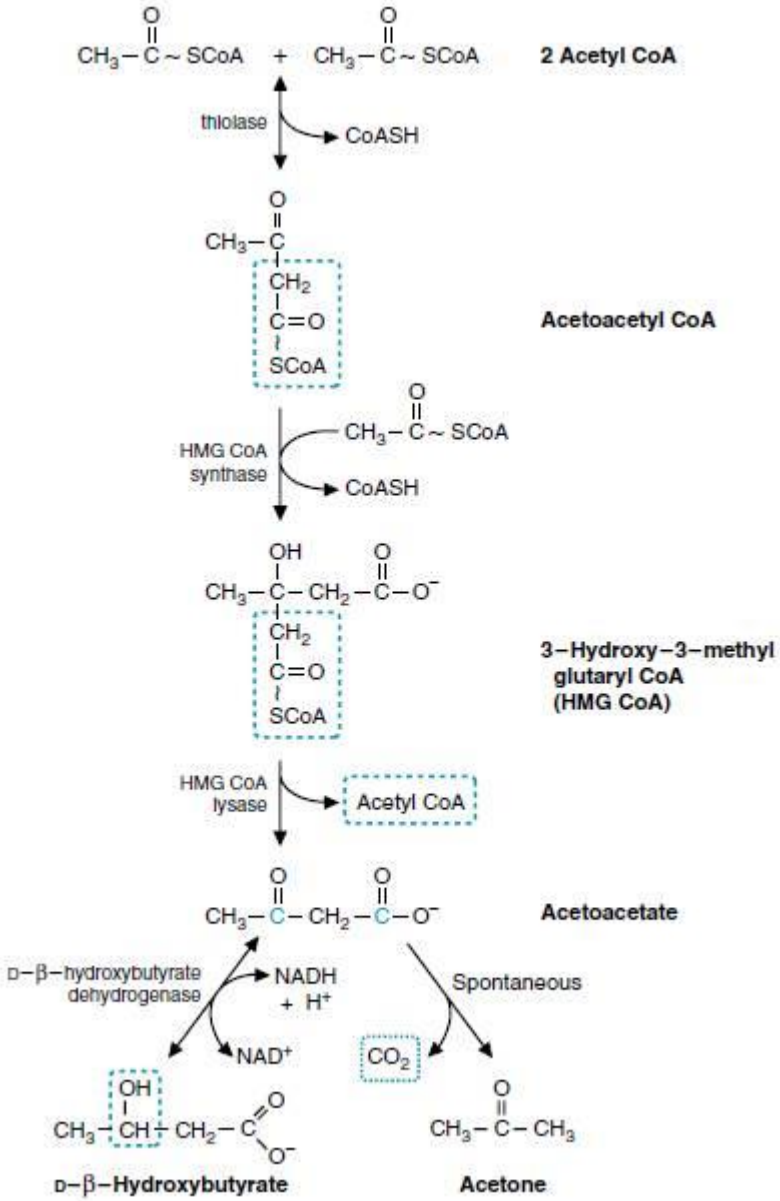


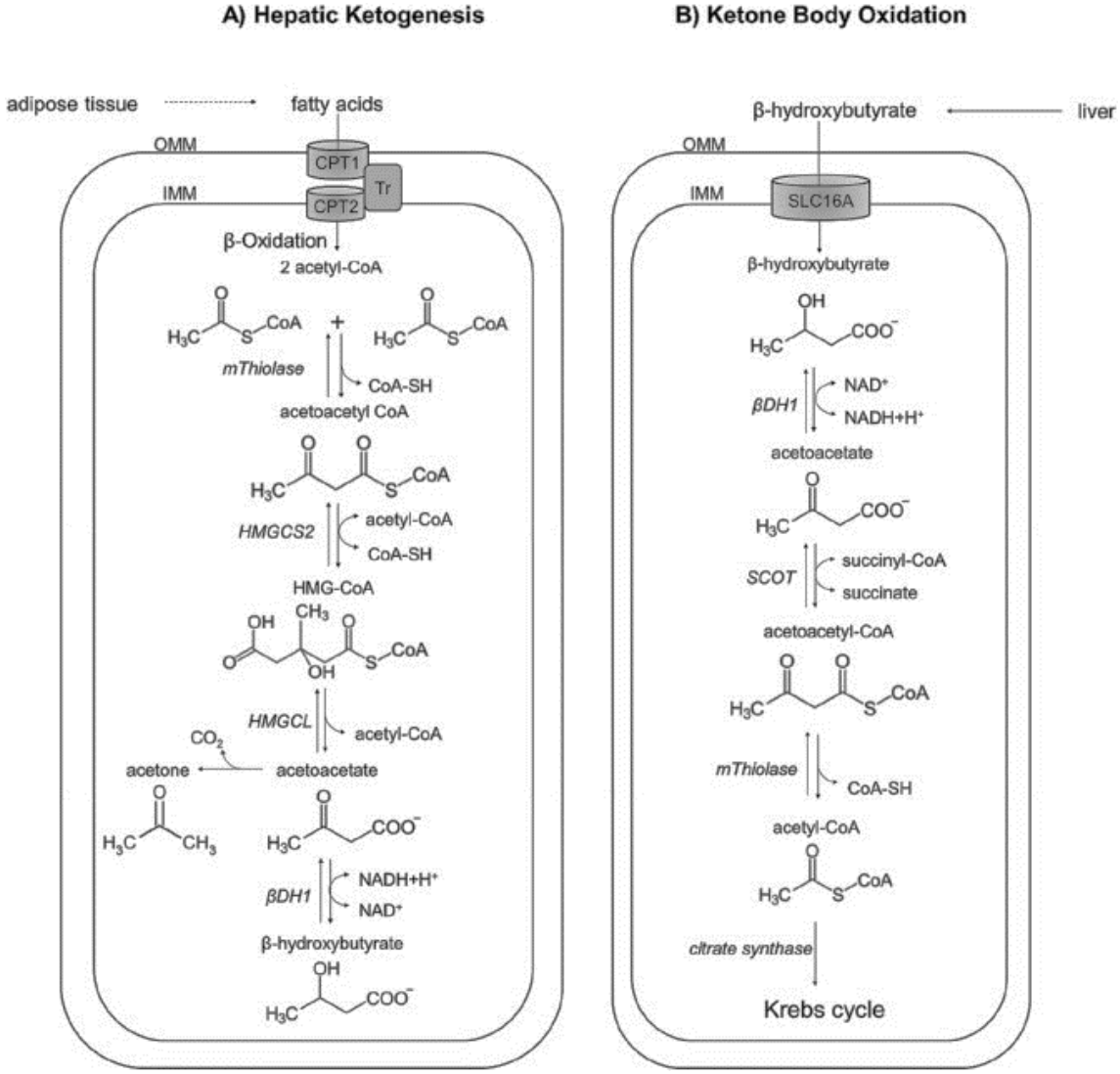
Ketone bodies and its metabolism



Ketone bodies can cross BBB

In short ketone bodies are reserve fuel for energy production in body specially for brain

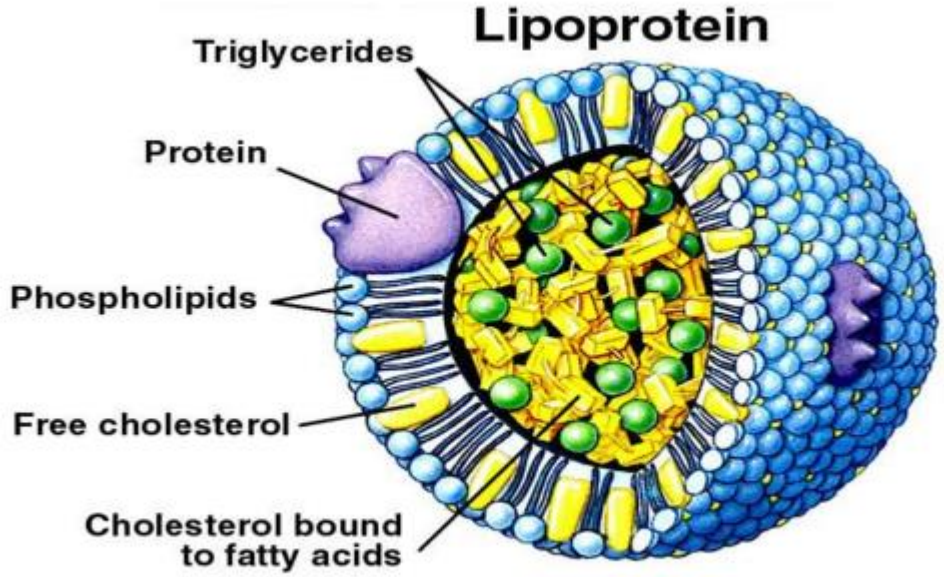

2 Acetyl CoA
thiolase
CoASH
Acetoacetyl CoA
HMG CoA synthase
CoASH
3–Hydroxy–3–methyl glutaryl CoA (HMG CoA)
HMG CoA lysase
Acetyl CoA
Acetoacetate
D–β–hydroxybutyrate dehydrogenase
NADH + H+
NAD+
Spontaneous
CO2
D–β–Hydroxybutyrate
Acetone



Plasma Lipoproteins

- What are plasma lipoproteins?
- Spherical macromolecular complexes of:
- Lipids + specific proteins (apo-proteins)
- They includes:

1. Chylomicron
2. Very low density lipoproteins (VLDL)
3. Low density lipoproteins (LDL)
4. High density lipoproteins (HDL)



Lipoproteins

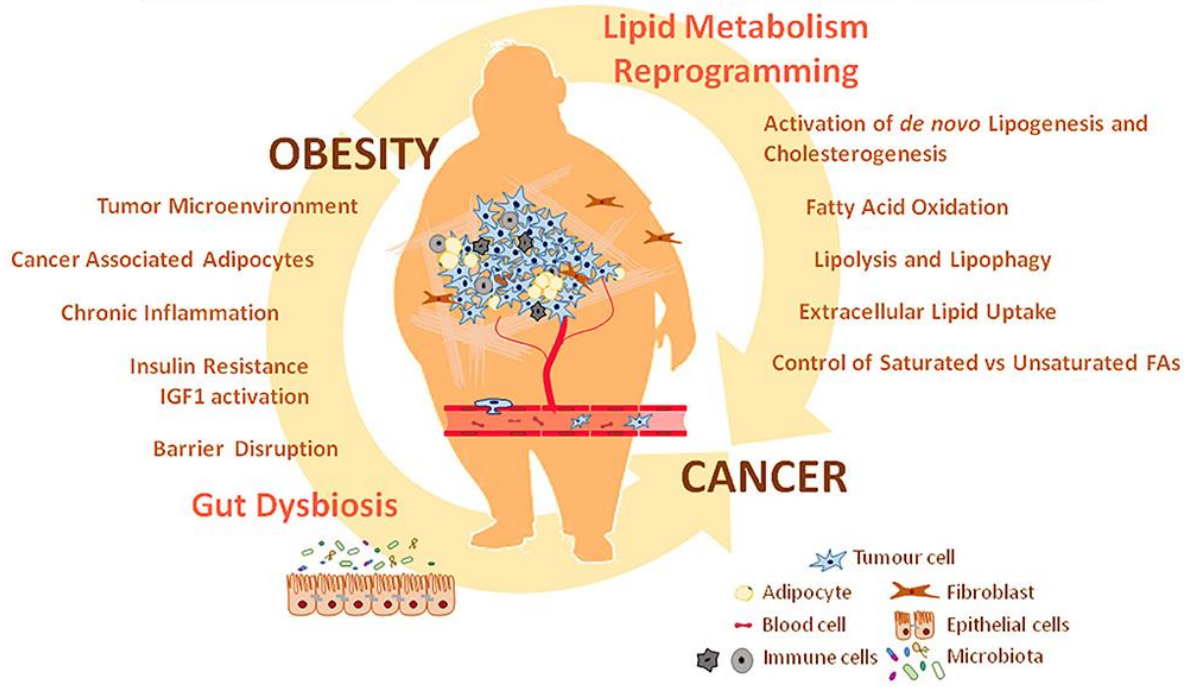
1. Chylomicrons - carry triacylglycerol from the intestines to the liver and adipose tissue.
2. Very low density lipoproteins (VLDL) - carry triacylglycerol from the liver to adipose tissue.
3. Low density lipoprotein (LDL) - carry cholesterol from the liver to the tissues. "Bad cholesterol"
4. High density lipoprotein (HDL) - collects cholesterol from the tissues, and transport it back to the liver. "Good cholesterol"

Types of Lipoprotein

(all contain characteristic amounts TAG, cholesterol, cholesterol esters, phospholipids and Apoproteins – NMR Spectroscopy)

Increasing density ↓

Class	Diameter (nm)	Source and Function	Major Apoliproteins
Chylomicrons (CM)	500 Largest	Intestine. Transport of *dietary* TAG	A, B48, C(I,II,III) E
Very low density lipoproteins (VLDL)	43	Liver. Transport of *endogenously* synthesised TAG	B100, C(I,II,III) , E
Low density lipoproteins (LDL)	22	Formed in circulation by partial breakdown of IDL. Delivers cholesterol to peripheral tissues	B100
High density lipoproteins (HDL)	8 Smallest	Liver. Removes "used" cholesterol from tissues and takes it to liver. Donates apolipoproteins to CM and VLDL	A, C(I,II,III), D, E



Modified from Aguirre-Portolès C, Fernández LP, Ramirez de Molina A. Precision Nutrition for Targeting Lipid Metabolism in Colorectal Cancer. Nutrients (2017) 9: doi:10.3390/nu9101076

General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Catabolism of triacylglycerols in adipocytes of adipose tissue: sequence of reactions, mechanisms of regulation of triglyceride lipase activity.

2. Neurohumoral regulation of lipolysis with the participation of adrenaline, norepinephrine, glucagon and insulin).
3. Fatty acid oxidation reactions (β-oxidation); the role of carnitine in the transport of fatty acids in mitochondria.
4. Energy cost of β-oxidation of fatty acids in cells.
5. Glycerol oxidation: enzymatic reactions, bioenergetics.
6. Ketone bodies. Reactions of biosynthesis and disposal of ketone bodies, physiological significance.
7. Violation of the metabolism of ketone bodies under pathological conditions (diabetes mellitus, starvation).

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture №9

Topic: Metabolism of amino acids. General pathways of amino acid conversion (deamination, transamination, decarboxylation). Ammonia metabolism: urea biosynthesis and its disorders. Specialized ways of converting amino acids; hereditary enzymopathies of amino acid metabolism.

Relevance of the topic: Deamination reactions together with transamination reactions are the central link of the intracellular metabolism of amino acids. As a result of deamination processes, ammonia is formed - a toxic substance that is subject to temporary and final detoxification processes and is excreted in the urine in the form of end products of nitrogenous metabolism, one of which are ammonium salts. The synthesis of ammonium salts in the kidneys ensures the maintenance of acid-base balance and the stability of the ionic composition of the body. During the decarboxylation of amino acids, biogenic amines are formed, which are mediators of the central nervous system and have a hormonal effect.

Purpose: study of the main mechanisms of amino acid metabolism, which is a prerequisite for the development of methods and means of pharmacological correction of amino acid metabolism disorders and formation of the scientific outlook of the future doctor.

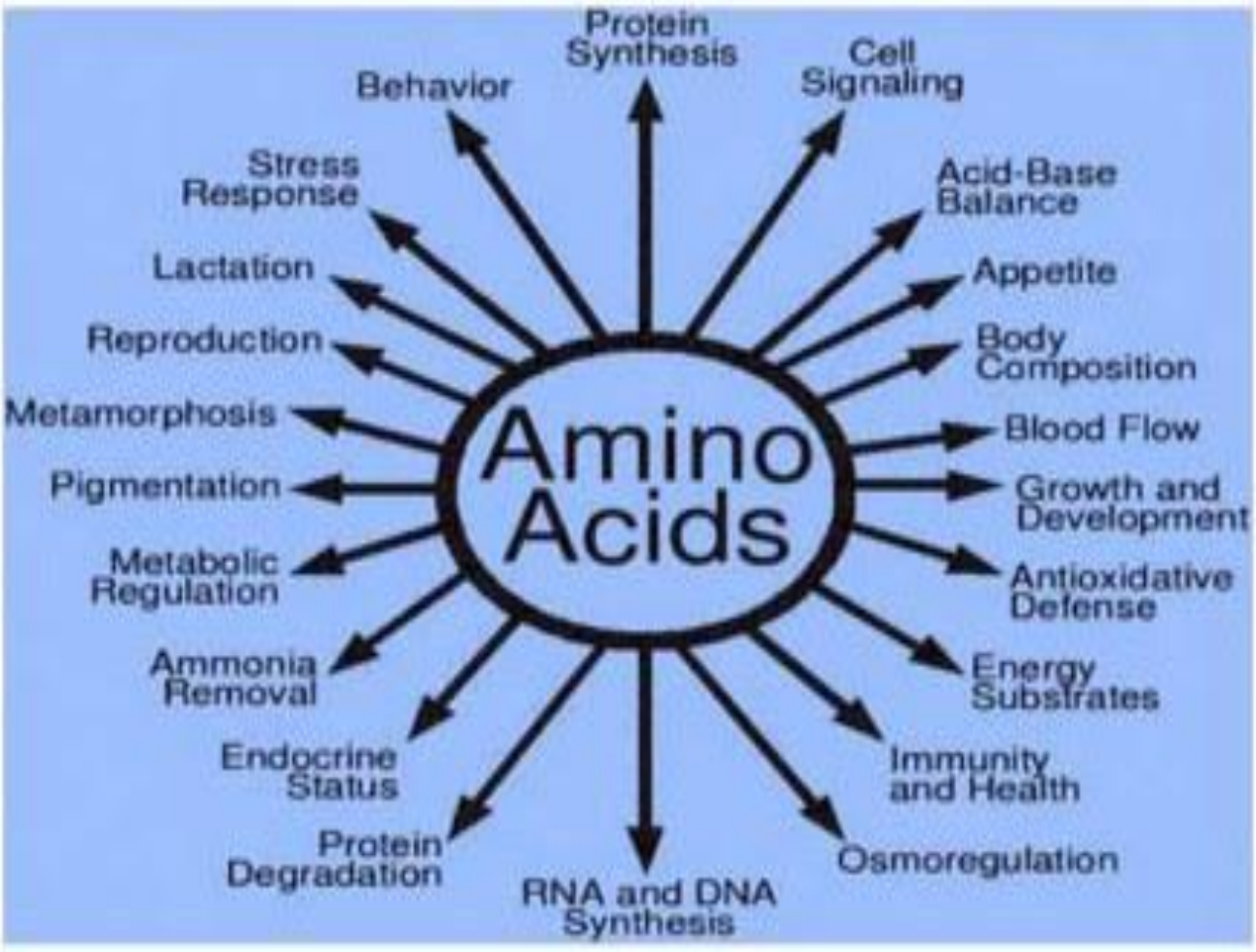
Basic concepts:

1. Deaminating dehydrogenases.
2. Transaminases.
3. Mediators of inflammation, allergies.
4. Arginine-succinic aciduria.
5. "Maple syrup" disease
6. Phenylketonuria.
7. Alkaptonuria.
8. Albinism.

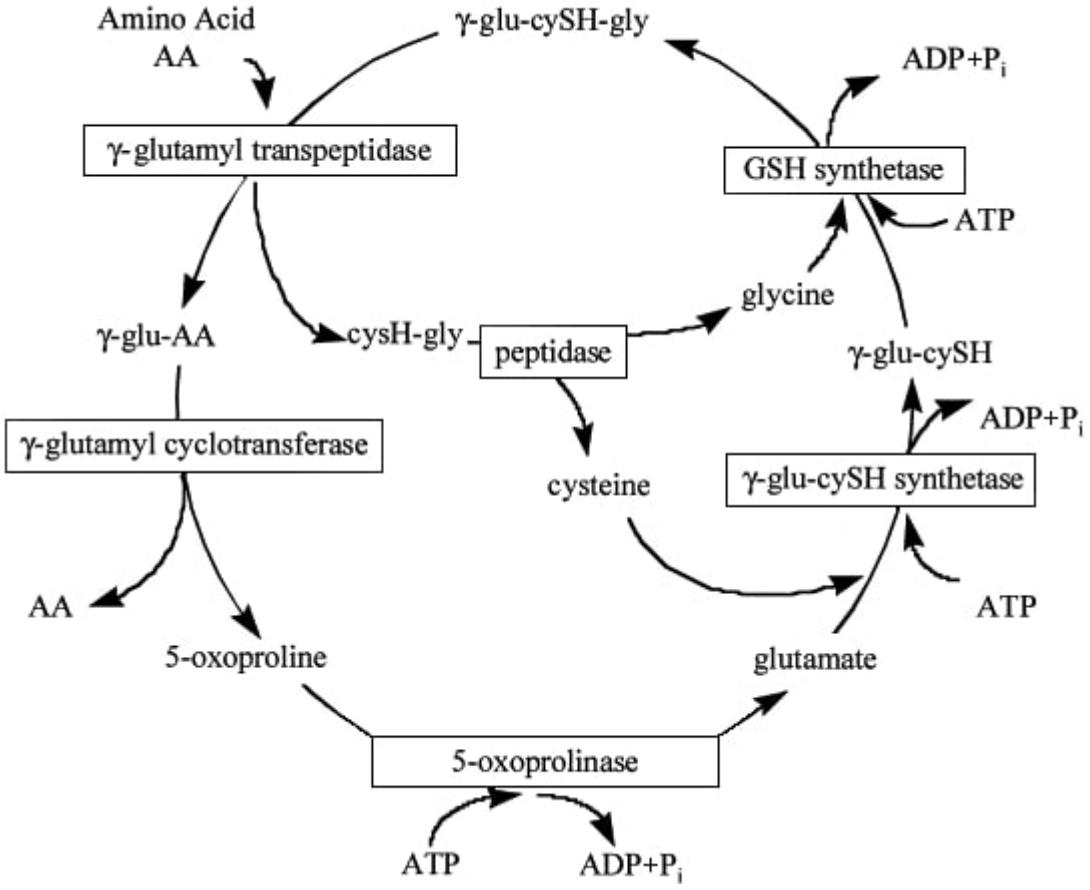
Plan and organizational structure of the lecture:

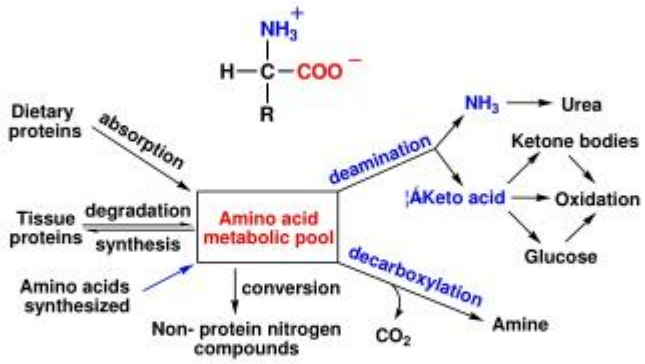
1. Deamination, decarboxylation of amino acids.
2. Transamination.
3. Urea biosynthesis. Sequence of reactions and characteristics of enzymes.
4. Pathology of urea synthesis.
5. Specific ways of exchange of acyclic and cyclic amino acids.
6. Violation of amino acid metabolism.

Content of the lecture material



Glutathione (GSH)





Deamination of amino acids

Deamination - ***elimination of amino group from amino acid with ammonia formation.***

Four types of deamination:

- ***oxidative*** (the most important for higher animals
- ***Reduction deamination:***

$$\mathbf{R\text{-}CH(NH_2)\text{-}COOH + 2H^+ \rightarrow R\text{-}CH_2\text{-}COOH + NH_3}$$

amino acid *fatty acid*

Hydrolytic deamination:

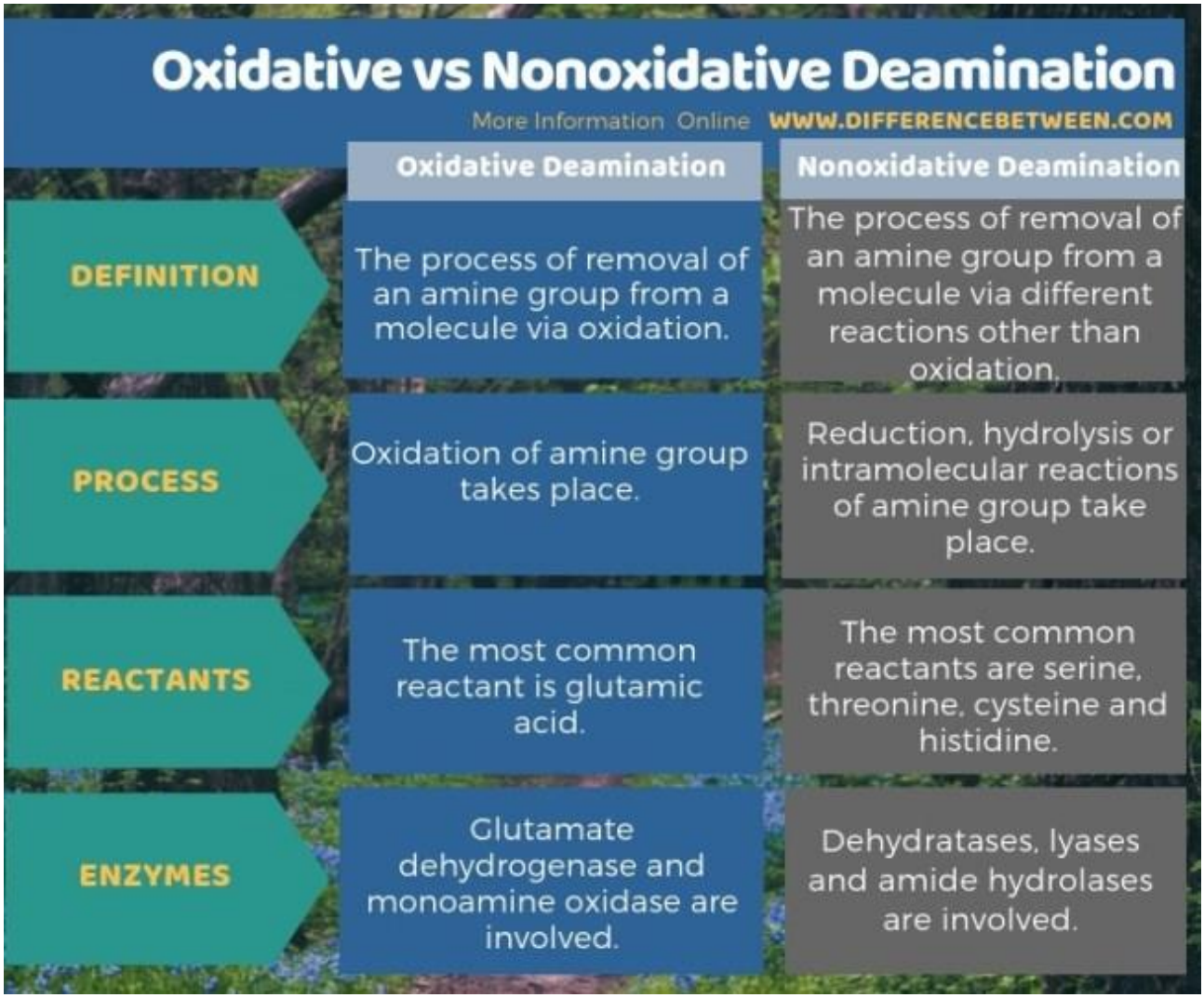
$$\mathbf{R\text{-}CH(NH_2)\text{-}COOH + H_2O \rightarrow R\text{-}CH(OH)\text{-}COOH + NH_3}$$

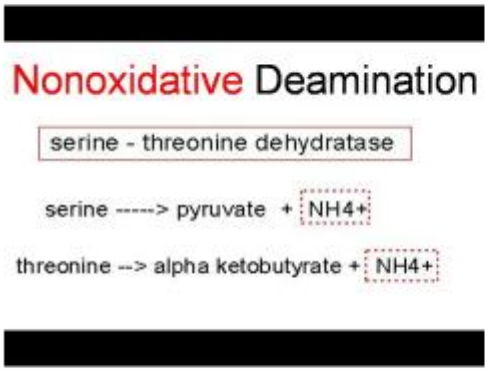
amino acid *hydroxyacid*

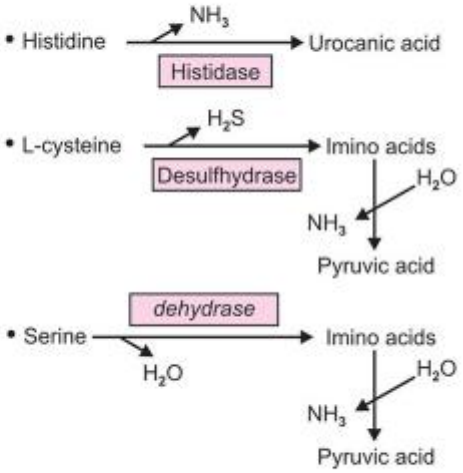
Intramolecular deamination:

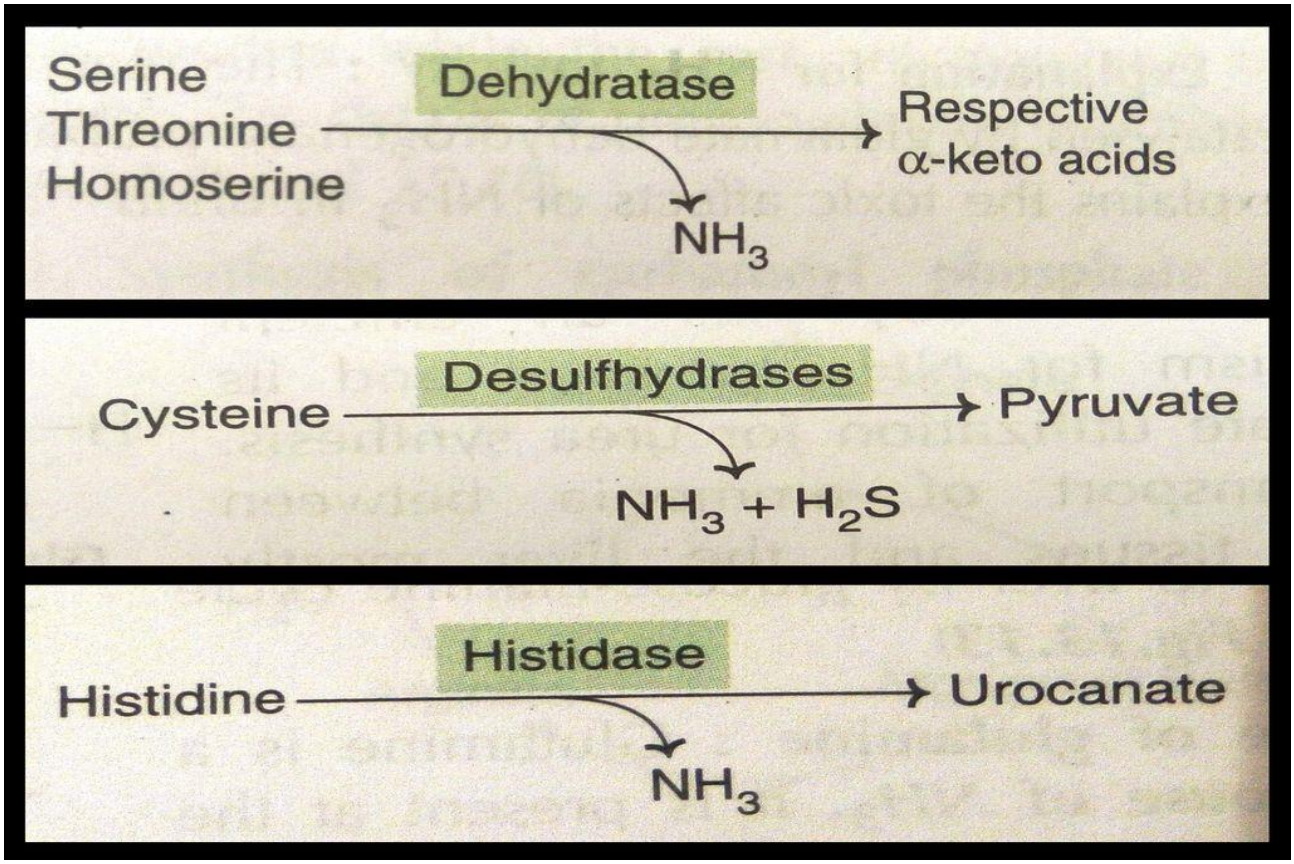
$$\mathbf{R\text{-}CH(NH_2)\text{-}COOH \rightarrow R\text{-}CH\text{-}CH\text{-}COOH + NH_3}$$

amino acid *unsaturated fatty acid*


Oxidative vs Nonoxidative Deamination
More Information Online WWW.DIFFERENCEBETWEEN.COM
Oxidative Deamination
Nonoxidative Deamination
DEFINITION
The process of removal of an amine group from a molecule via oxidation.
The process of removal of an amine group from a molecule via different reactions other than oxidation.
PROCESS
Oxidation of amine group takes place.
Reduction, hydrolysis or intramolecular reactions of amine group take place.
REACTANTS
The most common reactant is glutamic acid.
The most common reactants are serine, threonine, cysteine and histidine.
ENZYMES
Glutamate dehydrogenase and monoamine oxidase are involved.
Dehydratases, lyases and amide hydrolases are involved.

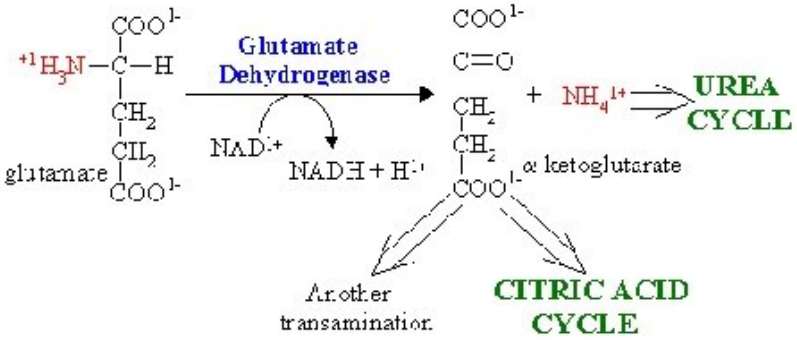

Nonoxidative Deamination
serine - threonine dehydratase
serine -----> pyruvate + NH4+
threonine --> alpha ketobutyrate + NH4+


• Histidine
NH3
Urocanic acid
Histidase
• L-cysteine
H2S
Imino acids
Desulfhydrase
H2O
NH3
Pyruvic acid
dehydrase
• Serine
Imino acids
H2O
H2O
NH3
Pyruvic acid



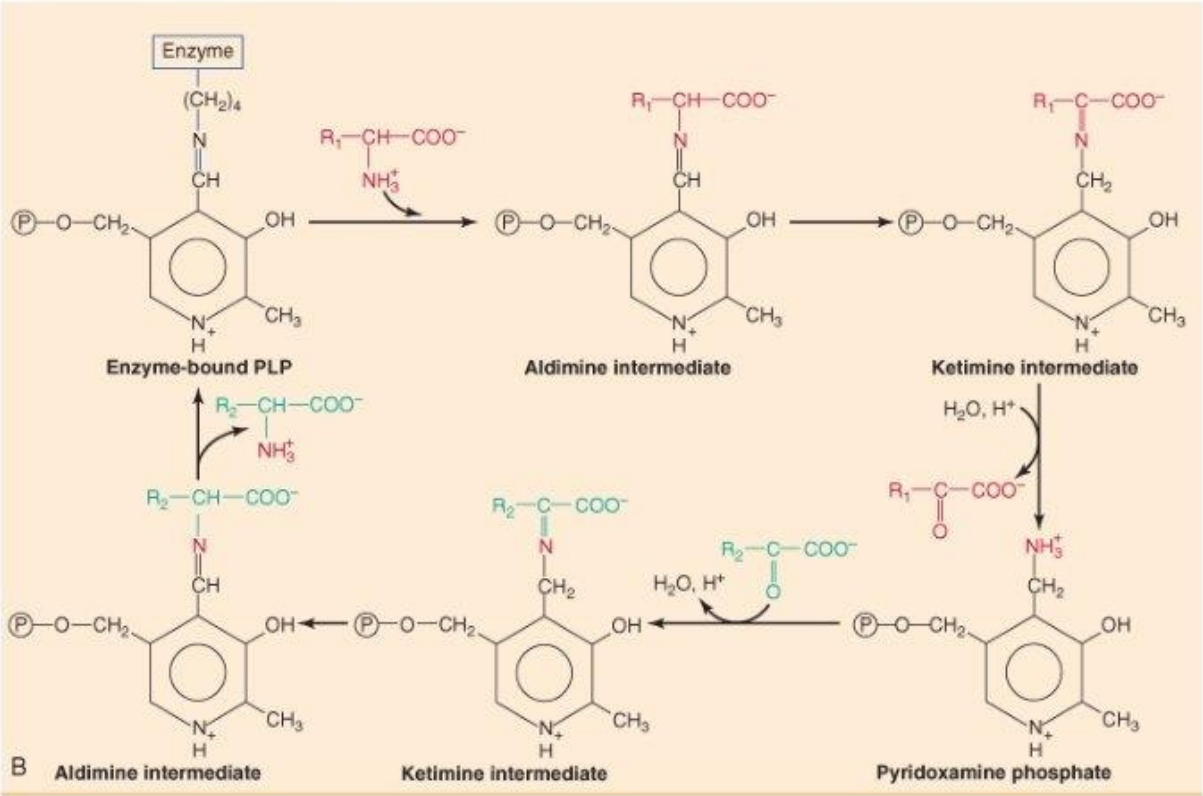
Oxidative Deamination

- Glutamate formed by transamination reactions is deaminated to α-ketoglutarate
- Glutamate dehydrogenase - NAD^+ or $NADP^+$ is coenzyme



- Other AA oxidases - (liver, kidney) low activity

Mechanism of transamination reaction: PPL complex with enzyme accept an amino group to form pyridoxamine phosphate, which can donate its amio group to an α-keto acid.



DECARBOXYLATION OF AMINO ACIDS

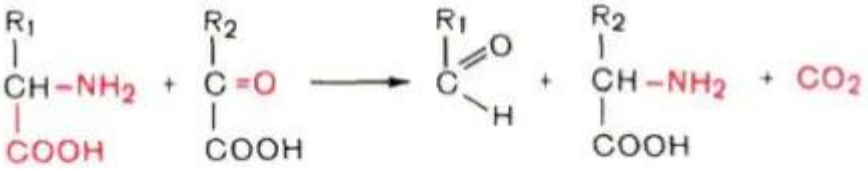
α-decarboxilation

$$R-CH(NH_2)-COOH \longrightarrow R-CH_2-NH_2 + CO_2$$

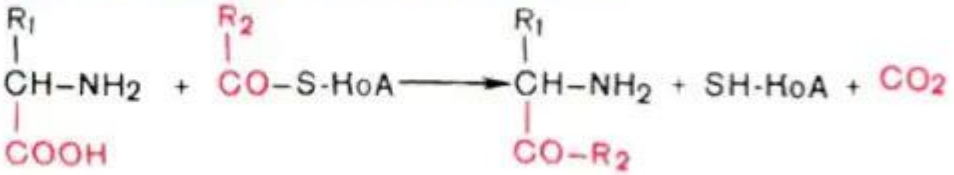
ω-decarboxilation

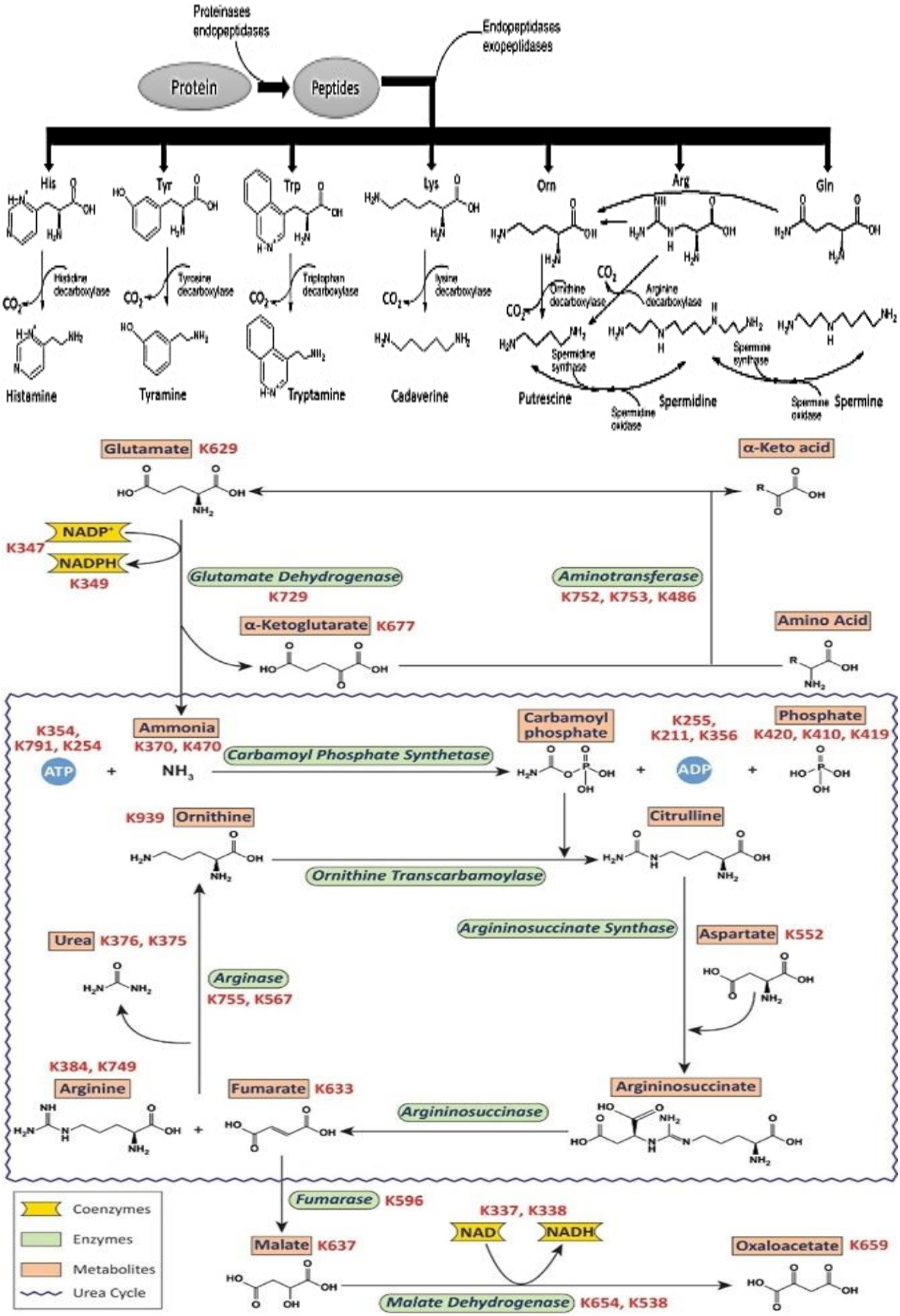
$$HOOC-CH_2-CH(NH_2)-COOH \longrightarrow CH_3-CH(NH_2)-COOH + CO_2$$

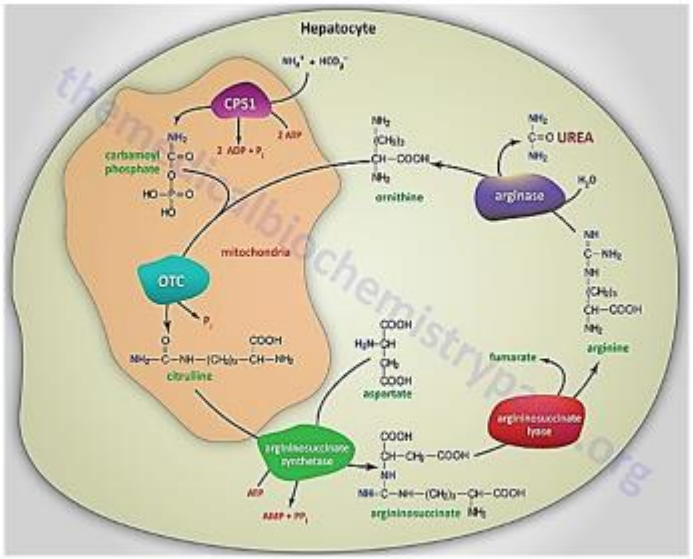
Decarboxilation with transamination

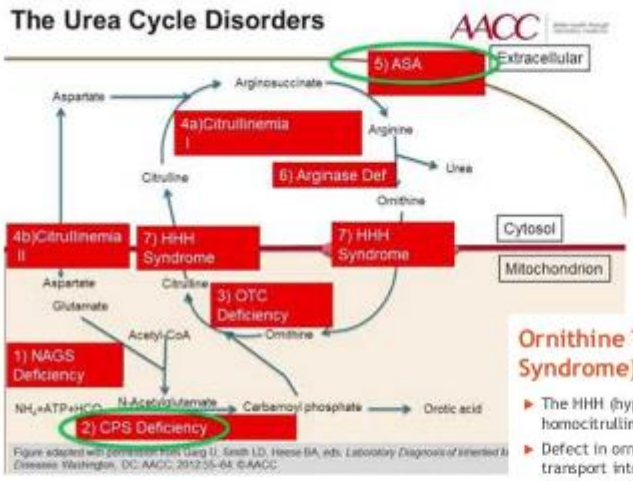


Decarboxilation with conjugation of two molecules




Proteinases
endopeptidases
Protein
Peptides
Endopeptidases
exopeptidases
His
Tyr
Trp
Lys
Orn
Arg
Gln
Histamine
Tyramine
Tryptamine
Cadaverine
Putrescine
Spermidine
Spermine
Glutamate K629
α-Keto acid
NADP+
K347
NADPH
K349
Glutamate Dehydrogenase
K729
Aminotransferase
K752, K753, K486
α-Ketoglutarate K677
Amino Acid
K354,
K791, K254
ATP
Ammonia
K370, K470
NH3
Carbamoyl Phosphate Synthetase
Carbamoyl phosphate
K255,
K211, K356
ADP
Phosphate
K420, K410, K419
K939 Ornithine
Ornithine Transcarbamoylase
Citrulline
Argininosuccinate Synthase
Aspartate K552
Urea K376, K375
Arginase
K755, K567
K384, K749
Arginine
Fumarate K633
Argininosuccinase
Argininosuccinate
Fumarase K596
K337, K338
NAD
NADH
Malate K637
Oxaloacetate K659
Malate Dehydrogenase K654, K538
Coenzymes
Enzymes
Metabolites
Urea Cycle





Ornithine Translocase Deficiency (HHH Syndrome)

- The HHH (hyperornithinemia, hyperammonemia, homocitrullinuria)
- Defect in ornithine translocase results in diminished ornithine transport into the mitochondria with ornithine accumulation in the cytoplasm and reduced intramitochondrial ornithine causing impaired ureagenesis and orotic aciduria
- Homocitrulline is thought to originate from transcarbamylation of lysine.

HHH syndrome

- One year & 1mth old boy with severe failure to thrive , excessive sleepiness .
- The condition started at the age of 4mths after feeding baby with yogurt . He started to have recurrent attacks of vomiting sometimes with diarrhea ,admitted to hospital for IV fluids.
- On examination:

  Fair complexion , apathy , GDD (motor &mental) . Normal abd. Exam.
  Pt . weighted 6 kg , H.C. 42 cm , length 69 cm (all< 3rd percentile for age)

Hereditary disorders of Urea cycle

Disease	Enzyme	Mode of inheritance	Clinical presentations	Blood metabolites
Hyper-ammonemia, type I	Carbamoyl phosphate synthetase I	Autosomal recessive	Coma, and death within 24-48 hours after birth	Gln Ala NH_3
Hyper-ammonemia, type II	Ornithine carbamoyl-transferase	X-linked	Hypotension, reduced tolerance to proteins	Gln Ala NH_3

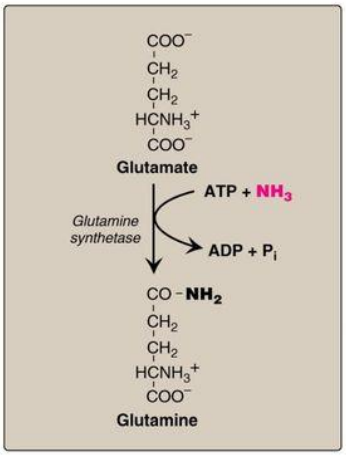
B: Transport of NH3 from peripheral tissues into the liver

- NH3 needs to reach the liver because the liver contains the enzyme required to convert ammonia to urea
- Ammonia is produced by all tissues and the main disposal is via formation of urea in liver
- Blood level of NH3 must be kept very low, otherwise, hyperammonemia and CNS toxicity will occur (NH3 is toxic to CNS)
- To solve this problem, NH3 is transported from peripheral tissues to the liver via formation of:

✓ Glutamine (most tissues)
✓ Alanine (muscle)

Ammonia should not be left alone in the blood because it can cause CNS toxicity Therefore it is transported to the liver in the form of Glutamine and alanine

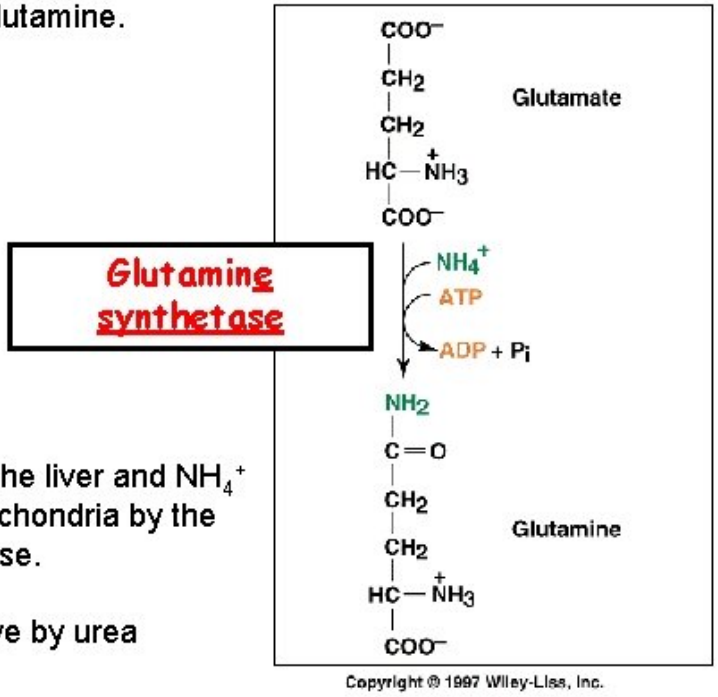
Transport of NH3 from peripheral tissues into the liver



From most peripheral tissues NH3 is transported Into the liver through forming glutamine by glutamine synthetase

Ammonia transport in the form of glutamine

Excess ammonia is added to glutamate to form glutamine.

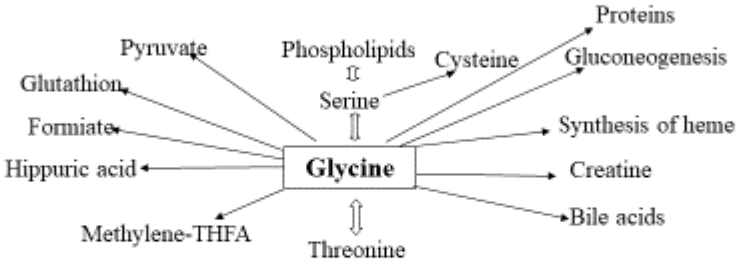


Copyright © 1997 Wiley-Liss, Inc.

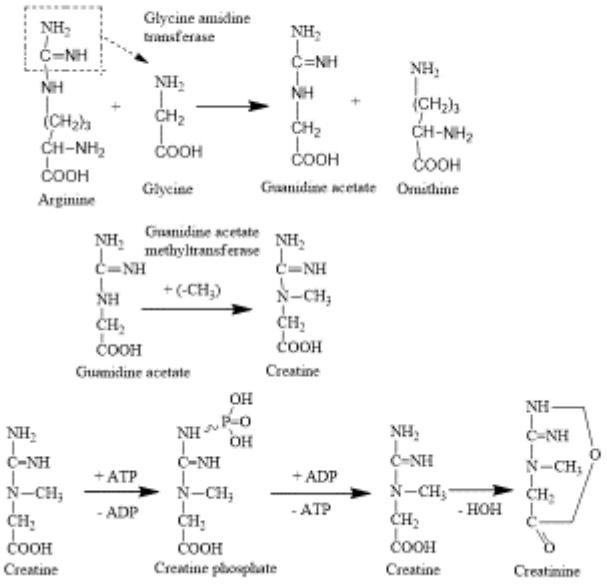
Glutamine enters the liver and NH_4^+ is liberated in mitochondria by the enzyme glutaminase.

Ammonia is remove by urea synthesis.

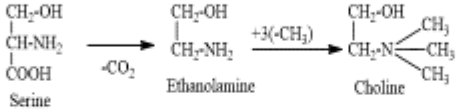
Metabolism of glycine

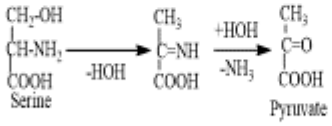


Synthesis of creatine

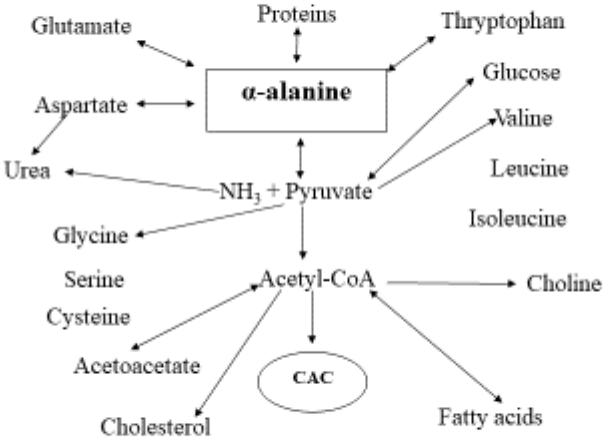


Metabolism of serine

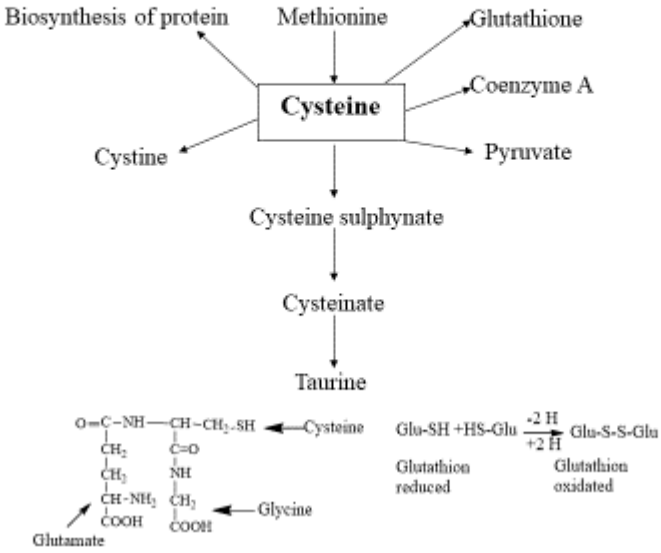




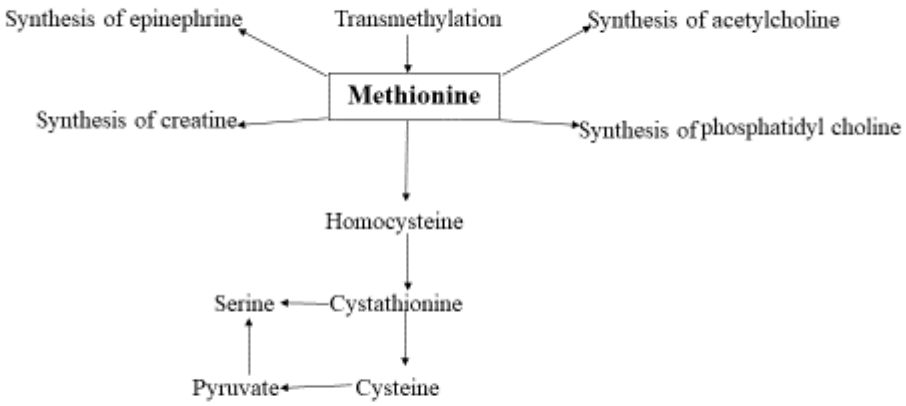
Metabolism of α-alanine



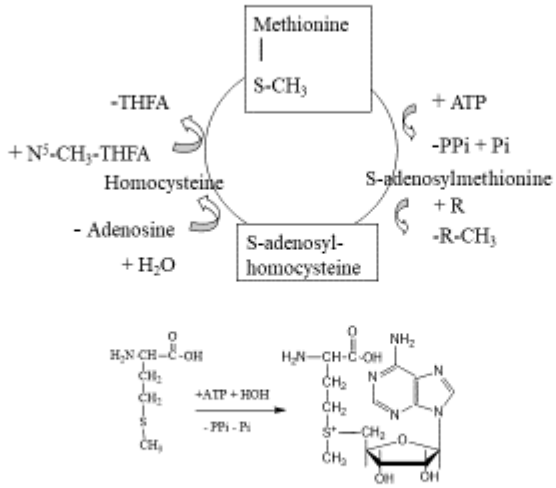
Metabolism of cysteine



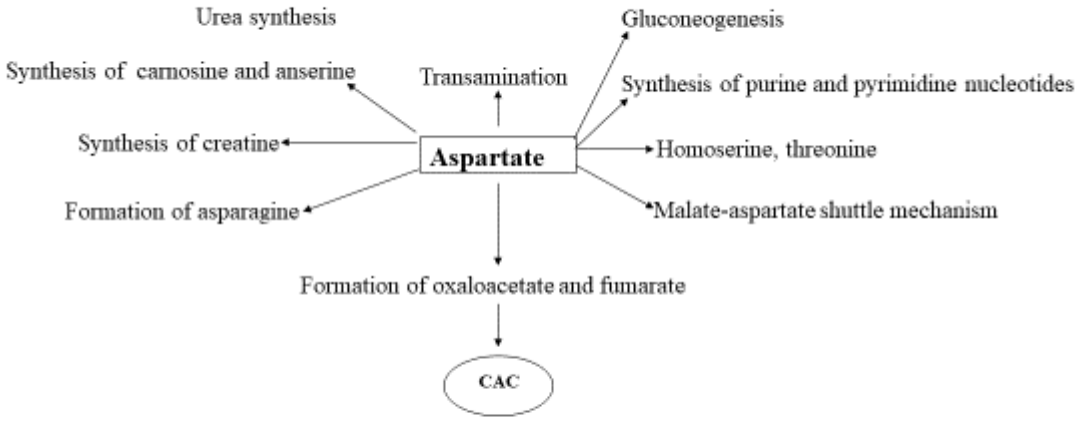
Metabolism of methionine



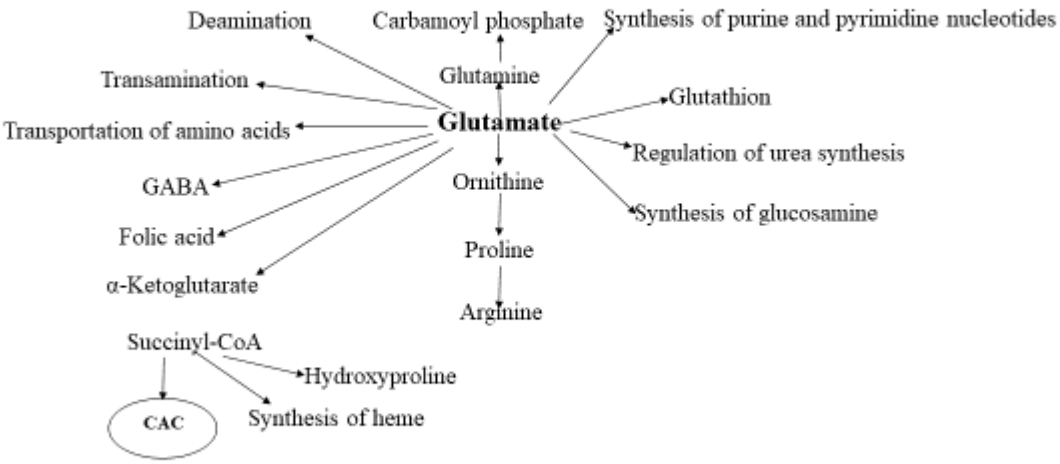
Scheme of S-adenosylmethionine synthesis



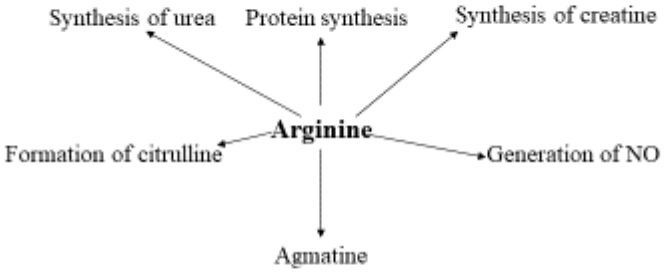
Metabolism of aspartate



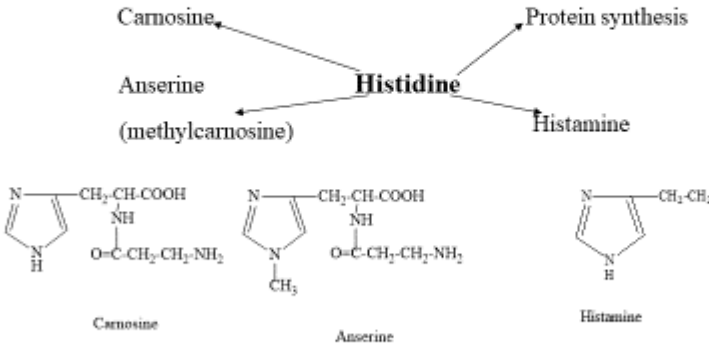
Metabolism of glutamate

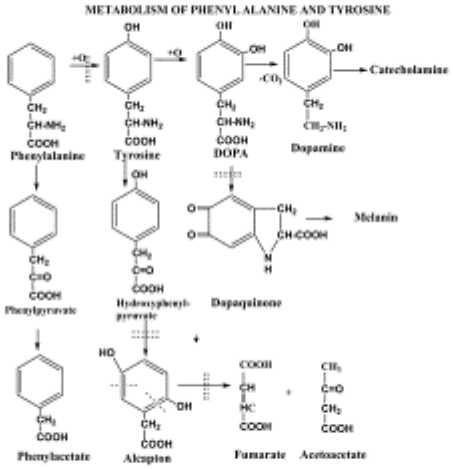


Metabolism of arginine

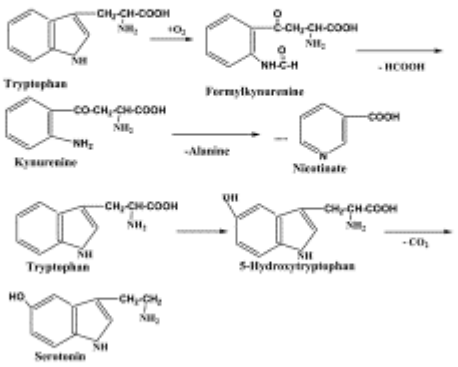


Metabolism of histidine





METABOLISM OF TRYPTOPHAN



Aminoacidopathies

- They are rare inherited disorders of amino acid metabolism.
- Hereditary disorders of amino acid processing can be the result of :
  - defects either in the breakdown of amino acids (activity of a specific enzyme)
  - or in the body's ability to get the amino acids into cells (membrane transport system).
- More than 100 diseases have been identified that result from inborn errors of amino acid metabolism.
- Because these disorders produce symptoms early in life, newborns are routinely screened for several common ones.

Dr. Mazen Alkahema CC 2008/3

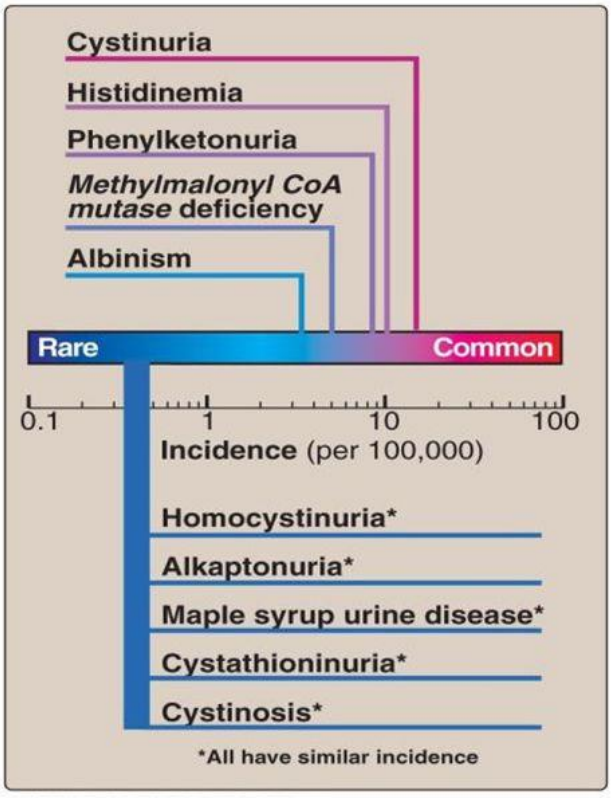
TABLE 18–2 Some Human Genetic Disorders Affecting Amino Acid Catabolism

Medical condition	Approximate incidence (per 100,000 births)	Defective process	Defective enzyme	Symptoms and effects
Albinism	<3	Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (tyrosinase)	Lack of pigmentation; white hair, pink skin
Alkaptonuria	<0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	<1.5	Urea synthesis	Argininosuccinase	Vomiting; convulsions
Carbamoyl phosphate synthetase 1 deficiency	<0.5	Urea synthesis	Carbamoyl phosphate synthetase 1	Lethargy; convulsions; early death
Homocystinuria	<0.5	Methionine degradation	Cystathionine β-synthase	Faulty bone development; mental retardation
Maple syrup urine disease (branched-chain ketoaciduria)	<0.4	Isoleucine, leucine, and valine degradation	Branched-chain α-keto acid dehydrogenase complex	Vomiting; convulsions; mental retardation; early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting; convulsions; mental retardation; early death
Phenylketonuria	<8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation

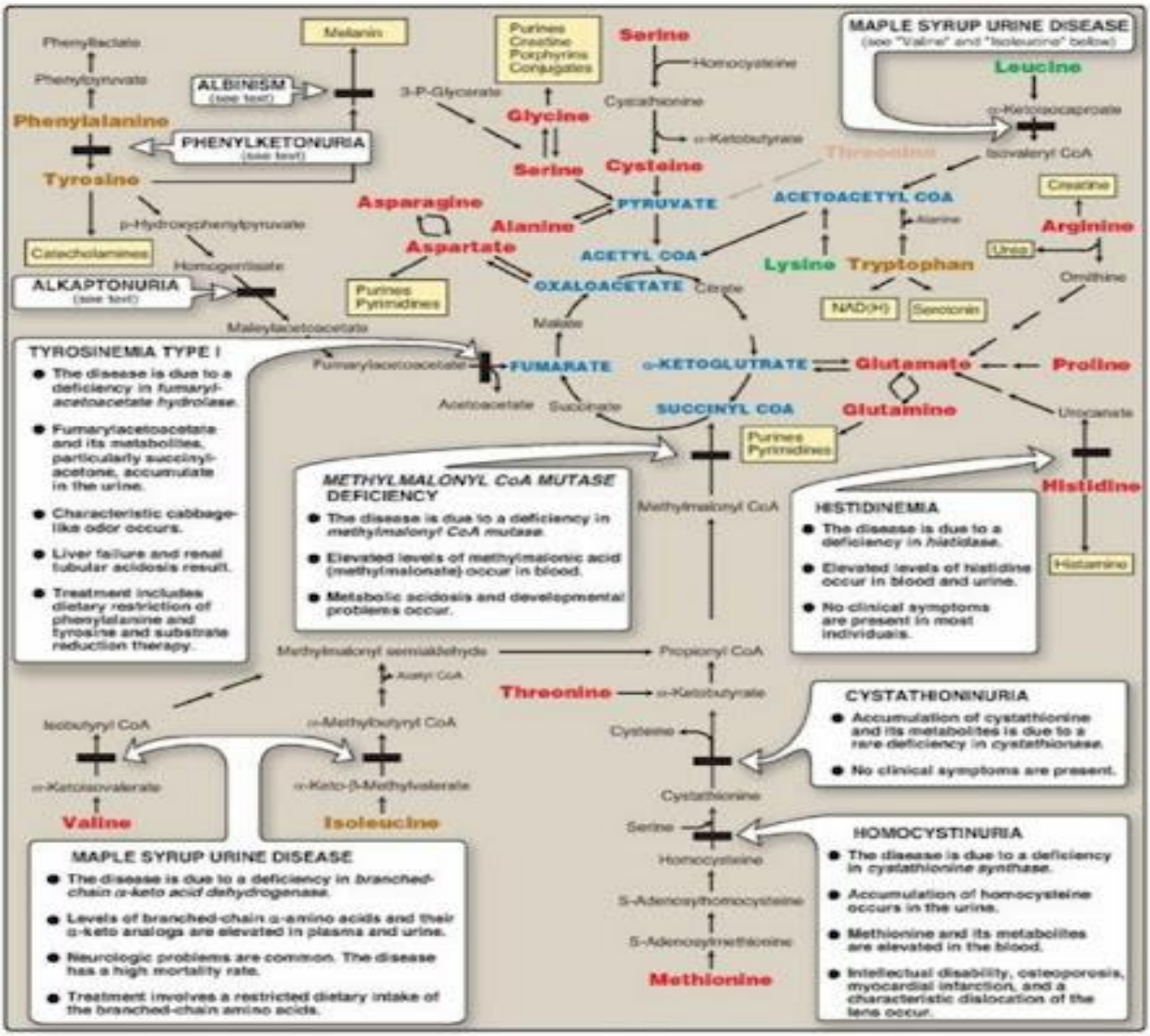
Table 18-2
Lehninger Principles of Biochemistry, Fifth Edition

Metabolic defects in amino acid metabolism

- Inborn errors of metabolism are commonly caused by mutant genes that generally result in abnormal proteins, most often enzymes. The inherited defects may be expressed as a total loss of enzyme activity or as a partial deficiency in activity.
- Newborn screening and timely initiation of treatment are essential. By law, all states must screen for over 20 disorders. All states screen for PKU.
- Treatment: diets low in the amino acids whose catabolism is impaired.



3



General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. The pool of free amino acids in the body: ways of obtaining and using free amino acids in tissues.
2. Transamination of amino acids: reactions and their biochemical significance, mechanisms of action of aminotransferases.
3. Direct and indirect deamination of free L-amino acids in tissues.
4. Decarboxylation of L-amino acids in the human body. Physiological significance of the products formed. Oxidation of biogenic amines.

5. Ways of formation and neutralization of ammonia in the body.
6. Urea biosynthesis: sequence of biosynthesis enzyme reactions, genetic anomalies of urea cycle enzymes.
7. General ways of metabolism of carbon skeletons of amino acids in the human body. Glucogenic and ketogenic amino acids.
8. Biosynthesis and biological role of creatine and creatine phosphate.
9. Glutathione: structure, biosynthesis and biological functions of glutathione
10. Specialized ways of metabolism of cyclic amino acids - phenylalanine and tyrosine.
11. Metabolism of the cyclic amino acid tryptophan and its hereditary enzymopathies.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture No. 10

Topic: Biosynthesis and catabolism of purine and pyrimidine nucleotides. Biosynthesis of nucleic acids: DNA replication; RNA transcription. Protein synthesis in ribosomes. Regulation of protein biosynthesis.

Relevance of the topic: Nucleotides are structural monomers of nucleic acids, the main custodians and carriers of hereditary information. In addition, cyclic nucleotides play the role of secondary mediators in the action of hormones on target cells, and nucleoside triphosphates are the main macroergs in the human body. Nucleic acids, like proteins, are the main substrate of life. The role of nucleic acids in the processes of protein biosynthesis is particularly significant, and in connection with this, in the formation of hereditary traits of the organism, in reproduction, growth and development, as well as in the adaptation (adaptation) of the organism to various conditions of existence. Nucleic acids are part of cells, as a rule, in combination with proteins, in the form of nucleoproteins, and it is precisely in the form of nucleoproteins that their functions are realized.

Purpose: to study the structure of the constituent parts of nucleic acids (DNA and RNA) - mononucleotides and to know the formation of end products of purine and pyrimidine metabolism, including changes in pathology; to acquaint higher education applicants with the biological role of nucleic acids and nucleoproteins, to create an idea of nucleic acids as carriers of genetic information. Mastering the mechanisms of matrix synthesis of proteins and its regulation.

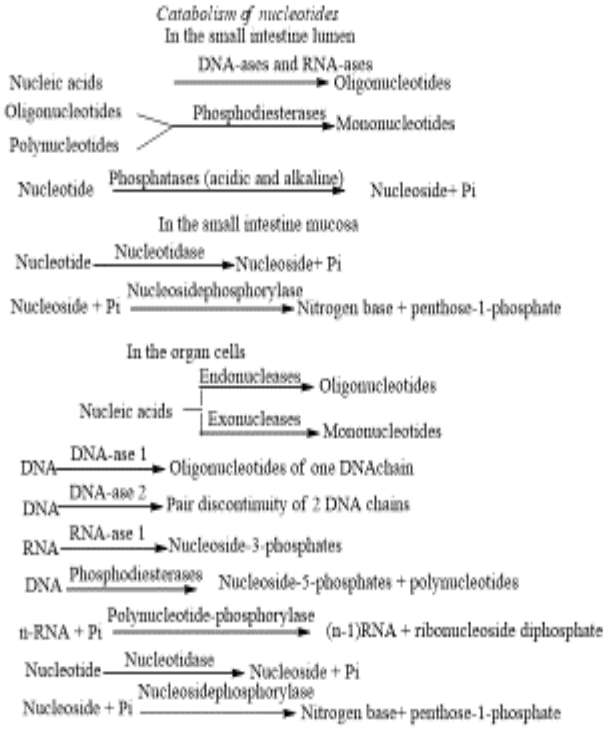
Basic concepts:

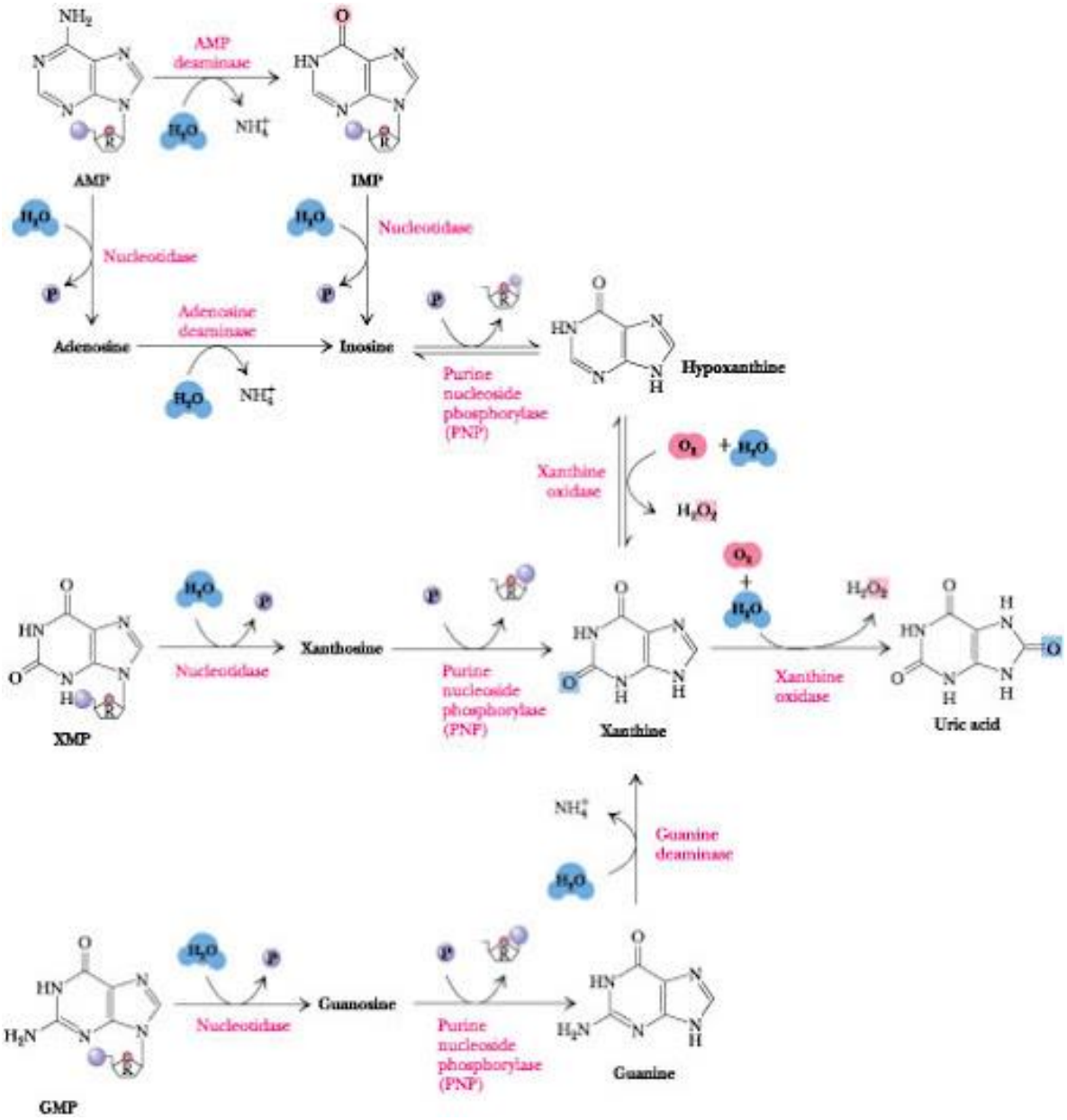
1. Nitrogen bases
2. Nucleoside.
3. Nucleotide.
4. Methotrexate.
5. Gout.
6. Lesch-Nyhan syndrome.
7. Orotaaciduria.
8. Chargaff rules, Watson-Crick model.
9. Anti-parallelism of chains.
10. Gene expression.
11. Processing.
12. Genetic (biological) code.
13. Recognition.
14. Folding.
15. Mutations.
16. Recombinant DNA.

Plan and organizational structure of the lecture:

1. The structure of nucleotides, their biological role.
2. Catabolism of purine nucleotides.
3. Catabolism of pyrimidine nucleotides.
4. Biosynthesis of purine nucleotides. Regulation.
5. Biosynthesis of pyrimidine nucleotides. . Regulation.
6. Pathology of purine and pyrimidine metabolism.
7. Replication. Stages.
8. Transcription. Stages.
9. Processing. Stages.
10. Protein biosynthesis. Stages.
11. Inhibitors of transcription and translation in prokaryotes and eukaryotes: antibiotics and interferons - their use in medicine; diphtheria toxin.
12. Regulation of gene expression.
13. Mutations.
14. Genetic engineering.

Content of the lecture material

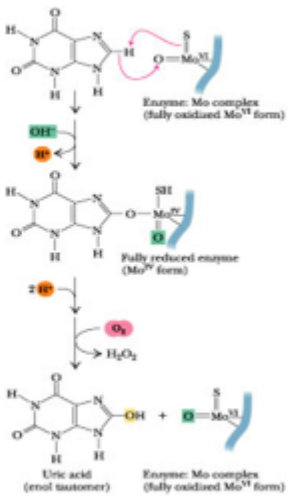




Xanthine Oxidase

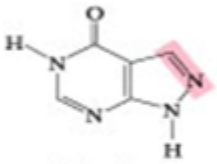
is present in large amounts in liver, intestinal mucosa, and milk. Xanthine oxidase is using molecular oxygen to oxidize a wide variety of purines, pteridines, and aldehydes, producing H_2O_2 as a product. It possesses FAD, nonheme Fe-S centers, and a *molybdenum cofactor* as electron-transferring prosthetic groups.

In humans and other primates, uric acid is the end product of purine catabolism and is excreted in the urine.

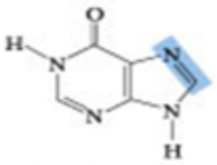


Gout: An Excess of Uric Acid

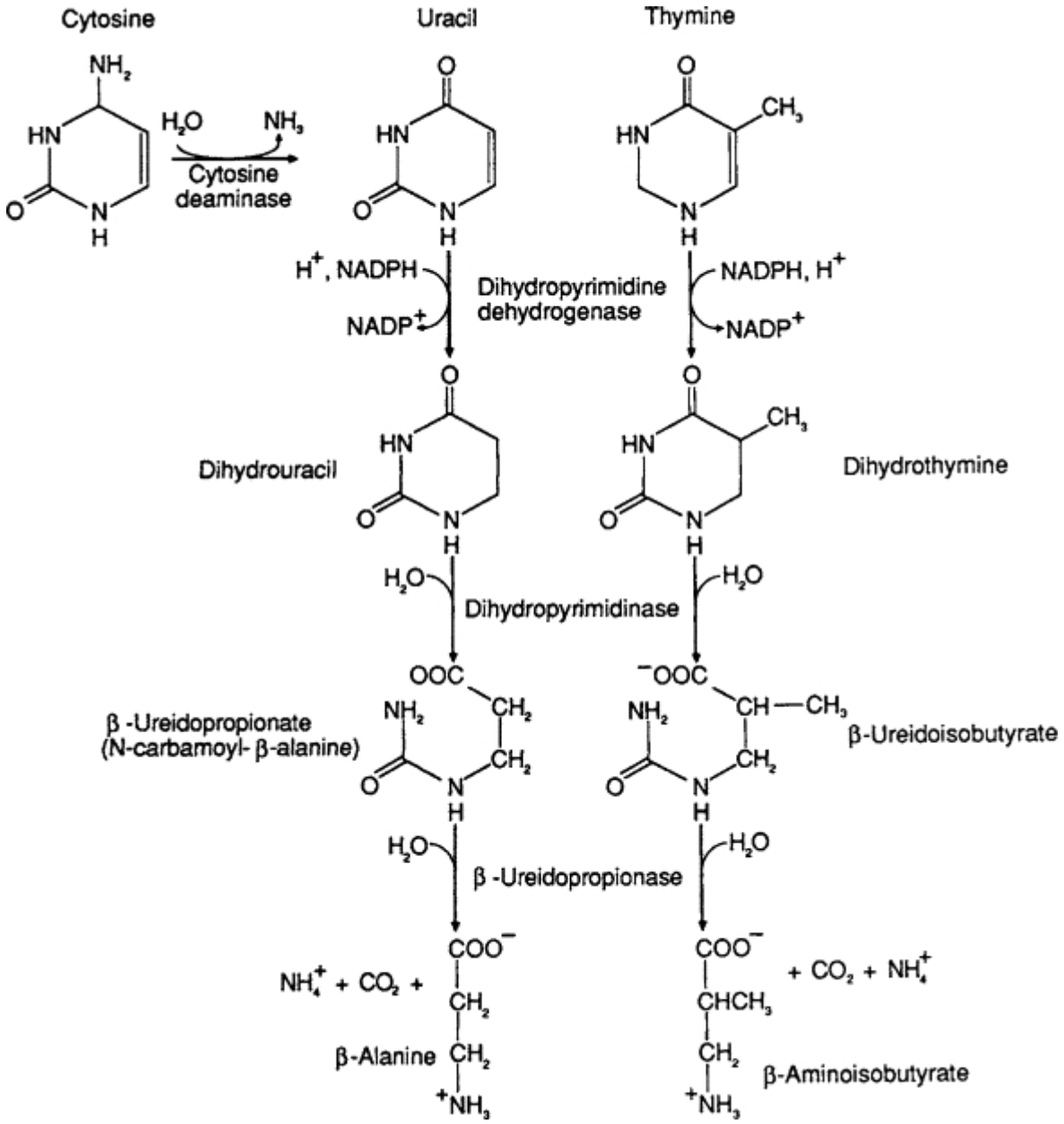
Gout is the clinical term describing the physiological consequences accompanying excessive uric acid accumulation in body fluids. Uric acid and urate salts are rather insoluble in water and tend to precipitate from solution if produced in excess. The most common symptom of gout is arthritic pain in the joints as a result of urate deposition in cartilaginous tissue. The joint of the big toe is particularly susceptible. Urate crystals may also appear as kidney stones and lead to painful obstruction of the urinary tract. **Hyperuricemia,** chronic elevation of blood uric acid levels, occurs in about 3% of the population as a consequence of impaired excretion of uric acid or overproduction of purines. Purine-rich foods (such as caviar—fish eggs rich in nucleic acids) may exacerbate the condition. The biochemical causes of gout are varied. However, a common treatment is *allopurinol*. This hypoxanthine analog binds tightly to xanthine oxidase, thereby inhibiting its activity and preventing uric acid formation. Hypoxanthine and xanthine do not accumulate to harmful concentrations because they are more soluble and thus more easily excreted.

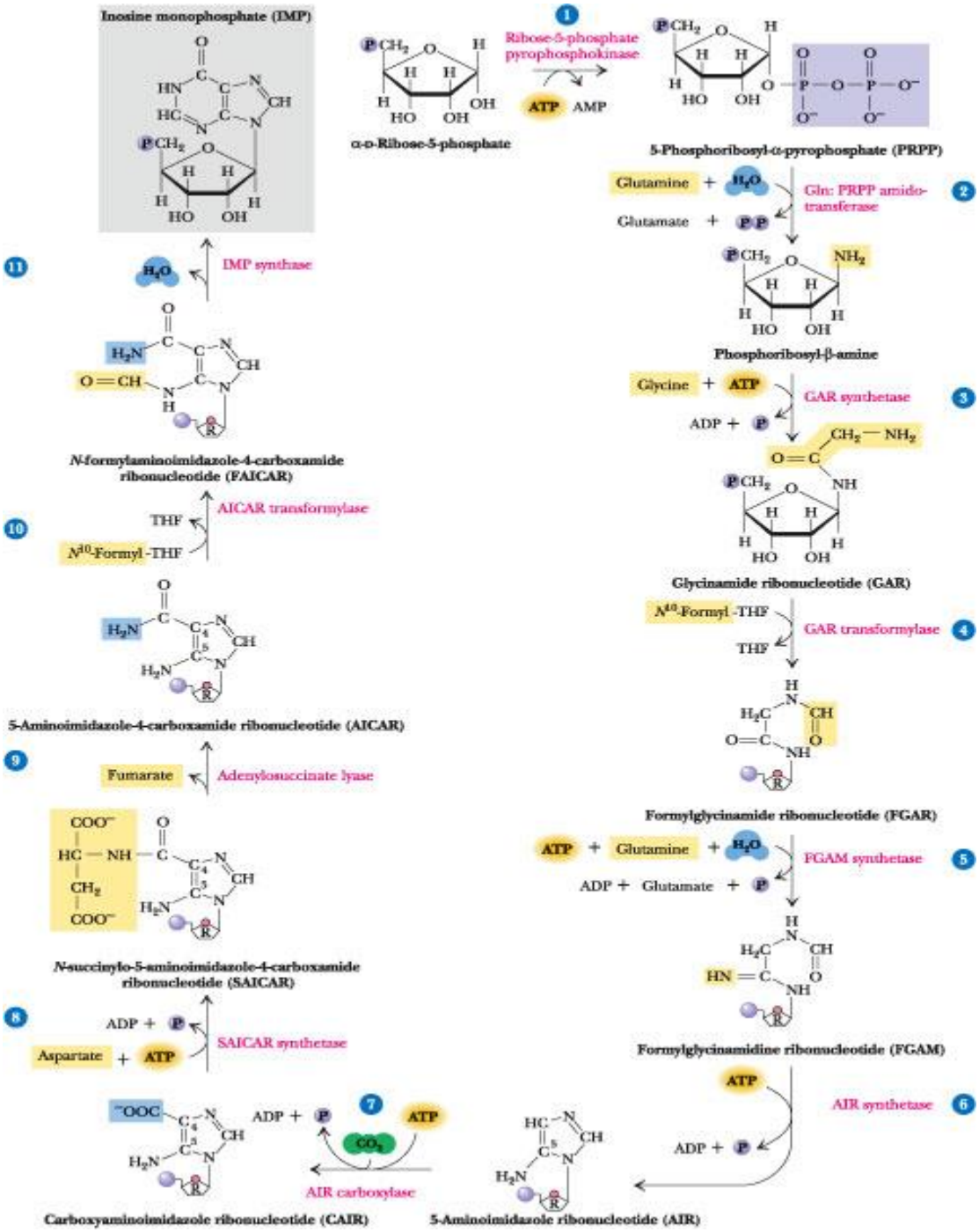


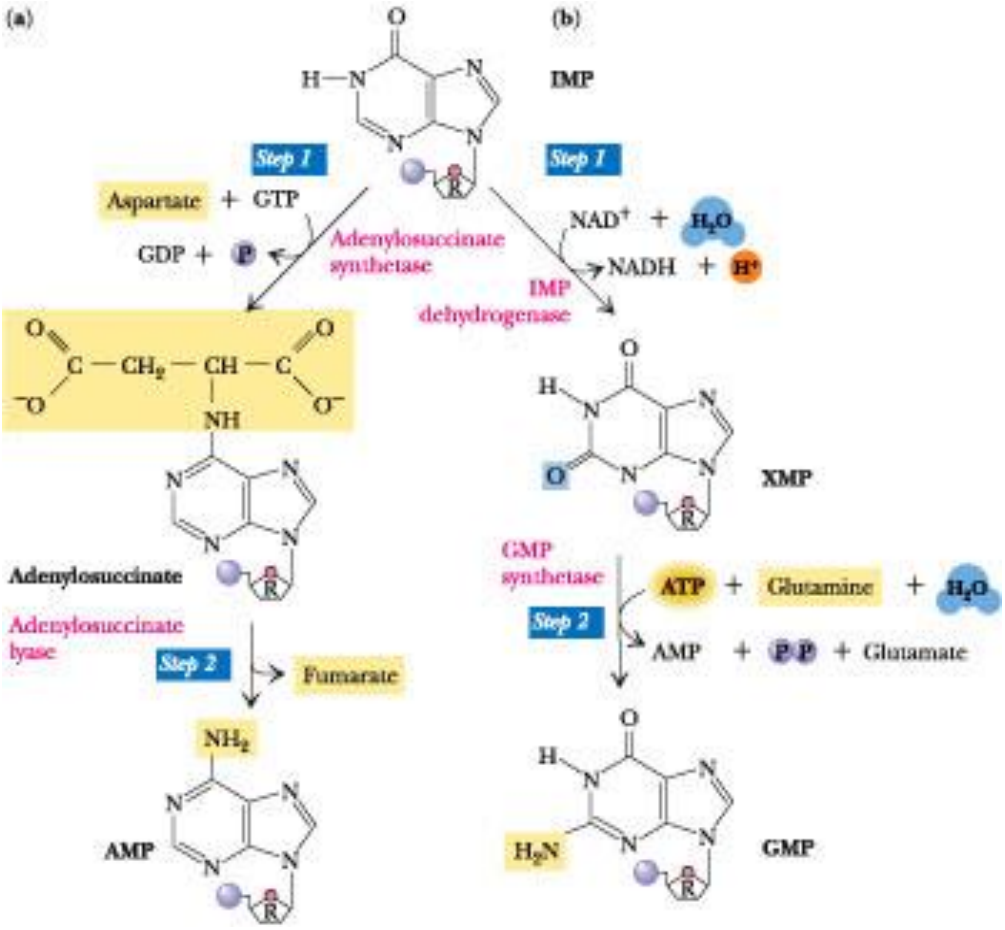
Allopurinol



Hypoxanthine




Inosine monophosphate (IMP)
1
Ribose-5-phosphate pyrophosphokinase
ATP
AMP
α-D-Ribose-5-phosphate
5-Phosphoribosyl-α-pyrophosphate (PRPP)
Glutamine + H2O
Gln: PRPP amido-transferase
2
Glutamate + PP
Phosphoribosyl-β-amine
Glycine + ATP
GAR synthetase
3
ADP + P
Glycinamide ribonucleotide (GAR)
N10-Formyl-THF
GAR transformylase
4
THF
Formylglycinamide ribonucleotide (FGAR)
ATP + Glutamine + H2O
FGAM synthetase
5
ADP + Glutamate + P
Formylglycinamidine ribonucleotide (FGAM)
ATP
AIR synthetase
6
ADP + P
5-Aminoimidazole ribonucleotide (AIR)
HC
AIR carboxylase
7
ADP + P
ATP
CO2
Carboxyaminoimidazole ribonucleotide (CAIR)
Aspartate + ATP
SAICAR synthetase
8
ADP + P
N-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)
Fumarate
Adenylosuccinate lyase
9
5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)
N10-Formyl-THF
AICAR transformylase
10
THF
N-formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)
H2O
IMP synthase
11



REGULATION OF PURINE METABOLISM

- **Inosine monophosphate (IMP)** is the parent nucleotide of purine from which both **AMP** and **GMP** are formed.

- Synthesis of IMP from the amphibolic intermediate such as **glycine**, **glutamine**, **tetrahydrofolate** derivatives, **aspartate** and **ATP**.

- The pathway then branches , one path leading from **IMP to AMP**, the other from **IMP to GMP**.

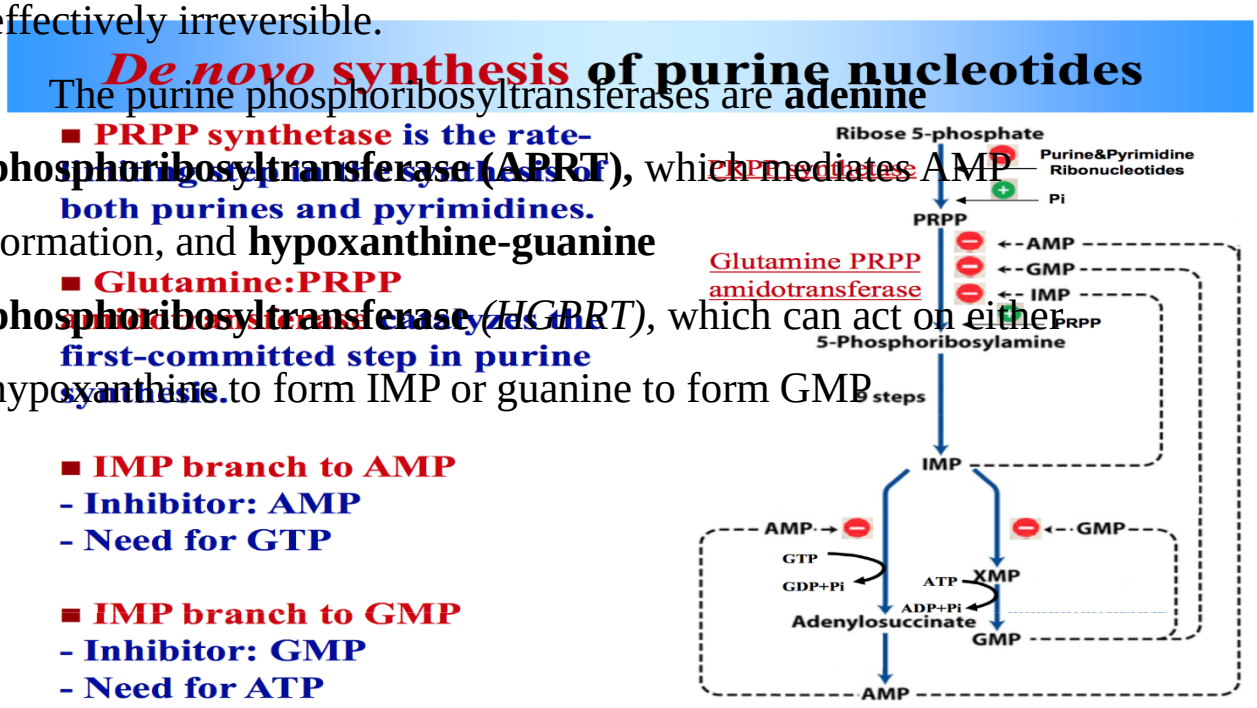
Purine Salvage

Nucleic acid turnover (synthesis and degradation) is an ongoing metabolic process in most cells. Messenger RNA in particular is actively synthesized and degraded. These degradative processes can lead to the release of free purines in the form of adenine, guanine, and hypoxanthine (the base in IMP). These substances represent a metabolic investment by cells. So-called salvage pathways exist to recover them in useful form. Salvage reactions involve resynthesis of nucleotides from bases via **phosphoribosyltransferases.**

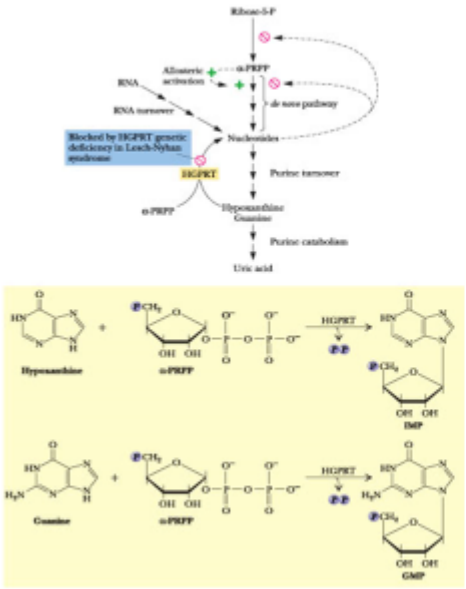
$$\text{Base} + \text{PRPP} \rightarrow \text{nucleoside-5'-phosphate} + PP_i$$

The subsequent hydrolysis of PP_i to inorganic phosphate by pyrophosphatases renders the phosphoribosyltransferase reaction effectively irreversible.

The purine phosphoribosyltransferases are **adenine phosphoribosyltransferase (APRT),** which mediates AMP formation, and **hypoxanthine-guanine phosphoribosyltransferase** *(HGPRT)*, which can act on either hypoxanthine to form IMP or guanine to form GMP.



Lesch-Nyhan Syndrome: HGPRT Deficiency Leads to Severe Clinical Disorder

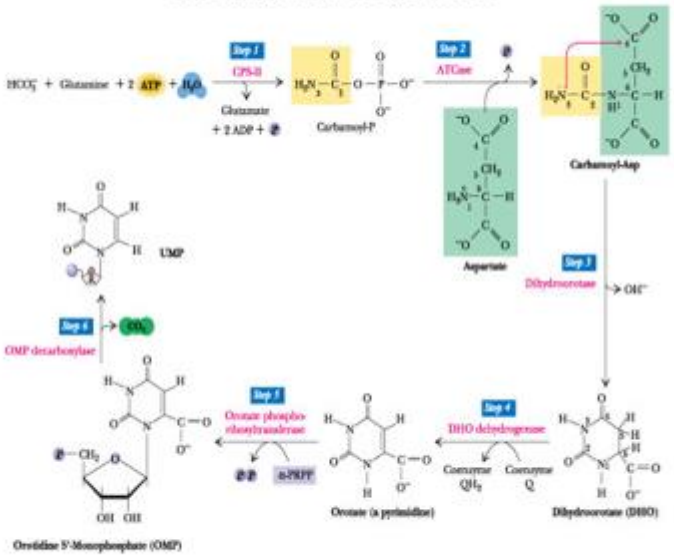


The symptoms of Lesch-Nyhan syndrome are tragic: a crippling gouty arthritis due to excessive uric acid accumulation and severe malfunctions in the nervous system that lead to mental retardation, spasticity, aggressive behavior, and self-mutilation.

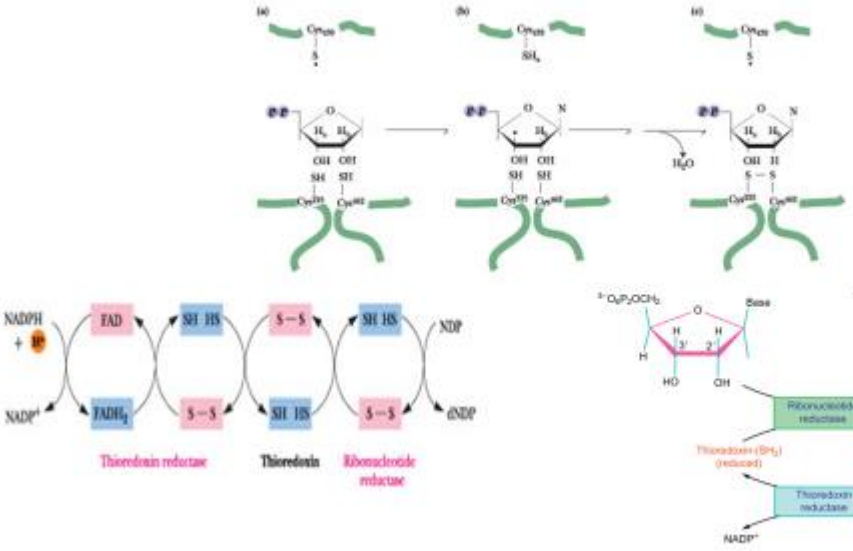
Lesch-Nyhan syndrome results from a complete deficiency in HGPRT activity. The structural gene for HGPRT is located on the X chromosome, and the disease is a congenital, recessive, sex-linked trait manifested only in males. Absence of HGPRT leads to: dramatically increased biosynthesis de novo purine and elevated levels of the uric acid in the blood.

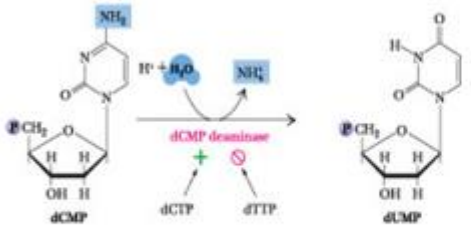
Deficiencies in HGPRT activity in fetal cells can be detected following amniocentesis.

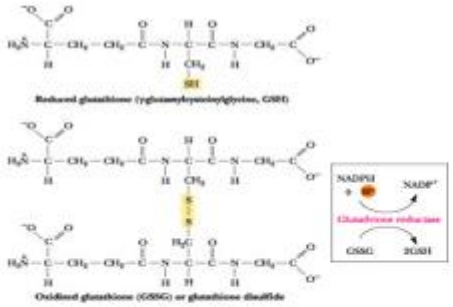
The Biosynthesis of Pyrimidines

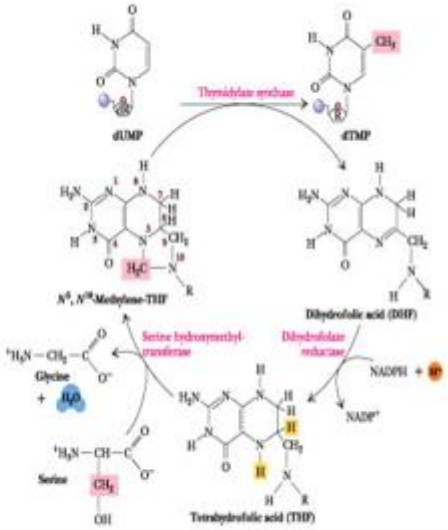


Deoxyribonucleotide Biosynthesis

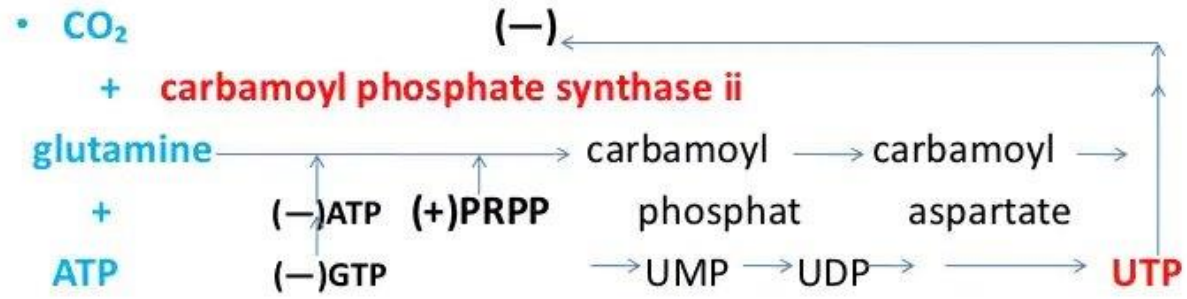








REGULATION OF PYRIMIDINE METABOLISM



*carbamoyl phosphate synthase ii is **inhibited by UTP** and **purine nucleotide** particularly **ATP and GTP.**

*carbamoyl phosphate synthase ii is **activated** by **PRPP.**

Inhibitors of Purine and Pyrimidine Nucleotide Synthesis

6-Mercaptopurine
Thioguanine — **used in the treatment of leukaemia**

Methotrexate — **used as anticancer drug**

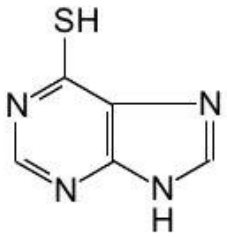
Sulfonamides — **used as antibiotic**

Azaserine and **diazonorleucine** are glutamine analog and inhibit the enzymes that utilize glutamine as amino group donor in the biosynthesis of **purine nucleotides**.

5-Fluorouracil is an anti cancer drug which inhibits thymidylate synthase enzyme

Inhibitors of purine synthesis (antineoplastic agents)

- inhibitors of dihydrofolate reductase
- glutamine analogs (azaserine)
- 6-mercaptopurine- inhibition of conversion of IMP to AMP and GMP



mercaptopurine

33

Synthetic Inhibitors of Purine Nucleotides

Some synthetic inhibitors of purine synthesis are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions (e.g sulfonamides). Sulfonamides are structural analogues of PABA (para-aminobenzoic acid) that competitively inhibit bacterial synthesis of folic acid, and hence they reduce the synthesis of "tetrahydrofolate" which is an essential co-enzyme for purine synthesis leading to slow down this synthetic pathway in bacteria.

12

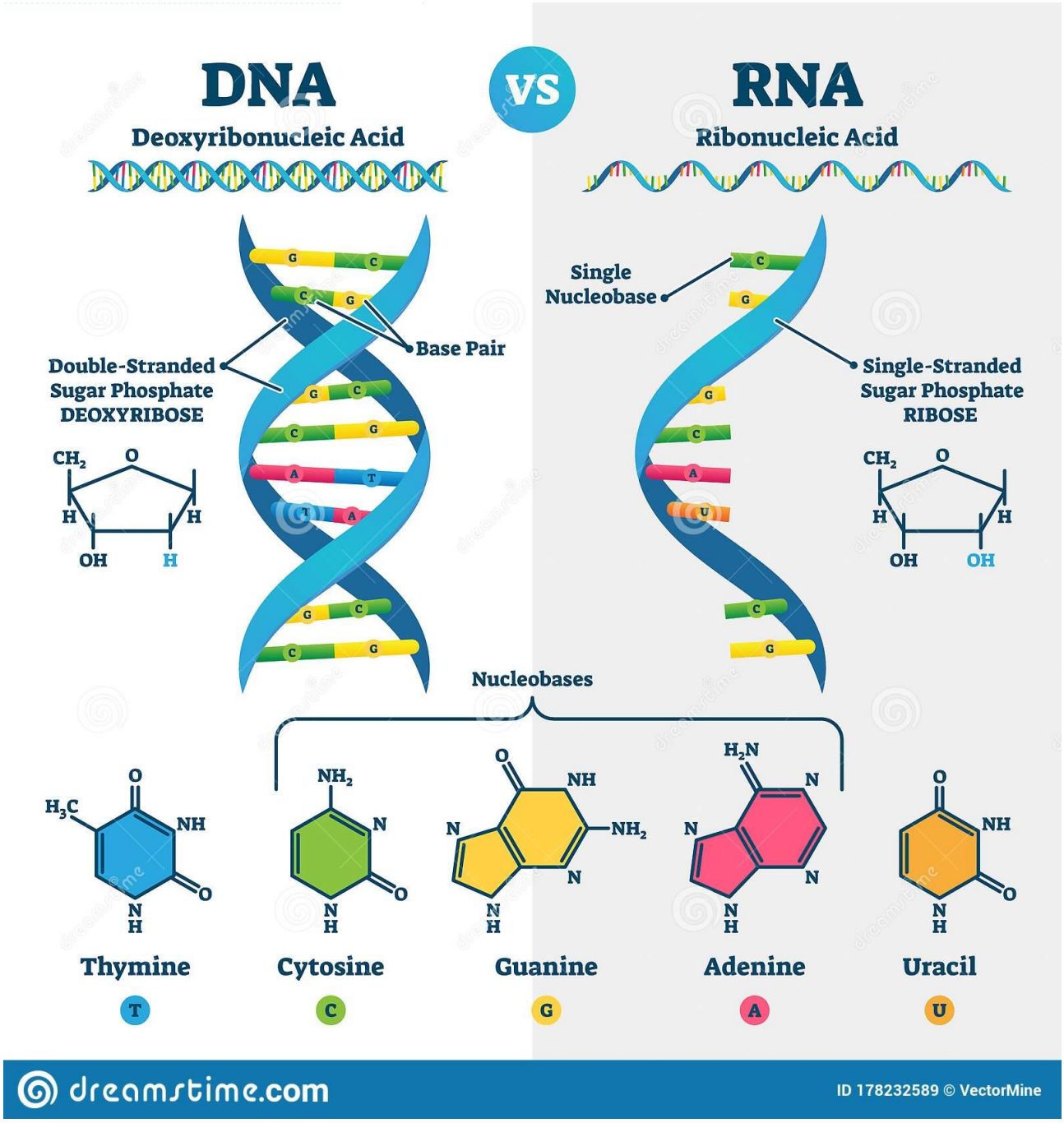
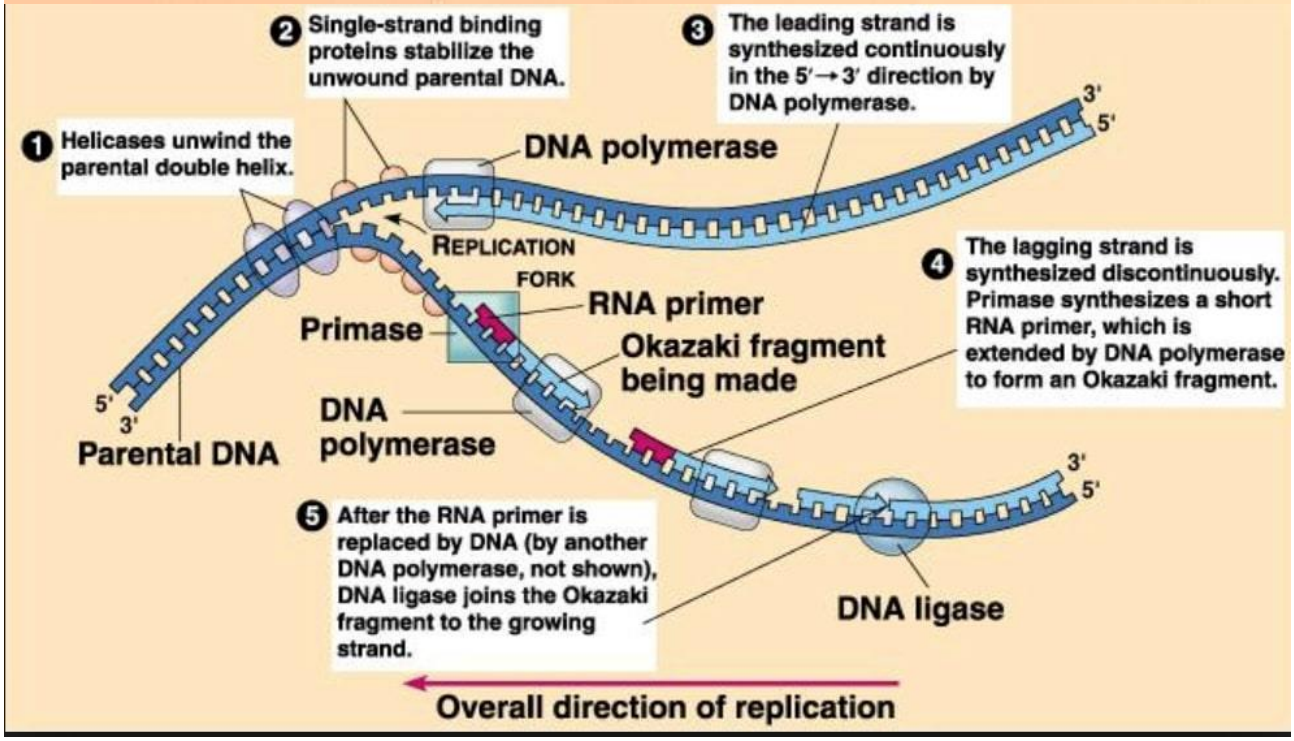

DNA
VS
RNA
Deoxyribonucleic Acid
Ribonucleic Acid
Single Nucleobase
Base Pair
Double-Stranded Sugar Phosphate DEOXYRIBOSE
Single-Stranded Sugar Phosphate RIBOSE
Nucleobases
Thymine
Cytosine
Guanine
Adenine
Uracil
T
C
G
A
U
dreamstime.com
ID 178232589 © VectorMine

Table 3 Comparison of DNA and RNA

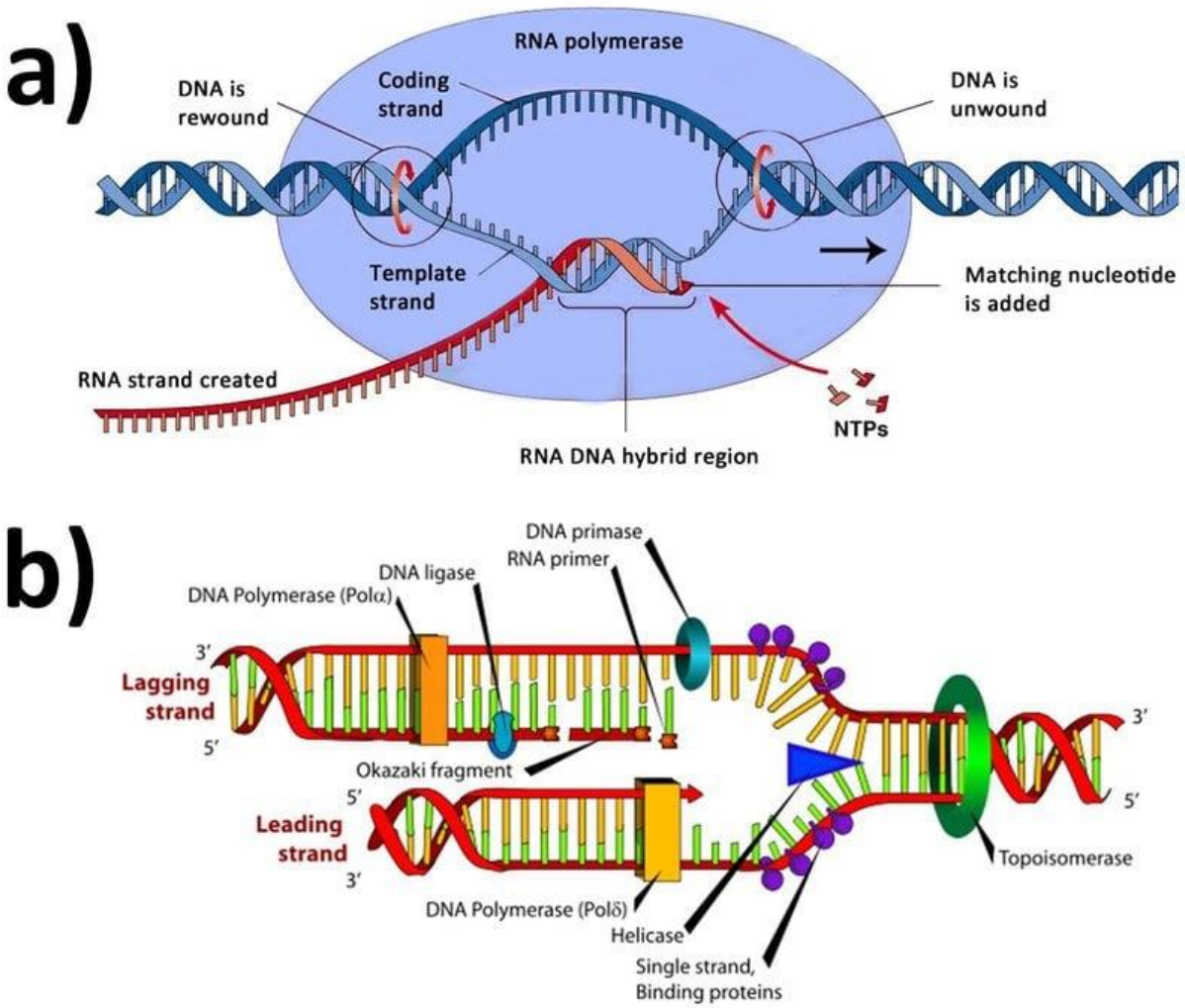
	DNA	RNA
Location	Almost entirely in the nucleus	Almost entirely in the cytoplasm
Composition	Nucleotides: adenine (A), guanine (G), cytosine (C), thymine (T) Sugar: deoxyribose	Nucleotides: adenine (A), guanine (G), cytosine (C), uracil (U) Sugar: ribose
Structure	Double-stranded helix formed by nucleotide pairing A-T; G-C	Single strand
Function	Makes up the chromosomes, hereditary units that control all cell activities; divided into genes that carry the nucleotide codes for the manufacture of proteins	Manufacture proteins according to the nucleotide codes carried in the DNA; three types: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA)



DNA Polymerases

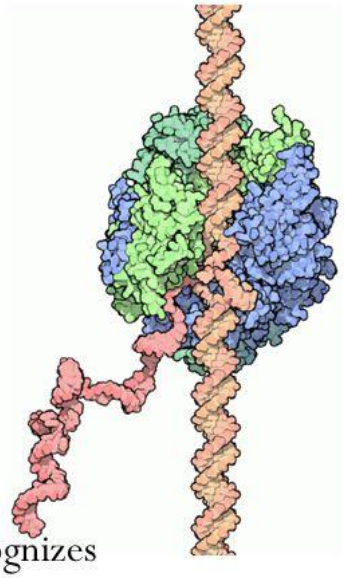
3

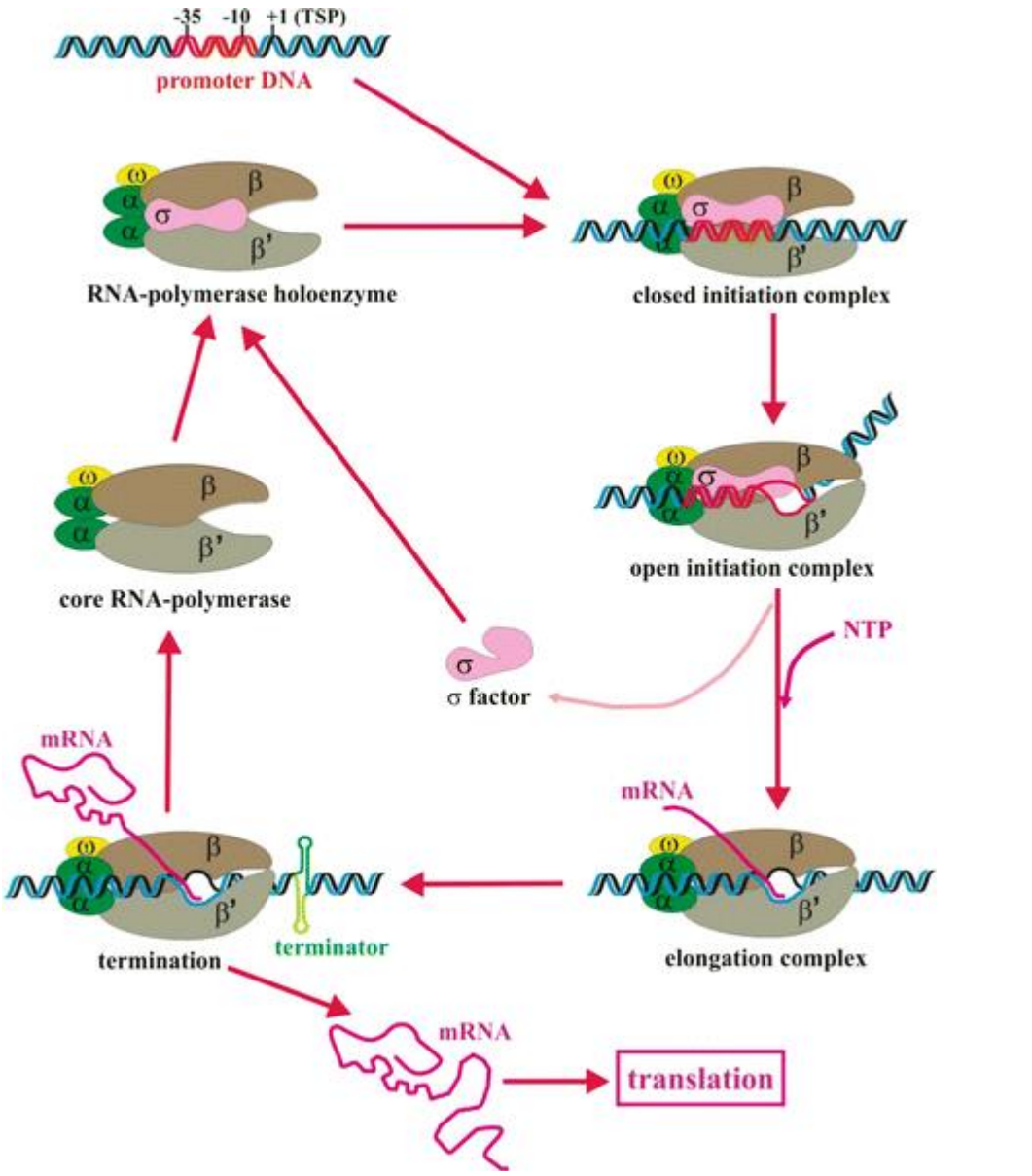
- The enzymes that create **DNA** molecules by assembling nucleotides
- These enzymes are essential to **DNA** replication
- They usually work in pairs to create two identical **DNA** strands from a single original **DNA** molecule
- There are three main types of prokaryotic DNA polymerases
- **DNA polymerases I**
- **DNA polymerases II**
- **DNA polymerases III**

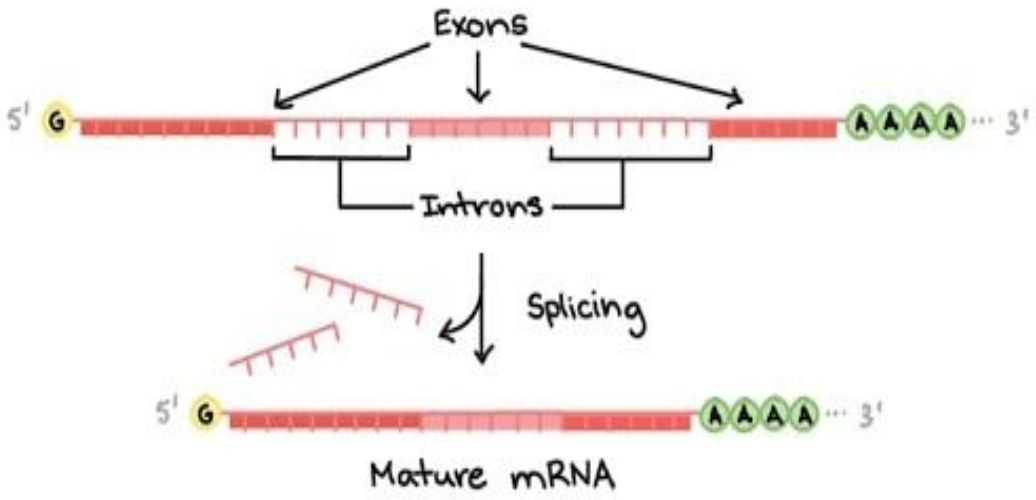

a)
RNA polymerase
DNA is rewound
Coding strand
DNA is unwound
Template strand
Matching nucleotide is added
RNA strand created
NTPs
RNA DNA hybrid region
b)
DNA primase
RNA primer
DNA ligase
DNA Polymerase (Polα)
3′
Lagging strand
5′
Okazaki fragment
5′
Leading strand
3′
DNA Polymerase (Polδ)
Helicase
Single strand, Binding proteins
Topoisomerase
3′
5′

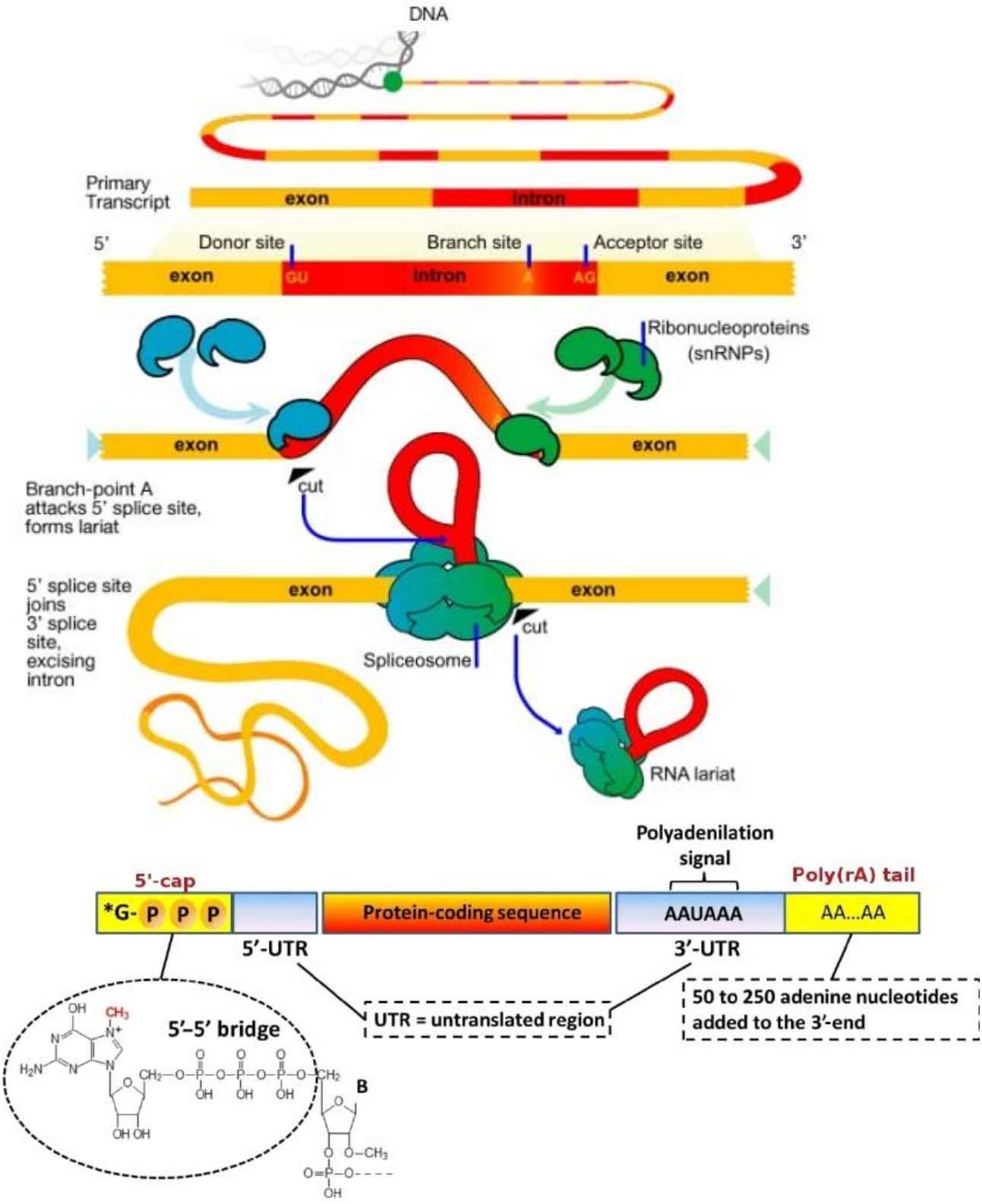
RNA polymerases

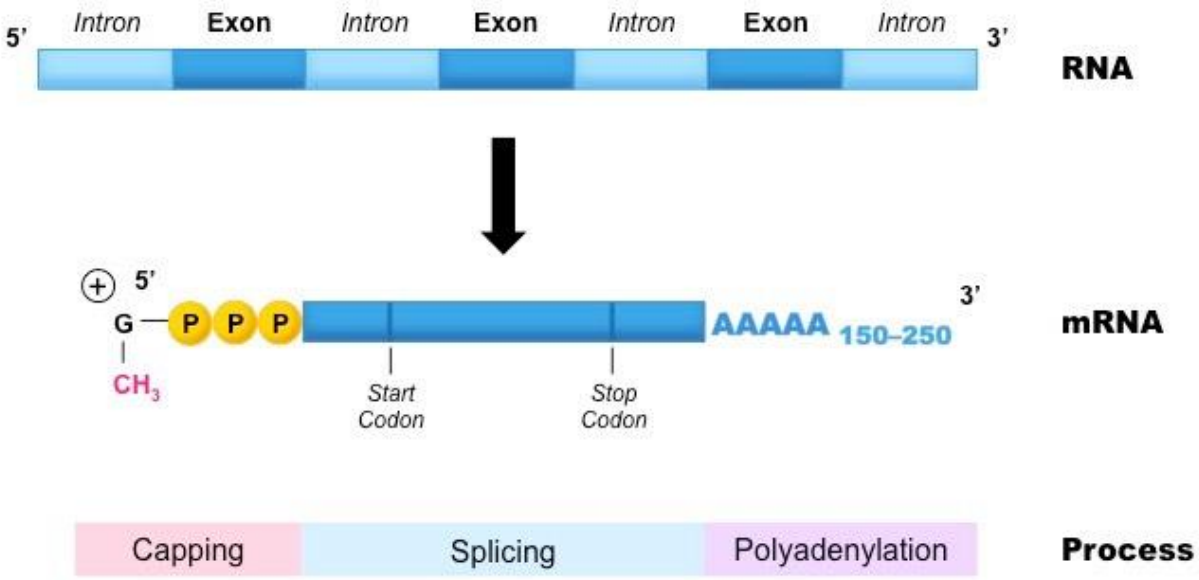
- 3 RNA polymerase enzymes
  - RNA polymerase 1
    - only transcribes rRNA genes
    - makes ribosomes
  - RNA polymerase 2
    - transcribes genes into mRNA
  - RNA polymerase 3
    - only transcribes tRNA genes
  - each has a specific promoter sequence it recognizes

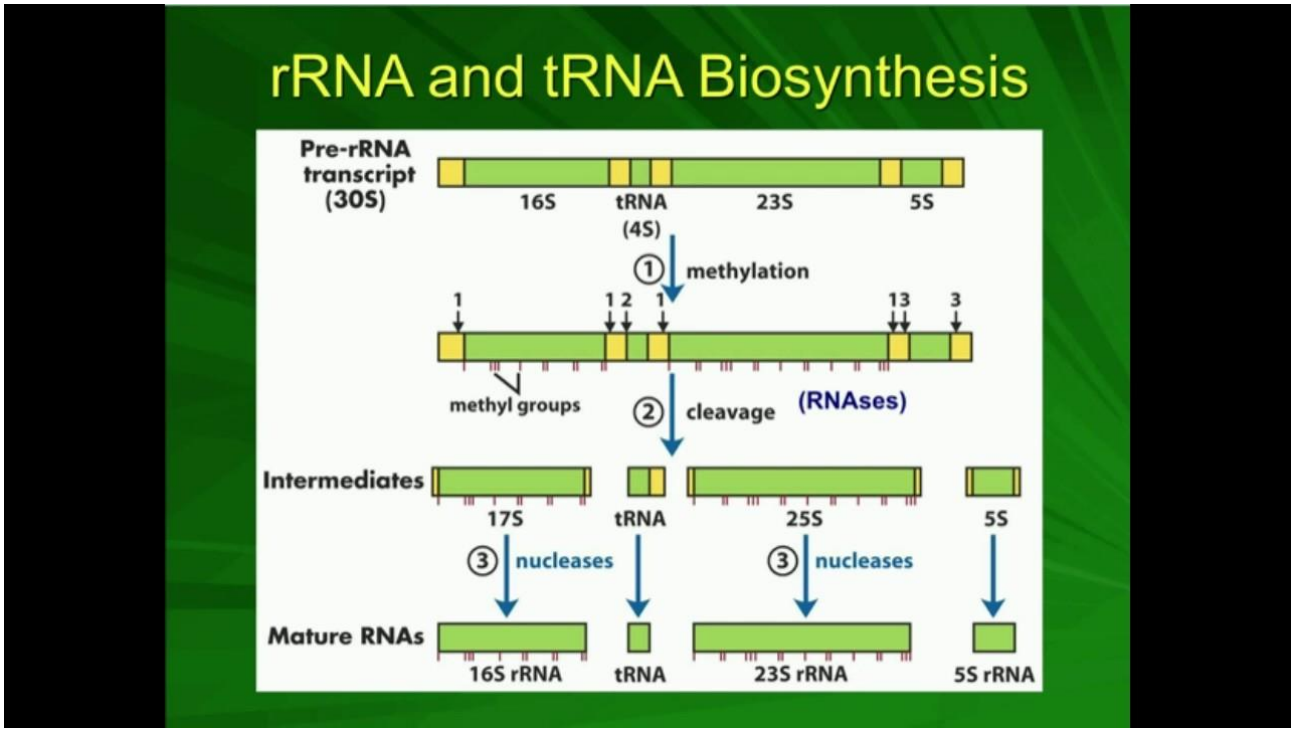



-35 -10 +1 (TSP)
promoter DNA
RNA-polymerase holoenzyme
closed initiation complex
open initiation complex
NTP
σ factor
core RNA-polymerase
mRNA
mRNA
termination
terminator
elongation complex
mRNA
translation


Exons
5'
G
A A A A
3'
Introns
Splicing
5'
G
A A A A
3'
Mature mRNA


DNA
Primary Transcript
exon
intron
5'
Donor site
Branch site
Acceptor site
3'
exon
GU
intron
A
AG
exon
Ribonucleoproteins (snRNPs)
exon
exon
Branch-point A attacks 5' splice site, forms lariat
cut
5' splice site joins 3' splice site, excising intron
exon
exon
cut
Spliceosome
RNA lariat
Polyadenilation signal
Poly(rA) tail
5'-cap
*G- P P P
Protein-coding sequence
AAUAAA
AA...AA
5'-UTR
3'-UTR
UTR = untranslated region
50 to 250 adenine nucleotides added to the 3'-end
5'–5' bridge
B


5'
Intron
Exon
Intron
Exon
Intron
Exon
Intron
3'
RNA
5'
G
P
P
P
CH3
AAAAA 150–250
3'
mRNA
Start Codon
Stop Codon
Capping
Splicing
Polyadenylation
Process


rRNA and tRNA Biosynthesis
Pre-rRNA transcript (30S)
16S
tRNA (4S)
23S
5S
1 methylation
1
1 2
1
13
3
methyl groups
2 cleavage
(RNAses)
Intermediates
17S
tRNA
25S
5S
3 nucleases
3 nucleases
Mature RNAs
16S rRNA
tRNA
23S rRNA
5S rRNA

General tRNA Structure

Shows the significant parts of tRNA.

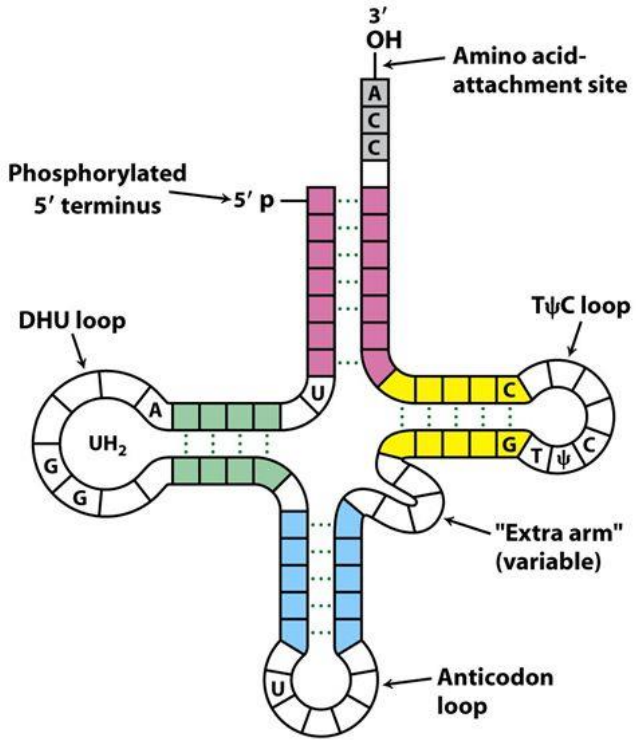
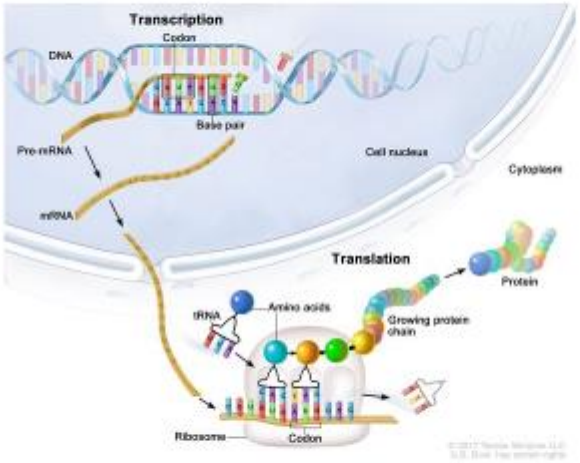
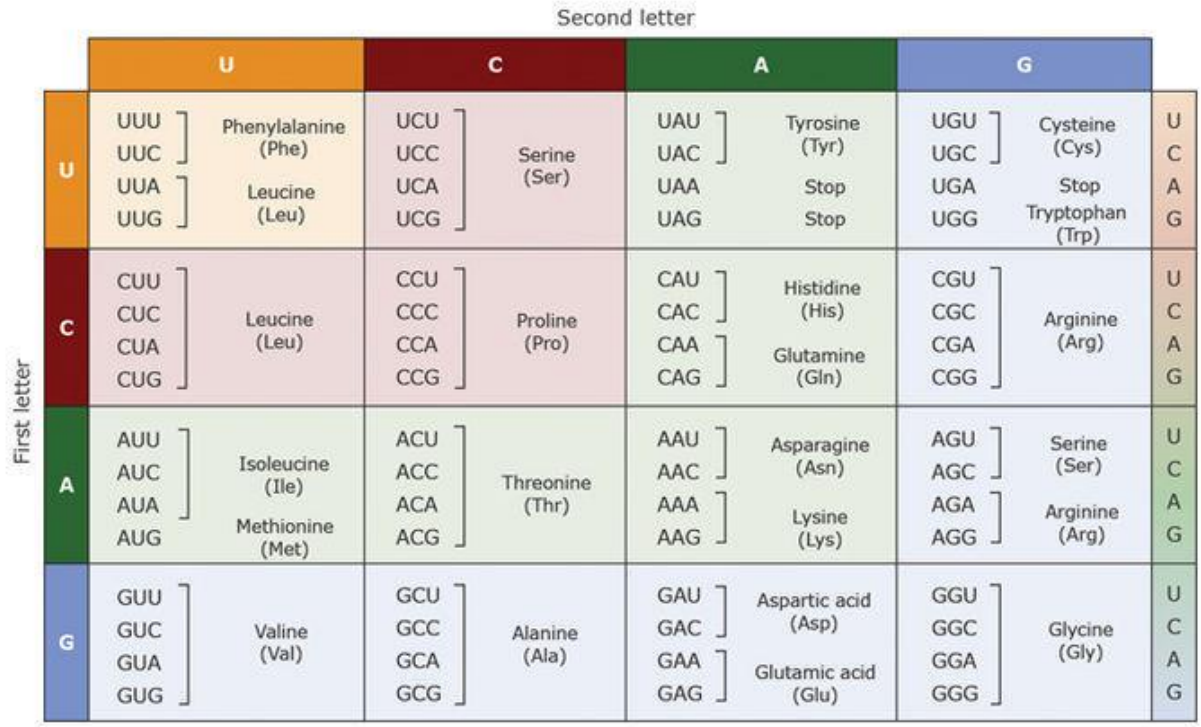


Figure 30-3
Biochemistry, Sixth Edition





The Genetic Code

Properties of the Genetic code

1- The code is written in a linear form using the nucleotides that comprise the mRNA

2- The code is a triplet: THREE nucleotides specify ONE amino acid

3- The code is degenerate: more than one triplet specifies a given amino acid

4- The code is unambiguous: each triplet specifies only ONE amino acid

5- The code contains stop signs- There are three different stops

6- The code is comma less

7- The code is non-overlapping

8- The code is universal: The same "dictionary" is used by viruses, prokaryotes, invertebrates and vertebrates.

55

- **6. Universal:**

The codons are the same for the same amino acid in all species; the same for "Elephant and E.coli".

The genetic code has been highly preserved during evolution.

- **7. Terminator codons:**

There are three codons which do not code for any particular amino acids. They are "**nonsense codons**", more correctly termed as **punctuator codons or terminator codons.** They put "**full stop**" to the protein synthesis. These three codons are **UAA, UAG, and UGA**.

- **8. Initiator codon:**
- In most of the cases, **AUG** acts as the initiator codon.

18

Properties of Genetic Code

2. The Code is Degenerate:

The occurrence of more than **one codon** for a **single amino acid** is referred to as degenerate. A review of genetic code dictionary will reveal that most of the amino acids have more than one codon. Out of 61 functional codons, AUG and UGG code to one amino acid each. But remaining 18 amino acids are coded by 59 codons.

- a given amino acid may be coded for by more than one codon

64 codons and only 20 amino acids:

so some amino acids are coded for by several codons – exceptions [next slide]:

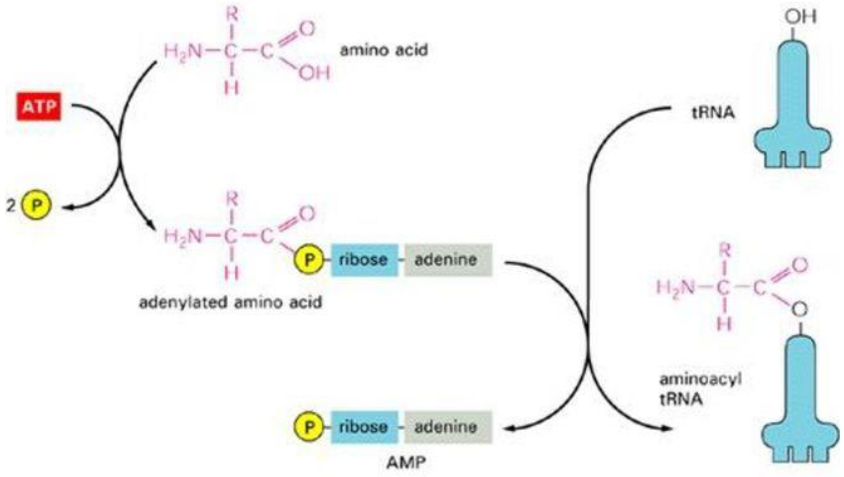
Valine
GUU
GUC
GUA
GUG

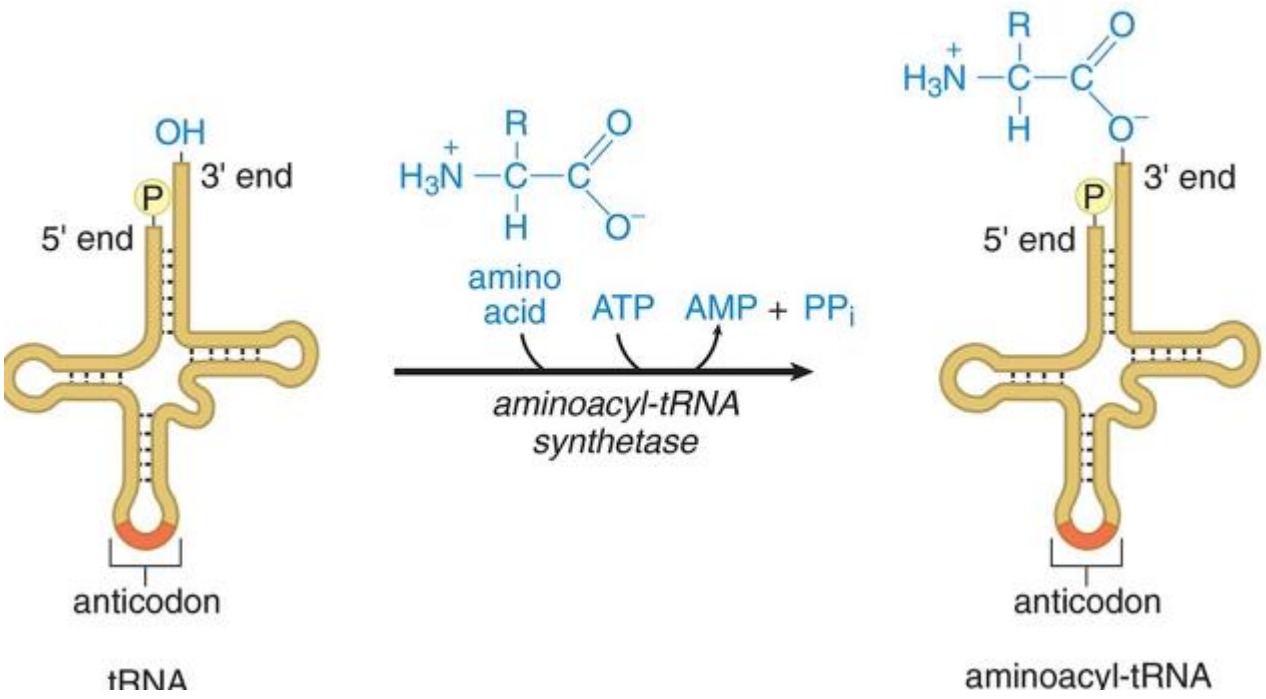
Lysine
AAA
AAG

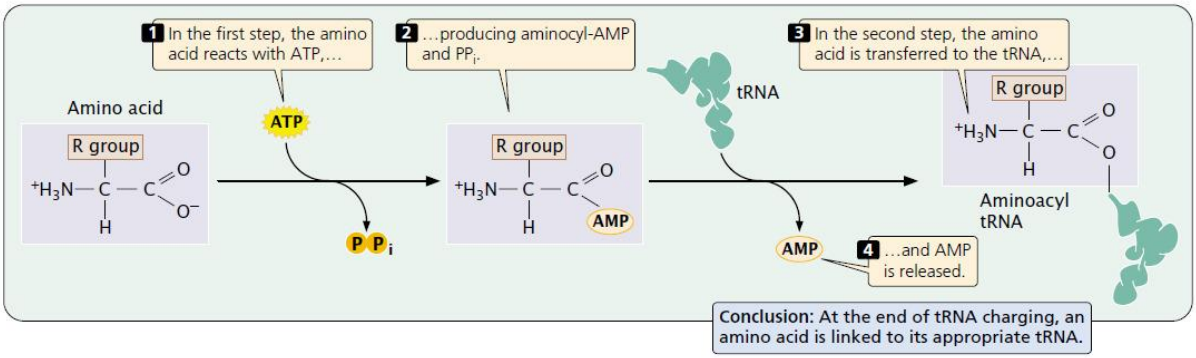
Tyrosine
UAU
UAC

Amino Acid Activation

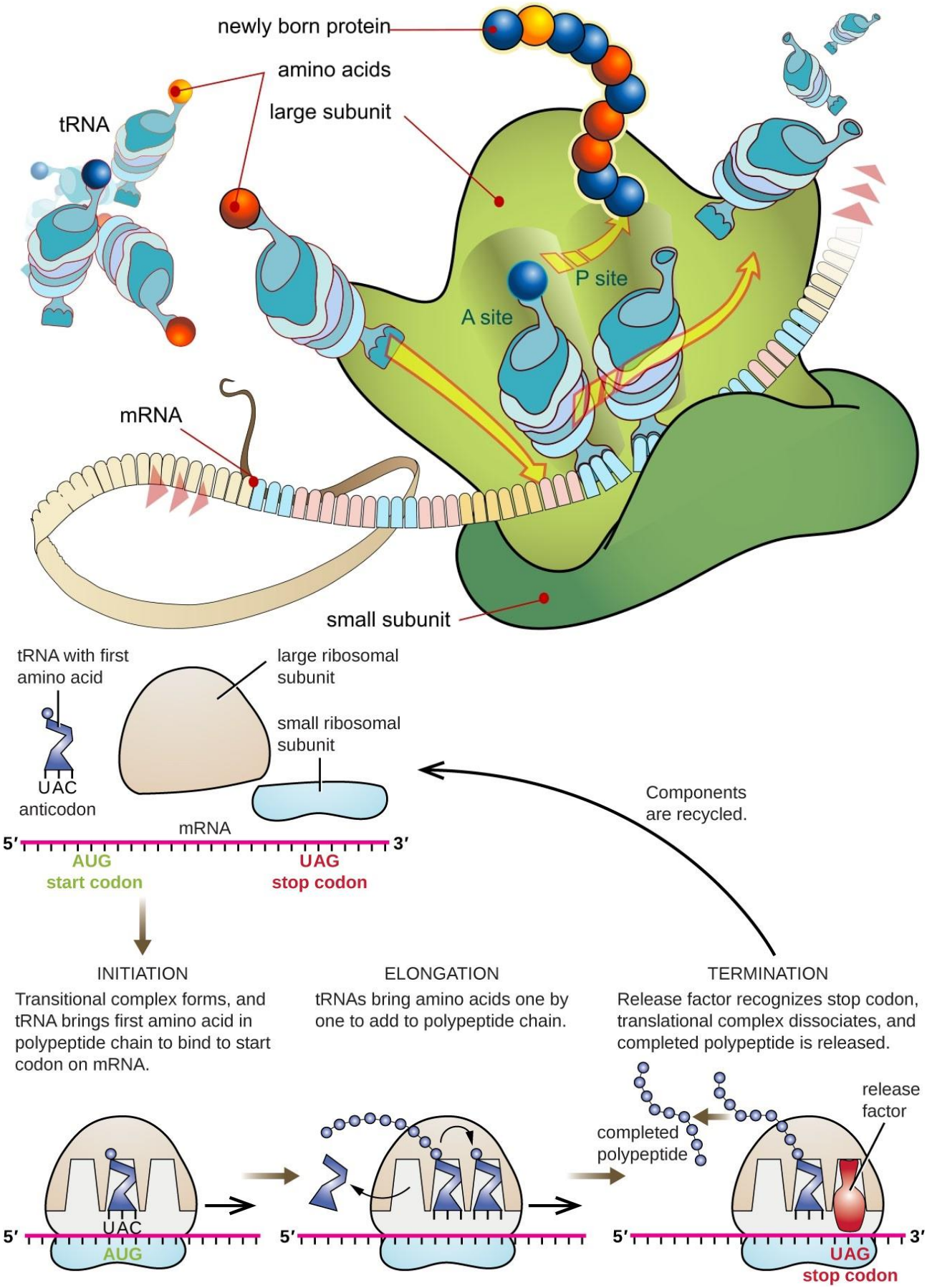
- You now know that tRNAs carry specific amino acids.
- However, you need to know that a tRNA cannot pick up an amino acid **unless the amino acid is activated**.
- The **amino acid attachment site** of a tRNA will bind to a **specific amino acid**, if energy is supplied.

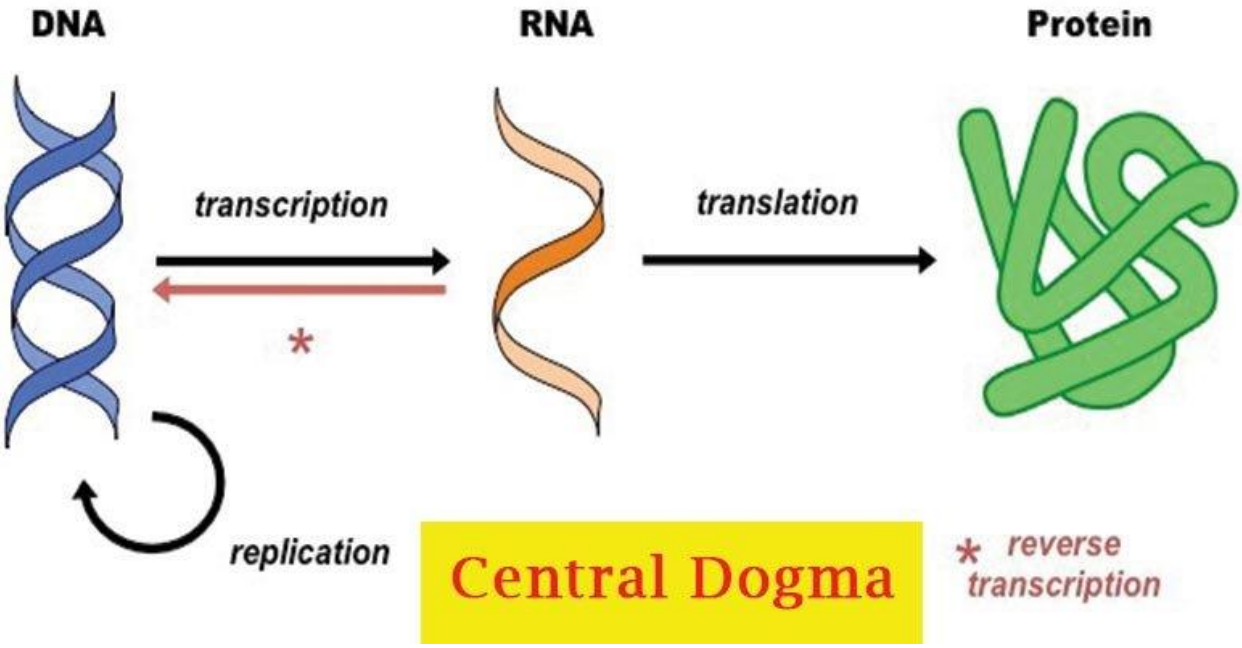






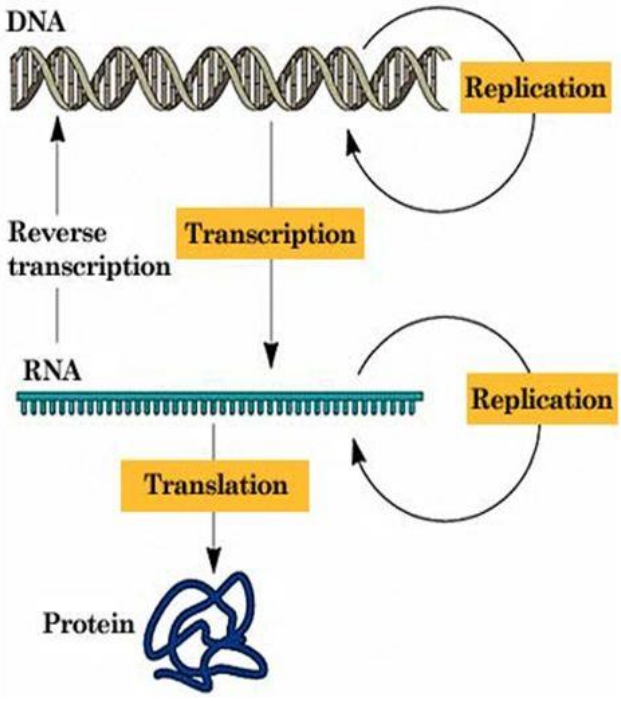
15.18 An amino acid becomes attached to the appropriate tRNA in a two-step reaction.


newly born protein
amino acids
large subunit
tRNA
P site
A site
mRNA
small subunit
tRNA with first amino acid
large ribosomal subunit
small ribosomal subunit
UAC
anticodon
mRNA
5′
3′
AUG
start codon
UAG
stop codon
Components are recycled.
INITIATION
Transitional complex forms, and tRNA brings first amino acid in polypeptide chain to bind to start codon on mRNA.
ELONGATION
tRNAs bring amino acids one by one to add to polypeptide chain.
TERMINATION
Release factor recognizes stop codon, translational complex dissociates, and completed polypeptide is released.
release factor
completed polypeptide
UAC
AUG
UAG
stop codon



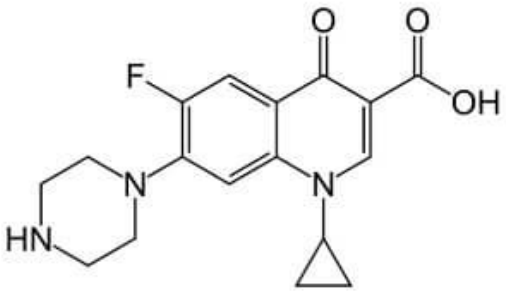
The Central Dogma

- The Central Dogma traces the flow of genetic information
- DNA Replication, Transcription, and Translation take place in human cells as part of their normal lifecycle
- Some viruses make use of RNA replication to reproduce
- Retroviruses, such as HIV, engage in reverse transcription, which is the process of inserting RNA into DNA

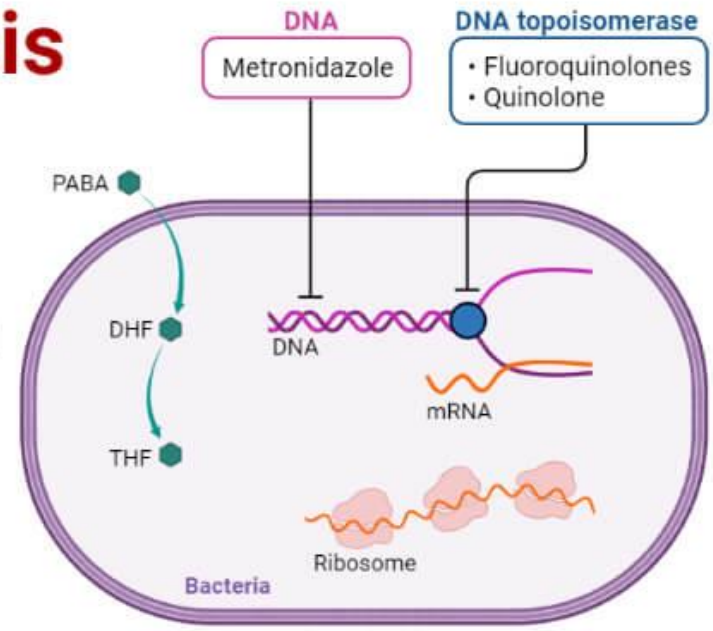


Source: http://cats.med.uvm.edu/cats_teachingmod/microbiology/courses/genomics/images_new/1_centraldogma_wisc_13.jpg

DNA Synthesis Inhibitors



Ciprofloxacin



Antibiotics That Inhibit Nucleic Acid Biosynthesis/ DNA Replication

Antibiotic	Site of action	Specific effect
Quinolones		Inhibition of DNA replication
Nalidixic acid	DNA gyrase (subunit A)	
Ciprofloxacin	DNA gyrase (subunit A)	
Norfloxacin	DNA gyrase (subunit A)	
Novobiocin	DNA gyrase (subunit B)	
Metronidazole	Production of free radicals (O_2^-)	Inhibition of DNA replication
Rifamycins		Inhibition of transcription
Rifampicin	RNA polymerase (subunit B)	
Sulfonamides	Enzymes in nucleic acid synthesis	Inhibition of tetrahydrofolic acid (folic acid - vitamin B9 or M) synthesis

1. Inhibitor of prokaryotic initiation - **Rifamycin**
2. Inhibitor of RNA chain elongation(prokaryotes)- **Cordycepin**
3. Inhibitor of chain elongation- **Streptoglydin** (Prokaryotes)
4. Inhibitor of transcription in vitro - **heparin** (prokaryotes)
5. Intercalating agents inhibit RNA polymerase (Pro Eu) – **Actinimycin D,Ethidium bromide andAcridine**
6. Inhibitors of RNA chain termination (Prokaryotes) – **Inosine triphosphate**

Inhibitors of Transcription

- **Actinomycin D**: It binds with DNA template strand and blocks the movement of RNA polymerase. It is widely used in the treatment of tumors
- **Rifamycin**: It binds to the beta subunit of RNA polymerase and inhibits its activity
- **Amantin**: It tightly binds to the RNA polymerase II in eukaryotes and stops the transcription process
- **Heparin**: It binds to the beta subunit & inhibits transcription in vitro.

59

4. CLASSIFICATION OF PROTEIN SYNTHESIS INHIBITORS

TETRACYCLINES 1

Demeclocycline DECLOMYCIN
Doxycycline VIBRAMYCIN
Minocycline MINOCIN
Tetracycline SUMYCIN

GLYCYLCYCLINES 2

Tigecycline TYGACIL

AMINOGLYCOSIDES 3

Amikacin AMIKIN, OTHERS
Gentamicin GARAMYCIN
Neomycin NEO-FRADIN
Streptomycin STREPTOMYCIN
Tobramycin TOBREX

MACROLIDES/KETOLIDES 4

Azithromycin ZITHROMAX
Clarithromycin BIAXIN
Erythromycin E-MYCIN
Telithromycin KETEK

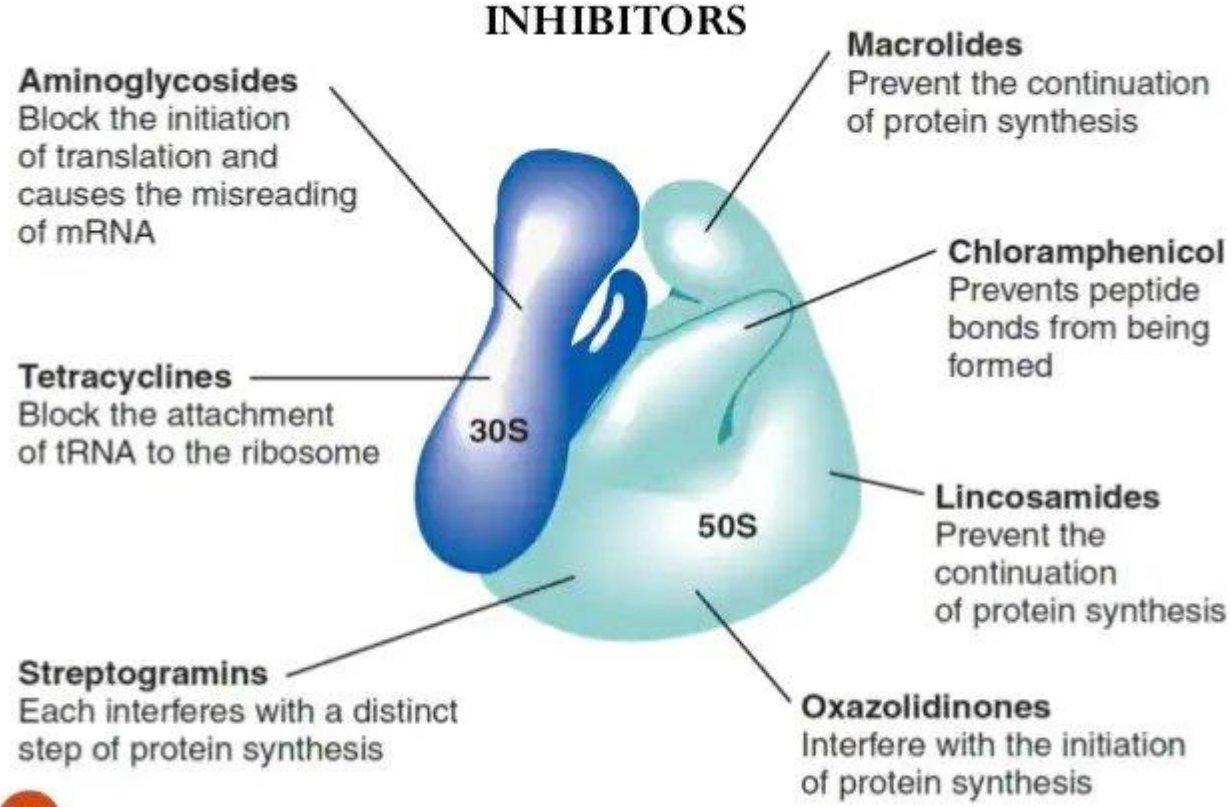
OTHERS 5

Chloramphenicol CHLOROMYCETIN
Clindamycin CLEOCIN
Linezolid ZYVOX
Quinupristin/Dalfopristin SYNERCID

7

Dr.K.Saminathan.M.Pharm, M.B.A, Ph.D

9.GENERAL MECHANISM OF PROTEIN SYNTHESIS INHIBITORS

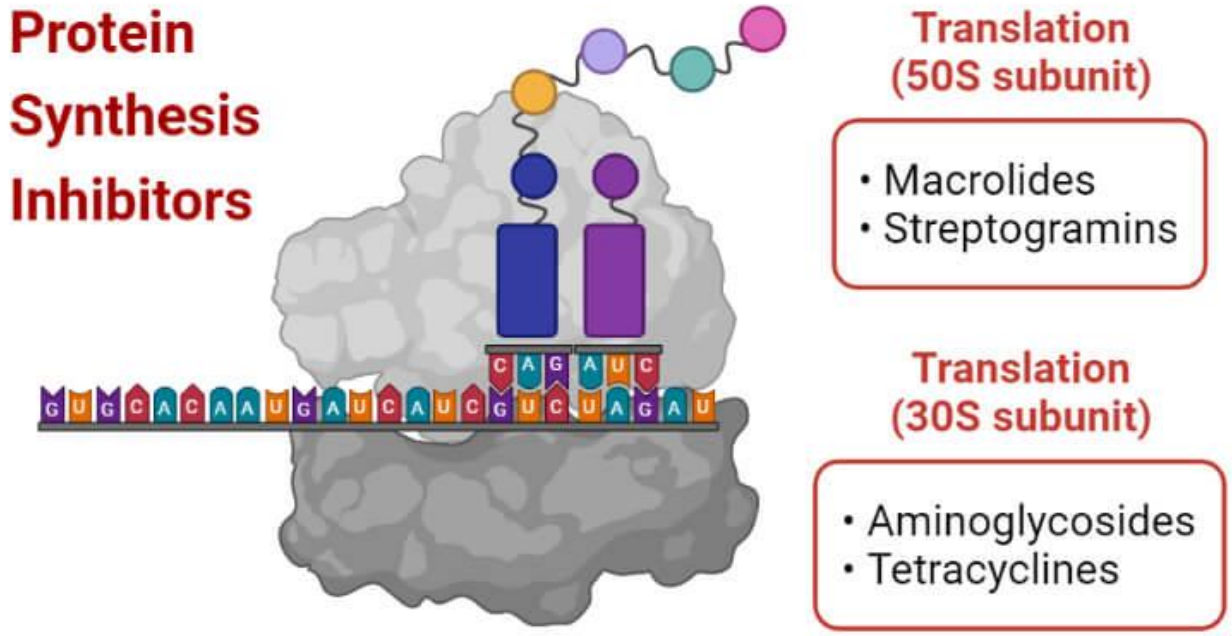


12

Dr.K.Saminathan.M.Pharm, M.B.A, Ph.D

Con...

Inhibitor	*Comments*
Chloramphenicol	Inhibits prokaryotic peptidyltransferase
Streptomycin	Inhibits prokaryotic peptide chain initiation, also induces mRNA misreading
Tetracycline	Inhibits prokaryotic aminoacyl-tRNA binding to the ribosome small subunit
Neomycin	Similar in activity to streptomycin
Erythromycin	Inhibits prokaryotic translocation through the ribosome large subunit
Fusidic acid	Similar to erythromycin only by preventing prokaryotic elongation factor G (EF-G, also called translocase) from dissociating from the large subunit
Puromycin	Resembles an aminoacyl-tRNA, interferes with peptide transfer resulting in premature termination in both prokaryotes and eukaryotes
Diphtheria (diphtheria) toxin	Protein from *Corynebacterium diphtheriae*, which causes diphtheria; catalyzes ADP-ribosylation and inactivation of eEF-2; eEF-2 contains a modified His residue known as **diphthamide** (it is this residue that is the target of the toxin)
Ricin	Found in castor beans, catalyzes cleavage of the eukaryotic large subunit rRNA
Cycloheximide	Inhibits eukaryotic peptidyltransferase



Inhibitors of gene expression

1. Inhibitors of prokaryotic transcription:

- Actinomycin D binds to DNA and blocks transcription
- Rifampicin binds to RNA polymerase and blocks initiation of RNA synthesis
- Streptolydigin inhibits elongation of transcription by binding to RNA polymerase

2. Inhibitors of eukaryotic transcription:

- α-Amanitin inhibits RNA polymerase II and RNA polymerase III (at high doses). RNA polymerase I is insensitive

General material and educational and methodological support of the lecture:
- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Nitrogenous bases, nucleosides and nucleotides are constituent components of nucleic acid molecules. Minor nitrogenous bases and nucleotides.
2. Free nucleotides (ATP, NAD, NADP, FAD, FMN, CTF, UTF; 3',5'-AMP, 3',5'-HMF) and their biochemical functions.
3. Biosynthesis of purine nucleotides: scheme of IMF synthesis reactions; formation of AMP and HMF; regulation mechanisms.
4. Biosynthesis of pyrimidine nucleotides: scheme of reactions; synthesis regulation.

5. Biosynthesis of deoxyribonucleotides. Formation of thymidyl nucleotides; dTMF biosynthesis inhibitors as antitumor agents.
6. Catabolism of purine nucleotides; hereditary disorders of uric acid metabolism.
7. Scheme of catabolism of pyrimidine nucleotides.
8. Pathology of nucleotide exchange.
9. Nucleic acids. General characteristics of DNA and RNA, their biological significance in the preservation and transmission of genetic information.
10. Features of the primary structure of DNA and RNA. Bonds forming the primary structure of nucleic acids.
11. Secondary structure of DNA, the role of hydrogen bonds in its formation (Chargaf rules, Watson-Crick model), antiparallelism of chains.
12. Tertiary structure of DNA. Physicochemical properties of DNA: interaction of DNA with cationic ligands, formation of nucleosomes.
13. Molecular organization of nuclear chromatin of eukaryotes: nucleosome organization; histones and non-histone proteins.
14. Structure, properties and biological functions of RNA. Types of RNA: mRNA, tRNA, rRNA. Features of the structural organization of different types of RNA.
15. Nucleoproteins: structure, biological functions.
16. DNA replication: biological significance; semi-conservative mechanism of replication.
17. Sequence of stages and enzymes of DNA replication in prokaryotes and eukaryotes.
18. RNA transcription: RNA polymerases of prokaryotes and eukaryotes, transcription signals (promoter, initiator and terminator regions of the genome).
19. Processing - post-transcriptional modification of newly synthesized mRNAs.
20. Genetic (biological) code; triplet code structure, its properties.
21. Transport - tRNA and activation of amino acids. Aminoacyl-tRNA-syn¬¬-tetases.
22. Stages and mechanisms of translation (protein biosynthesis) in ribosomes: initiation, elongation and termination.
23. Post-translational modification of peptide chains. Broadcast regulation.
24. Inhibitors of transcription and translation in prokaryotes and eukaryotes: antibiotics and interferons - their use in medicine; diphtheria toxin.
25. Regulation of prokaryotic gene expression: regulatory and structural regions of the lactose (Lac-) operon (regulatory gene, promoter, operator).
26. Mutations: genomic, chromosomal, gene; mechanisms of action of mutagens; the role of induced mutations in the occurrence of human enzymopathies and hereditary diseases.
27. Biological significance and mechanisms of DNA repair. Repair of UV-induced gene mutations: xeroderma pigmentosum.
28. Genetic engineering: construction of recombinant DNA; gene cloning; genetic engineering synthesis of enzymes, hormones, interferons, etc.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.

3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
Електронні інформаційні ресурси:
1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture №11

Topic: Biochemical and molecular biological mechanisms of hormone action; hierarchy of hormones. Hormones of protein-peptide nature.
Relevance of the topic: Hormonal regulation is one of the levels that ensure homeostasis. Under the influence of various internal and external stimuli, impulses arise that reach the central nervous system, from there to the hypothalamus, where releasing factors are synthesized, which reach specific cells of the pituitary gland, where tropic hormones are secreted. Tropic hormones reach the endocrine glands and contribute to the production of a certain hormone in the gland. These hormones affect specific organs and tissues /target organs/, causing appropriate chemical and physiological reactions of the body in response.

Purpose: formation of concepts about the hormonal regulation of the human body, its types and the role of establishing a connection between the body and the environment.

Basic concepts:
1. Hormones.
2. Membrane mechanism of action.
3. Cytoplasmic mechanism of action.
4. Diabetes insipidus
5. Gigantism.
6. Pituitary dwarfism.
7. Hashimoto's disease.
8. Endemic goiter.
9. Flayani's disease.

Plan and organizational structure of the lecture:
1. General laws of hormonal regulation.
2. Classification of hormones.

3. Mechanisms of hormone action on target cells.
4. Hormones of the hypothalamus.
5. Tropic hormones of the pituitary gland.
6. Thyroid hormones. Pathology.
7. Hormones of the parathyroid gland. Regulation of phosphorus-calcium metabolism.

Content of the lecture material

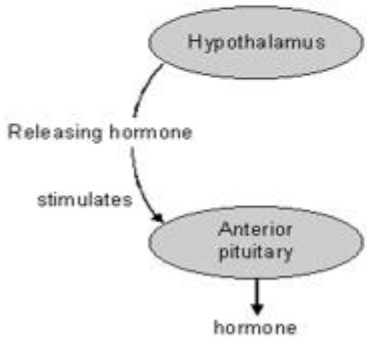
Hormones are biologically active organic compounds that enter the bloodstream and have a regulatory effect on metabolism and physiological functions.

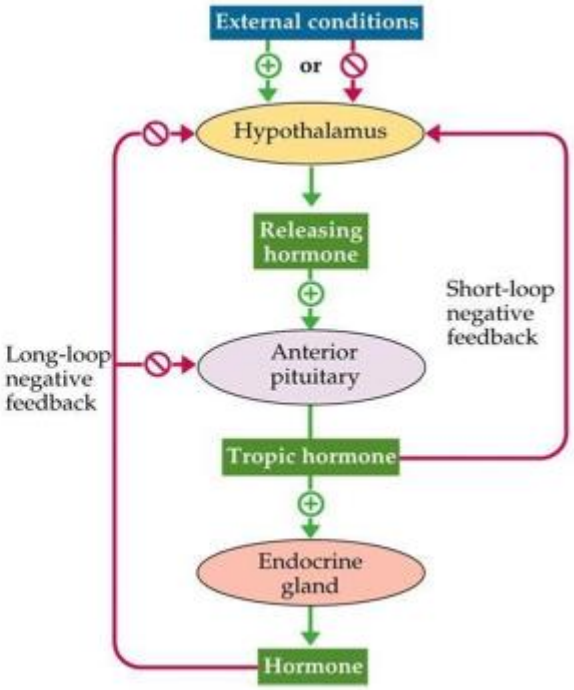
- Hormones realize their action by changing the rate of enzymatic catalysis or by increasing the rate of the synthesis of enzyme de novo .

- The secretion of hormones is stimulated by external and internal signals entering the central nervous system.

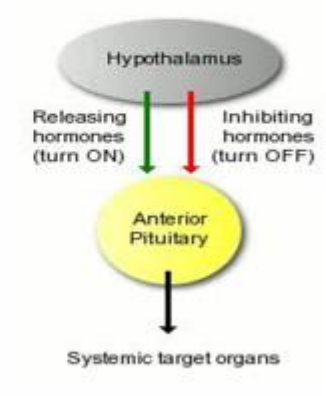
- Signals enter the hypothalamus, where they stimulate the synthesis of releasing hormones: liberins (7), statins (3).

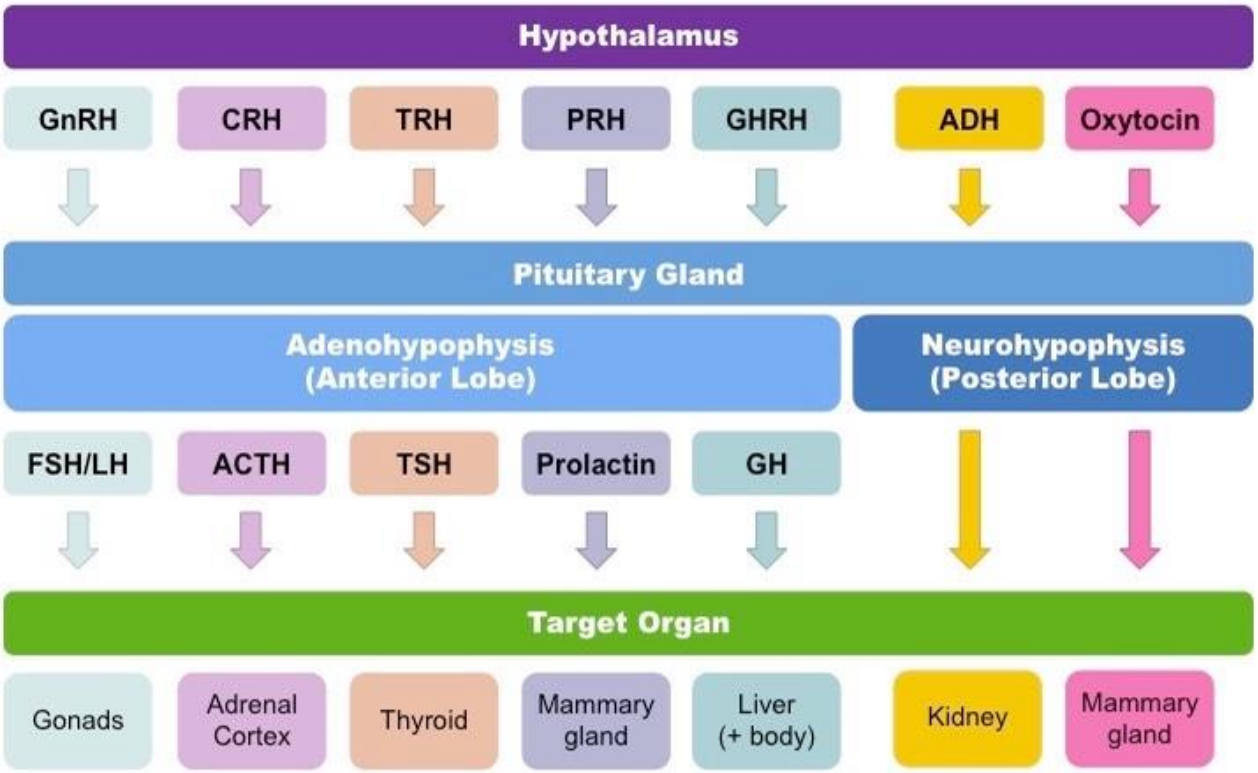
- Releasing hormones stimulate the synthesis of pituitary tropic hormones, which stimulate the synthesis and secretion of hormones of the endocrine glands.
- **Tropic hormones** are hormones that have other endocrine glands as their target.





- A change in the concentration of metabolites in target cells suppresses the synthesis of hormones by acting on the endocrine glands or on the hypothalamus. The synthesis of tropic hormones is suppressed by hormones of the peripheral glands.





- Anterior pituitary gland (adenohypophysis)
  - Names of major hormones
    - Thyroid-stimulating hormone (TSH)
    - Adrenocorticotropic hormone (ACTH)
    - Follicle-stimulating hormone (FSH)
    - Luteinizing hormone (LH)
    - Growth hormone (GH)
    - Prolactin (lactogenic hormone)

13

So what do the pituitary hormones do?

The four tropic ones regulate the function of other hormones:

- TSH stimulates the thyroid to produce thyroid hormone
- ACTH stimulates the adrenal cortex to produce corticosteroids: aldosterone and cortisol
- FSH stimulates follicle growth and ovarian estrogen production; stimulates sperm production and androgen-binding protein
- LH has a role in ovulation and the growth of the corpus luteum; stimulates androgen secretion by interstitial cells in testes

10

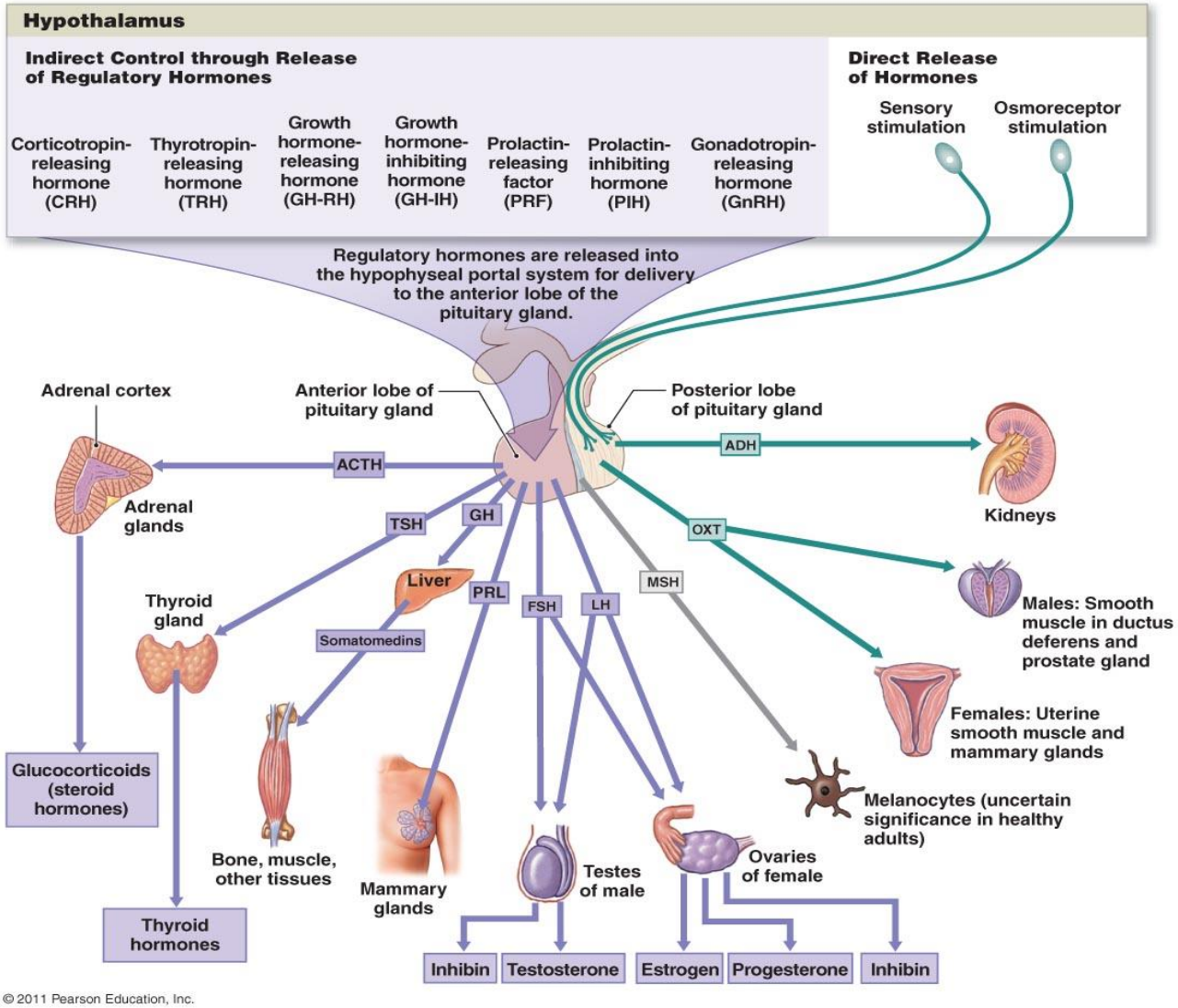
The others from the anterior pituitary…

- GH (aka somatrotropic hormone) stimulates growth of skeletal epiphyseal plates and body to synthesize protein
- PRL stimulates mammary glands in breast to make milk
- MSH stimulates melanocytes; may increase mental alertness

From the posterior pituitary (neurohypophysis)
structurally part of the brain

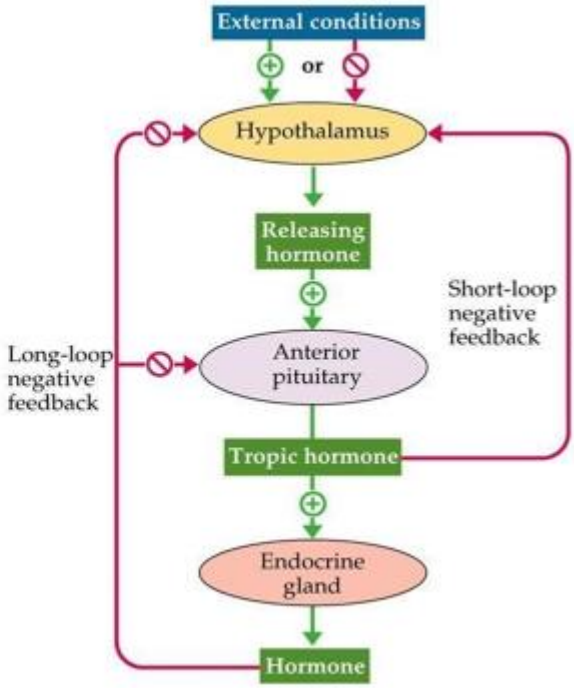
- ADH (antidiuretic hormone AKA vasopressin) stimulates the kidneys to reclaim more water from the urine, raises blood pressure
- Oxytocin prompts contraction of smooth muscle in reproductive tracts, in females initiating labor and ejection of milk from breasts

An overview of the relationships between hypothalamic and pituitary hormones, and some effects of pituitary hormones on target tissues



Regulation of Hormone Secretion

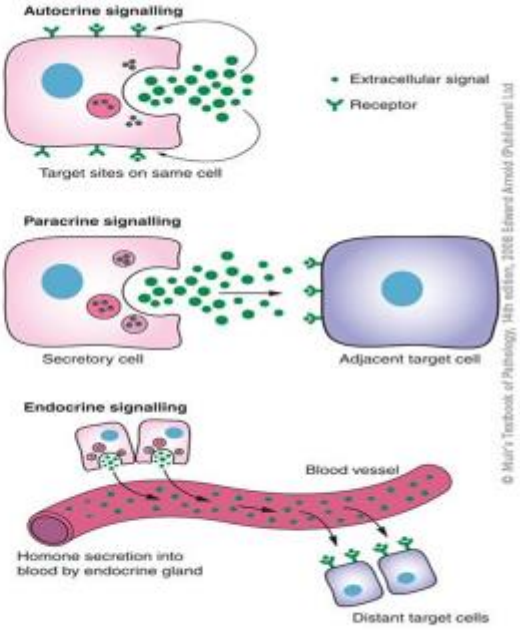
- Hormone secretion is controlled by homeostatic feedback
- Negative feedback—mechanisms that reverse the direction of a change in a physiological system
- Positive feedback—(uncommon) mechanisms that amplify physiological changes



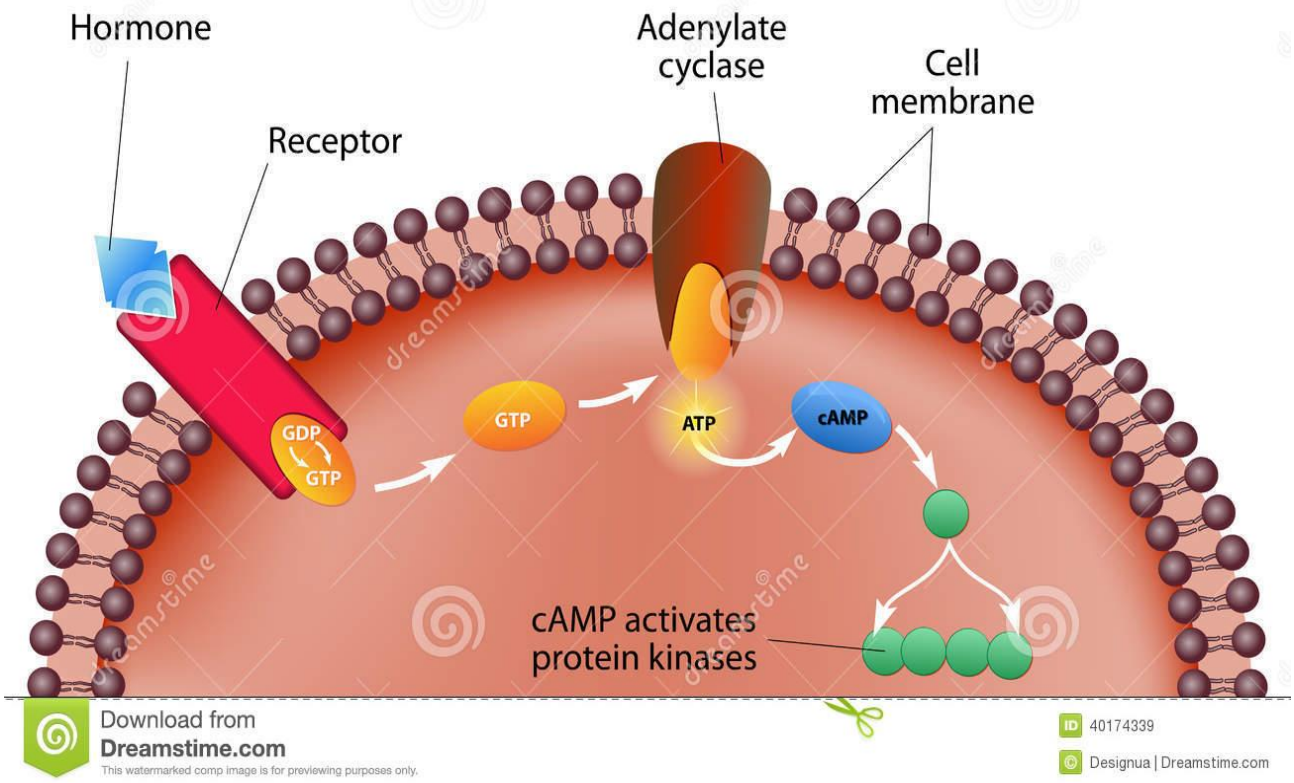
Hormones of the endocrine glands act on cells located far from the allocation of these hormones.

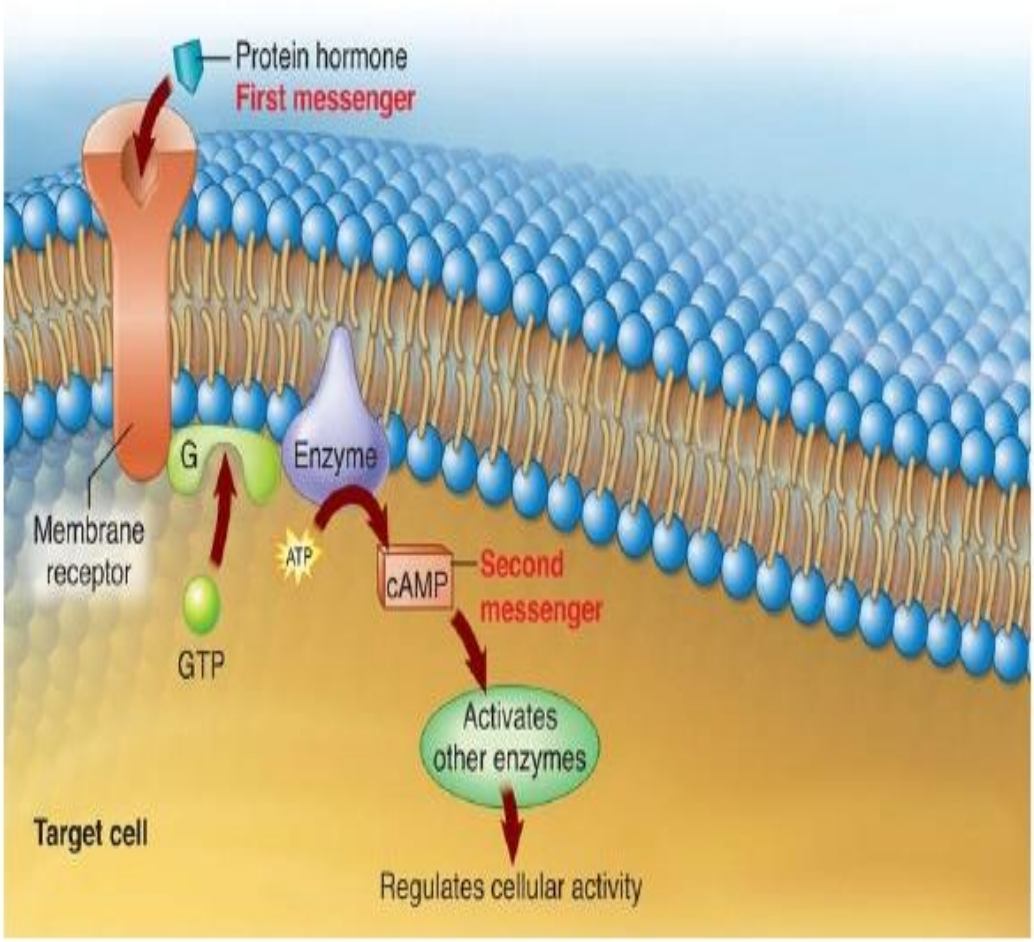
Some hormones can be synthesized by different organs and tissues.

- autocrine hormones affect those cells that produce them.
- paracrine hormones affect only the cells located in the vicinity. Autocrine and paracrine hormones belong to the local hormones.

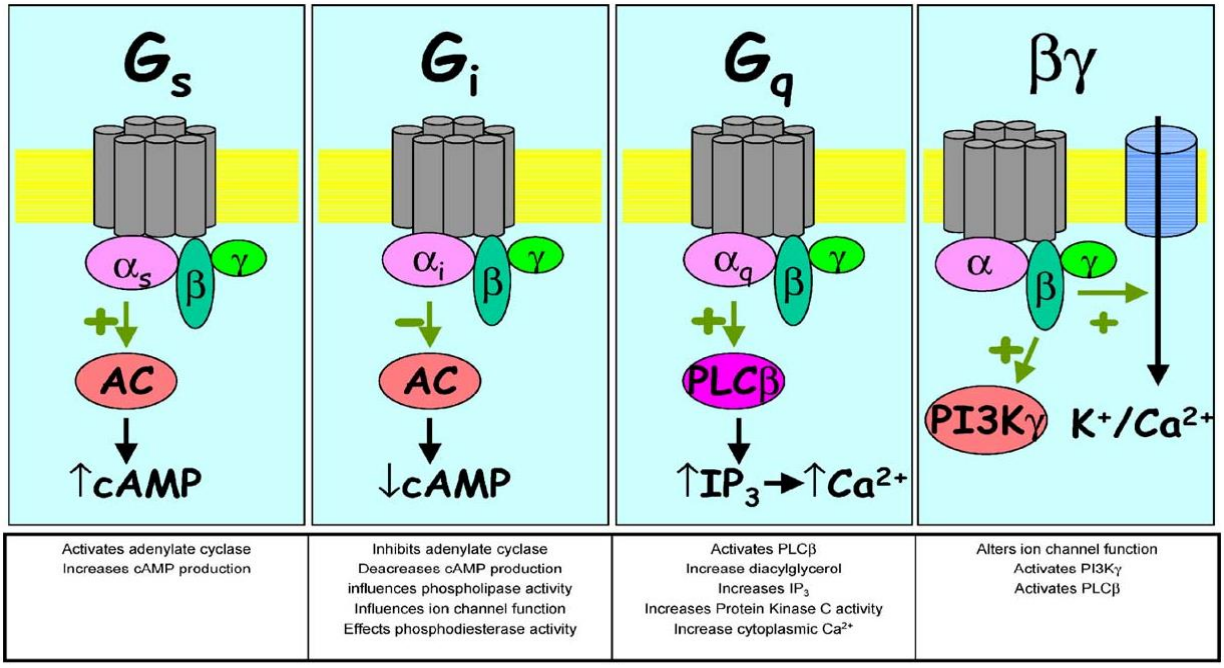


MECHANISMS OF HORMONE ACTION





7



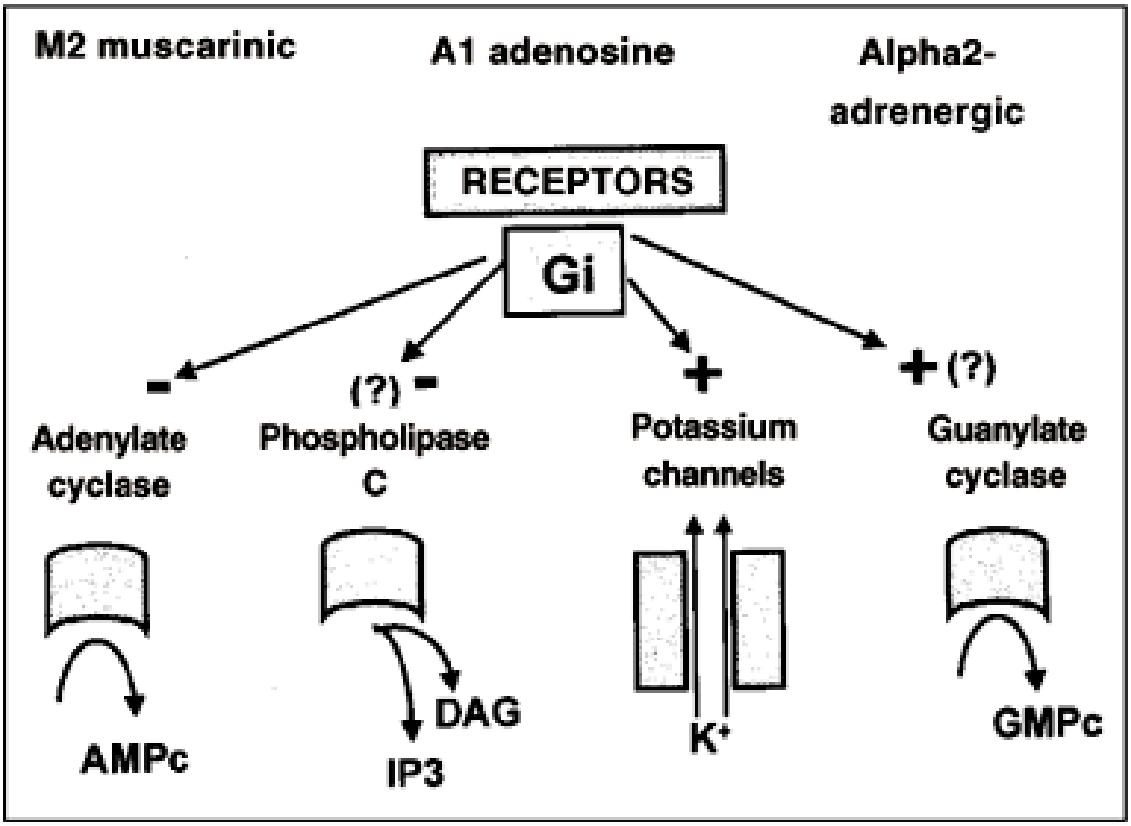
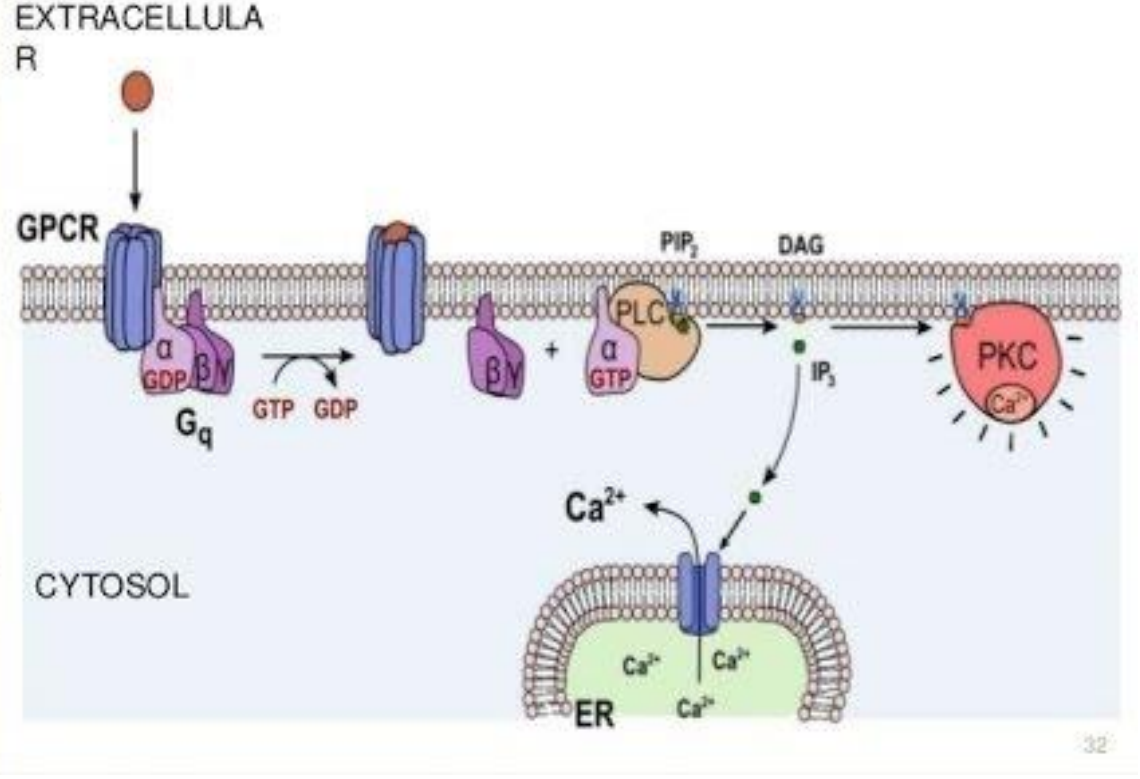
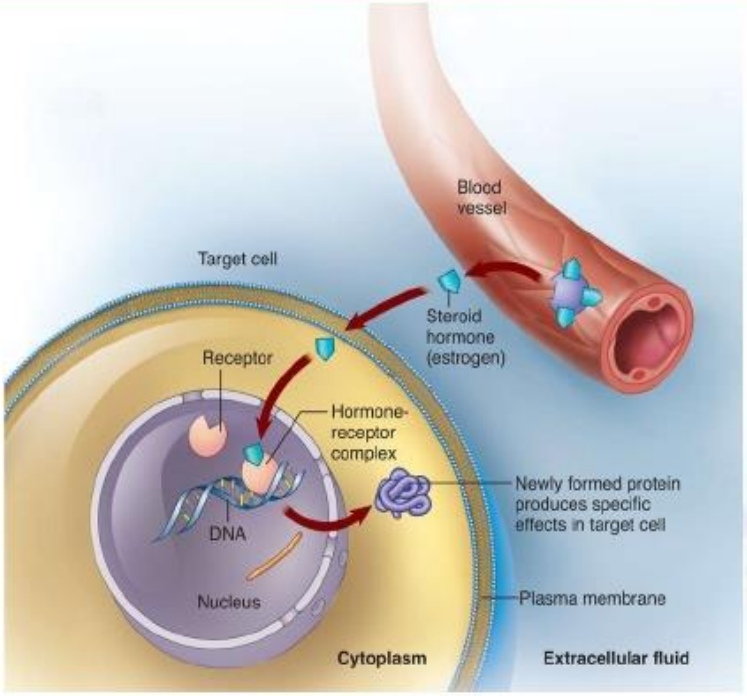


Fig. 3 - Inhibiting G-proteins and their relation with varied types of receptors and cellular responses. Gi - inhibiting G-protein; DAG - diacylglycerol; IP3 - inositol triphosphate.

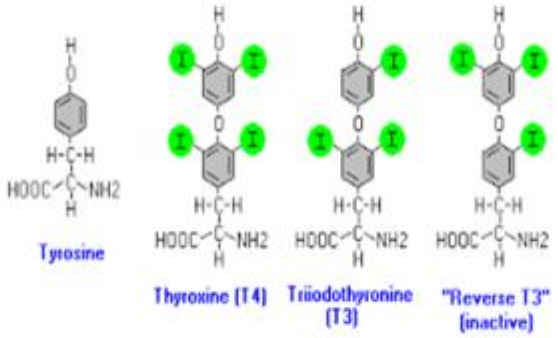
Gq Protein Coupled Receptor

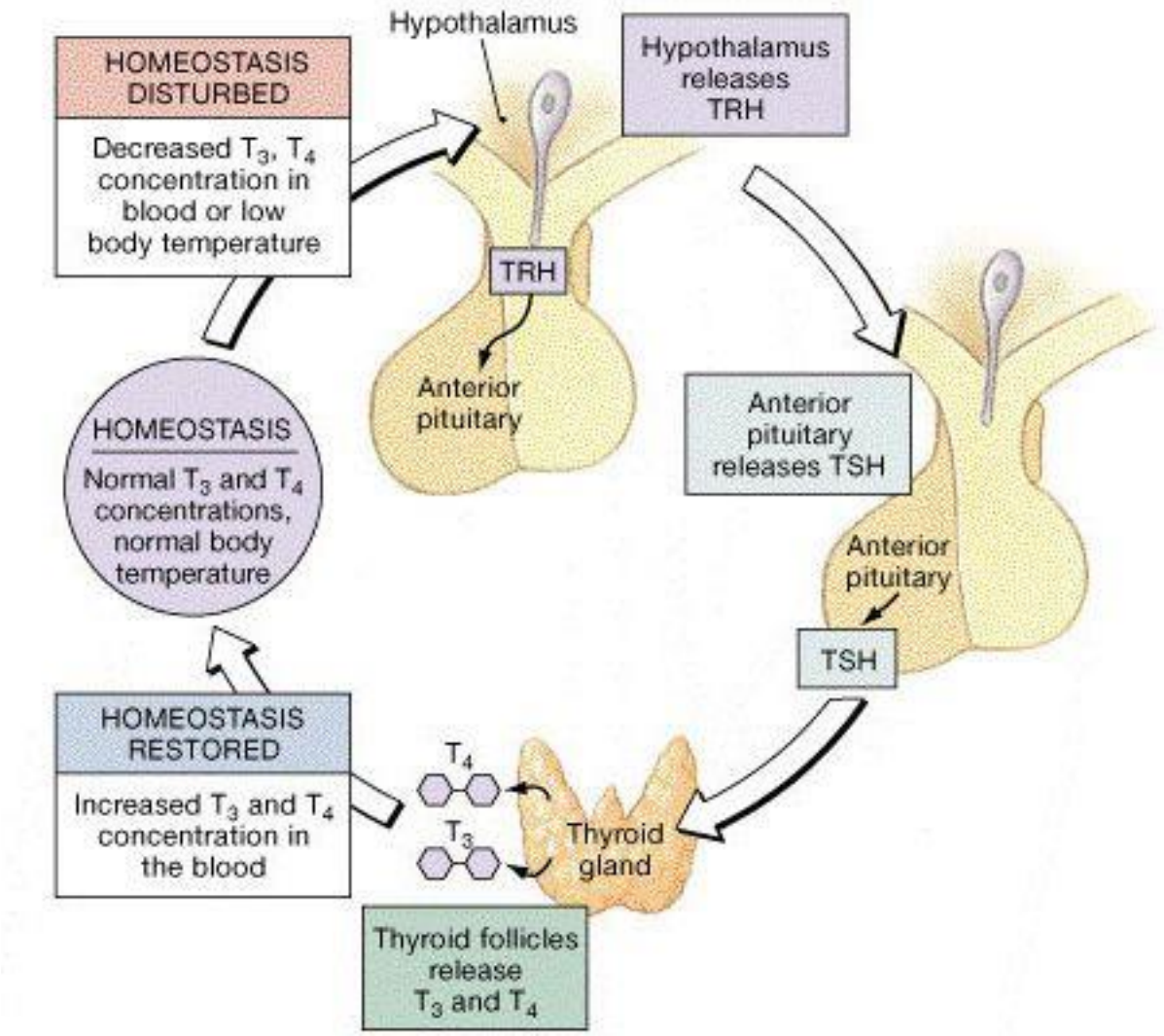


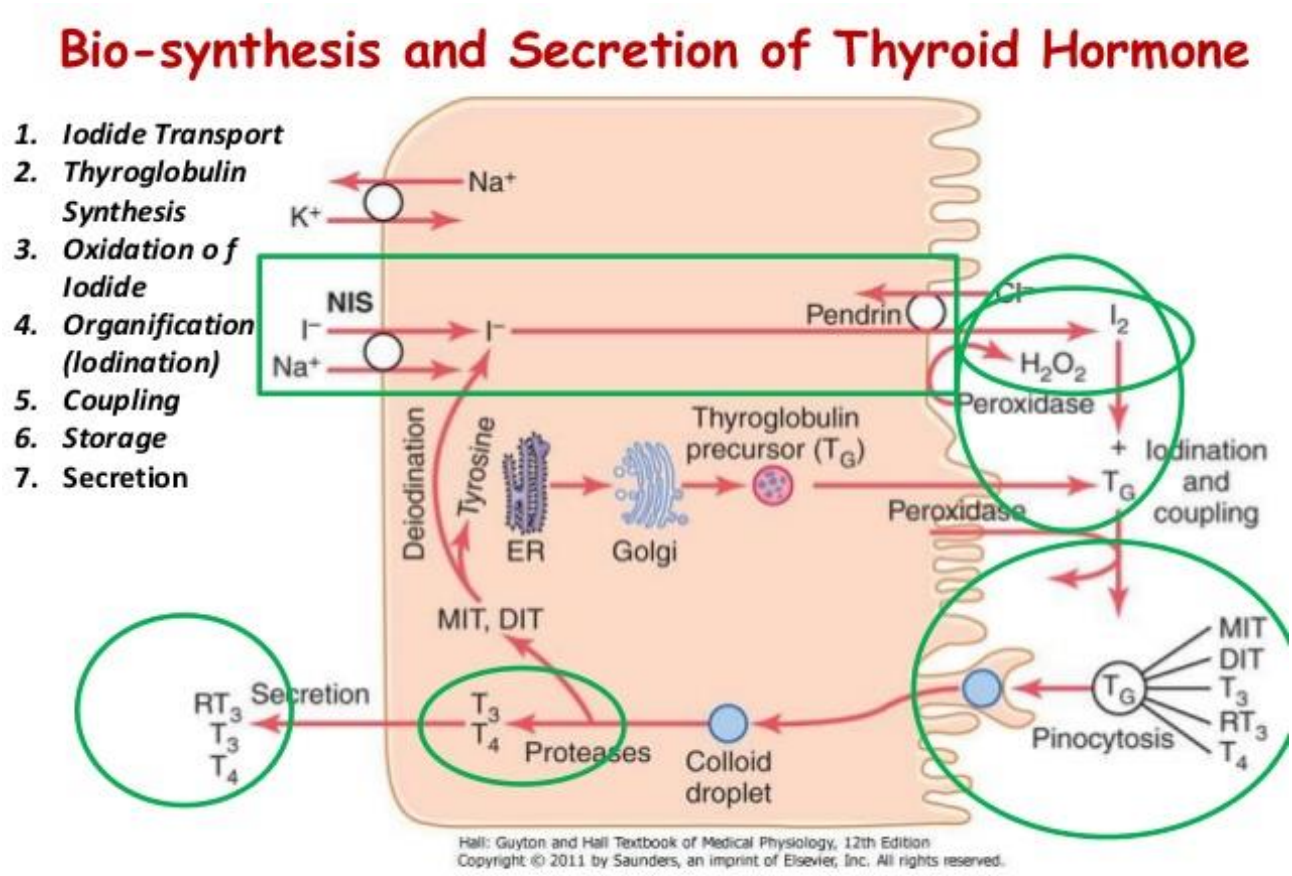


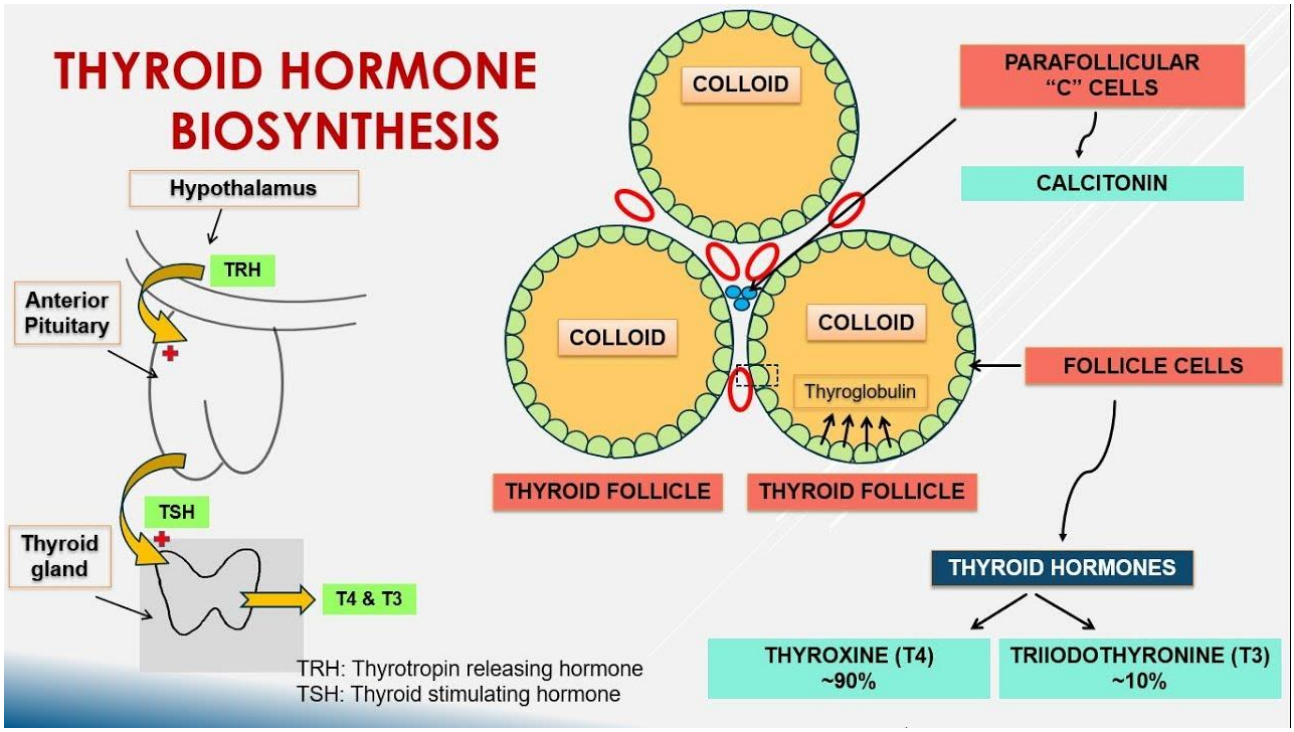
8

Hormones of thyroid gland

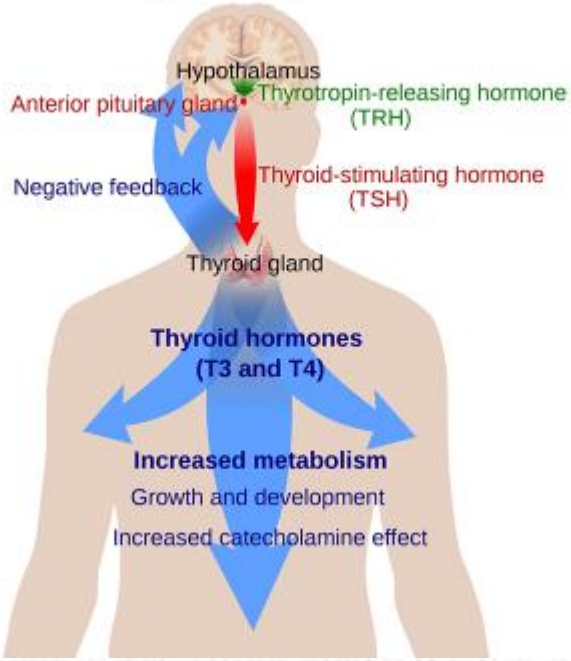



HOMEOSTASIS DISTURBED
Decreased T_3, T_4 concentration in blood or low body temperature
Hypothalamus
Hypothalamus releases TRH
TRH
Anterior pituitary
Anterior pituitary releases TSH
Anterior pituitary
TSH
HOMEOSTASIS
Normal T_3 and T_4 concentrations, normal body temperature
HOMEOSTASIS RESTORED
Increased T_3 and T_4 concentration in the blood
T_4
T_3
Thyroid gland
Thyroid follicles release T_3 and T_4

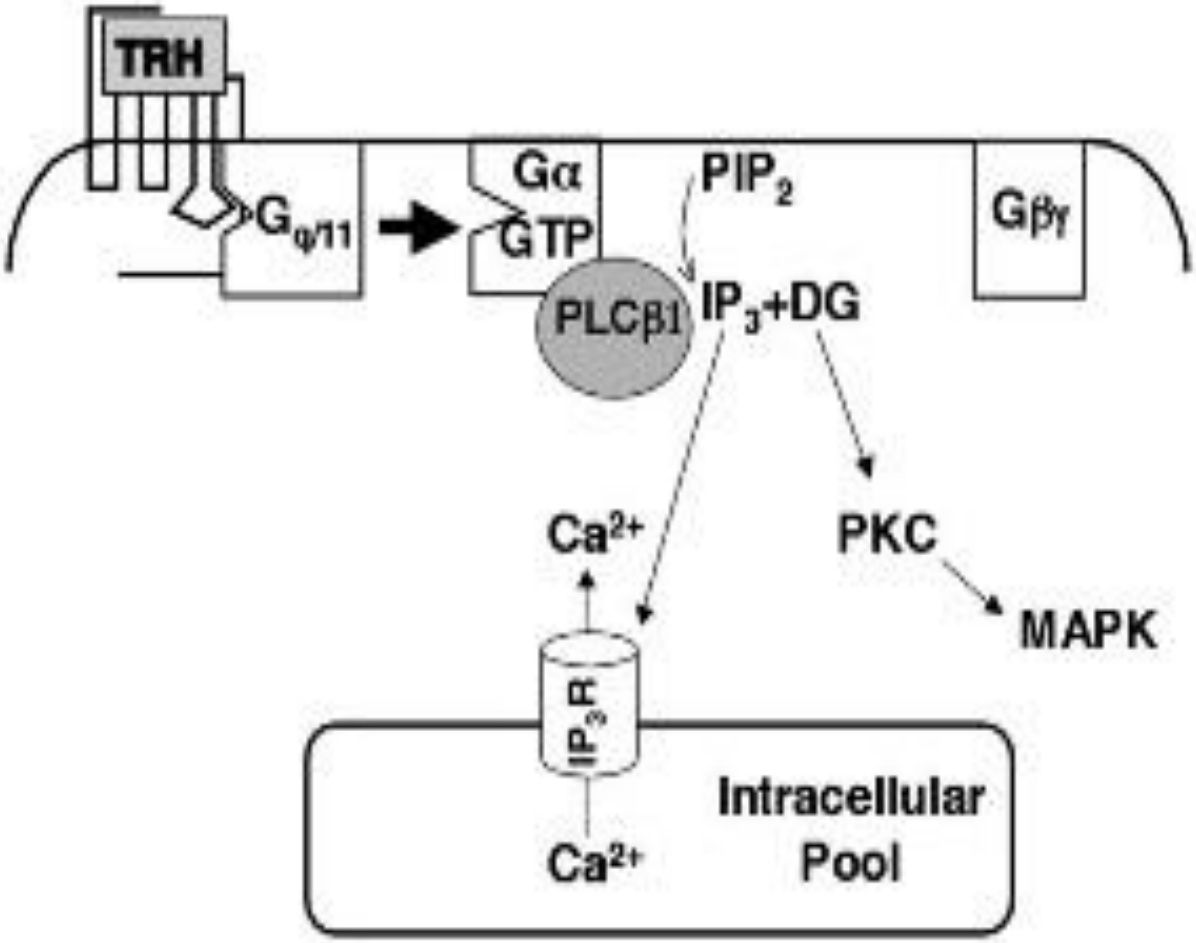

Bio-synthesis and Secretion of Thyroid Hormone
1. Iodide Transport
2. Thyroglobulin Synthesis
3. Oxidation o f Iodide
4. Organification (Iodination)
5. Coupling
6. Storage
7. Secretion
Na+
K+
NIS
I−
Na+
Pendrin
Cl−
I2
H2O2
Peroxidase
Deiodination
Tyrosine
ER
Golgi
Thyroglobulin precursor (TG)
+ Iodination and coupling
TG
Peroxidase
MIT, DIT
MIT
DIT
T3
RT3
T4
Pinocytosis
RT3
T3
T4
Secretion
T3
T4
Proteases
Colloid droplet
Hall: Guyton and Hall Textbook of Medical Physiology, 12th Edition
Copyright © 2011 by Saunders, an imprint of Elsevier, Inc. All rights reserved.


THYROID HORMONE BIOSYNTHESIS
Hypothalamus
TRH
Anterior Pituitary
TSH
Thyroid gland
T4 & T3
TRH: Thyrotropin releasing hormone
TSH: Thyroid stimulating hormone
COLLOID
COLLOID
COLLOID
Thyroglobulin
THYROID FOLLICLE
THYROID FOLLICLE
PARAFOLLICULAR "C" CELLS
CALCITONIN
FOLLICLE CELLS
THYROID HORMONES
THYROXINE (T4) ~90%
TRIIODOTHYRONINE (T3) ~10%

Thyroid system



G Protein-Dependent TRH Signaling



Mechanism of action of iodothyronines

Secretion of iodothyronines realizes by the help of hydrolysis of thyroglobuline and is under the control of thyrotropine.

Biological effect of T_3 is in 3 - 5 times higher than effect of T_4.

Thyroid hormones effect on the differentiation of the cells and on the energetical metabolism:

a) Through the nuclear receptors they effect to the chromatin and induce the synthesis of more than 100 oxidative-reductive enzymes, increase the number of mitochondria;
b) Through the activation of adenylate cyclase they activate lipolysis in the fat tissue and glycogenolysis in the liver and muscles.

Iodothyronines uncouple respiration and phosphorylation, increase free oxidation and production of heat.

PHYSIOLOGICAL EFFECTS
OF THYROID GLAND HORMONES

- Increase the size and number of mitochondria, activation of oxidative enzymes
- Increase an activity of Na + - K + - pumps and excitability
- Activation of energy metabolism in tissues and basal metabolism
- Increase thermogenesis in tissues and body temperature
- Increase gene expression, mRNA synthesis, and protein synthesis
- Provide the synthesis of beta-adrenergic receptors, suppressing MAO activity, increasing the effects of sympathetic regulation
- Promote bone growth and brain maturation

METABOLIC EFFECTS
OF THYROID HORMONES

Carbohydrate metabolism:

- activation of glucose absorption in the intestine
- activation of gluconeogenesis in the liver
- activation of glucose utilization by activating of key glycolysis enzymes in muscle and adipose tissue

Protein metabolism:

- activation of protein synthesis in the myocardium and skeletal muscles, positive nitrogen balance
- suppression of the synthesis of glycosaminoglycans

Lipid metabolism:

- activation of lipolysis in adipose tissue

METABOLIC EFFECTS OF EXCESS OF THYROID HORMONES

- ACTIVATION OF PROTEOLYSIS (protein breakdown)
- HYPERGLYCEMIA
- LIPOLYSIS ACTIVATION
- HYPERLIPACIDEMIA (violation of the ratio of blood plasma lipoproteins, an increase of free fatty acids in plasma)

Symptoms of thyrotoxicosis – rising of body temperature, losing of weight, intoxication, damage of heart and nervous system.

With increased secretion of thyroxin by an enlarged thyroid gland, hyperthyroidism develops. The extreme degree of hyperthyroidism is thyrotoxicosis or Graves' disease.

It is characterized by an increase of the basal metabolic rate, the rate of synthesis and catabolism of proteins, fats, carbohydrates, thermoregulation disorders - increased heat production, water-salt metabolism, and intracellular ATP deficiency.

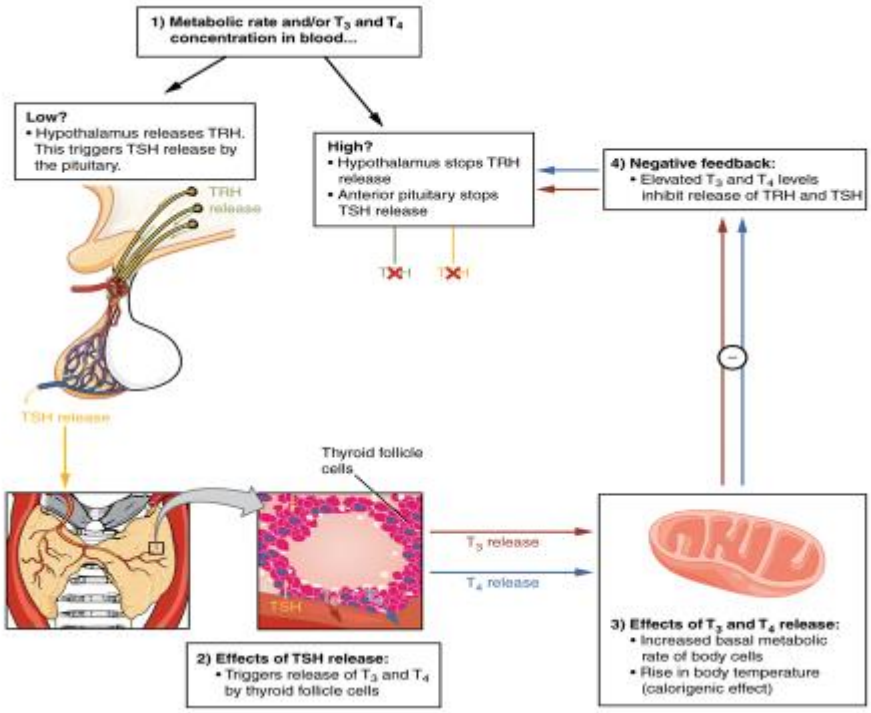
An insufficiency of thyroid gland leads to:

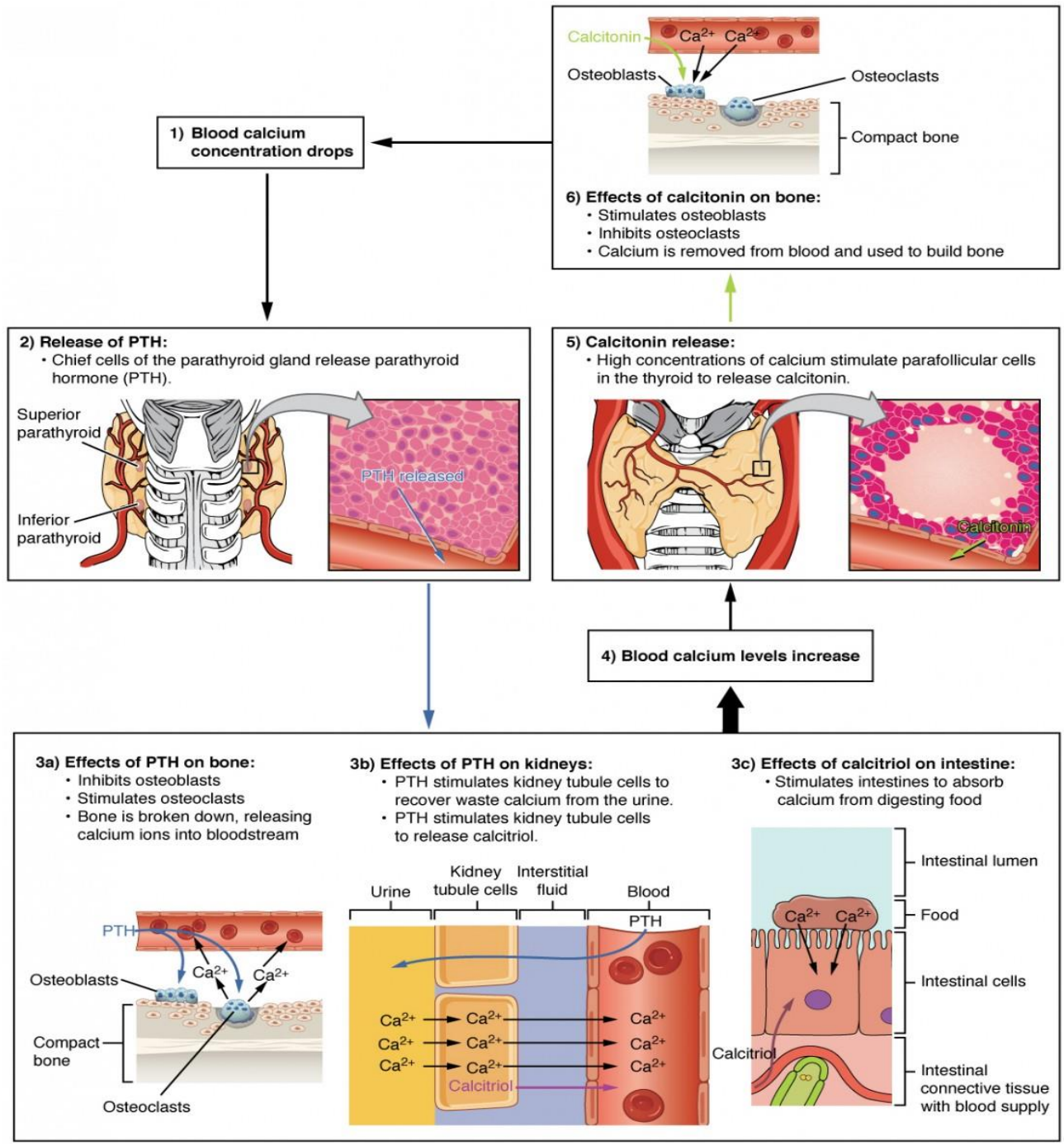
1. CRETINISM (up to idiocy) - congenital hypothyroidism
2. MIXEDEMA - a disease in adulthood, manifests itself in a decrease in the intensity of basal metabolism, oxygen consumption, pulmonary ventilation, heart biting rate, body temperature, mucous edema

Primary hypothyroidism (autoimmune thyroiditis Hashimoto) – insufficiency of thyroid gland.

Secondary hypothyroidism - insufficiency of pituitary gland.

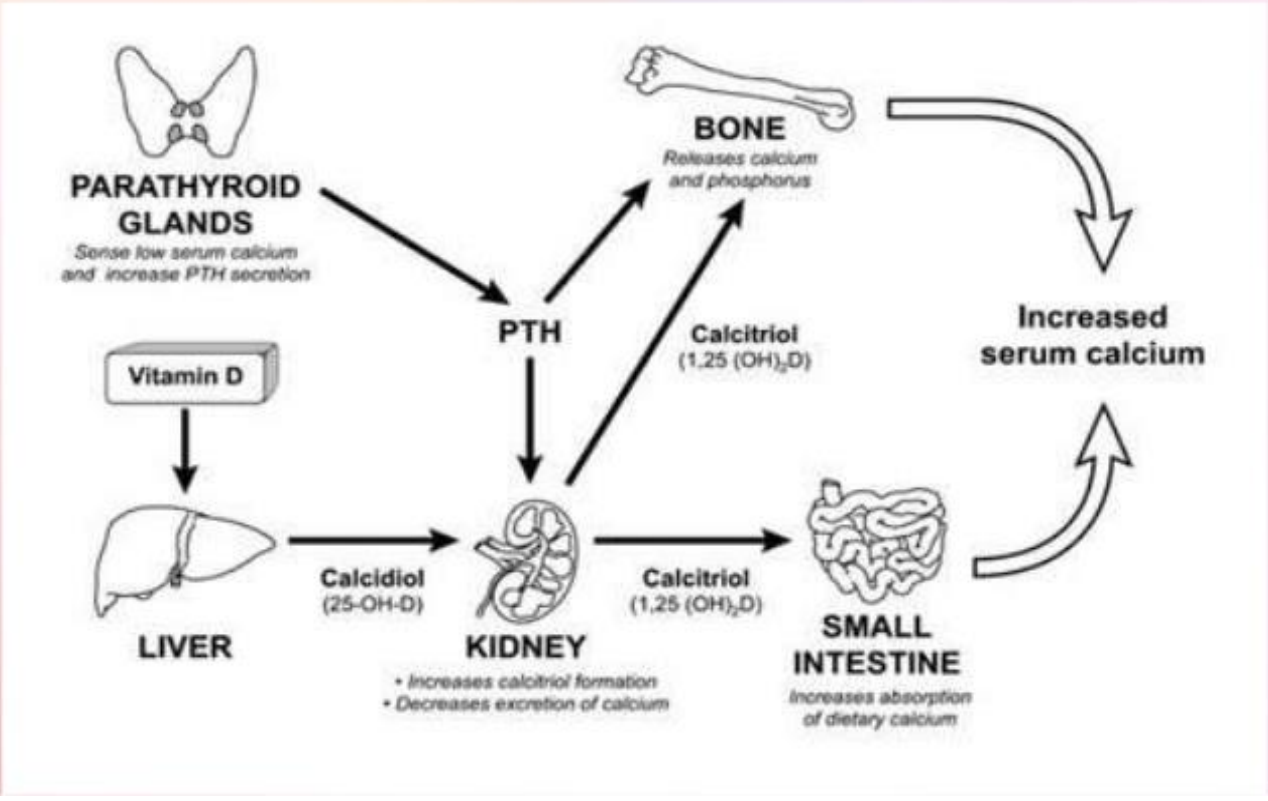
Tertiary hypothyroidism - insufficiency of hypothalamus.



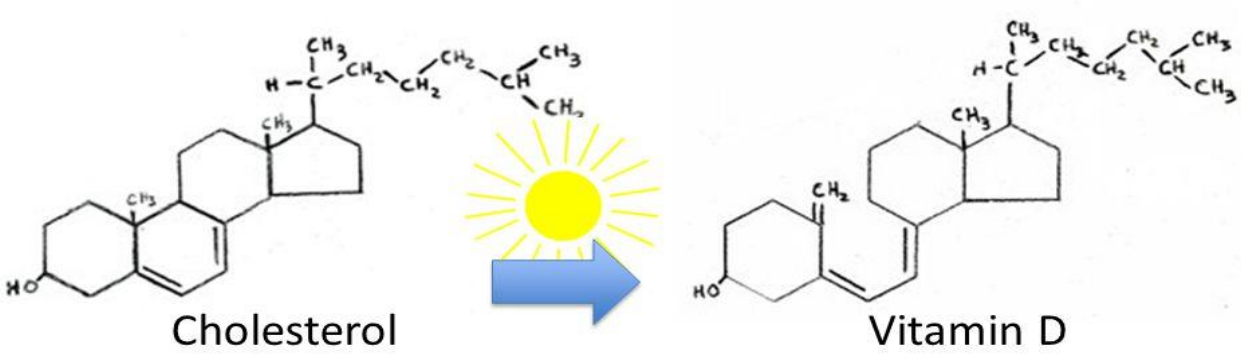

Calcitonin
Ca^{2+} Ca^{2+}
Osteoblasts
Osteoclasts
Compact bone
1) Blood calcium concentration drops
6) Effects of calcitonin on bone:
• Stimulates osteoblasts
• Inhibits osteoclasts
• Calcium is removed from blood and used to build bone
2) Release of PTH:
• Chief cells of the parathyroid gland release parathyroid hormone (PTH).
Superior parathyroid
Inferior parathyroid
PTH released
5) Calcitonin release:
• High concentrations of calcium stimulate parafollicular cells in the thyroid to release calcitonin.
Calcitonin
4) Blood calcium levels increase
3a) Effects of PTH on bone:
• Inhibits osteoblasts
• Stimulates osteoclasts
• Bone is broken down, releasing calcium ions into bloodstream
PTH
Ca^{2+}
Ca^{2+}
Osteoblasts
Compact bone
Osteoclasts
3b) Effects of PTH on kidneys:
• PTH stimulates kidney tubule cells to recover waste calcium from the urine.
• PTH stimulates kidney tubule cells to release calcitriol.
Urine
Kidney tubule cells
Interstitial fluid
Blood
PTH
Ca^{2+}
Calcitriol
3c) Effects of calcitriol on intestine:
• Stimulates intestines to absorb calcium from digesting food
Intestinal lumen
Food
Intestinal cells
Calcitriol
Intestinal connective tissue with blood supply

Calcium Metabolism

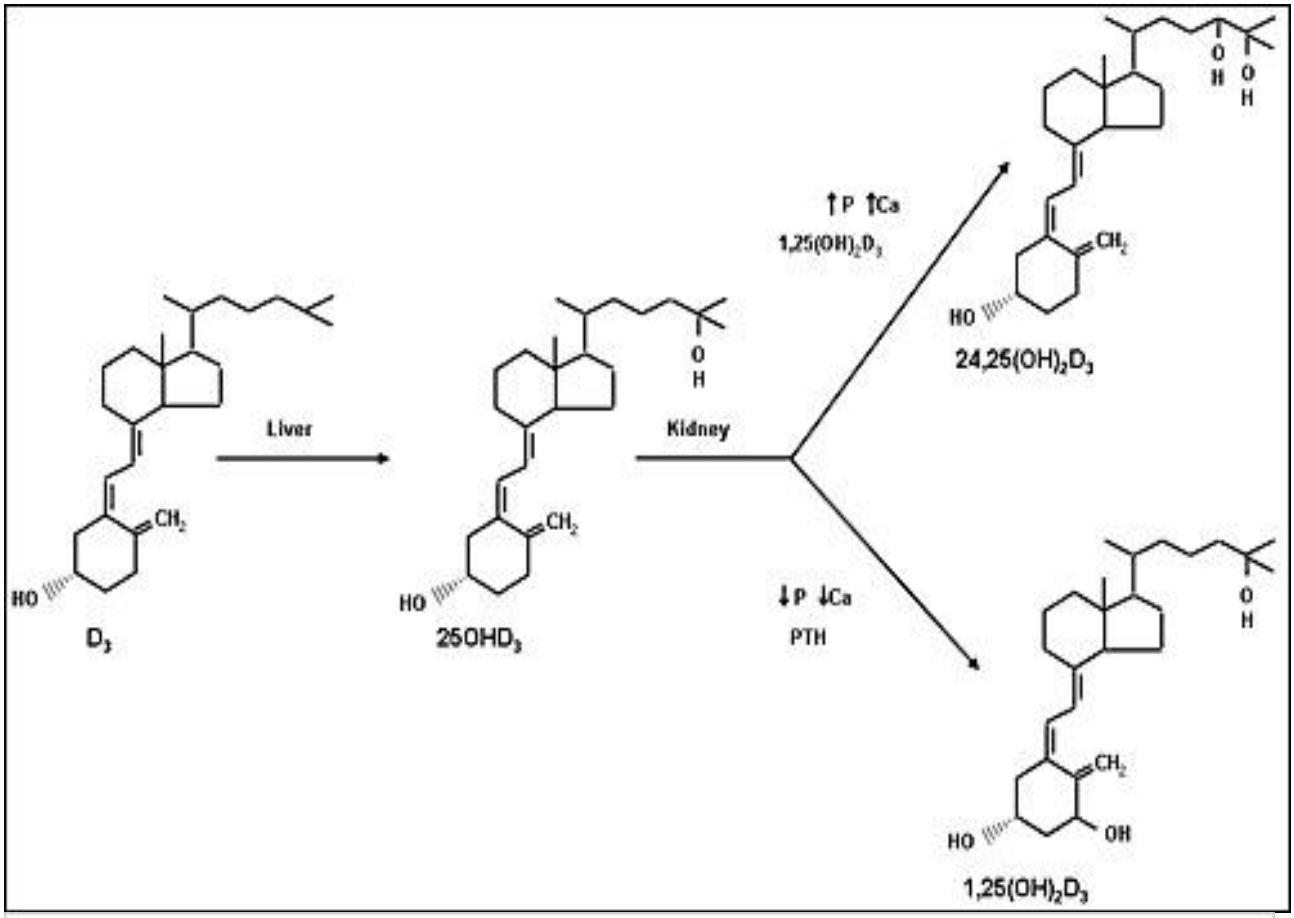
- Dr. Chintan

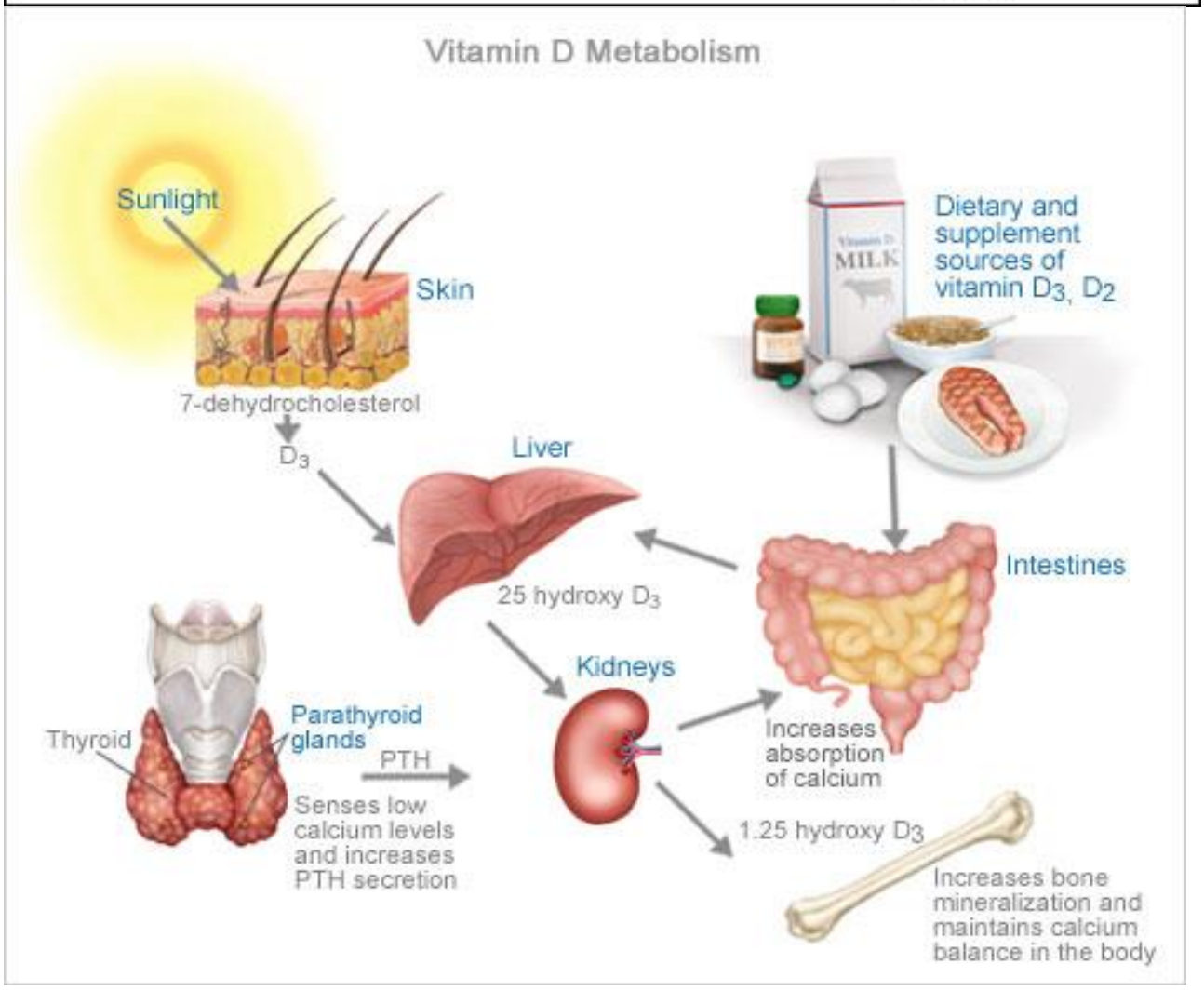


Cholesterol and Vitamin D



- Cholesterol and Vitamin D are nearly identical in chemical structure
- Vitamin D is synthesized from cholesterol in the skin upon exposure to sunlight


Liver
Kidney
↑P ↑Ca
1,25(OH)2D3
↓P ↓Ca
PTH
HO
CH2
OH
D3
25OHD3
24,25(OH)2D3
1,25(OH)2D3


Vitamin D Metabolism
Sunlight
Skin
7-dehydrocholesterol
D3
Liver
25 hydroxy D3
Kidneys
Dietary and supplement sources of vitamin D3, D2
MILK
Intestines
Increases absorption of calcium
1.25 hydroxy D3
Increases bone mineralization and maintains calcium balance in the body
Thyroid
Parathyroid glands
PTH
Senses low calcium levels and increases PTH secretion

REGULATION OF Ca – P METABOLISM

Parathormone through the Gs and Gq proteins activates adenylate cyclase and phosphoinositol system.
It inhibits isocitrate dehydrogenase and alkaline phosphatase and it leads to the formation of Ca citrate and mobilization of it into the blood.

Parathormone decreases the reabsorption of P and increases the reabsorption of Ca.

Calcitonin is responsible for the transfer of Ca from the blood to the bones, for the inhibition of the resorption of the bones and reabsorption of the P in kidney.

Calcitriols (1, 25- and 24, 25) – are the forms of hydroxylated vitamin D_3.

Their responsibility – synthesis of protein-transporter of Ca from intestine to the blood, activation of the reabsorption of Ca and P in kidney.

Glucocorticoids lead to the decreasing of the protein synthesis in the osteoblasts, to the activation of the resorption of bones, to the decreasing of absorption of Ca and P from intestine and activation of excretion of them by kidney.

Growth hormone increases the intestinal absorption of Ca.

Insulin like factor of growth 1 – is responsible for the stimulation of synthesis of osteoblasts.

Thyroid hormones lead to the osteoporosis, calcemia, calciuria.

Estrogens lead to the activation of the synthesis of collagen in the osteoblasts, and prevention of osteoporosis.

Insulin is responsible for the activation of osteosynthesis.

General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Hormones: general characteristics; the role of hormones and other bio-regulators in the system of intercellular integration of human body functions.
2. Classification of hormones and bioregulators: compliance of the structure and mechanisms of action of hormones.
3. Reaction of target cells to the effect of hormones. Membrane (ionotropic, metabotropic) and cytosolic receptors.
4. Biochemical systems of intracellular transmission of hormonal signals: G-proteins, secondary mediators (cAMP, Ca2+/calmodulin, IF3, DAH).
5. Molecular and cellular mechanisms of action of steroid and thyroid hormones.
6. Hormones of the hypothalamus - liberins and statins.
7. Hormones of the anterior lobe of the pituitary gland: somatotropin (STH), prolactin. pathological processes associated with dysfunction of these hormones.
8. Hormones of the posterior lobe of the pituitary gland. Vasopressin and oxytocin: structure, biological functions.
9. Hormonal regulation of calcium homeostasis in the body. Parathyroid hormone, calcitonin, calcitriol.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 12

Topic: Hormones and bioregulators - derivatives of amino acids; hormones and physiologically active compounds of lipid origin. Local hormones

Relevance of the topic: The interaction of the nervous and endocrine systems allows us to talk about a single neuroendocrine system of regulation of body functions. In the process of evolution, humoral regulation arose first than nervous regulation, but it did not lose its importance, but developed and improved. Most of the visceral functions of the human body are triggered and adjusted primarily by humoral rather than nervous regulation mechanisms. During humoral regulation, information is transmitted using a complex of biologically active compounds that are carried throughout the body by blood, lymph, or by diffusion into the intercellular fluid and form the endocrine system. Modern knowledge about the functioning of the human body in normal and pathological conditions is based exclusively on neurohumoral regulation. Knowing the basic patterns of humoral regulation and the mechanisms of hormone action on body cells, one can understand the mechanisms of development of most pathological conditions of the body, prevent their occurrence, and achieve success in the treatment of the visceral systems of the body.

Purpose: study of the effect of hormones of endocrine glands and tissue hormones on proliferation and metabolism in the tissues of the human body. Study of pathological conditions associated with hormonal dysregulation.

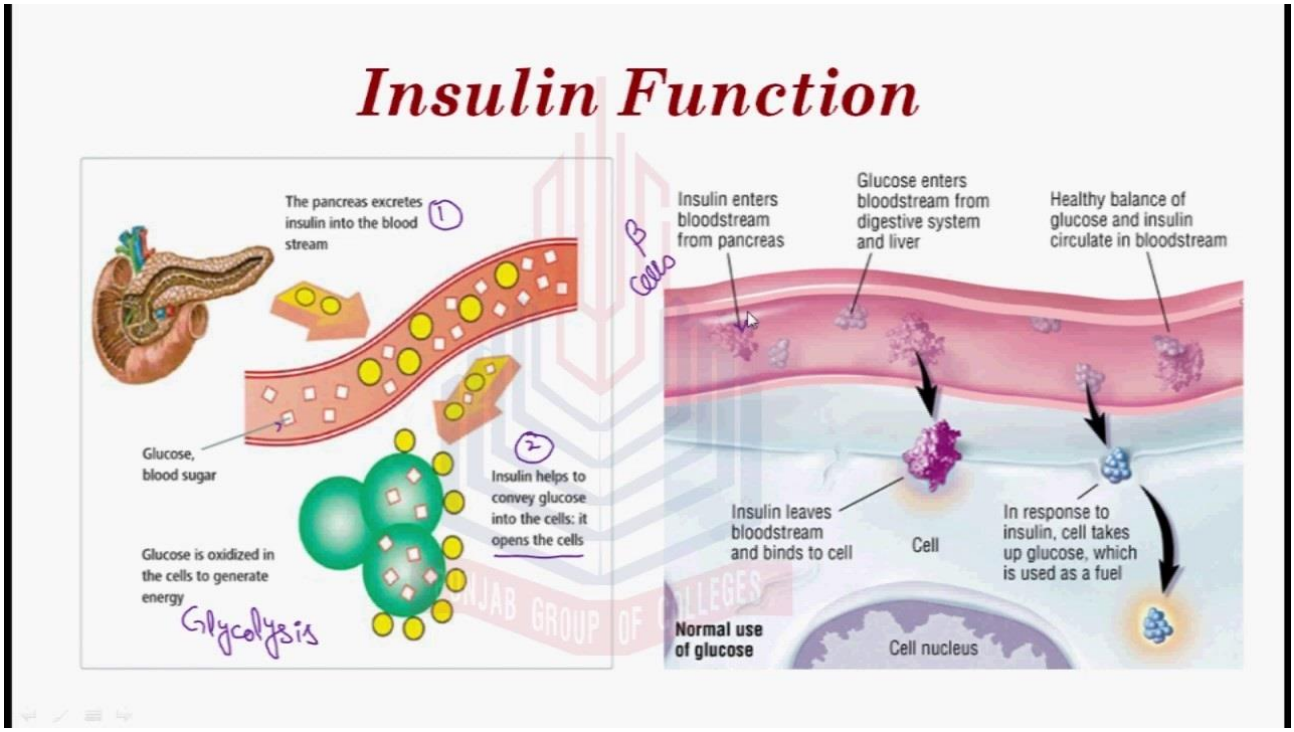
Basic concepts:

1. Insulin-dependent diabetes.
2. Non-insulin-dependent diabetes mellitus.
3. Itsenko-Cushing syndrome.
4. Itsenko-Cushing's disease.
5. Addison's disease.

Plan and organizational structure of the lecture:

1. Insulin. Synthesis, forms of insulin in the blood, mechanisms of action. Pathology.
2. Glucagon. Mechanisms of action.
3. Hormones, derivatives of amino acids.
4. Steroid hormones. Mechanisms of action. Pathology.
5. Eicosanoids. Mechanisms of action. Pathology.

Content of the lecture material



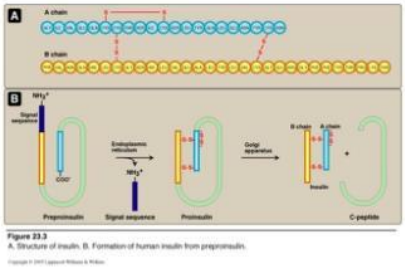
Biosynthesis of Insulin – 3 major steps

- Site; β- cells of Islets

1. Synthesis of Preproinsulin.
2. Conversion of preproinsulin to proinsulin.
3. Conversion of proinsulin to insulin.

Insulin is synthesized by ribosomes of the rough ER as a larger precursor peptide that is then converted to the mature hormone prior to secretion

Synthesis & secretion of insulin



Preproinsulin (inactive precursor)

↓ in ER

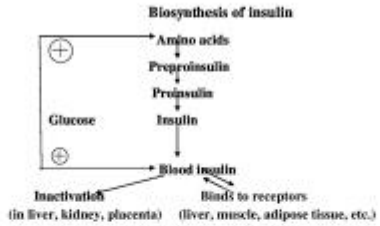
Proinsulin (inactive precursor)

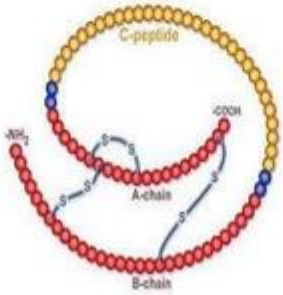
↓ Golgi apparatus

Insulin + C-peptide

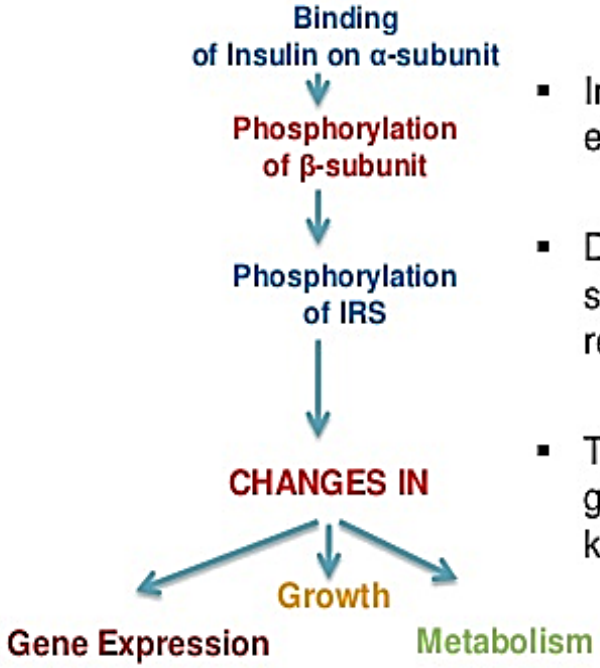
Insulin & C-peptide are stored in cytosol as granules
To be released by certain stimulus by exocytosis to blood

C-peptide has a longer half-life in the plasma more than insulin.
& hence is a good indicator of insulin production & secretion in early cases of DM





Mechanism of action of insulin



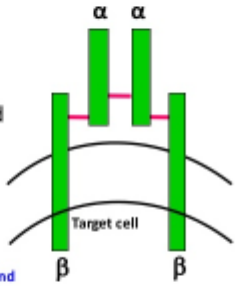
- Insulin regulates both metabolic enzymes and gene expression.
- Does not enter cells, but initiates a signal that travels from the cell surface receptor to -cytosol and to the nucleus.
- The insulin receptor (INS-R) is a glycoprotein receptor with tyrosine kinase activity.

Introduction: Insulin Receptor

- Insulin receptor belongs to a family of receptor-tyrosine kinases (RTKs), which phosphorylate their substrate proteins on tyrosine residues.
- The insulin receptor comprises of two subunits: the extracellular α-subunit and the transmembrane β-subunit. The functional receptor exists as a dimer/heterotetrameric complex: α2β2
- The α-subunit is contains the ligand binding site, as it is the only subunit identified by affinity labelling protocols.
- The β subunit is involved in intracellular signalling.

Insulin receptor

- It has two α & two β subunits, held by disulphide bridges
- α subunits bind insulin
- β subunits
  - have tyrosine kinase activity (**Kinase-phosphatase system, 2nd messenger**)
  - Contain autophosphorylation sites

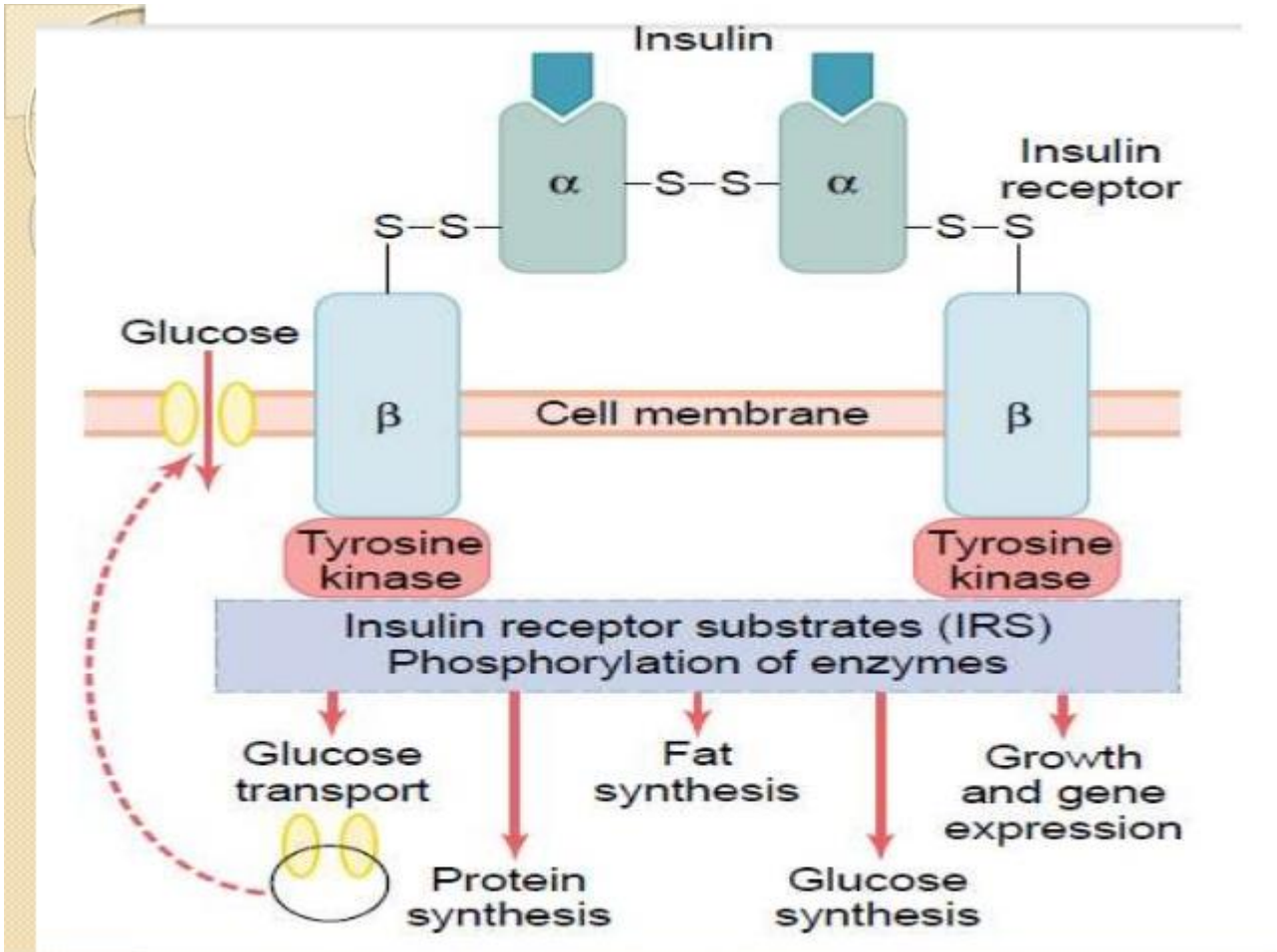


Mechanism of Action of Insulin

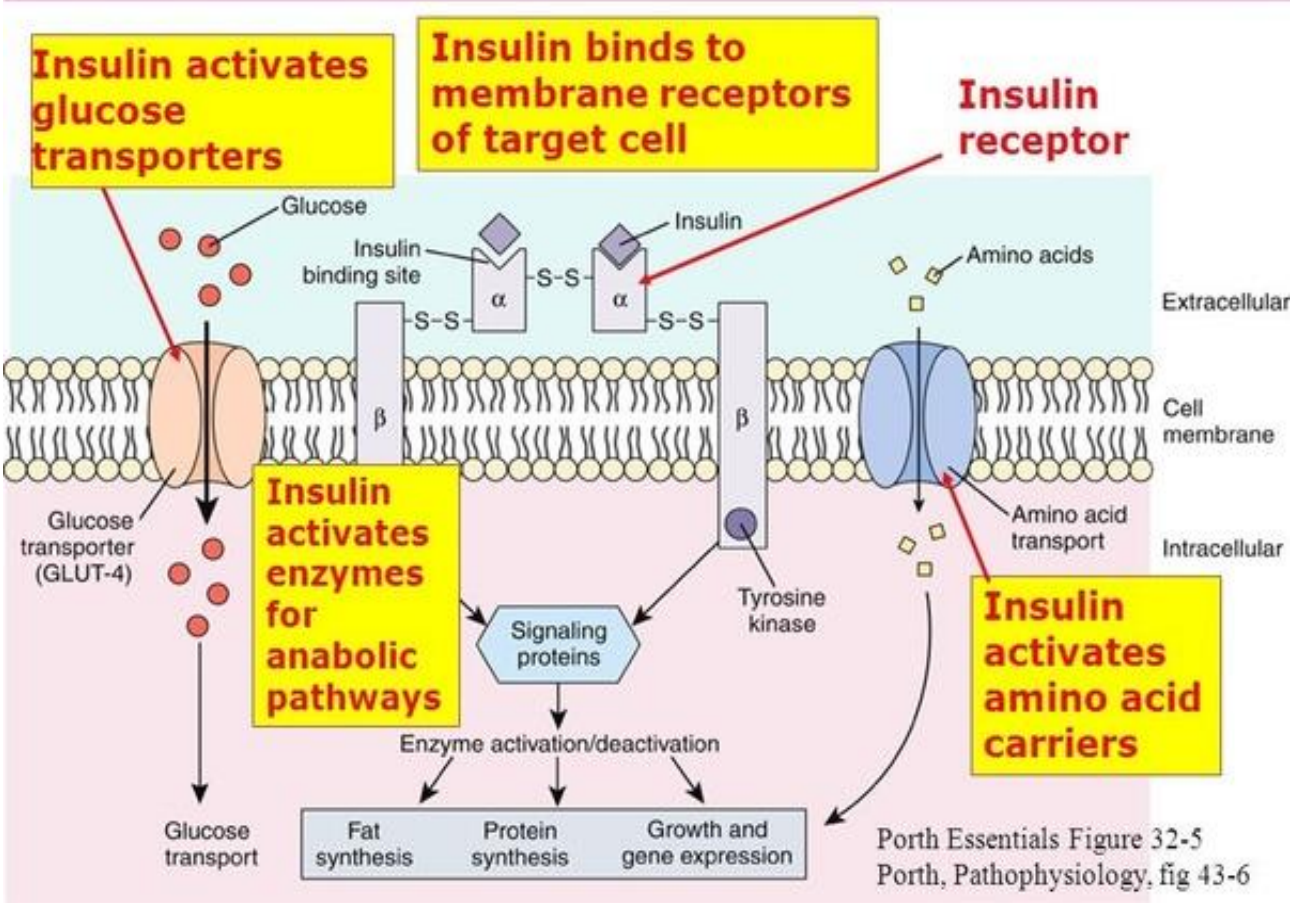
The insulin receptor

- A specific transmembrane ***tyrosine-kinase linked receptor*** located in cell membranes of most tissues.
- Activation of this receptor, triggers the phosphorylation of a ***tyrosine kinase*** enzyme which in turn leads to the following two cascade pathways:

1. ***Insulin receptor substrate-1 (IRS-1) pathway:*** Leading to
   a. Regulation of proliferation and differentiation of several cell types
   b. Regulation of DNA synthesis
2. ***Insulin receptor substrate-2 (IRS-2) pathway:*** Leading to
   a. Increased glucose uptake by the lipid and muscle cells
   b. Increased glycogen formation
   c. Regulation of gene transcription



Insulin Receptors on Target Cells



Insulin Mechanism of Action

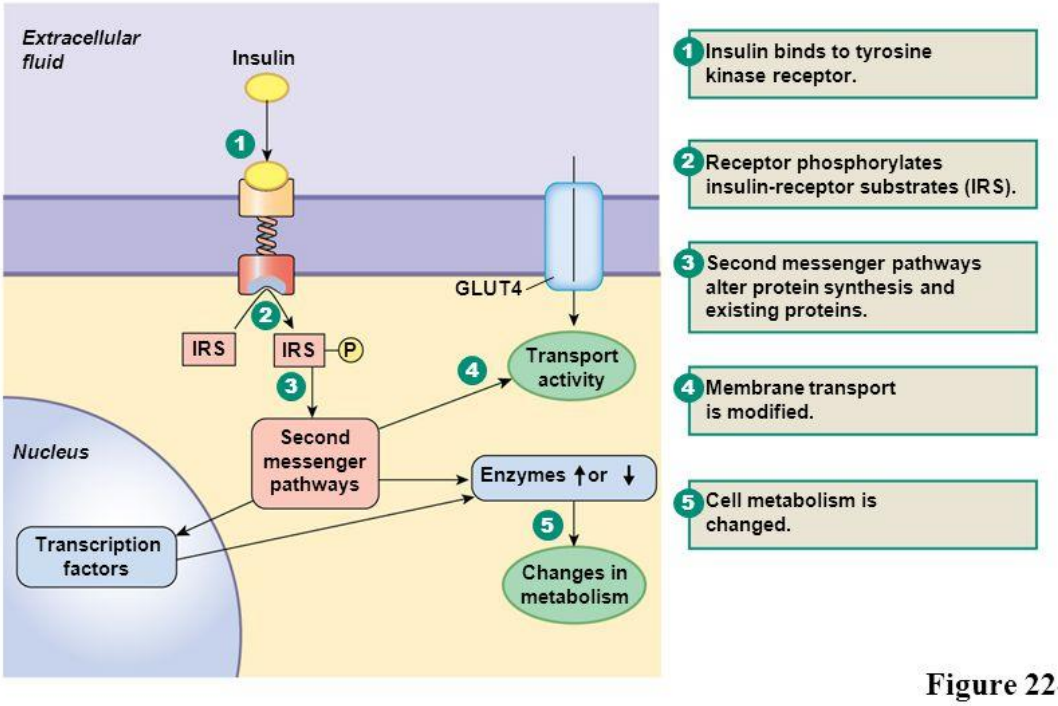
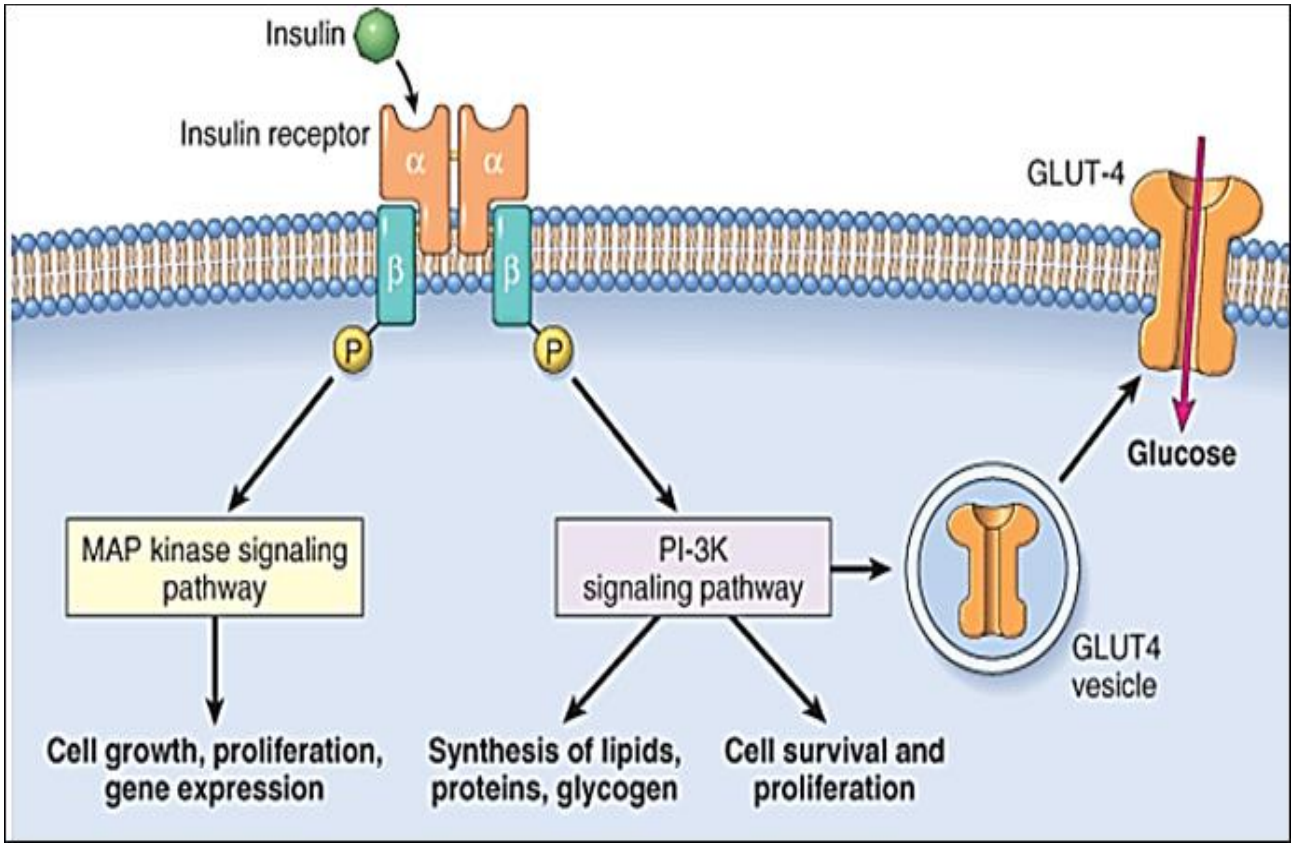
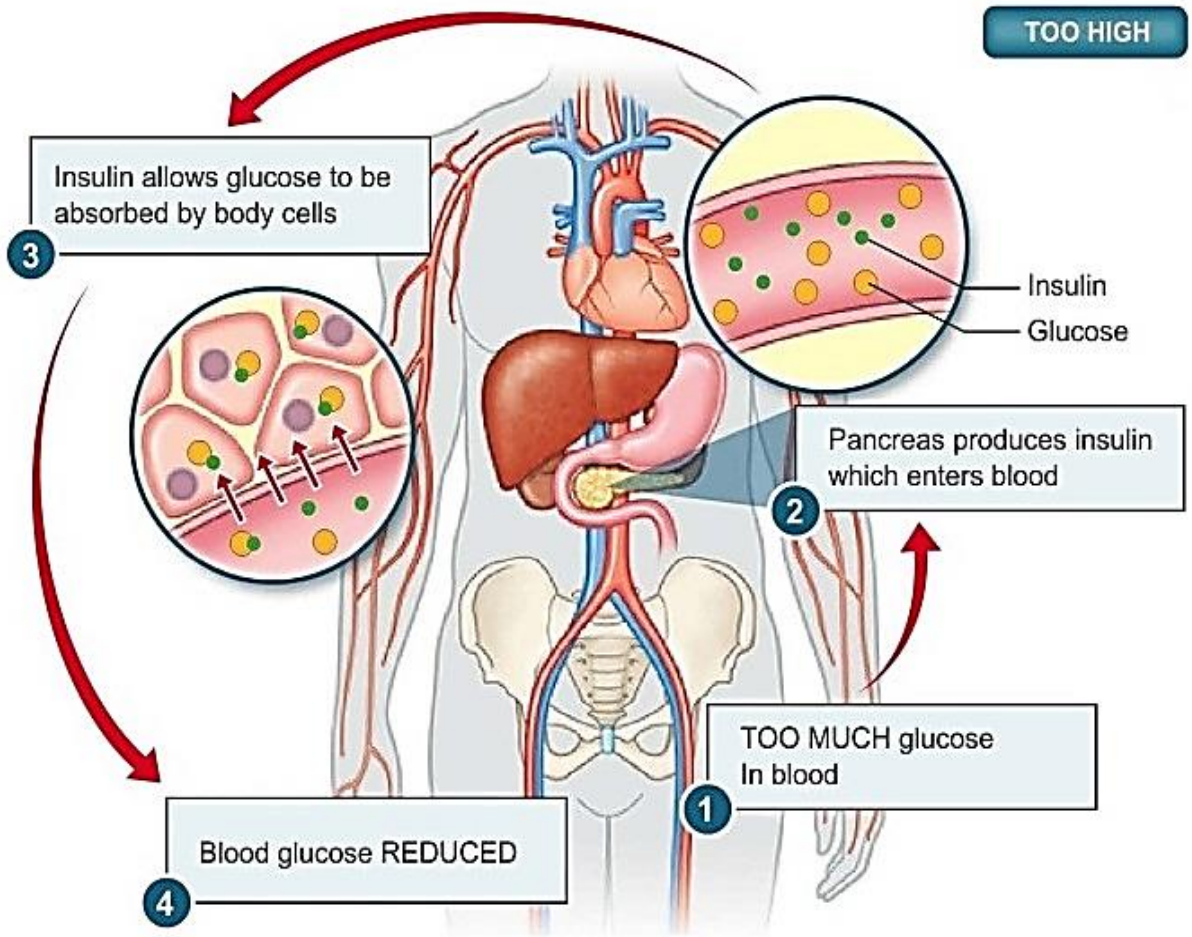
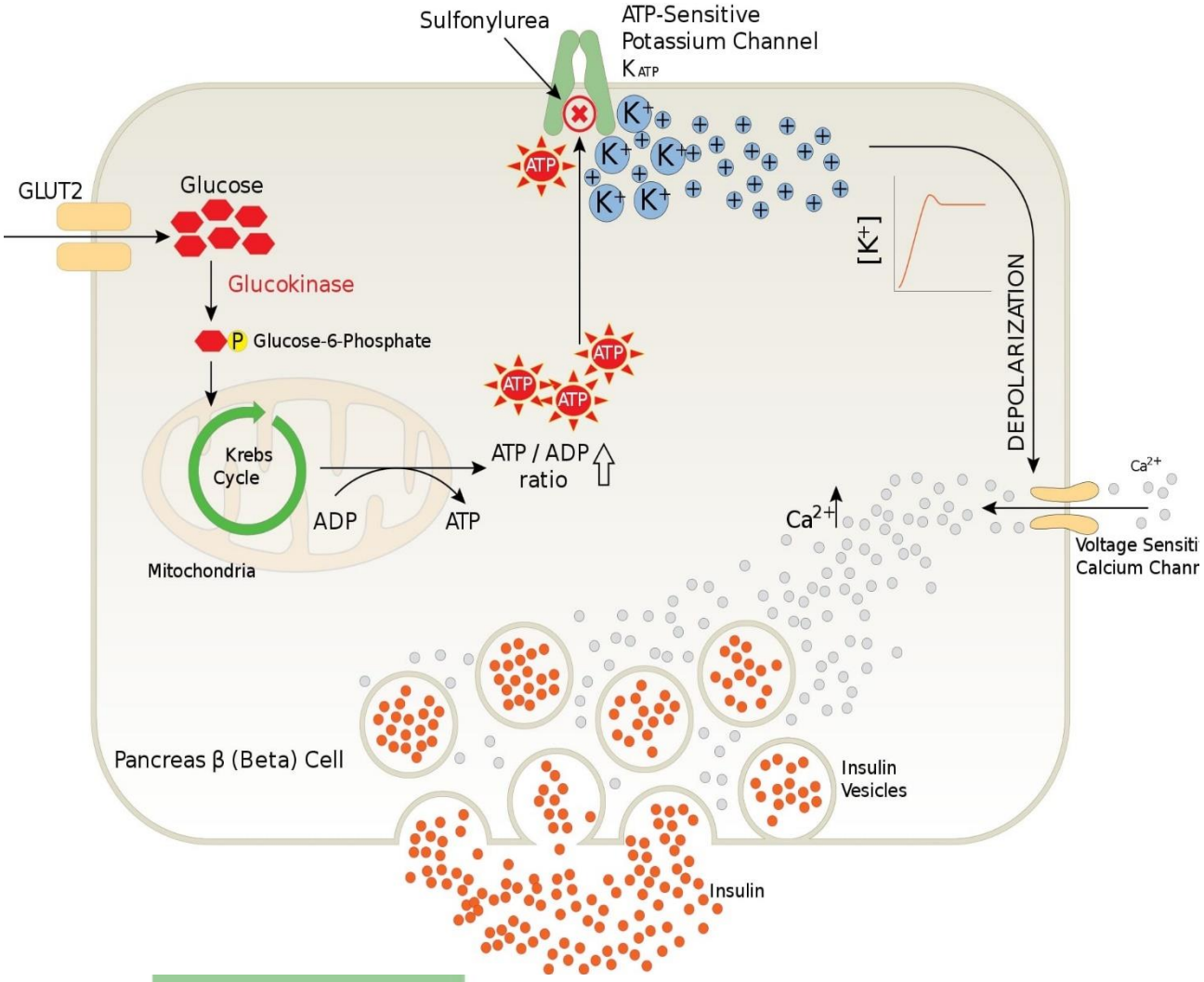


Figure 22-11




TOO HIGH
Insulin allows glucose to be absorbed by body cells
3
Insulin
Glucose
Pancreas produces insulin which enters blood
2
TOO MUCH glucose In blood
1
Blood glucose REDUCED
4



Mechanism of action of sulfonylureas:

- Stimulate insulin release from functioning B cells by blocking of ATP-sensitive K channels which causes depolarization and opening of voltage- dependent calcium channels, which causes an increase in intracellular calcium in the beta cells, which stimulates insulin release.

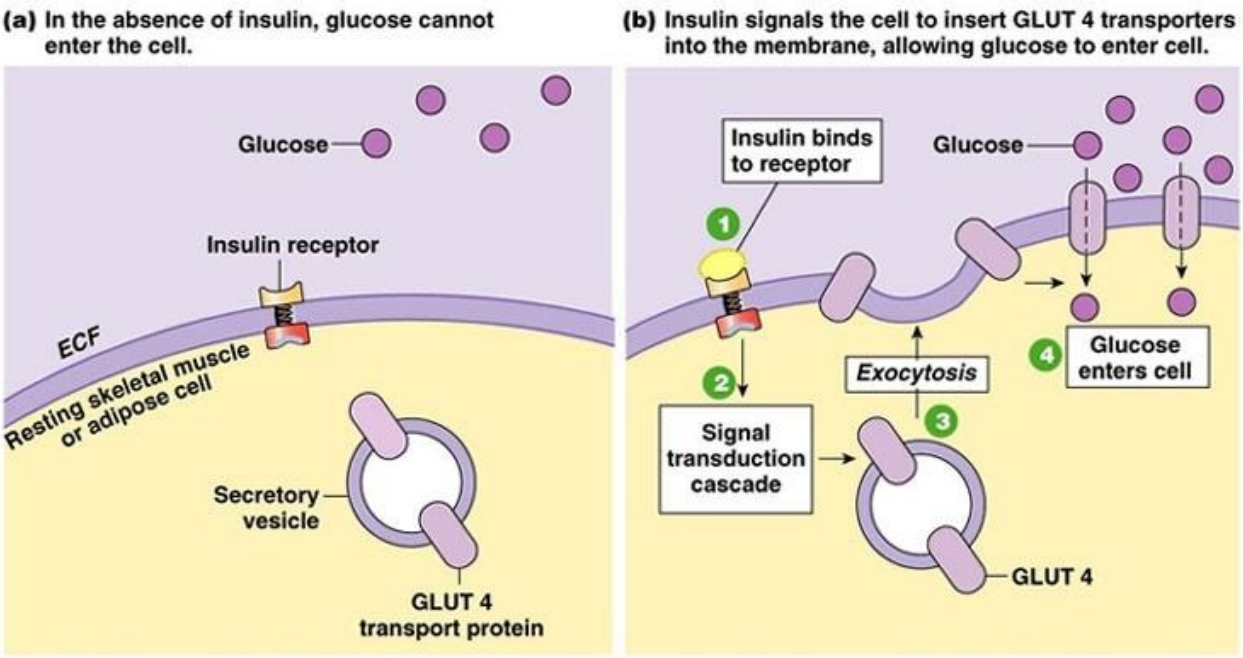
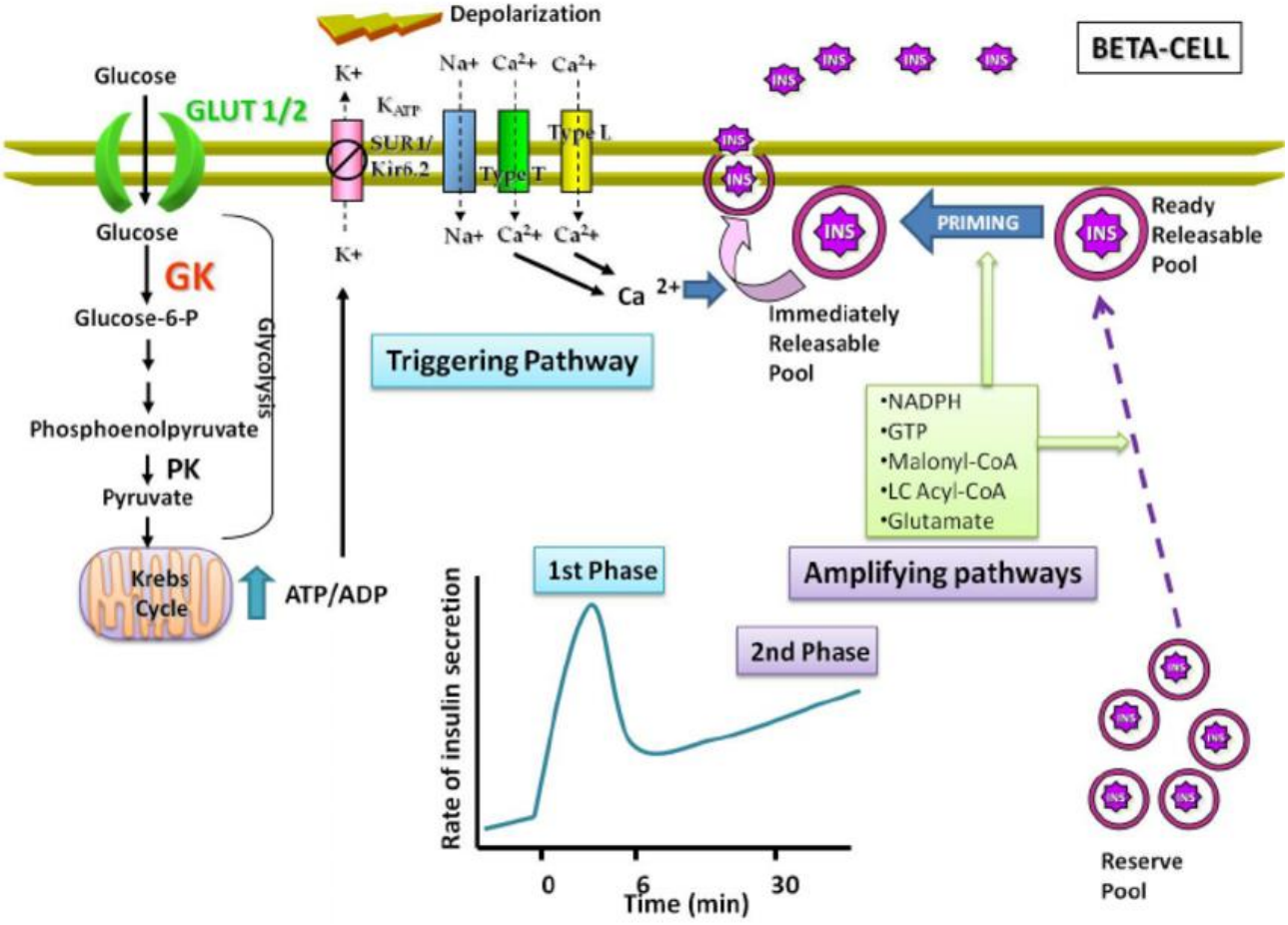


Fig. 22-12

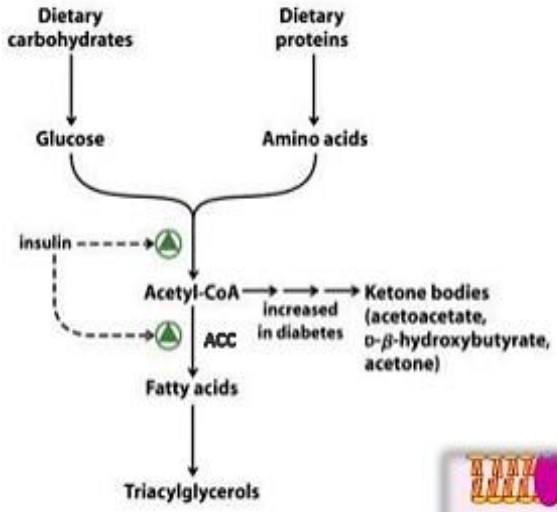


The Pharmacological Action of Insulin

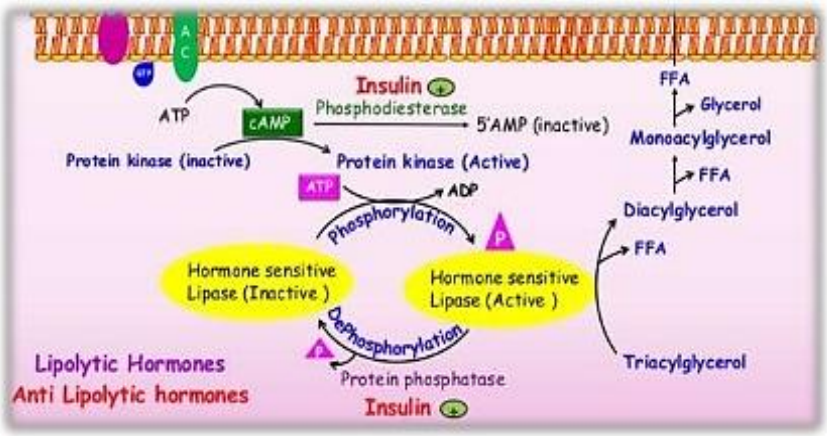
- It allows the active uptake of glucose and its utilisation in muscle and fat cells.
- It stimulates synthesis of glycogen in the liver.
- It inhibits formation of glucose (gluconeogensis) in the liver.
- It inhibits breakdown of lipids.
- It stimulates protein synthesis.
- It stimulates some cell ion transport mechanisms (e.g. Na^+/K^+-ATPase). Increase K+ inflow.

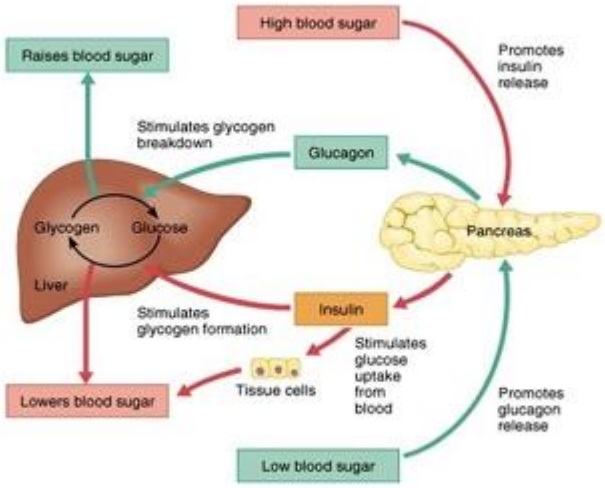
20

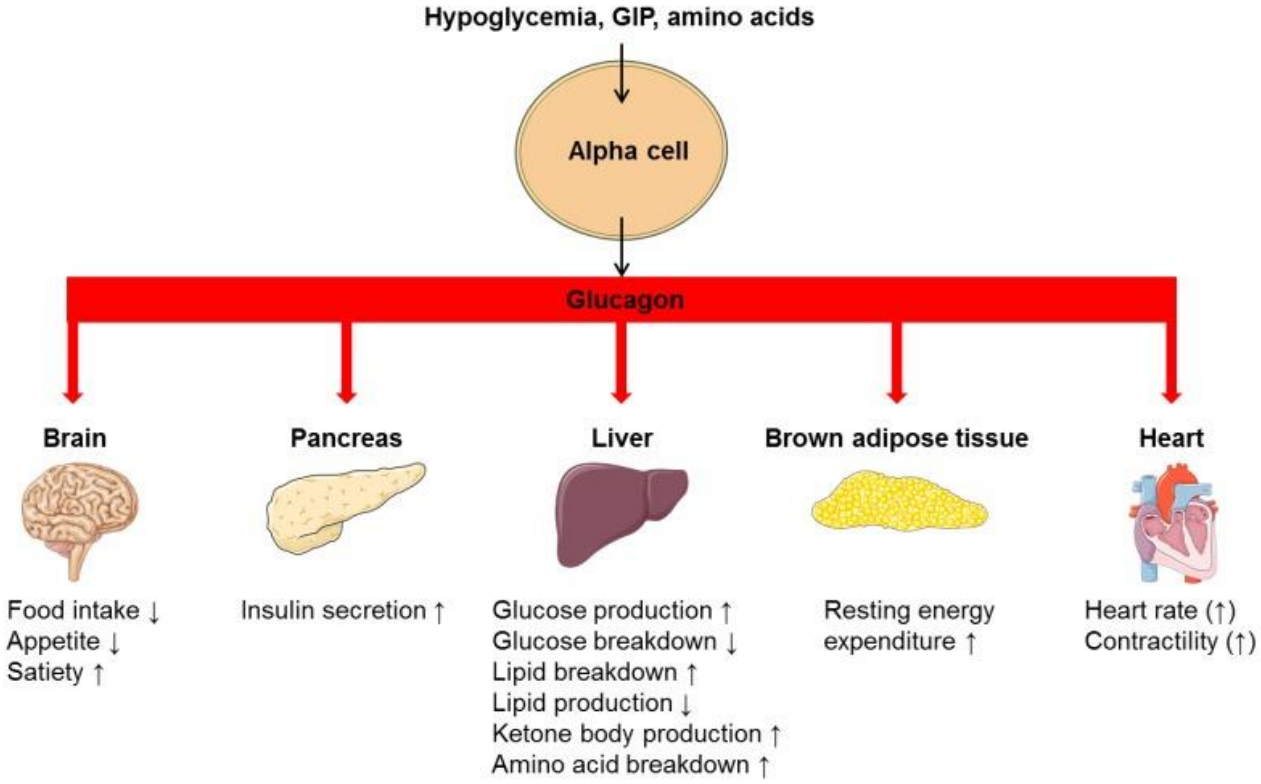
Effect on lipogenesis:

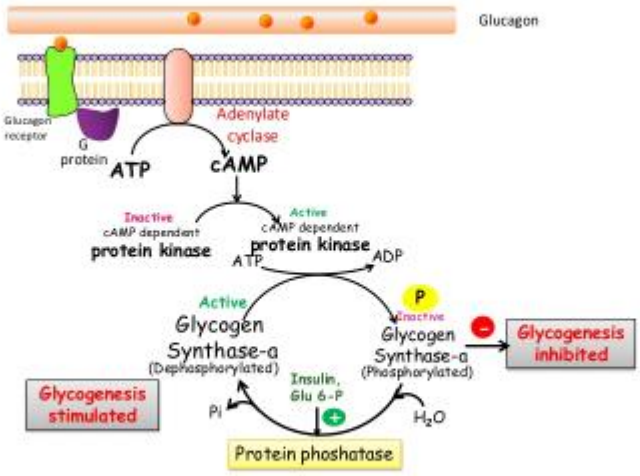


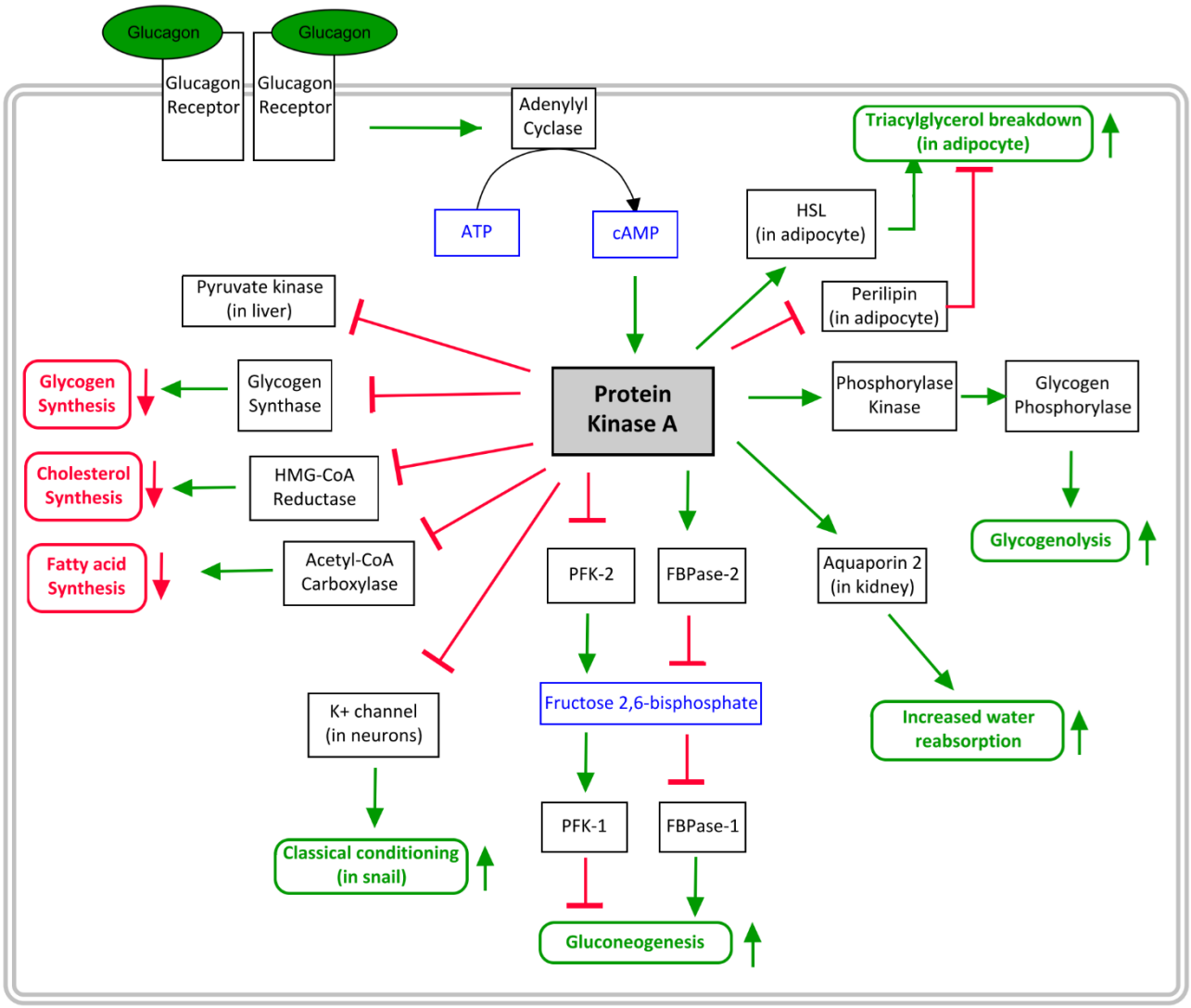
Effect on lipolysis:



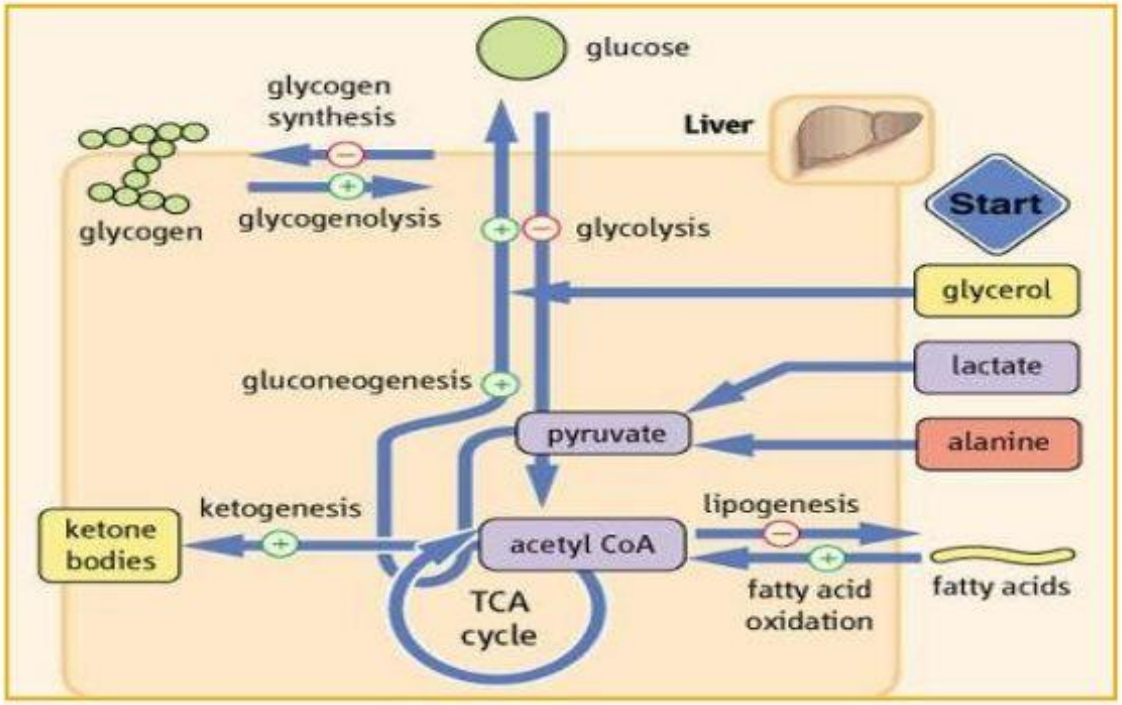

High blood sugar
Raises blood sugar
Promotes insulin release
Stimulates glycogen breakdown
Glucagon
Glycogen
Glucose
Liver
Pancreas
Insulin
Stimulates glycogen formation
Stimulates glucose uptake from blood
Tissue cells
Lowers blood sugar
Promotes glucagon release
Low blood sugar


Hypoglycemia, GIP, amino acids
Alpha cell
Glucagon
Brain
Pancreas
Liver
Brown adipose tissue
Heart
Food intake ↓
Appetite ↓
Satiety ↑
Insulin secretion ↑
Glucose production ↑
Glucose breakdown ↓
Lipid breakdown ↑
Lipid production ↓
Ketone body production ↑
Amino acid breakdown ↑
Resting energy expenditure ↑
Heart rate (↑)
Contractility (↑)

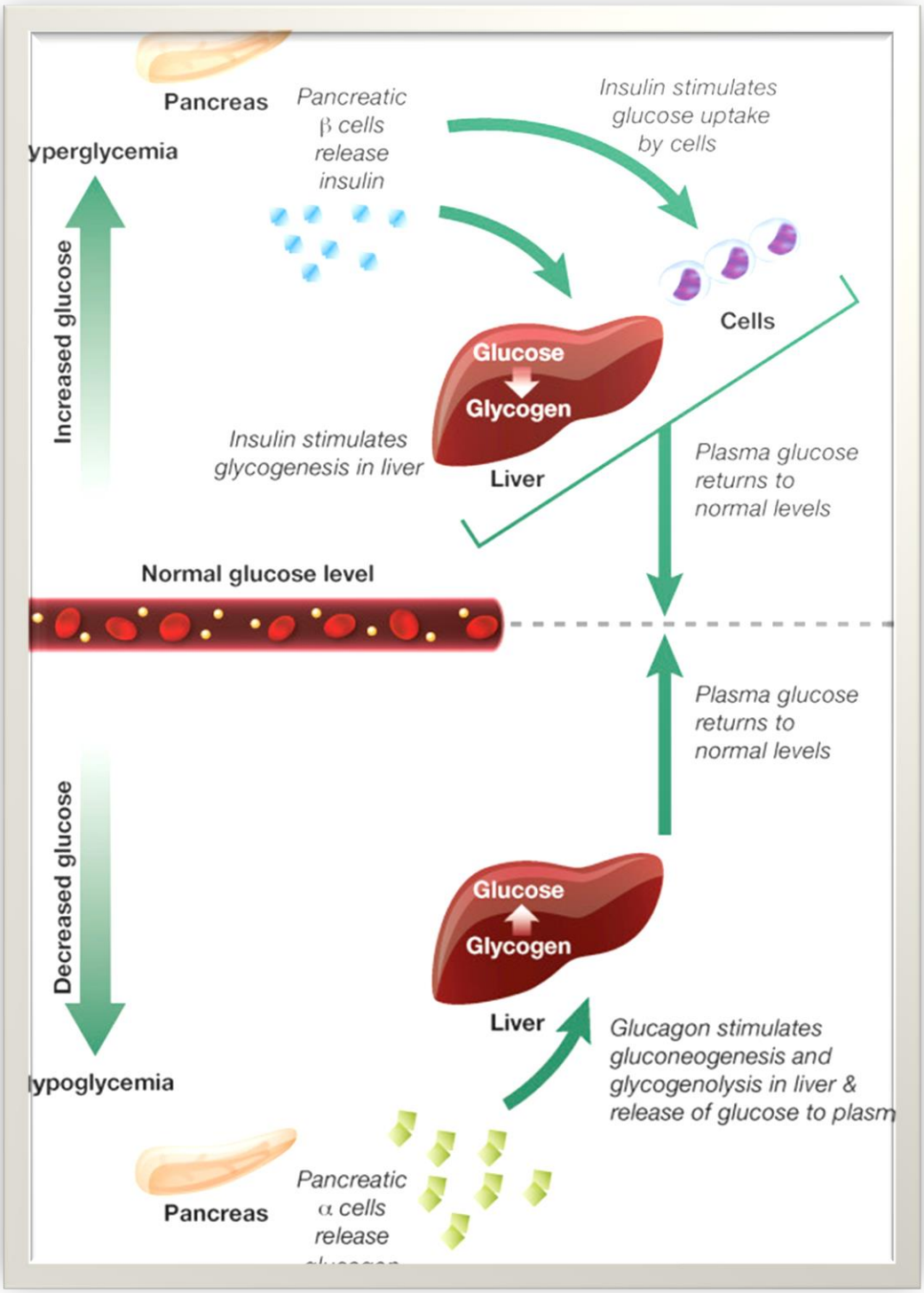

Glucagon
Glucagon receptor
G protein
Adenylate cyclase
ATP
cAMP
Inactive cAMP dependent protein kinase
Active cAMP dependent protein kinase
ATP
ADP
Active Glycogen Synthase-a (Dephosphorylated)
Inactive Glycogen Synthase-a (Phosphorylated)
P
Glycogenesis inhibited
Glycogenesis stimulated
Insulin, Glu 6-P
Pi
H2O
Protein phoshatase

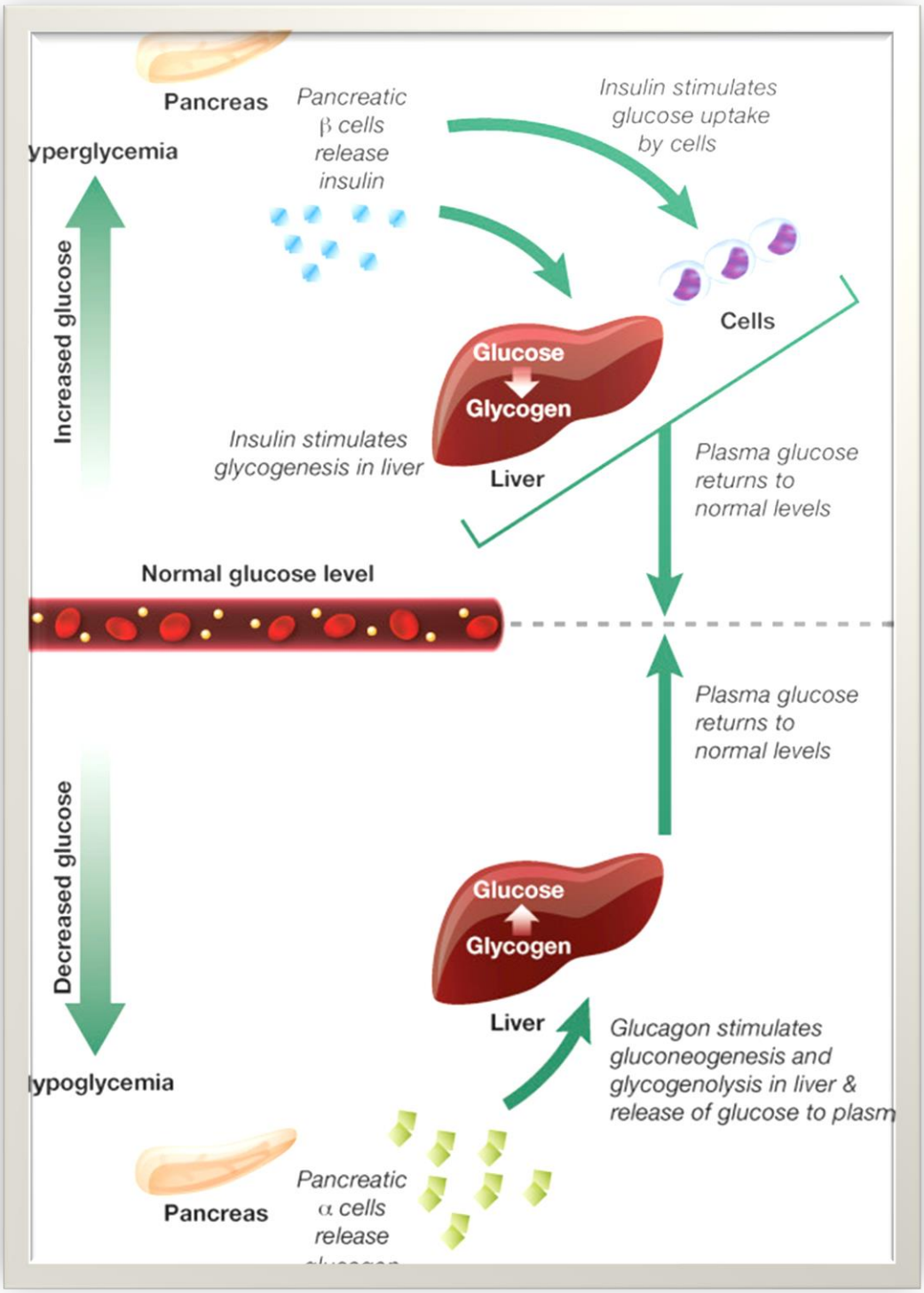

Glucagon
Glucagon
Glucagon Receptor
Glucagon Receptor
Adenylyl Cyclase
ATP
cAMP
Triacylglycerol breakdown (in adipocyte)
HSL (in adipocyte)
Perilipin (in adipocyte)
Pyruvate kinase (in liver)
Glycogen Synthesis
Glycogen Synthase
Protein Kinase A
Phosphorylase Kinase
Glycogen Phosphorylase
Cholesterol Synthesis
HMG-CoA Reductase
Fatty acid Synthesis
Acetyl-CoA Carboxylase
Glycogenolysis
PFK-2
FBPase-2
Aquaporin 2 (in kidney)
K+ channel (in neurons)
Fructose 2,6-bisphosphate
Increased water reabsorption
PFK-1
FBPase-1
Classical conditioning (in snail)
Gluconeogenesis

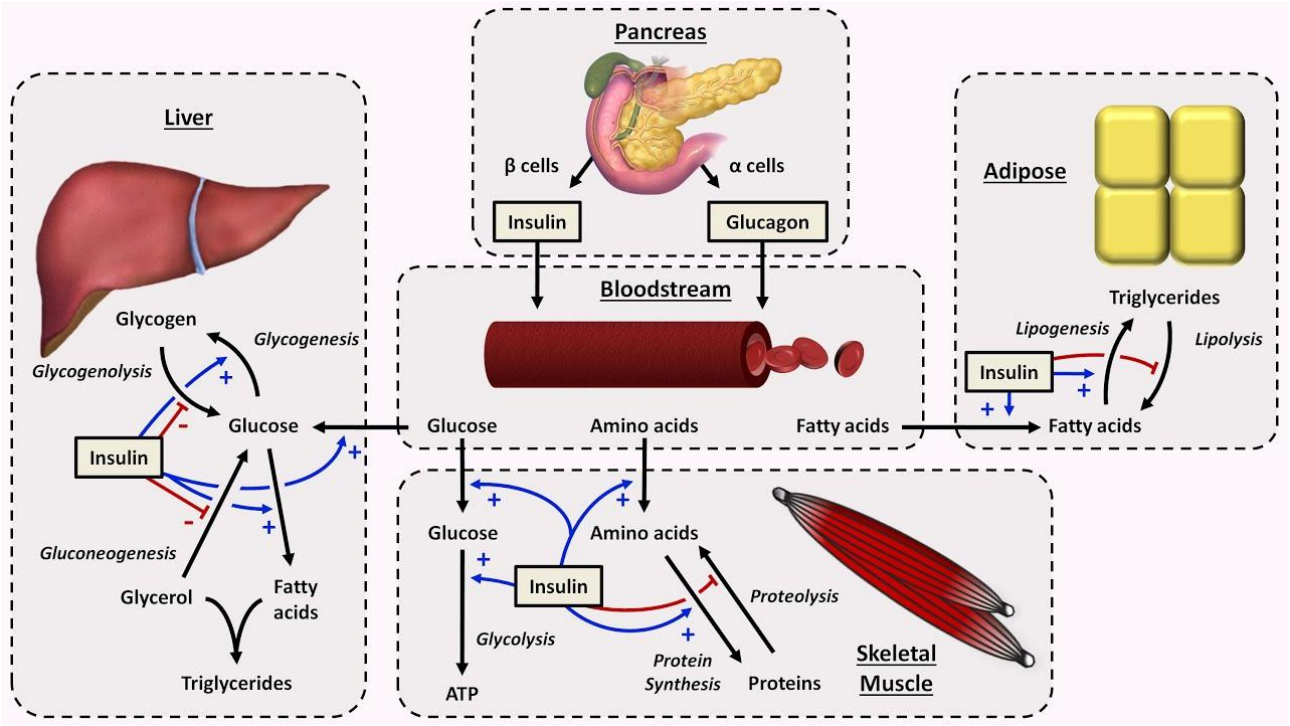
Metabolic Effects of Glucagon



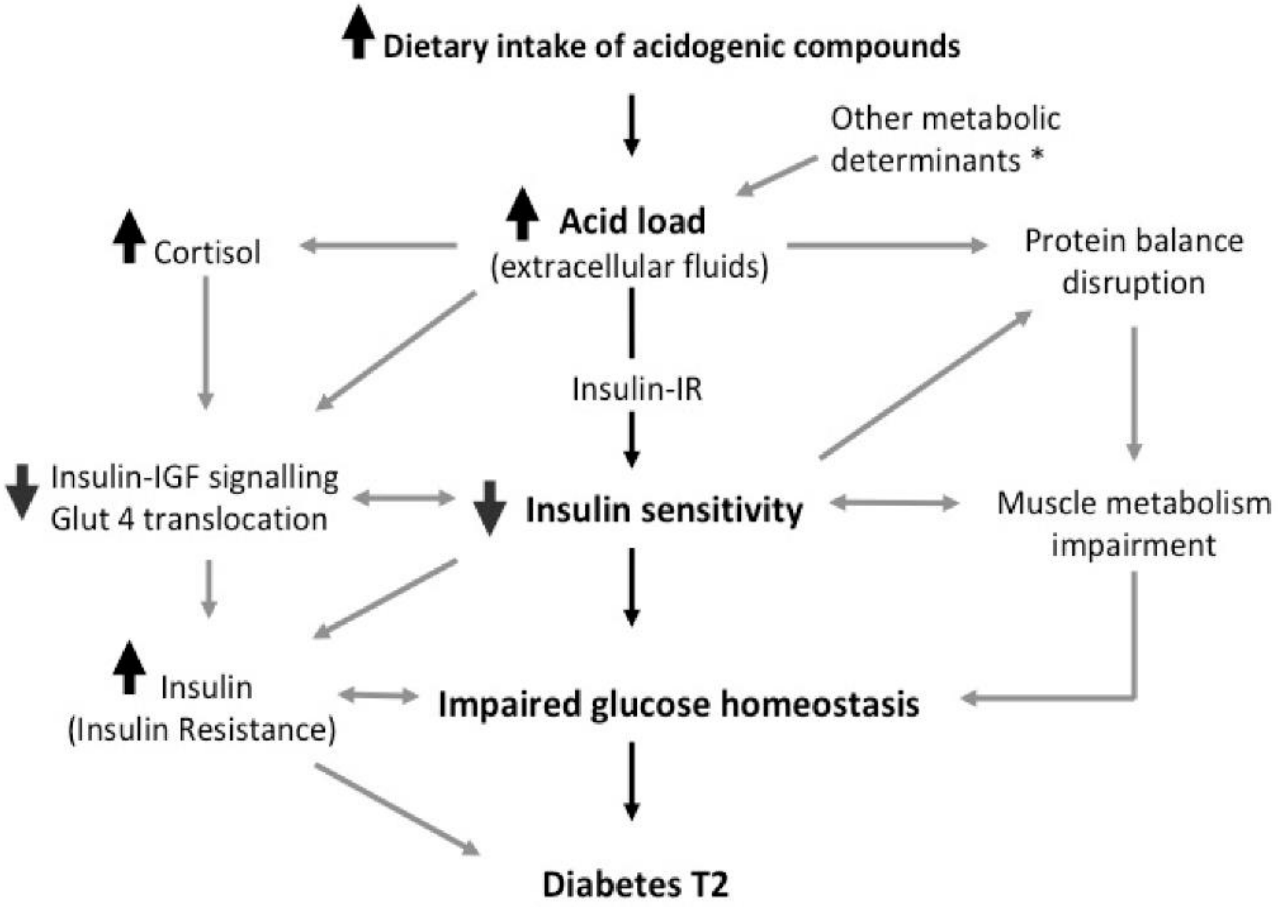
20

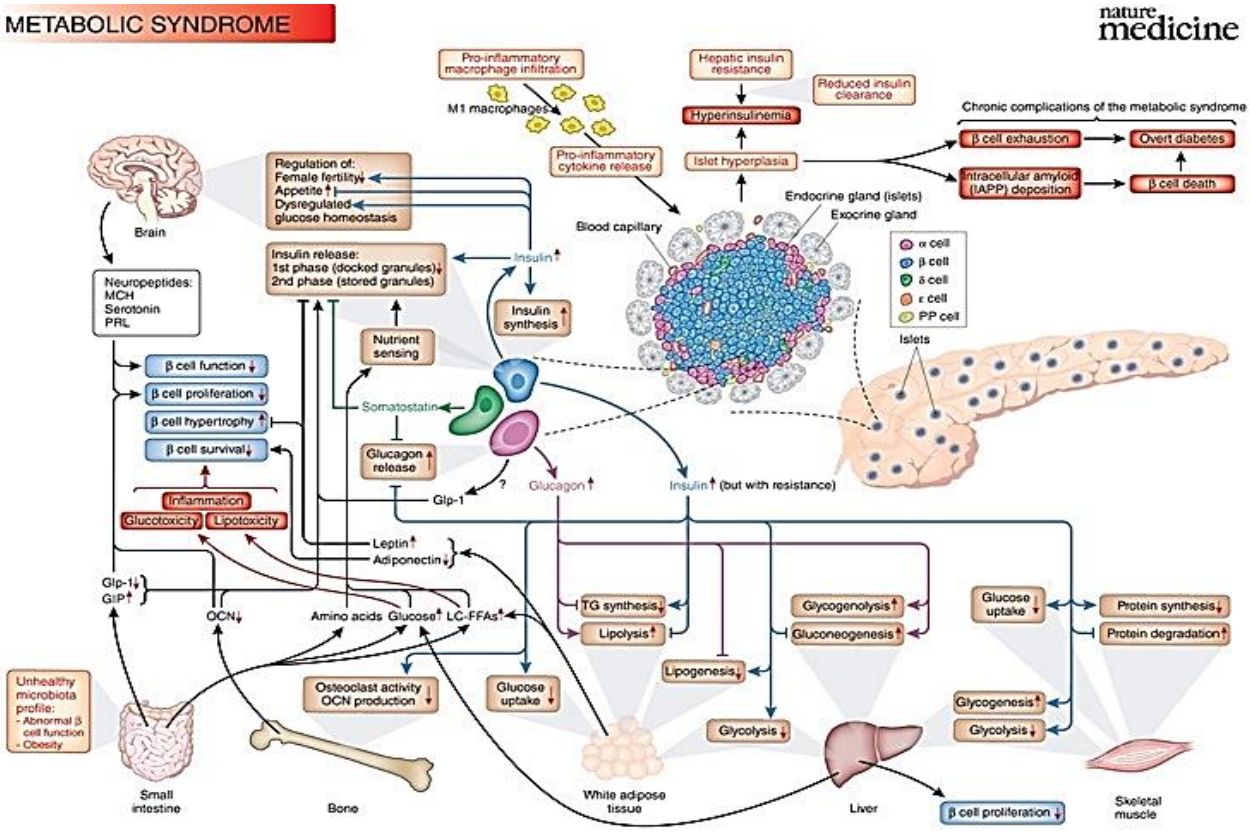

Pancreas
Pancreatic β cells release insulin
Insulin stimulates glucose uptake by cells
yperglycemia
Increased glucose
Cells
Glucose
Glycogen
Liver
Insulin stimulates glycogenesis in liver
Plasma glucose returns to normal levels
Normal glucose level
Plasma glucose returns to normal levels
Decreased glucose
Glucose
Glycogen
Liver
ypoglycemia
Glucagon stimulates gluconeogenesis and glycogenolysis in liver & release of glucose to plasm
Pancreas
Pancreatic α cells release


Pancreas
Pancreatic β cells release insulin
Insulin stimulates glucose uptake by cells
yperglycemia
Increased glucose
Cells
Glucose
Glycogen
Liver
Insulin stimulates glycogenesis in liver
Plasma glucose returns to normal levels
Normal glucose level
Plasma glucose returns to normal levels
Decreased glucose
Glucose
Glycogen
Liver
ypoglycemia
Glucagon stimulates gluconeogenesis and glycogenolysis in liver & release of glucose to plasm
Pancreas
Pancreatic α cells release


Pancreas
β cells
α cells
Insulin
Glucagon
Liver
Glycogen
Glycogenesis
Glycogenolysis
Glucose
Insulin
Gluconeogenesis
Glycerol
Fatty acids
Triglycerides
Bloodstream
Glucose
Amino acids
Fatty acids
Adipose
Triglycerides
Lipogenesis
Lipolysis
Insulin
Fatty acids
Glucose
Amino acids
Insulin
Glycolysis
ATP
Proteolysis
Protein Synthesis
Proteins
Skeletal Muscle


↑Inflammation
↑Testosterone (women)
↓Liver detoxification pathways
↑Estrogen (men)
INSULIN
↑Blood pressure
↑Oxidative Stress
↑Cholesterol
↑↓Neurotransmitters

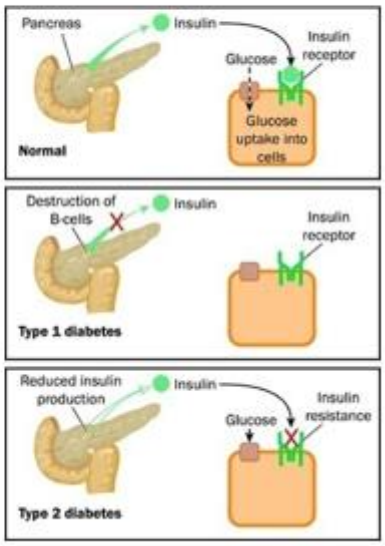

Dietary intake of acidogenic compounds
Other metabolic determinants *
Acid load
(extracellular fluids)
Cortisol
Protein balance disruption
Insulin-IR
Insulin-IGF signalling
Glut 4 translocation
Insulin sensitivity
Muscle metabolism impairment
Insulin
(Insulin Resistance)
Impaired glucose homeostasis
Diabetes T2

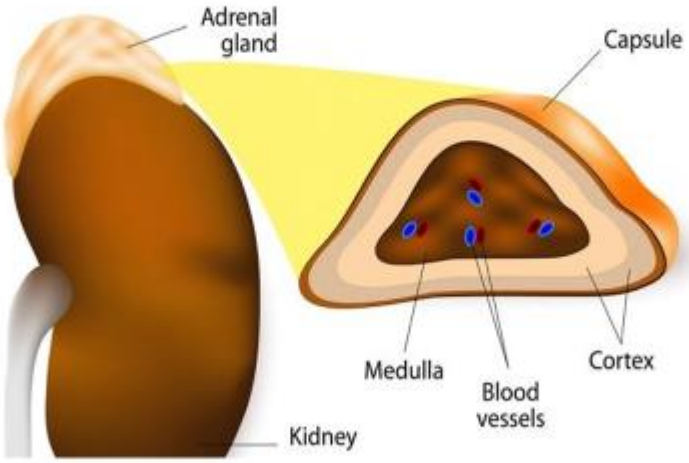


Diabetes mellitus

Type 1 – insufficiency of pancreas to produce insulin

Type 2 – insufficiency of receptors to insulin




Adrenal gland
Capsule
Medulla
Blood vessels
Cortex
Kidney

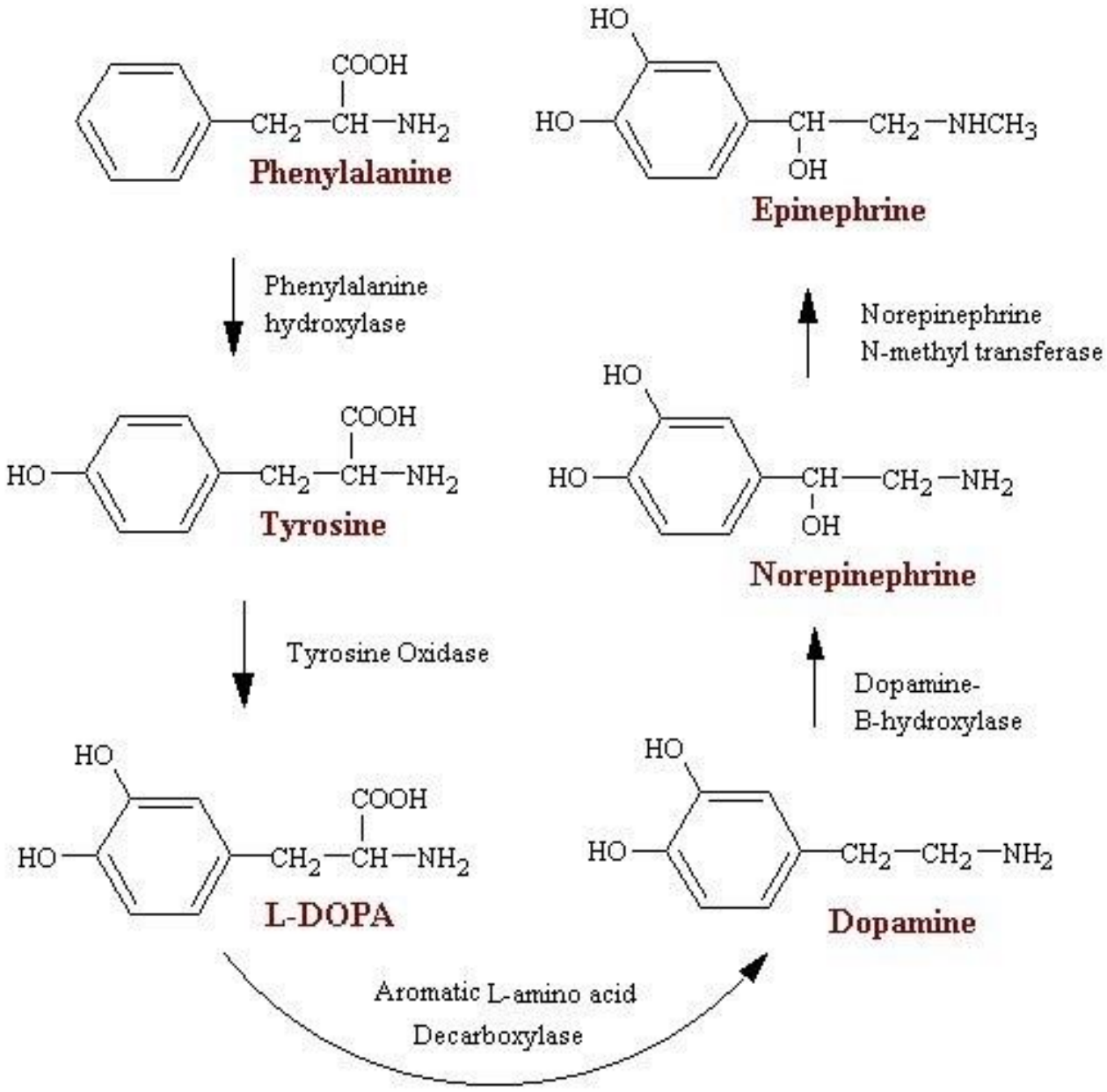
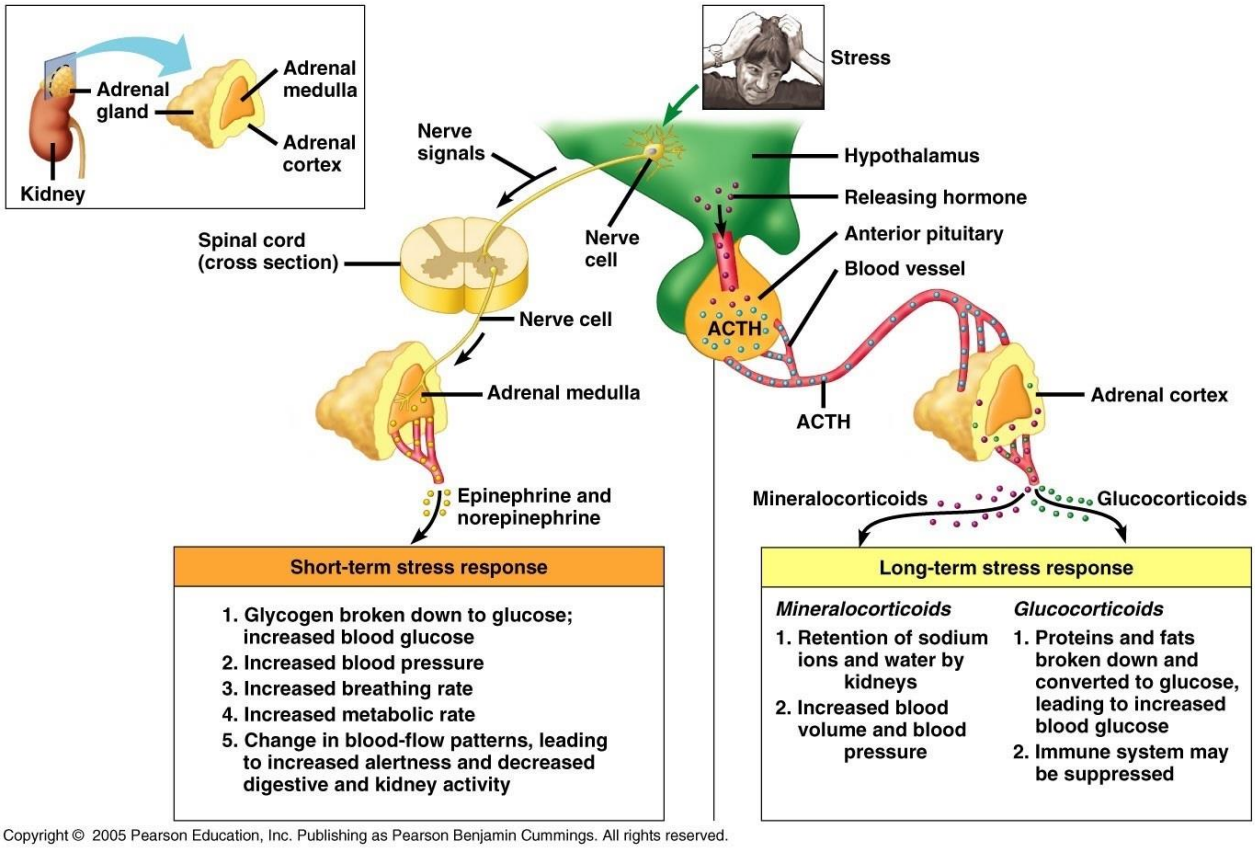
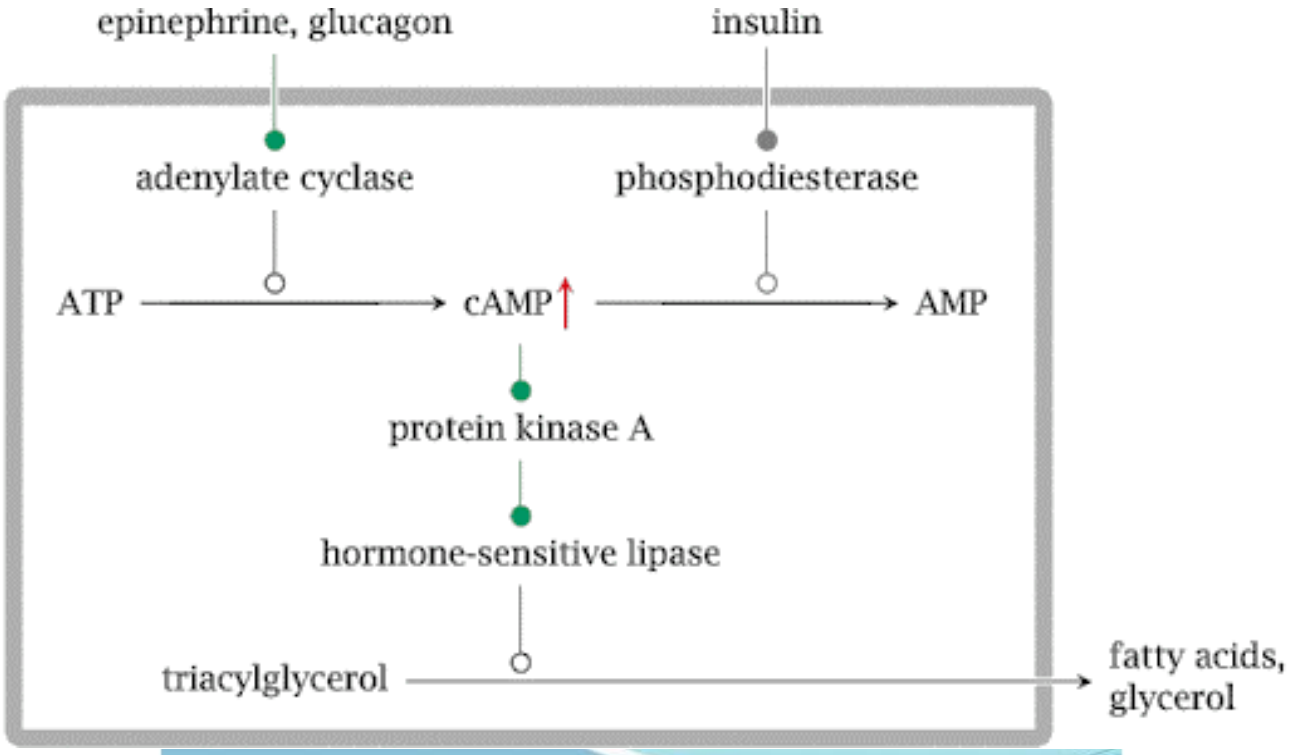

COOH
CH2—CH—NH2
Phenylalanine
HO
HO
CH—CH2—NHCH3
OH
Epinephrine
Phenylalanine
hydroxylase
Norepinephrine
N-methyl transferase
HO
COOH
HO
CH2—CH—NH2
Tyrosine
HO
HO
CH—CH2—NH2
OH
Norepinephrine
Tyrosine Oxidase
Dopamine-
B-hydroxylase
HO
COOH
HO
CH2—CH—NH2
L-DOPA
HO
HO
CH2—CH2—NH2
Dopamine
Aromatic L-amino acid
Decarboxylase

Table 22–3 Physiological and metabolic effects of epinephrine: preparation for vigorous activity

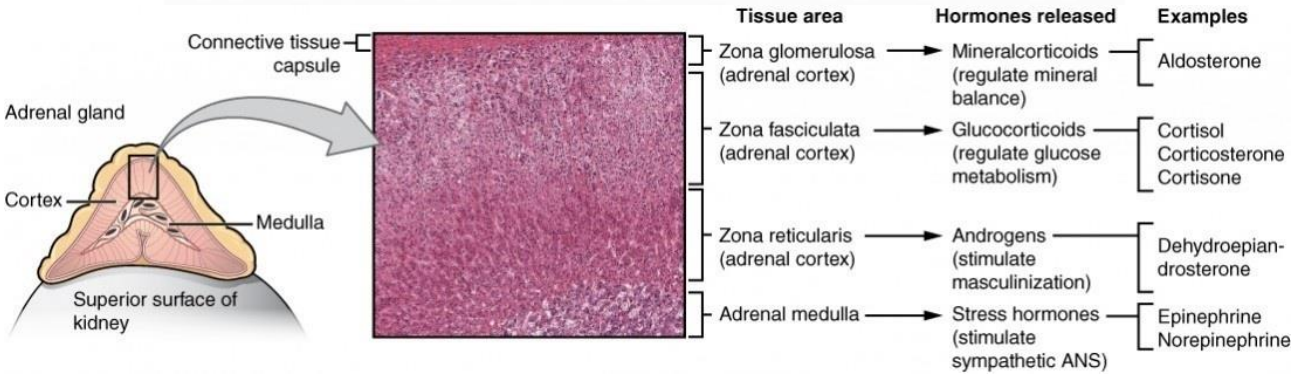
Physiological	
↑ Heart rate ↑ Blood pressure ↑ Dilation of respiratory passages	Increased delivery of O_2 to tissues (muscle)
Metabolic	
↑ Glycogen breakdown (muscle, liver) ↓ Glycogen synthesis (muscle, liver) ↑ Gluconeogenesis (liver)	Increased production of glucose for fuel
↑ Glycolysis (muscle)	Increased ATP production in muscle
↑ Fatty acid mobilization (adipose tissue)	Increased availability of fatty acids as fuel
↑ Glucagon secretion ↓ Insulin secretion	Reinforce metabolic effects of epinephrine



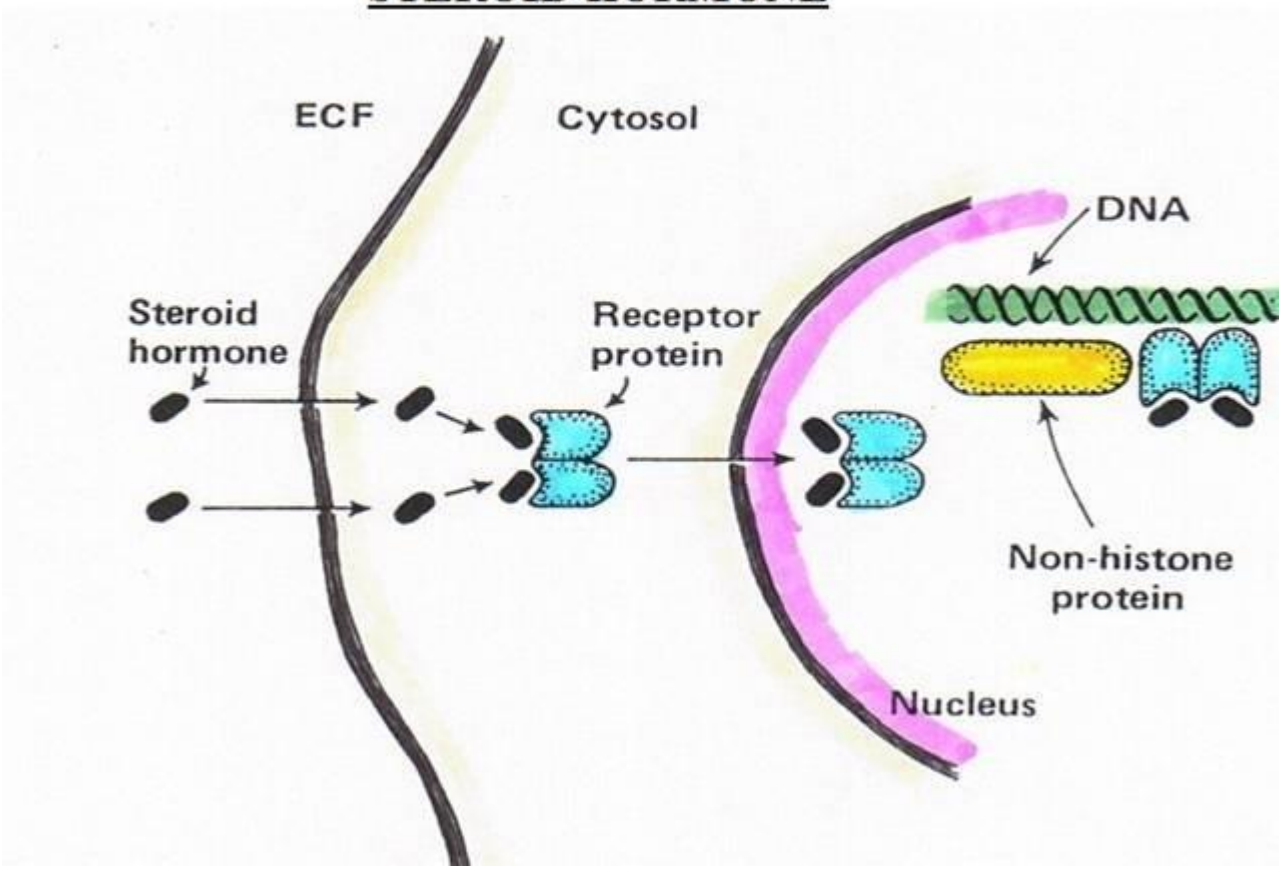


Adrenal Cortex

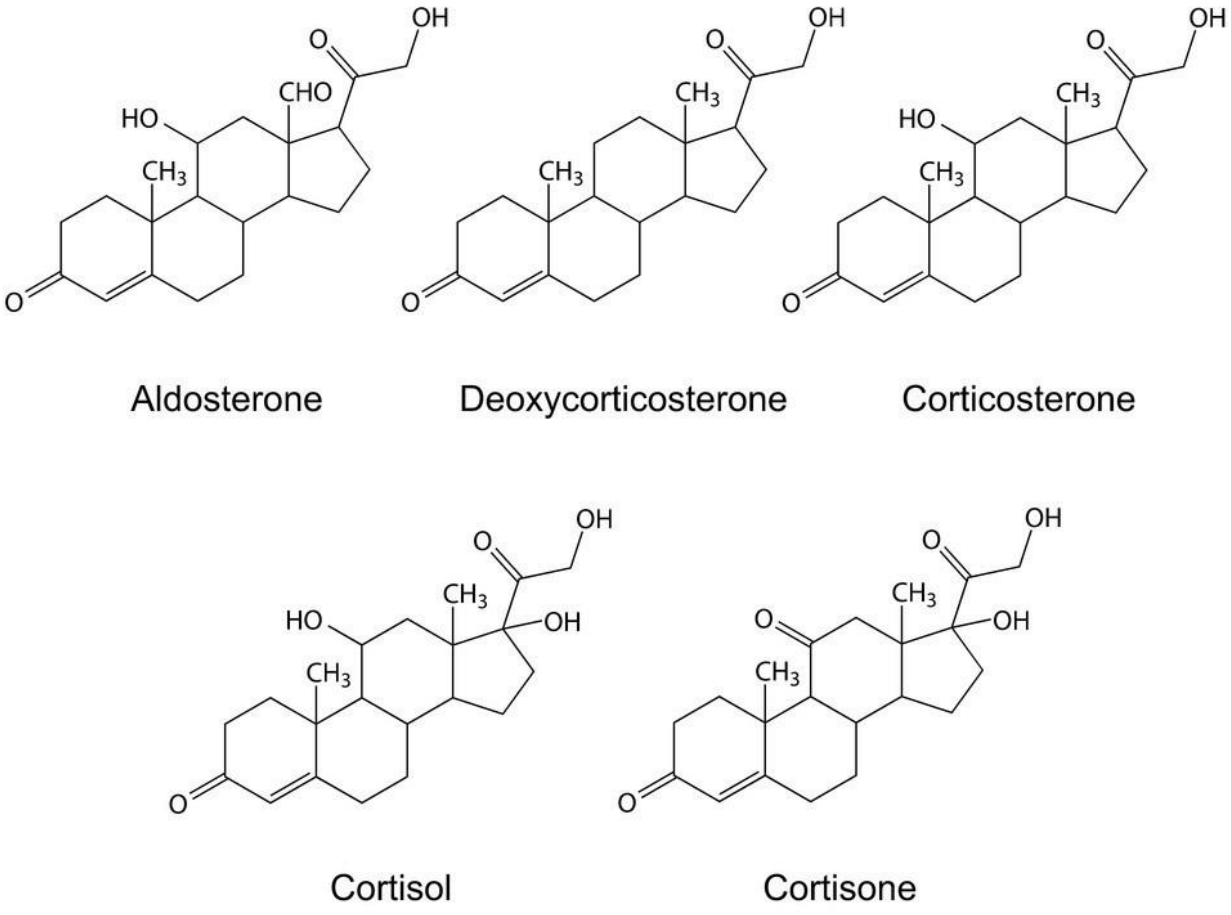
- Hormones produced by the adrenal cortex are referred to as corticosteroids.
- These comprise mineralocorticoids, glucocorticoids and androgens.
- The cortex is divided into three regions:
  - zona glomerulosa
  - zona fasciculata
  - zona reticularis

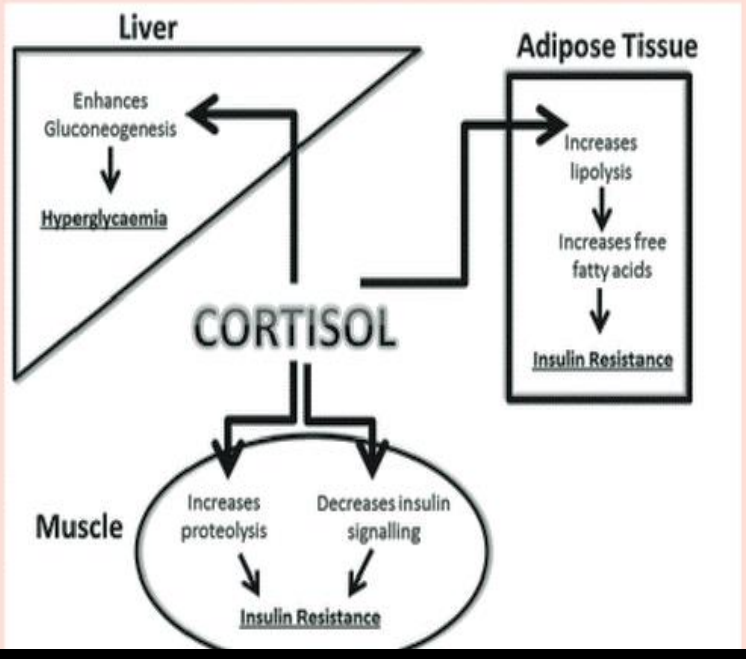


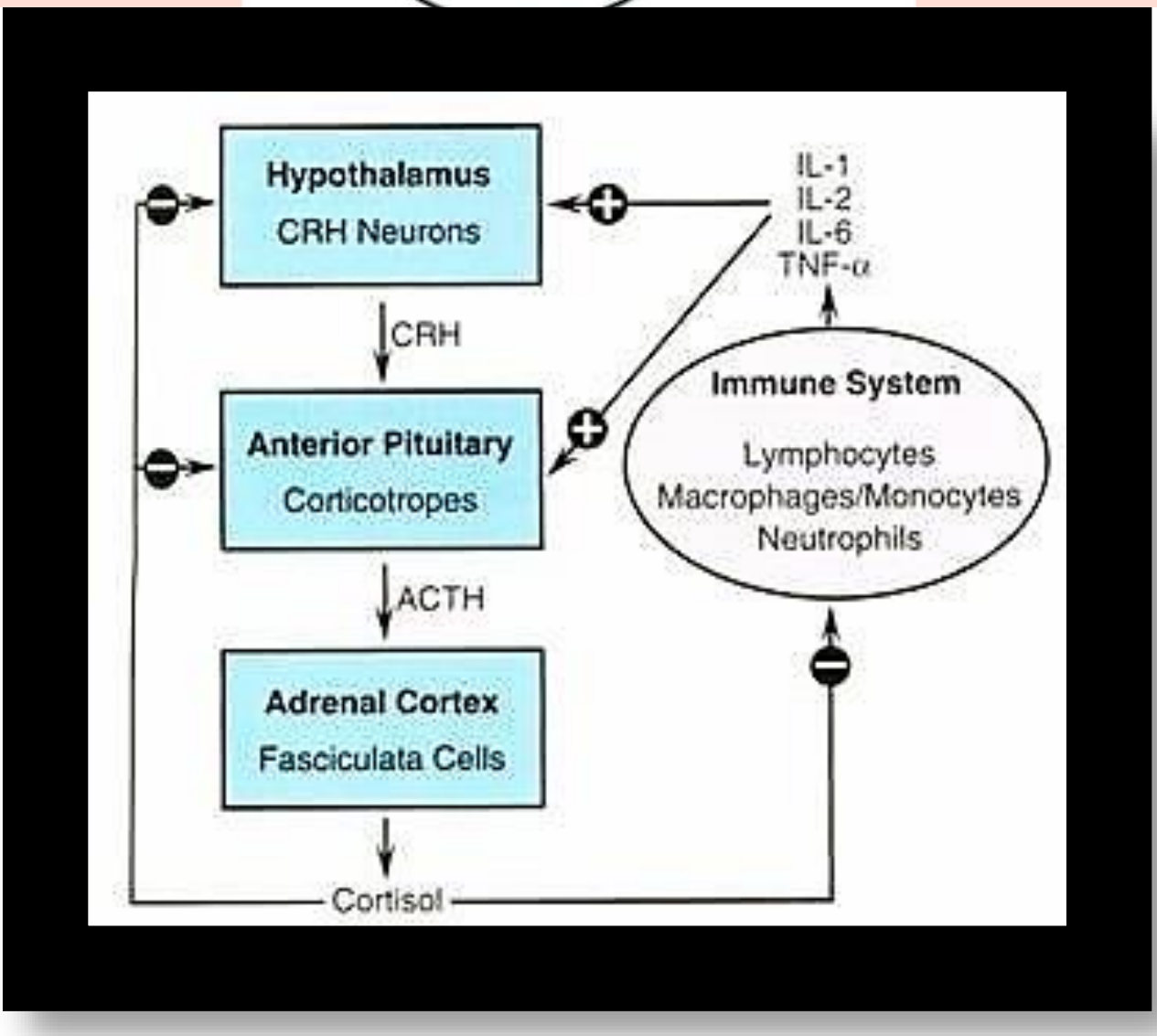
THE ACTION OF GLUCOCORTICOIDS, A TYPE OF STEROID HORMONE



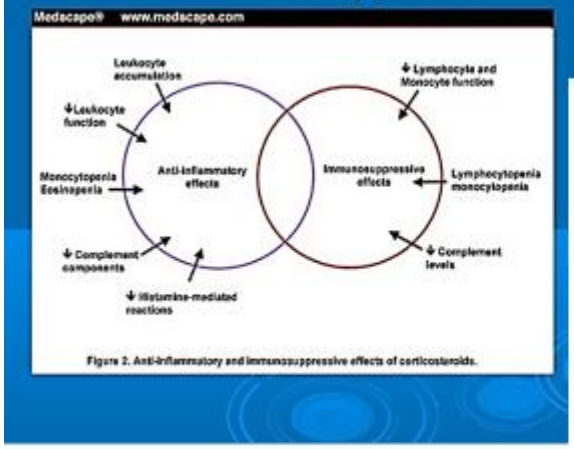
Natural mineralocorticoids and glucocorticoids




Liver
Enhances Gluconeogenesis
Hyperglycaemia
Adipose Tissue
Increases lipolysis
Increases free fatty acids
Insulin Resistance
CORTISOL
Muscle
Increases proteolysis
Decreases insulin signalling
Insulin Resistance


Hypothalamus
CRH Neurons
IL-1
IL-2
IL-6
TNF-α
CRH
Immune System
Lymphocytes
Macrophages/Monocytes
Neutrophils
Anterior Pituitary
Corticotropes
ACTH
Adrenal Cortex
Fasciculata Cells
Cortisol

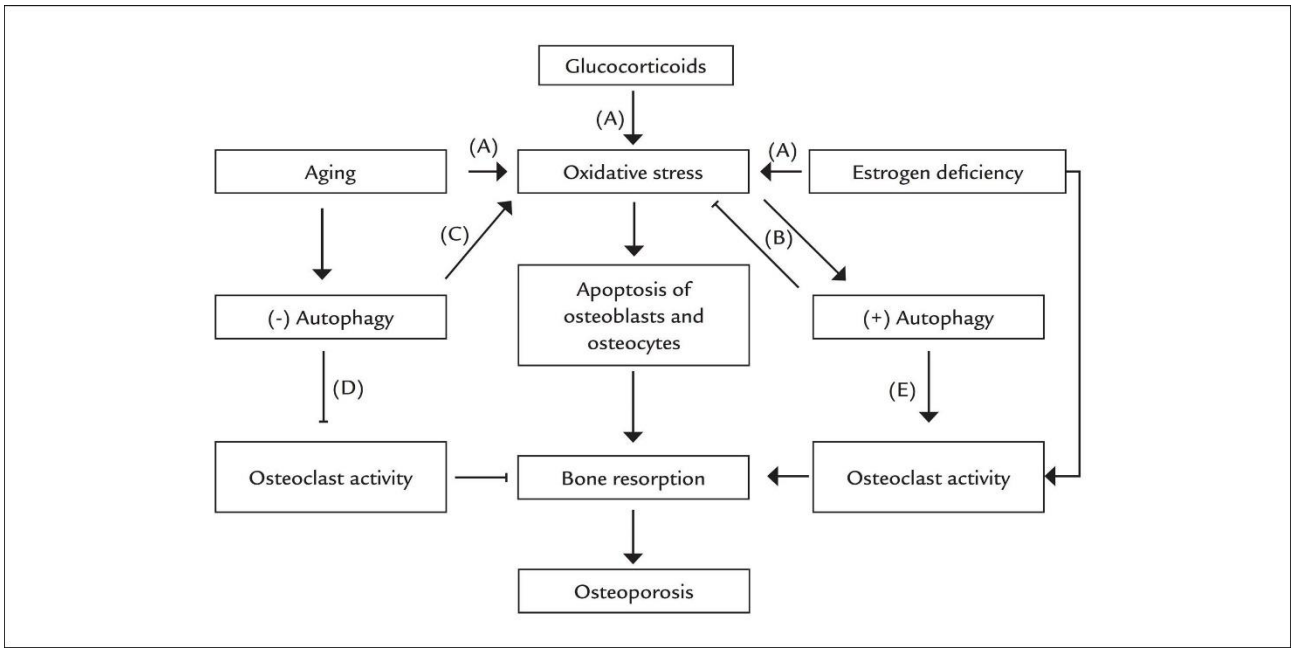
Glucocorticoids – Anti-inflammatory and Immunosuppressive effects

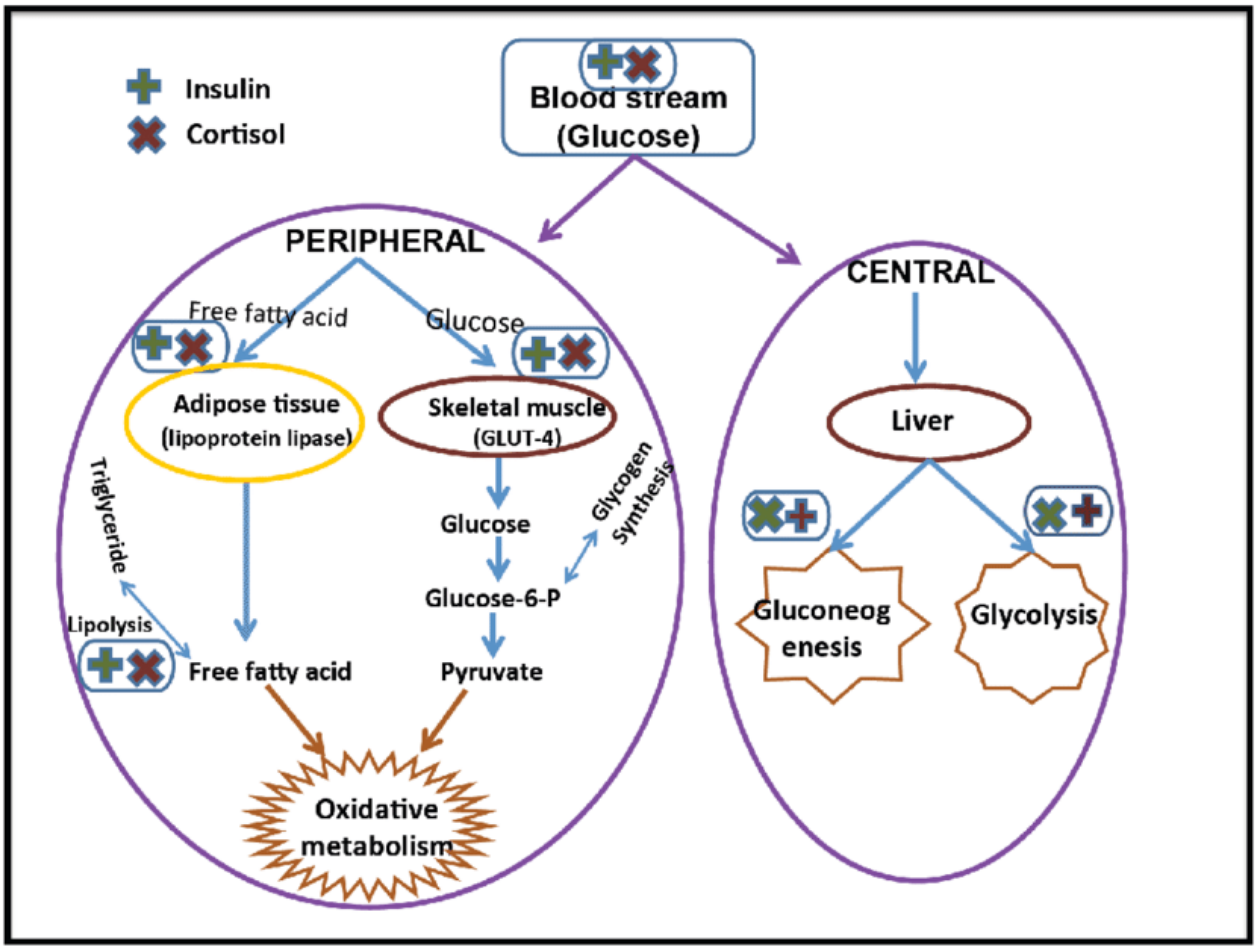


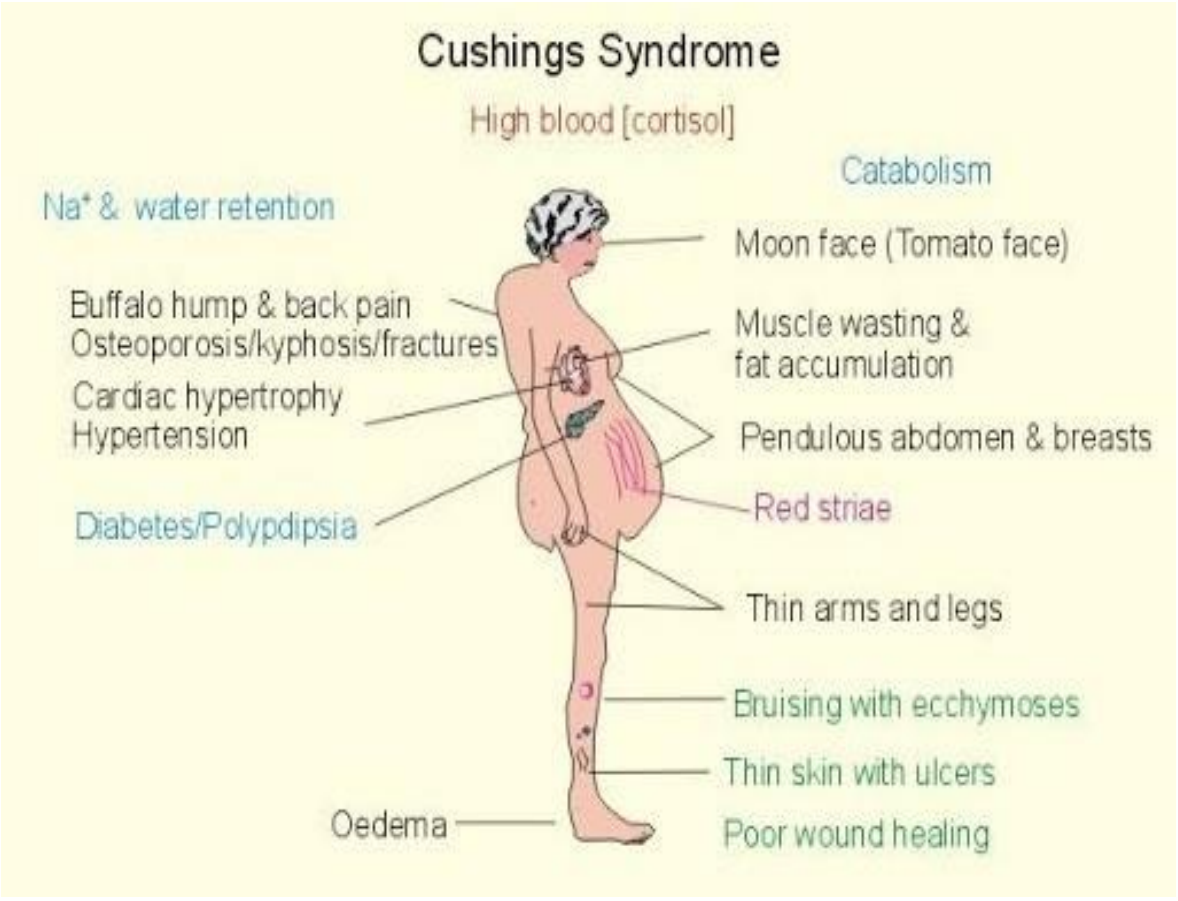
Anti-inflammatory and Anti- allergic effects of glucocorticoids

- Release from the damaged tissue cells of chemical substances
- An increase in blood flow in the inflamed area, leakage of large quantities of plasma followed by clotting of tissue fluid
- Infiltration of area by leukocytes
- Growth of fibrous tissue (Healing process)

- Stabilizes the lysosomal membranes
- Decreases the permeability of capillaries
- Decreases both migration of WBC's and phagocytosis of damaged cells, suppresses lymphocyte reproduction
- Attenuates fever (IL-1from WBC)




Insulin
Cortisol
Blood stream
(Glucose)
PERIPHERAL
CENTRAL
Free fatty acid
Glucose
Adipose tissue
(lipoprotein lipase)
Skeletal muscle
(GLUT-4)
Liver
Triglyceride
Glycogen
Synthesis
Glucose
Glucose-6-P
Lipolysis
Free fatty acid
Pyruvate
Gluconeog
enesis
Glycolysis
Oxidative
metabolism


Cushings Syndrome
High blood [cortisol]
Catabolism
Na⁺ & water retention
Moon face (Tomato face)
Buffalo hump & back pain
Osteoporosis/kyphosis/fractures
Muscle wasting &
fat accumulation
Cardiac hypertrophy
Hypertension
Pendulous abdomen & breasts
Diabetes/Polydipsia
Red striae
Thin arms and legs
Bruising with ecchymoses
Thin skin with ulcers
Oedema
Poor wound healing

Adrenal Glands

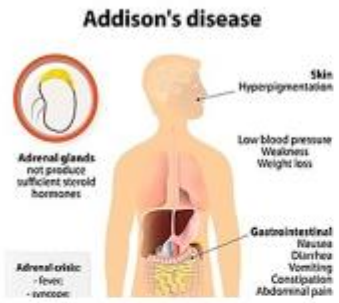
- Adrenal abnormalities
  - Hypersecretion of glucocorticoids causes Cushing syndrome: moon face, hump on back, elevated blood sugar levels, frequent infections
  - Hypersecretion of adrenal androgens may result from a virilizing tumor and cause masculinization of affected women

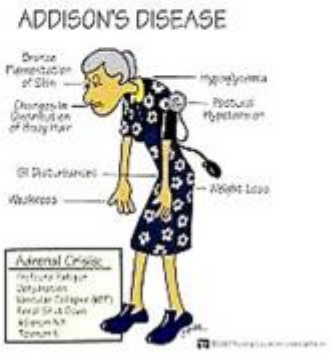
33

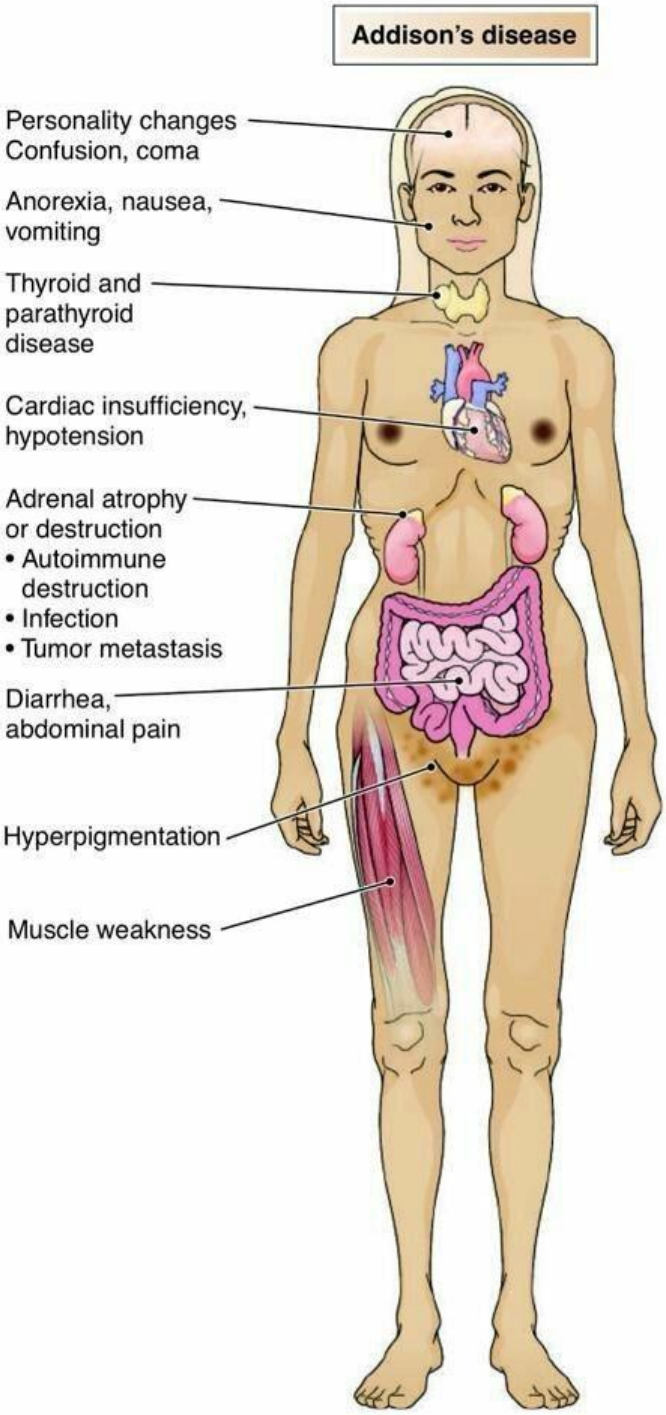
Adrenal Glands

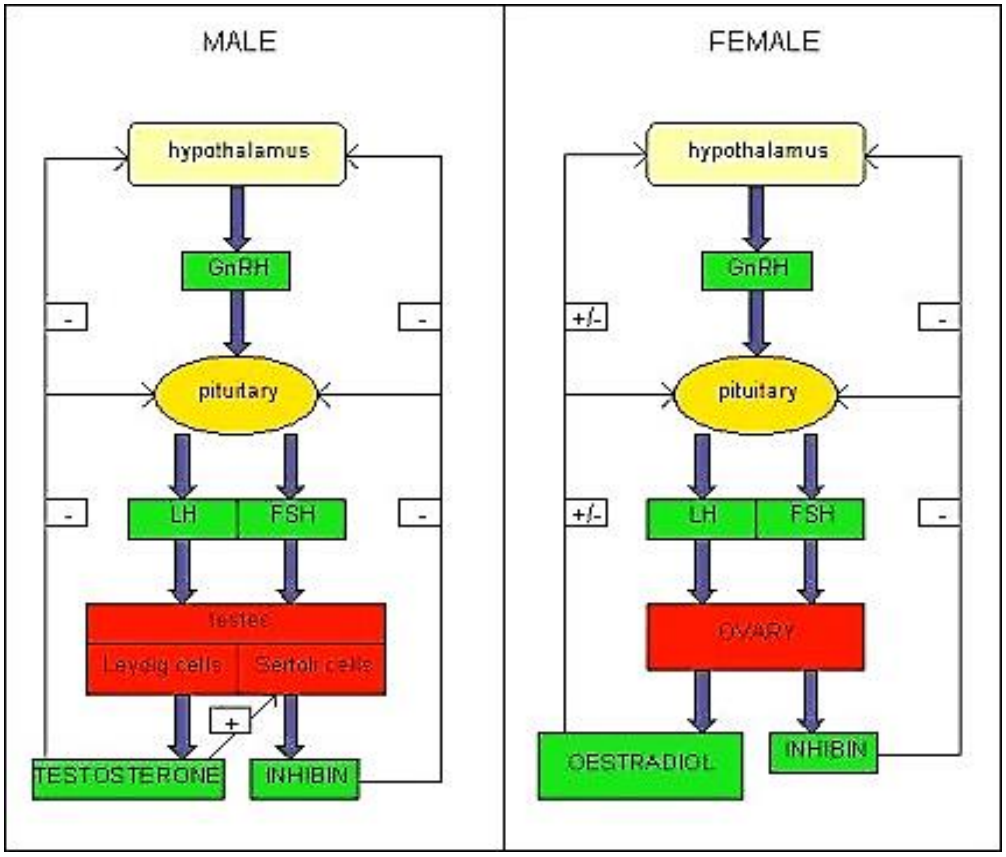
- Hyposecretion of cortical hormones may result in Addison disease: muscle weakness, reduced blood sugar, nausea, loss of appetite, and weight loss

34

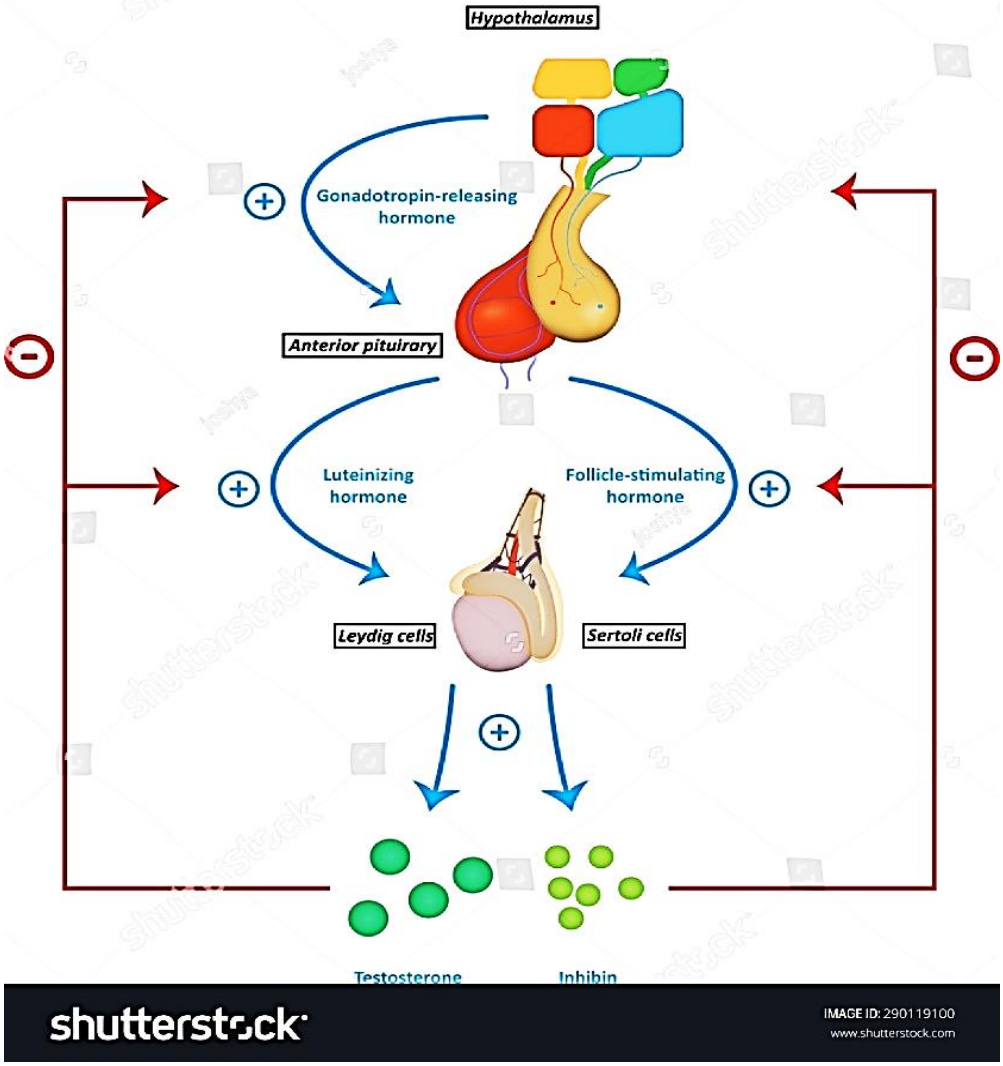

Addison's disease
Skin
Hyperpigmentation
Low blood pressure
Weakness
Weight loss
Adrenal glands
not produce
sufficient steroid
hormones
Gastrointestinal
Nausea
Diarrhea
Vomiting
Constipation
Abdominal pain
Adrenal crisis:
- fever;

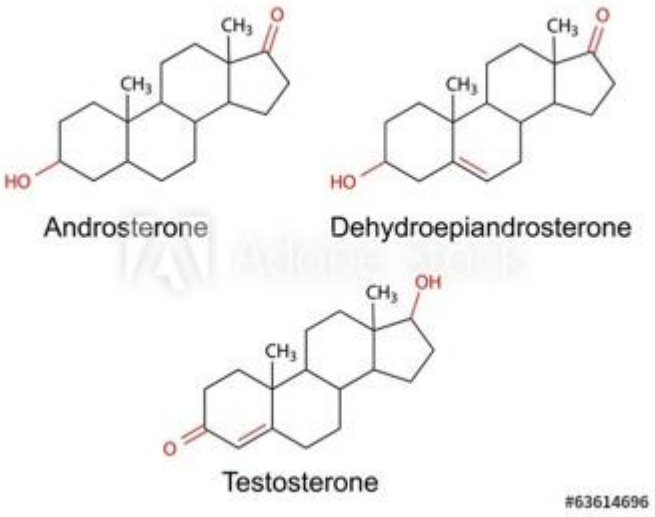

ADDISON'S DISEASE
Bronze Pigmentation of Skin
Hypoglycemia
Changes in Growth or Quality of Body Hair
Postural Hypotension
GI Disturbances
Weight Loss
Weakness
Adrenal Crisis:

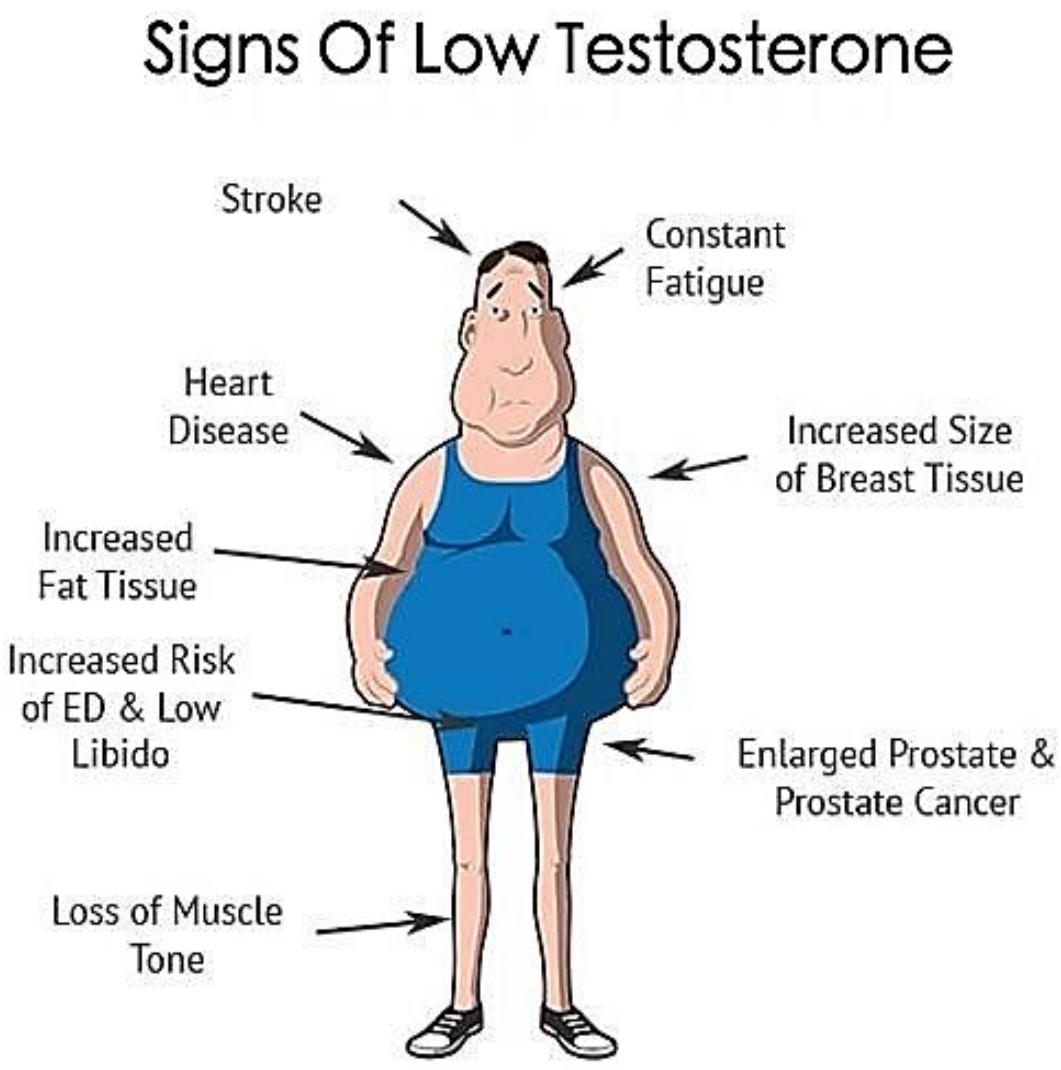

Addison's disease
Personality changes
Confusion, coma
Anorexia, nausea,
vomiting
Thyroid and
parathyroid
disease
Cardiac insufficiency,
hypotension
Adrenal atrophy
or destruction
• Autoimmune
destruction
• Infection
• Tumor metastasis
Diarrhea,
abdominal pain
Hyperpigmentation
Muscle weakness

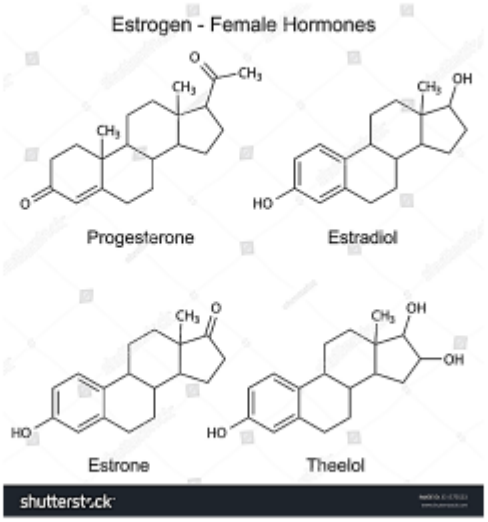

MALE
hypothalamus
GnRH
-
-
pituitary
-
LH
FSH
-
testes
Leydig cells
Sertoli cells
+
TESTOSTERONE
INHIBIN
FEMALE
hypothalamus
GnRH
+/-
-
pituitary
+/-
LH
FSH
-
OVARY
OESTRADIOL
INHIBIN

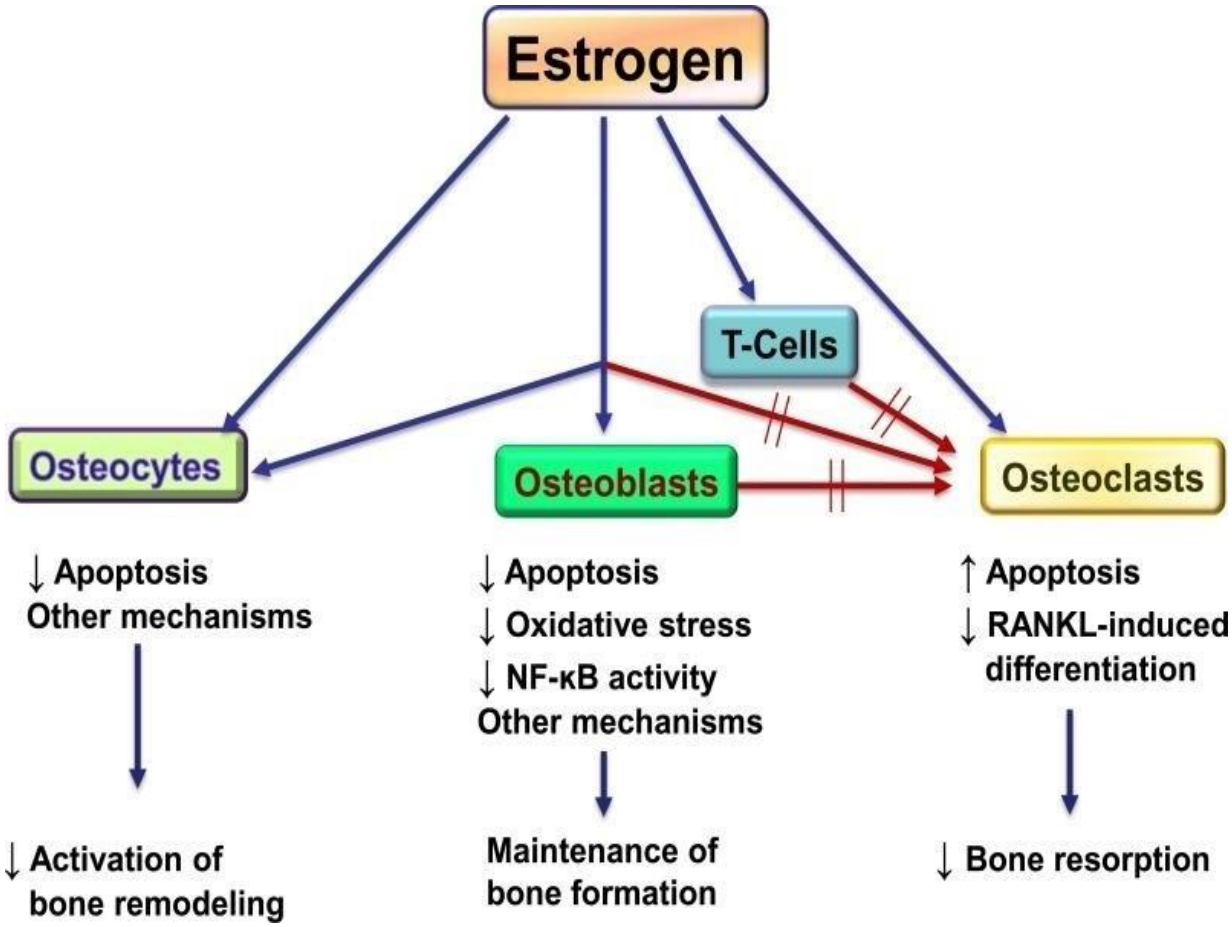
Male Hypothalamic-Pituitary-Gonadal Axis

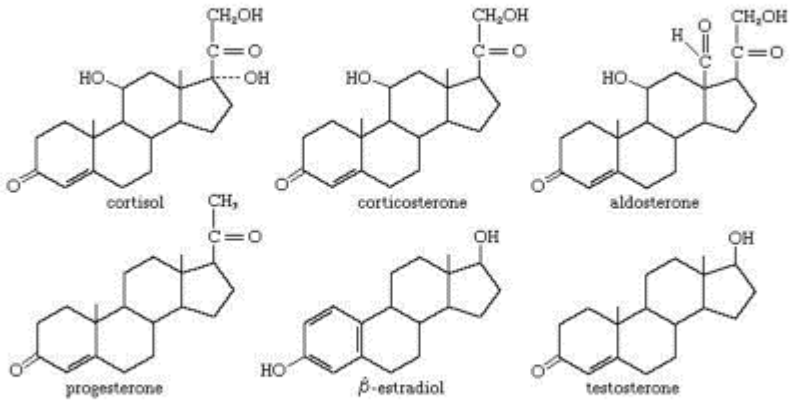


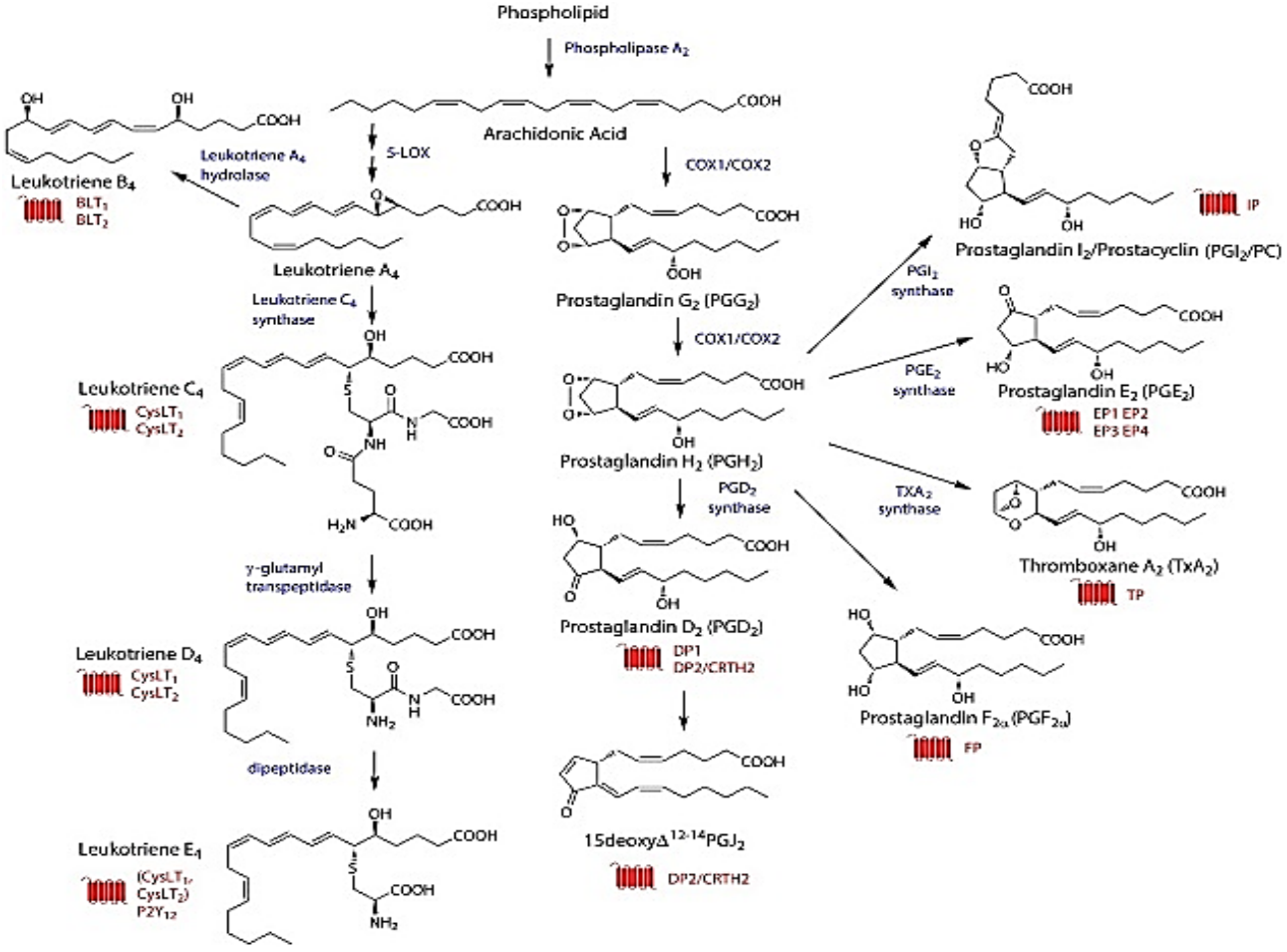

Androsterone
Dehydroepiandrosterone
Testosterone
#63614696


Signs Of Low Testosterone
Stroke
Constant Fatigue
Heart Disease
Increased Size of Breast Tissue
Increased Fat Tissue
Increased Risk of ED & Low Libido
Enlarged Prostate & Prostate Cancer
Loss of Muscle Tone


Estrogen - Female Hormones
Progesterone
Estradiol
Estrone
Theelol
shutterstock


Estrogen
T-Cells
Osteocytes
Osteoblasts
Osteoclasts
↓ Apoptosis
Other mechanisms
↓ Activation of bone remodeling
↓ Apoptosis
↓ Oxidative stress
↓ NF-κB activity
Other mechanisms
Maintenance of bone formation
↑ Apoptosis
↓ RANKL-induced differentiation
↓ Bone resorption





General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks

- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Insulin: structure, biosynthesis and secretion; influence on the metabolism of carbohydrates, lipids, amino acids and proteins. Growth-stimulating effects of insulin.
2. Glucagon: regulation of carbohydrate and lipid metabolism.
3. Thyroid hormones: structure, biological effects of T4 and T3. Disruption of metabolic processes in hypo- and hyperthyroidism.
4. Catecholamines (adrenaline, norepinephrine, dopamine): structure, biosynthesis, physiological effects, biochemical mechanisms of action.
5. Steroid hormones of the adrenal cortex (C21-steroids) – glucocorticoids and mineralocorticoids; structure, properties.
6. Female sex hormones: estrogens, progesterone. Physiological and biochemical effects; connection with the phases of the ovulatory cycle.
7. Male sex hormones (C19-steroids). Physiological and biochemical effects of androgens; regulation of synthesis and secretion.
8. Eicosanoids: structure, biological and pharmacological properties. Aspirin and other nonsteroidal anti-inflammatory drugs as inhibitors of prostaglandin synthesis.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture №13

Topic: Biochemistry of human nutrition. Vitamins and trace elements as components of human nutrition. Water-soluble vitamins.

Relevance of the topic: In addition to proteins, fats, carbohydrates, minerals and water, vitamins are needed to maintain the normal functioning of the body. This term refers to a group of additional food substances that belong to different classes of organic compounds and, with rare exceptions, are not synthesized in the human body. They have a strong and to some extent specific effect on exchange processes, and in very small quantities.
Purpose: generalization of information about the properties, the history of the discovery of vitamins, their modern classification, sources of entry into the body, daily consumption by the population of different age groups and their importance for the human body are given.

Basic concepts:
1. Cocarboxylase.
2. Beri-Beri disease.
3. Scurvy.
4. Addison-Birmer's disease.
5. Folate cycle.

Plan and organizational structure of the lecture:

1. Digestion and absorption of proteins, fats, carbohydrates.
2. Vitamins and trace elements as components of human nutrition
3. Characteristics of vitamins.
4. Classification of vitamins.
5. Water-soluble vitamins. Properties, mechanisms of action, daily need, content in food products.
6. Pathological conditions with a lack of water-soluble vitamins.

Content of the lecture material

Vitamins.

Definition - Organic compound required in small amounts.

Vitamin B1, B2, B3, B5, B6, B7, B9, B12

Vitamin A

Vitamin D

Vitamin E

Vitamin K

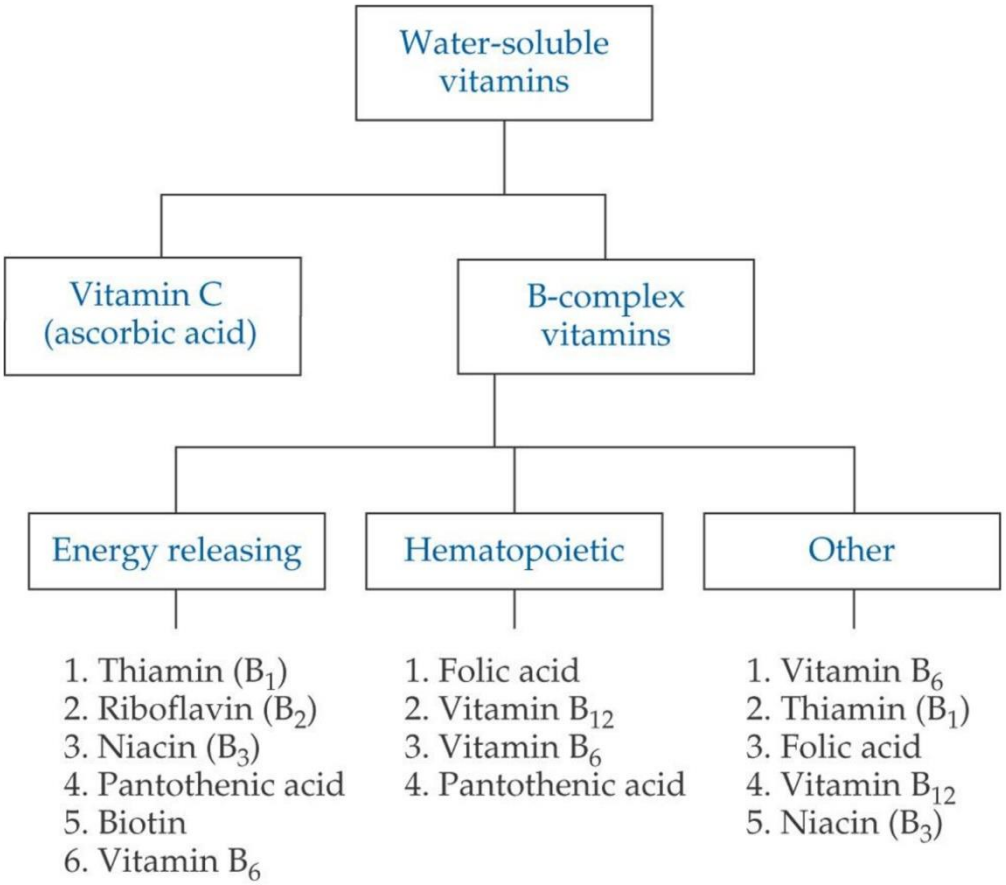
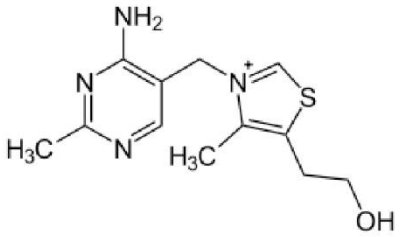


Fig. 9-1, p. 260

Vitamin B1 - Thiamine



Thiamine

Some uses:

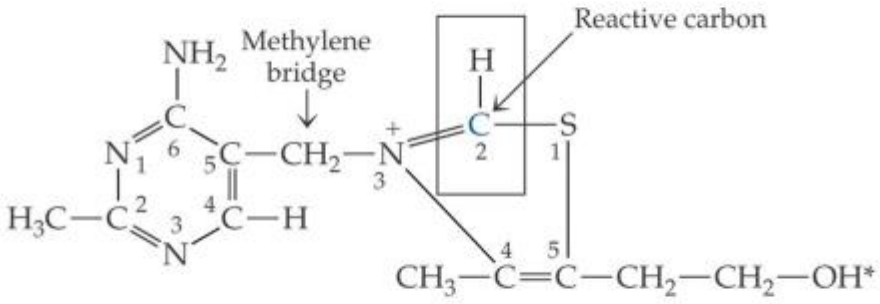
Cofactor for several enzymes (a precursor for thiamine pyrophosphate, one of the cofactors used by the pyruvate decarboxylase complex (PDC).

Also, a cofactor for branched chain a-keto dehydrogenase.

Deficiency causes beriberi (muscle atrophy, neurological problems).

Transport:

- Free form on albumin (10%) or inside RBCs as thiamin monophosphate (TMP; 90%)
- Active transport into tissues - skeletal muscle, liver, heart, kidney and brain



*Diphosphate addition occurs here to form the active coenzyme thiamin diphosphate (TDP).

Fig. 9-10, p. 275

Found in: meat, legumes, and whole, fortified, or enriched grain products, cereals and breads. Yeast and wheat germ. Pork, fish, liver, nuts.

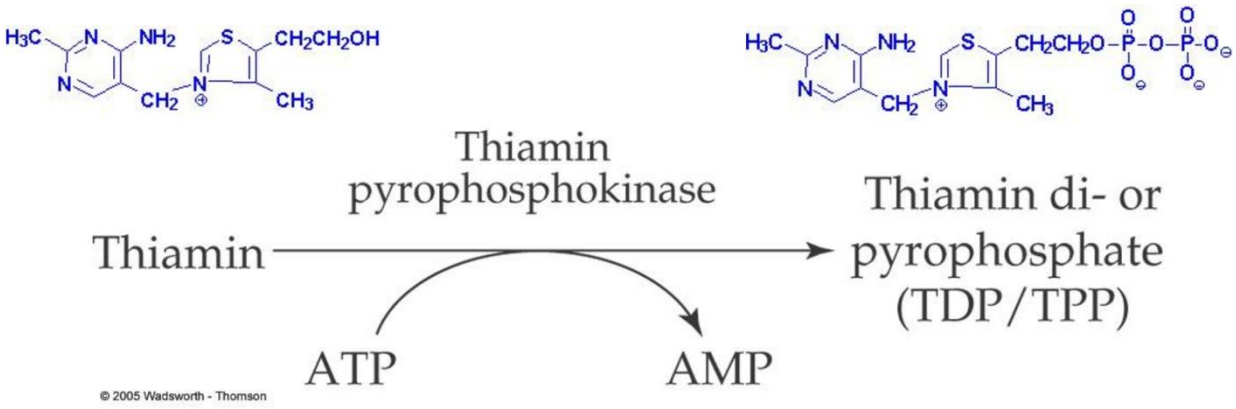
Exists in a non-phosphorylated form in plant sources, but in a phosphorylated form (thiamin pyrophosphate or TPP) in meats.

Absorption:

- Intestinal phosphatases hydrolyze the phosphates prior to absorption.
- Can be absorbed either actively, or move through membrane passively depending on concentration of vitamin present.
- Transport into blood is energy dependent and requires sodium co-transport. Inhibited by ethanol.

Functions:

- As a coenzyme in energy transformation reactions
- Synthesis of pentose sugars and NADPH as a coenzyme
- Maintenance of membrane and nerve conduction (not as a coenzyme)



p. 276a

Energy Transformation Reactions

- Pyruvate dehydrogenase complex
- A keto dehydrogenase complex
- Transketolase

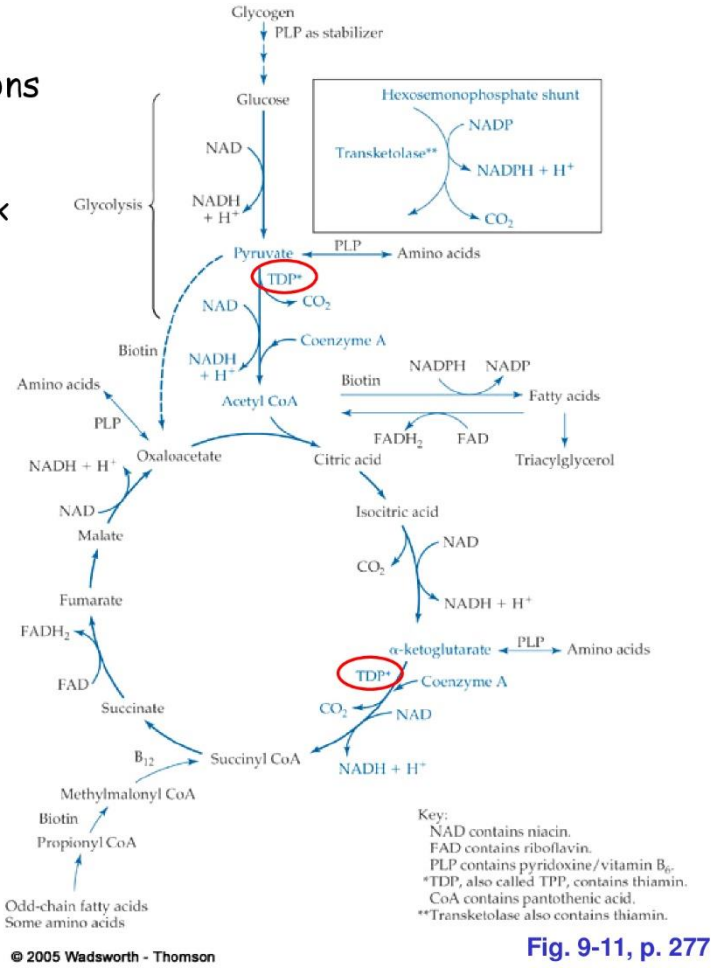


Fig. 9-11, p. 277

B-1 Thiamine

- Important in:
  - Producing energy from carbohydrates
  - proper nerve function
  - stabilizing the appetite
  - promoting growth and good muscle tone
  - ATP production

Recommendations

- Men 14+
  1.2 mg/day
- Women 14-18
  1.0 mg/day
- Women 19+
  1.1 mg/day

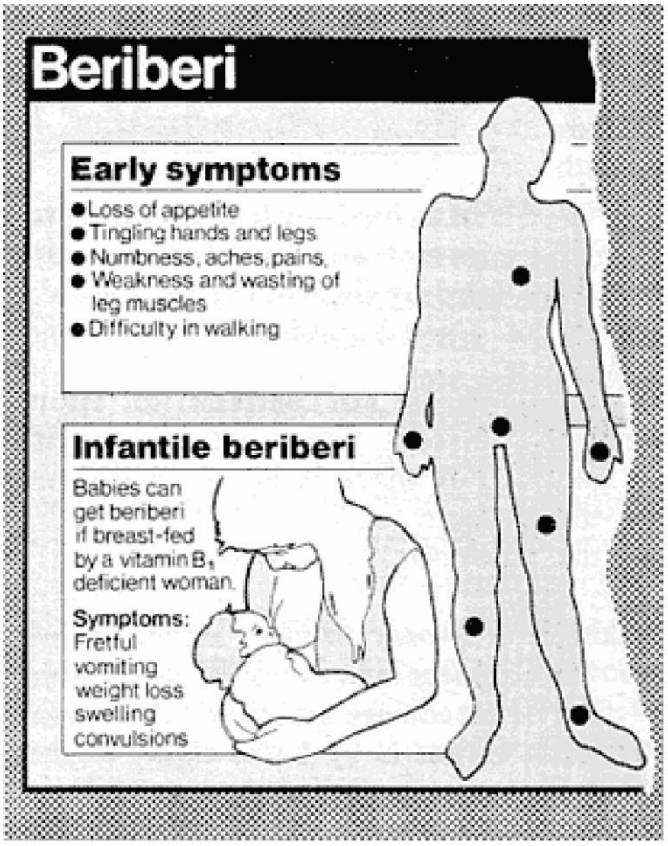
- 1 broiled pork chop,
- 1.25 cups corn flakes

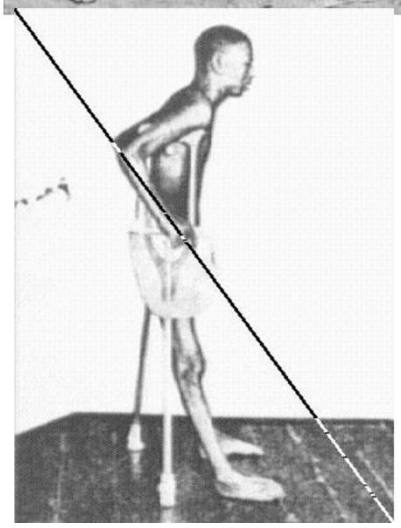
OR

- 1 baked potato (w/ skin)
- 0.5 cup of lentils
- 1 cup raisin bran

B-1 Deficiency

- Loss of appetite
- Weakness & Feeling tired
- Insomnia
- Loss of weight
- Depression
- Heart & Gastrointestinal problems



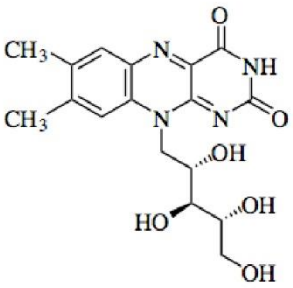


Toxicity??? Problems at 100X dose

Who's at Risk?

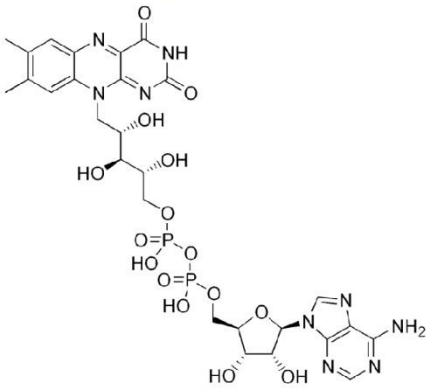
- Homeless & Malnourished
- Alcoholics
- People with malabsorption conditions

Vitamin B2 - riboflavin



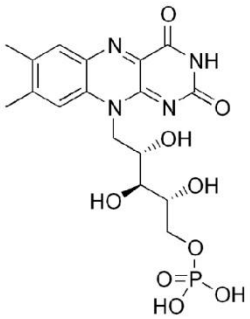
Riboflavin is a precursor for FAD and FMN.

FAD -
flavin adenine dinucleotide.



FAD is a cofactor for pyruvate dehydrogenase complex (PDC), and succinate dehydrogenase in TCA cycle.

FMN - Flavin mononucleotide



FMN is an electron carrier in the electron transport chain.

Riboflavin (B2)

Found In: milk and milk products, eggs, meat and legumes

Freed by HCl in stomach, absorbed by a saturable, energy requiring carrier in the duodenum.

Phosphorylated to form FMN, enters portal system. Transported by a variety of proteins.

Tissues take it up via carrier mediated processes – not brain!

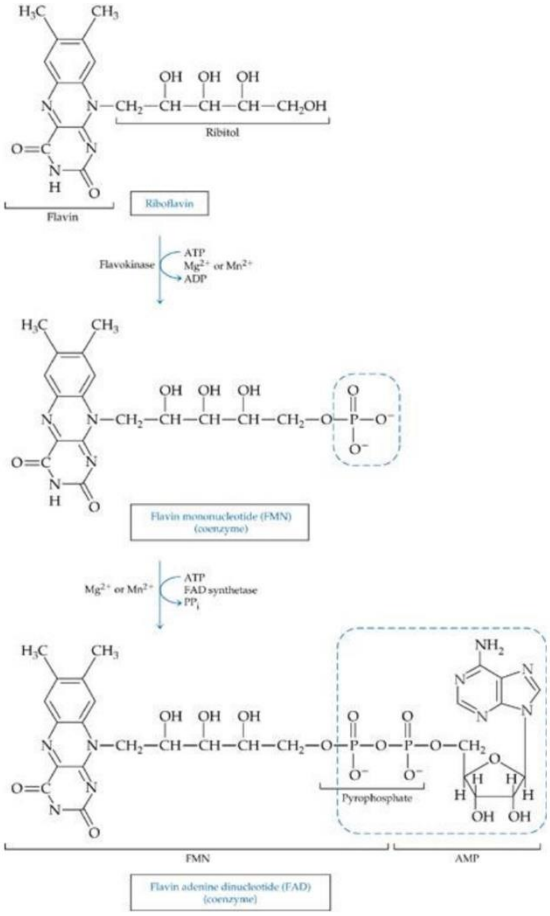
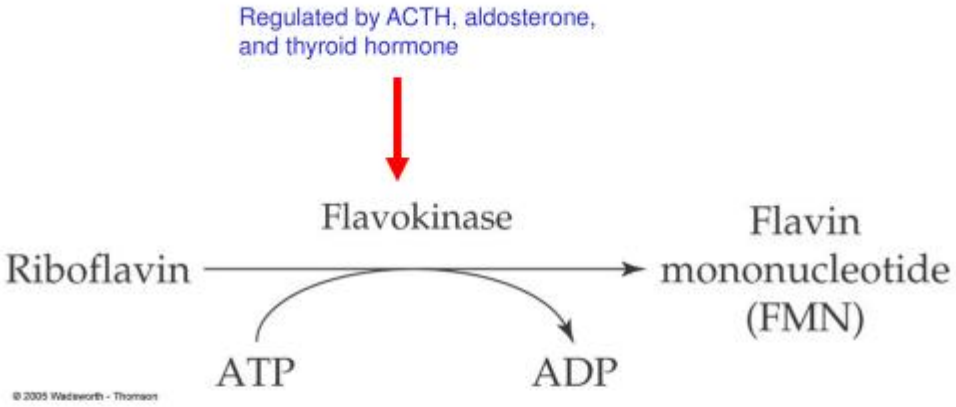
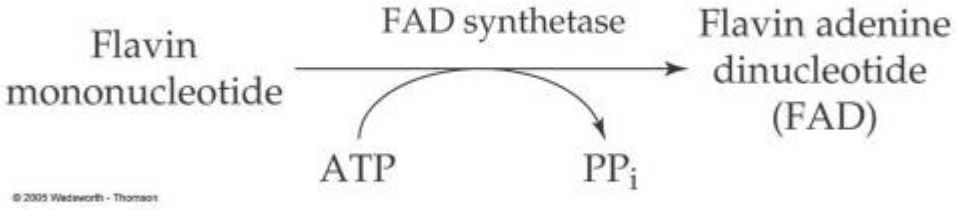


Fig. 9-14, p. 282



p. 283b



p. 283c

Functions:

- Electron transport chain (see below) or as intermediate electron carriers
  - In succinate dehydrogenase
  - In fatty acyl dehydrogenase (beta oxidation)
  - In metabolism of drugs or toxins
- As a coenzyme for: xanthine oxidase, aldehyde oxidase, pyridoxine phosphate oxidase, glutathione reductase
- Synthesis of folate
- Monoamine oxidase metabolism – neurotransmitters
- Prevention of cataracts
- Treatment of migraine headaches

284

Important in:

energy production

carbohydrate, fat, and protein metabolism

formation of antibodies and red blood cells

cell respiration

maintenance of good vision, skin, nails, and hair

alleviating eye fatigue

Recommendations

- Men 14-70
  1.3 mg/day
- Women 14-70
  1.0 mg/day
- 71+
  Larger doses

- 1 cup raisin bran
- 1 cup milk
- 1 egg

OR

- 1 small extra lean hamburger
- 1 cup plain yogurt
- 0.5 cup fresh cooked spinach
- 1 cup cottage cheese

Nutrient Interactions:

- Other B-complex vitamins
- Iron

Drug Interactions

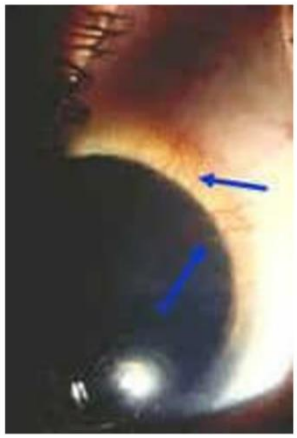
- Antipsychotic medications, chlorpromazine, and tricyclic antidepressants
- Antimalarial drugs (quinacrine)
- Chemotherapy agents (adriamycin)
- Anti-convulsant drug (phenobarbitol)

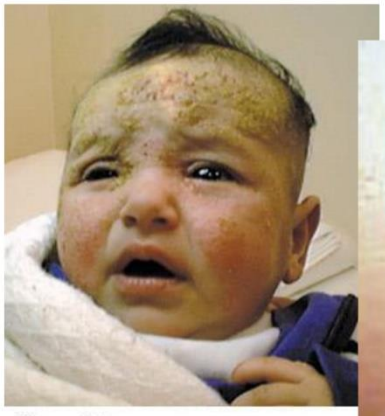
B-2 Deficiency symptoms

- Itching and burning eyes
- Cracks and sores in mouth and lips
- Bloodshot eyes
- Dermatitis
- Oily skin
- Digestive disturbances

Deficiency

- Ariboflavinosis
  - Cheliosis, angular stomatitis, magenta tongue, seborrheic dermatitis, vascularization of the cornea
  - Normochromic normocytic anemia
- Preeclampsia





c Dermatitls

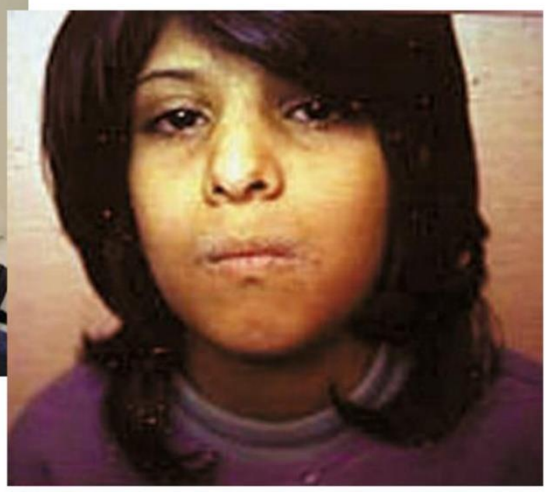
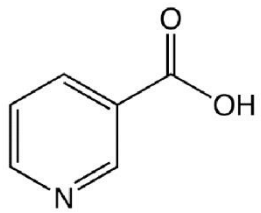
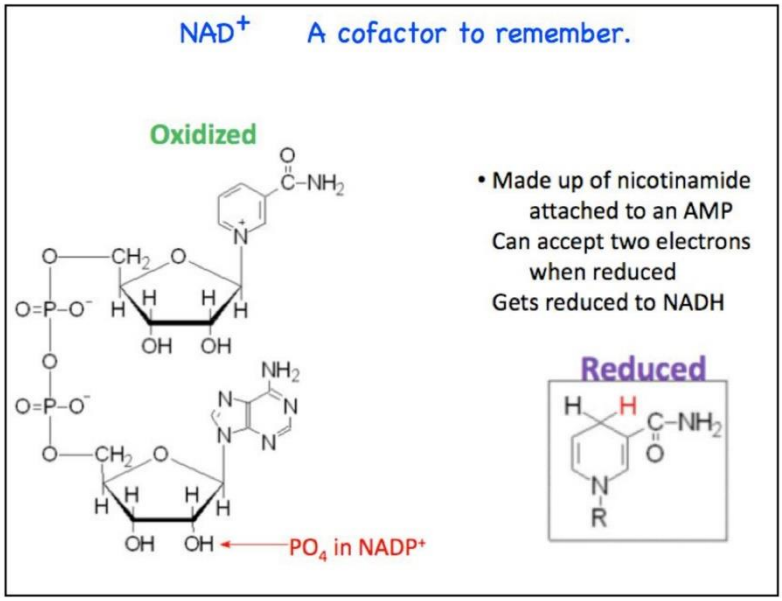


Fig. 41. Angular cheilitis

Vitamin B3 - nicotinic acid
(a precursor for NAD).
Also known as niacin.



nicotinic acid

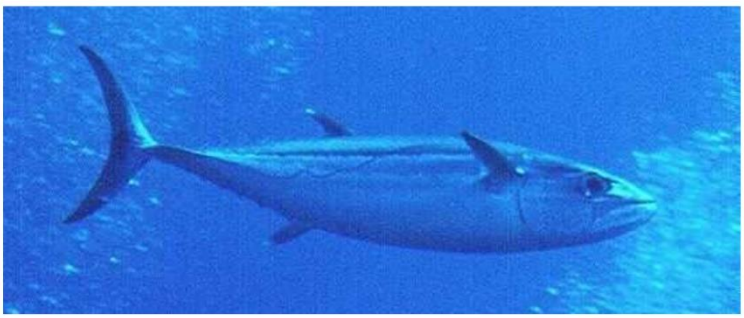


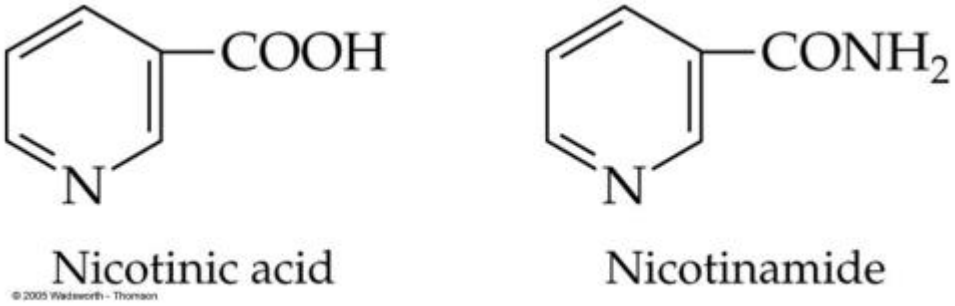
NAD^+ is needed for glycolysis, NADH gets oxidized in electron transport chain, etc.

Niacin (B3); nicotinamide, nicotinic acid

Sources:

- Fish (tuna and halibut), beef, chicken, turkey, pork (nicotinamide).
- Enriched cereals and breads, whole grains, seeds, and legumes (bound to carbs as niacytin; bound to peptides as nyacinogen).
- Coffee and tea!!!
- Synthesized in liver from tryptophan





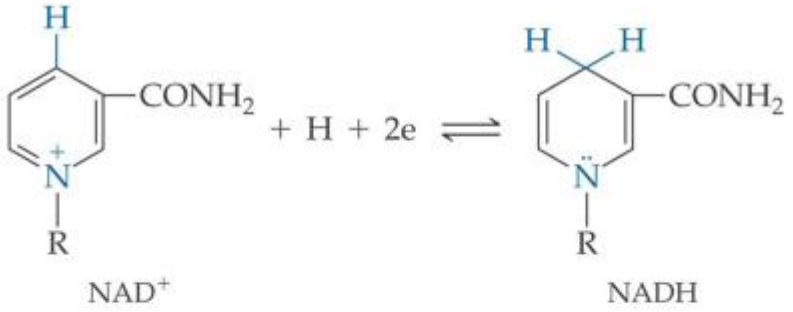
Nicotinic acid Nicotinamide

Absorption: stomach and small intestine; cotransport using sodium; saturable system at low concentrations, passive at high concentrations

In plasma as nicotinamide bound primarily to plasma proteins, then into tissues via passive diffusion

Functions: 200 enzymes require NAD or NADP as a coenzyme; act as hydrogen donors or acceptors

Fig. 9-16, p. 286



(a)

(b)

Fig. 9-19, p. 288

Recommendations

- Men 14+
  16 mg/day
- Women 14+
  14 mg/day

- 1 cup rice
- 4 oz. broiled salmon
- 1 tbsp peanut butter
- 1 bagel
- OR
- 1 small extra lean hamburger
- 0.5 cup grape nuts cereal

B-3 Deficiency

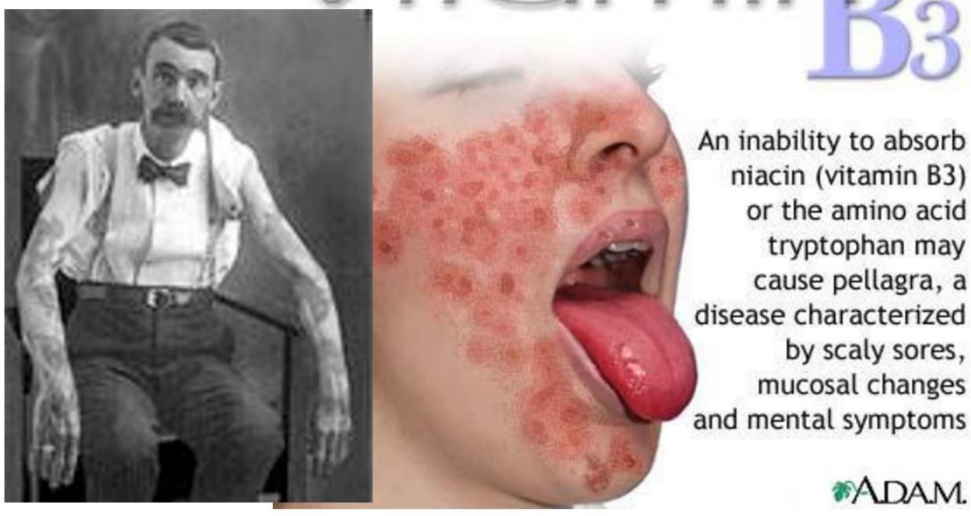
- Pellegra
  - disease caused by B-3 deficiency
  - rare in Western societies
- gastrointestinal disturbance, loss of appetite
- headache, insomnia, mental depression
- fatigue, aches, and pains
- nervousness, irritability

Breakdown and Excretion:

- Degraded by glucohydrolase into nicotinamide and ADP-ribose
- Released nicotinamide is oxidized in the liver into a variety of products; excreted in urine

Deficiency: Pellagra

- Dermatitis
- Dementia
- Diarrhea
- Death



Who's at Risk?

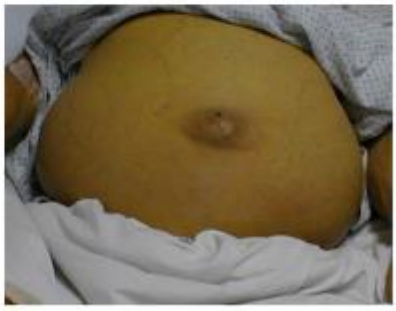
- Most people get plenty of B-3 from their diet because it is added to white flour.

Warnings

- In doses of only 50-100 mg nicotinic acid can cause dilation of blood vessels and potentially painful tingling ("niacin flush"), diarrhea, nausea, vomiting, and long term liver damage.
- Nicotinamide is almost always safe to take, although a few cases of liver damage have been reported in doses of over 1000 mg/day.

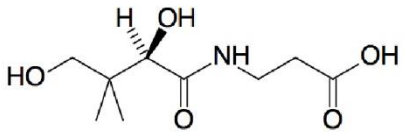
Toxicity (> 35 mg/day):

- Used to treat high cholesterol but often results in several unpleasant side effects including;
  - ·Flushing
  - ·Heartburn, nausea, vomiting
  - ·Liver injury, jaundice, hepatitis, liver failure
  - ·Hyperuricemia and gout
  - ·Glucose intolerance

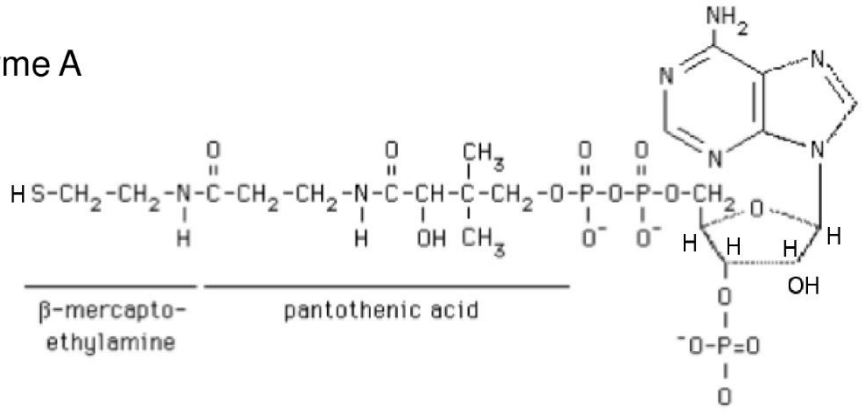


Vitamin B5 - pantothenic acid (needed for making CoA)

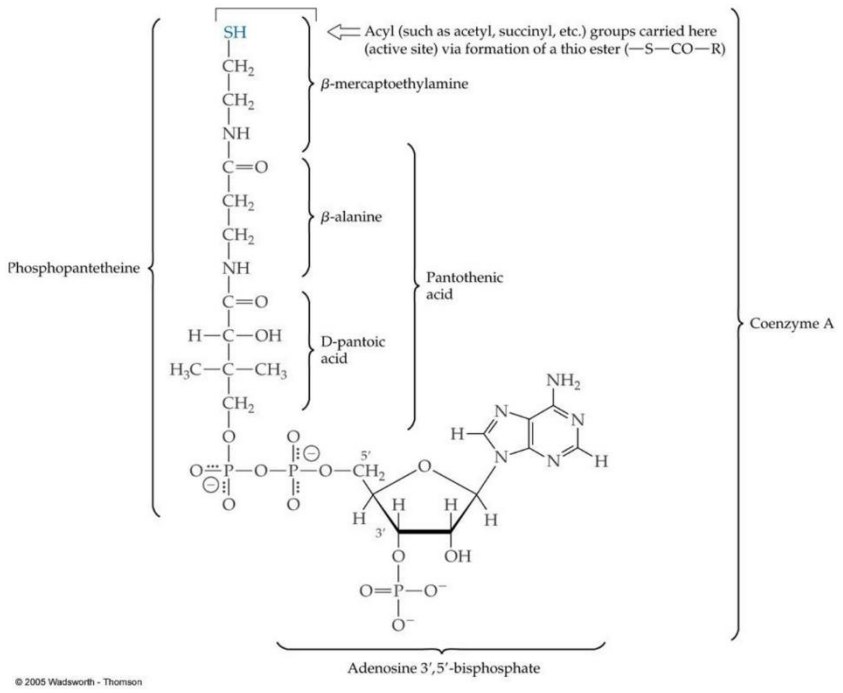
pantothenic acid



Coenzyme A



We get pantothenic acid in our diet as CoA, which must be broken down to pantothenic acid to be absorbed in intestine. We then use the pantothenic acid in making our own CoA.



Differential uptake by different tissues

Fig. 9-21, p. 293

Pantothenic Acid (pantothenate; B5)

Sources: virtually all foods; high in meats, egg yolk, potatoes, mushrooms, legumes and whole grain cereals, royal jelly from bees

Found in both free and bound forms – 85% occurs bound as a part of coenzyme A (CoA)

Absorption: CoA is hydrolysed to pantothenate; passive absorption in the jejunum, but at low concentrations shares a multivitamin transporter with biotin and lipoic acid.

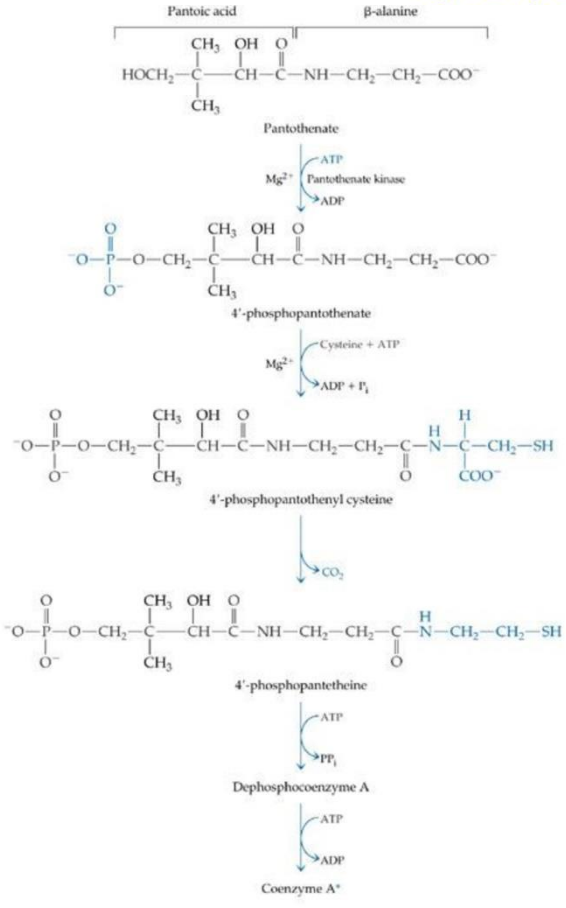


Fig. 9-20, p. 292

Functions:

- A part of coenzyme A
  - Acetylates sugars, proteins, fatty acid metabolites
  - Oxidative decarboxylation of pyruvate
  - Synthesis of cholesterol, bile salts, ketones, fatty acids, steroid hormones
- A part of 4'-phosphopantetheine
  - Prosthetic group for acyl carrier protein (ACP) in fatty acid synthesis
- Wound healing
- Cholesterol metabolism; pantethine

Excretion – primarily urine

Toxicity – rare; tingling hands and feet

Nutrient and Drug Interactions:

- Oral contraceptives containing both estrogen and progesterone

Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine and their phosphate derivatives)

Sources: Pyridoxine in plant sources; pyridoxal phaophate and pyridoxamine phosphate primarily in animal products

Overall found in meats, whole-grain products, vegies, bananas, and nuts, also fortified cereals; processing influences availability

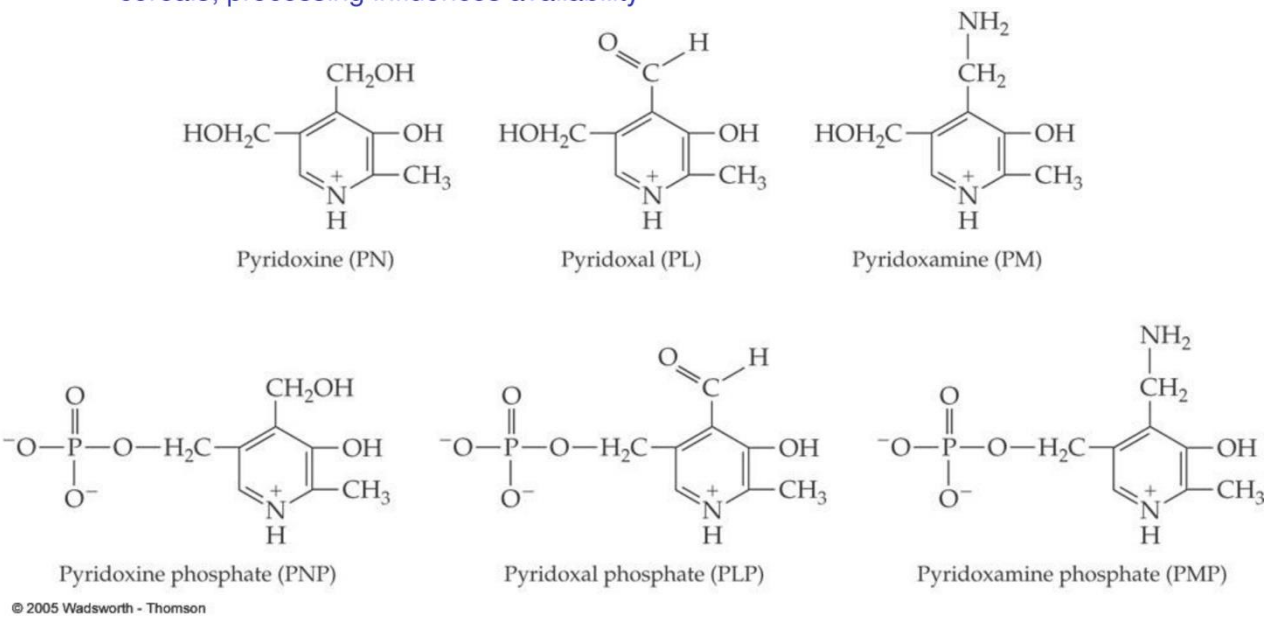


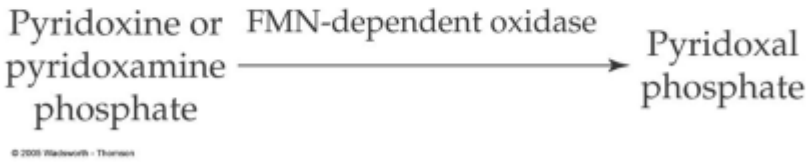
Fig. 9-38, p. 316

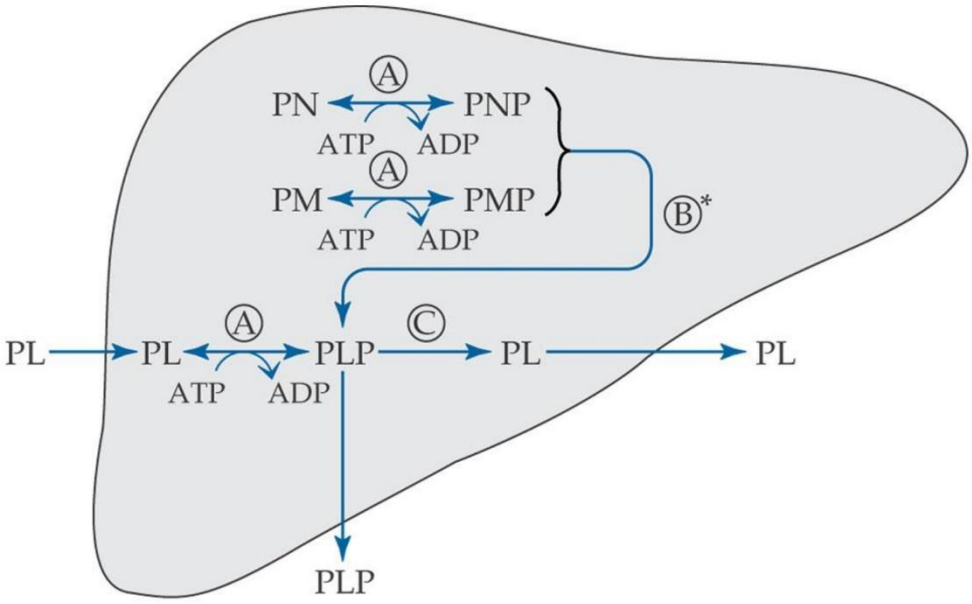
Absorption: dephosphorylation by alkaline phosphatase in the brush border; absorption in the jejunum by passive diffusion; overall efficiency about 75%

Dephosphorylated forms move into blood and taken up by the liver where it is converted by pyridoxal phosphate. This requires riboflavin.

Pyridoxial phosphate is bound to albumin for circulation in the systemic blood

Tissues only take up unphosphorylated forms (alkaline phosphatase in plasma), they are then rephosphorylated intracellularly.





Enzymes

Ⓐ Kinase (ATP dependent)
Ⓑ PMP and PNP oxidase* (FMN dependent)
Ⓒ Phosphatase

*Oxidase is found mainly in the liver and enterocytes.

Fig. 9-39, p. 316

Functions:

Coenzyme (>100 reactions) most in amino acid metabolism

- Transamination
- Decarboxylation

Initial step in glycogen metabolism

- Glycogen phosphorylase

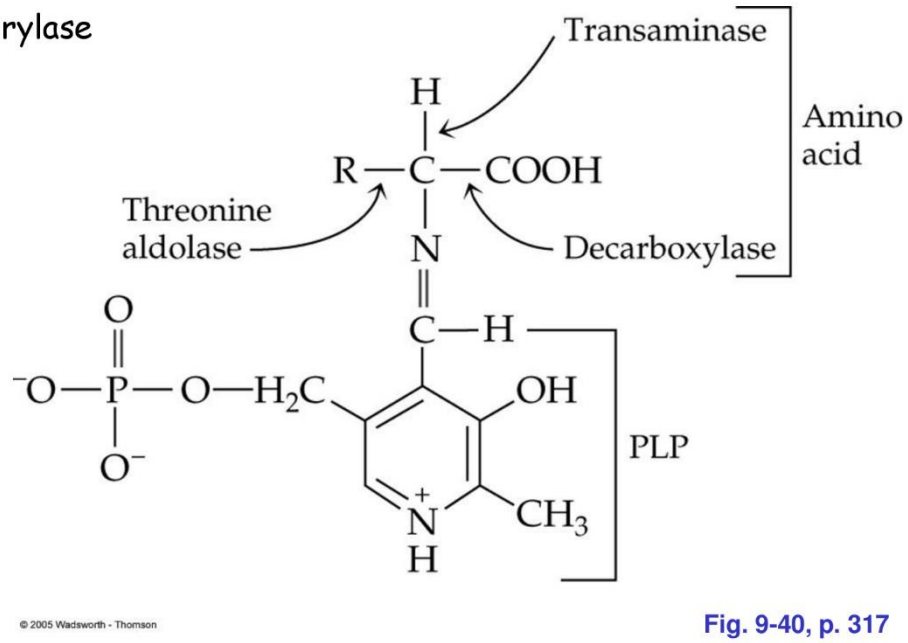


Fig. 9-40, p. 317

B-6 Pyridoxine

- Important in:
  - Production of red blood cells
  - conversion of tryptophan to niacin (B-3)
  - immunity
  - nervous system functions
  - reducing muscle spasms, cramps, and numbness
  - maintaining proper balance of sodium and phosphorous in the body

Recommendations

- Men 14-50
  1.3 mg/day
- Men 50+
  1.7 mg/day
- Women 14-18
  1.2 mg/day
- Women 19-50
  1.3 mg/day
- Women 50+
  1.5 mg/day

- 1 chicken breast
- 0.5 cup cooked spinach
- 1 cup brown rice

OR

- 1 baked potato with skin
- 1 banana
- 4 oz. lean sirloin

Deficiency: relatively rare

Symptoms include: sleepiness, fatigue, cheilosis, glossitis, stamatitis, and neurological problems; alteration of magensium and calcium metabolism; impairs niacin synthesis; inhibits homocysteine metabolism nervousness, insomnia, loss of muscle control, muscle weakness arm and leg cramps, water retention, skin lesions

Toxicity: sensory and peripheral neuropathy (100 mg/day);

High doses used for hyperhomocysteinemia, carpal tunnel syndrome, premenstrual syndrome, depression, muscular fatigue, and autism.

Warnings:

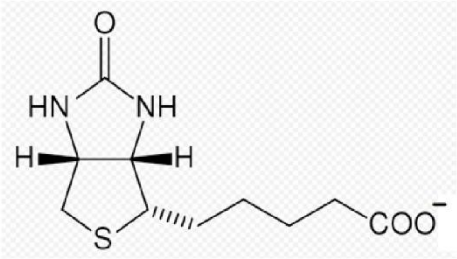
High doses of B-6 may be recommended to treat PMS, carpal tunnel syndrome, and sleep disorders, but continued use of high and doses may result in permanent nerve damage.

Who's at Risk?

- very rare
- alcoholics
- patients with kidney failure
- women using oral contraceptives

Vitamin B7 – Biotin (vitamin H).

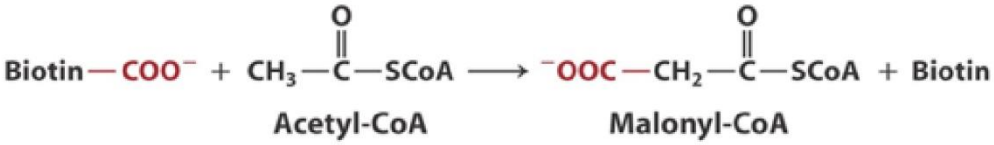
Used in fatty acid synthesis, as a cofactor of acetyl-CoA-carboxylase.



Acetyl-CoA is converted to malonyl-CoA for use in FA synthesis.

Acetyl – CoA Carboxylase

$$\text{Biotin} + HCO_3^- + ATP \longrightarrow \underset{\text{activated biotin}}{\text{Biotin} - COO^-} + ADP + P_i$$



Biotin (B7)

Sources: liver, soybeans, egg yolk, cereals, legumes, nuts; found combined to lysine (biocytin) or other proteins; intestinal bacteria can synthesize biotin

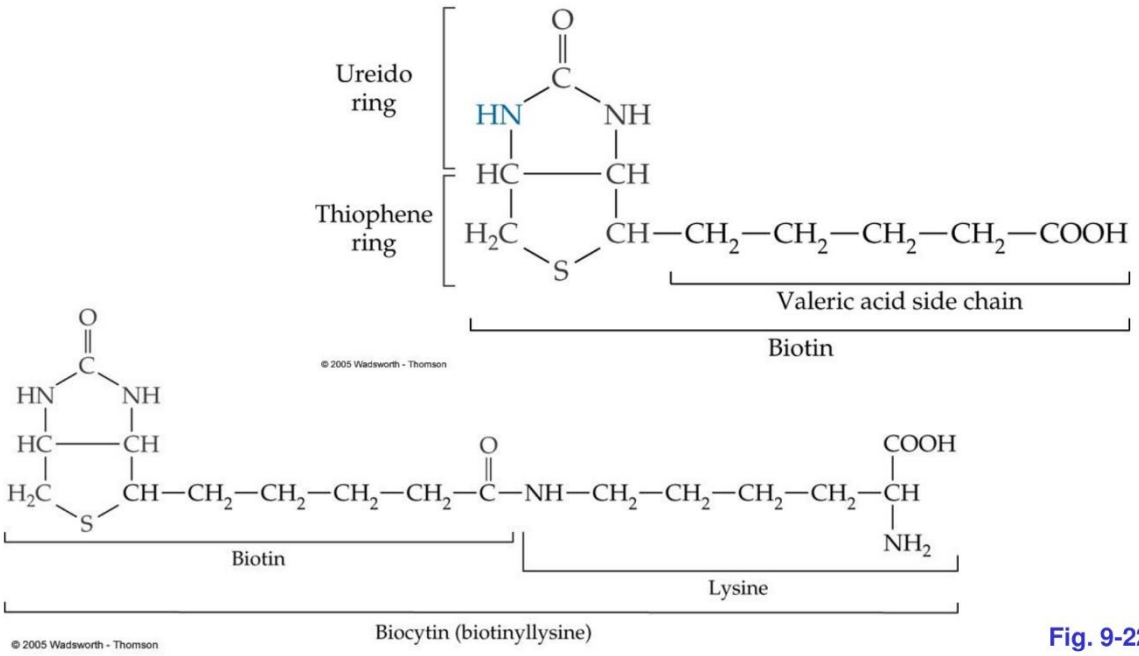


Fig. 9-22, p. 295

Absorption:

- Protein portion digested off by peptidases or biotinidase
- 100% absorption in the jejunum and ileum
- Passive diffusion into enterocytes; carrier mediated into plasma; cotransport with sodium into tissues
- Stored in small quantities in muscle, liver, and brain

Functions:

- Coenzyme for carboxylases; move a carboxyl group from one compound to another

Table 9.2 Biotin-Dependent Enzymes

Enzyme	Role	Significance
Pyruvate carboxylase	Converts pyruvate to oxaloacetate	Replenishes oxaloacetate for Krebs cycle. Necessary for gluconeogenesis
Acetyl CoA carboxylase	Forms malonyl CoA from acetate	Commits acetate units to fatty acid synthesis
Propionyl CoA carboxylase	Converts propionyl CoA to methylmalonyl CoA	Provides mechanism for metabolism of some amino acids and odd-numbered–chain fatty acids.
β-methylcrotonyl CoA carboxylase	Converts β-methylcrotonyl CoA to β-methylglutaconyl CoA	Allows catabolism of leucine and certain isoprenoid compounds

Table 9-2, p. 296

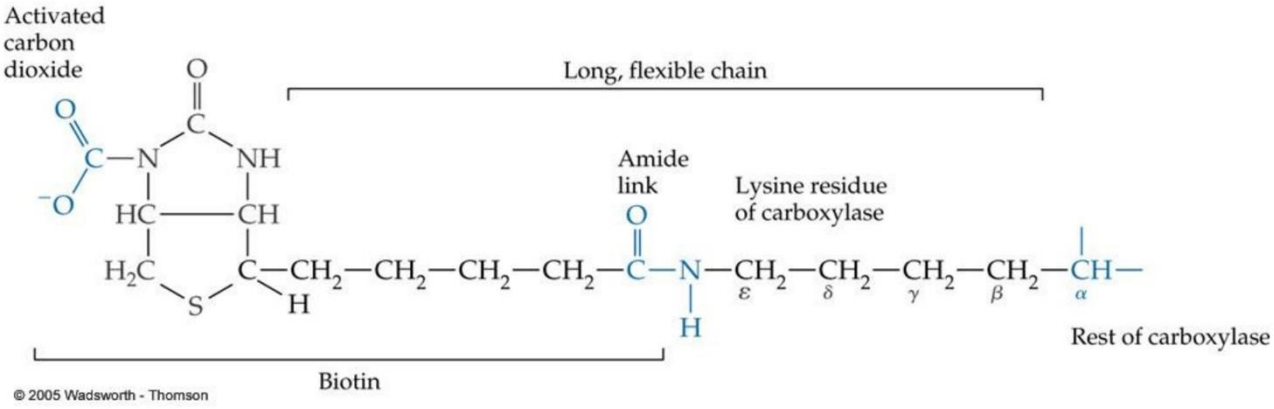


Fig. 9-24, p. 297

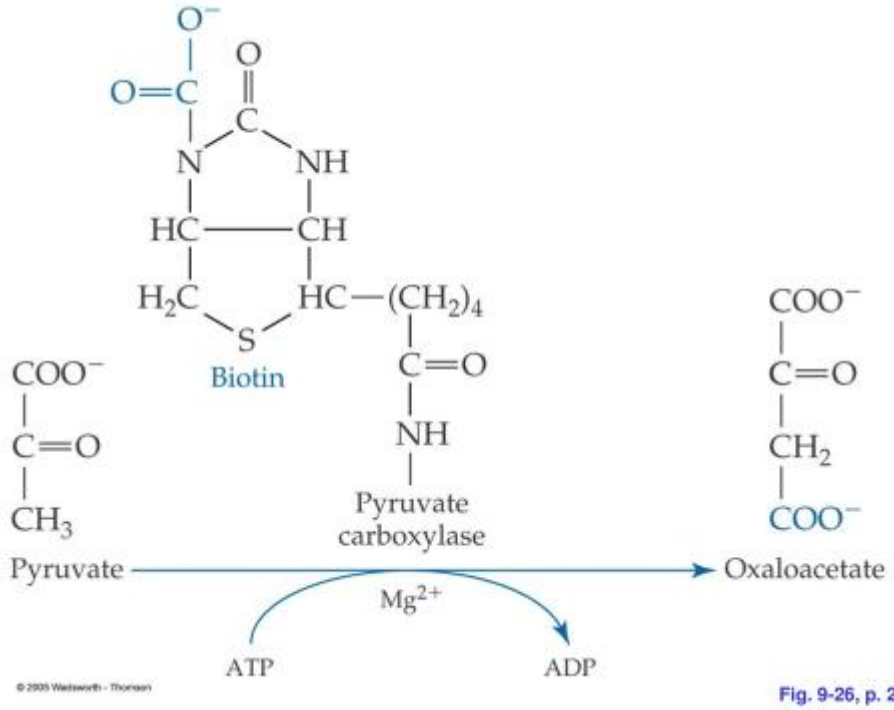
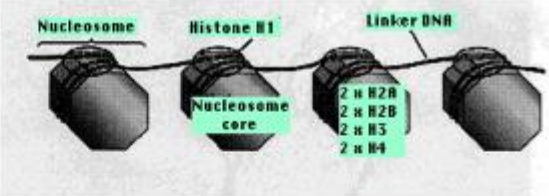
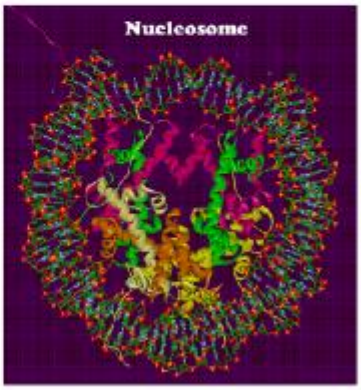


Fig. 9-26, p. 298



- **DNA maintenance/cell cycle**
- **Gene expression**
  - ↑ Insulin, insulin receptor, glucokinase
  - ↓ PEP carboxykinase



Deficiency: Lethargy, depression, hallucinations, muscle pain, parathesia, anorexia, nausea, alopecia, dermatitis

- May occur during pregnancy
- May occur with excessive consumption of alcohol
- Is known as a teratogen in mammals

Folic Acid (folacin; folate; pteropolyglutamate)

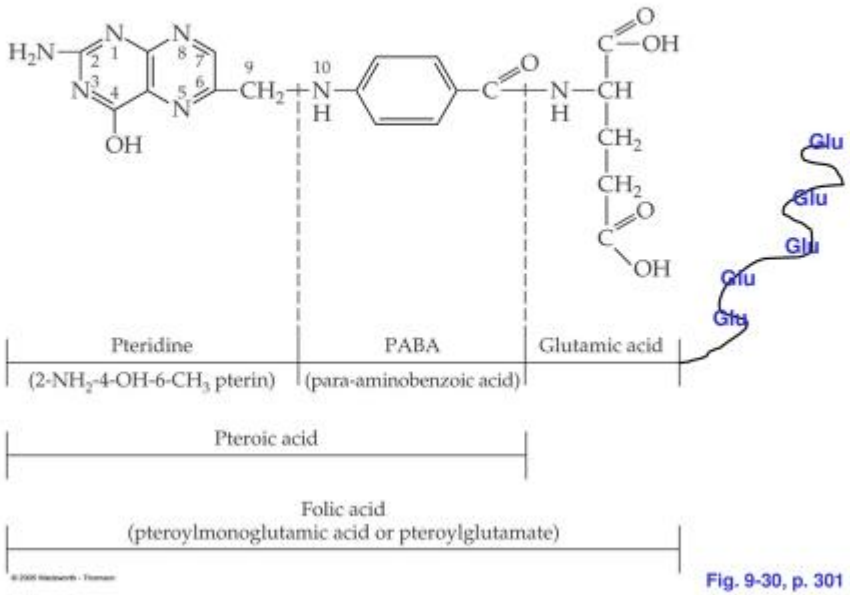
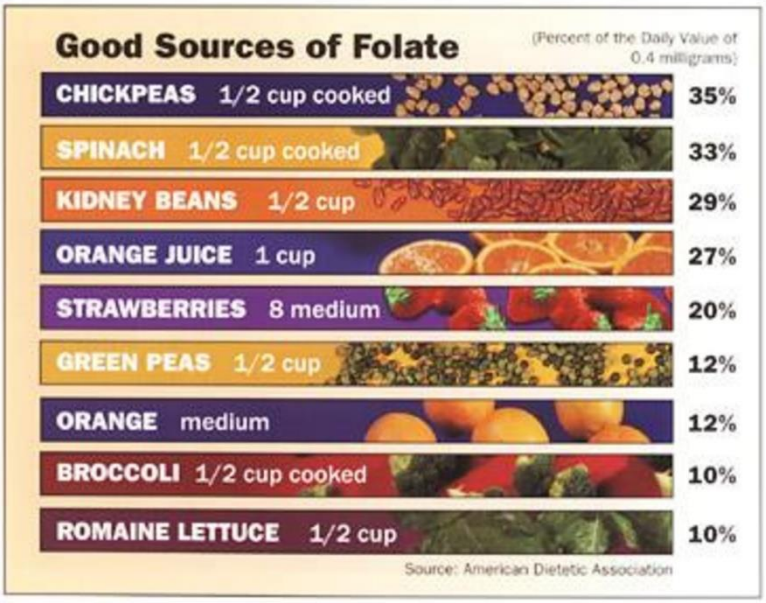


Fig. 9-30, p. 301

Sources: mushrooms, green leafy vegies, legumes, citrus fruits, and liver; raw foods have more than cooked



Absorption: brush border enzymes cut off the glutamates (requires Zinc; inhibited by alcohol, legumes, lentils, cabbage and oranges); transporter is pH and sodium dependent; mostly in jejunum; efficiency estimated to be about 50%

Functions:

- Coenzyme – accepts one-carbon groups then donates them in synthetic reactions (histidine, serine, glycine, methionine)
  - Plays a role in reducing blood homosysteine levels

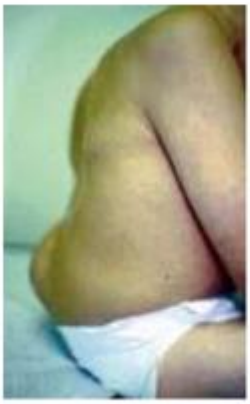
5-formimino THF	—HC=NH—
5,10-methenyl THF	=CH—
5,10-methylene THF	—CH_2—
5-methyl THF	—CH_3

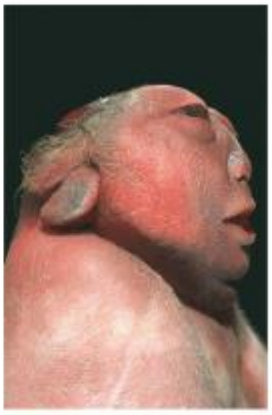
p. 303

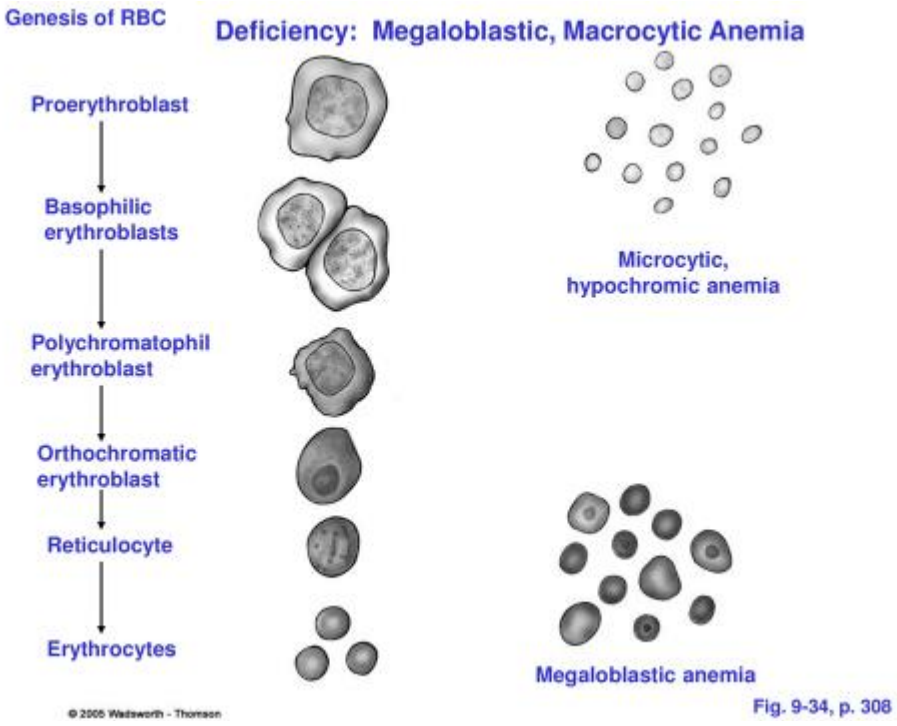
Dementia

Colon cancer

Neural tube defects







Interaction with other nutrients: vitamin B12 needed and visa versa; oversupply of one can mask a deficiency of the other

Excretion: in urine and in feces; some intact, some in metabolite forms

Also excreted as a part of bile, however it is reabsorbed

Toxicity: rare; in reported cases you can have insomnia, irritability and GI distress

Vitamin B12 - cobalamin.
(also hydroxycobalamin, adenosylcobalamin).

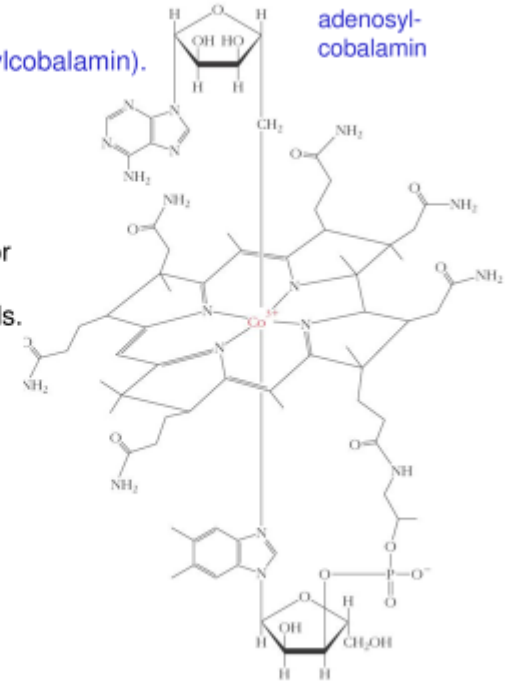
We saw cobalamine in fatty acid oxidation.

Cobalamin is needed in making adenosylcobalamin, a cofactor for "methyl malonyl mutase", which breaks down odd-chain fatty acids.

B12 is also used in regenerating folate

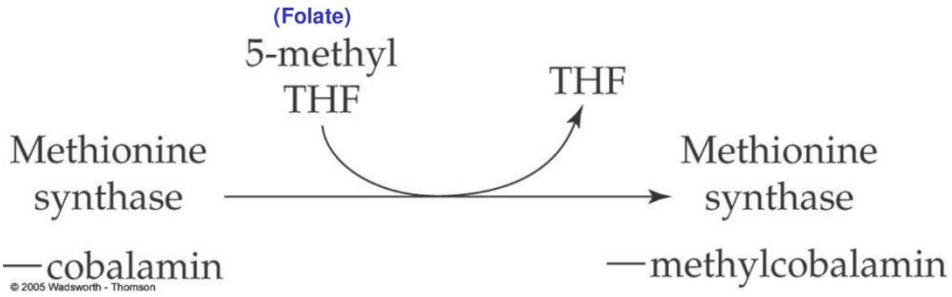
Sources: meat, milk and eggs.

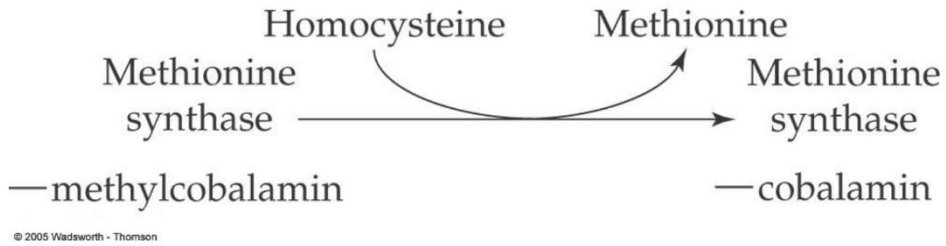
Vegetarians who eat dairy products are OK, but non-dairy vegans may not get enough.



Functions: Coenzyme in two reactions

- Conversion of homocysteine to methionine; "methyl-folate trap"





p. 312a

- Important in:
  - proper nerve function
  - production of red blood cells
  - metabolizing fats and proteins
  - prevention of anemia
  - DNA reproduction
  - energy production?

Recommendations:

Men and Women
14+
2-3 mcg/day

1 chicken breast
1 hard boiled egg
1 cup plain low fat yogurt
OR
1 cup milk
1 cup raisin bran

- Coenzyme in production of Krebs cycle intermediates from some amino acids, and odd chain fatty acids
  - Deficiency causes build-up of methylmalonyl CoA which is useful in diagnosis of B12 deficiency

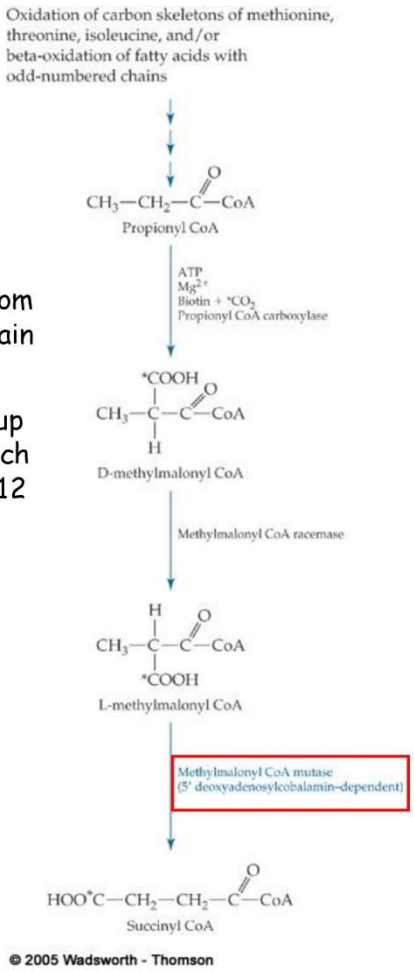


Fig. 9-37, p. 313

Deficiency: megaloblastic, macrocytic anemia;

symptoms include: pallor, fatigue, sortness of breath, palpitations, insomnia, tingling and numbness in extremities, abnormal gait, loss of concentration, memory loss, and possibly dementia.

Anemia can be corrected with large doses of folate, but the neuropathies cannot.

Associated with risk factors for coronary heart disease via homocysteine production

Most deficiencies associated with:

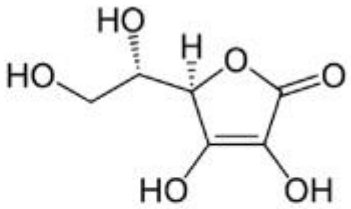
- malabsorption (long term vegetarians; vegetarian children and infants; changes in intrinsic factor production;
- GI diseases such as sprue;
- prolonged use of H blockers and proton pump inhibitors

Excretion: not extensively degraded prior to excretion; almost all excreted in bile

Toxicity: none known

Vitamin C - asorbic acid

Required for collagen synthesis, and as a cofactor for several enzymes. Also scavenges oxygen radicals.



In almost all organisms, ascorbic acid is synthesized from glucose in 4 steps.

A relatively recent (40 million years ago) mutation in the ancestor of humans made us unable to make ascorbic acid. So for us, and some closely related primates, it's a vitamin.

Guinea pigs can't make ascorbic acid, either.

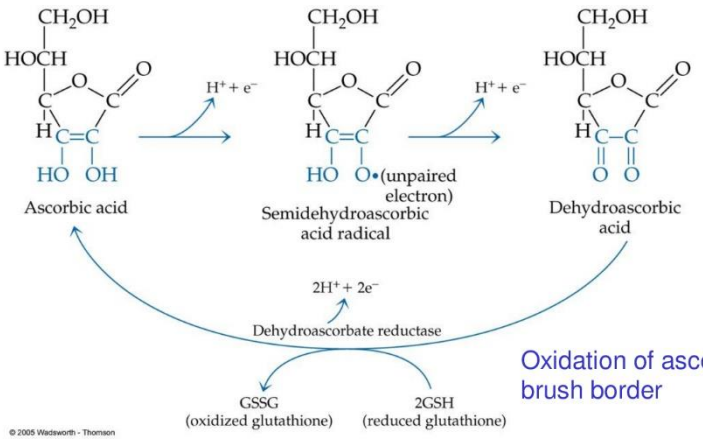
Sources of vitamin C are fruit and fresh meat. Vitamin C deficiency causes scurvy, and in human history vitamin C deficiency may have been an impediment to spreading northward.

Common foods with high Vitamin C content

- Asparagus
- Oranges
- Cauliflower
- Green peppers
- Lemons
- Kale
- Strawberries

Absorption:

- Undergoes oxidation prior to absorption
- Co-transport across intestinal membrane (sodium dependent); carrier mediate transport from intestinal cell into blood
- Saturable transport
- Absorption inhibited by pectin and zinc; interferes with iron and copper
- May be destroyed before absorption by high iron concentrations in diet



Oxidation of ascorbate at the intestinal brush border

- Transported by blood to tissues with ascorbate transporters; stored in non-fatty tissues
  - Adrenal gland, pituitary gland, lens of the eye
  - Liver, spleen heart, kidneys, lungs, pancreas, leukocytes

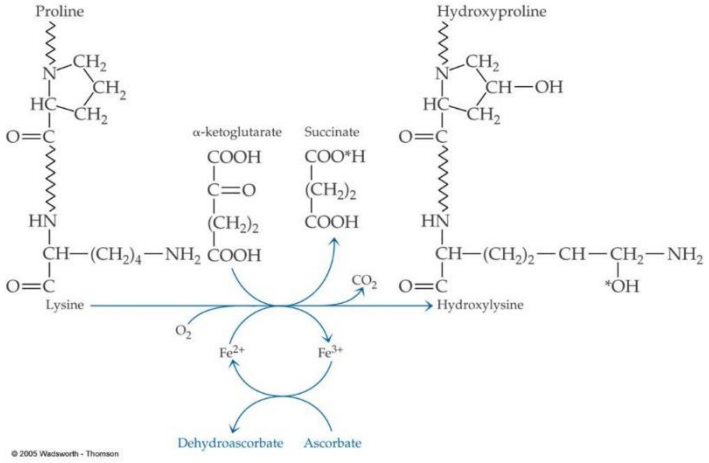
Fig. 9-3, p. 262

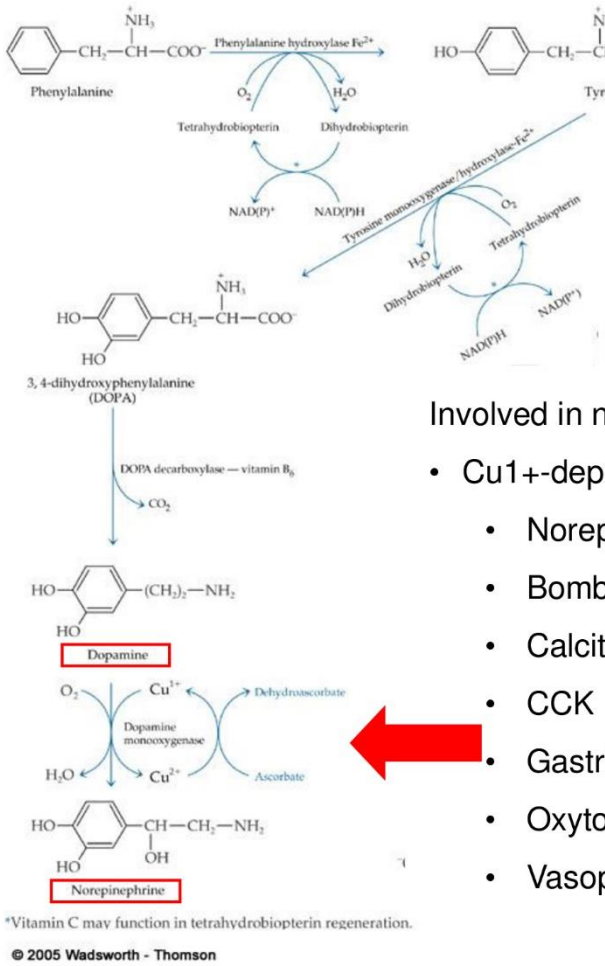
Major Functions of Ascorbic Acid

- Collagen synthesis
- Tyrosine synthesis and catabolism – epinephrine, norephinephrine, serotonin, dopamine
- Maturation of glial cells in developing nervous system
- Iron absorption and storage
- Bile acid formation/cholesterol degradation
- Vasodilation and anticlotting (via activation of NO release)
- Aids in prevention of cancers of oral cavity and pancreas
- General antioxidant
- Pro-oxidant (not necessarily good)
- Common cold??????
- Immune function

Collagen Synthesis

- Collagen is a structural protein in almost all connective tissues, including bone, cartilage, skin, tendons
- Composed of a triple helix of helical proteins
- Vitamin C is necessary for the helical proteins to crosslink posttranslationally
- Indirectly used because Fe2+ is a cofactor for the enzyme prolyl hydroxylase, and ascorbate is needed to recycle the iron from the Fe3+ to the Fe2+ state

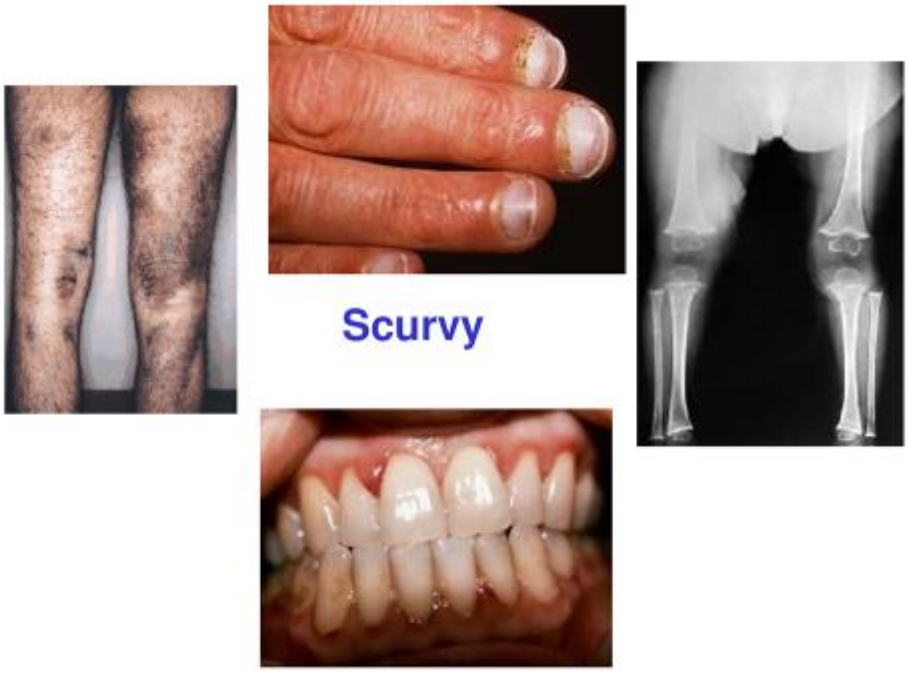




Involved in neurotransmitter synthesis

- Cu1+-dependent monooxygenases
  - Norepinephrine (shown)
  - Bombesin
  - Calcitonin,
  - CCK
  - Gastrin
  - Oxytocin
  - Vasopressin

Fig. 9-6, p. 267



Toxicity: (max dose without adverse effects 2g/day)

- GI problems including abdominal pain and diarrhea
- Increased risk of kidney stones in susceptible populations
- Increased risk of iron toxicity in susceptible populations
- Can interfere with clinical lab tests including glucose in urine, fecal occult blood, and urine blood.

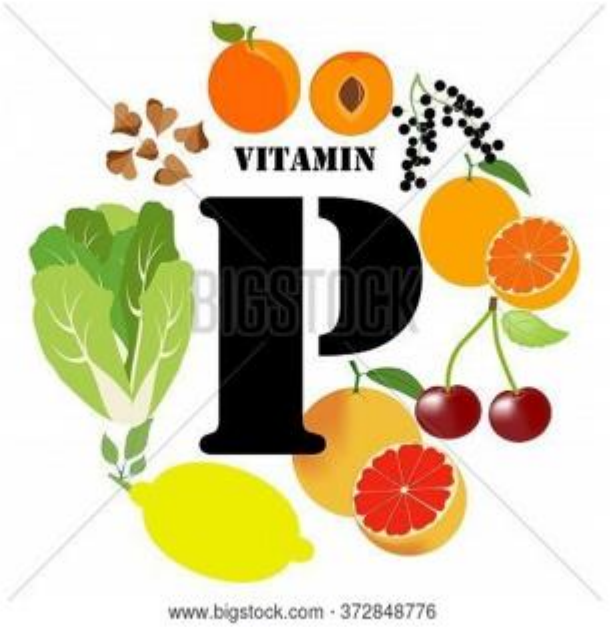


Table 9.1 The Water-Soluble Vitamins: Discovery, Functions, Human Deficiency Syndromes, Food Sources, and Recommended Intake

Water-Soluble Vitamins						
Vitamin	Discovery	Main Coenzymes	Biochemical or Physiological Function	Deficiency Syndrome or Symptoms	Good Food Sources	RDA* or AI†
Thiamin (vitamin B_1)	Casimir Funk (1912)	Thiamin diphosphate (TDP) or thiamin pyrophosphate (TPP)	Oxidative decarboxylation of α-keto acids and 2-keto sugars	*Beriberi*, muscle weakness, anorexia, tachycardia, enlarged heart, edema	Yeast, pork, sunflower seeds, legumes	1.1 mg* 1.2 mg
Riboflavin (vitamin B_2)	Kuhn, Szent-György, and Wagner-Jaunergy (1933)	Flavin adenine dinucleotide (FAD); flavin mononucleotide (FMN)	Electron (hydrogen) transfer reactions	*Ariboflavinosis*, cheilosis, glossitis, hyperemia and edema of pharyngeal and oral mucous membranes, angular stomatitis, photophobia	Beef liver, braunschweiger sausage, steak, mushrooms, ricotta cheese, nonfat milk, oysters	1.1 mg* 1.3 mg
Niacin (vitamin B_3) (nicotinic acid, nicotinamide)	Elvehjem et al. (1937)	Nicotinamide adenine dinucleotide (NAD); nicotinamide adenine dinucleotide phosphate (NADP)	Electron (hydrogen) transfer reactions	*Pellagra*, diarrhea, dermatitis, mental confusion, or dementia	Tuna, beef liver, chicken breast, beef, halibut, mushrooms	14 mg* 16 mg
Pantothenic acid	R. J. Williams (1933)	Coenzyme A (CoA)	Acyl transfer reactions	Deficiency very rare; numbness and tingling of hands and feet, vomiting, fatigue	Widespread in foods; exceptionally high amounts in egg yolk, liver, kidney, yeast	5 mg†
Biotin	Szent-György (1940)	N-carboxybiotinyl lysine	CO_2 transfer/ carboxylation reactions	Deficiency very rare, anorexia, nausea, glossitis, depression, dry scaly dermatitis	Synthesized by microflora of digestive tract; yeast, liver, kidney	30 μg†

Table 9-1a, p. 261

Vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine)	Szent-György, Kuhn (1938)	Pyridoxal phosphate (PLP)	Transamination and decarboxylation reactions	Dermatitis, glossitis, convulsions	Steak, navy beans, potato, salmon, banana, whole grains	1.3 mg*
Folic acid (folacin)	Mitchell et al. (1941)	Derivatives of tetrahydrofolic acid: 5,10-methylidyne THF, 10-formyl THF, 5-formimino THF, 5,10-methylene THF, 5-methyl THF	One-carbon transfer reactions	*Megaloblastic anemia*, diarrhea, fatigue, depression, confusion	Brewer's yeast, spinach, asparagus, turnip greens, lima beans, beef liver	400 μg*
Vitamin B_{12} (cobalamin)	Riches, Folkers, et al. (1948)	Methyl cobalamin, adenosyl cobalamin (cobalamides)	Methylation of homocysteine to methionine; conversion of methylmalonyl CoA to succinyl CoA	*Megaloblastic anemia*, degeneration of peripheral nerves, skin hypersensitivity, glossitis	Meat, fish, shellfish, poultry, milk	2.4 μg*
Ascorbic acid (vitamin C)	Szent-György‡ (1928) King‡ (1932)	None	Antioxidant, cofactor of hydroxylating enzymes involved in synthesis of collagen, carnitine, norepinephrine	*Scurvy*, loss of appetite, fatigue, retarded wound healing, bleeding gums, spontaneous rupture of capillaries	Papaya, orange juice, cantaloupe, broccoli, brussels sprouts, green peppers, grapefruit juice, strawberries	75 mg* 90 mg

*Adults age 19 to 50 years, females and males respectively.
†Adequate intake.
‡Szent-György and King are considered to be codiscoverers of vitamin C.

Table 9-1b, p. 261

General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Biochemistry of human nutrition: components and nutritional compounds of normal nutrition; biological value of individual nutrients.
2. Mechanisms of conversion of nutrients (proteins, carbohydrates, lipids) in the digestive tract. Enzymes of the stomach and intestines.
3. Violation of digestion of certain nutrients in the stomach and intestines; hereditary enzymopathies of digestive processes.
4. Microelements in human nutrition. Biological functions of individual trace elements; manifestations of trace element deficiency.
5. Vitamins in human nutrition. Water-soluble and fat-soluble vitamins; exogenous and endogenous causes of vitamin deficiency.
6. Vitamin B1 (thiamine): structure, biological properties, mechanism of action, sources, daily requirement.

7. Vitamin B2 (riboflavin): structure, biological properties, mechanism of action, sources, daily requirement.
8. Vitamin PP (nicotinic acid, nicotinamide): structure, biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
9. Vitamin B6 (pyridoxine): structure, biological properties, mechanism of action, sources, daily requirement.
10. Vitamin B12 (cobalamin): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
11. Vitamin B (folic acid): biological properties, mechanism of action, sources, daily requirement.
12. Vitamin H (biotin): biological properties, mechanism of action, sources, daily requirement.
13. Vitamin B3 (pantothenic acid): biological properties, mechanism of action, sources, daily requirement.
14. Vitamin C (ascorbic acid): structure, biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
15. Vitamin P (flavonoids): structure, biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 14

Topic: Fat-soluble vitamins, bioantioxidants. Exogenous and endogenous hypo- and avitaminosis, hypervitaminosis

Relevance of the topic: Fat-soluble vitamins are not part of enzymes and affect metabolism indirectly, creating conditions for optimal action of enzymes on membrane structures. They play the role of modulators of the structure and functions of membranes. In this regard, fat-soluble vitamins in the body also perform an antimutagenic function, protecting the genetic apparatus from damage by chemical and physical factors. This is due to the pronounced antioxidant properties of fat-soluble vitamins: they are able to neutralize active forms of oxygen and free radicals and inhibit the processes of peroxide oxidation of biopolymers (nucleic acids, proteins, lipoprotein complexes).

They also affect the processes of tissue respiration (directly or indirectly), stabilize cell membranes, and regulate their selective permeability to substances. For some fat-soluble vitamins, specific receptors have been found in the nucleus of cells, with the help of which they activate gene expression, which leads to cell differentiation. Vitamins A, D and E work according to this principle. The latter activates the biosynthesis of hemsynthesizing enzymes.

Purpose: generalization of information about the properties and mechanisms of action of fat-soluble vitamins. Study of pathological conditions associated with disturbances in the metabolism of fat-soluble vitamins. The concept of exogenous and endogenous hypo- and avitaminosis, hypervitaminosis.

Basic concepts:
1. Chicken blindness.
2. Xerophthalmos.
3. Keratomalacia.
4. Rickets.

Plan and organizational structure of the lecture:

1. Vitamin A (retinol, retinal, retinoic acid): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
2. Vitamin K (phylloquinone, farnoquinone): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
3. Vitamin E (a-tocopherol): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
4. Vitamin D3 (cholecalciferol): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
5. Exogenous and endogenous hypo- and avitaminosis, hypervitaminosis.

Content of the lecture material

Lipid-soluble vitamins

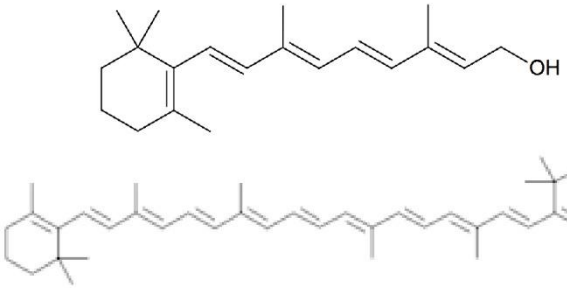
Vit.A

Vit.D

Vit.E

Vit.K

Vitamin A - Retinol



Retinol (vitamin A)

β-carotene (2 μg β-carotene considered equivalent to 1 μg retinol as a vitamin A source)

Sources in diet - Many plants (photoreceptors), also meat, especially liver. Fat soluble, so you can get too much, or too little if absorption is a problem.

Some uses:

Vision (11-cis-retinol bound to rhodopsin detects light in our eyes).

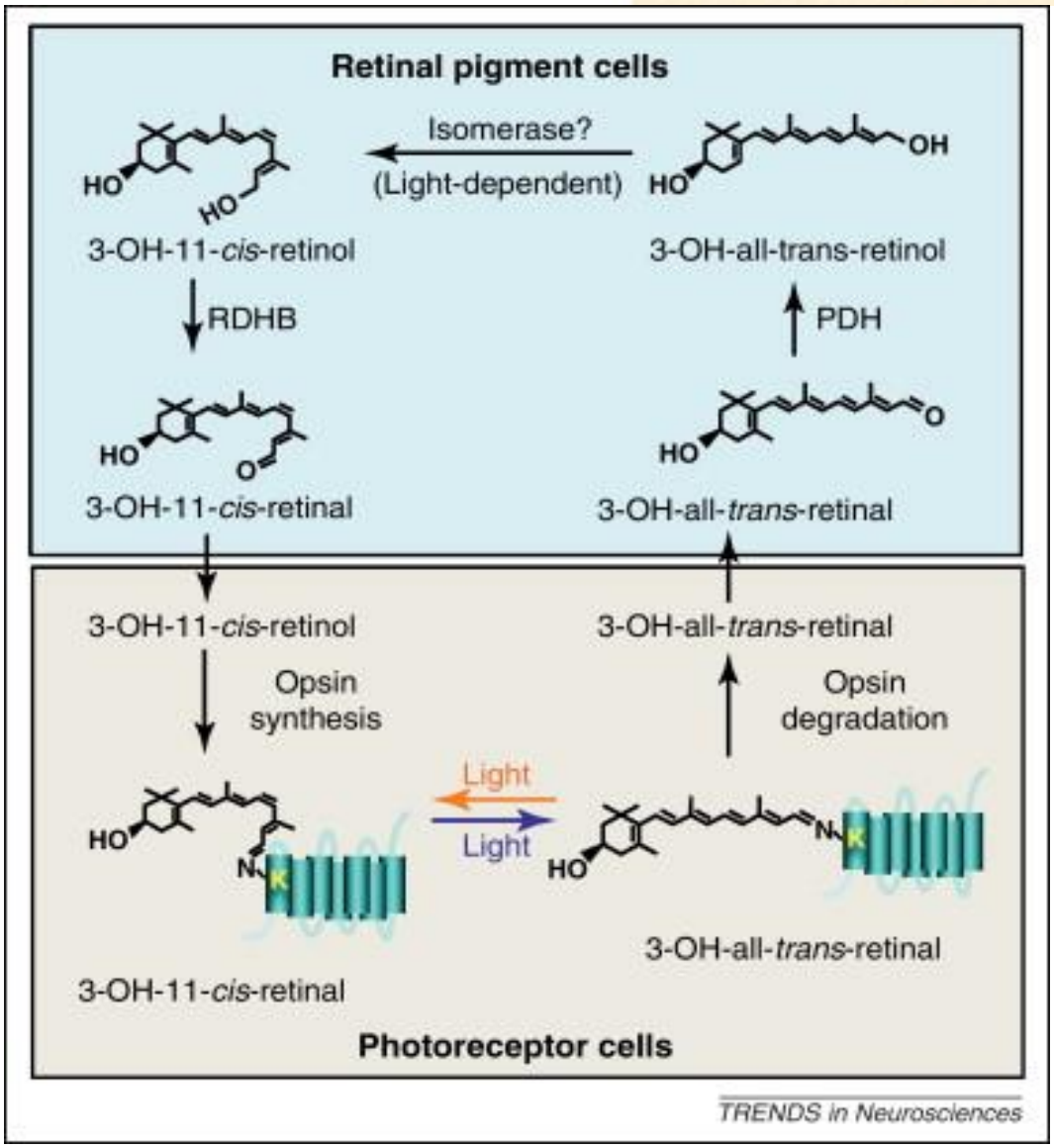
Regulating gene transcription (retinoic acid receptors on cell nuclei are part of a system for regulating transcription of mRNAs for a number of genes).

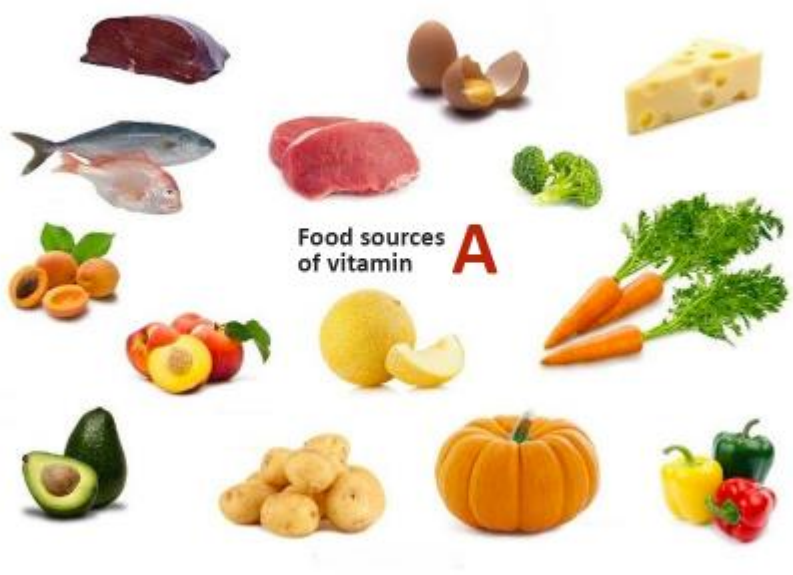
Biochemical functions of vitamin A

- Vision in dim light
- Necessary for maintenance of normal epithelium: Synthesis of goblet cells in epithelial tissue which secrete mucous having antimicrobial component.
- Embryonic development & reproduction: During fetal development, retinoic acid allows for development of lungs , hearts, eyes and ears & regulates expression of growth hormone gene.

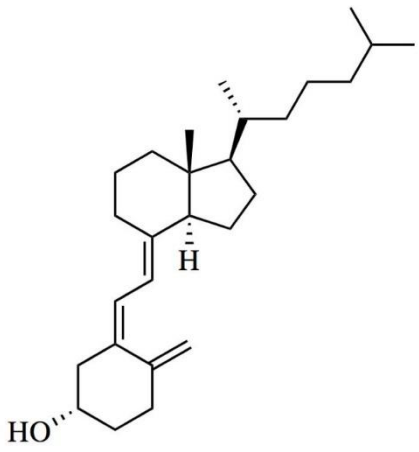
Other biochemical functions of vitamin A

- Retinol and retinoic acid function like steroid hormones
- They regulate protein synthesis and involved in cell growth and differentiation
- Vitamin A is essential to healthy epithelial tissue
- Vitamin A is considered to be essential for maintenance of proper immune system

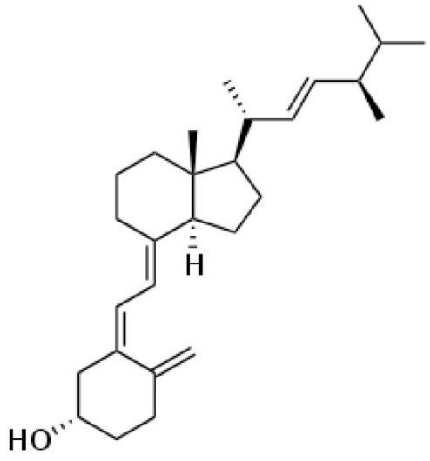




Vitamin D refers to a group of similar lipid-soluble molecules (major forms are D2 and D3, also D1, D4, D5).

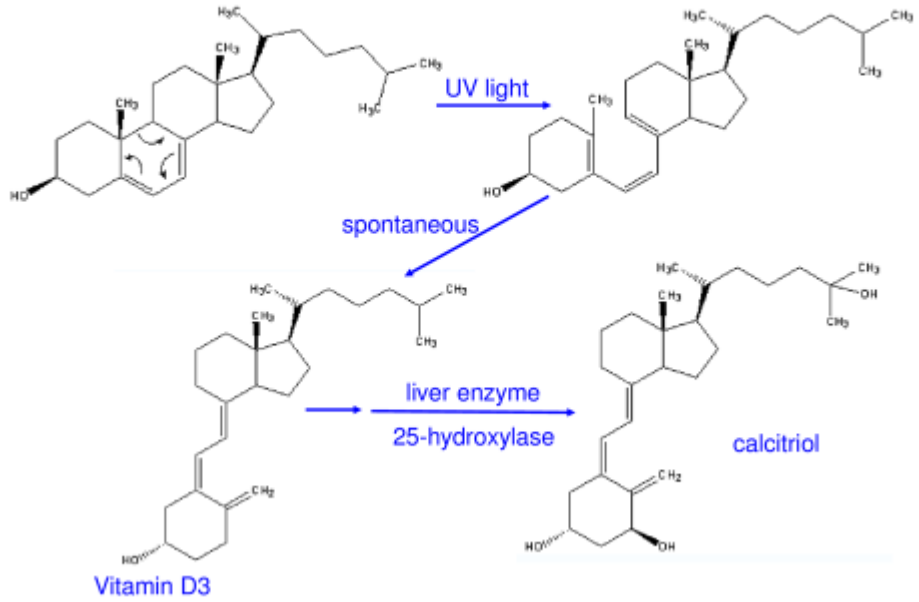


Vitamin D3 (cholecalciferol)



Vitamin D2 (ergocalciferol)

Vitamin D3 can be obtained in diet, or derived from cholesterol in a reaction that requires UV light.



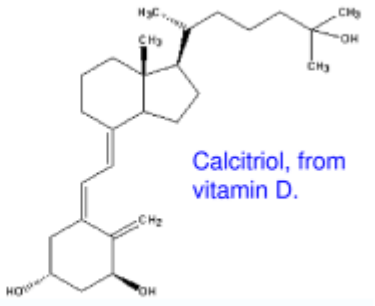
Vitamin D binds to a "vitamin D binding protein" (VDP) for transport to target organs.

Vitamin D is not active itself (it's a prohormone); it is modified to yield biologically active forms, such as calcitriol.

Calcitriol (derived from vitamin D) is a transcription factor, influencing expression of proteins involved in calcium absorption and transport.

Vitamin D is also important for immune system function.

Deficiency causes rickets, bone loss.

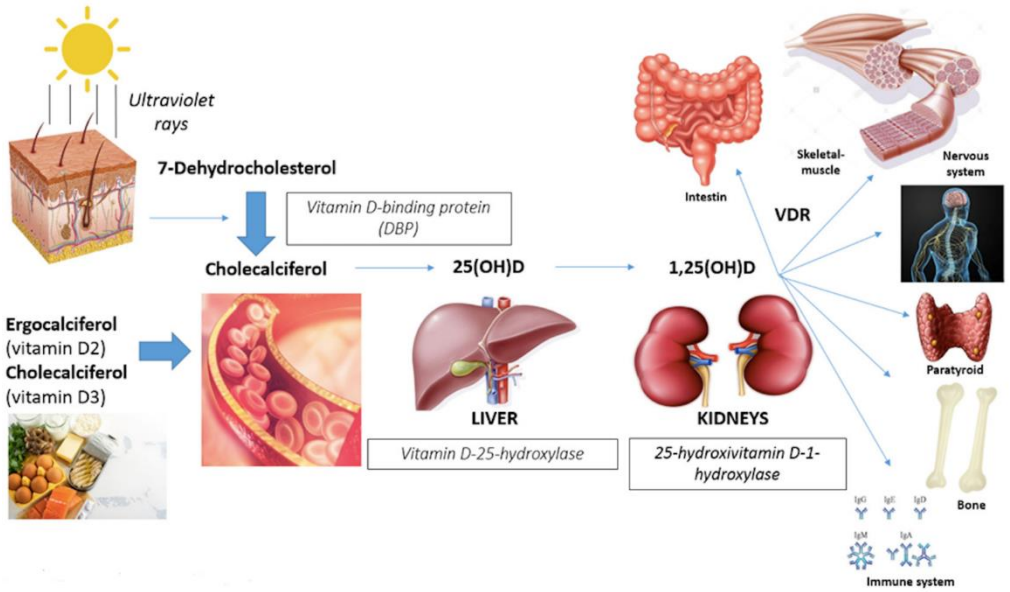


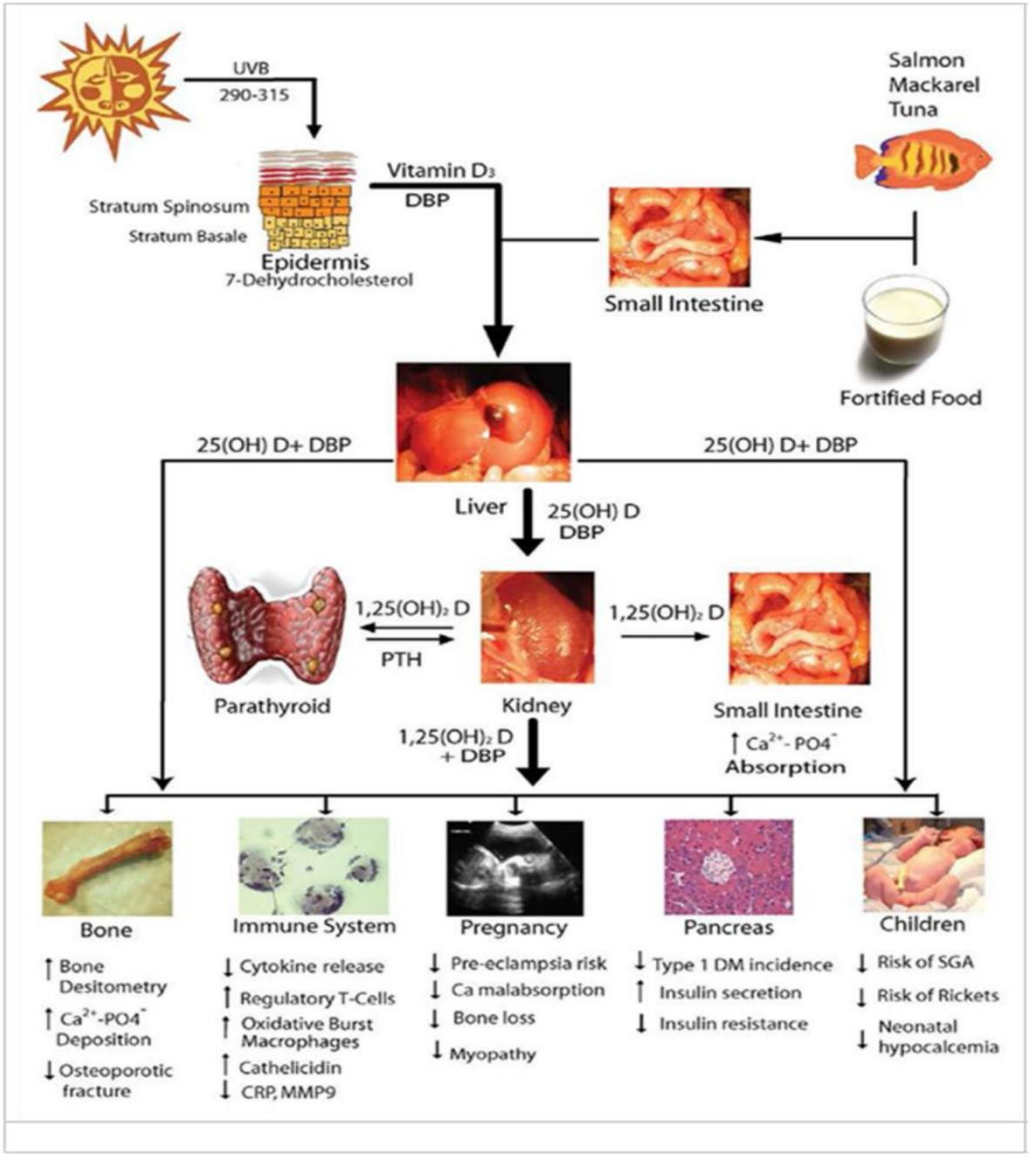
Vitamin D production requires UV light (sunlight).

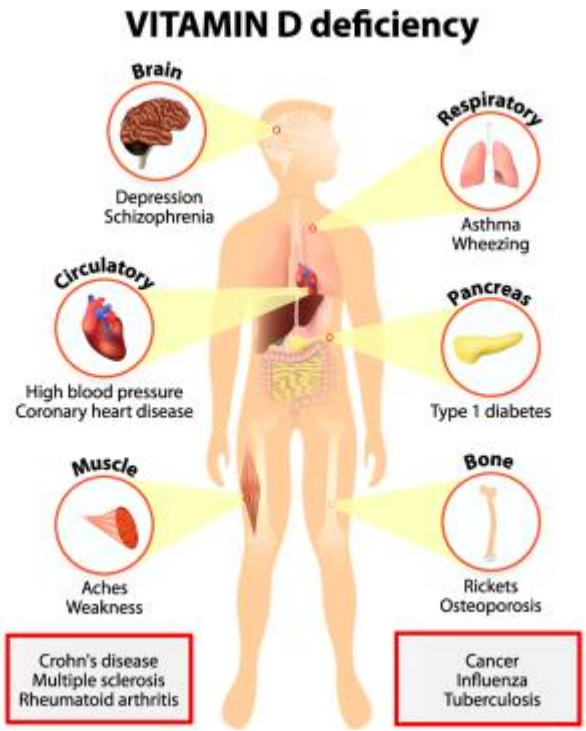
Sometime after humans migrated north out of Africa about 50,000 years ago, mutations appeared that reduced melanin (pigment) production in the skin, permitting vitamin D production with less sunlight.

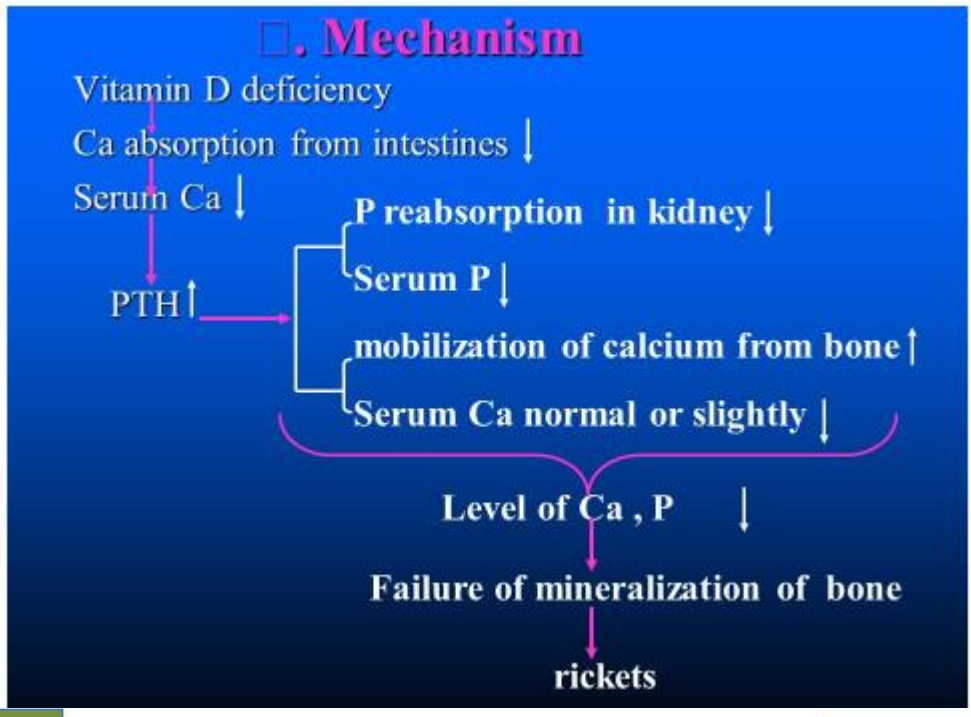
Disadvantages of less melanin production are skin that is easily damaged by the sun, skin cancer risk, and loss of folic acid due to UV damage.

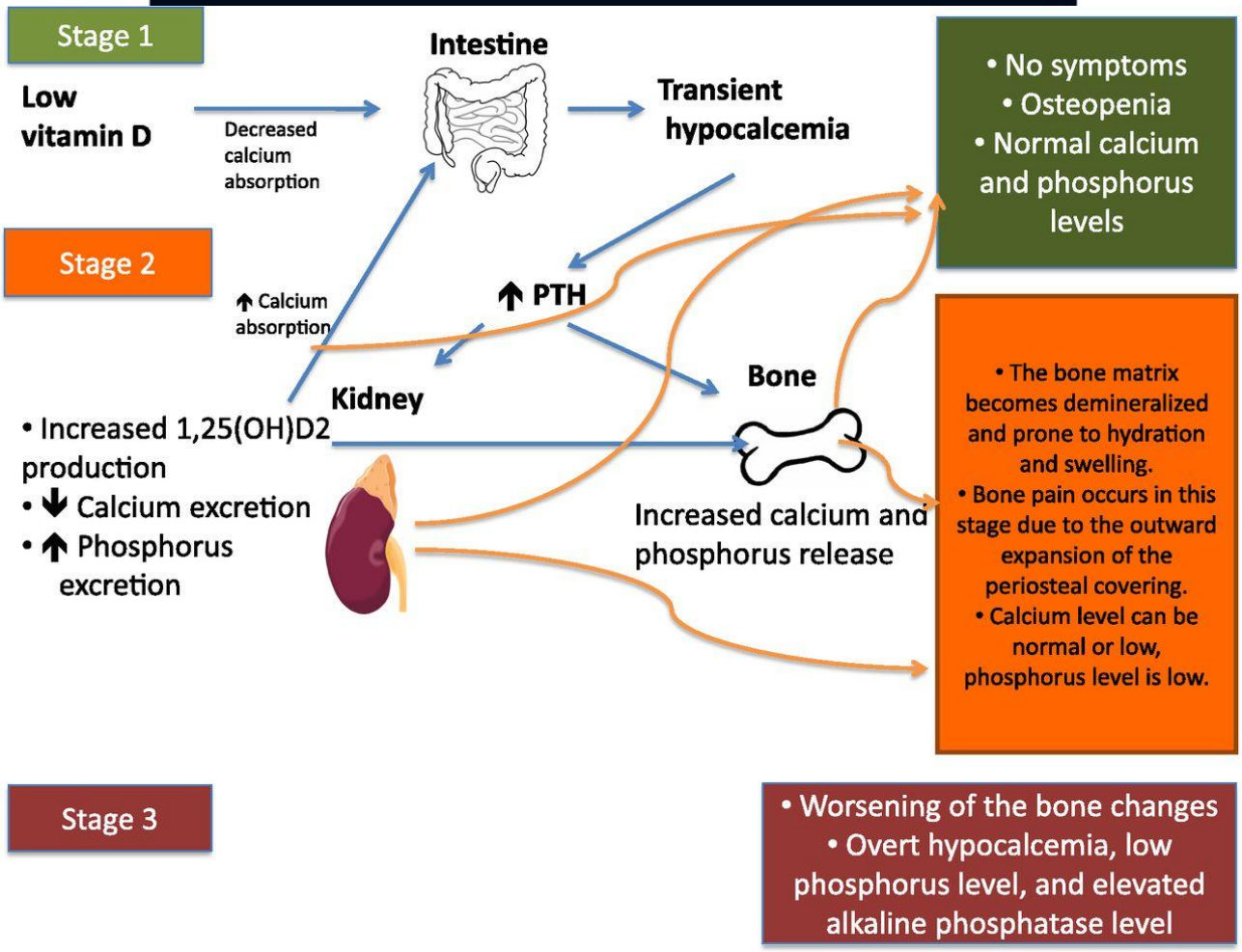
The melanin-reducing mutations helped early humans make vitamin D in northern europe in winter.




UVB
290-315
Salmon
Mackarel
Tuna
Vitamin D3
DBP
Stratum Spinosum
Stratum Basale
Epidermis
7-Dehydrocholesterol
Small Intestine
Fortified Food
25(OH) D+ DBP
25(OH) D+ DBP
Liver
25(OH) D
DBP
1,25(OH)2 D
PTH
1,25(OH)2 D
Parathyroid
Kidney
Small Intestine
↑ Ca2+- PO4-
Absorption
1,25(OH)2 D
+ DBP
Bone
↑ Bone Desitometry
↑ Ca2+-PO4- Deposition
↓ Osteoporotic fracture
Immune System
↓ Cytokine release
↑ Regulatory T-Cells
↑ Oxidative Burst Macrophages
↑ Cathelicidin
↓ CRP, MMP9
Pregnancy
↓ Pre-eclampsia risk
↓ Ca malabsorption
↓ Bone loss
↓ Myopathy
Pancreas
↓ Type 1 DM incidence
↑ Insulin secretion
↓ Insulin resistance
Children
↓ Risk of SGA
↓ Risk of Rickets
↓ Neonatal hypocalcemia


VITAMIN D deficiency
Brain
Depression
Schizophrenia
Respiratory
Asthma
Wheezing
Circulatory
High blood pressure
Coronary heart disease
Pancreas
Type 1 diabetes
Muscle
Aches
Weakness
Bone
Rickets
Osteoporosis
Crohn's disease
Multiple sclerosis
Rheumatoid arthritis
Cancer
Influenza
Tuberculosis

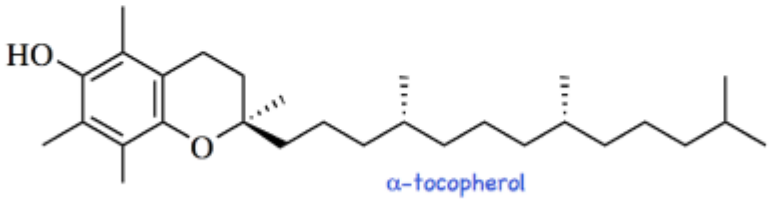

. Mechanism
Vitamin D deficiency
Ca absorption from intestines
Serum Ca
P reabsorption in kidney
Serum P
PTH
mobilization of calcium from bone
Serum Ca normal or slightly
Level of Ca , P
Failure of mineralization of bone
rickets


Stage 1
Low vitamin D
Decreased calcium absorption
Intestine
Transient hypocalcemia
• No symptoms
• Osteopenia
• Normal calcium and phosphorus levels
Stage 2
Calcium absorption
PTH
Kidney
Bone
• Increased 1,25(OH)D2 production
• Calcium excretion
• Phosphorus excretion
Increased calcium and phosphorus release
• The bone matrix becomes demineralized and prone to hydration and swelling.
• Bone pain occurs in this stage due to the outward expansion of the periosteal covering.
• Calcium level can be normal or low, phosphorus level is low.
Stage 3
• Worsening of the bone changes
• Overt hypocalcemia, low phosphorus level, and elevated alkaline phosphatase level

Vitamin E - Collectively refers to 8 related tocopherols.

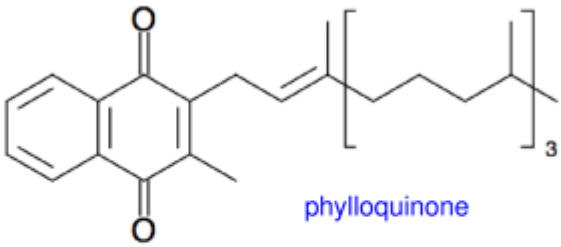
It is essential, roles: suggestions include
neural membrane component, antioxidant.

Obtained in diet: fat, deficiency is rare.



α-tocopherol

Vitamin K - Refers to phylloquinonone (vitamin K-1), and several structurally similar molecules.

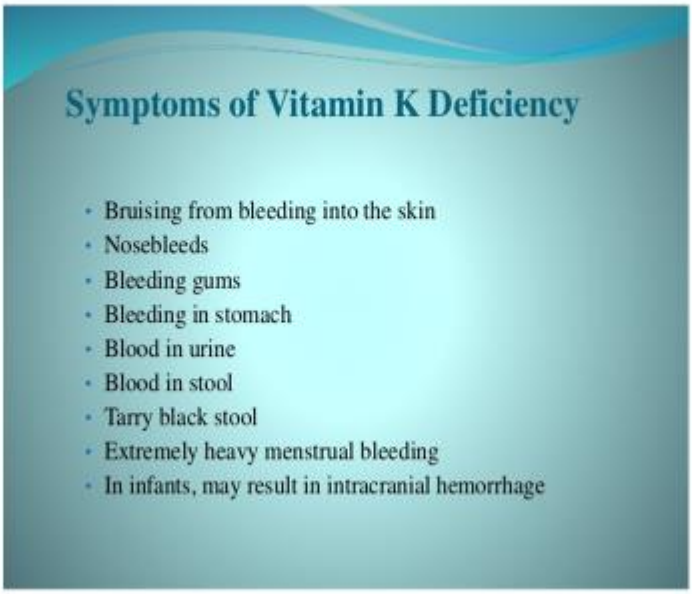


phylloquinone

Vitamin K is required for proper blood clotting.

It is used in synthesizing gamma carboxy glutamate, a post-translationally modified amino acid in prothrombin.

Sources are vegetables and fruits, cabbage, spinage
Deficiency is observed in the antibiotics use.



General material and educational and methodological support of the lecture:
- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Vitamin A (retinol, retinal, retinoic acid): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
2. Vitamin K (phylloquinone, farnoquinone): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
3. Vitamin E (a-tocopherol): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
4. Vitamin D3 (cholecalciferol): biological properties, mechanism of action, manifestations of deficiency, sources, daily requirement.
5. Exogenous and endogenous hypo- and avitaminosis, hypervitaminosis

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.

5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.
Електронні інформаційні ресурси:
1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

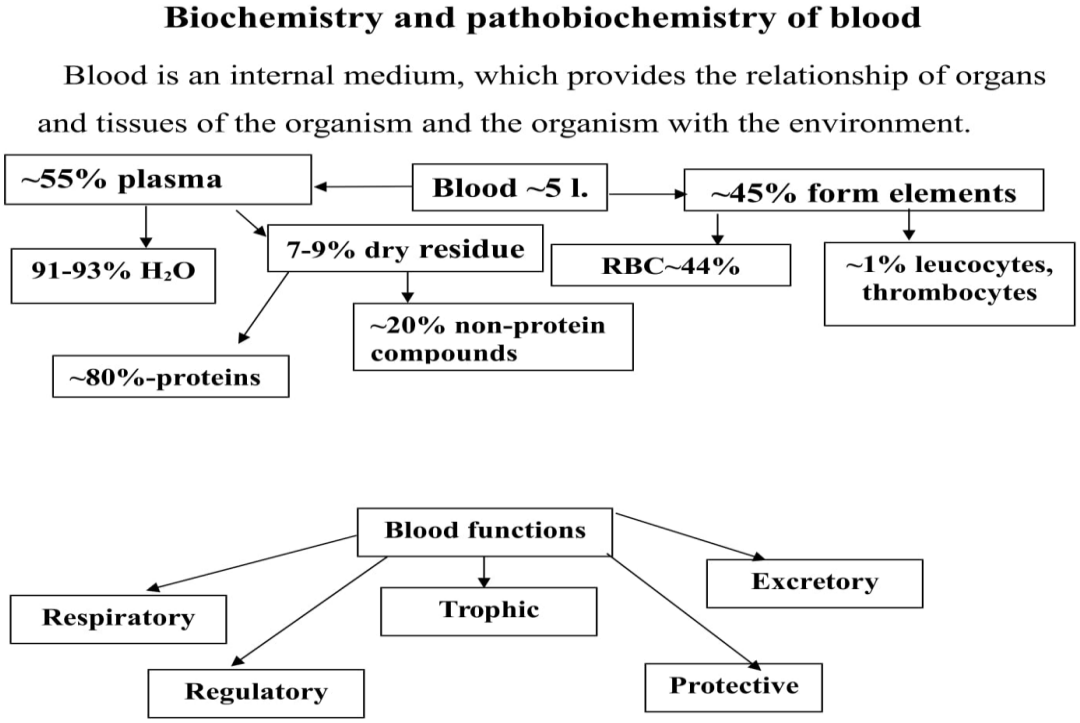
Lecture №15

Topic: Chemical composition and functions of blood. Transport of gases by blood. Biochemistry and pathobiochemistry of hemoglobins. Biosynthesis of porphyrins, heme catabolism. Exchange of bile pigments.
Relevance of the topic: Blood is a liquid tissue that carries out the transport of chemical substances in the body, thanks to which the biochemical processes occurring in various cells and intercellular spaces are integrated into a single system that determines the necessary mode of their existence, as well as to a large extent communication organism with the environment. Blood in the body performs a variety of life support functions, the most important of which are: respiratory, nutritional, excretory, protective, regulatory. Blood plasma proteins represent a genetically determined heterogeneous system that differs in physicochemical and functional properties. Among them are enzymes, enzyme inhibitors, hormones, transport proteins, coagulation and anticoagulation factors, antibodies, antitoxins, etc. With a number of diseases, not only the quantitative ratio changes, but also the qualitative composition of individual groups of blood plasma proteins, and therefore the determination of the protein composition of the blood plasma has important diagnostic and prognostic value.
Purpose: to generalize information about the main biochemical indicators of blood composition in healthy people and in a number of diseases, to study the mechanisms of disturbance and compensation of the acid-base state. Learn the structure and functions of hemoglobin, the main factors and mechanisms of humoral and cellular immunity. Carry out differential diagnosis of jaundice.
Basic concepts:
1. The cooperative effect of Oxygen.
2. Bohr effect.
3. Azotemia.
4. Dysproteinemia.
5. Paraproteinemia.

Plan and organizational structure of the lecture:
1. Biochemical composition of blood.
2. Respiratory function of erythrocytes.

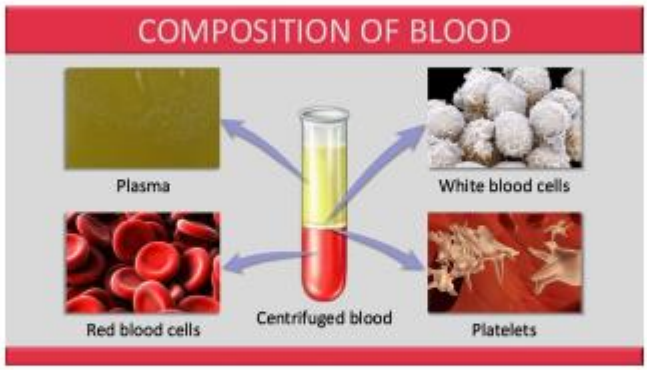
3. Blood plasma enzymes; value in enzymodiagnosis of diseases of organs and tissues.
4. Mediators and hormones of the immune system.
5. Hemoglobin: mechanisms of participation in the transport of oxygen and carbon dioxide. Variants and pathological forms of human hemoglobins
6. Hemoglobin breakdown. Differential diagnosis of jaundice.

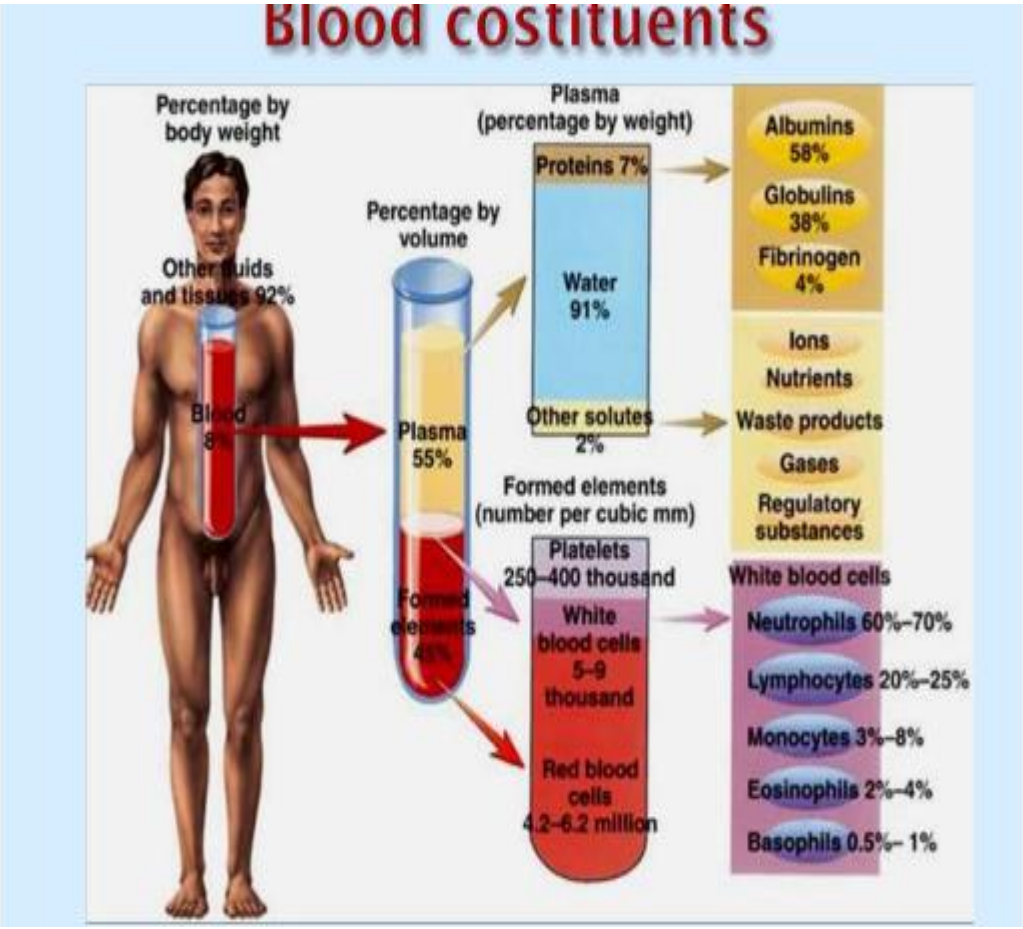
Content of the lecture material

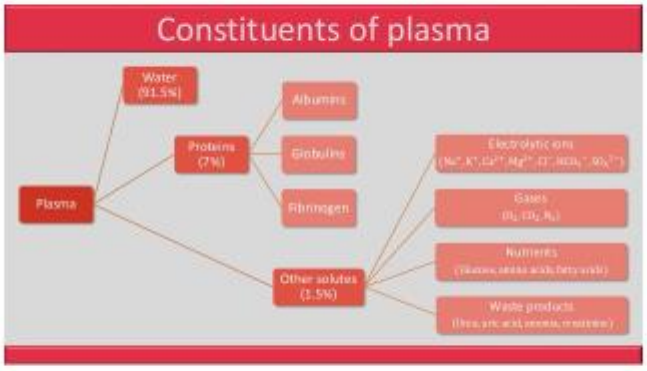


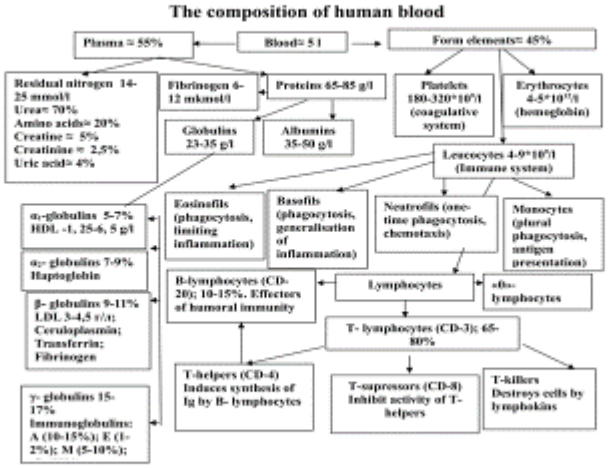
Major Functions of Blood

- Transport of O_2, CO_2, nutrients, hormones, metabolic wastes
- Thermoregulation
- pH regulation
- Protection against blood loss
- Protection against diseases through phagocytic blood cells and antibodies

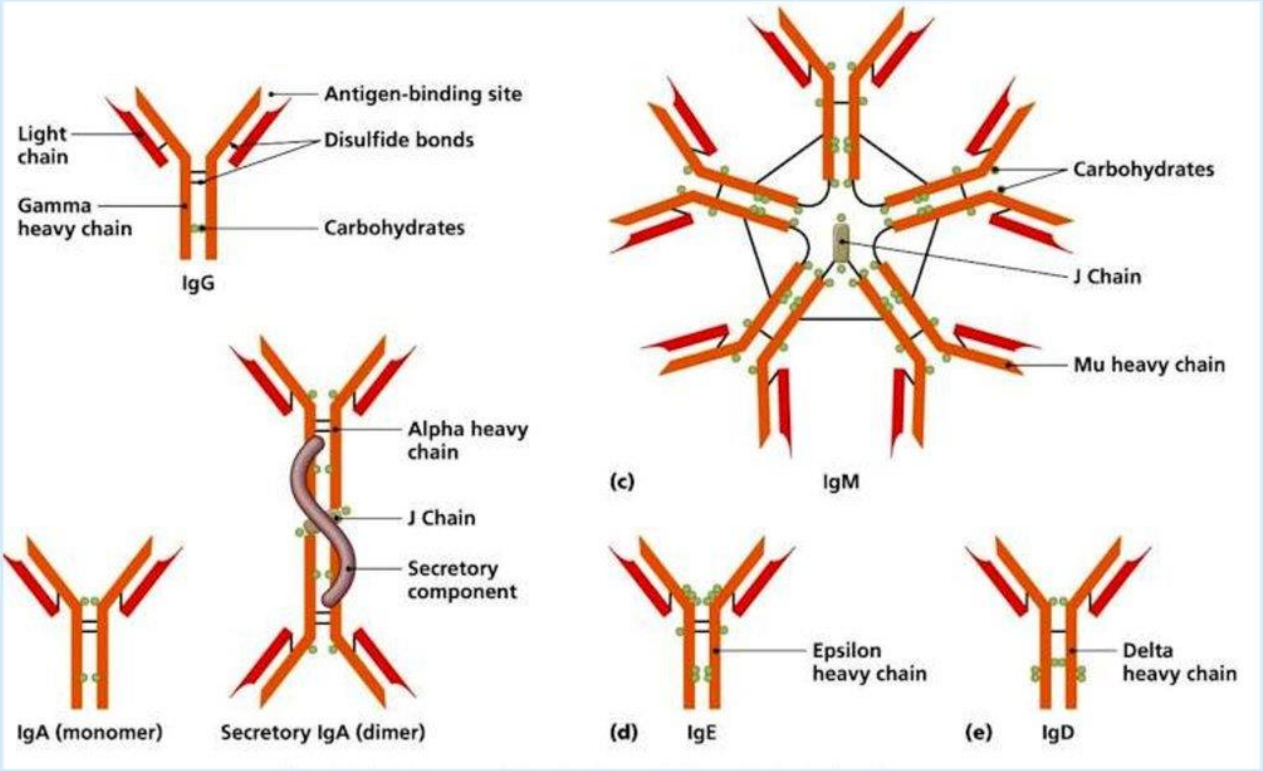



Blood costituents
Percentage by body weight
Other fluids and tissues 92%
Blood 8%
Percentage by volume
Plasma 55%
Formed elements 45%
Plasma (percentage by weight)
Proteins 7%
Water 91%
Other solutes 2%
Albumins 58%
Globulins 38%
Fibrinogen 4%
Ions
Nutrients
Waste products
Gases
Regulatory substances
Formed elements (number per cubic mm)
Platelets 250–400 thousand
White blood cells 5–9 thousand
Red blood cells 4.2–6.2 million
White blood cells
Neutrophils 60%–70%
Lymphocytes 20%–25%
Monocytes 3%–8%
Eosinophils 2%–4%
Basophils 0.5%– 1%


Constituents of plasma
Plasma
Water (91.5%)
Proteins (7%)
Albumins
Globulins
Fibrinogen
Other solutes (1.5%)
Electrolytic ions
Gases
Nutrients
Waste products



Immunoglobulins



Plasma II. – Proteins

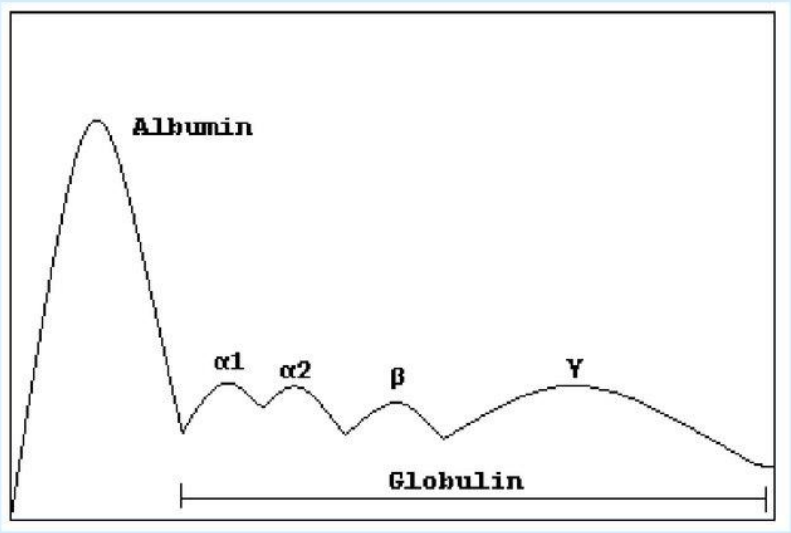
Group	Protein	Mr (thousands)	Function
albumins	pre–albumin	50–66	Transport of thyroxin
	albumin	67	Osmotic pressure of blood, transport of lipophilic compounds
α_1–globulins	antitrypsin	51	Trypsin inhibition
	antichymotrypsin	58–68	Chymotrypsin inhibition
	lipoprotein HDL	200–400	Lipid transport
	prothrombin	72	Coagulation factor
	transcortin	51	Transport of C_{21}–steroids
	acidic glycoprotein	44	Progesterone transport
	TBG (Thyroxin binding globulin)	54	Thyroxin transport
α_2–globulins	ceruloplasmin	135	Transport Cu^{2+}
	antithrombin III	58	Inhibition of coagulation
	haptoglobin	100	Haemoglobin binding
	cholinesterase	350	Hydrolisis of choline esters
	plasminogen	90	Plasmin precursor
	macroglobulin	725	Zn^{2+} transport
	RBP (Retinol Binding Protein)	21	Retinol trasnport
	vitamin D binding protein	52	Vitamin D transport
β–globulins	lipoprotein LDL	2000–4500	Lipid transport
	transferrin	80	Transport of iron ionts
	fibrinogen	340	Coagulation factor I.
	protein binding C_{19}– and C_{18}–steroid hormones	65	Transport of sex steroid hormones.
	transcobalamine	38	Vitamin B_{12} transport
	C–reactive protein (CRP)	110	Complement activator.
γ–globulins	IgG	150	Immune system
	IgA	162	
	IgM	900	
	IgD	172	
	IgE	196	

Chemical composition of blood

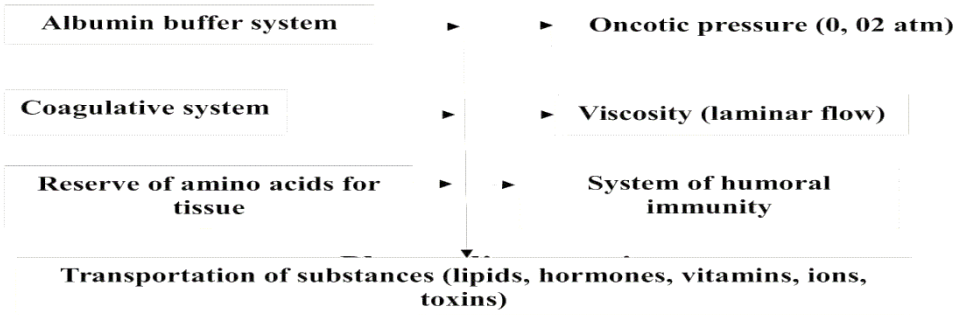
Proteins

Functions:

- π, V, pH
- Viscosity
- Transport
- Blood clotting
- Immunity
- Amino acid source



Physiological role of blood plasma proteins



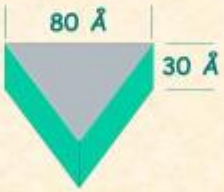
Characteristics of Plasma Proteins

- Most plasma proteins are synthesized in the liver, however, certain proteins are synthesized in other sides
- Generally synthesized on membrane –bound polyribosomes
- With the exception of albumin, almost all plasma proteins are glycoproteins
- Many plasma proteins exhibit polymorphism
- Each plasma protein has a characteristic half-life in the circulation
- The levels of certain proteins in plasma increase during acute inflammatory states or secondary to certain types of tissue damage

- Transport of ions, fatty acids, steroids, hormones etc.
  - Albumin (fatty acids), ceruloplasmin (Cu^{2+}), transferrin (Fe), lipoproteins (LDL, HDL)
- Nutritional source of amino acids for tissues
- Hemostasis (coagulation proteins)
- Prevention of thrombosis (anticoagulant proteins)
- Defense against infection (antibodies, complement proteins)

Albumin

- MW 66 000
- Single chain, 580 amino acids, sequence is known
- Dimensions - Heart shaped molecule
- 50% a helix [He and Carter, Nature, 358 209 (1992)]
- Modeled as:



Functions

- **Maintaining colloid osmotic pressure of blood (80% due to albumin)**
  - Colloid osmotic pressure is generated by plasma proteins
  - The most abundant of the plasma proteins
  - The lowest molecular weight of the major protein molecules in the plasma
  - High negative charge
  - Regulates water distribution
- **Transportation**
  - Albumin can act as a carrier molecule for bilirubin, fatty acids, trace elements and many drugs

Chemical composition of blood

- Non-protein nitrogen compounds
- Nitrogen-free organic compounds
- Electrolytes
- Micronutrients

- Hypoproteinemia - kidneys and liver lesions, protein deficiency
- Hyperproteinemia - severe diarrhea, vomiting, burns
- Disproteinemia – change in protein ratio

G/A

means

Globulin/Albumin Ratio

Blood buffer systems

Bicarbonate

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Phosphate

$$H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-}$$

Blood buffer systems

Hemoglobin

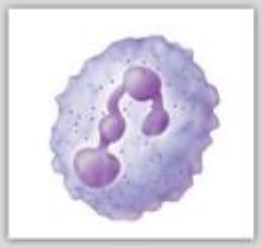
$$KHb + H_2CO_3 \rightarrow KHCO_3 + HHb$$

Protein

$$NH_2\text{–Protein –COOH}$$

White blood cells

Shape	Amoeboid nucleated
Size	12 – 15 µm
Colour	Colourless & translucent
Count	5000 – 10000 WBCs/µL
Life span	10 – 13 days



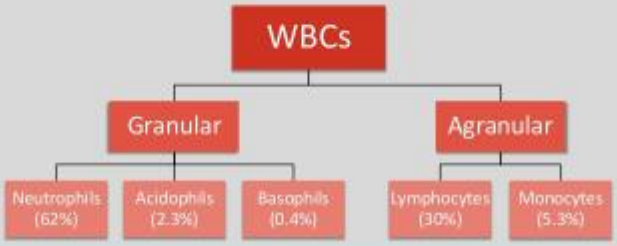
Leucopoiesis

The production of WBCs is known as leucopoiesis.

Adult	Liver, spleen, tonsils, bone marrow
Foetus	Liver, spleen

- Increase in number of WBCs is known as **leucocytosis**
- Decrease in number of WBCs is known as **leucopenia**
- Pathological increase in number of WBCs is known as **leukemia (blood cancer)**

Types of WBCs



Granular WBCs

Type	Appearance	Features	Functions	Location produced
Neutrophils		• Nucleus with 3-4 lobes • Stain with neutral dye (hematoxylin)	Destroy bacteria by phagocytosis	Bone marrow
Acidophils (eosinophils)		• Nucleus with 2 lobes • Stain with acidic dye (eosin)	Combat the effect of histamine in allergic reactions	Bone marrow
Basophils		• Nucleus with indistinct lobes • Stain with basic dye (methylene blue)	Liberate heparin and histamine in allergic reactions to intensify inflammatory response	Bone marrow

Agranular WBCs

Type	Appearance	Features	Functions	Location produced
Lymphocyte		• Smallest of WBCs • Large round nucleus	Produce antibodies	Bone marrow, spleen, tonsils
Monocyte		• Largest of WBCs • Large kidney shaped nucleus	Ingest microorganisms	Bone marrow

Platelets

Shape	Circular biconvex non-nucleated
Size	2 – 4 µm
Count	1,50,000 – 4,00,000 platelets/µL
Life span	5 – 9 days
Function	Blood clotting

Thrombopoiesis

The production of platelets is known as thrombopoiesis.

Platelets are the fragments of large cells called megakaryocytes that remain in the bone marrow.

- Increase in number of platelets is known as **thrombocytosis**
- Decrease in number of platelets is known as **thrombocytopenia**

Red blood cells

Shape	Circular biconcave non-nucleated
Size	Diameter = 7 – 8 µm Thickness = 2.5 µm
Colour	Red (haemoglobin pigment)
Count	Adult male = 5.4 million RBCs/µL Adult female = 4.8 million RBCs/µL
Life span	120 days

Erythropoiesis

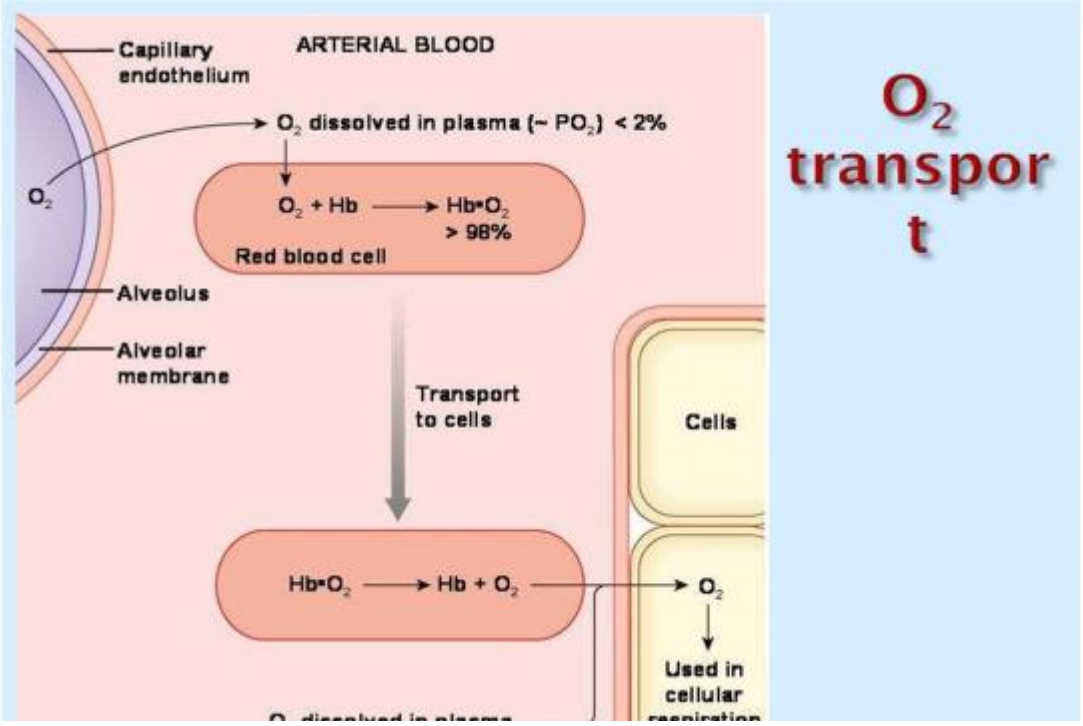
The production of RBCs is known as erythropoiesis.

Adult	Red bone marrow of long bones (hip bone, breast bone & ribs)
Child (upto 5 year)	Bone marrow of all the bones
Foetus	Liver & spleen

- Increase in number of RBCs is known as **polycythemia**
- Decrease in number of RBCs is known as **erythropenia**

Eritrocyte metabolism

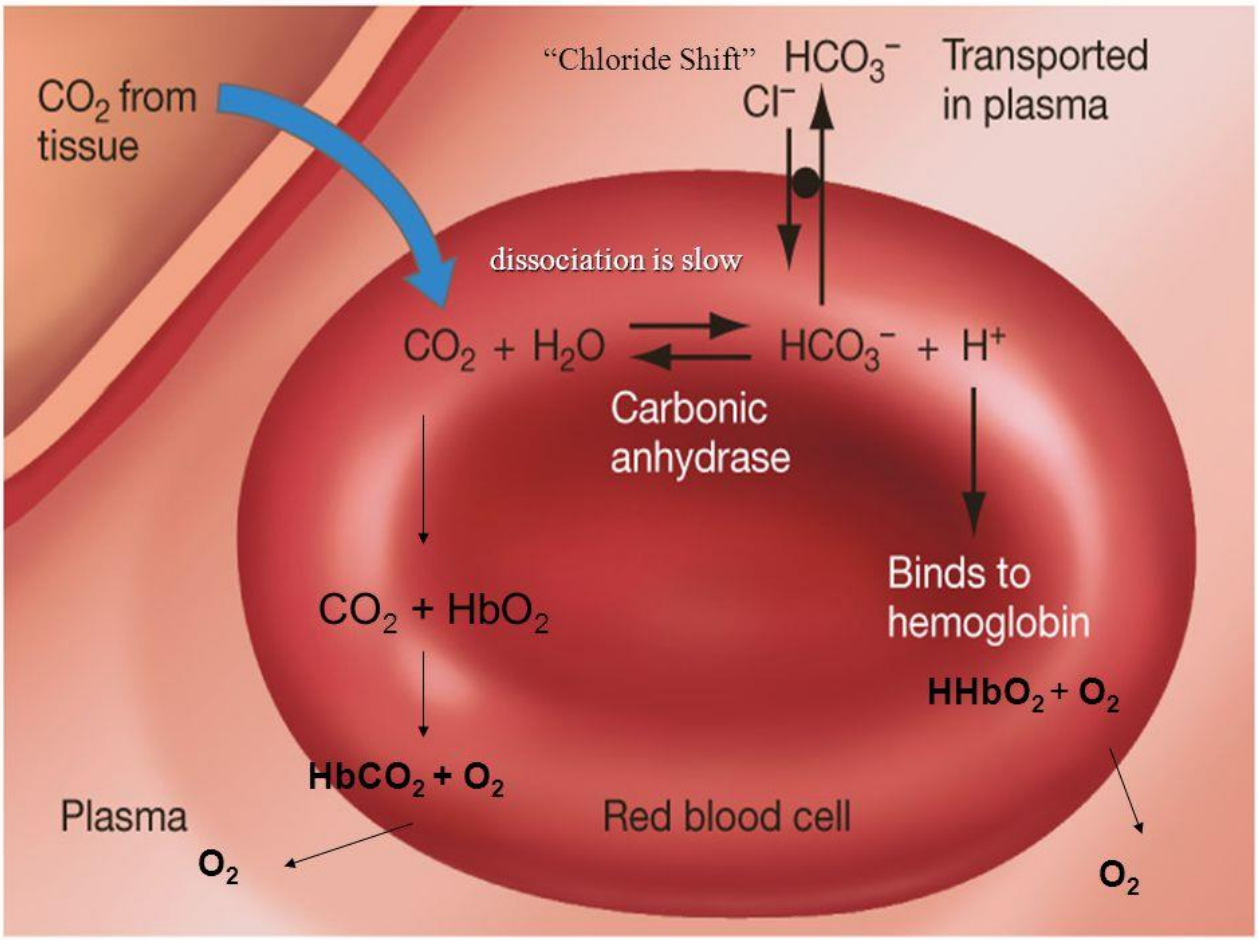
- No nucleus, ribosome, mitochondria
- Anaerobic glycolysis
- Pentose phosphate pathway
- 2,3-biphosphoglicerate regulates Hb affinity to oxygen
- Protection of Hb from oxidation:
  - Methemoglobin reductase
  - NADPH, glutatione



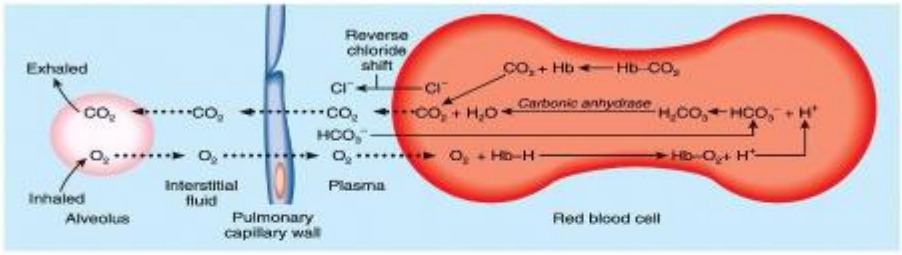
O_2 transport

Oxygen release helps to maintain pH in tissues

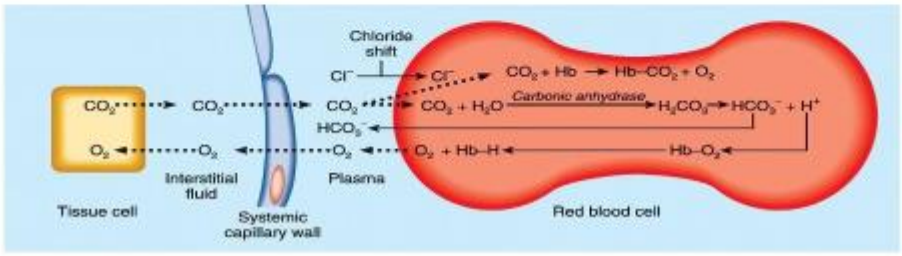
- Lungs: *HHb + O2 = HbO2 + H+*
- CO_2 is formed from plasmatic bicarbonate and proton released from Hb
- Tissues: CO_2 forms proton and bicarbonate:
  - Proton is bound to Hb, when O_2 is released
  - Bicarbonate leaves RBC
- Carboanhydrase plays a key role…
- Cl- / HCO3- interchange is Hamburger effect



Summary



(a) Exchange of O_2 and CO_2 in pulmonary capillaries (external respiration)



(b) Exchange of O_2 and CO_2 in systemic capillaries (internal respiration)

3. The Bohr Effect

i. The influence of pH and pCO_2 to facilitate oxygenation of Hb in the lungs and deoxygenation at the tissues is known as the Bohr effect (1904).

ii. Binding of CO_2 forces the release of O_2

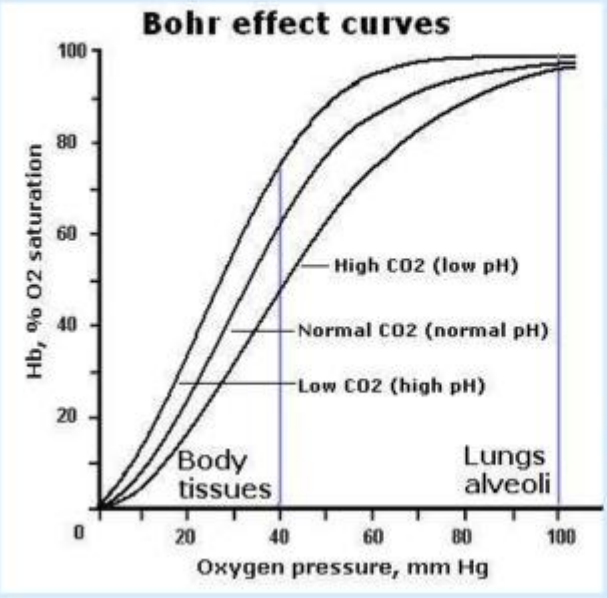
iii. When the pCO_2 high, CO_2 diffuses into the RBCs

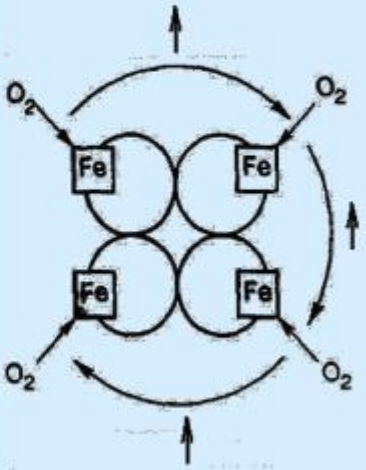
$$CO_2 + H_2O \xrightarrow[\text{Carbonic Anhydrase}]{} H_2CO_3 \rightarrow H^+ + HCO_3^-$$

Iv. When carbonic acid is ionizes, the intracellular pH falls. The affinity of Hb for oxygen is decreased and oxygen is unloaded

8/15/2014

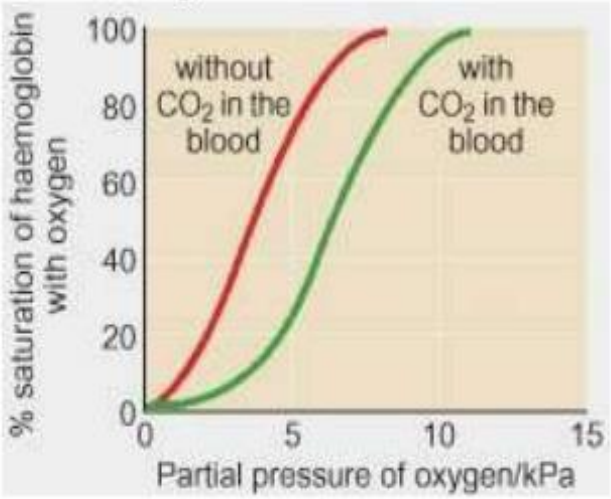
Bohr effect





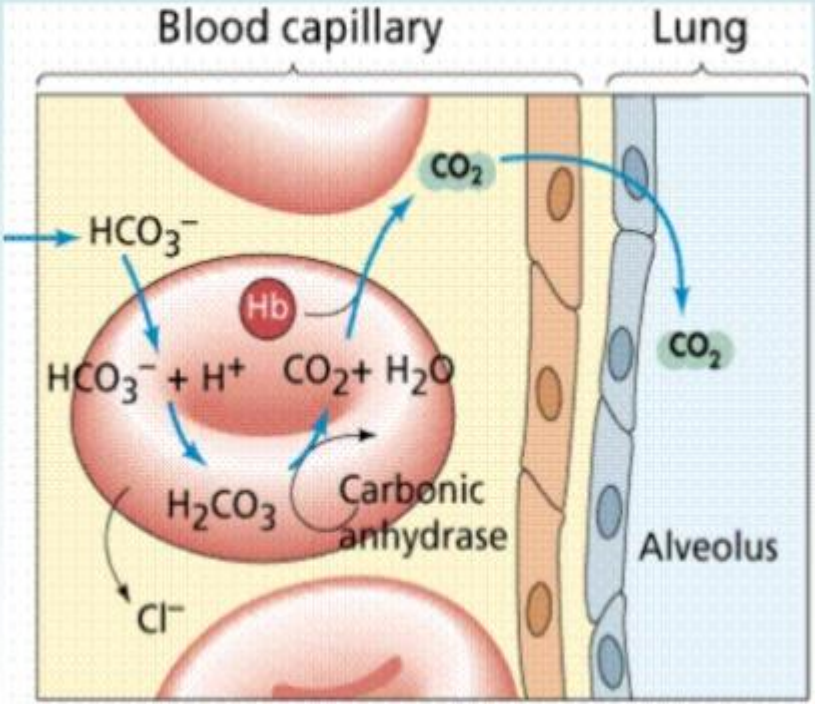
Bohr effect

- Increased carbon dioxide levels lowers the pH of the blood
- This affects the ability of the haemoglobin subunits to transport oxygen
- A lower pH causes the Haemoglobin to release more oxygen
- A higher pH causes the Haemoglobin to hold onto more oxygen



- Haldane effect :

Haldane effect results from the fact that combination of O2 with Hb causes Hb to be a stronger acid. Thus displaces CO2



Metabolic Characteristics of Mature Erythrocytes

- Can not carry out synthesis of nucleic acid and proteins
- Can not obtain energy by oxidative phosphorylation of the mitochondria
- ATP is synthesized from glycolysis and is important in process that help the red blood cell maintain its biconcave shape and also in the regulation of the transport of ions and of water in and out of the cell
- The principal modes of glucose metabolism are anaerobic glycolysis and the pentose-phosphate pathway

Glycolysis

- **Obtain energy by glycolysis of glucose**

  Utilize 2ATP moleculars, produces 4ATP moleculars with a net gain of 2ATP

- **The function of ATP**

  To maintain the correct ion balance, brought about by the pumping out of sodium in exchange for potassium

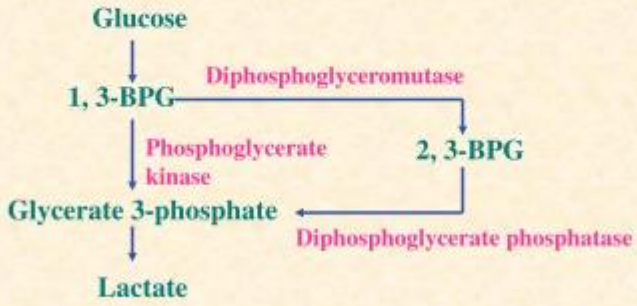
  To maintain the correct conformation of the cell

  To protect against the formation of methaemoglobin

  To synthesize NAD+ and glutathione

The pathway of 2,3-bisphosphoglycerate (2,3-BPG)

- **Formation of 2,3-BPG**



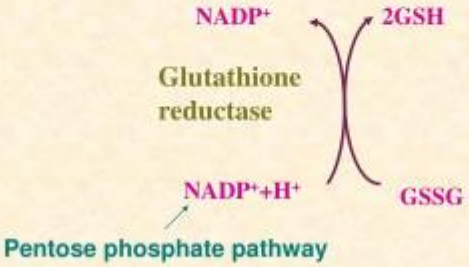
The role of 2,3-BPG

- Plays an important role in the binding of oxygen to hemoglobin in erythrocytes
- Combine with hemoglobin, causing a decrease affinity of hemoglobin for oxygen

$$\underset{(Hb)}{\text{Hemoglobin}} \underset{pO_2\downarrow\ 2,3\text{-DPG}\uparrow(\text{tissues})}{\overset{pO_2\uparrow\ 2,3\text{-DPG}\downarrow(\text{lungs})}{\rightleftharpoons}} \underset{(HbO_2)}{\text{Oxyhemoglobin}}$$

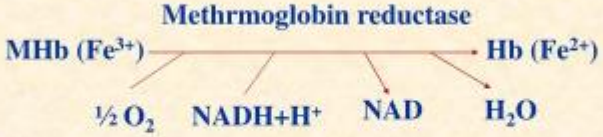
The role of the pentose phosphate pathway

- Produce the NADH which is essential for the regeneration of reduced glutathione from oxidized glutathione



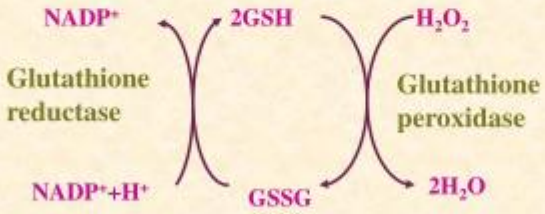
Reduction of methemoglobin

- Methemoglobin does not combine with molecular oxygen and does not have the function of transporting oxygen
- Normally, methemoglobin is reduced to the ferrous state by the NADH-dependent methrmoglobin reductase



The role of glutathione are as follows

The role in the destruction of hydrogen peroxide (H_2O_2) in erythrocytes



Genetic abnormality-deficiency of glucose-6-phosphate dehydrogenase

- glucose-6-phosphate dehydrogenase is the first enzyme of the pentose phosphate pathway
- A deficiency of this enzyme will lead to failure of restoring GSSG to GSH in the erythrocytes, a step essential for the removel of H_2O_2
- Cell damage is likely to result from oxidation of the membranes by the H_2O_2, leading to hemolytic anemia

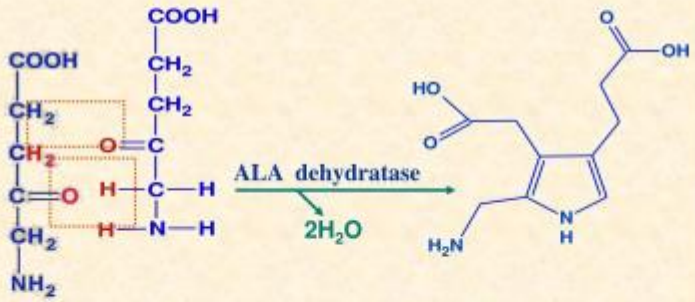
Hemoglobin synthesis

- Heme synthesis starts with the condensation of glycine and succinyl coenzyme A under the action of a rate limiting enzyme δ-aminolevulinic acid (ALA) synthase.
- δ -ALA will be formed.
- Pyridoxal phosphate (vit. B_6) is a coenzyme for this reaction.

This step takes place in the mitochondria

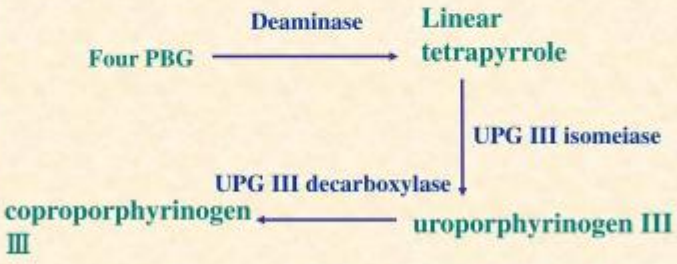
A series of biochemical reactions will follow.

Two molecules of δ-ALA condense to form a pyrrole called porphobilinogen (PBG)



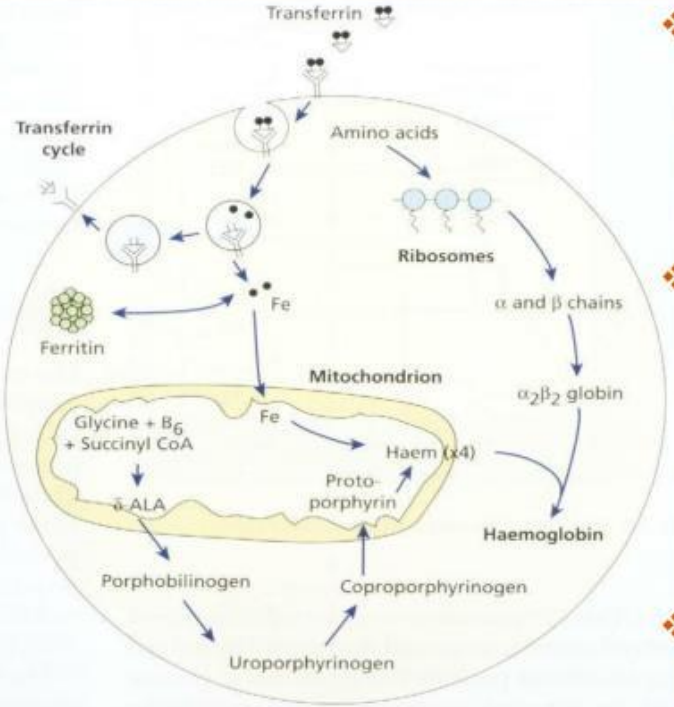
This step occurs in the cytoplasm

- Four PBG condense to form a tetrapyrrole uroporphyrinogen III.
- UPG III is then converted to coproporphyrinogen.



This step occurs in the cytoplasm

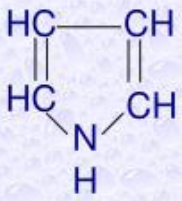
Haemoglobin synthesis

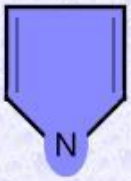


- CPG then changes to protoporphyrin which ultimately combines with iron in the ferrous state (Fe^{2+}) to form haem.
- Iron is brought to the developing red cells by a carrier protein (transferrin) which attaches to special binding sites on the surface of these cells.
- Transferrin releases iron and returns back to circulation.

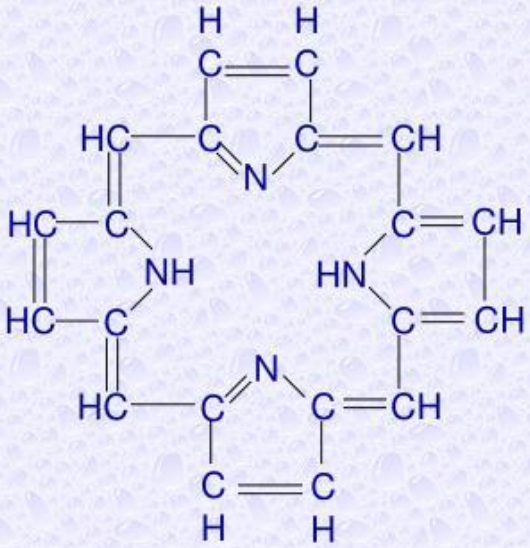
What is Porphyrin ?

Pyrrole

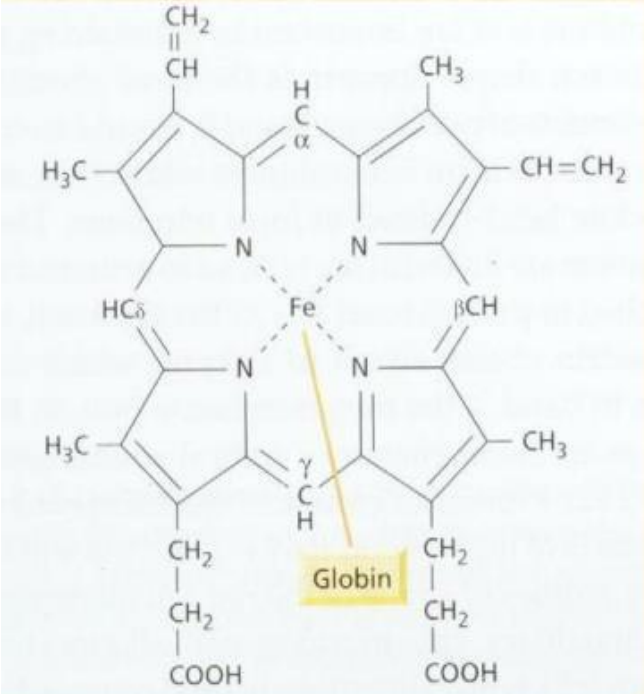




Porphyrin

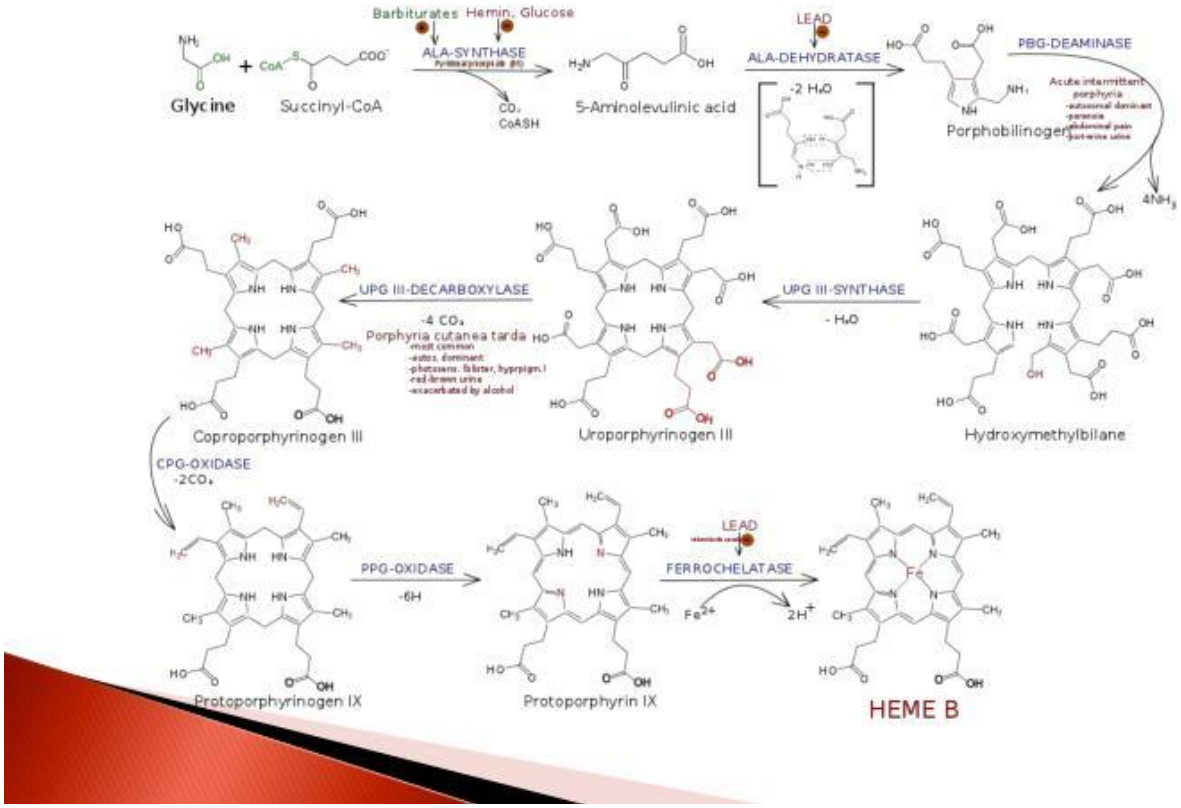


Haemoglobin structure



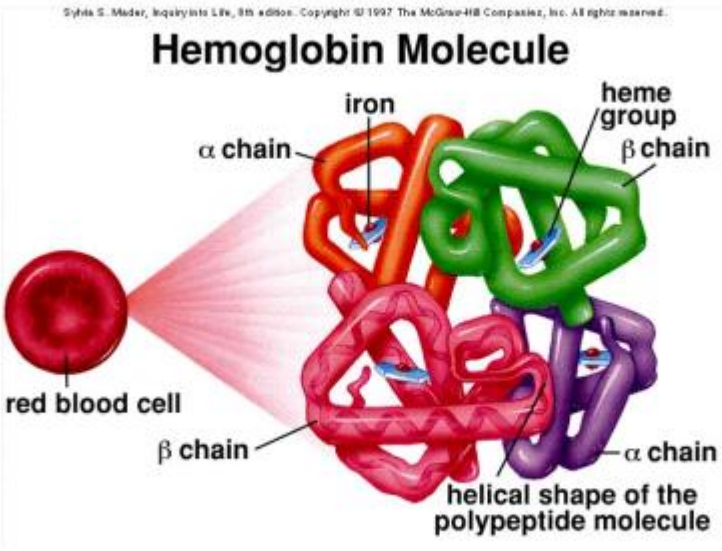
- **Haem consists of a protoporphyrin ring with an iron atom at its centre.**
- **The protoporphyrin ring consists of four pyrrole groups which are united by methane bridges (=C-).**
- **The hydrogen atoms in the pyrrole groups are replaced by four methylene (CH3-), two vinyl (-C=CH2) and two propionic acid (-CH2-CH2-COOH) groups.**

Erythrocytes – Heme synthesis



Gunther disease - congenital erythropoietic porphyria, is one of the least common porphyrias. It results from a deficient activity of uroporphyrinogen III synthase (URO-synthase).

Gilbert Syndrome - a mild genetic disorder in which the liver does not properly process a substance called bilirubin. Gilbert Syndrome is more common in men than women.



Further forms of Hb

- HbA (2α 2β): 90% of Hb in adult
- HbA_2 (2α2σ): 2-3% of Hb in adult
- HbA_{IC}: glycated Hb – important marker of long-term diabetes compensation
- HbF (2α2γ) - fetal Hb, high affinity to O_2
- Hemoglobinopathies: rare monogenic diseases (HbS –anemia).

Hemoglobine derivates unable to transport CO_2

- Methemoglobine: contains Fe 3+ instead of Fe 2+ (e.g. nitrate/nitrite containing food or water)
- Carboxyhemoglobine – CO poisoning, smokers (cherry red colour)
- Sulfhemoglobine – green

Factors with influence on Hb affinity to O_2

- *Right shift* means higher ability of Hb to release O_2 , but lower ability to bind it.
- Is useful in tissues (site of O_2 release):
  - higher temperature
  - lower pH (Bohr effect)
  - higher 2,3 BPG level

ESSENTIALITY OF STRUCTURE AND METABOLISM OF ERYTHROCYTES

1. **Erythrocytes – cells without nucleus, they haven't ribosomes and mitochondria**
2. **The main protein – hemoglobin (more than 95% of all organic substances in the RBC). 1 erythrocyte contains till 400 millions molecule of hemoglobin.**
3. **Hemoglobin contains Fe^{2+}. Conversion of Fe^{2+} to Fe^{3+} leads to the formation of methemoglobin, which cannot join O_2**

   $$Hb(Fe^{2+}) + O_2 \longrightarrow Hb(Fe^{3+}) + O_2^-$$

4. **Hb consists of 4 polypeptide chains (2α, 141 and 2β, 146). Presence of γ-chain in HbF. Changing in β-chain – HbS.**
5. **ATP in RBC is formed by anaerobic glycolysis (90%), active is PPP (10%).**
6. **NADPH·H^+ is necessary for the maintaining of the integrity of membranes of RBC and for the antioxidant systems (glutathione system)**
7. **Activity of Na/K-АТФ-ase, supports the gradient of concentration of Na/K, is high.**
8. **2,3-bisphosphoglycerate (forms from the metabolyte of glycolysis) is present in RBC in the equimolar amount with Hb. It decreases the affinity of Hb to the O_2 in 26 times and helps in the transfer of O_2 from the blood into the tissues.**
9. **Hb joins to O_2 bad, but it is enough to connect the 1 subunit with O_2, and all the other 3 subunits will join to O_2 (cooperative effect).**
10. **CO_2 decreases the affinity of Hb to O_2, it releases an additional amount of O_2 (Bohr's effect)**

    ≈ 10% of CO_2 join with amino groups of globin, ≈ 10% of CO_2 are transported in blood plasma in water-soluble form and ≈ 80% CO_2 are transported by blood in the form of hydrocarbonates

Haemoglobin abnormalities

There are mainly two types of abnormalities, these are :

- Quantitative abnormalities: where there is reduction in the production of certain types of globins e.g. α thalassaemia
  β thalassaemia
- Qualitative abnormalities: where there is production of abnormal haemoglobin e.g. sickle cell anaemia.

Haemoglobinopathies

Haemoglobinopathy

- abnormal structure of the haemoglobin (mutation)
- large number of haemoglobin mutations, a fraction has deleterious effects
- sickling, change in O_2 affinity, heme loss or dissociation of tetramer
- **haemoglobin M and S**, and **thalassemias**

Haemoglobin M

- replacement of the histidine (E8 or F7) in α or β-chain by the tyrosine
- the iron in the heme group is in the Fe^{3+} state (methaemoglobin) stabilized by the tyrosine
- methaemoglobin can not bind oxygen

Thalassemias

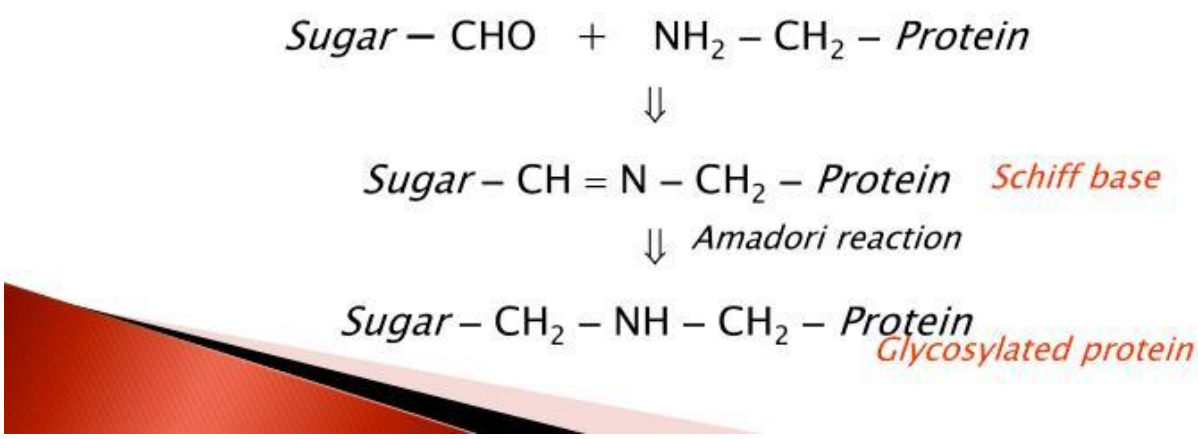
- genetic defects- α or β-chains are not produced (α or β-thalassemia)

Haemoglobin S (sickle-cell)

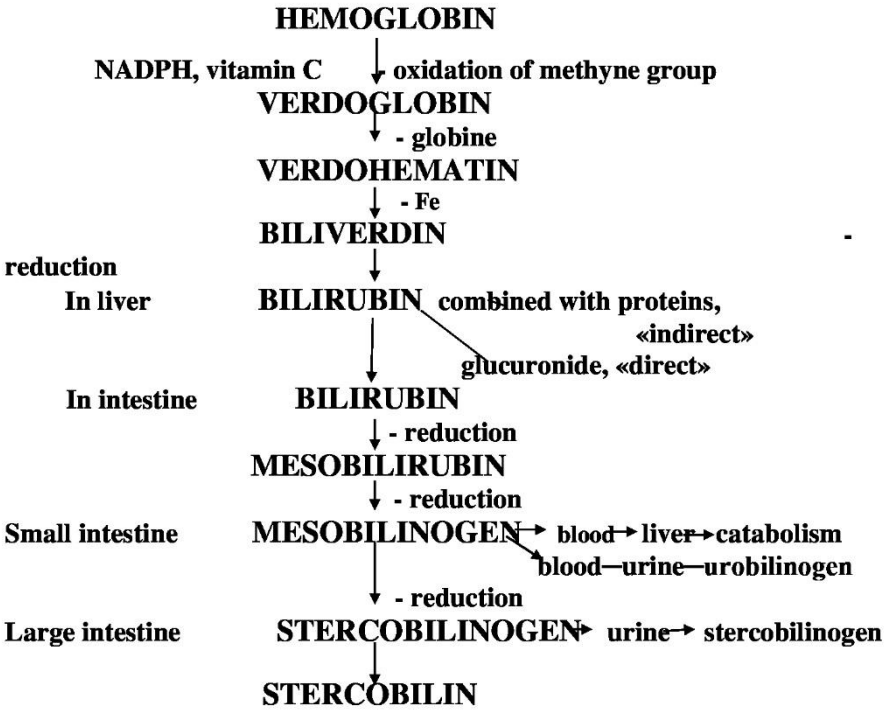
- Causes a sickle-cell anemia
- Erythrocytes adopt an elongated sickle shape due to the aggregation of the haemoglobin S

Glycosylated haemoglobin (HbA1)

- formed by hemoglobin's exposure to high plasma levels of glucose
- non-enzymatic glycolysation (glycation)- sugar bonding to a protein
- normal level HbA_1- 5%; a buildup of HbA_1- increased glucose concentration
- the HbA_1 level is proportional to average blood glucose concentration over previous weeks; in individuals with poorly controlled diabetes, increases in the quantities of these glycated hemoglobins are noted (patients monitoring)



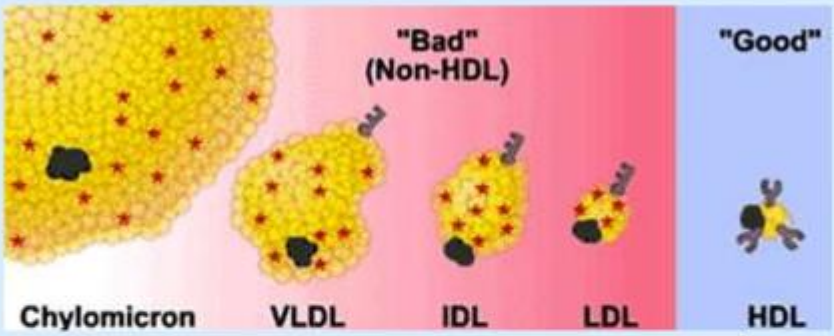
FORMATION OF BILE PIGMENTS



DIFFERENTIAL DIAGNOSTICS OF JAUNDICE BY THE DATA OF INVESTIGATION OF PIGMENT METABOLISM

Type of jaundice	Blood		Urine			Faeces
	Bilirubin		Sterco-bilin (ogen)	Uro-bilin (ogen)	Bili-rubin (direct)	Sterco-bilin (ogen)
	Direct	Indirect				
Normal	±	+	+	—	—	+
Obstractive	↑↑↑	↑	—	—	↑	—
Parenshymatic	↑↑↑	↑↑	—	↑	↑↑↑	—
Hemolytic	+	↑↑↑	↑↑	—	—	↑↑

Blood lipoproteins



Blood Cholesterol Ratios		
	Protective	Warning
Total Cholesterol / HDL	Less than 4.2	4.3 and higher
LDL / HDL	Less than 2.5	2.6 and higher

The main function of plasma lipoproteins is lipid transport.

According to chemical composition, movement in an electric field,

the difference in density, they are divided into several groups:

Characteristics	Chylomicrons	LDL	VLDL	HDL
Protein %	2	10	25	50
Phospholipids	5	15	25	30
Cholesterol	5	15	40	15
Triacylglycerols	85	60	10	5
Transportation	Exo-TAG	Endo-TAG	Cholesterol	Phospholipids
Ratio proteins/lipids	1:50	1:10	1:4	1:1
Blood concentration	1-2,5 g/l	1-1,5 g/l	3-4,5 g/l	1, 5-4 g/l

Classification of hyperlipoproteinemia by Fredrikksen (WHO)

Type of GLP	Level of lipoproteins	Chole sterol	Triacyl glycerols	Atherogenicity	Frequency
I	Chylomicrons	Norma	++++	Wasn't prove	< 1%
IIa	LDL	++	Norma	+++	10%
IIв	LDL and VLDL	++	++	+++	40%
III	IDL	++	+++	+++	< 1%
IV	VLDL	N or +	++	+	45%
V	VLDL and chylomicrons	++	++++	+	5%

General material and educational and methodological support of the lecture:

- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Biochemical and physiological functions of blood in the human body. Respiratory function of erythrocytes.
2. Hemoglobin: mechanisms of participation in the transport of oxygen and carbon dioxide. Variants and pathological forms of human hemoglobins.
3. Blood buffer systems. Violation of the acid-base balance in the body (metabolic and respiratory acidosis, alkalosis).
4. Biochemical composition of human blood. Blood plasma proteins and their clinical and biochemical characteristics.
5. Blood plasma enzymes; value in enzymodiagnosis of diseases of organs and tissues.
6. Kallikrein-kinin system of blood and tissues. Medicines are antagonists of kinin formation.
7. Non-protein organic compounds of blood plasma. Inorganic components of plasma.
8. Metabolism of porphyrins: heme structure; Scheme of biosynthesis reactions of protoporphyrin IX and heme.
9. Hereditary disorders of porphyrin biosynthesis, types of porphyrias.
10. Catabolism of hemoglobin and heme (scheme); formation and structure of bile pigments.
11. Pathobiochemistry and types of jaundice; biochemical diagnosis of jaundice.
12. Conjugation reactions in hepatocytes: biochemical mechanisms, functional significance.
13. The role of the liver in the exchange of bile pigments. Pathobiochemistry of jaundice; types of jaundice; hereditary (enzymatic) jaundice.
14. Immunoglobulins; biochemical characteristics of individual classes of human immunoglobulins.
15. Mediators and hormones of the immune system: interleukins; interferons; protein-peptide factors of cell growth and proliferation regulation.
16. Complement system; biochemical components of the human complement system; classical and alternative ways of activation.
17. Biochemical mechanisms of immunodeficiency states: primary (hereditary) and secondary immunodeficiencies.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php

Lecture № 16

Topic: Biochemistry of coagulation, anticoagulation and fibrinolytic systems. Violation of coagulation hemostasis

Relevance of the topic: Blood coagulation is a complex physiological and biochemical process, which is a protective reaction of our body to blood loss. Knowledge of the biochemical characteristics of the coagulation, anticoagulation and fibrinolytic systems of blood is necessary for understanding the mechanisms of maintaining the aggregate state of blood under normal conditions and with numerous diseases, as well as for their timely correction with pharmaceuticals

Purpose: to study the molecular mechanisms of blood coagulation and anticoagulation systems, the role of the liver in this process. Summarize information about the molecular structure of coagulants and anticoagulants, mechanisms of hemophilia of various genesis. To know the role of the components of the coagulation, anticoagulation and fibrinolytic systems in the pathochemistry of atherosclerosis and hypertension.

Basic concepts:
1. Hemostasis.
2. Fibrinolysis.
3. DIC - syndrome.

Plan and organizational structure of the lecture:

1. Biochemical and functional characteristics of the hemostasis system.
2. Blood coagulation system; characteristics of individual factors; mechanisms of functioning of the cascade blood coagulation system.
3. The role of vitamin K in coagulation reactions; medicines - agonists and antagonists of vitamin K.
4. Anticoagulant blood system; characteristics of anticoagulants. Hereditary disorders of the blood coagulation process.
5. Fibrinolytic blood system.
6. DIC-syndrome

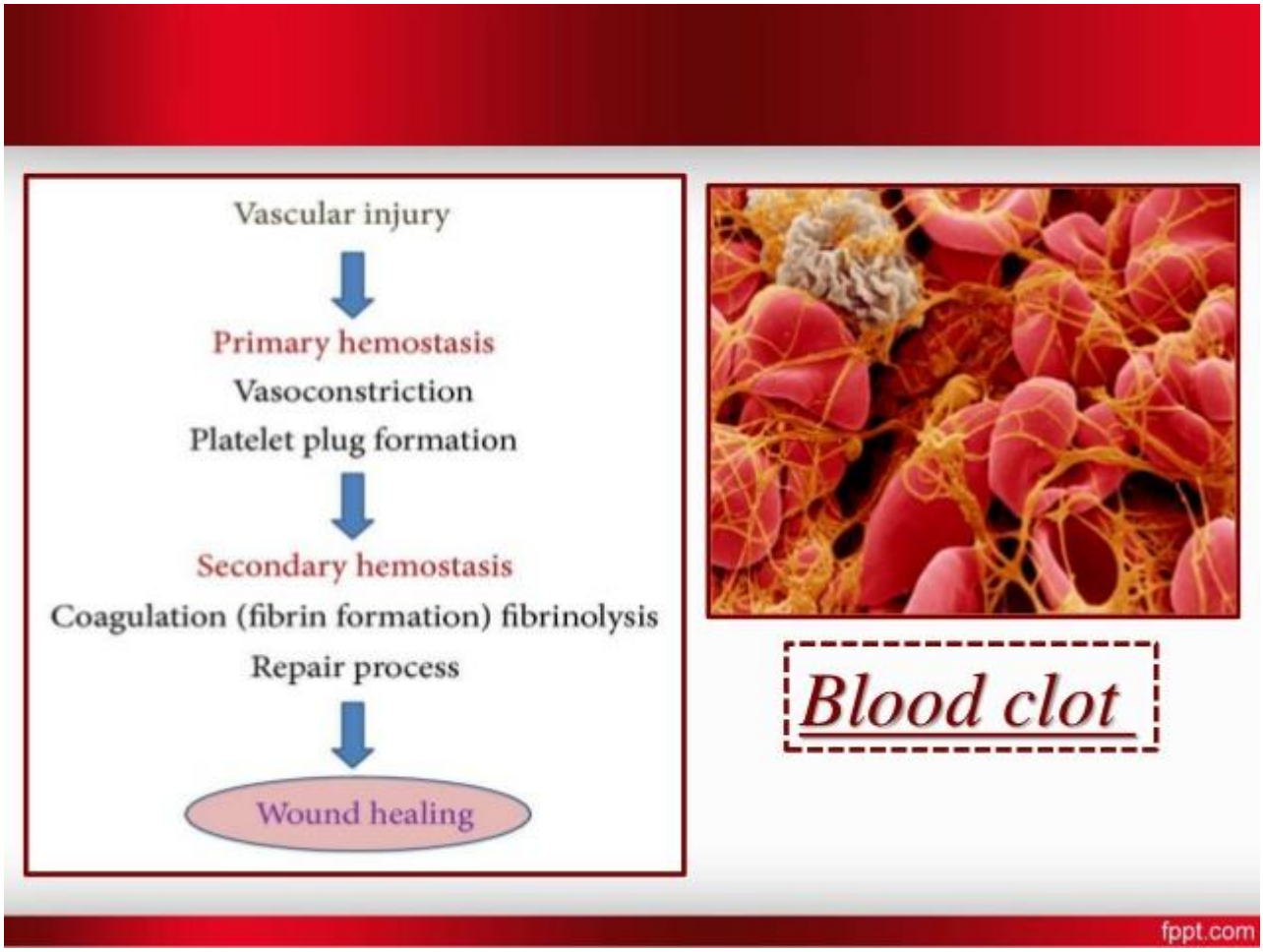
Content of the lecture material

HEMOSTASIS OVERVIEW

- DEFINITION
  - Heme = blood
  - stasis = to halt

- It is the process of forming clots in the wall of damaged blood vessels & preventing blood loss while maintaining blood in a fluid state with in the vascular system.

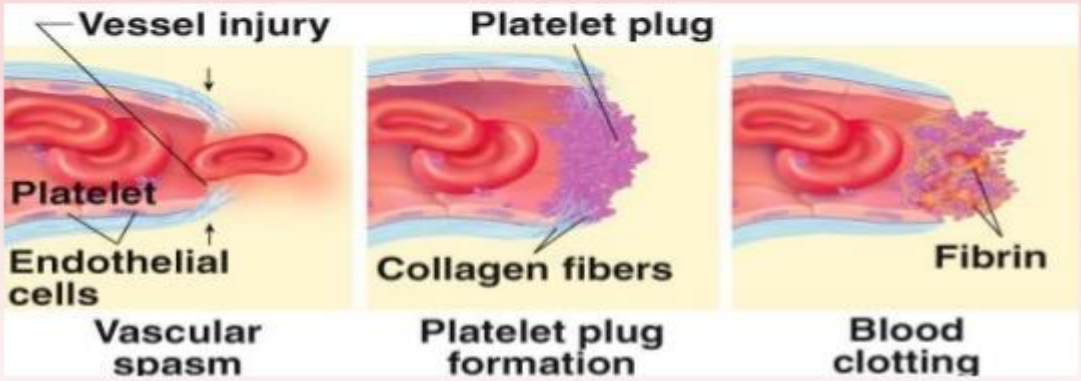
- Spontaneous arrest of bleeding by physiological process.



STAGES OF HEMOSTASIS:

When a blood vessel is injured, the injury initiates a series of reactions, resulting in hemostasis. It occurs in three stages.

1. Vasoconstriction.
2. Platelet plug formation.
3. Coagulation of blood.



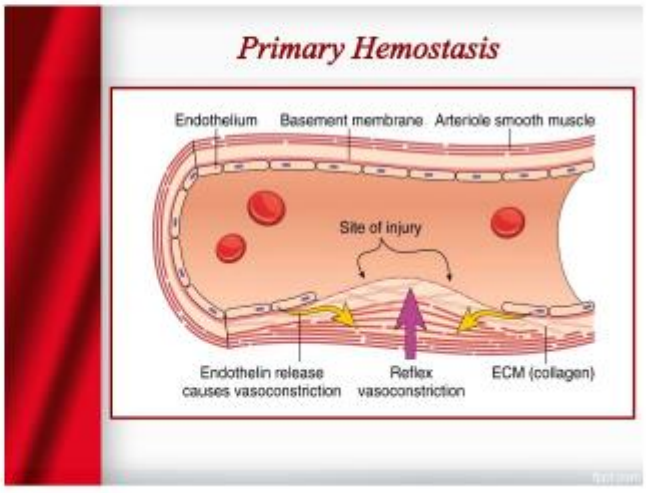
I-Vascular spasm

Reduces flow of blood from injured vessel.

Cause:

1- Sympathetic reflex

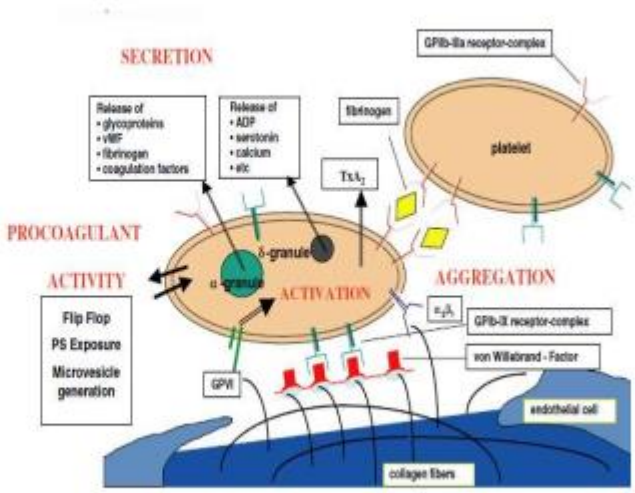
2- Release of vasoconstrictors (TXA_2 and serotonin) from platelets that adhere to the walls of damaged vessels.



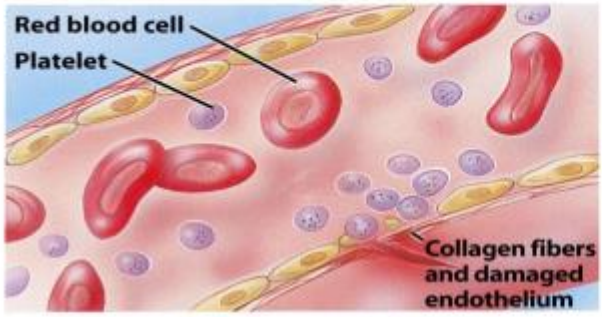
II- Platelet plug formation

Mechanism:

Platelet adherence
Platelet activation
Platelet aggregation

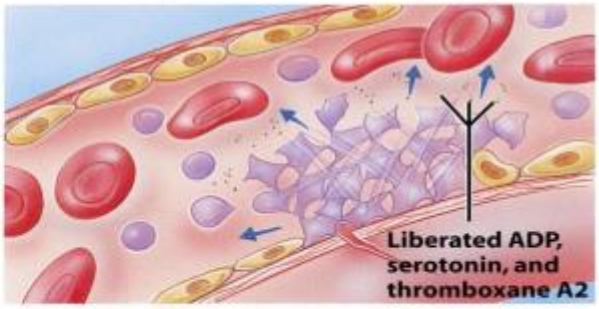


platelet adhesion



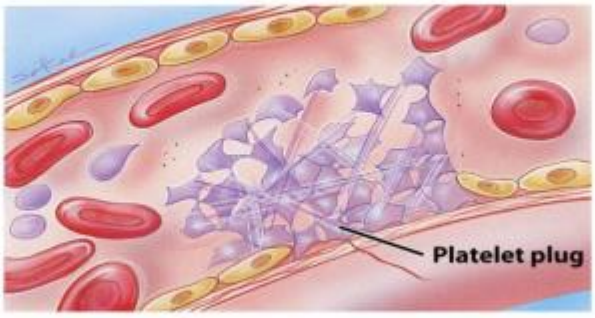
1 Platelet adhesion

Platelet activation : platelet release action



2 Platelet release reaction

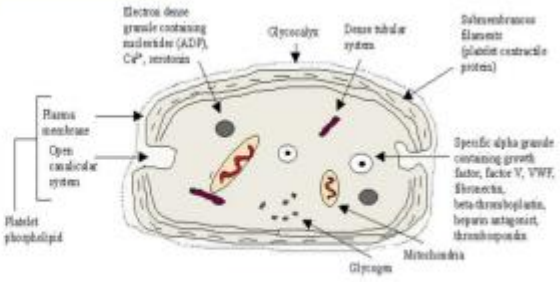
Platelet aggregation



3 Platelet aggregation

Functional characteristics of platelets

- The cell membrane of platelets contains:
  - A coat of glycoprotein (receptors) that cause adherence to injured endothelial cells and exposed collagen.
  - Phospholipids, that play an important role in blood clotting.

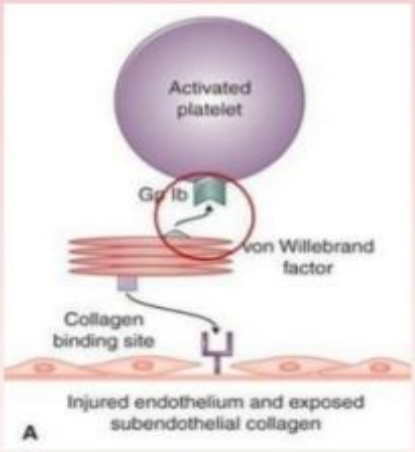


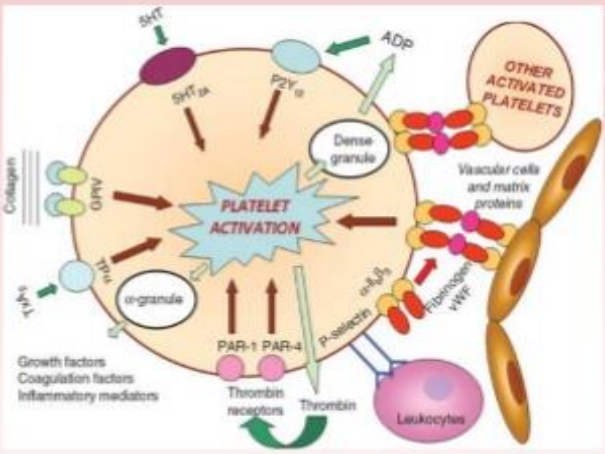
Substances present in platelet granules	
Alpha granules	**Dense granules**
Clotting factors: fibrinogen, V and XIII Platelet-derived growth factor Vascular endothelial growth factor Basic fibroblast growth factor Endostatin Thrombospondin	Nucleotides Serotonin Phospholipid Calcium Lysosomes

PROPERTIES OF PLATELETS

Platelets have three important properties (three 'A's):

1. Adhesiveness
2. Aggregation
3. Agglutination.

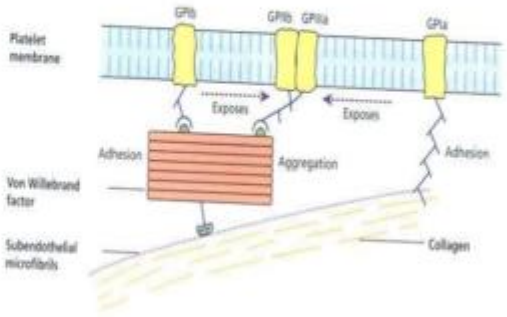




Mechanism of platelet plug formation

* *Platelet adhesion:* When a blood vessel wall is injured, platelets adhere to the exposed collagen and von Willebrand factor in the wall via platelet receptors → *Platelet activation.*

*Activated platelets release the contents of their granules including ADP and secrete TXA_2 → activates nearby platelets to produce further accumulation of more platelets (*platelet aggregation)* and forming a *platelet plug*.



18

ACTIVATORS AND INHIBITORS OF PLATELETS

ACTIVATORS OF PLATELETS:

- Collagen
- Von Willebrand factor
- Thromboxane A2
- Platelet-activating factor
- Thrombin
- ADP
- Calcium ions
- P-selectin
- Convulxin

INHIBITORS OF PLATELETS:

- Nitric oxide
- Clotting factors: II, IX, X, XI, XII
- Prostacyclin
- Nucleotidases

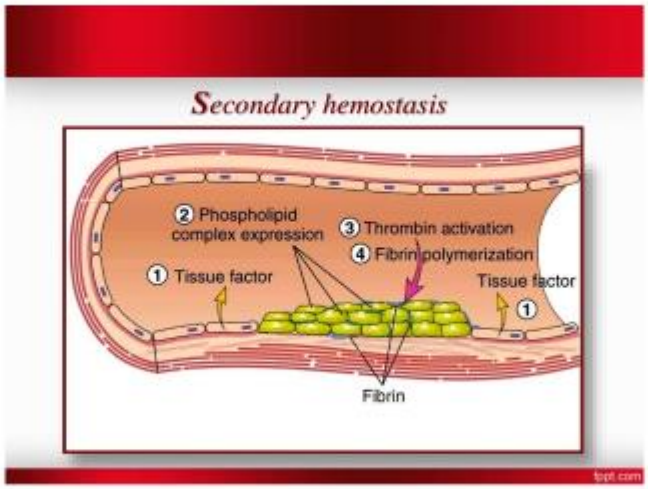
PLATELET DISORDERS

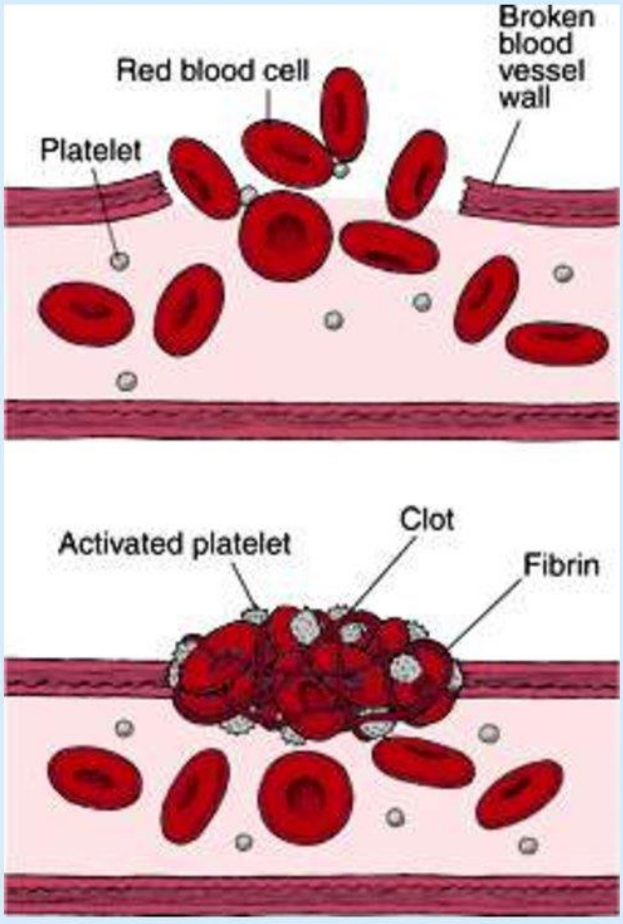
Thrombocytopenia	Thrombocytosis	Thrombocythemia	Glanzmann's thrombasthenia:
Acute infections Acute leukemia Aplastic and Pernicious anemia Chickenpox Smallpox Splenomegaly Scarlet fever Typhoid Tuberculosis Purpura Gaucher's disease.	Allergic conditions Asphyxia Hemorrhage Bone fractures Surgical operations Splenectomy Rheumatic fever Trauma	Carcinoma Chronic leukemia Hodgkin's disease.	Inherited disorder associated with structural or functional abnormalities in platelets.

Secondary hemostasis

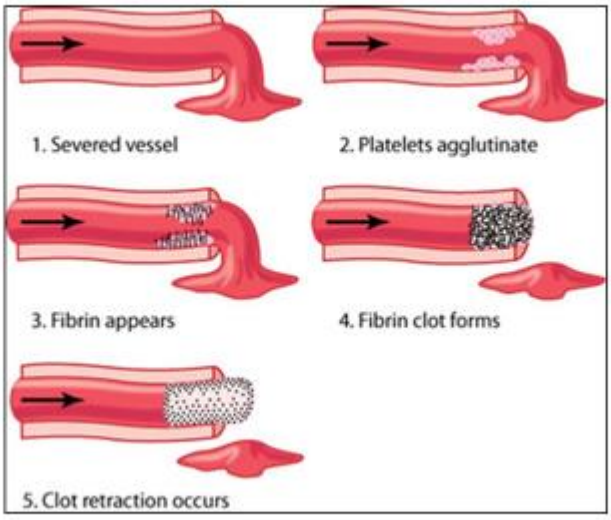
- "***Cascade of reactions***" by **Macfarlane, R.G.,1967**

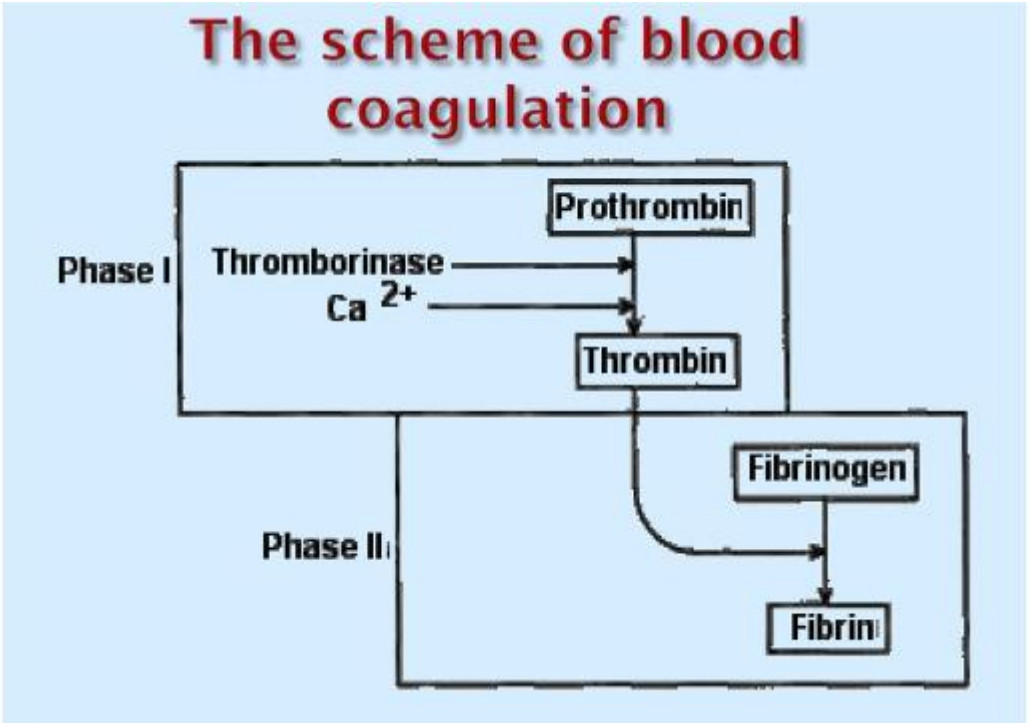
It states that '**inactive**' enzymes are **activated**, and the '**activated**' enzymes in turn **activates other inactive enzymes** until final step is reached.





Blood clotting


1. Severed vessel
2. Platelets agglutinate
3. Fibrin appears
4. Fibrin clot forms
5. Clot retraction occurs


The scheme of blood coagulation
Phase I
Thromborinase
Ca 2+
Prothrombin
Thrombin
Phase II
Fibrinogen
Fibrin

MECHANISM OF CLOTTING

- Coagulation of blood occurs through a series of reactions due to the activation of a group of substances.
- Substances necessary for clotting are called **clotting factors.**
- Thirteen clotting factors are identified.

Name	Description	Function
Fibrinogen (Factor I)	Molecular Weight (MW) = 340,000 daltons (Da); glycoprotein	Adhesive protein that forms the fibrin clot
Prothrombin (Factor II)	MW = 72,000 Da; vitamin K-dependent serine protease	Activated form is main enzyme of coagulation
Tissue factor (Factor III)	MW = 37,000 Da; also known as thromboplastin	Lipoprotein initiator of extrinsic pathway
Calcium ions (Factor IV)	Necessity of Ca++ ions for coagulation reactions described in 19th century	Metal cation necessary for coagulation reactions
Factor V (Labile factor)	MW = 330,000 Da	Cofactor for activation of prothrombin to thrombin
Factor VII (Proconvertin)	MW = 50,000 Da; vitamin K-dependent serine protease	With tissue factor, initiates extrinsic pathway
Factor VIII (Antihemophilic factor)	MW = 330,000 Da	Cofactor for intrinsic activation of factor X
Factor IX (Christmas factor)	MW = 55,000 Da; vitamin K-dependent serine protease	Activated form is enzyme for intrinsic activation of factor X
Factor X (Stuart-Prower factor)	MW = 58,900 Da; vitamin K-dependent serine protease	Activated form is enzyme for final common pathway activation of prothrombin
Factor XI (Plasma thromboplastin antecedent)	MW = 160,000 Da; serine protease	Activated form is intrinsic activator of factor IX
Factor XII (Hageman factor)	MW = 80,000 Da; serine protease	Factor that nominally starts aPTT-based intrinsic pathway
Factor XIII (Fibrin stabilizing factor)	MW = 320,000 Da	Transamidase that cross-links fibrin clot
High-molecular-weight kininogen (Fitzgerald, Flaujeac, or William factor)	MW = 110,000 Da; circulates in a complex with factor XI	Cofactor
Prekallikrein (Fletcher factor)	MW = 85,000 Da; serine protease	Activated form that participates at beginning of aPTT-based intrinsic pathway

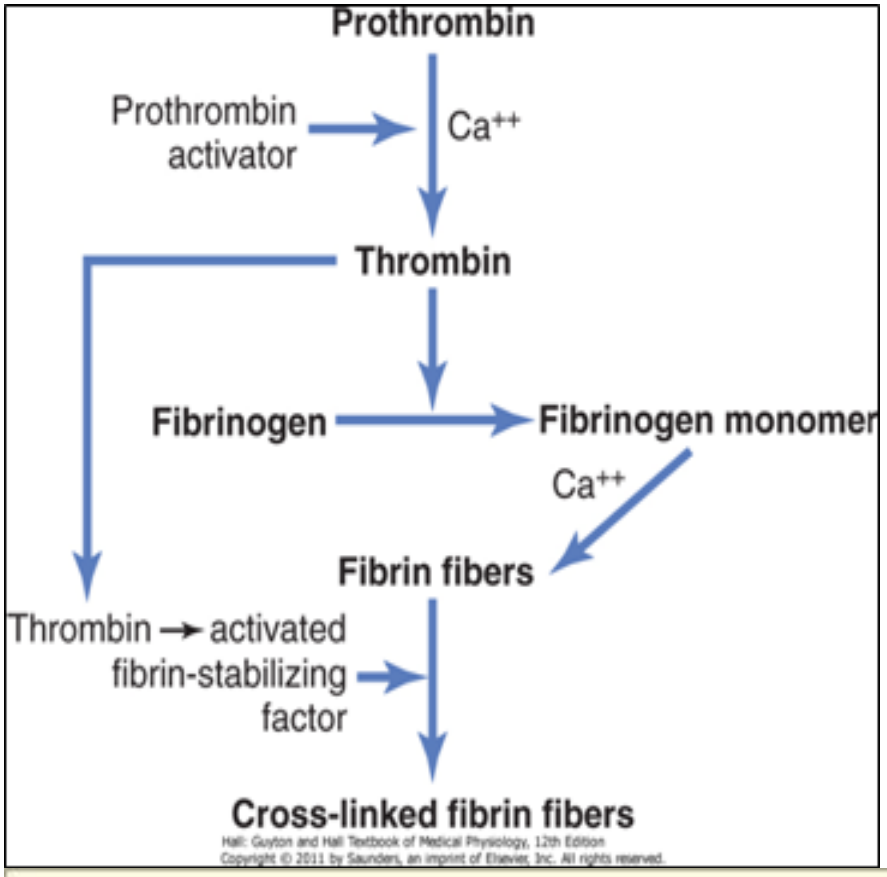
Blood Coagulation

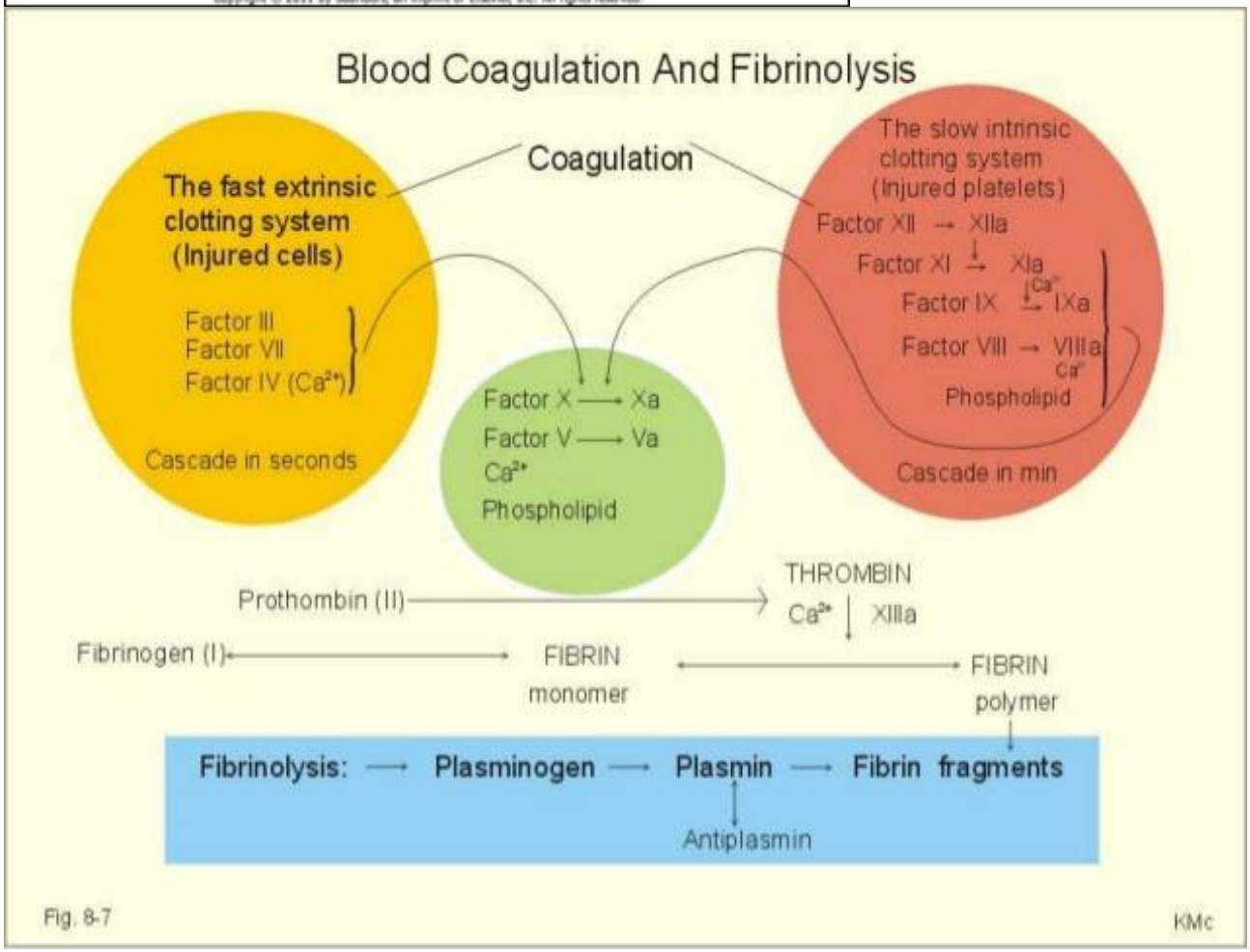
Coagulation Factors		
Factor	**Descriptive Name**	**Function/Active Form**
I	Fibrinogen	Fibrin
II	Prothrombin	Serine protease
III	Tissue factor	Receptor and cofactor
IV	Ca^{+2}	Cofactor
V	Proaccelerin, labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihemophilia factor A	Cofactor
IX	Antihemophilia factor B, Christmas factor	Serine protease
X	Stuart-Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
XIII	Fibrin stabilizing factor	Ca^{2+}-dependent transglutaminase
Prekallikrein		Serine protease
High-molecular-weight kininogen		Cofactor
Regulatory Proteins		
Thrombomodulin	Endothelial cell receptor, binds thrombin	
Protein C	Activated by thrombomodulin-bound thrombin; is a serine protease	
Protein S	cofactor; binds activated protein C	

Table 13.4 | The Plasma Clotting Factors

Factor	Name	Function	Pathway
I	Fibrinogen	Converted to fibrin	Common
II	Prothrombin	Converted to thrombin (enzyme)	Common
III	Tissue thromboplastin	Cofactor	Extrinsic
IV	Calcium ions (Ca^{2+})	Cofactor	Intrinsic, extrinsic, and common
V	Proaccelerin	Cofactor	Common
VII*	Proconvertin	Enzyme	Extrinsic
VIII	Antihemophilic factor	Cofactor	Intrinsic
IX	Plasma thromboplastin component; Christmas factor	Enzyme	Intrinsic
X	Stuart-Prower factor	Enzyme	Common
XI	Plasma thromboplastin antecedent	Enzyme	Intrinsic
XII	Hageman factor	Enzyme	Intrinsic
XIII	Fibrin stabilizing factor	Enzyme	Common

*Factor VI is no longer referenced; it is now believed to be the same substance as activated factor V.


Prothrombin
Prothrombin activator
Ca++
Thrombin
Fibrinogen
Fibrinogen monomer
Ca++
Fibrin fibers
Thrombin → activated fibrin-stabilizing factor
Cross-linked fibrin fibers
Hall: Guyton and Hall Textbook of Medical Physiology, 12th Edition
Copyright © 2011 by Saunders, an imprint of Elsevier, Inc. All rights reserved.

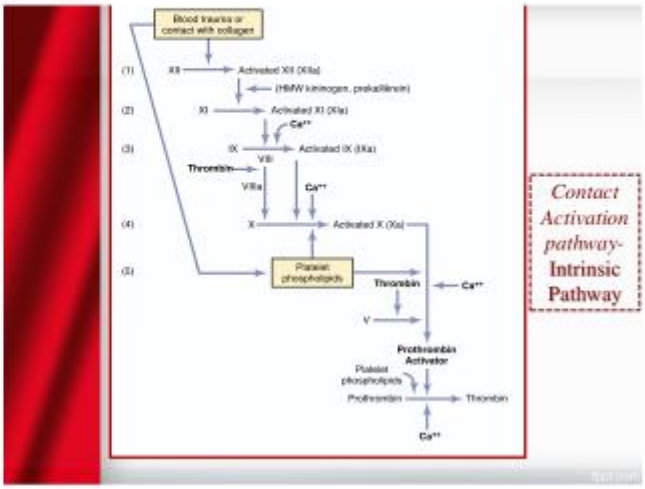

Blood Coagulation And Fibrinolysis
Coagulation
The fast extrinsic clotting system (Injured cells)
Factor III
Factor VII
Factor IV (Ca2+)
Cascade in seconds
Factor X → Xa
Factor V → Va
Ca2+
Phospholipid
The slow intrinsic clotting system (Injured platelets)
Factor XII → XIIa
Factor XI → XIa
Factor IX → IXa
Factor VIII → VIIIa
Phospholipid
Cascade in min
Prothombin (II)
THROMBIN
Ca2+ XIIIa
Fibrinogen (I)
FIBRIN monomer
FIBRIN polymer
Fibrinolysis: → Plasminogen → Plasmin → Fibrin fragments
Antiplasmin
Fig. 8-7
KMc

Intrinsic pathway

The initial reaction is the conversion of inactive factor XII to active factor XIIa.

Factor XII is activated in vitro by exposing blood to foreign surface (glass test tube).

Activation in vivo occurs when blood is exposed to collagen fibers underlying the endothelium in the blood vessels.



27

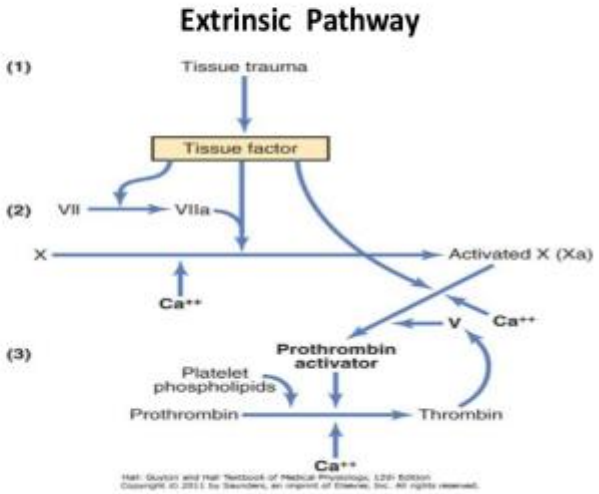
Extrinsic pathway

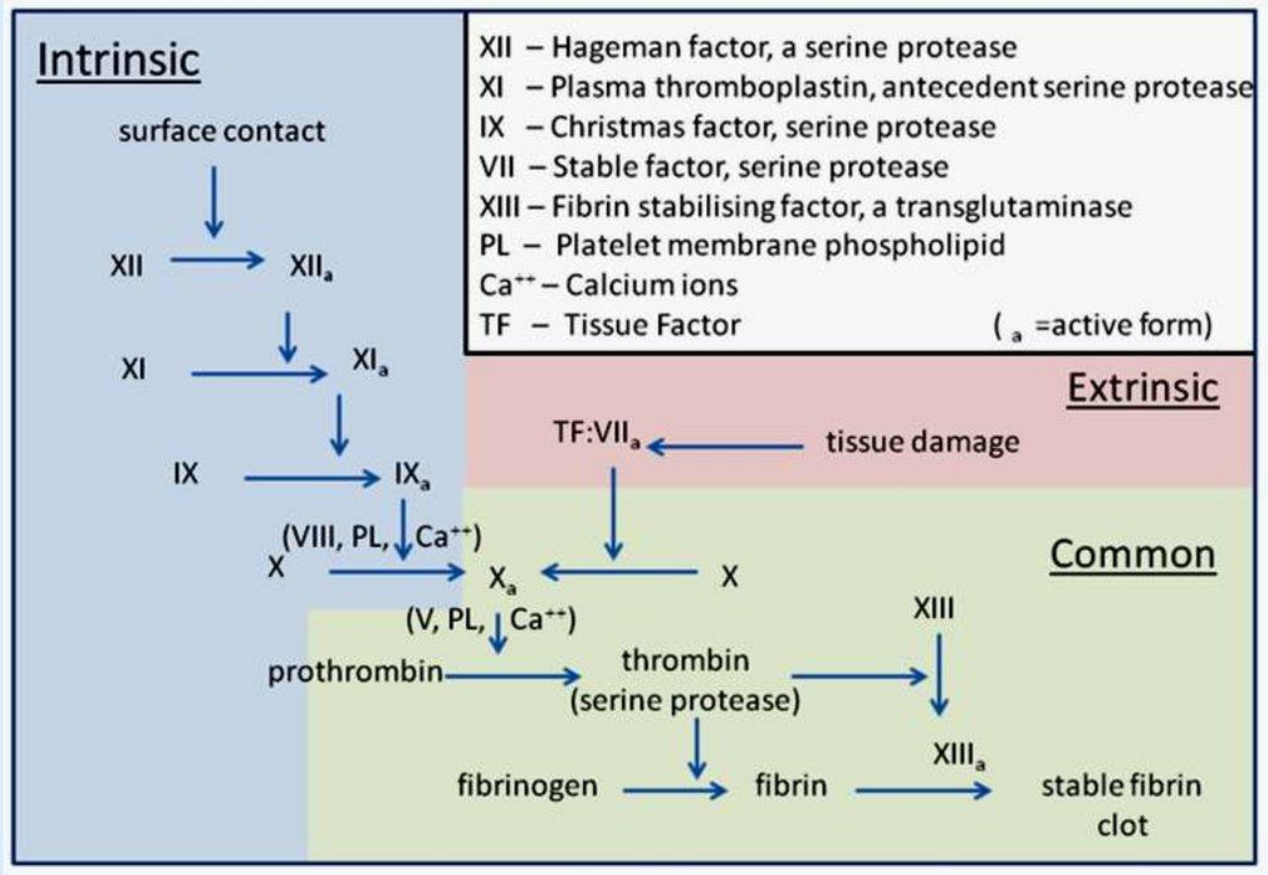
Requires contact with tissue factors external to blood.

This occurs when there is trauma to the vascular wall and surrounding tissues.

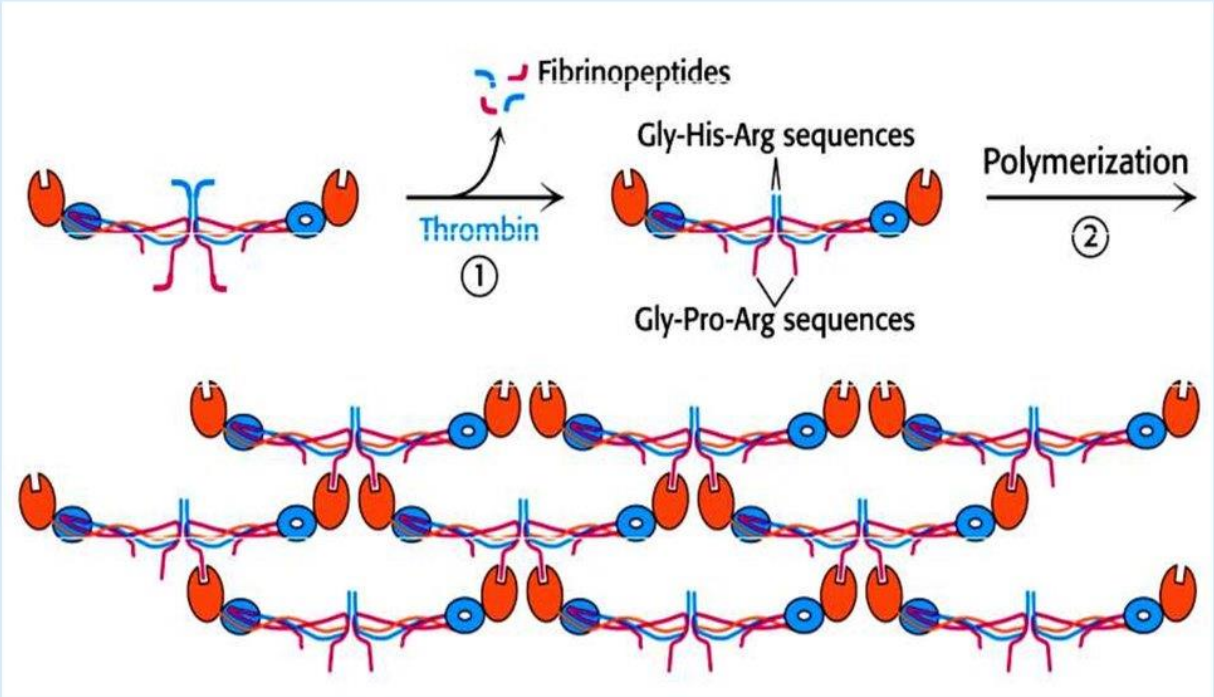
The extrinsic system is triggered by the release of tissue factor (thromboplastin from damaged tissue), that activates factor VII.

The tissue thromboplastin and factor VII activate factor X.

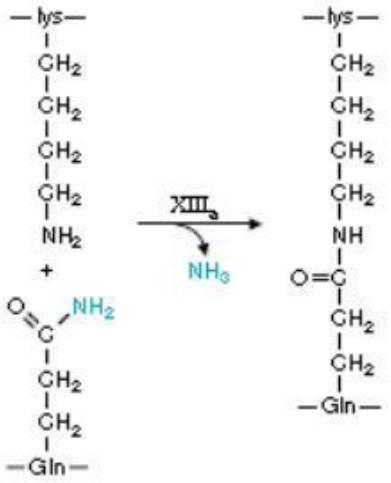




Fibrin polymerisation



Blood Coagulation Cascade

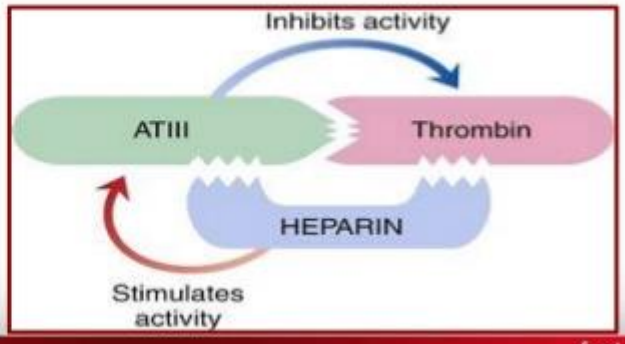


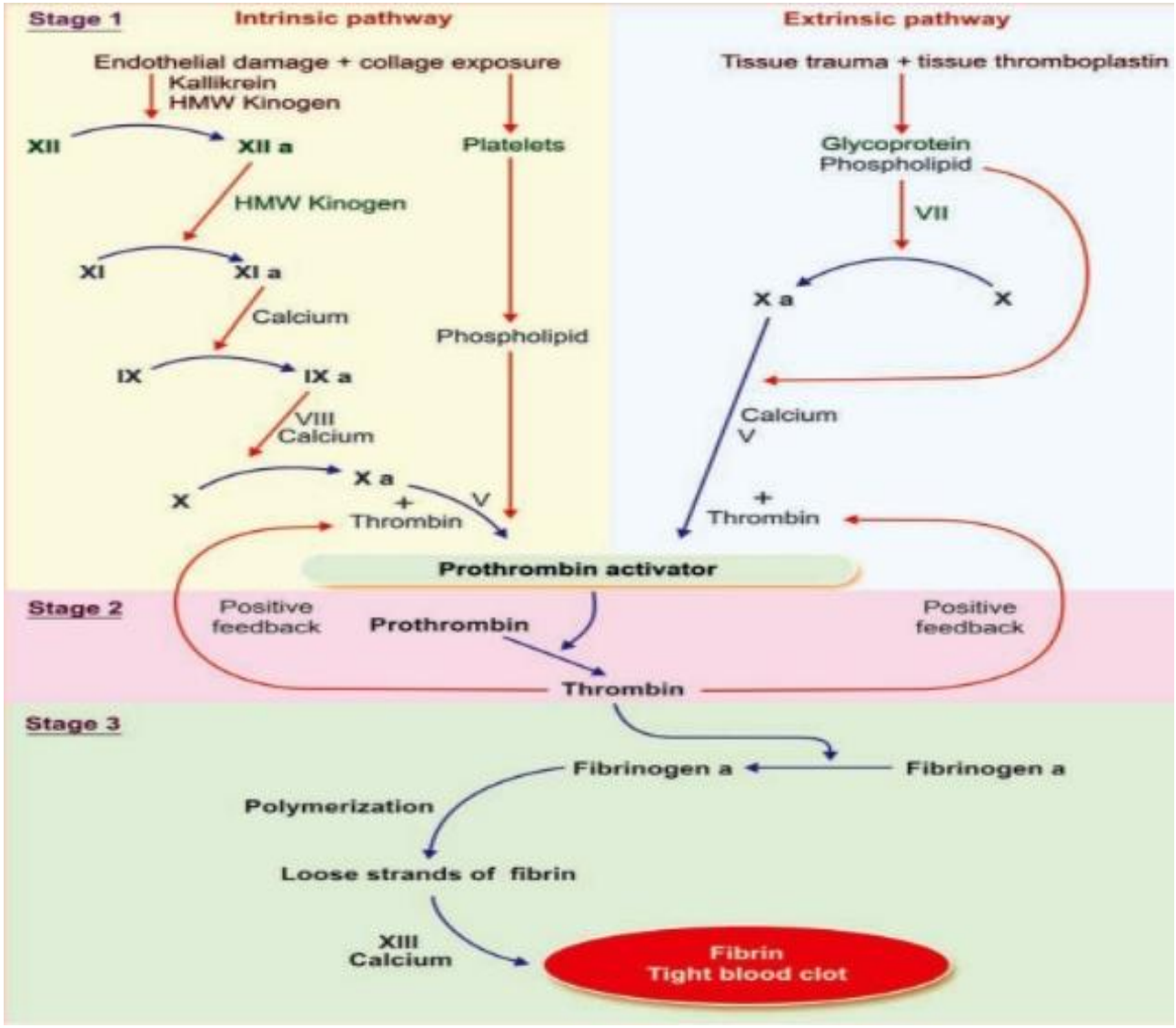
Transamidation by factor XIIIa / transglutamidase

Regulation of Thrombin activity

The activation of thrombin is also regulated by specific thrombin inhibitors.

- *Antithrombin III* is the most important since it can also inhibit the activities of factors IXa, Xa, XIa and XIIa, plasmin, and kallikrein. The activity of antithrombin III is potentiated in the presence of heparin by the following means: heparin binds to a specific site on antithrombin III, producing an altered conformation of the protein, and the new conformation has a higher affinity for thrombin as well as its other substrates. This effect of heparin is the basis for its clinical use as an anticoagulant. The naturally occurring heparin activator of antithrombin III is present as heparan and heparan sulfate on the surface of vessel endothelial cells.





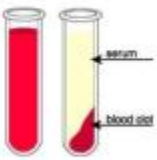
Clot retraction

Clot formation is fully developed in 3-6 min

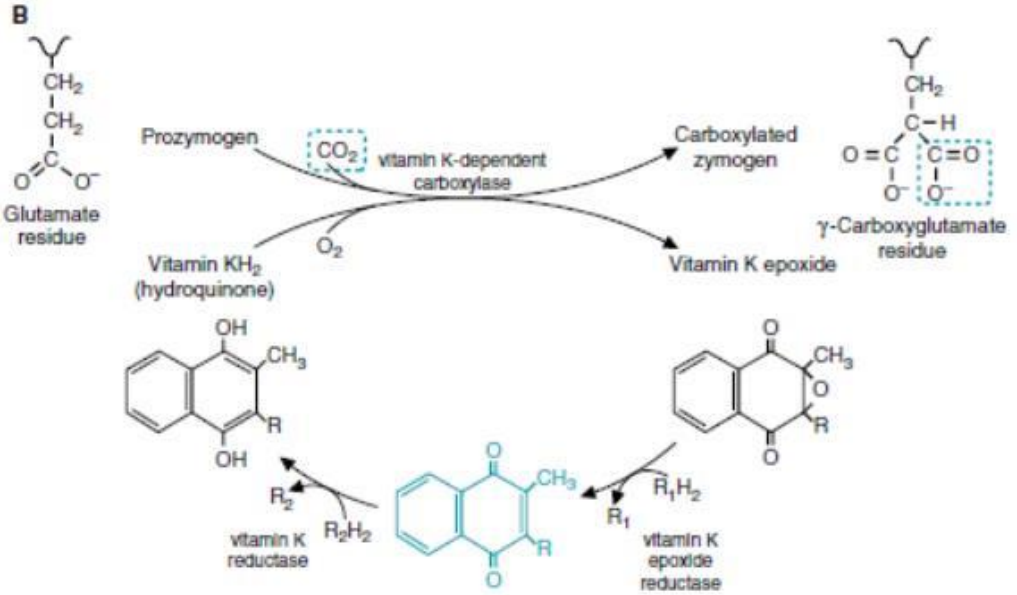
Contraction of platelets trapped within the clot shrinks the fibrin meshwork pulling the edges of the damaged vessel closer together.

During clot retraction serum is squeezed from the clot.

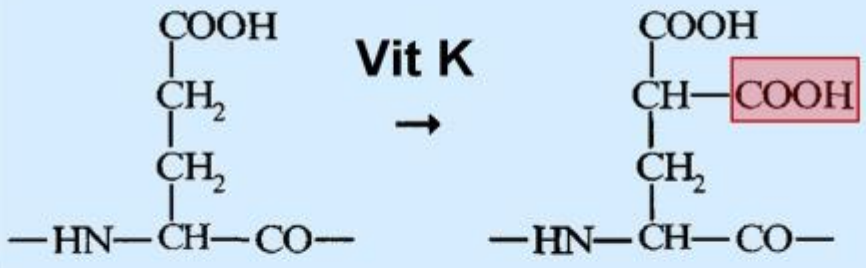
- Release of fibrin stabilizing factor
- Contractile protein of platelets
- Activated and accelerated by thrombin and Ca ions.



Blood Coagulation – Vitamine K

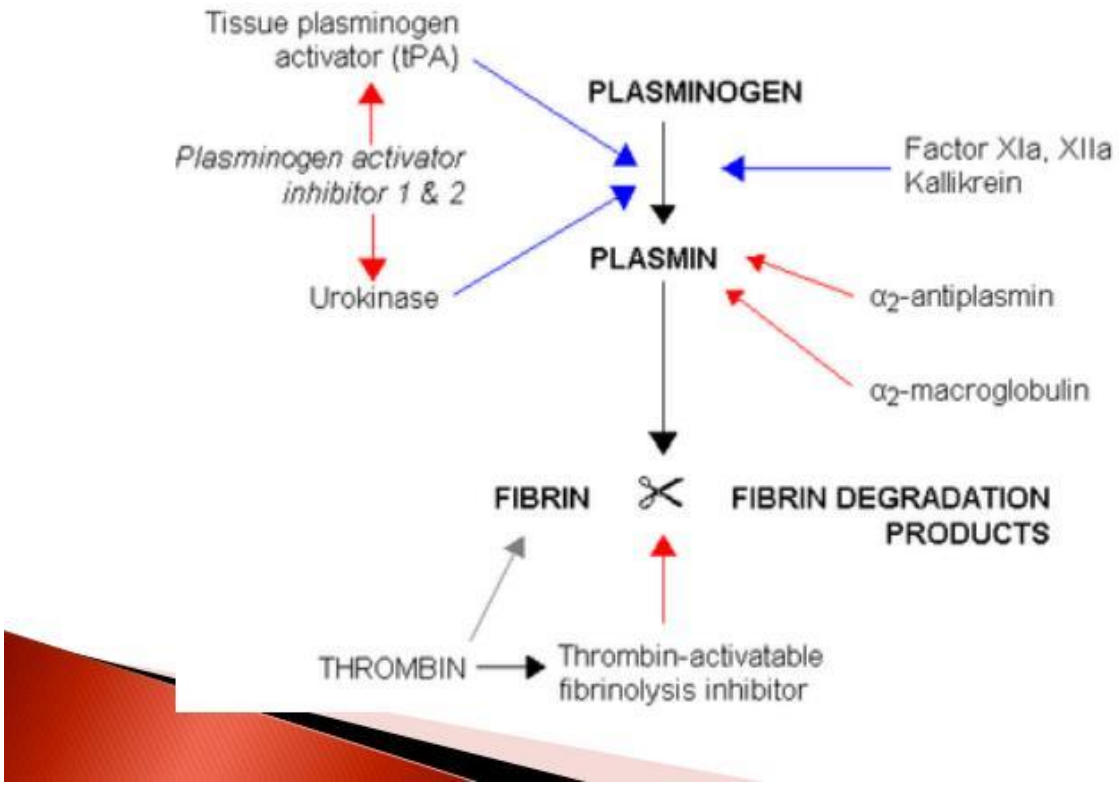


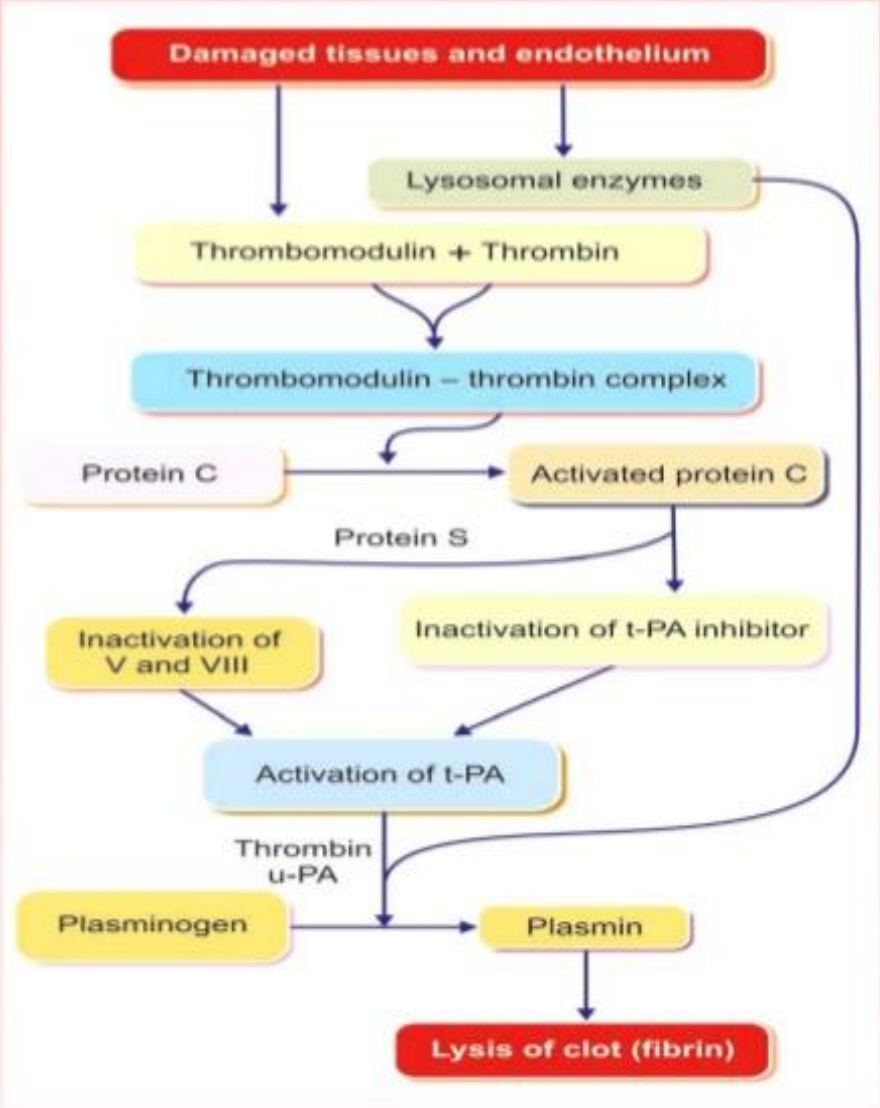
The formation of the γ-carboxyglutamate residue



II, VII, IX, X

Blood Coagulation – Fibrinolysis




Damaged tissues and endothelium
Lysosomal enzymes
Thrombomodulin + Thrombin
Thrombomodulin – thrombin complex
Protein C
Activated protein C
Protein S
Inactivation of V and VIII
Inactivation of t-PA inhibitor
Activation of t-PA
Thrombin u-PA
Plasminogen
Plasmin
Lysis of clot (fibrin)

ANTICLOTTING MECHANISM IN THE BODY

Under physiological conditions, intravascular clotting does not occur. It is because of the presence of some physicochemical factors in the body.

1. ***Physical Factors***

   i. Continuous circulation of blood.

   ii. Smooth endothelial lining of the blood vessels.

2. ***Chemical Factors – Natural Anticoagulants***

   i. Presence of natural anticoagulant called heparin produced by liver.

   ii. Production of thrombomodulin by endothelium

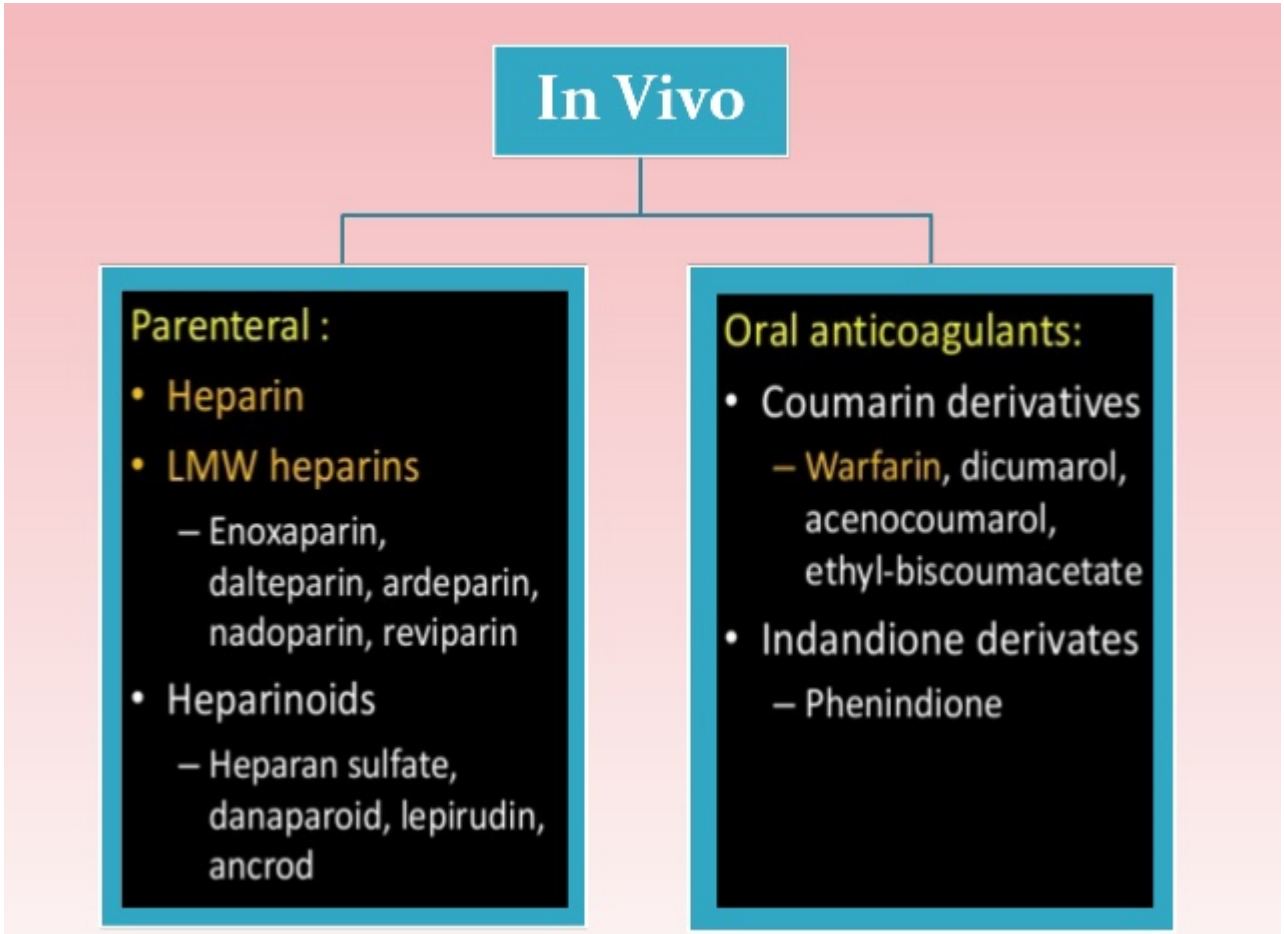
   iii. All the clotting factors are in inactive state.

ANTICOAGULANTS

Substances which prevent or postpone coagulation of blood are called anticoagulants.

Anticoagulants are of three types:

1. Anticoagulants used to prevent blood clotting inside the body, i.e. **in vivo.**
2. Anticoagulants used to prevent clotting of blood that is collected from the body, i.e. **in vitro.**
3. Anticoagulants used to prevent blood clotting both **in vivo and in vitro.**


In Vivo
Parenteral :
• Heparin
• LMW heparins
– Enoxaparin, dalteparin, ardeparin, nadoparin, reviparin
• Heparinoids
– Heparan sulfate, danaparoid, lepirudin, ancrod
Oral anticoagulants:
• Coumarin derivatives
– Warfarin, dicumarol, acenocoumarol, ethyl-biscoumacetate
• Indandione derivates
– Phenindione


In Vitro
Heparin
Calcium Complexing Agents: Sodium Citratre
Sodium Oxalate
EDTA
In Vitro & In Vivo
Heparin
Dextran Sulphate
Ancord

BLEEDING DISORDERS

Bleeding disorders are the conditions characterized by prolonged bleeding time or clotting time.

Bleeding disorders are of Four types:

1. **Vessel wall disorders**
2. **Platelet disorders**
3. **Coagulation disorders**
4. **Fibrinolytic disorders**

Bleeding Disorders – vessel wall abnormalities-Causes

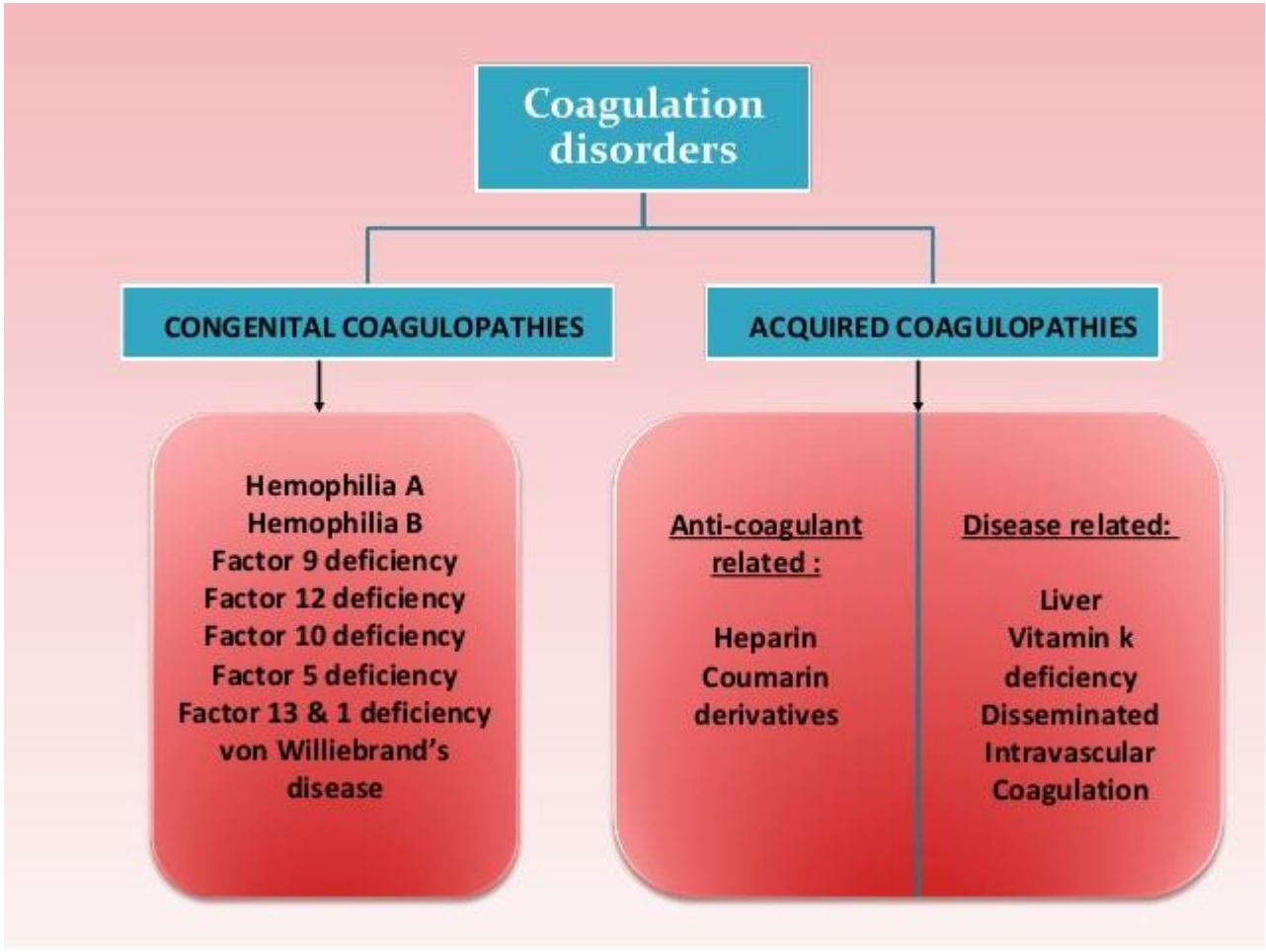
- **Infections**=Meningococcemia, infective endocarditis, rickettsioses (either vasculitis or DIC)
- **Drug reactions**=immune leucocytoclastic vasculitis
- **Scurvy & Ehlers-Danlos syndrome** — **Lack of collagen support**
- **Senile purpura & Cushing's Syndrome**
- **Henoch – Schonlein purpura (immune complex disease)**
  - Colicky abdominal pain, polyarthralgia & acute glomerulonephritis
- **Hereditary hemorrhagic telangiectasia**
  - AD disorder with tortuous thin blood vessels. Nose bleeds, bleeding in oral cavity, eyes etc
- **Systemic Amyloidosis**

DISORDERS OF COAGULATION

- **Defective blood clotting**
  - deficiency of clotting factors (I, II, V, VIII, IX, X)
  - deficiency of Vit- K
  -von Willebrand disease
  - anticoagulant overdose
- **Defective capillary contractility**
  - Purpura
- **Thrombosis**

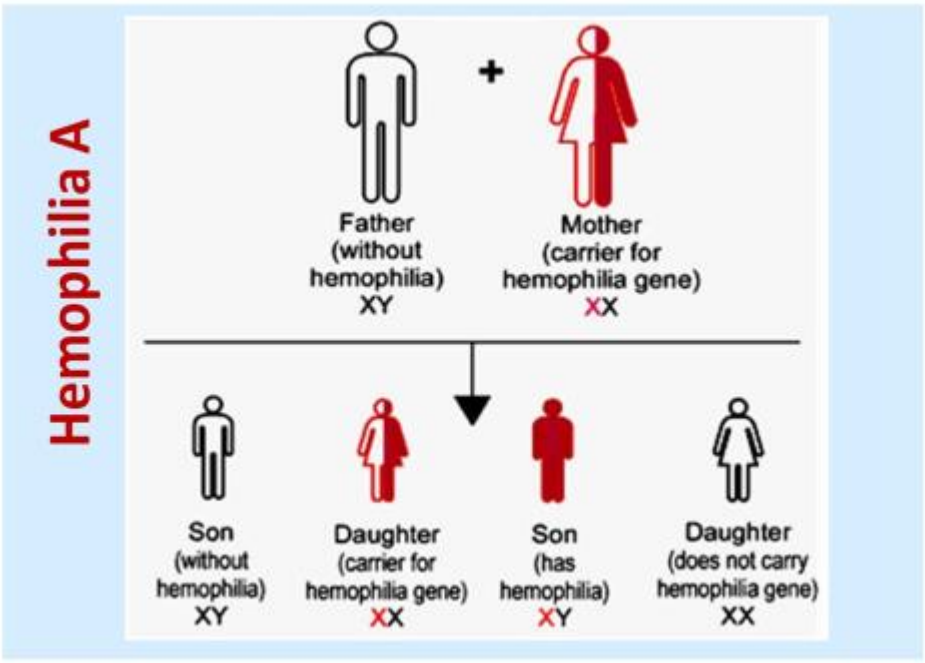
DEFICIENCY OF FACTORS

Deficiency of Factor:	Clinical Syndrome	Cause
I	Afibrinogenemia	Depletion during pregnancy with premature separation of placenta; also congenital (rare)
II	Hypoprothrombinemia (hemorrhagic tendency in liver disease)	Decreased hepatic synthesis, usually secondary to vitamin K deficiency
V	Parahemophilia	Congenital
VII	Hypoconvertinemia	Congenital
VIII	Hemophilia A (classic hemophilia)	Congenital defect due to various abnormalities of the gene on X chromosome that codes for factor VIII; disease is therefore inherited as sex-linked characteristic
IX	Hemophilia B (Christmas disease)	Congenital
X	Stuart-Prower factor deficiency	Congenital
XI	PTA deficiency	Congenital
XII	Hageman trait	Congenital



Hemophilia

- Factor – VIII deficiency
- Inheritance – Sex linked,
  -X-chromosome, females are carrier
- Diagnosis - CT increased, BT- normal
- Treatment
  - Fresh blood transfusion
  - Injecting factor – VIII and IX
  - Injecting thrombin or thromboplastin



Hemophilia – B
(Christmas disease)

❖ **Factor – IX deficiency**

Hemophilia - C

❖ **Factor – XI**
(Plasma thromboplastin anticedent) deficiency.

Hemophilia - D

❖ Factor – **XII**
(Hageman factor) deficiency

Purpura

- Purple coloured petechial hemorrhages and bruises in the skin.

- Characterized by spontaneous hemorrhages beneath the skin, mucous membrane and internal organ.
- Capillary abnormality
- Types
  - Primary (Idiopathic) –congenital or heriditary , seen in children
  - Secondary (Symptomatic)
    - allergies, infections, drugs, cancer

Fibrinolytic disorders:

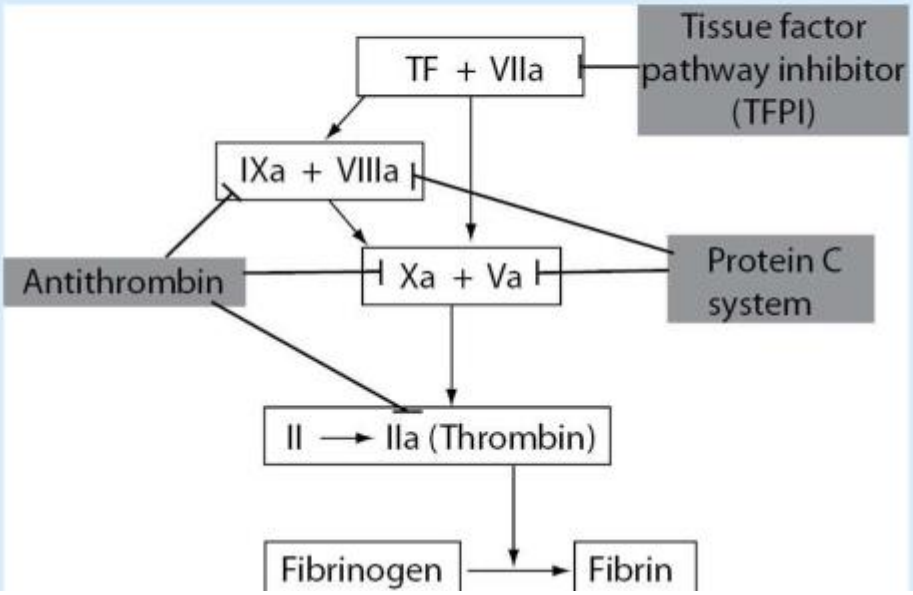
Disorders of the fibrinolytic system can lead to hemorrhage when clot breakdown is enhanced, or excessive clotting and thrombosis when clot breakdown mechanisms are retarded.

- Disseminated Intravascular Coagulation
- Thrombosis

Definition

- Disseminated intravascular coagulation (DIC) is a syndrome in which either the extrinsic or intrinsic or both pathways are activated to produce multiple fibrin clots in small blood vessels. The resultant reduction of the coagulation factors and platelets results in bleeding.

Anticoagulant system



Anticoagulant Properties of the Endothelium

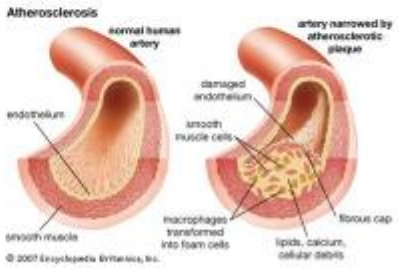
Anti-platelet properties

Covers highly thrombogenic basement membrane

Uninjured endothelium does not bind platelets

PGI2 (prostacyclin) and NO from uninjured endothelium inhibit platelet binding

ADPase counters the platelet aggregating effects of ADP



Anticoagulant properties of the endothelium

* **HEPARIN-LIKE MOLECULES:** activate anti-thrombin III (inactivates active proteases)

* **THROMBOMODULIN**: changes specificity of thrombin (activates protein C , which inactivates factors Va and VIIIa

* Endothelial cells produce **t- PA** which activates fibrinolysis via plasminogen to plasmin

General material and educational and methodological support of the lecture:
- Working program of the academic discipline
- Syllabus
- Methodical recommendations for independent work of higher education applicants
- Multimedia presentations
- Situational clinical tasks
- Electronic bank of test tasks by subdivisions of the discipline

Questions for self-control:

1. Biochemical and functional characteristics of the hemostasis system.

2. Blood coagulation system; characteristics of individual factors; mechanisms of functioning of the cascade blood coagulation system.
3. The role of vitamin K in coagulation reactions; medicines - agonists and antagonists of vitamin K.
4. Anticoagulant blood system; characteristics of anticoagulants. Hereditary disorders of the blood coagulation process.
5. Fibrinolytic blood system. Medicines affecting fibrinolysis processes.

Literature

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
2. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
3. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
4. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
5. Donald Voet, Judith G. Voet, Charlott W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. ISBN: 978-1-118-91840-1 February 2016, 1184 p.
6. William Marshall, Marta Lapsley, Andrew Day, Kate Shipman. Clinical Chemistry. Elsevier, 2020. 432 p.

Електронні інформаційні ресурси:

1. https://info.odmu.edu.ua/chair/biology/
2. http://libblog.odmu.edu.ua/
3. https://moodle.odmu.edu.ua/login/index.php