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
"1" 09 2024

METHODOLOGICAL DEVELOPMENT TO THE LECTURES ON THE EDUCATIONAL DISCIPLINE

Faculty, course	International faculty, 1st, 2d year
Specialty	221 "Dentistry"
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.

Lecture № 1

Topic: **«General provisions of bioorganic chemistry. Features of the structure of bioorganic compounds.»**

Actuality of theme: In medical universities, chemistry is a fundamental general theoretical discipline. Knowledge of chemistry largely determines the theoretical basis of a highly qualified doctor or a doctor who works in the field of medical science. Chemistry lays the physico-chemical basis for studying the functioning of biological systems at different levels of organization, determines the possibility of an approach to the molecular level of processes that occur in the body normally and with various pathologies. Chemistry is closely related to the following disciplines: pharmacology, pathophysiology, biochemistry, military medicine, physiology, anesthesiology, therapy.

Aims: To form systematic knowledge about the structure of organic molecules, their classification, the influence of individual groups of atoms on chemical properties, as well as on the biological role of organic compounds. Explanation of the mechanisms of homogeneous and heterogeneous bond breaking. To learn the mechanism of organic reactions, necessary for determining the behavior of compounds and preliminary orientation of possible products.

Basic concepts: organic chemistry, bioorganic chemistry, Butlerov's theory, classification of organic compounds, types of reagents, electronic effects, inductive and mesomeric effects, types of organic reactions.

Plan and organizational structure of the lecture:

1. The theory of the structure of organic substances.
2. Classification of organic compounds.
3. Nomenclature of organic compounds: trivial, rational, IUPAC.
4. isomerization of organic compounds.
5. Electron shifts in molecules of organic compounds: inductive (I) and mesomeric (M) effects.
6. Classification of reagents: nucleophilic, electrophilic, radical.
7. Mechanisms of organic reactions.

Content of lecture material (lecture text)

The study of the relationship between the structure and properties of various natural and medicine has proved that their biological activity is largely determined by the spatial distribution of atoms and molecules that make up these compounds. Complementarity underlies the processes of food hydrolysis under the influence of enzymes (structural correspondence of enzyme and substrate configurations) and the synthesis of new biopolymers specific for a given organism (matrix syntheses of proteins and nucleic acids), protective (immune) reactions of the body, etc.

The mechanisms of action of drugs on the human body (on the corresponding receptors of cell membranes) are also due to the spatial structure of these compounds

and their corresponding receptors. The determination of the spatial distribution of atoms in the molecules of organic compounds that are biologically active consists in the sequence of revealing their structure, configuration, and conformation.

The structure of biologically active compounds is proved on the basis of elemental analysis (content of C, H, N, O, S, P, etc.) The spatial configuration of the molecules of biologically active substances is determined by the hybridization of the electronic orbitals of atoms involved in the formation of chemical bonds.

Nomenclature of Organic Compounds

Nomenclature is a system of rules, the language of organic chemistry, which is used to convey the names of organic compounds in their structure.

Currently, the **systematic nomenclature of IUPAC** is generally accepted (**IUPAC** - International Union of Theoretical and Applied Chemistry). The IUPAC rules allow the use of especially ingrained trivial names that were historically the first (acetone, glycerol, ascorbic acid, etc.)

The most widely represented in the rules of the systematic nomenclature of IUPAC is the **replacement and radical-functional nomenclature.**

Substitution Nomenclature

To form a name according to the replacement nomenclature, the following order must be followed:

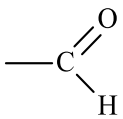
1. Select the main carbon chain or main cyclic structure.
2. Identify the senior functional group.
3. Number the atoms of the main chain or cycle.
4. Build the name of the organic compound.

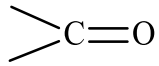
The choice of the main carbon chain is carried out taking into account the following criteria: maximum length, maximum number of functional groups, multiple bonds.

The definition of a senior functional group is carried out in accordance with the table in which these groups are indicated in descending order of seniority from top to bottom. The older group is reflected in the title ending.

Table 1.

The order of precedence of functional groups, indicated in the prefix and in the end

Function Group	Prefix	End
-(C)OOH*	-	-oic acid
-COOH	Carboxy-	
$-SO_3H$	Sulpho-	Sulfonic acid
-(C)≡N	Cyano-	Nitrile
	Oxo-	-al

	Oxo-	-one
-OH	Hydroxy-	-ol
-SH	Mercapto-	-thiol
$-NH_2$	Amino-	-amine

*- The carbon atom, enclosed in brackets, is part of the main carbon chain.

Building the name of the organic compound

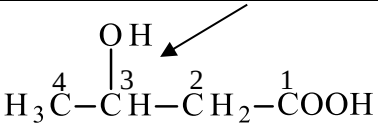
The name of the main chain is determined and the ending is formed depending on the senior functional group. The degree of saturation of the main chain is reflected by suffixes: an - saturated carbon skeleton, en - the presence of double and triple bonds. The name of the substituents is determined - the lower functional groups, carbon radicals, which are indicated by prefixes in a single alphabetical order.

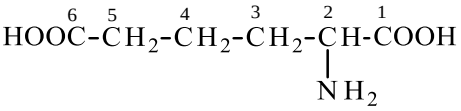
The position of each substituent and each multiple bond is indicated by a number corresponding to the number of the carbon atom to which the substituent is associated. For a multiple bond, the smallest carbon atom number at which this bond is located is indicated. The numbers are prefixed and after suffixes or endings.

If the compound has several identical substituents or multiple bonds, then the multiplication prefix is placed in front of the corresponding designation: di, three, tetra, etc.

Table 2.

The following are some examples of names for the IUPAC substitute nomenclature

		Substituent: **hydroxy**		
The main carbon chain is **butane**.	→	 3-hydroxybutanoic acid	←	Senior Functional Group – **oic acid**

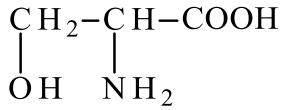


2-aminohexane-1,6-dioic acid

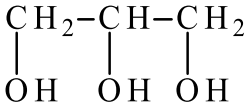
The trivial (historical) nomenclature is the first nomenclature that arose at the beginning of the development of organic chemistry, when there was no classification and theory of the structure of organic compounds.

Organic compounds were given random names by source of production (oxalic acid, malic acid, vanillin), color or smell (aromatic compounds), less often - by chemical properties (paraffins).

Many such names are often used to this day. For example, urea, toluene, xylene, acetic acid, butyric acid, valerianic acid, glycerin, alanine, etc.



2-amino-3-hydroxypropanoic acid (serine)



Propane-1,2,3-triol (glycerol)

In organic compounds, the carbon atom can reside in three valence states; they correspond to sp^3, sp^2, sp-hybridization. In all cases, the type of bond is the same – covalent non-polar or low-polar. The course of any chemical reaction is accompanied by the breaking of old and the formation of new bonds. The direction of the reaction is determined by a change in the Gibbs free energy (ΔG). The speed of the process is determined by the speed of the slowest limiting stage. Since breaking a covalent bond requires a significant expenditure of energy, often the first stage is the slowest, and therefore the rate of organic reactions is much lower than the rate of ionic reactions, therefore, their course in time can be studied. The mechanism that describes this move is a hypothesis of the sequence of stages and may change with a change in our understanding of this process.

The nature of the chemical bond also provides a mechanism for the mutual influence of atoms that are not directly related to one another. Electronic clouds can be displaced in a molecule so that the electrons "serve" not only a pair of atoms that form a bond, but also other atoms. It should be borne in mind that electron clouds have no boundaries and, accordingly, can overlap and influence one on one through space (field effect).

To a large extent, the spatial influence occurs if the atoms are sufficiently close together, since the interaction is weakened in proportion to the square of the distance.

The ability of a molecule to one or another type of transformation is determined primarily by the distribution and mobility of electrons, which depends on the following factors:

1. The polarity of the bonds.
2. The polarizability of the bonds.
3. Conjugation.
4. Hyperconjugation.

An ideal covalent bond can exist only between homogeneous atoms or groups of atoms. If atoms are characterized by different affinities for the electron, then the bond between them will always be more or less polar. The affinity for the electron depends on the position of the element in D. I. Mendeleev periodic system of elements. It increases in the period from left to right (I), in the group – from bottom to top (II).

$$C < N < O < F \qquad Br < Cl < F$$
$$\text{I} \qquad\qquad \text{II}$$

The mutual influence of atoms

The reactivity of the compounds largely depends on the nature of the distribution of electron density in the reacting molecules. The uneven distribution of electron density is a consequence of the electronic effects of substituents, divided into inductive and mesomeric effects.

Inductive effect

The covalent bond is non-polar (its electron density is distributed evenly) only when atoms of the same or similar electronegativity are bonded. When atoms with different electronegativity are joined, the electron density of the covalent bond is shifted toward a more electronegative atom. Such a connection is polarized. The polarization is not limited to only one σ-bond, but propagates along the chain and leads to the appearance of partial charges (δ) on atoms:

$$\delta^{III}+ \quad \delta^{II}+ \quad \delta^{I}+ \quad \delta+ \quad \delta-$$
$$C \rightarrow C \rightarrow C \rightarrow C \rightarrow X$$

Thus, the substituent X causes the polarization of not only "his" σ-bond with the carbon atom, but also transfers the effect (exerts an effect) to neighboring σ-bonds. This type of electronic influence is called inductive and is denoted by I.

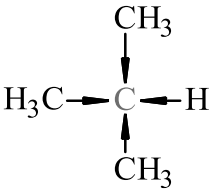
The inductive effect is the transfer of the electronic influence of the substituent along the σ-bond chain.

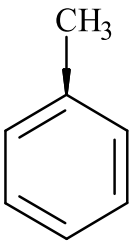
It is customary to qualitatively evaluate the direction of the inductive effect of a substituent by comparison with a hydrogen atom, the inductive effect of which is taken as 0 (the CH bond is considered almost nonpolar)

$$\overset{\delta+\ \ \delta-}{R \rightarrow CH_2 \rightarrow X} \qquad R \rightarrow CH_2 — H \qquad \overset{\delta-\ \ \delta+}{R \rightarrow CH_2 \leftarrow У}$$

X: -I H: I = 0 У: +I

The substituent X, which attracts the electron density of the σ bond stronger than the hydrogen atom, exhibits a negative inductive effect, -I. If the substituent Y, in comparison with the H atom, increases the electron density in the chain, then it exhibits a positive inductive effect, + I. Graphically, the I-effect is depicted by an arrow coinciding with the position of the valence line and directed by the tip towards a more electronegative atom. + I-effect have alkyl groups.





CH_3 : +I CH_3 : +I

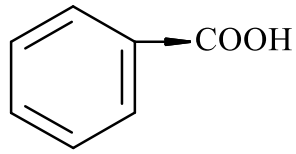
Isobutane (2-methylpropane) Toluene (methylbenzene)

Most substituents have an -I effect and the greater in case, the higher the electronegativity of the atom forming a covalent bond with the carbon atom.

$$\delta+ \quad \delta- \qquad \delta^{I}+ \quad \delta^{II}+ \quad \delta-$$

$H_3C \rightarrow Cl$ $H_3C \rightarrow CH_2 \rightarrow OH$

Cl: -I OH: -I



COOH: -I

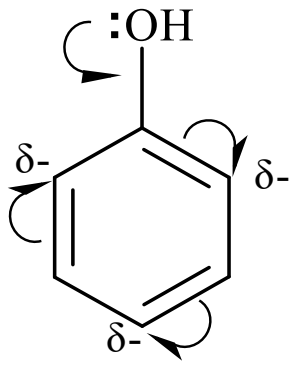
Due to the weak polarizability of the σ bond, the inductive effect decays after 3-4 σ bonds in the chain. Its action is most pronounced on the two carbon atoms closest to the substituent.

Mesomeric effect

If the inductive effect always occurs when there are atoms in the molecule with different electronegativity, then for the manifestation of the mesomeric effect it is necessary to have a conjugated site in the molecule (the presence of alternating single and double bonds). The mesomeric effect is also called the conjugation effect, since the transfer of influence occurs through a system of π-bonds.

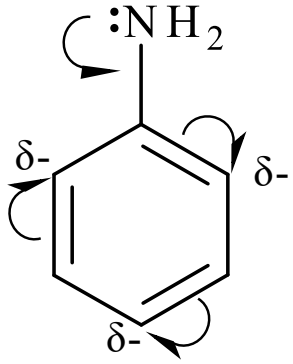
The mesomeric effect (M-effect) is the transfer of the electronic influence of a substituent through a system of π-bonds.

Substituents that increase the electron density in the conjugated system exhibit a positive mesomeric effect, + M. The substituents containing atoms with a lone electron pair or a whole negative charge have a positive mesomeric effect.



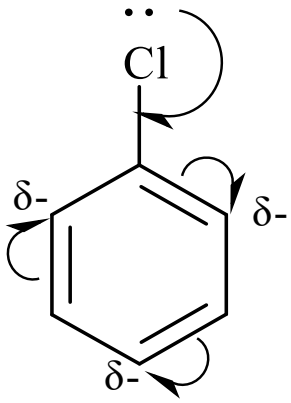
OH: +M

Phenol



H_2N: +M

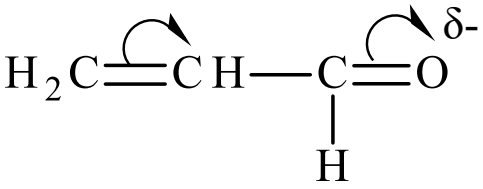
Aniline



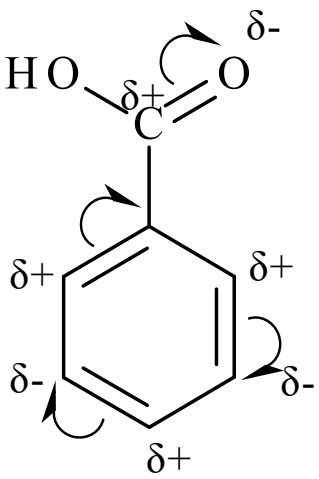
Cl: +M

Chlorobenzene

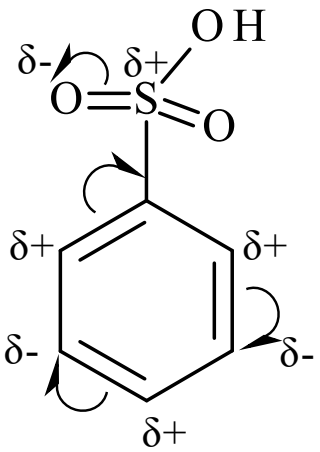
Substituents pulling electron density from the conjugated system exhibit a negative mesomeric effect, -M. These include unsaturated groups, positively charged atoms.



CHO: -M



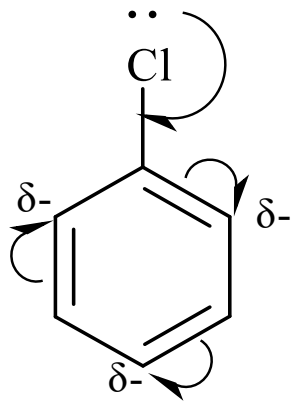
COOH: -M



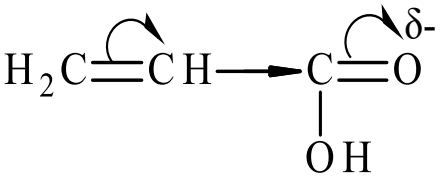
SO_3H: -M

Redistribution (displacement) of the common electron cloud under the influence of the M-effect is graphically indicated by curved arrows, the beginning of which shows which p- or π-electrons are displaced, and the end is the bond or atom to which they are displaced. Partial charges are usually indicated on terminal atoms of a conjugated chain. In contrast to the inductive mesomeric effect, it is transmitted through a system of conjugated bonds to a much greater distance.

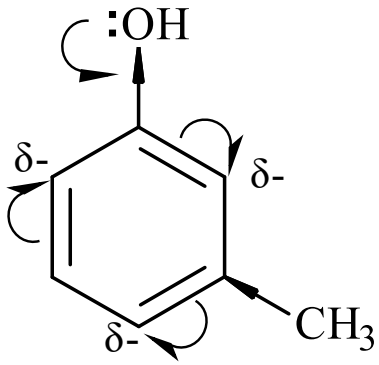
In general, when assessing the effect of substituents on the distribution of electron density in a molecule, it is necessary to take into account the total effect of inductive and mesomeric effects.



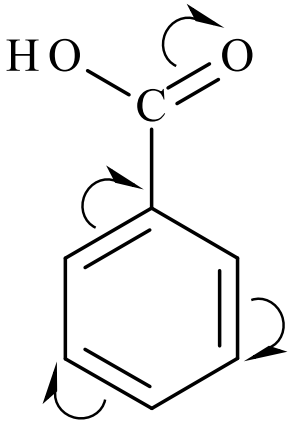
Chlorobenzene
Cl: -I > +M

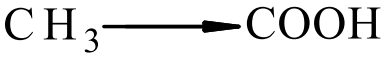


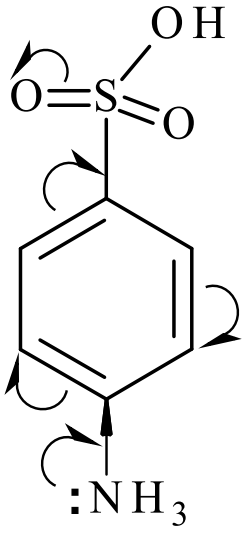
Acrylic acid
COOH: -I, -M



m-Cresol
CH_3: +I; OH: +M > -I







Benzoic acid
COOH: -I, -M

Acetic acid
COOH: -I

Sulfanilic acid
NH_2: +M>> -I
SO_3H: -I, -M

All substituents, depending on whether they increase or decrease the electron density in the molecule, are considered as electron-donating - alkyls, NH_2-, OH-, -OR or electron-withdrawing - halogens, $-NO_2$, -COOH, $-SO_3H$, >C=O.

Reagents for Organic Reactions

According to the number of molecules participating in the elementary act of chemical transformation, organic reactions are most often monomolecular or bimolecular.

Monomolecular reactions include decyclization, dissociation, intramolecular rearrangements, etc.

Bimolecular reactions proceed in solutions and in the gas phase, suggest the presence of two components in the organic mixture.

For instance,

$$CH_3I + NaOH \rightarrow CH_3OH + NaI$$

A substance that undergoes transformation during the course of a chemical reaction is called a substrate (CH_3I). The substance under the influence of which there was a change in the substrate is called a reagent (NaOH). The course of the reaction depends on the nature of both the substrate and the reagent.

In the molecules of organic substances, the main type is a covalent non-polar bond. There are two ways to break the covalent bond:

1. Homolytic fission (free radicals are obtained)

$$A : B \rightarrow A\cdot + B\cdot$$

2. Heterolytic fission (ions are formed)

$$A : B \rightarrow [A:]^- + [B]^+$$

Most organic reactions occur between molecules, molecules and ions, molecules and free radicals.

The reaction mechanism (free radical or ionic) can be assumed, given the nature of the substrate and reagent.

Nucleophilic reagents are particles with electron-donating properties:
- negatively charged ions;
- compounds that contain unshared electron pairs (Lewis bases);
- compounds with double bonds;
- aromatic compounds, etc.

Electrophilic reagents include particles with electron-withdrawing properties:
- positively charged ions;
- compounds that are electron acceptors (Lewis acids);
- halogens;
- compounds that contain a carbonyl group;
- acetylene derivatives, etc.

The main types and mechanisms of organic reactions

According to the final result, organic reactions are divided into:

1. Substitution reactions (S).
2. Addition reactions (A).
3. Elimination reactions (E).
4. Rearrangement reactions.

Given the nature of the attacking reagent, the reaction mechanisms may be as follows: S_R - radical substitution; S_E - electrophilic substitution; S_N — nucleophilic substitution; A_E - electrophilic addition; A_N — nucleophilic addition.

The course of the reaction depends on the type of covalent bonds, electronic effects in the substrate, the nature of the attacking particle, and external factors (hν, P, T).

Radical S_R substitution at a saturated carbon atom (saturated hydrocarbons)

In saturated aliphatic hydrocarbons (alkanes), there are only sp^3-hybridized carbon atoms. These compounds are characterized by nonpolar Csp^3-Csp^3 σ bonds and practically nonpolar Csp^3-H σ bonds, which are of sufficient strength and are not prone to heterolytic rupture. As a result, saturated hydrocarbons are inert in most heterolytic reactions. Possible for them are radical processes, radical substitution reactions (S_R).

Halogenation is a typical example of a radical substitution reaction proceeding by a free-radical chain mechanism:

$$CH_4 + Cl_2 \xrightarrow{h\nu} CH_3Cl + HCl$$

Methane *methyl chloride*

The mechanism of this reaction includes several stages:

1. Chain initiation. Under the action of a quantum of light, mutual repulsion of chlorine atoms occurs and the bond between them breaks down with the formation of chlorine radicals (atomic chlorine):

$$Cl_2 \xrightarrow{h\nu} Cl\cdot + Cl\cdot$$

2. Chain propagation. A chlorine atom attacks a methane molecule. The CH bond in this molecule breaks homolytically. In this case, HC1 and the methyl radical - CH_3 • are formed. The latter further reacts with a chlorine molecule, giving methyl chloride and a chlorine atom, which continues the process:

$$Cl\cdot + H : CH_3 \rightarrow HCl + CH_3\cdot$$
$$CH_3\cdot + Cl : Cl \rightarrow CH_3Cl + Cl\cdot$$

Such processes are called chain processes, since the initially formed single chlorine radical can initiate the chlorination of many methane molecules. The methyl radical CH3 • is the simplest organic free radical, extremely reactive, due to the desire to build the external electronic level to a stable octet.

3. Chain termination may occur as a result of the following reactions:

$$Cl\bullet + Cl\bullet \rightarrow Cl_2;$$
$$CH_3\bullet + CH_3\bullet \rightarrow CH_3\text{-}CH_3;$$
$$CH_3\bullet + Cl\bullet \rightarrow CH_3Cl$$

Hydrocarbon oxidation

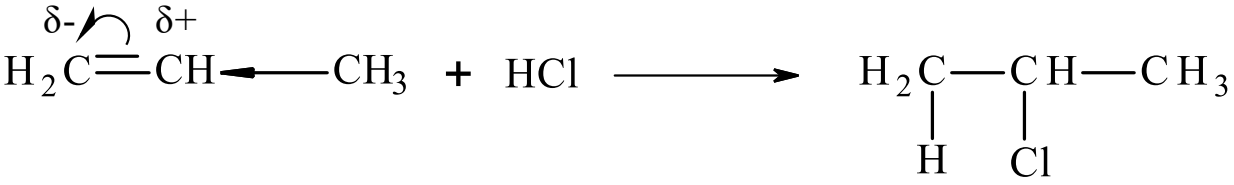
An important type of radical process is the interaction of organic compounds with oxygen. The oxygen molecule is a biradical •O-O• and can react with compounds containing CH bonds by the radical mechanism with the formation of hydroperoxides or products of their further transformations:

$$R\text{-}H + O_2 \rightarrow R\text{-}O\text{-}O\text{-}H$$

Oxidation of organic compounds with oxygen can occur under fairly mild conditions in the body (in vivo). These processes include lipid peroxidation, which proceeds as a free radical multistep process, resulting in mono- and dicarboxylic acids with shorter carboxylic chains. Lipid peroxidation causes damage to cell membranes (for example, in radiation sickness).

Electrophilic addition A_E unsaturated compounds (alkenes, diene hydrocarbons).

Addition of hydrogen halides to asymmetric alkenes. Markovnikov Rule



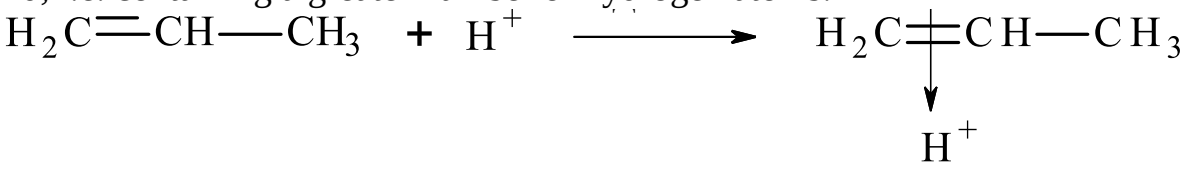
Propene *2-Chloropropane*

This reaction proceeds according to a heterolytic electrophilic mechanism. The electrophilic particle here is the simplest electrophile - the proton H^+. The process includes two main stages:

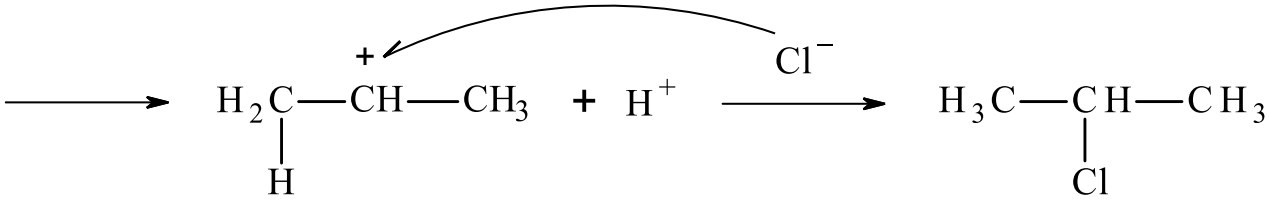
1. Electrophilic attack with an alkene proton to form a carbocation. This slow stage determines the speed of the process as a whole.

2. Nucleophilic attack by the chlorine anion of the resulting carbocation, leading to the final product (fast stage).

For this reaction, the Markovnikov rule will be fulfilled: in the case of asymmetric alkenes when interacting with reagents of the HX type (HCl, HBr, H_2O, H_2SO_4, etc.), the hydrogen joins the most hydrogenated carbon atom of the double bond, i.e. containing a greater number of hydrogen atoms.



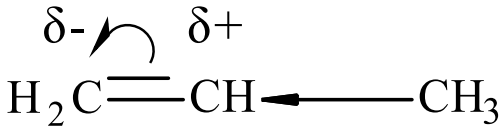
π- complex



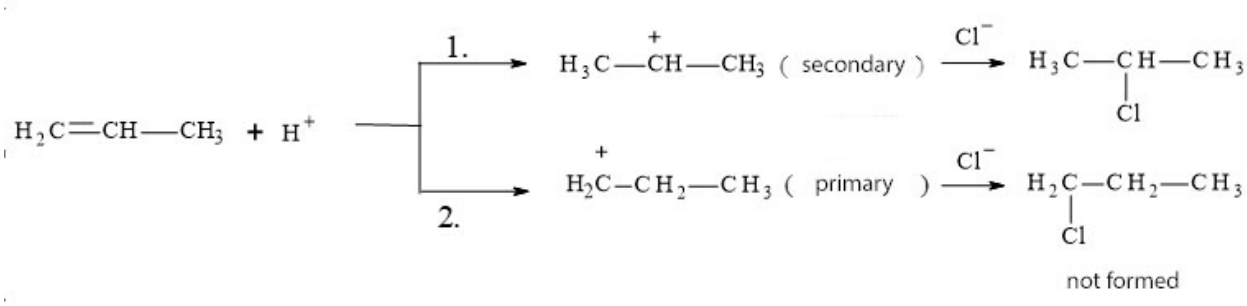
Carbbcation (σ- complex) *2-chloropropane*

This reaction course is due to two reasons:

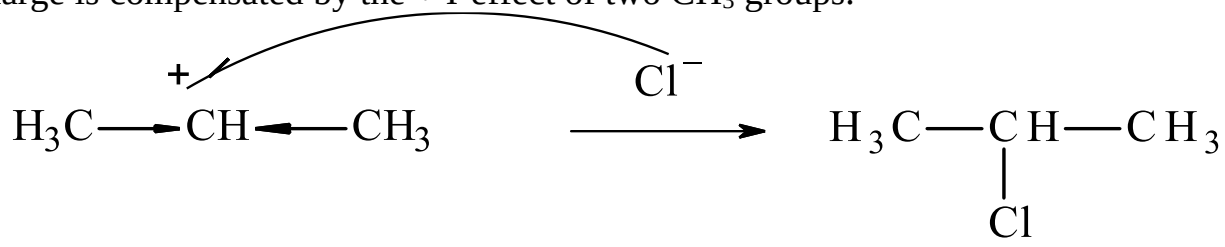
1. The π-bond in the propene molecule is polarized due to the positive inductive effect of the (+ I) methyl group. Therefore, the proton (H^+) is attached to that of the carbon atoms, which has a partial negative charge (δ-).



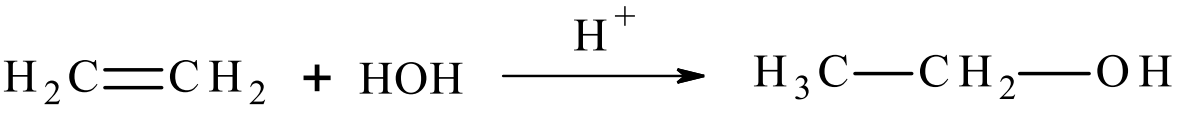
2. As a result of the addition of a proton, it is theoretically possible to form carbocations of two types:



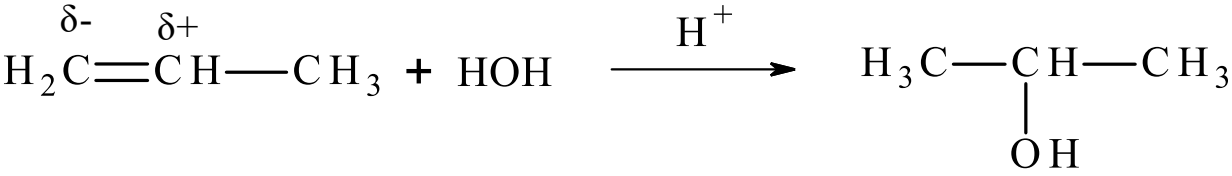
The first of them is much more stable than the second, since in it the positive charge is compensated by the + I-effect of two CH_3 groups.



Hydration reaction (water addition)



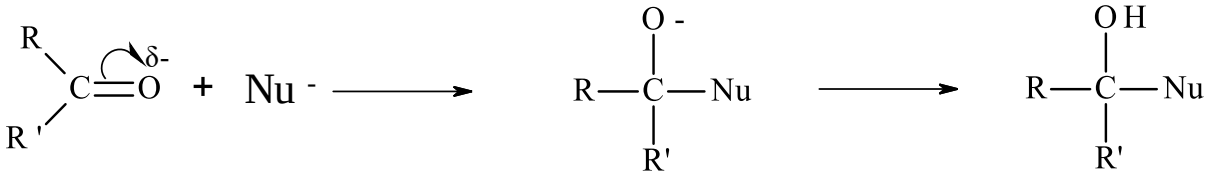
ethanol



Propan-2-ol

Nucleophilic addition reactions A_N

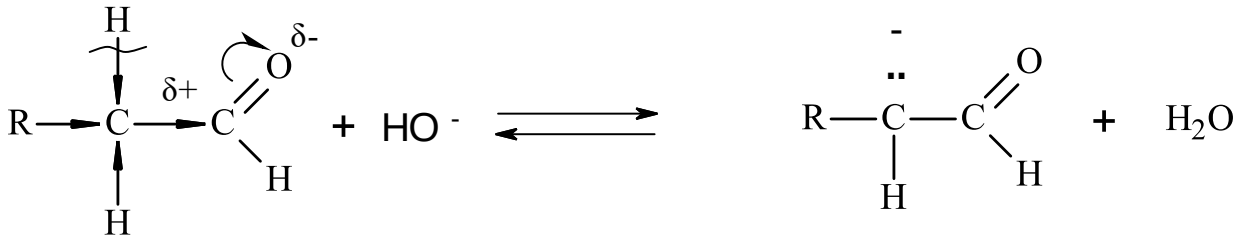
Based on the distribution of electron density in the molecule of the aldehyde or ketone, we can conclude that the most characteristic reaction for aldehydes and ketones is the nucleophilic addition reaction A_N, which can be depicted in general in the form of such a scheme:



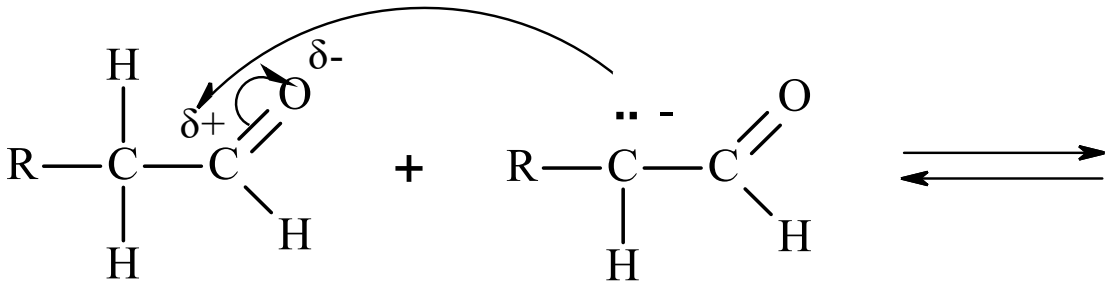
The attack of the nucleophilic reagent depends on the magnitude of the fractional positive charge on the carbonyl atom of the carbon. A significant effect on the value of δ^+ has a hydrocarbon radical. Since alkyl groups exhibit + I effect, aliphatic aldehydes are always more reactive in A_N reactions than aliphatic ketones.

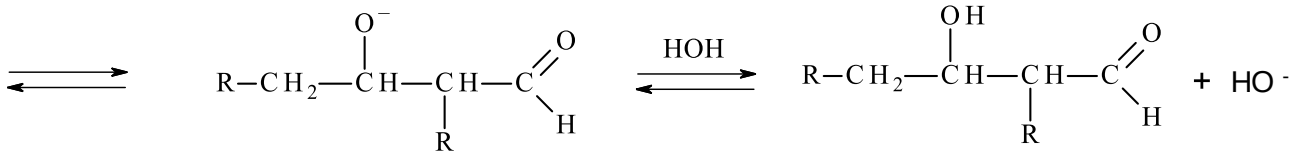
The aldehyde group has a strong -I-effect, due to which the hydrogen atoms at the α-carbon atom acquire increased mobility. Aldehydes enter into an aldol condensation reaction, the catalysts of which can be K_2CO_3, K_2SO_3, CH_3COOK (compounds with weak alkaline properties).

The aldol condensation mechanism is as follows:

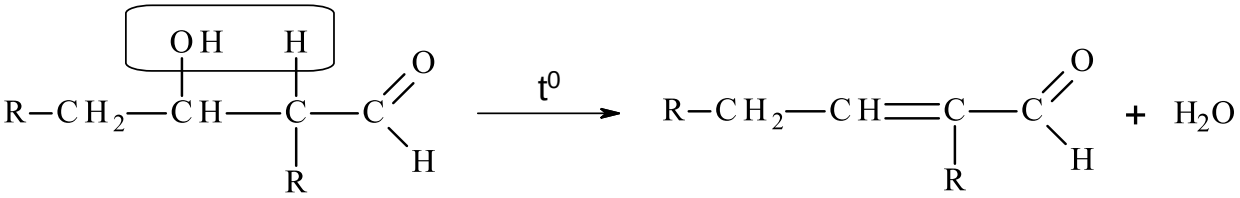


carbanion





Aldol, when heated without a dewatering agent, cleaves water with the formation of an unsaturated aldehyde. This process is called croton condensation.

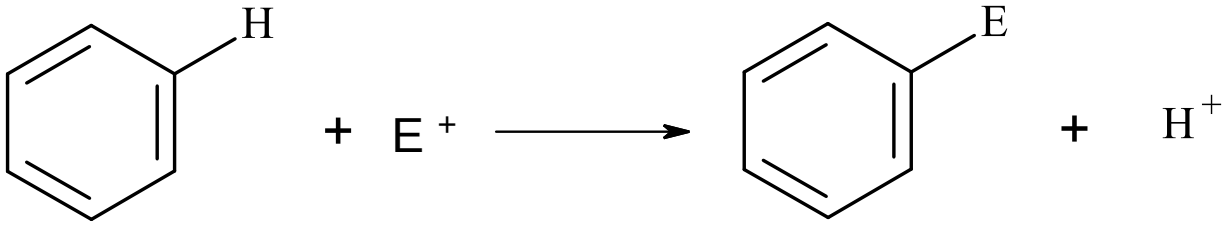


Aromatic electrophilic substitution reactions

The electrophilic substitution reactions S_E are most typical for aromatic compounds.

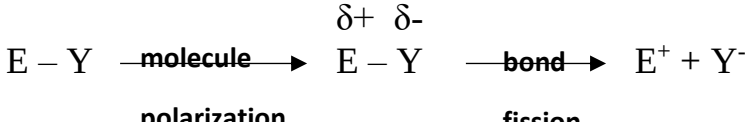
The mechanism of electrophilic substitution reactions

The presence of π-electron density on both sides of the flat aromatic cycle leads to the fact that aromatic compounds of the benzene series (arena) are nucleophiles and, therefore, are prone to electrophilic attack. In general terms, for benzene, the reaction of proton substitution with other electrophiles can be represented as follows:



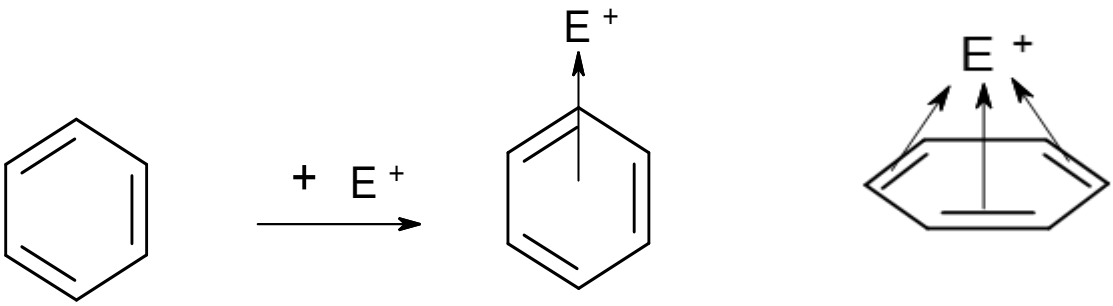
The general mechanism of most of these reactions involves the following stages:

1. Generation of an electrophilic particle in the presence of a catalyst.



2. The formation of π-complex (π-adduct).

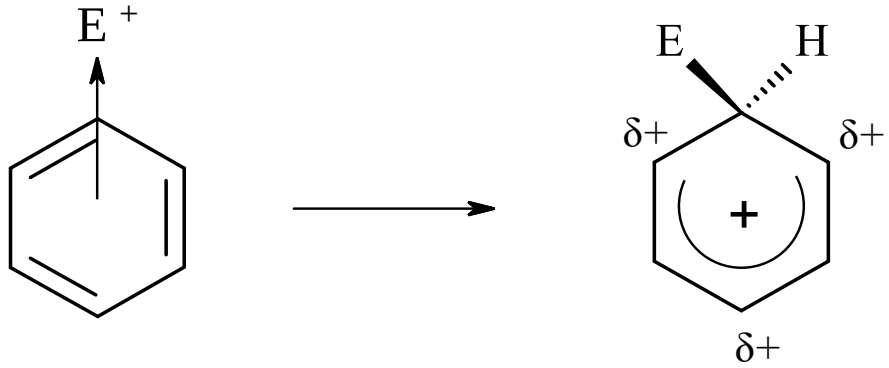
An electrophilic particle attacks the aromatic substrate, forming an unstable π-complex in which it is simultaneously connected with all π-electrons of the aromatic system.



OR

3. Transformation of the π-complex into a σ-complex (slow reaction stage)

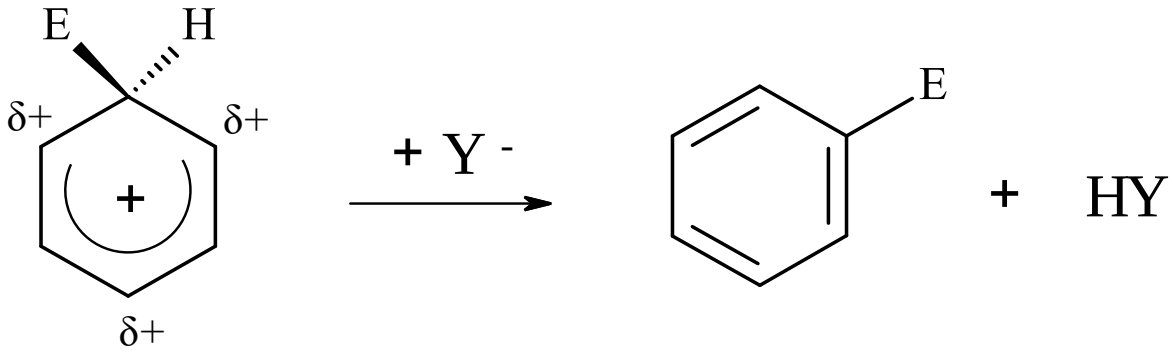
The electrophile picks up two electrons of the π-system, forming a σ-bond with one of the atoms of the carboxylic benzene ring:



In the σ-complex, the aromatic system is disrupted, since one of the carbon atoms of the ring has become sp^3-hybridized. The four remaining π-electrons are distributed between the five carbon atoms, with the largest electron density deficiency in the ortho and para positions relative to the substituent.

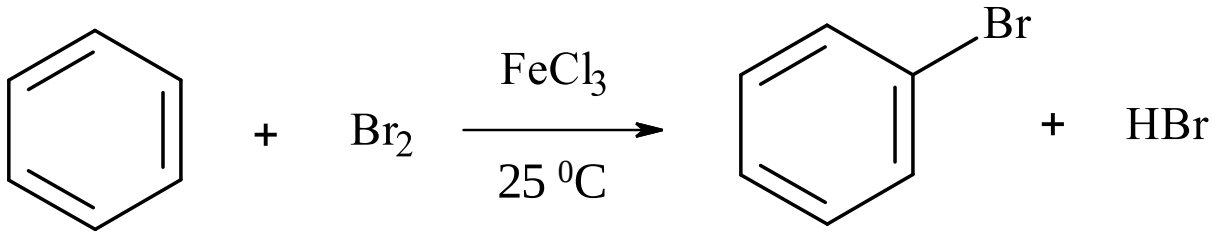
4. Cleavage of the proton from the σ-complex

The aromatic system is restored (a pair of electrons missing before sextet is returned to the nucleus), therefore this process is energetically beneficial. The cleaved proton binds to the nucleophile.

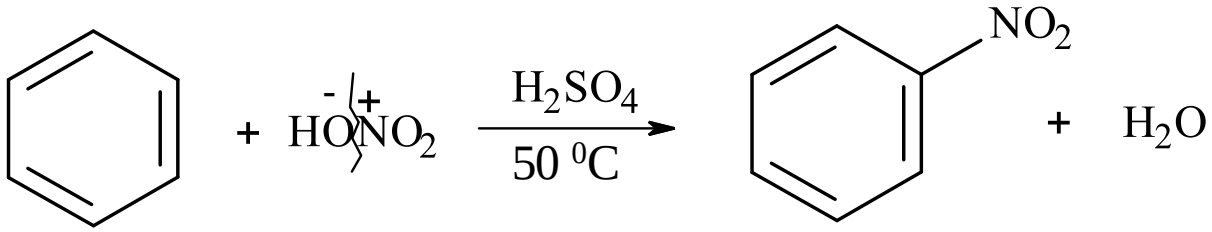


Electrophilic substitution reactions

Halogenation of benzene in the presence of a catalyst ($FeC1_3$ - Lewis acid). In the absence of a catalyst, benzene does not discolor bromine water.

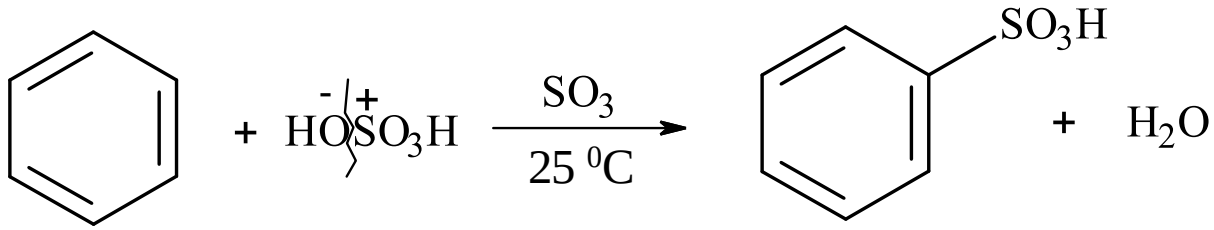


Nitration of benzene is carried out with a mixture of concentrated nitrate and sulfate acids in a ratio of 1: 2; as a result, a nitronium cation is generated (NO_2^+).



Nitrobenzene is widely used in industry as a starting product for the Zinin reaction (production of aniline).

Sulfonation of benzene is carried out by fuming sulfate acid in the presence of sulfuric oxide (VI).



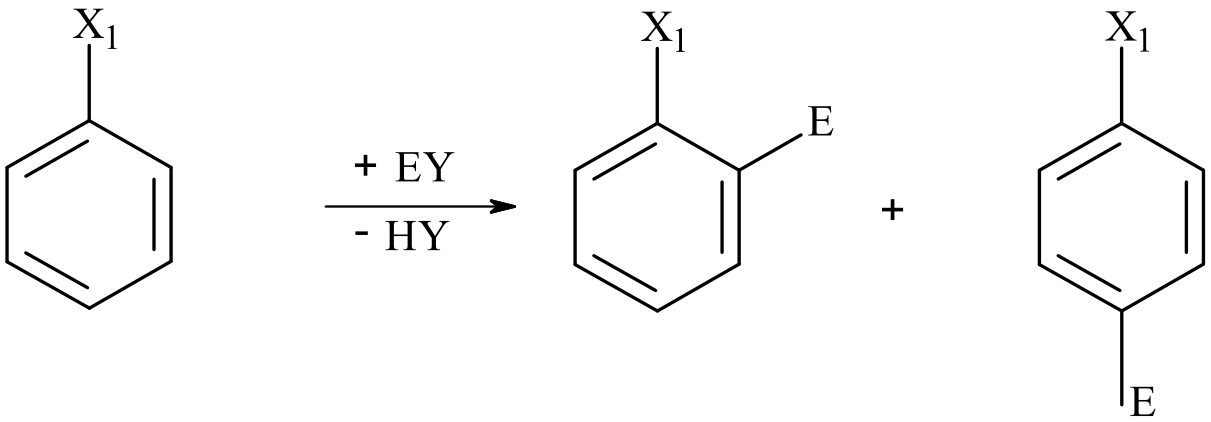
Orienting action of substituents in the benzene ring

According to the effect on electrophilic substitution reactions in arenas, substituents are divided into two groups:

1. **Substituents (orientants) of the first kind**

These include alkyl groups having a positive inductive effect (-R: $-CH_3$; $-C_2H_5$ and etc.); groups exhibiting a positive mesomeric effect: -OH, $-NH_2$, -OR, $-NR_2$, which have an electron-donating nature with respect to the benzene ring.

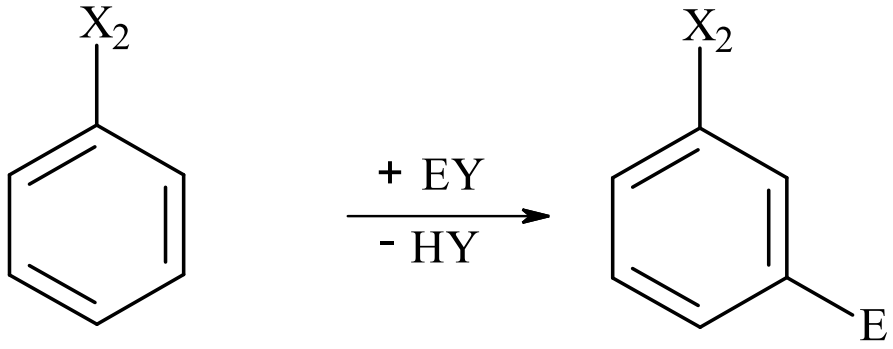
Type I (X_I) substituents facilitate electrophilic substitution in comparison with unsubstituted benzene and direct the incoming group to the ortho or para positions:



Substituents (orientants) of the second kind.

These groups include: NH_3^+, N^+R_3, NO_2, SO_3H, C ≡ N, CHO, COOH, which exhibit electron-withdrawing character with respect to the benzene ring due to negative inductive or negative mesomeric effects.

Substituents of the second kind (X_{II}) complicate the electrophilic substitution reaction in comparison with unsubstituted benzene. If under more severe conditions the reaction still passes, the incoming group enters the meta position:



Nucleophilic Substitution Reactions

The nucleophilic substitution reaction mechanism is best seen using halogenated saturated hydrocarbons as an example. The rate of substitution of the halogen atom in different halogenated derivatives is different and largely depends on the structure of the radical with which it is associated. If we compare the relative rate of alkaline hydrolysis of methyl bromide CH_3Br and tert-butyl bromide $(H_3C)_3CBr$, we can conclude that the reaction rate of hydrolysis of methyl bromide is proportional to both the concentration of OH- hydroxide ions and the concentration of methyl bromide (second-order reaction S_N2).

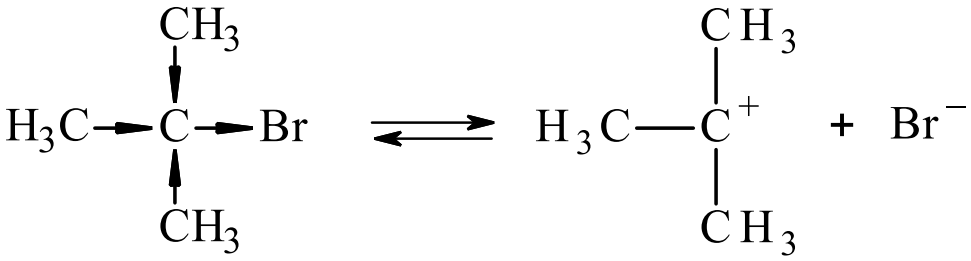
For tert-butyl bromide, the reaction rate depends only on the concentration $(H_3C)_3CBr$ and does not depend on the concentration of OH- (first-order S_{N1} reaction).

These facts can be explained by assuming that the substitution at the carbon atom can occur by different mechanisms.

The nucleophilic substitution reaction S_NI

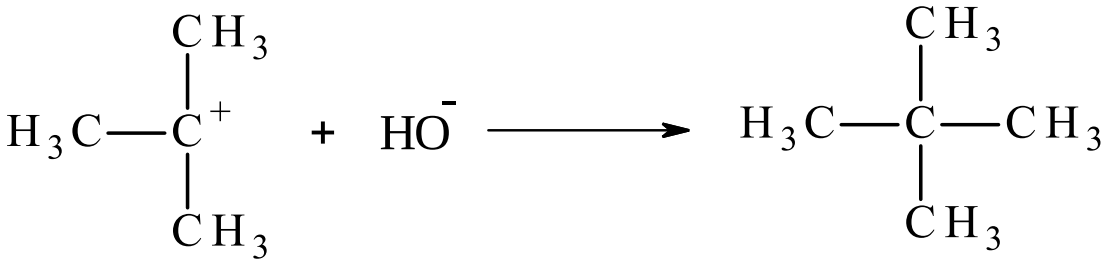
The hydrolysis of tert-butyl bromide proceeds in two stages:

Stage 1 - reversible dissociation of a haloalkane into ions (mono-molecular reaction)



Dissociation occurs slowly, resulting in carbocation.

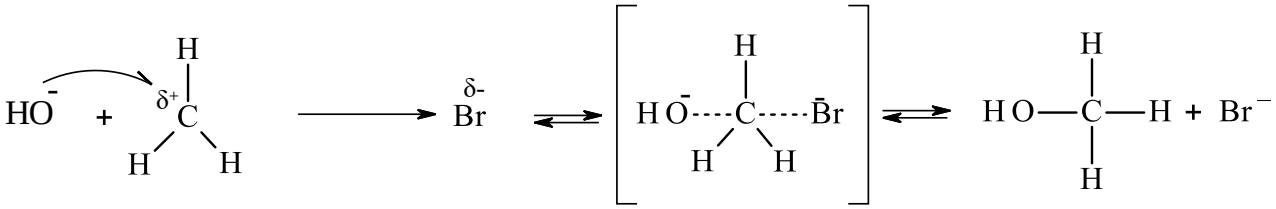
Stage II - the formed carbcation reacts with an attacking reagent, the ion reaction rate is very high.



The reaction rate as a whole is determined by the speed of the slowest process - the rate of dissociation, so the entire substitution process proceeds according to the kinetic equation of the first order reaction.

The reaction of nucleophilic substitution S_N2

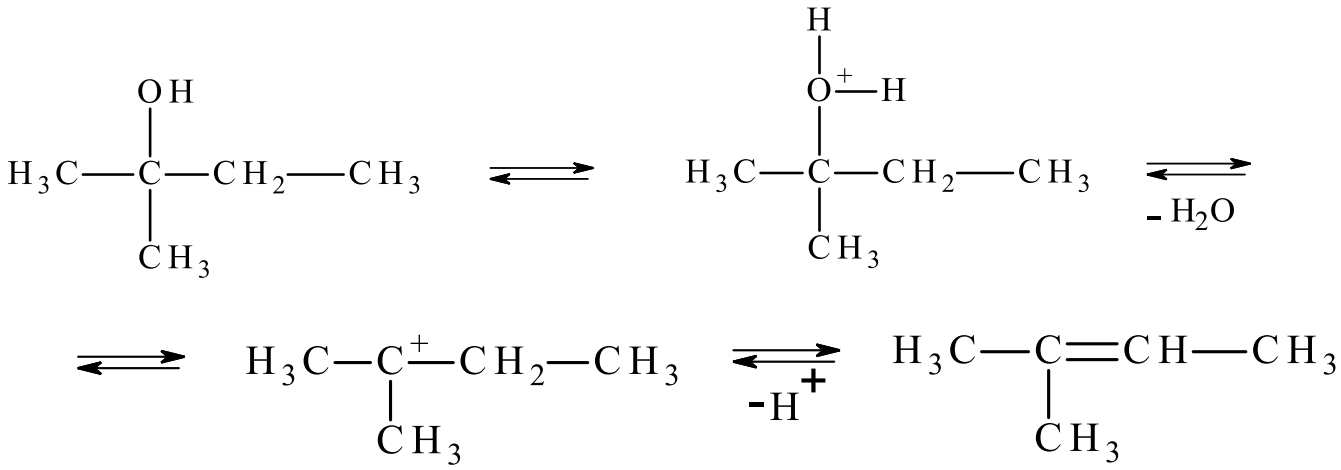
This mechanism is characteristic of the methyl bromide hydrolysis reaction. The hydroxide ion attacks the methyl bromide molecule, displacing bromine in the form of an anion, and the breaking of the C-Br bond and the formation of a new C-OH bond occur simultaneously through the formation of a transition state of the simultaneous coordination of five substituents. The hydrolysis rate is described by a second order equation.



Very often, nucleophilic substitution reactions are accompanied by a elimination reaction. This is due to the fact that both reactions proceed with the formation of the same intermediate product.

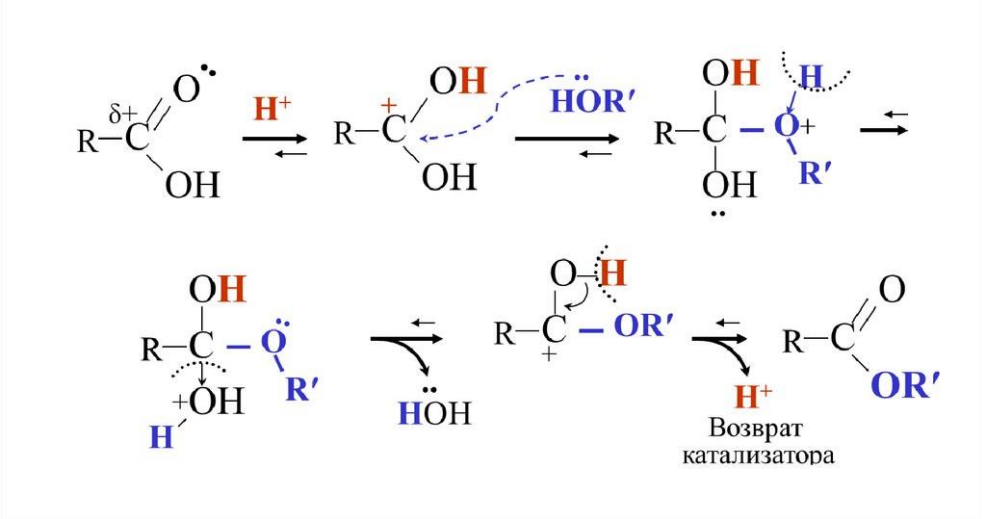
Let us consider the mechanism of the elimination reaction using the example of the dehydration reaction of 2-methylbutanol-2 in the presence of a catalyst. Most often, this role is played by acids (H_2SO_4, H_3PO_4), acidic salts ($KHSO_4$), oxides (Al_2O_3,

P_2O_5, etc.). The order of water cleavage most often meets the Zaitsev rule - when a water molecule is formed, the hydrogen is split off from the least hydrogenated carbon atom



At the first stage, the H^+ catalyst is attached to the lone electron pair of the OH group. An oxonium cation is formed, which cleaves the water molecule and turns into a carbcathion, the stabilization of which occurs by proton cleavage. This is how the catalyst regenerates. The considered transformations of organic substances concern compounds in which the carbon atom is in the first valence state (sp^3 hybridization).

Etherification reaction mechanism



(Regeneration of catalyst)

Thus, an understanding of exactly how reactions occur and what factors determine their direction is the most important achievement in organic chemistry, which is important for biology.

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:

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 № 2

“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.

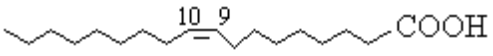
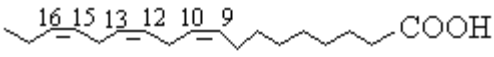
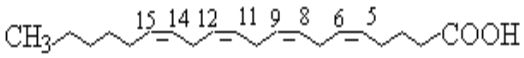
The main structural components of saponifiable lipids are alcohols and higher fatty acids. Saponifiable lipids of a more complex structure may contain residues of phosphoric acid, amino alcohols, as well as residues of mono- and oligosaccharides.

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}	$C_{19}H_{31}COOH$	

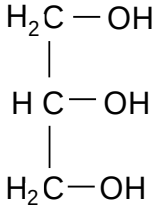
According to the position of the double bond relative to the last carbon atom, polyunsaturated fatty acids are divided by

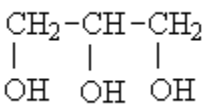
• ω-6 fatty acids – linoleic (C18:2; 9,12), γ-linolenic (C18:3; 6,9,12), arachidonic (C20:4; 5,8,11,14). These acids form vitamin F, and are found in vegetable oils.

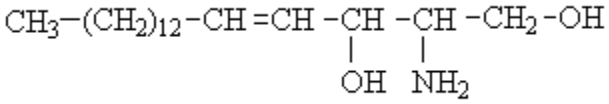
• ω-3-fatty acids – α-linolenic (C18:3; 9,12,15), timnodonic (eicosopentaenoic, C20:5; 5,8,11,14,17), clupanodonic (decosopentaenoic, C22:5; 7, 10,13,16,19), cervonic (decosohexaenoic, C22:6; 4,7,10,13,16,19). The most significant source of acids of this group is the fat of fish from the cold seas. An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

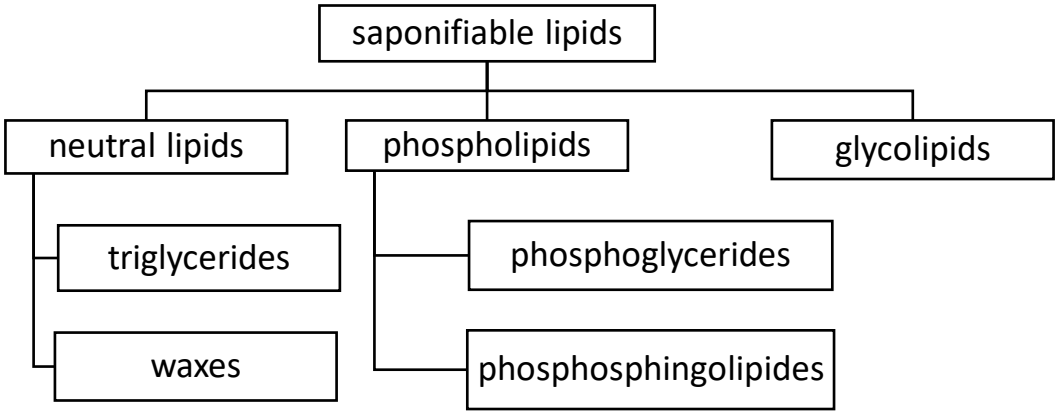






Glycerol glycerol sphingosine

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



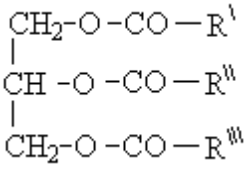
1.2. Neutral lipids

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

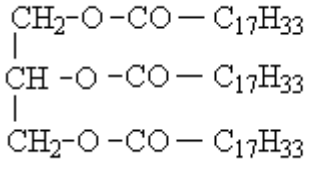
Triacylglycerides

Triacylglycerides are esters of glycerol and higher fatty acids.

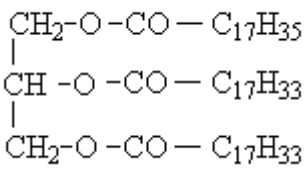
General formula:



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.

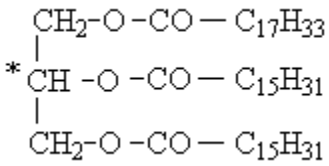


trioleinoglycerol



distearoyl-2-oleinoglycerol

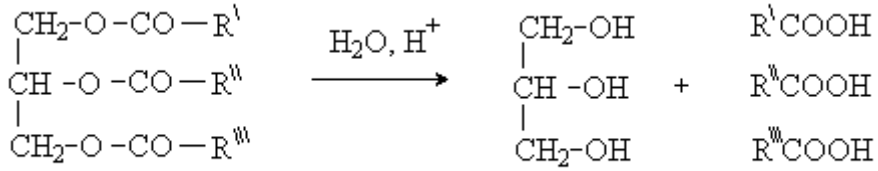
Mixed triacylglycerides may contain a chiral carbon atom in position 2 and have enantiomers, for example:

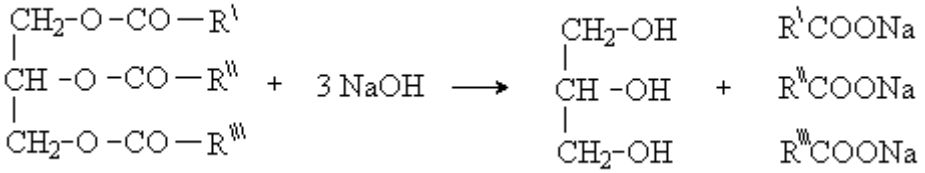


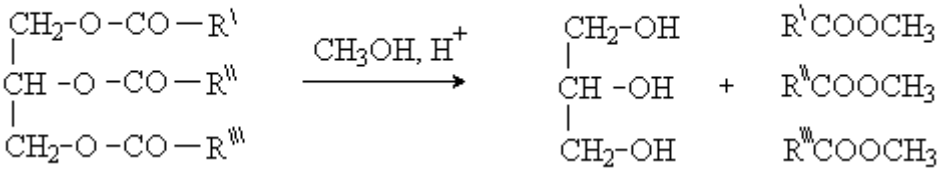
Triacylglycerides are low-polar, water-insoluble substances, since their molecules do not contain highly polar or charged groups. Triacylglycerides, containing predominantly unsaturated acid residues, are, under ordinary conditions, liquids, triglycerides of saturated acids are solids. They are part 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.

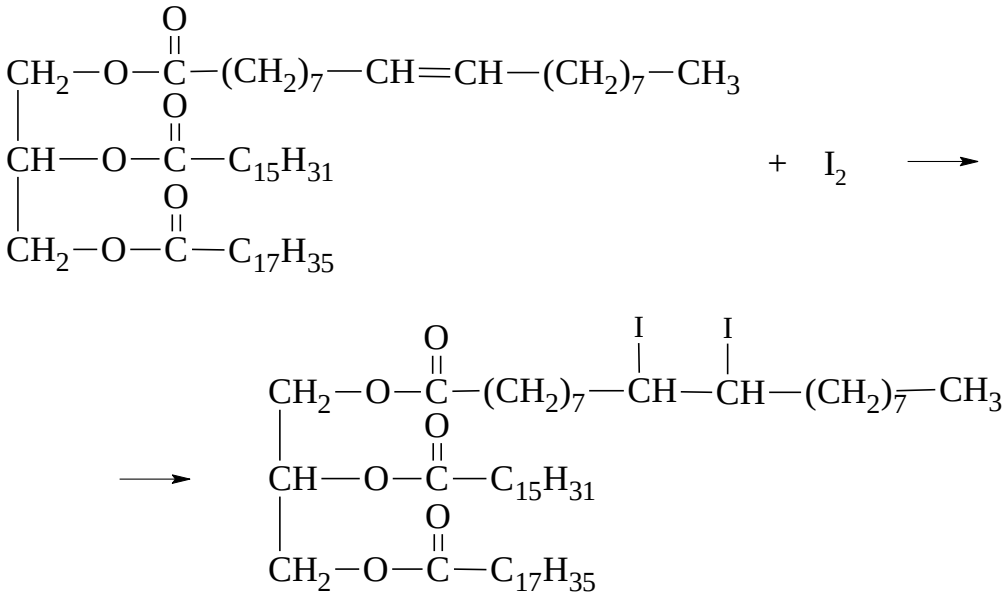






With alkaline hydrolysis (saponification) of fats, salts of fatty acids (soaps) are formed. Their molecules are diphilic (they contain a polar “head” and a non-polar “tail”), which causes their surface-active properties and a washing effect.

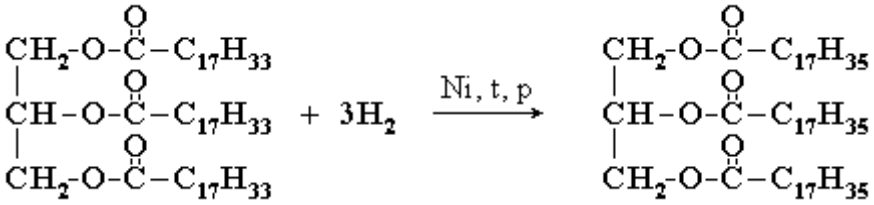
Triacylglycerides containing unsaturated fatty acid residues enter into double bond addition reactions.



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).



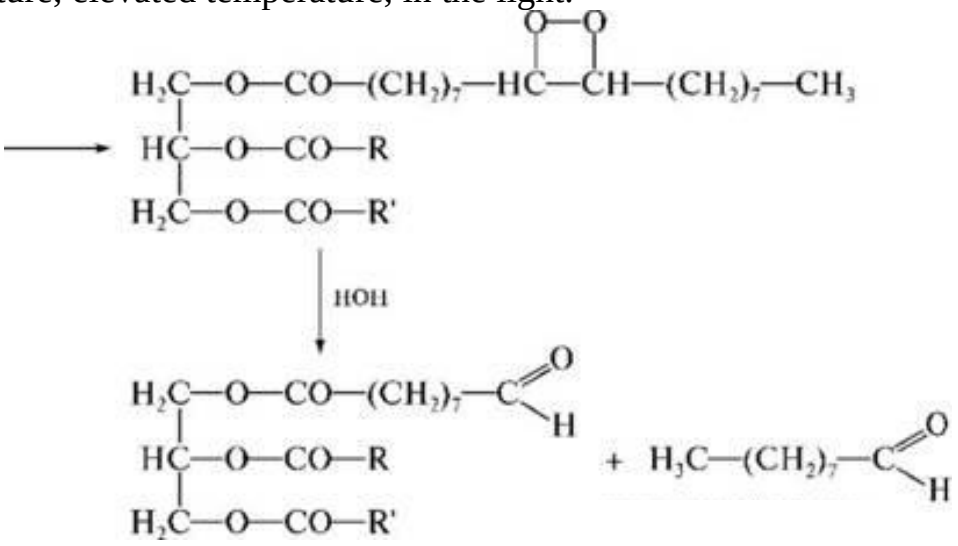
glycerol trioleate glycerol triostearate

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.

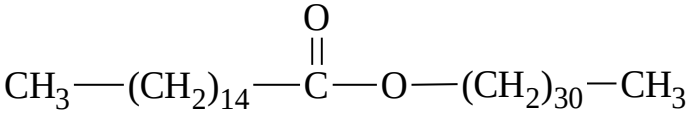
The regioselectivity of this process is explained by the formation of an allyl type radical, which is stabilized by the conjugation of an unpaired electron with double-bond electrons. The final oxidation products are short chain organic acids. They have an unpleasant smell of sweat or rancid oil.

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

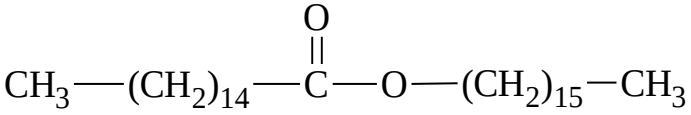
In cells under normal conditions, the self-oxidation of unsaturated fats is completely inhibited due to the presence of vitamin E, various enzymes, as well as ascorbic acid.

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

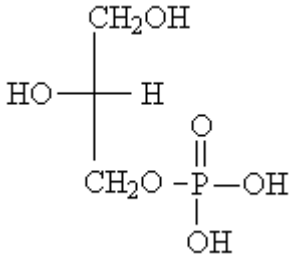
Phospholipids are complex lipids and are the main components of cell membranes in which lipid bilayers form. Alcohols are present in the PL composition: aminoethanol, choline, amino acids serine, threonine, polyhydric cyclic alcohol inositol.

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.

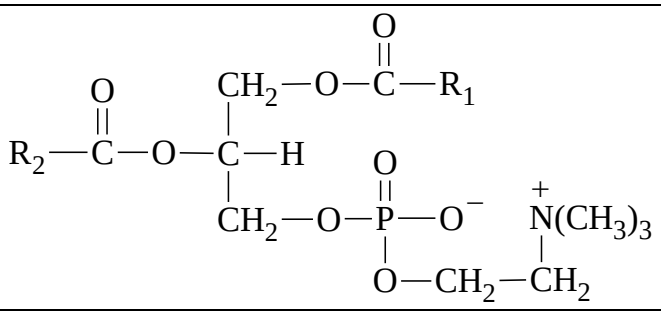
Phosphoglycerides

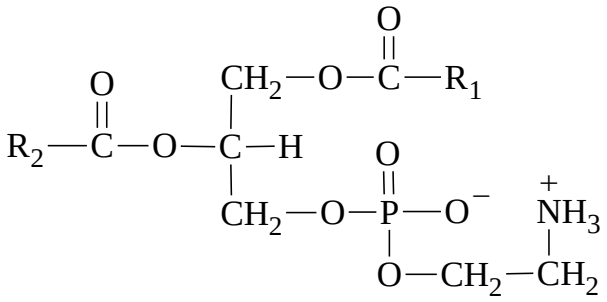
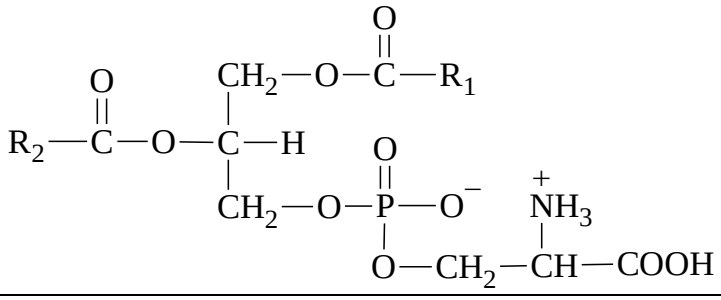
The main structural components that make up the phosphoglyceride molecule are glycerin, fatty acids, phosphoric acid, amino alcohols (ethanolamine or choline) or the amino acid serine. They are considered as derivatives of L-glycero-3-phosphate



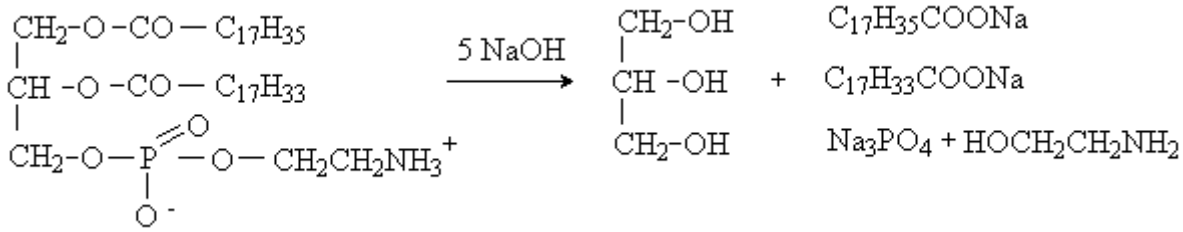
in which the alcohol groups are esterified with fatty acids and the residue of the phosphoric acid forms an ester bond with the amino alcohol. The general formula of phosphoglycerides:

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.

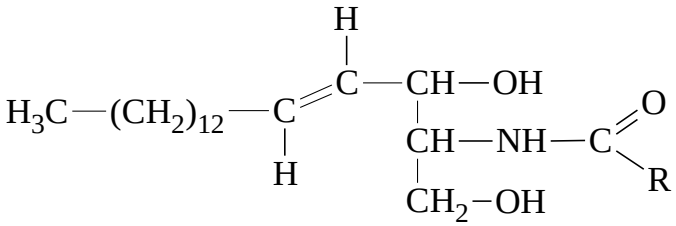


Sphingolipids

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:

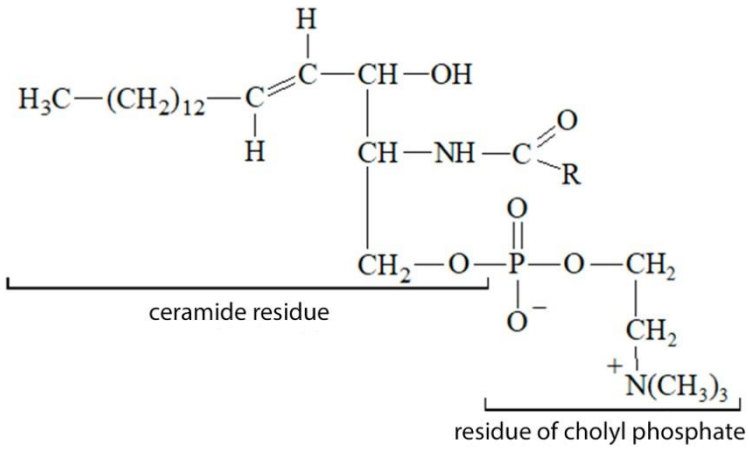


Ceramides are found in nature both in free form (in lipids of the liver, spleen, red blood cells), and as part of sphingolipids.

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:



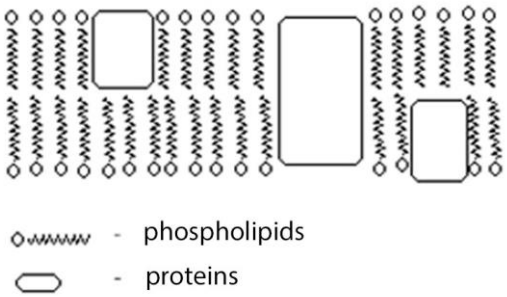
where R is the CO- residue of palmitic, stearic, lignoceric or nervonic acid.

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

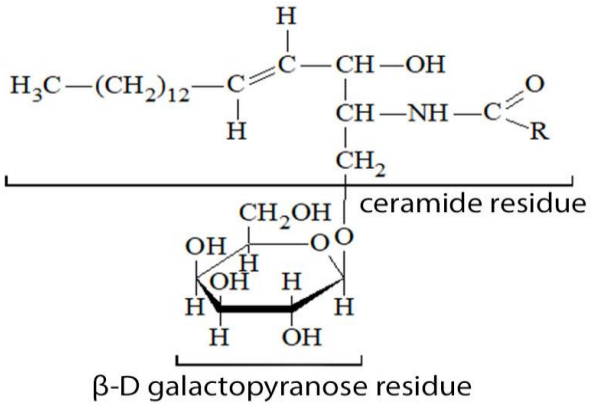


cell membrane structure

1.4. Glycolipids

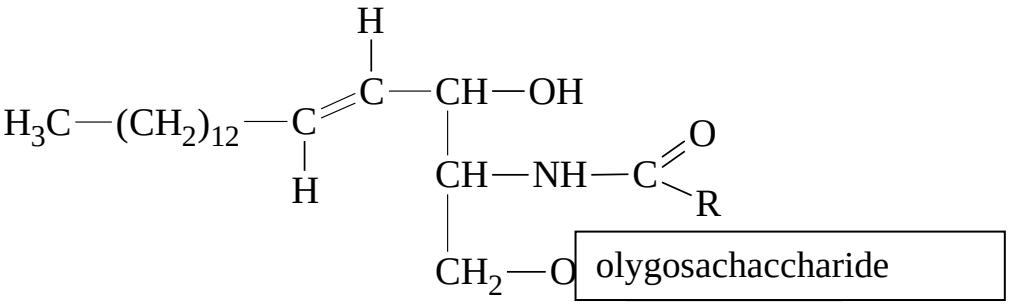
Among glycosphingolipids, **cerebrosides and gangliosides** are most common.

The general structure of cerebrosides can be represented as follows:



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.

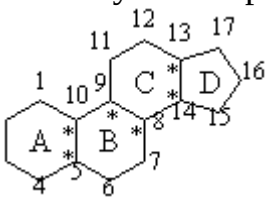
Lipid function

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.

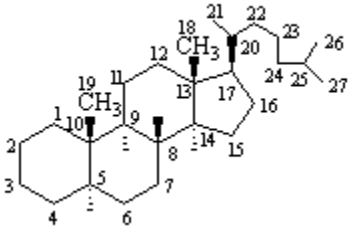


steran

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.

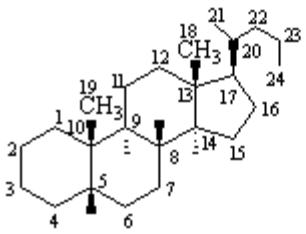
Cholesterol is the most common sterol in animals and humans. It is present in all animal lipids, blood and bile. The brain contains 7% cholesterol per dry weight.

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.

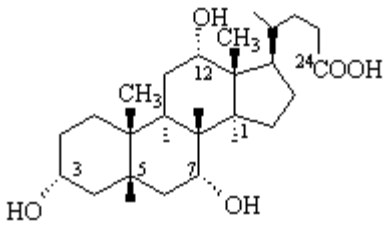
* Contrary to popular belief, cholesterol is not an "enemy of the human race," but serves as the basis for the synthesis of steroid hormones. In general, cholesterol is a rather important and moderately harmless metabolite, which plays an important role in ensuring the barrier function of cell membranes. 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

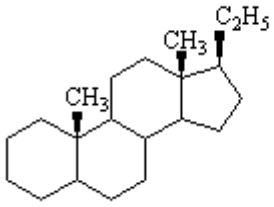


cholic acid

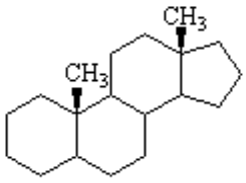
Bile acids are formed in the liver from cholesterol. Sodium and potassium salts of bile acids are surfactants. Emulsifying fats, they contribute to their absorption and digestion.

Steroid hormones

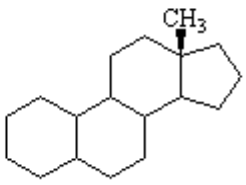
Steroid hormones are physiologically active substances of a number of steroids produced by the endocrine glands. According to the chemical structure and biological effect, hormones of the adrenal cortex (corticosteroids), male sex hormones (androgens) and female sex hormones (gestagens and estrogens) 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

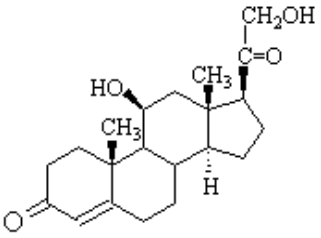


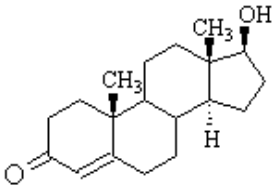
androstane

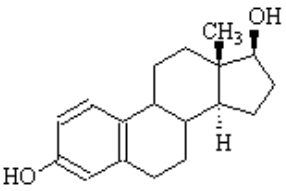


estran

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 № 3

"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.

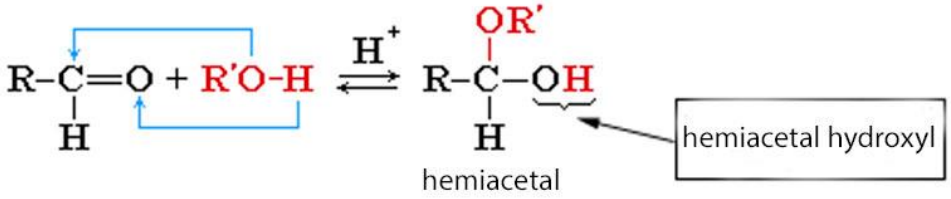
4. Protective – carbohydrates participate in maintaining the body's immunity. Thyroid-stimulating hormone controls the function and development of the thyroid gland, being a glycoprotein, i.e. a complex of carbohydrates with proteins.

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

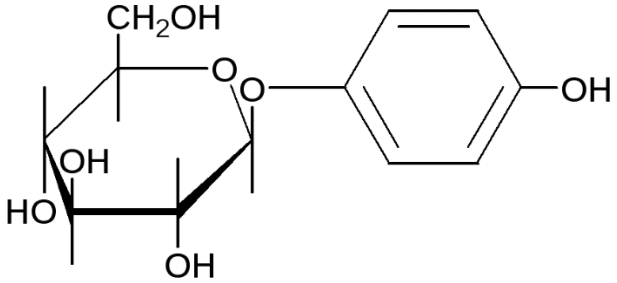
6. Regulatory – plant foods contain polysaccharide - cellulose, which improves bowel function and increases secretion in the stomach.

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_20) 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:

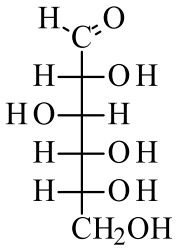
Simple carbohydrates, or simple sugars – monosaccharides, or monoses that are not able to hydrolysis.

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.

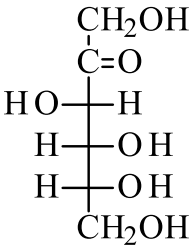
Monosaccharides are polyhydroxycarbonyl compounds. They are classified according to two characteristics: the length of the carbon chain and the nature of the oxo group.

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.

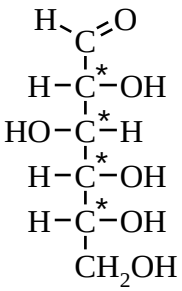
Monosaccharide molecules contain several chiral centers; therefore, several stereoisomers correspond to the same structural formula. The number of isomers is calculated by the formula: $N = 2^n$,

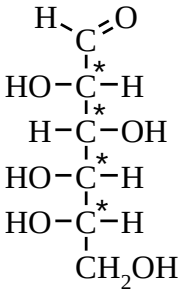
where N – number of isomers; n – number of chiral centers.

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

$$N = 2^4 = 16$$

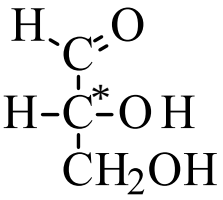
Thus, for each of the optical isomers there is one of its optical antipodes - the enantiomer, the rest - diastereomers. So, 16 aldoghexoses make up 8 pairs of antipodes that belong to the D and L rows.



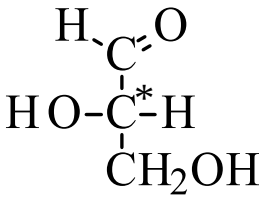


D - glucose L - idose

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)

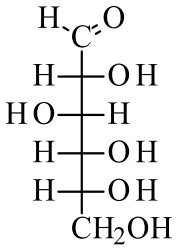
According to stoichiometric nomenclature, the D-, L-system in most cases is replaced by the R-, S-system, which is the main one when considering the spatial model of the molecule, in which seniority of substituents near the chiral center is taken into account. The seniority of the substituents is determined 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.

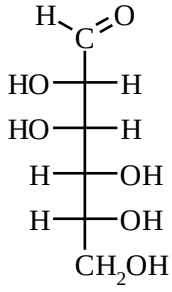
The optical activity of carbohydrates is indicated by a (+) sign for dextrorotatory compounds and a (-) sign for levorotatory compounds.

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

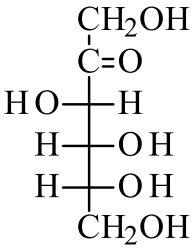
Fischer projection formulas are used to represent stereoisomers in monosaccharides.



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.

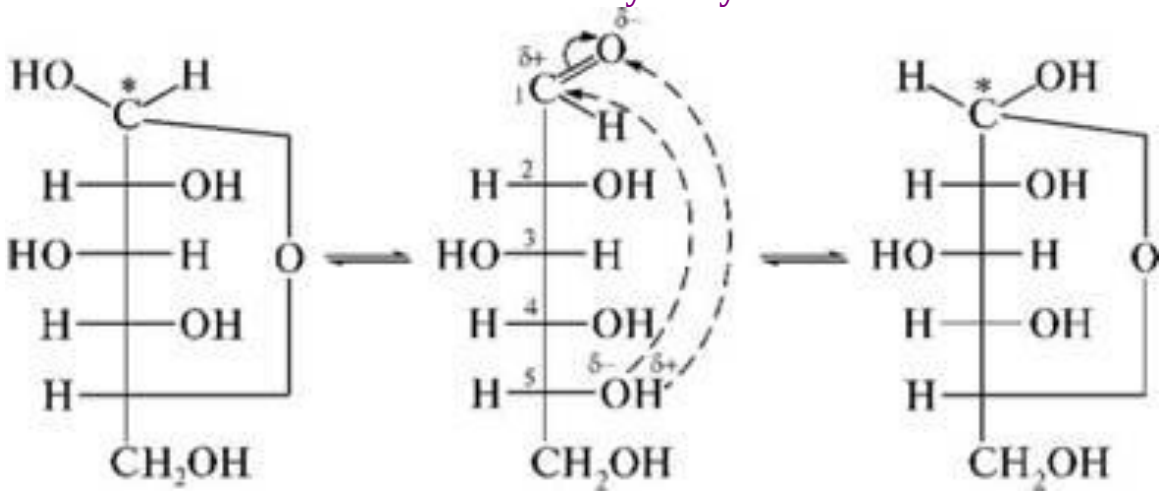
There are reactions that cannot be explained on the basis of the above structure of sugars, for example, the phenomenon of mutarotation and the formation of glycosides. The number of isolated isomers was twice as large as would be expected according to the formula $N = 2^n$. 32 isomers of aldoghexoses 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.

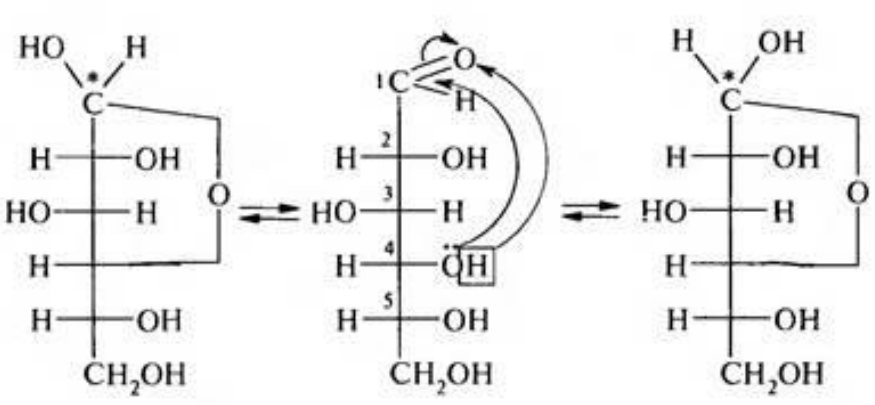
If a five-membered cycle is formed, closed to an oxygen atom, then such a cycle is called furanose, and if it is six-membered, it is called pyranose. The OH group that formed is called hemi-acetal, or glycoside. For example, glucose exists in five forms, four of them cyclic:

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.

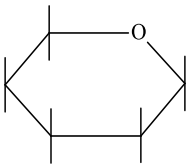


β-D – glucofuranose *D – glucose* *α-D - glucofuranose*

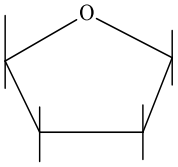
In the α-anomer, the configuration of the anomeric center is the same as the configuration of the "terminal" chiral center in the monosaccharide molecule, and in the β-anomer it is the opposite.

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

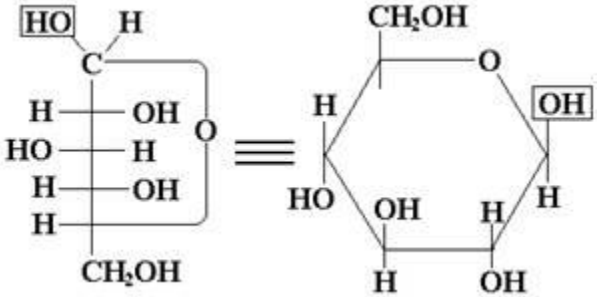


Pyranose cycle



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.

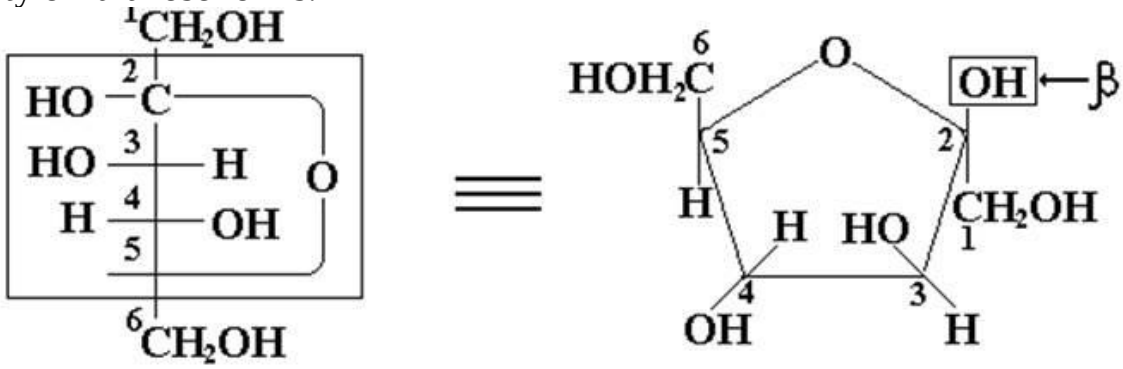


β-D-glucopyranose

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.

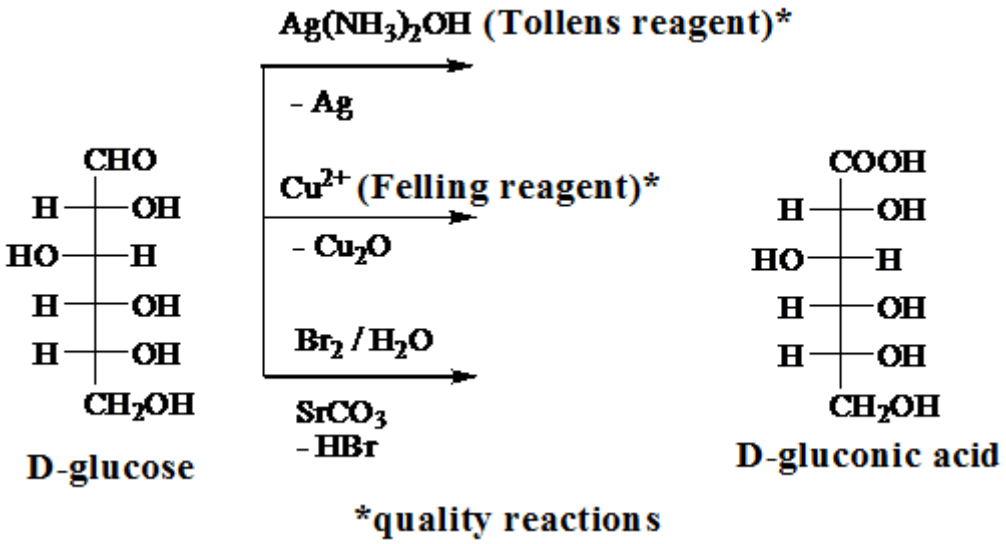
Due to the high reactivity of hemi-acetal hydroxyl, important metabolites are formed.

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

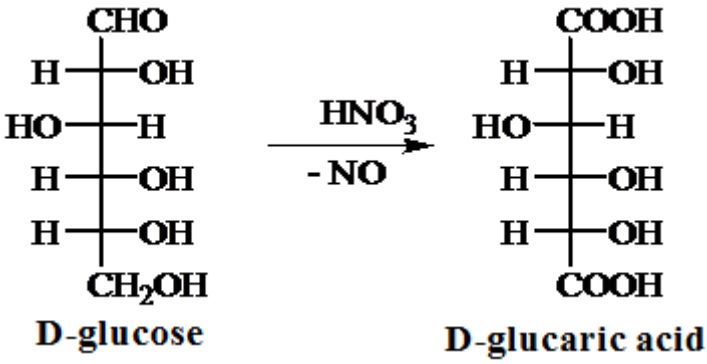
These reactions take place in polar solvents (often in aqueous solutions) with the obligatory participation of the carbonyl group.

a) Oxidation



*quality reactions

b) Concentrated Nitric Acid Oxidation.

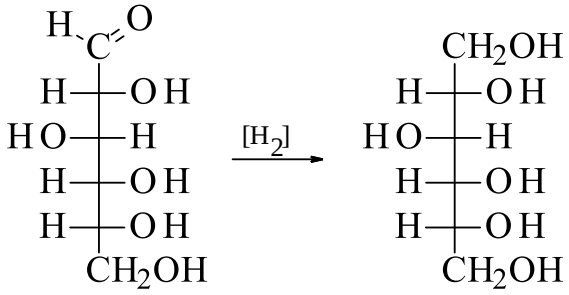


When concentrated HNO_3 is used, in addition to the carbonyl group, the terminal CH_2-OH group is oxidized and a number of glycaric (araic, aldaric, sugar) acids are formed.

D-mannaric acid → from D-mannose

D-galactaric acid →f rom D-galactose

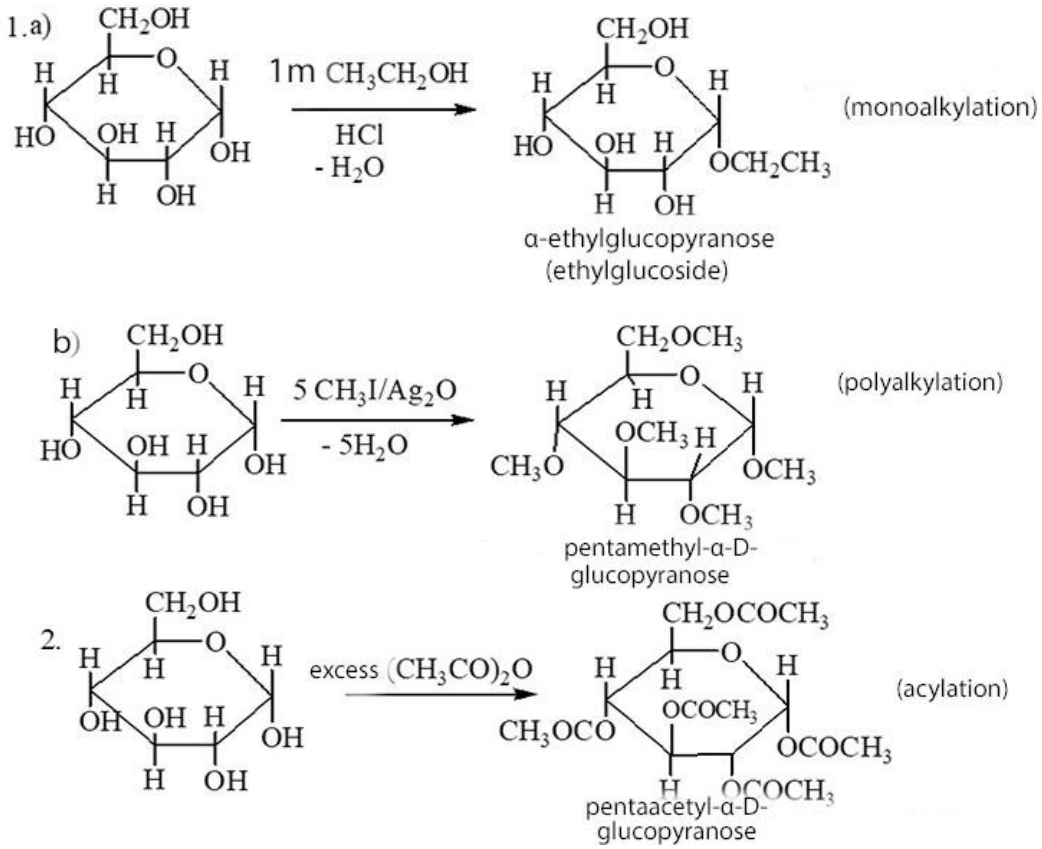
2. Reduction



glucose *sorbitol*

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);

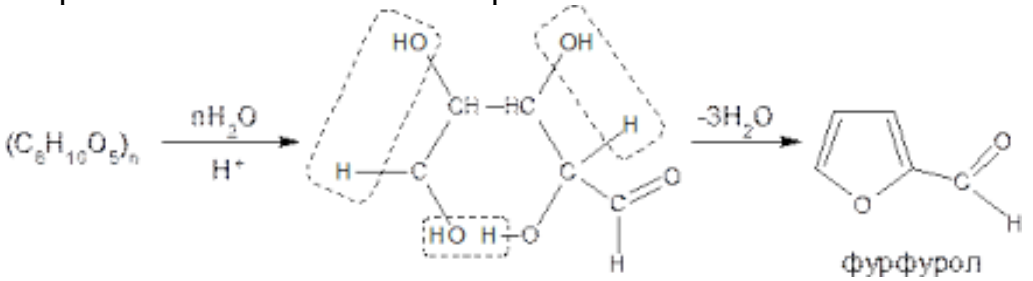
b) $C_6H_{12}O_6 \rightarrow 2CH_3\text{-}CH(OH)\text{-}COOH$ (lactic acid fermentation);

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);

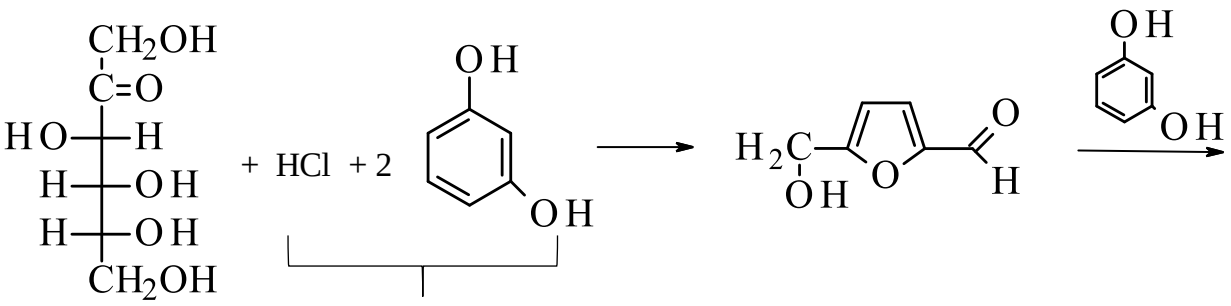
f) $2C_6H_{12}O_6 \rightarrow C_4H_9OH + CH_3\text{-}CO\text{-}CH_3 + 5CO_2 + 4H_2$ (acetone-butanol fermentation).

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

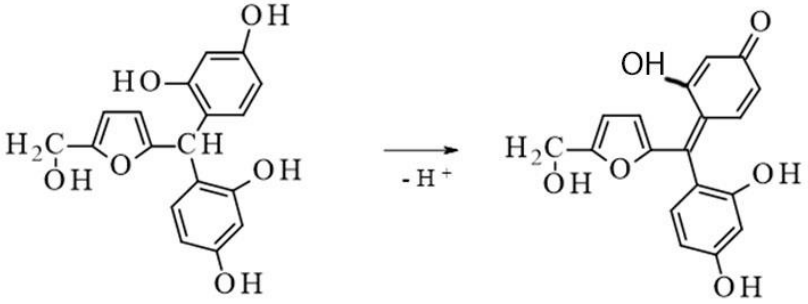


Aldopentose *furfural*

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*

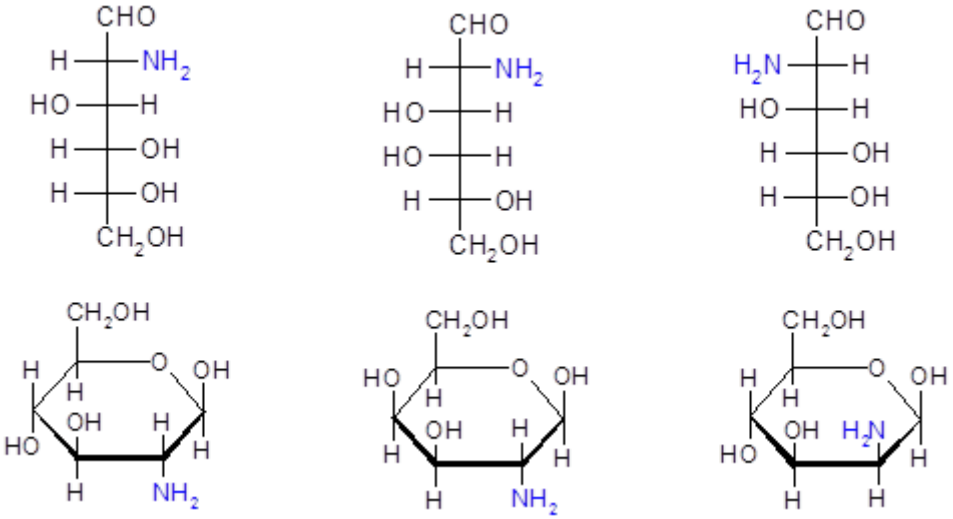


Amino sugars

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

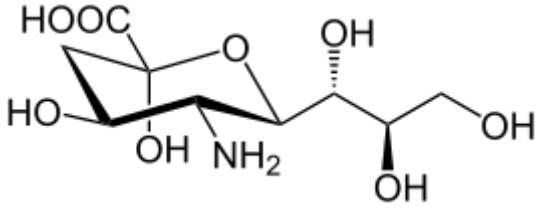
In the case of direct attachment of an amino group to the glycosidic carbon atom, such compounds are called glycosylamines or N-glycosides.

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.

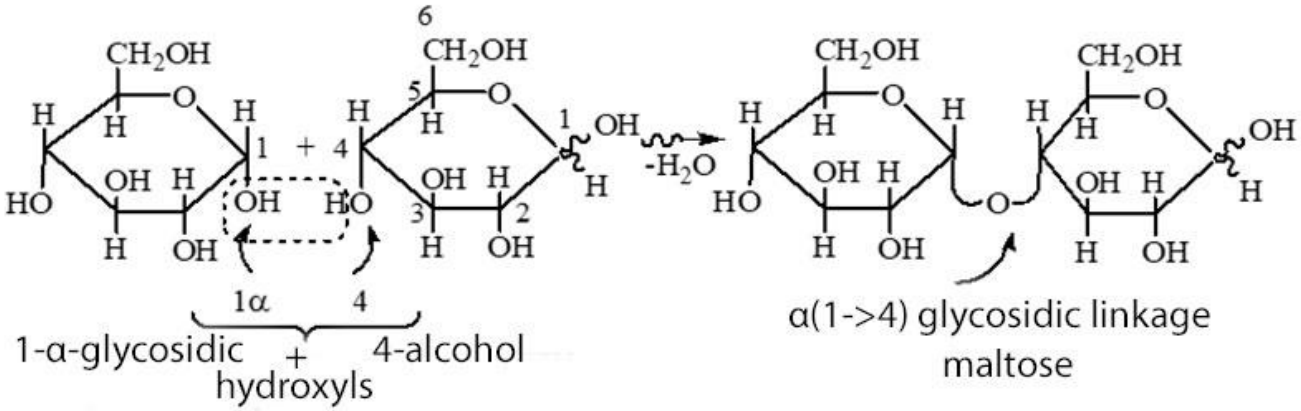


Neuraminic acid

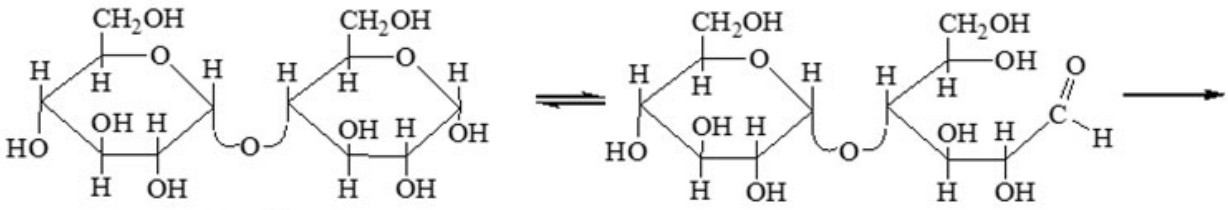
Amino sugars and their methyl derivatives are part of the molecules of a number of antibiotics: D-gulosamine (2-amino-2-deoxy-D-gulose) - streptomycin, deosamine (3-dimethylamino-3,4,6-trideoxy-D-glucose) - erythromycin, mycaminose (3-dimethylamino-3,6-dideoxy-D-glucose - carbomycin, etc.). N-acetylfucosamine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

Disaccharides

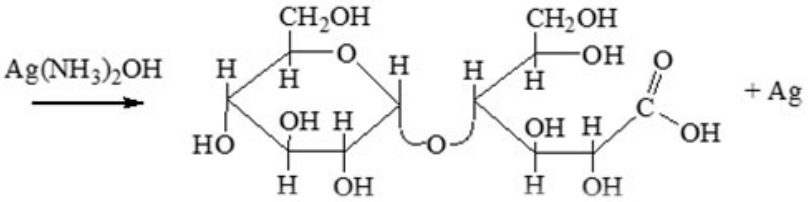
Disaccharides are carbohydrates, the molecules of which consist of two monosaccharide residues joined together by the interaction of hydroxyl groups (two hemi-acetal or one hemi-acetal and one alcohol). The absence or presence of glycosidic (hemi-acetal) hydroxyl is reflected in the properties of disaccharides. 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.

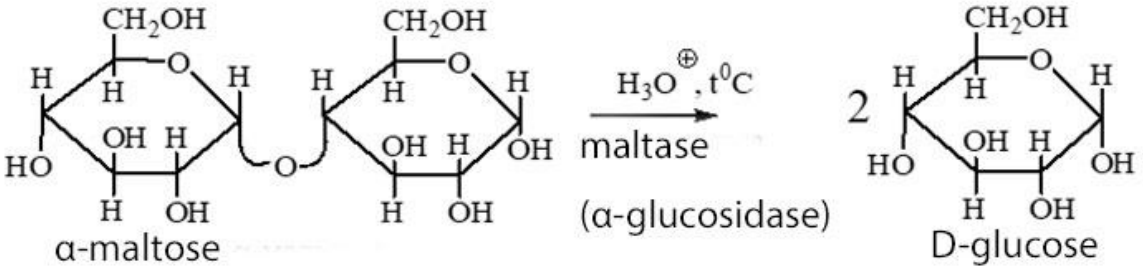


α-maltose



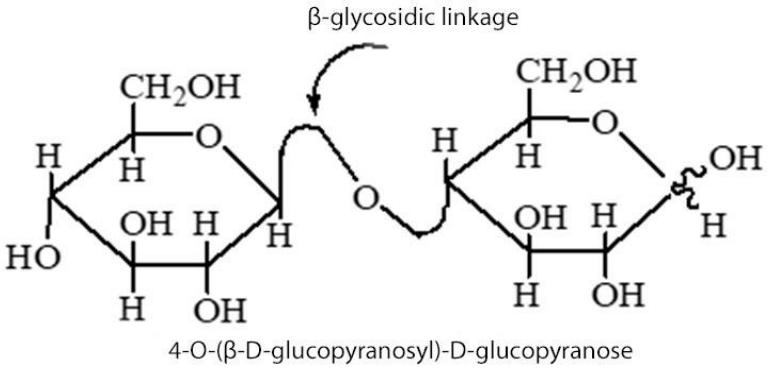
D-maltobionic acid

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



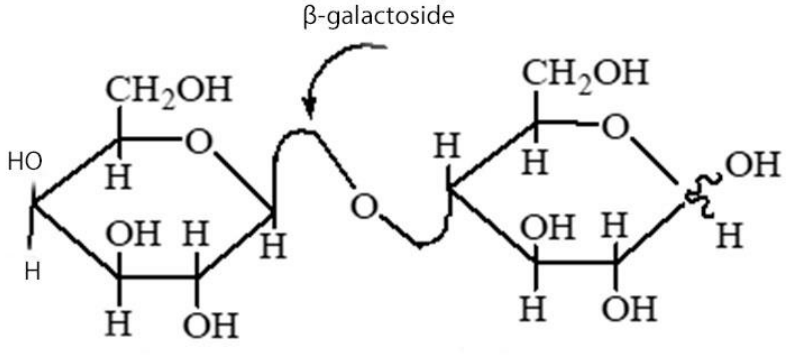
α-maltose

Cellobiose D-glucose 1β-4 D-glucose



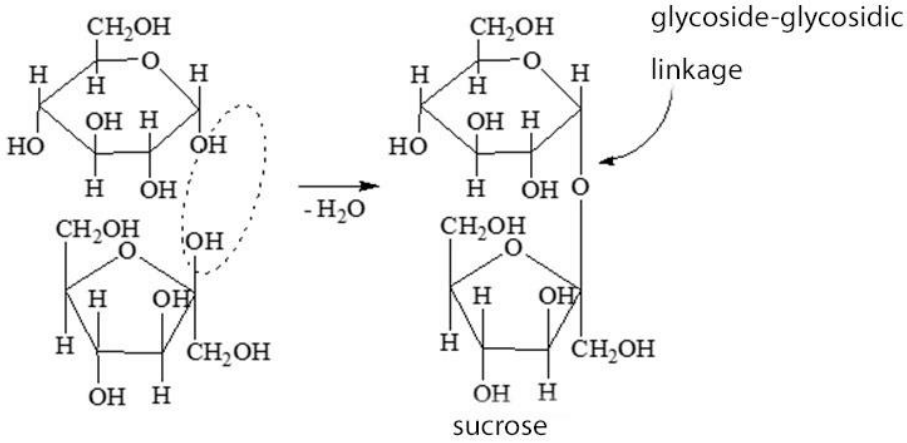
4-O-(β-D-glucopyranosyl)-D-glucopyranose

Lactose D-galactose 1β-4D-glucose

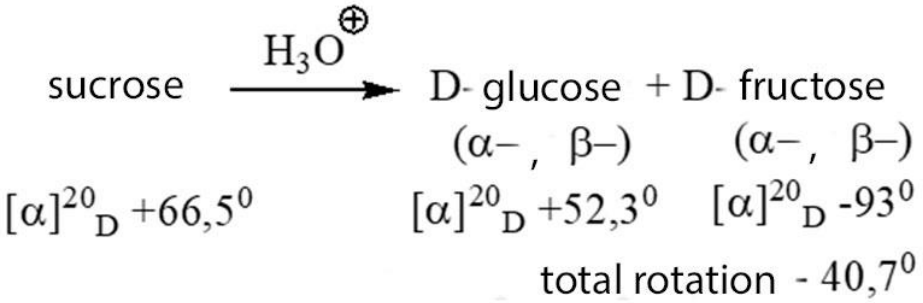


4-O-(β-D-galactopyranosyl)-D-glucopyranose

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.

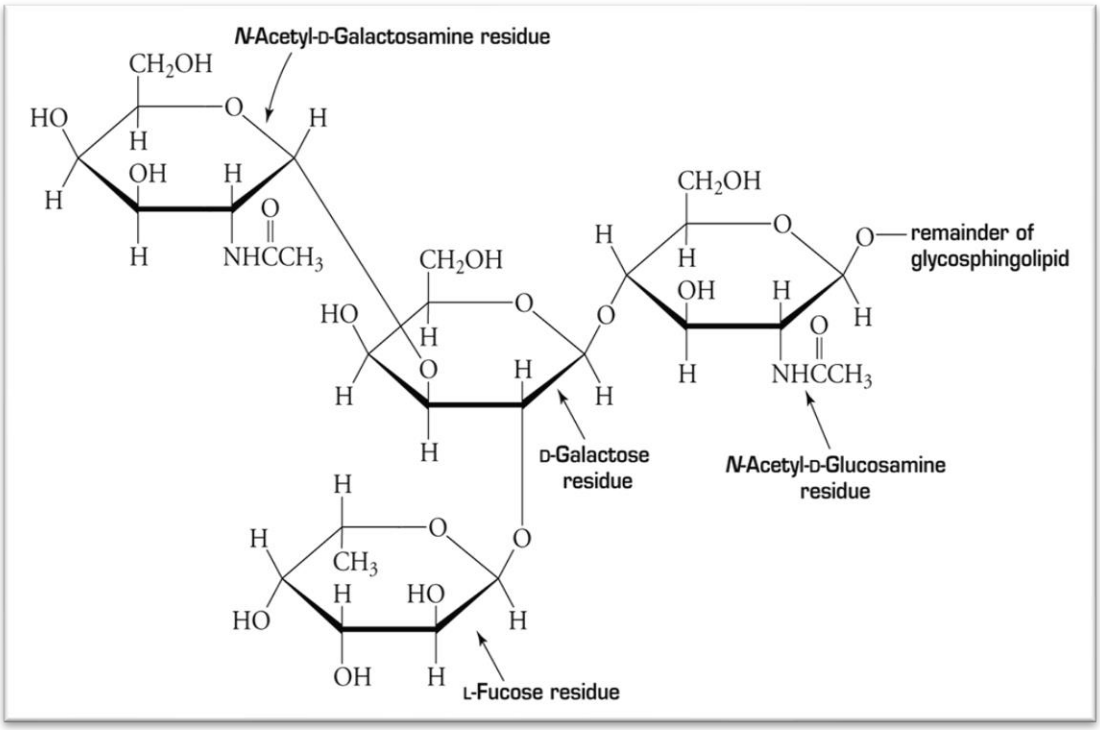


Oligosaccharides with 3 or more monosaccharides **N-Acetylgalactosamine (GalNAc)**, is an amino sugar derivative of galactose. ***The blood group antigens are oligosaccharides that are attached to lipids and proteins found on cell surfaces.***

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

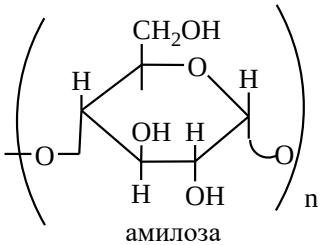
Homopolysaccharides contain a single type of monosaccharide unit.

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.

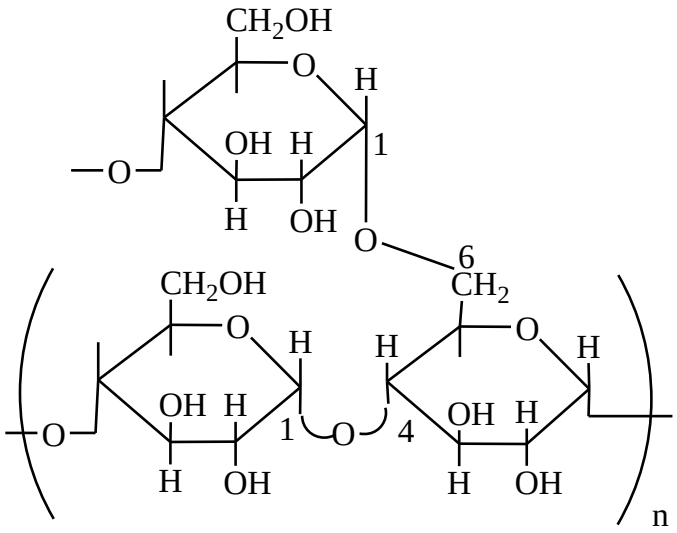
Starch is a reserve polysaccharide of plants, accumulates in the form of grains in the cells of seeds, bulbs, leaves, stems, is a white amorphous substance, insoluble in cold water. Starch is a mixture of amylose and amylopectin, which are built from α-D-glucopyranose residues.

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%.



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.

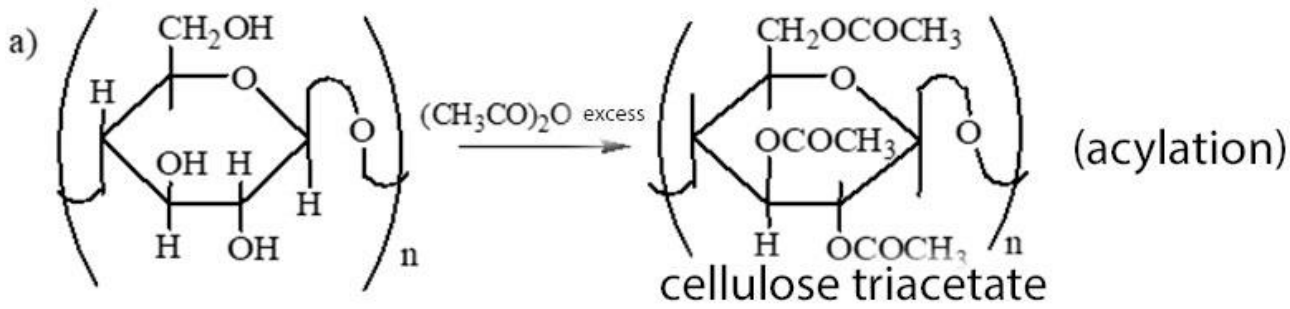
3. Acid hydrolysis of starch: starch → dextrins → maltose → α - D - glucose.

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.

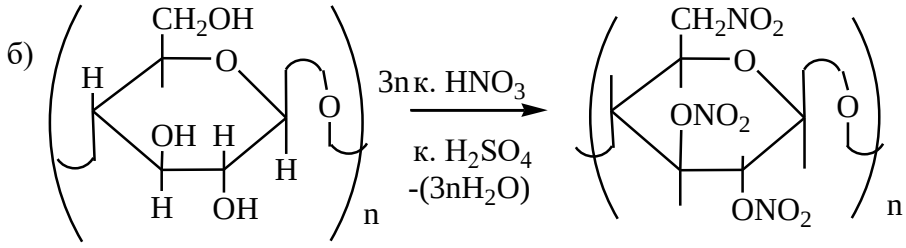
Chemical properties

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.

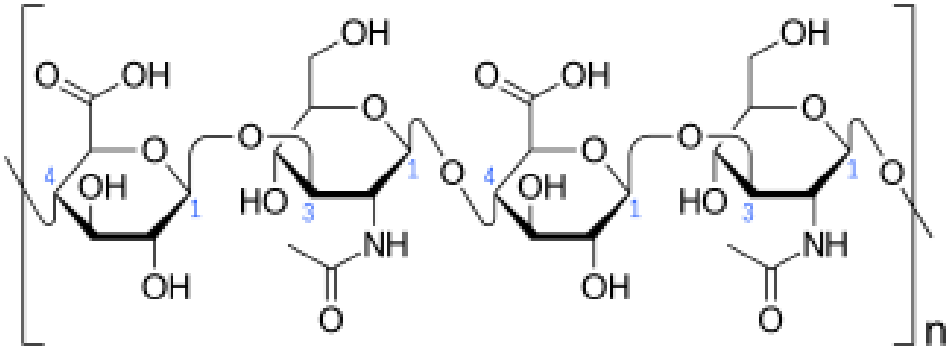
All glycosaminoglycans perform their biochemical and physiological functions being bound to proteins. Covalent complexes of connective tissue glycosaminglycans (hyaluronic acid, chondroitin sulfates, etc.) with proteins are called proteoglycans, which are representatives of mixed biopolymers (glycoconjugates).

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.

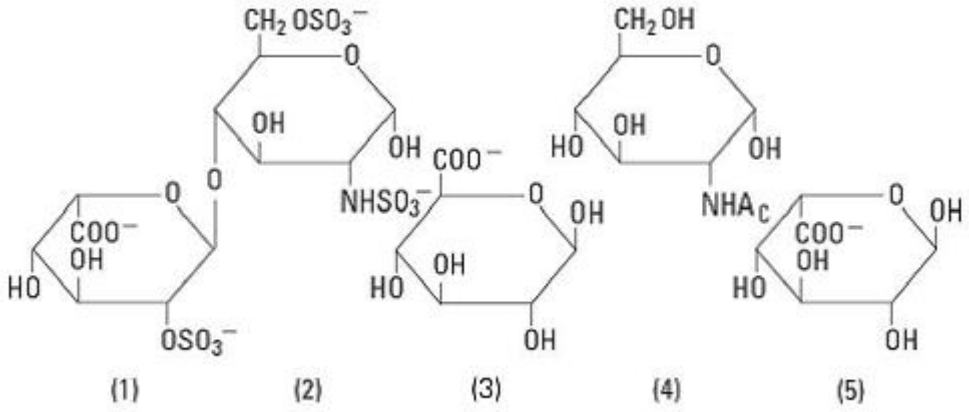


Hyaluronic acid has been used in various formulations to create artificial tears to treat dry eye. It is a common ingredient in skin care products, it is used as a dermal filler in cosmetic surgery. It is typically injected using either a classic sharp hypodermic needle or a micro-cannula. Some studies 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.

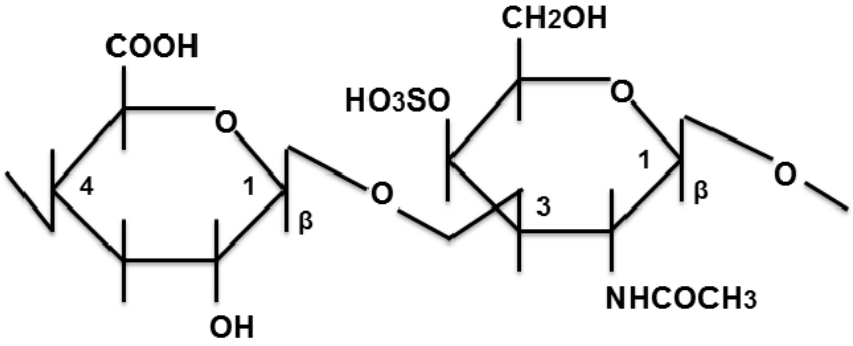
2. **Heparin:** It is made up of D-glucuronic acid, L-iduronic acid, N-sulfo-D-glucosamine and is largely distributed in mast cells and blood.

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.



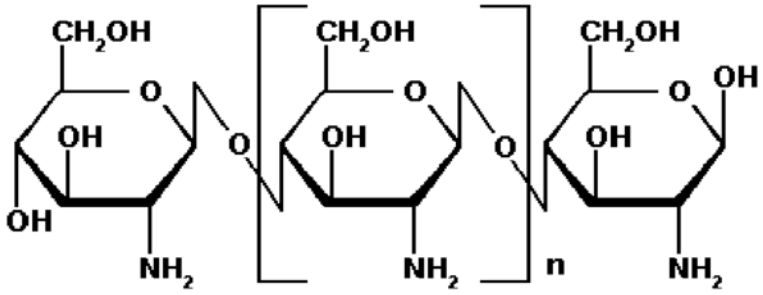
3. **Chondroitin-4-sulfate:** Its component sugars are D-glucuronic acid and N-acetyl-D-galactosamine-4-O-sulfate. It is present in the cartilages.



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.

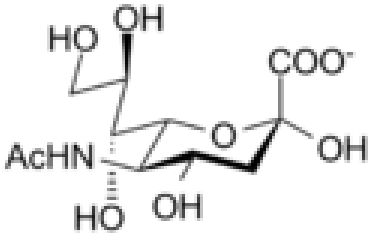
4. **Chitosan** is a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide.

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.

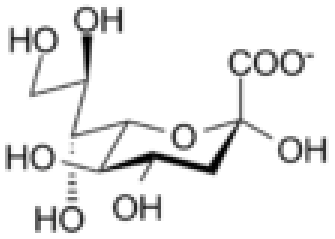


5. **Gamma globulin:** N-acetyl-hexosamine, D-mannose, D-galactose are the component sugars of this polysaccharide. It is found in the blood.

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 № 4

«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).

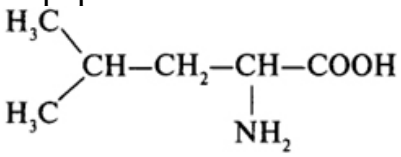
In addition, proteins are involved in the expression of genetic information, the transmission of nerve impulses, support the oncotic pressure of blood and cells, provide homeostasis pH of the internal medium of the body.

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.

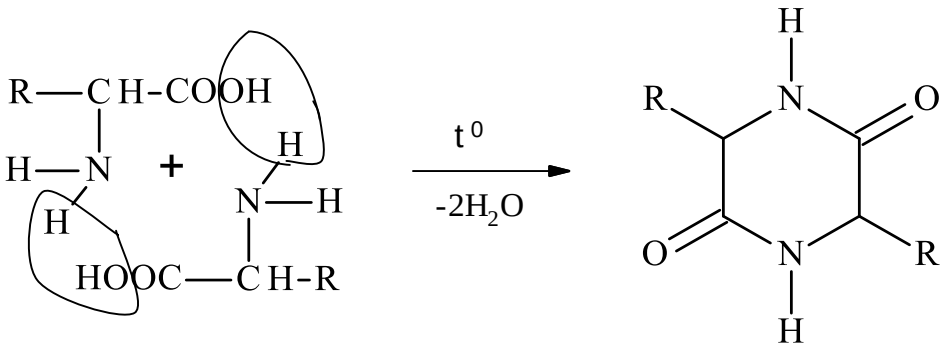


Leucine

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

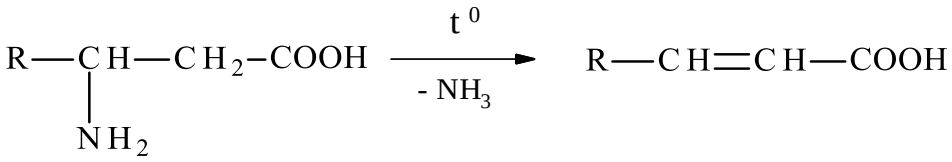
The specific properties of α, β, γ, δ are amino acids.

- **α-aminoacids**



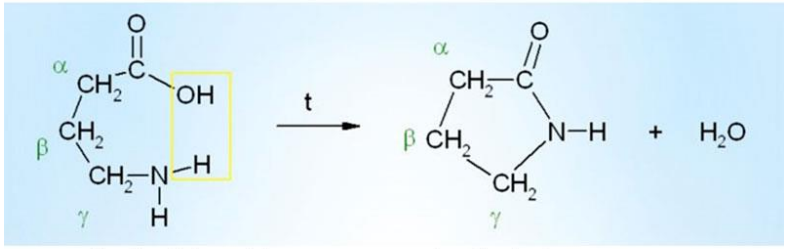
Diketopiperazine
(lactam modification)

- **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.

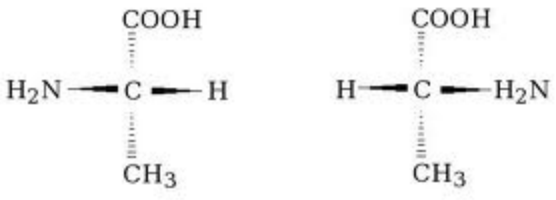
Usually about 20 natural amino acids are secreted, from which the whole set of natural proteins of plant and animal origin is formed.

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

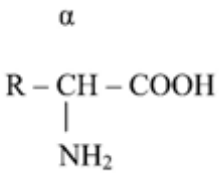
Natural amino acids are classified according to several characteristics:

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:



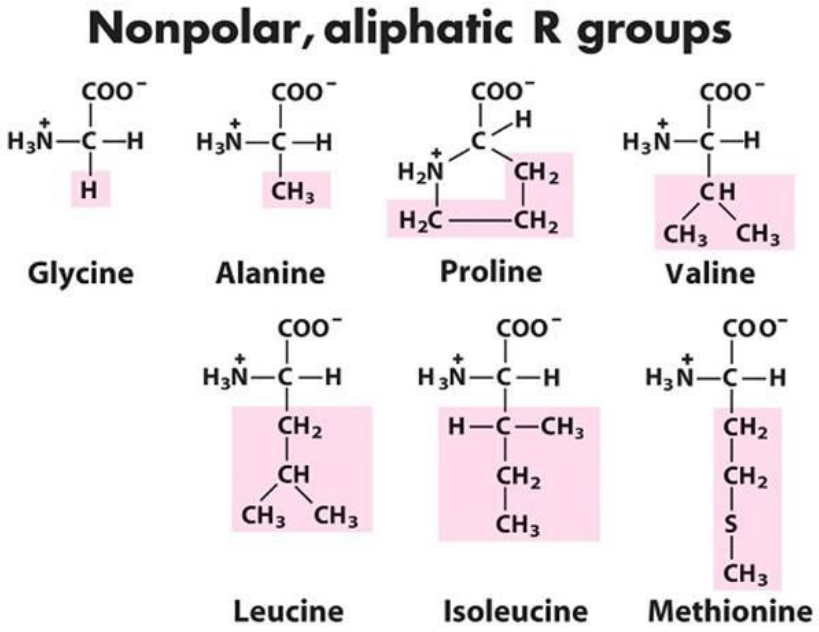
The radical R is a group of atoms that are not involved in the formation of a peptide bond. All structural features and functions of protein molecules are determined by the chemical nature of the radical R.

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

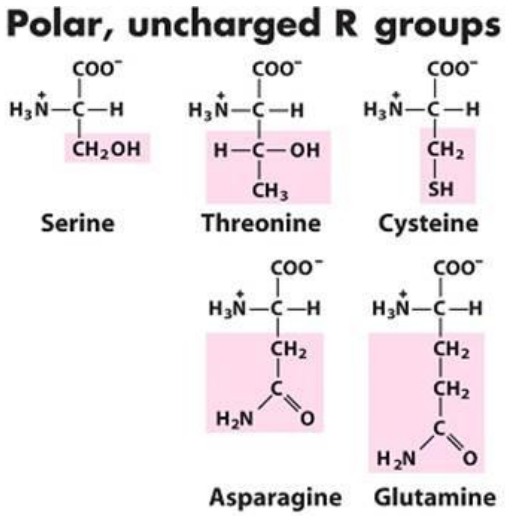
Modern rational classification is based on the polarity of radicals.

There are five classes of amino acids.

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



2. Amino acids with polar (hydrophilic) radicals:

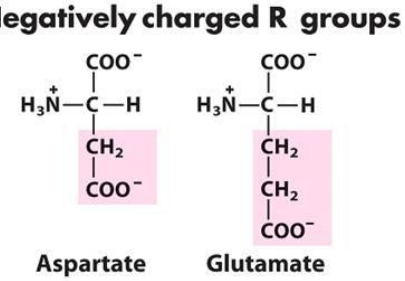


3. Amino acids with negatively charged radicals:

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

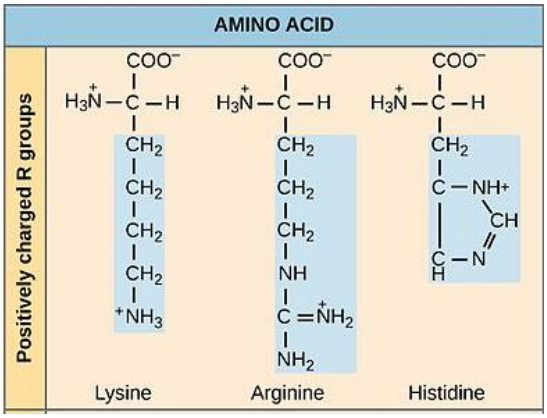
-SH→ -S^-+H^+ (the side chain acquires a negative charge);

Ar-OH→Ar-O^-+H^+ (the side chain acquires a negative charge)



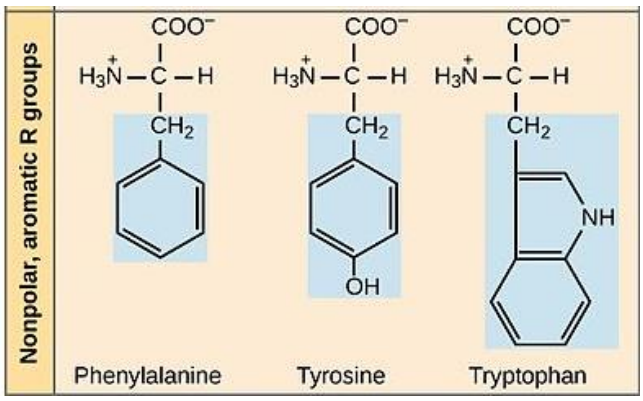
4. Amino acids with positively charged radicals:

-NH_2 + H^+ → -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.

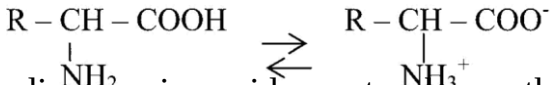


Acid-base properties of amino acids

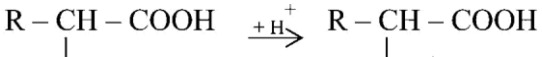
According to the protolytic theory of acids and bases, amino acids belong to ampholytes, because contain acid and base centers in the composition of the molecule. In an aqueous solution, the amino acid molecule exists in the form of a bipolar ion (zwitter-ions).

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

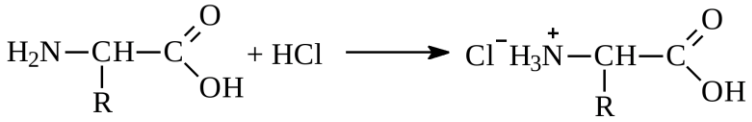
Ammonolysis of halogen acids (Emil Fischer) - the action of ammonia excess on α-halogen acids.

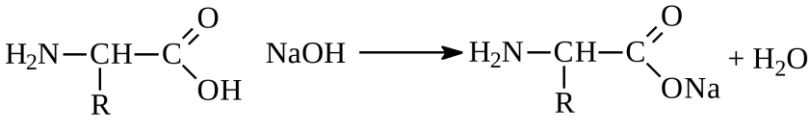
The Strecker-Zelinsky synthesis is the preparation of α-amino acids from aldehydes or ketones by the action of NH_3 and HCN followed by hydrolysis of the resulting α-aminonitriles.

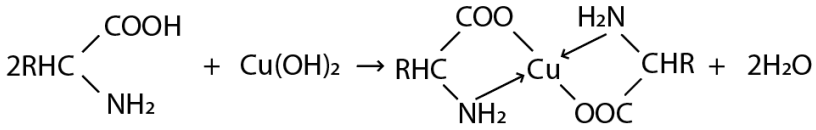
Chemical properties of amino acids

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.**





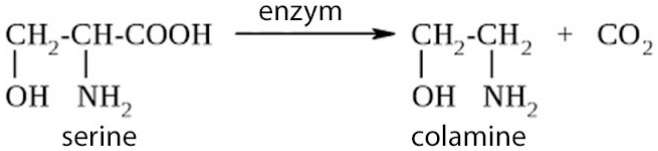


cupperglycinate
(chelate complex salt)

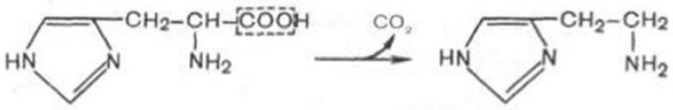
- **Decarboxylation**

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:

$$R{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH + Ba(OH)_2 \xrightarrow{t^0} R{-}CH_2NH_2 + BaCO_3 + H_2O$$

- **Deamination**

In vivo:

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

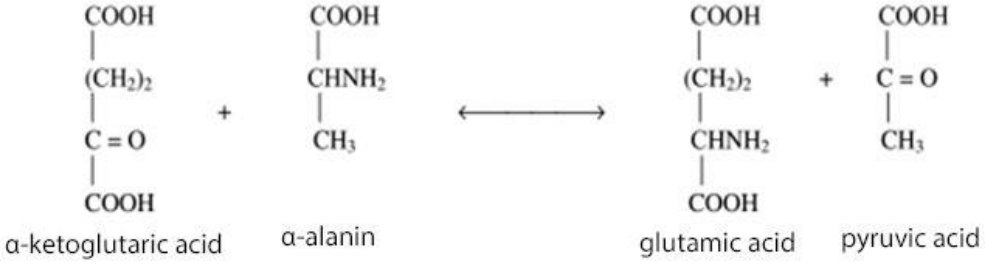
- **Non-oxidative deamination**

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.

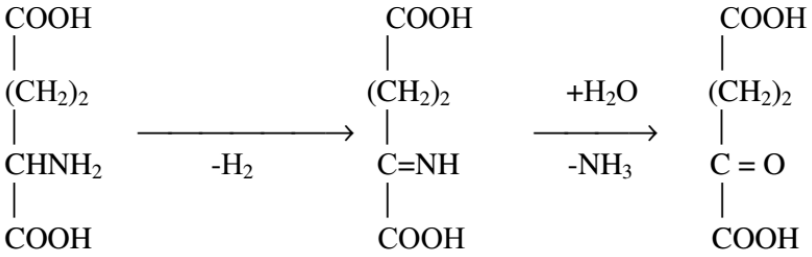


Elimination of the amino group

$$HOOC\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH_2\text{-}CH}}\text{-}COOH \xrightarrow{\text{aspartase}} \begin{matrix} H & COOH \\ | & | \\ C & = C \\ | & | \\ HOOC & H \end{matrix} + NH_3$$

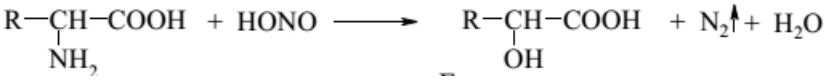
Aspartic acid fumaric acid (trans isomer)

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).

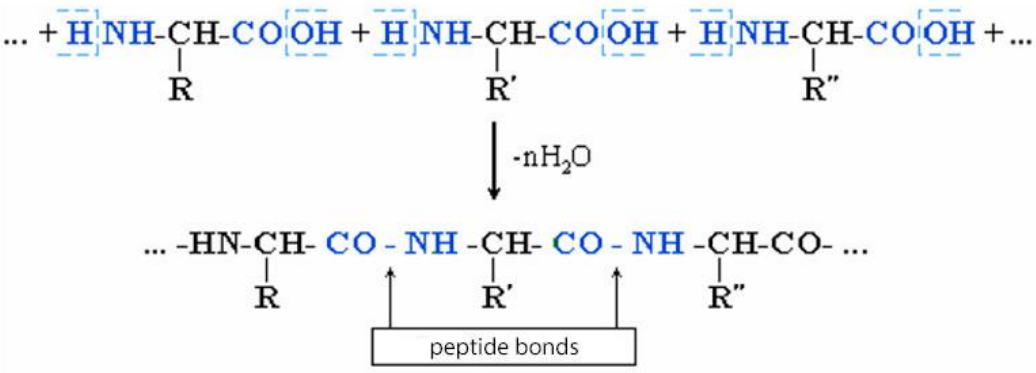
2. Additional activation of those groups that directly form a peptide bond.

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.

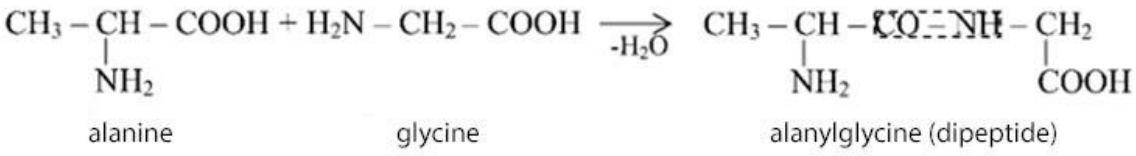
Protein renaturation is the reverse process with complete restoration of the structure and function of the protein molecule. It is possible with a short action of the denaturing agent. Denaturation is irreversible in disturbance of the quaternary, tertiary and secondary structures of the protein.

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.



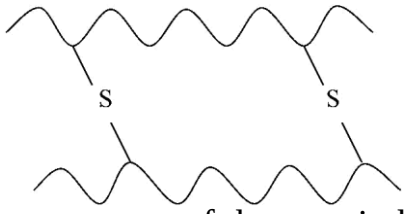
Other amino acids can attach to the dipeptide, forming a tri-, tetra-, pentapeptide, and so on up to the formation of a large polypeptide. The sequence of amino acids in the polypeptide is the primary structure of the protein.

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.



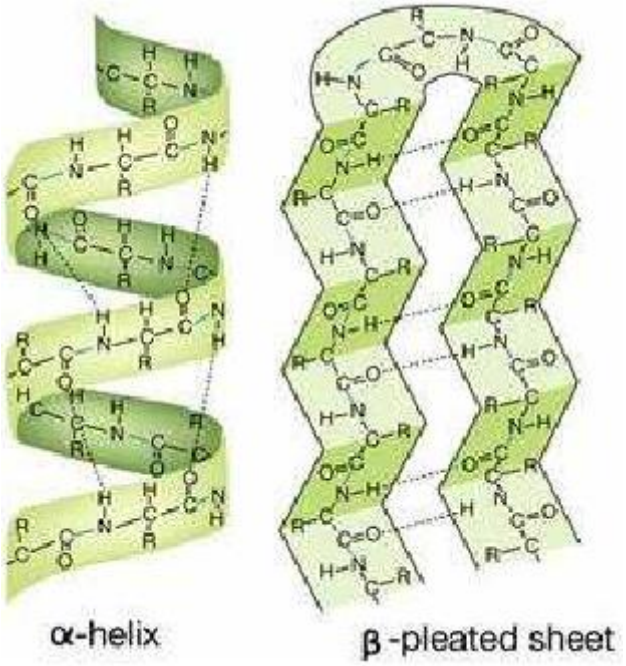
To determine the primary structure of the protein by hydrolysis, the quantitative ratio of amino acids in the protein molecule is determined. Then, the chemical nature of the terminal amino acids of the polypeptide chain, which contains one free NH_2 and COOH group, is determined. The Sanger method and 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);

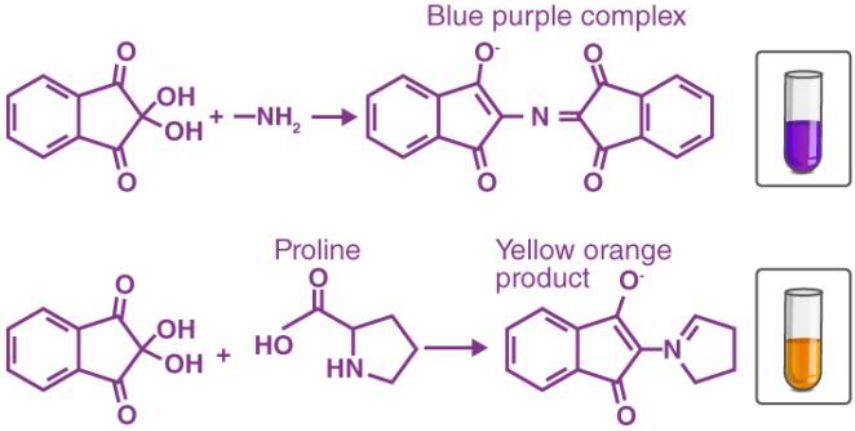
2) proteins - enzyme inhibitors;

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

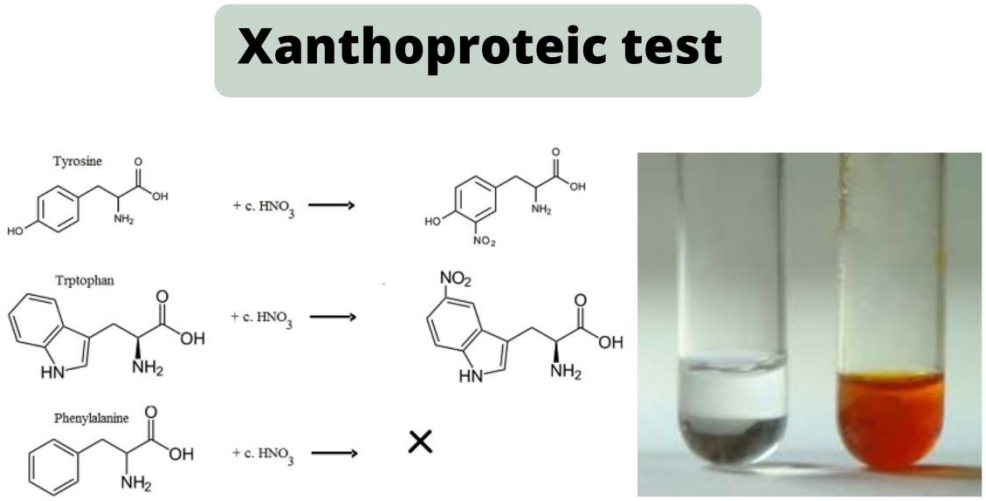
Amino acids can be detected using color reactions: ninhydrin, xanthoprotein, Fole, Milon, biuret test, etc. These reactions are nonspecific, because based on the detection of individual fragments in the structure of amino acids, which can occur in other compounds.

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.

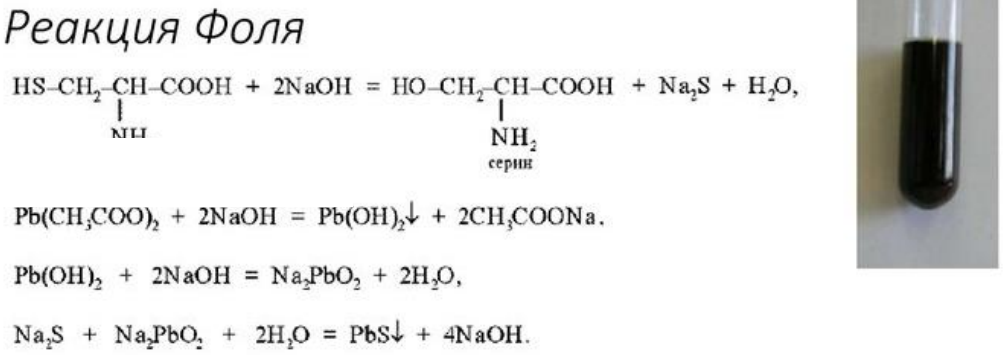
The xanthoprotein reaction allows you to detect aromatic amino acids (phenylalanine, tyrosine, histidine, tryptophan), based on the electrophilic substitution reaction in the aromatic nucleus (nitration).



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

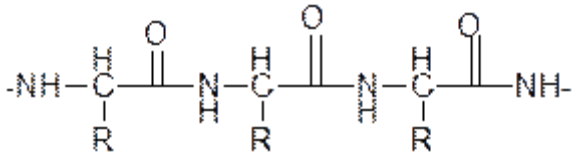
Biuret reaction may be used as a color reaction to proteins. In an alkaline medium in the presence of cuprum (II) salts, they give a violet color. The color is due to the formation of the complex compound cuprum (II), with peptide group —CO — NH—, which is characteristic of proteins. This reaction got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

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

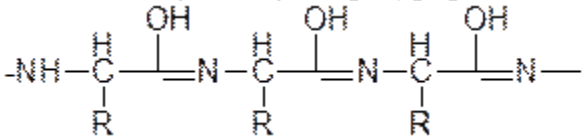
biuret

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.

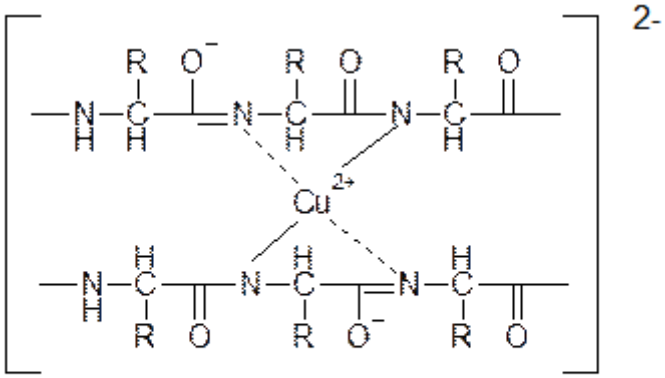


ketone form of the polypeptide



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:

1. Satyanarayana U. Biochemistry. 5th edition. India 2020. – 777 p.
3. Lehninger. Principles of Biochemistry. 7th edition. NY, United States. 2017.
4. Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto. Biochemistry. 8th Revised edition. 2015.
5. Lippincott Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer, 2017. 560 p.
6. 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.
7. 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

« 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. major function of nucleic acids involves the storage and expression of genomic information. Deoxyribonucleic acid, or DNA, encodes the information cells need to make proteins. A related type of nucleic acid, called ribonucleic acid (RNA), comes in different molecular forms that play multiple cellular roles, including protein synthesis.

Aims: To form knowledge about the structure and features of the chemical behavior of five- and six-membered heterocyclic compounds with biological activity, 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, barbituricacid, adenine, guanine, cytosine, uracil, thymine, nucleosides, nucleotides, ATP, DNA, structure of DNA, AMP, c-AMP, c-GMP, UMP

Plan and organizational structure of the lecture:

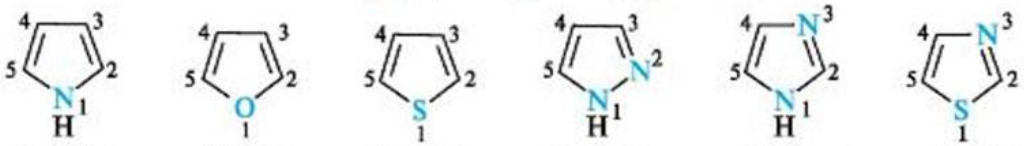
1. Classification of heterocycles by cycle size, number and type of heteroatoms.
2. Five-membered heterocycles with one and two heteroatoms.
3. six-membered heterocyclic compounds.
4. Pyrimidine and purine derivatives.
5. Imidazole and its derivatives
6. Pyrimidine and its derivatives
7. Purine and its derivatives
8. Tautomerism of barbituric acid
9. Structure of nucleotides - components of nucleic acids
10. Structure and significance of 3',5' - c-AMP, its role in the action of hormones on cells.
11. Phosphorylated nucleotide derivatives, biological significance of ADP and ATP.
12. Nucleic acids.
13. 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 or two heteroatoms

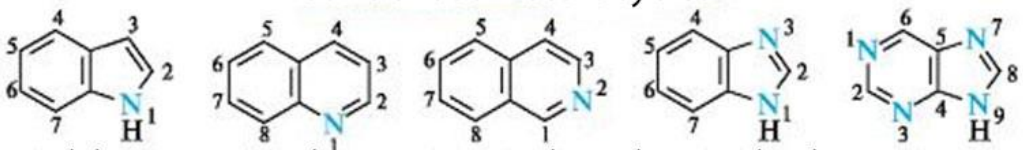


pyrrole furan thiophene pyrazol imidazole thiazole

Six membered heterocycles with nitrogen atoms

pyridine pyrimidine

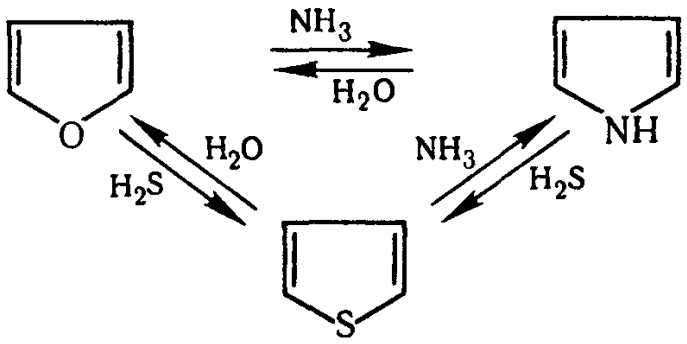
Condensed heterocycles



indole quinoline isoquinoline benzimidazol pyrine

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

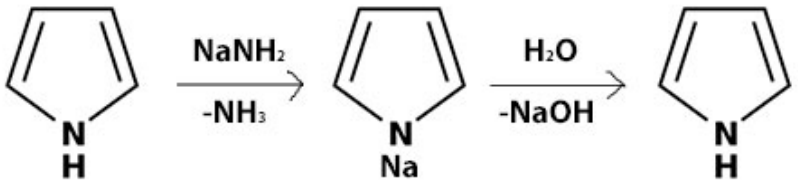
Pyrrole, furan and thiophene belong to the so-called **π-excess** heterocycles, i.e. to compounds with increased electron density inside the ring, since the six-electron π cloud is delocalized in them at 5 atoms of the cycle. These heterocycles have **aromaticity (Hückel rule):**

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

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

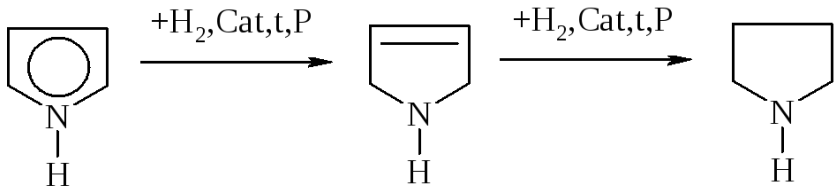
c) contains **(4n + 2)** π-generalized electrons, where n = 1, 2, 3, etc. which is determined by number of cycles.

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

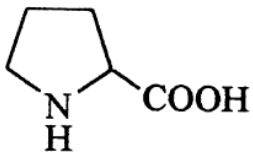
Pyrrole is able to **recover** in the presence of HI, the process is stepwise:



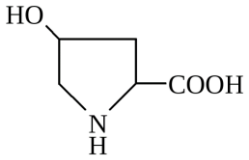
pyrroline pyrrolidine

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

pyrrolidine

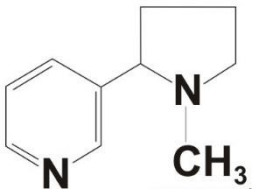


imino acid proline

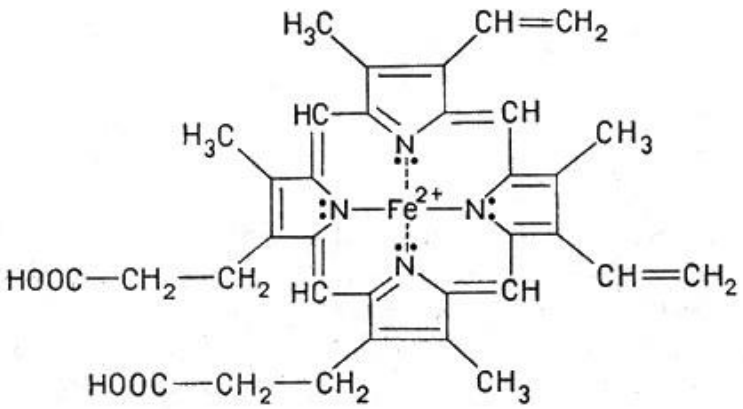


imino acid hydroxyproline

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



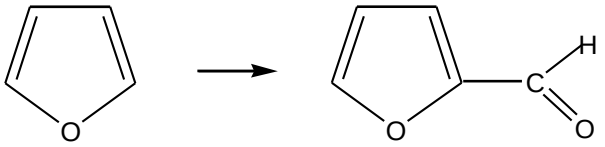
Ferroprotoporphyrin (heme)

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

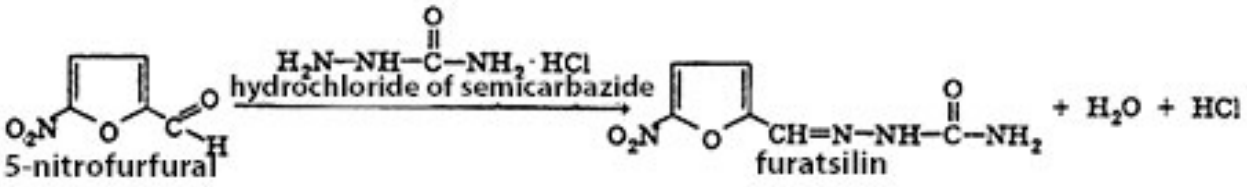
The complex of porphyrin with ferrum is part of a number of enzyme systems: cytochromes, catalase, peroxidase. The complex of porphyrin with magnesium is the basis of the chlorophyll molecule. Pyrrole nuclei connected with cobalt are part of vitamin B_{12} (cyancobalamine), which is necessary for normal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

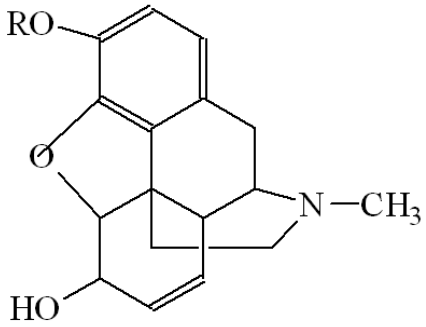
furan furfural



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

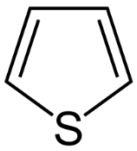


Furan is a part of narcotic substances - morphine, heroin, codeine, etc.



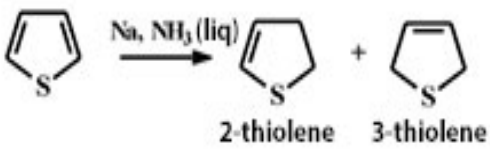
R=H – morphine
$R=CH_3$ - codeine

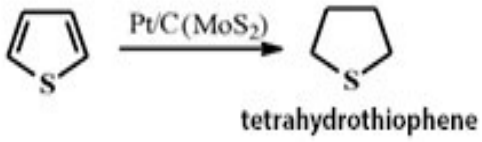
Morphine has a strong analgesic effect. It includes phenanthrene structure. The occurrence of addiction to morphine is known, which leads to the development of addiction.



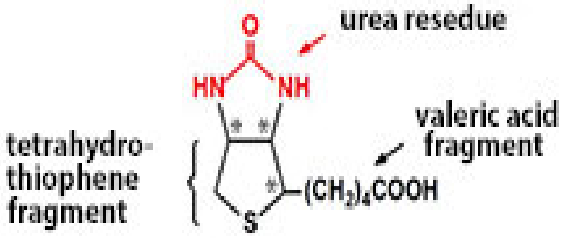
Thiophene – of the five-membered heterocyclic compounds under consideration with one heteroatom, it is closest to benzene in chemical and physical properties (boiling point of thiophene is 84 ° C, benzene 80 ° C). Thiophene is found in coal tar. Thiophene derivatives thereof are part of the ichthyol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

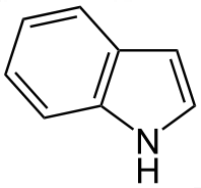




Biotin (vitamin H) is derivative of tetrahydrothiophene. Biotin consists of imidazoline ring that is cis-fused to a tetrahydrothiophene ring bearing a valerate side chain. The chirality at each of its three asimetric centers is indicated (*). Biotin is essential human nutrient. For lack of vitamin H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



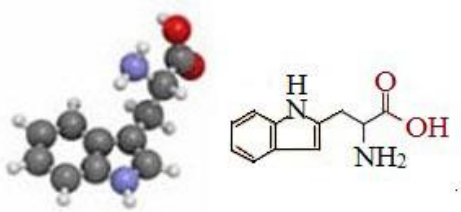
biotin (vitamin H)



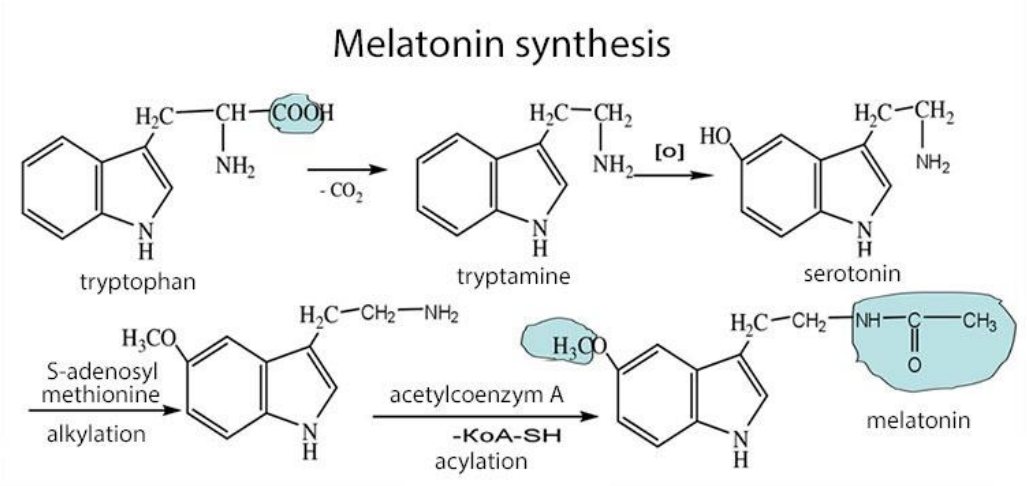
Indole (benzpyrrole)

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.

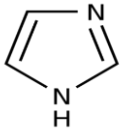


Serotonin is one of the mediators of the brain. Violation of its normal metabolism in the body leads to the development of schizophrenia. Serotonin is part of some biologically active substances that dramatically disrupt mental activity. So, psilocybin, lysergic acid diethylamide (LSD), which cause visual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

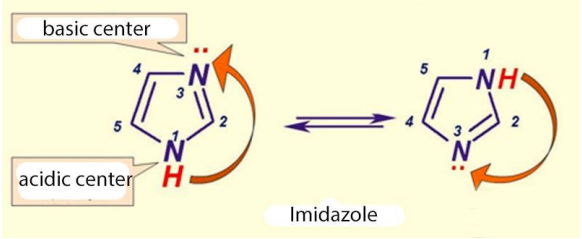


Imidazole is a five-membered heterocycle with two nitrogen atoms located in the first and third positions of the ring. One of these atoms is similar to the nitrogen atom in pyrrole and is responsible for the weakly acidic properties of imidazole, the other is similar to the "pyridine" nitrogen atom and 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.

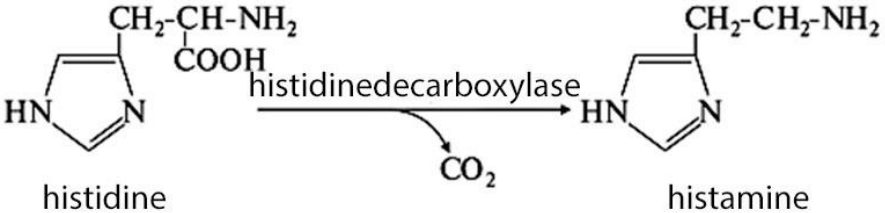
Prototropic (azole) tautomorism of heterocycles

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



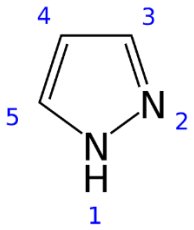
Hydrogen transfer is carried out so quickly (approximately 10 times per second) that it is not possible to isolate individual 4- or 5-monosubstituted imidazoles ("fast" tautomerism). Many imidazole derivatives are found in nature and have great biological significance. The most important are the amino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

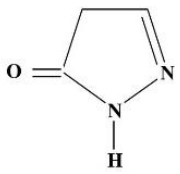


histidine histamine

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.



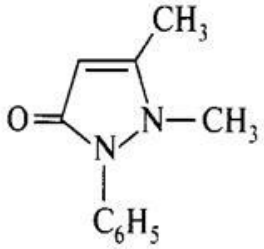
Pyrazole derivatives were not found in nature, but important drugs were created on its basis. Most of them are derivatives of pyrazolone-5



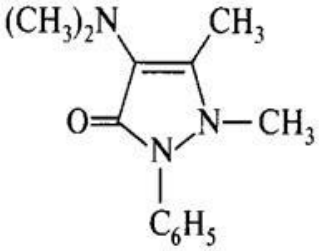
Pyrazolone-5

Pyrazolone-5 is the basic structure of analgetic drugs.

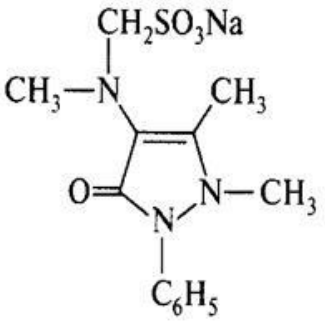
Pyrazolone medicines



Antipyrine
(1-phenylpyrazolone-5)



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



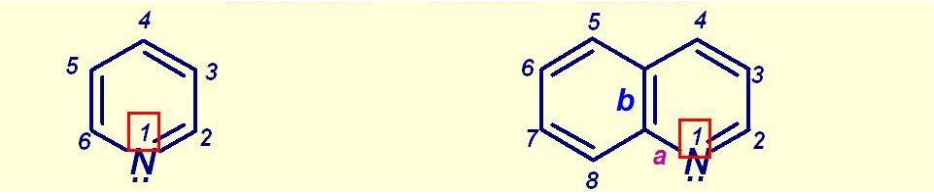
analgin

Antipyrine and amidopyrine are widely used in medicine as antipyretic, analgesics and sedatives. Analgin - is a sulfo derivative of amidopyrine. It may be used

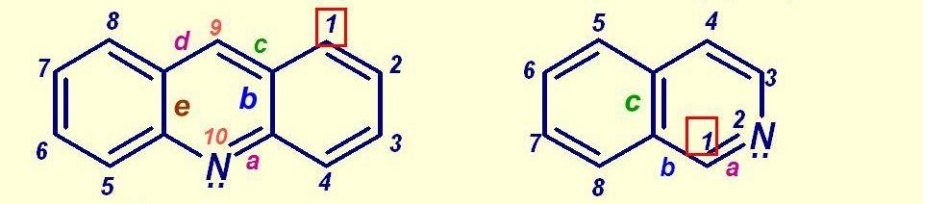
in liquid form. It is superior in activity and speed of reaction than amidopyrine and antipyrine.

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

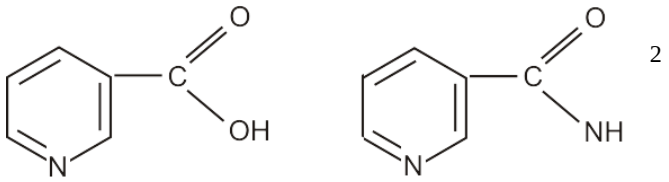


Acridine dibezo[b,e] pyridine Isoquinoline benzo[c]-pyridine

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.

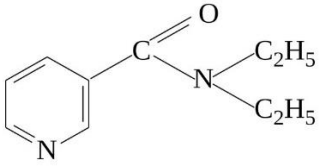
Nicotinic acid and nicotinamide

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



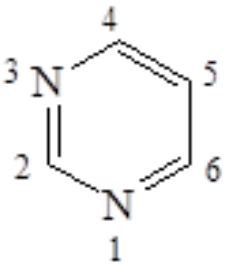
Nicotinic acid Nicotinamide

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:

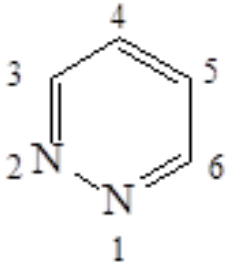


Six-membered heterocycles with two nitrogen atoms

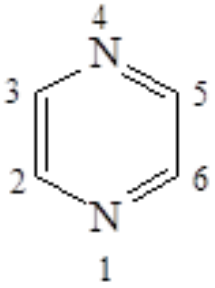
This group includes three isomeric six-membered heterocycles - pyridazine, pyrimidine, pyrazine.



Pyrimidine



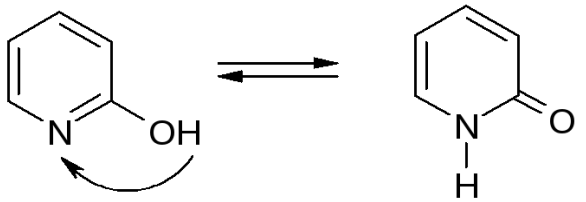
Pyridazine



Pyrazine

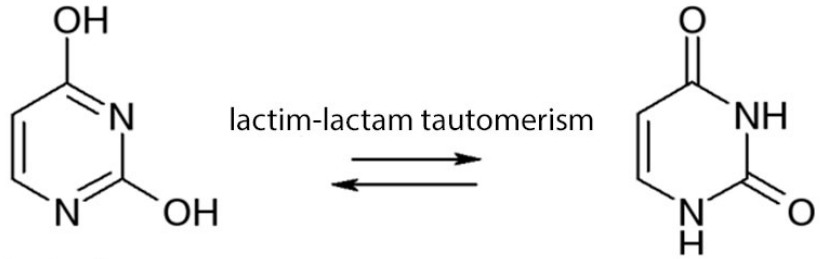
The introduction of the second nitrogen atom into the six-membered ring further reduces the activity of the heterocyclic nucleus (compared to pyridine) in electrophilic substitution reactions. The basicity of diazines also decreases. The introduction of electron-donating — OH and NH_2 groups into the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



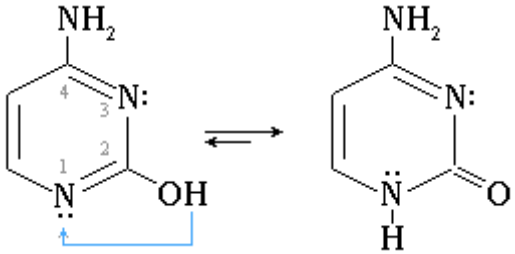
lactim form lactam form

2-hydroxypyridine



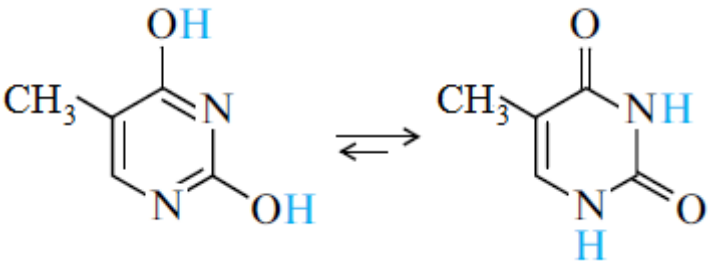
lactim form lactam form

uracil (2,4-dihydroxypyrimidine)



lactim form lactam form

cytosine (4-amino-2-hydroxypyrimidine)

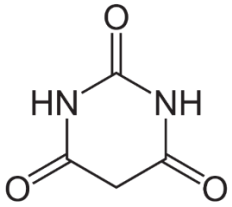


lactim form lactam form

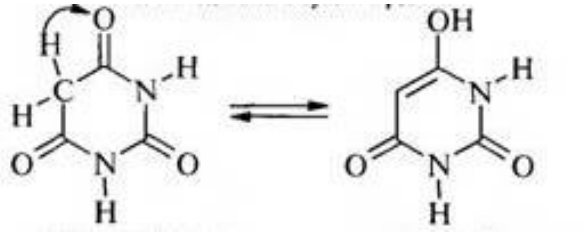
thymine (5-methyluracil)

Barbituric acid and its derivatives

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

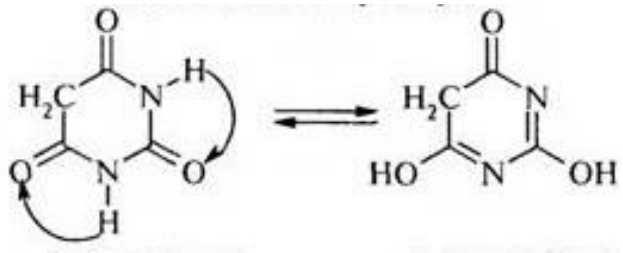


Keto-enol tautomerism



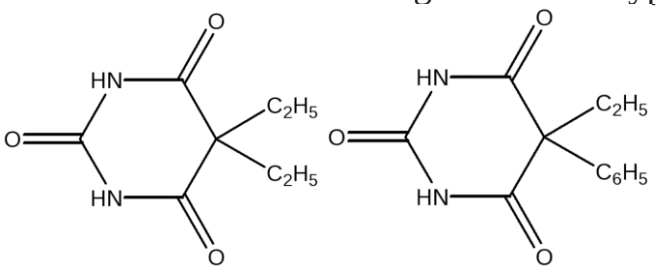
keto form enol form

lactim-lactam tautomerism



lactam form lactim form

Derivatives of barbituric acid belong to narcotic hypnotics.



Barbital (Veronal, 5.5-diethyl-barbituric acid)

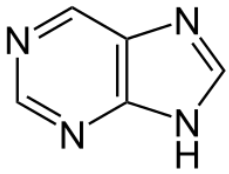
Phenobarbital (luminal, 5-ethyl-5-phenyl-barbituric acid)

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

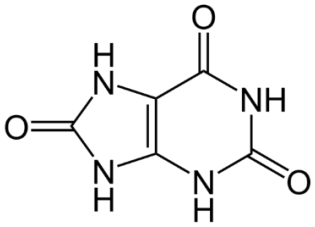
somatic disorders (withdrawal syndrome). There is anxiety, irritability, fear, vomiting, visual impairment, convulsions, etc. In severe cases, death may occur.

Purin and its derivatives

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



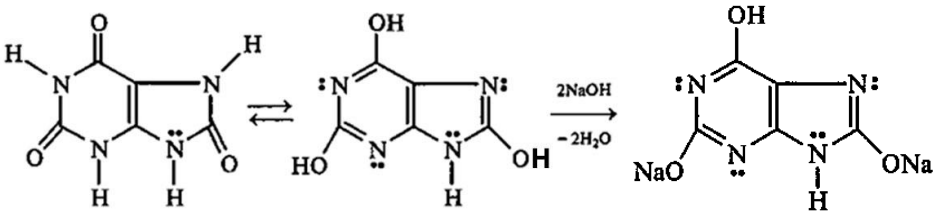
Purine



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.

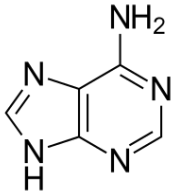
Uric acid is the end product of the metabolism of purine compounds in the body. Uric acid is dibasic, poorly soluble in water, but easily soluble in alkalis. Uric acid salts are called urates. With some disorders in the body, they are deposited in the joints, for example, with gout, as well as in the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



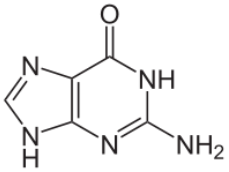
Uric acid Sodium urate

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.



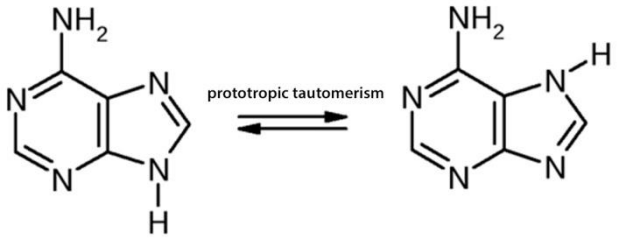
Adenine



Guanine

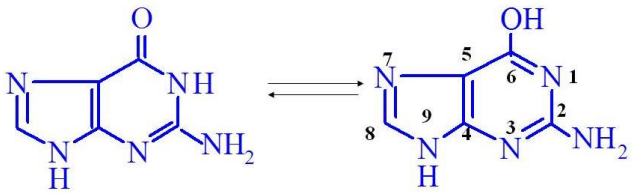
For adenine, prototropic tautomerism is possible due to the migration of hydrogen between N_7 and N_9 in the imidazole ring:

tautomeric forms of 6-aminopurine (adenine)



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

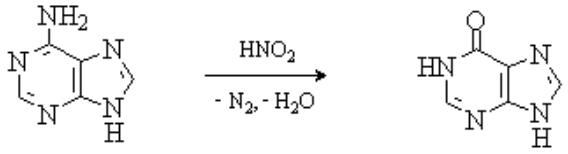
Lactim-lactam tautomerism



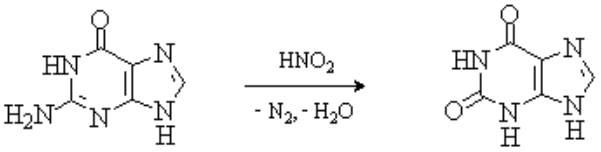
lactam form lactim form

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.

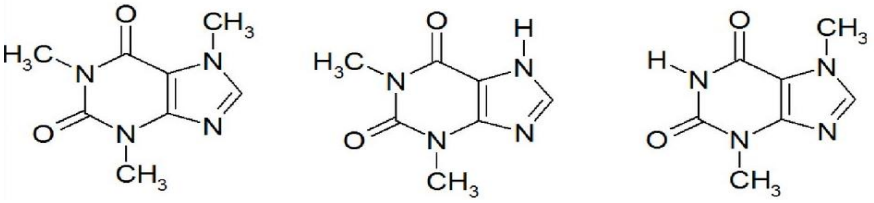


Adenine Hypoxanthine



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

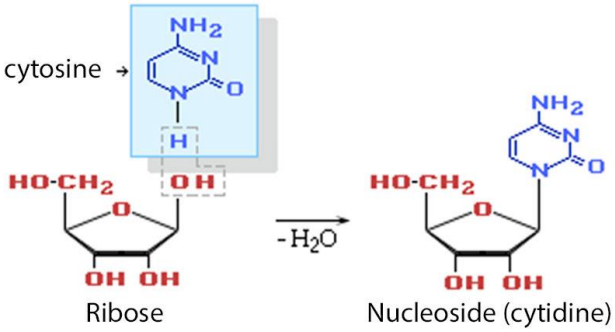
principal alkaloid of cacao bean, also in cola nuts and tea

small amounts in tea

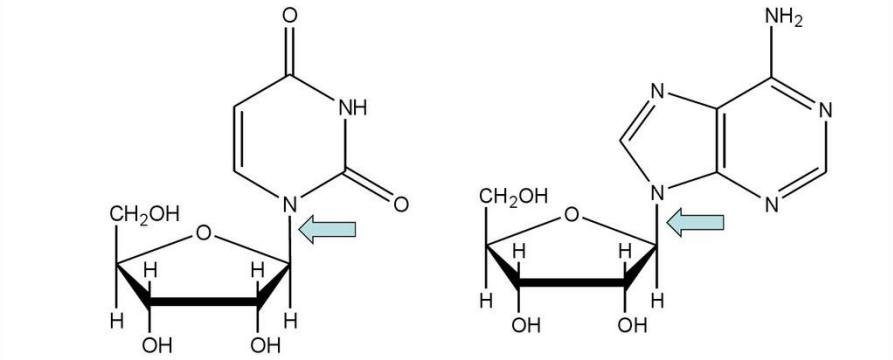
Nucleosides

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.

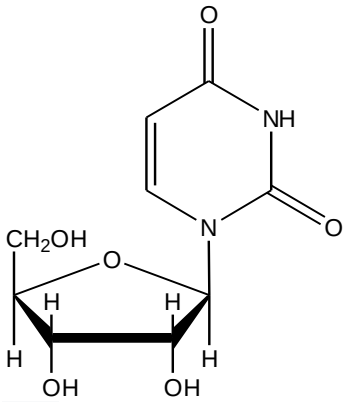
Thymine is the base of DNA, and if the nucleoside contains deoxyribose, then the name of the nucleoside (**thymidine**) does not need to emphasize the chemical nature of the carbohydrate. If thymine is associated with ribose, which is an atypical biological situation, then the name indicates the name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

The most common nucleosides

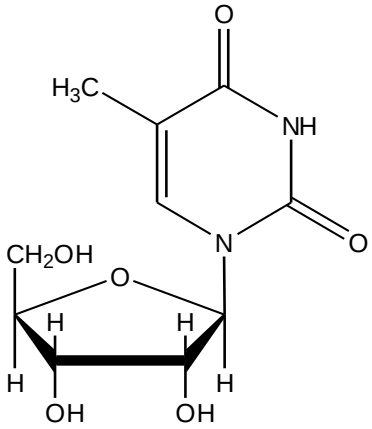
Type of bond - N-β-glycosidic linkage

Nitrogen base	Nucleoside carbohydrate ribose	Nucleoside carbohydrate deoxyribose

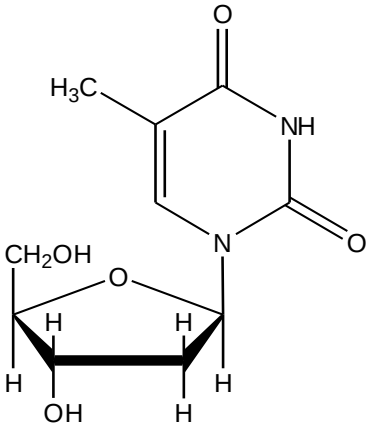
uracil	uridine	deoxyuridine
cytosine	cytidine	deoxycytodine
thymine	ribothymidine	thymidine
adenine	adenosine	deoxyadenosine
guanine	guanosine	deoxyguanosine
hypoxanthine	inosine	deoxyinosine



Uridine

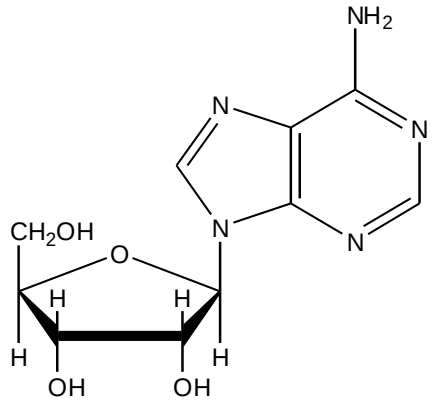


thymidine riboside

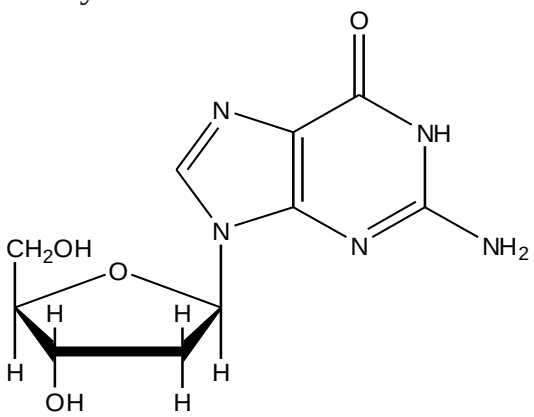


thymidine

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



Adenosine



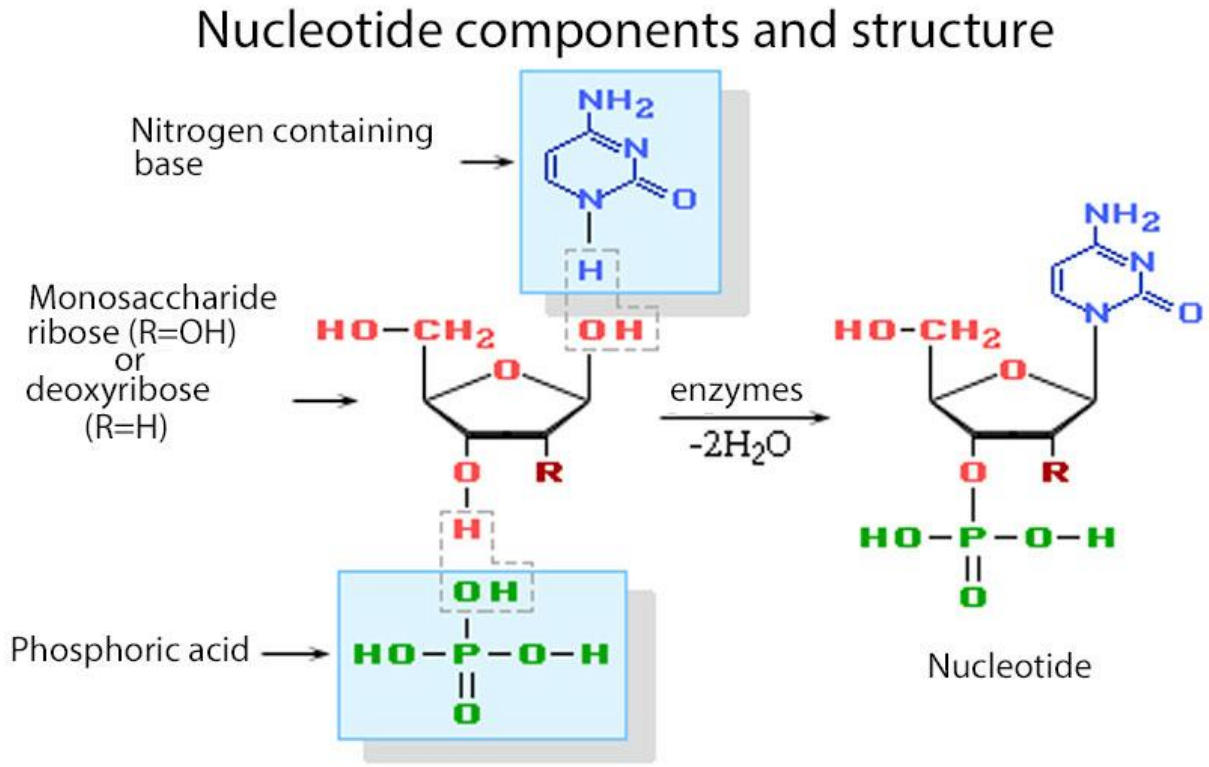
Deoxyguanosine

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

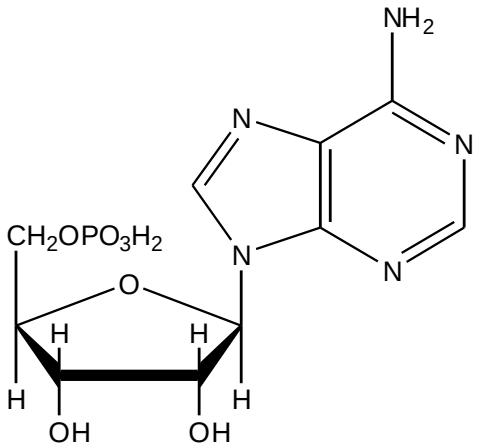
Nucleotides consisting of one nitrogen base molecule, pentose, phosphoric acid are called mononucleotides. Mononucleotides may contain one molecule of phosphoric acid, two or three molecules of phosphoric acid, connected to each other.

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.

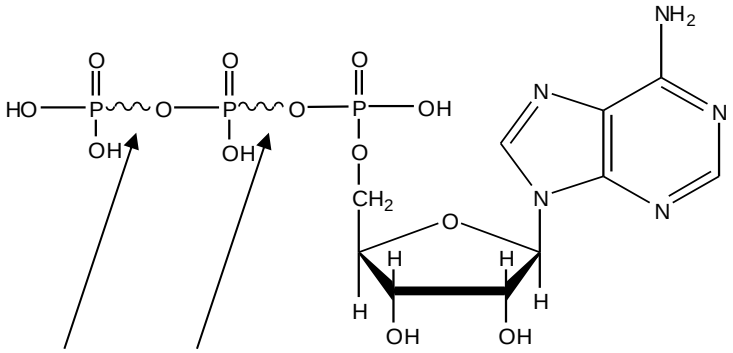
The absence of a hydroxy group in the second position has two important consequences: - the polarization of the glycosidic bond in DNA decreases and it becomes more resistant to hydrolysis. - 2-O-deoxyribose cannot undergo either epimerization or conversion to ketosis.

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)



ATP contains various types of chemical bonds:

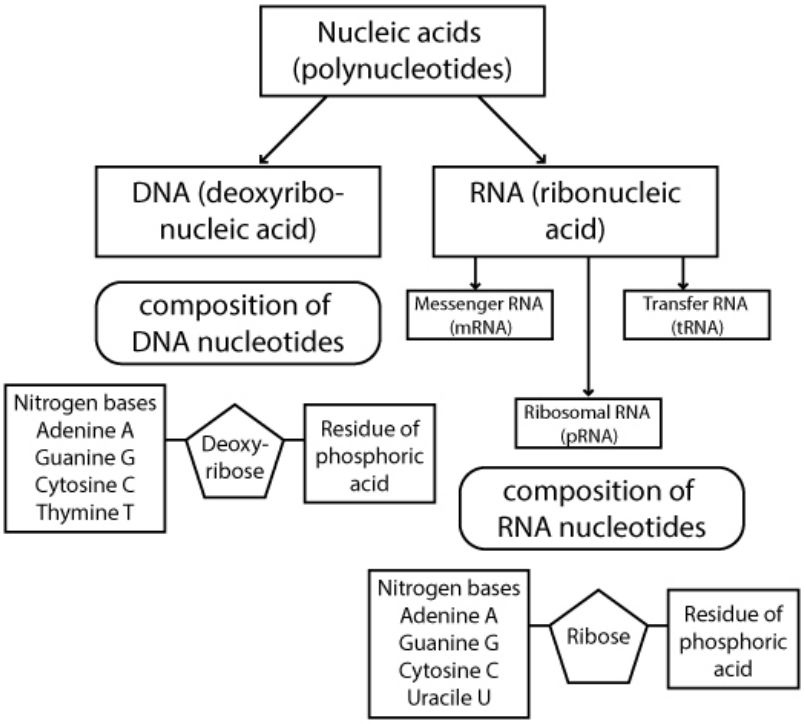
1. N-β-glycosidic
2. Ester
3. Two anhydride (biologically macroergic)

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°C 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:

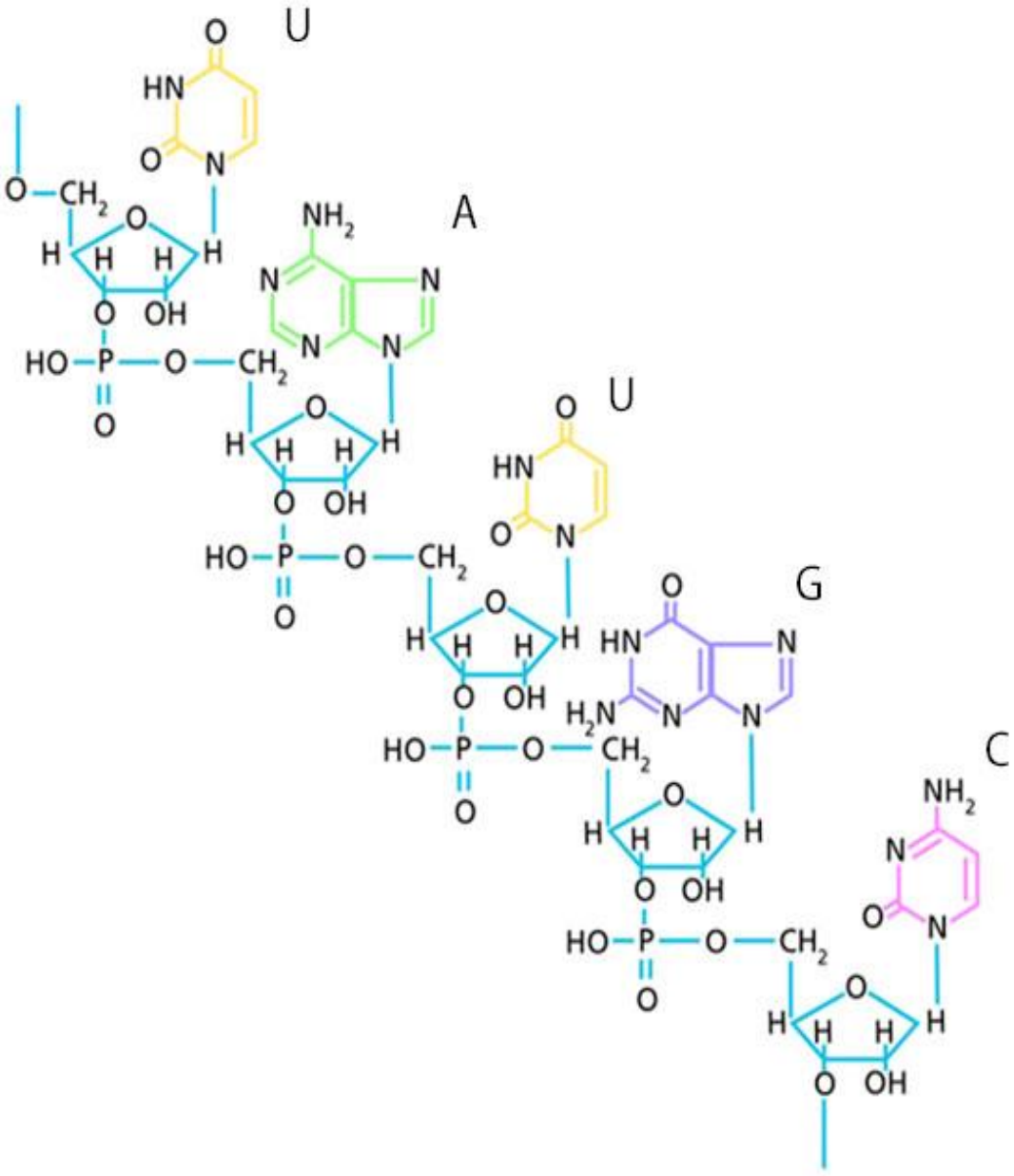
5', 3' 5', 3' 5', 3'

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.

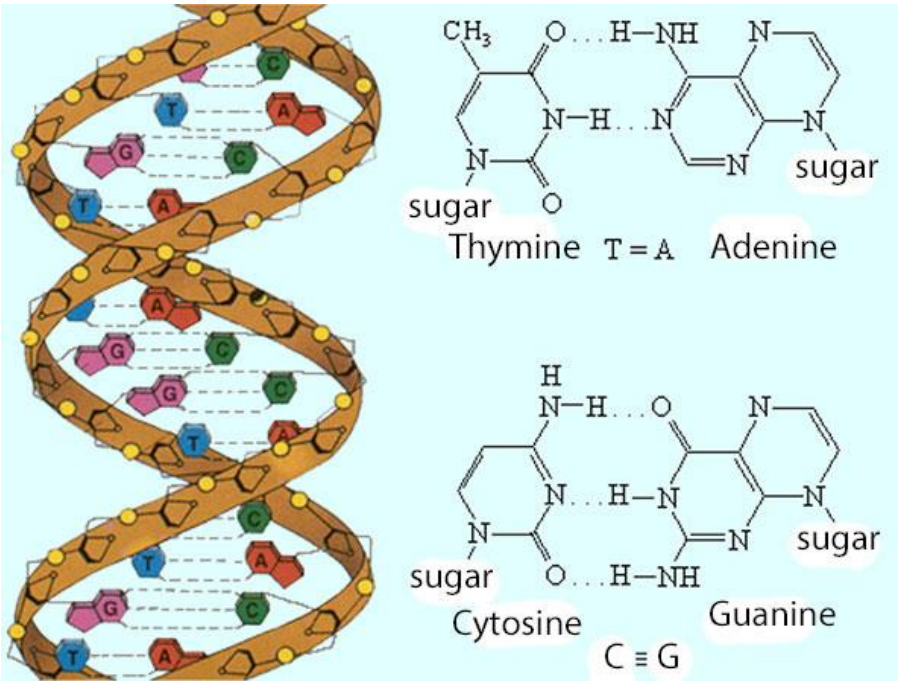
The discovery of the structure of DNA was facilitated by the research of E. Chargaff regarding the chemical composition of DNA. He found out:

- 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:

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: 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.

Topic relevance: An enzyme is a biocatalyst, which enhances the rate of thermodynamically favorable biological reactions to several thousand to million folds. Enzymes are highly specialized catalysts with extraordinary catalytic power and also with remarkable specificity, catalysing almost all cellular reactions. Therefore, they are known as the basis of life. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life.

Objective: knowledge about general patterns of enzymatic catalysis and using of enzyme activity to research in diagnosis of various pathological conditions.

Basic concepts:

1. Enzymes general concept
2. Active and allosteric cetes of enzymes

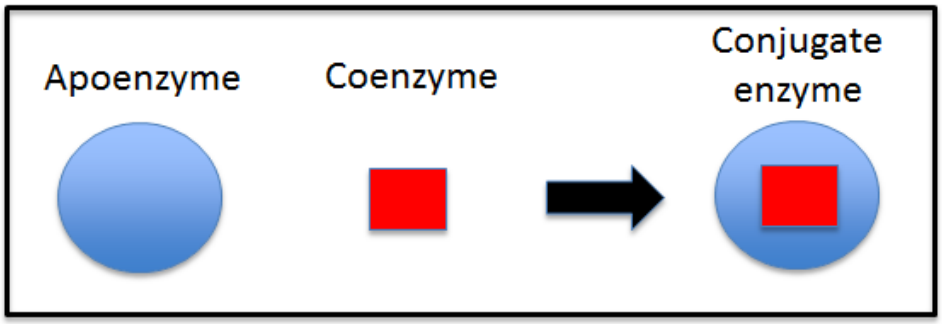
3. Nomenclature, classification of enzymes
4. Regulation of enzymes activity
5. Coenzymes, cofactors general meaning

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

General Characteristics of Enzymes
• ENZYME– Usually a protein, acting as catalyst in specific biochemical reaction
• Each cell in the human body contains 1,000s of different enzymes
– Every reaction in the cell requires its own specific enzyme
• Most enzymes are globular proteins – A few enzymes are made of RNA
• Catalyze biochemical reactions involving nucleic acids
• Enzymes undergo all the reactions of proteins
– Enzymes denaturation due to pH or temperature change
• A person suffering high fever runs the risk of denaturing certain enzymes
Enzyme Structure
SIMPLE ENZYMES
Composed only of protein
CONJUGATED ENZYMES
Composed of:
– Apoenzyme
- Conjugate enzyme without its cofactor
- Protein part of a conjugated enzyme

– Coenzyme (Cofactor)
• Non-protein part of a conjugated enzyme



• The apoenzyme can't catalyze its reaction without its cofactor.
– The combination of the apoenzyme with the cofactor makes the conjugated enzyme functional.
• Holoenzyme = apoenzyme + cofactor
– The biochemically active conjugated enzyme.

Coenzymes and cofactors

• Coenzymes provide additional chemically reactive functional groups besides those present in the amino acids of the apoenzymes
– Are either small organic molecules or inorganic ions

• Metal ions often act as additional cofactors (Zn^{2+}, Mg^{2+}, Mn^{2+} & Fe^{2+}) – A metal ion cofactor can be bound directly to the enzyme or to a coenzyme
• COENZYME
– A small organic molecule, acting as a cofactor in a conjugated enzyme •
Coenzymes are derived from vitamins or vitamin derivatives
– Many vitamins act as coenzymes, esp. B-vitamins

Enzyme definitions

Enzyme (simple)	Protein only enzyme that facilitates a chemical reaction
Coenzyme	Compound derived from a vitamin (e.g. NAD^{+}) that assists an enzyme in facilitating a chemical reaction
Cofactor	Metal ion (e.g. Mg^{2+}) that that assists an enzyme in facilitating a chemical reaction
Apoenzyme	Protein only part of an enzyme (e.g. isocitrate dehydrogenase) that requires an additional coenzyme to facilitate a chemical reaction (not functional alone)
Holoenzyme	Combination of the apoenzyme and coenzyme which together facilitating a chemical reaction (functional)

Enzyme Nomenclature

- Enzymes are named according to the type of reaction they catalyze and/or their substrate
- Substrate = the reactant upon which the specific enzyme acts

– Enzyme physically binds to the substrate

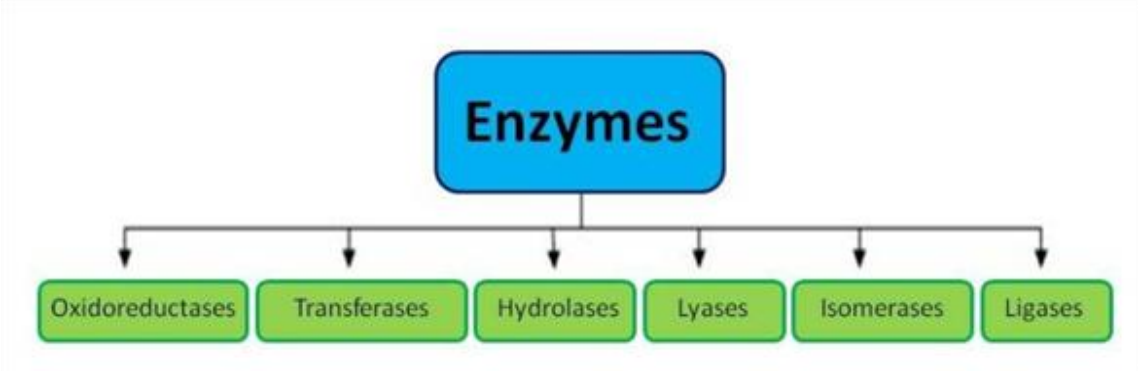
Enzyme Substrate Enzyme/substrate complex

• Suffix of an enzyme –ase
– Lactase, amylase, lipase or protease

• Denotes an enzyme
• Some digestive enzymes have the suffix –in
– Pepsin, trypsin & chymotrypsin
• These enzymes were the first ones to be studied
- Prefix denotes the type of reaction the enzyme catalyzes
  - – Oxidase: redox reaction
  - – Hydrolase: Addition of water to break one component into two parts
- Substrate identity is often used together with the reaction type

– Pyruvate carboxylase, lactate dehydrogenase

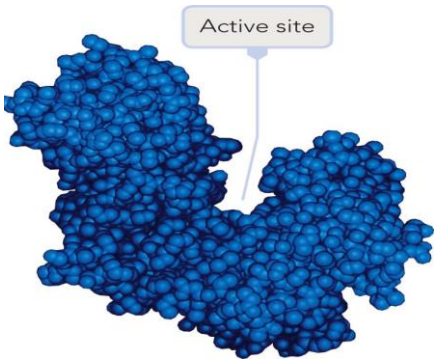
Major Classes of Enzymes



Types	Biochemical Property
Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other. These catalyze oxidation and reduction reactions, e.g. pyruvate dehydrogenase, catalysing the oxidation of pyruvate to acetyl coenzyme A.
Transferases	The Transferases enzymes help in the transportation of the functional group among acceptors and donor molecules. These catalyze transferring of the chemical group from one to another compound. An example is a transaminase, which transfers an amino group from one molecule to another.
Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it. They catalyze the hydrolysis of a bond. For example, the enzyme pepsin hydrolyzes peptide bonds in proteins

Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds. These catalyze the breakage of bonds without catalysis, e.g. aldolase (an enzyme in glycolysis) catalyzes the splitting of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.
Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule. They catalyze the formation of an isomer of a compound. Example: phosphoglucomutase catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate (phosphate group is transferred from one to another position in the same compound) in glycogenolysis (glycogen is converted to glucose for energy to be released quickly).
Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process. They catalyze the association of two molecules. For example, DNA ligase catalyzes the joining of two fragments of DNA by forming a phosphodiester bond.

Enzyme Active Site



• Active site

– The specific portion of an enzyme (location) where the substrate binds while it undergoes a chemical reaction

• The active site is a 3-D 'crevice-like' cavity formed by secondary & tertiary structures of the protein part of the enzyme

– Crevice formed from the folding of the protein

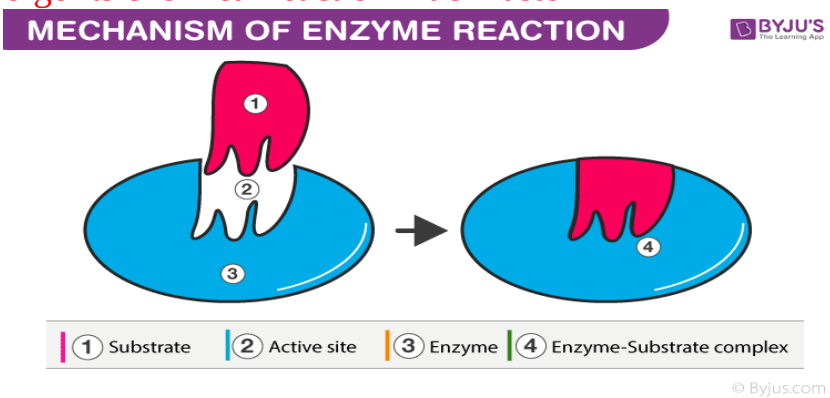
• Aka binding cleft

- – An enzyme can have more than only one active site
- – The amino acids R-groups (side chain) in the active site are important for determining the specificity of the substrate

Enzyme – Substrate Complex

• When the substrate binds to the enzyme active site an Enzyme-Substrate Complex is formed temporarily

– Allows the substrate to undergo its chemical reaction much faster



The enzyme action basically happens in two steps:

Step1: Combining of enzyme and the reactant/substrate.

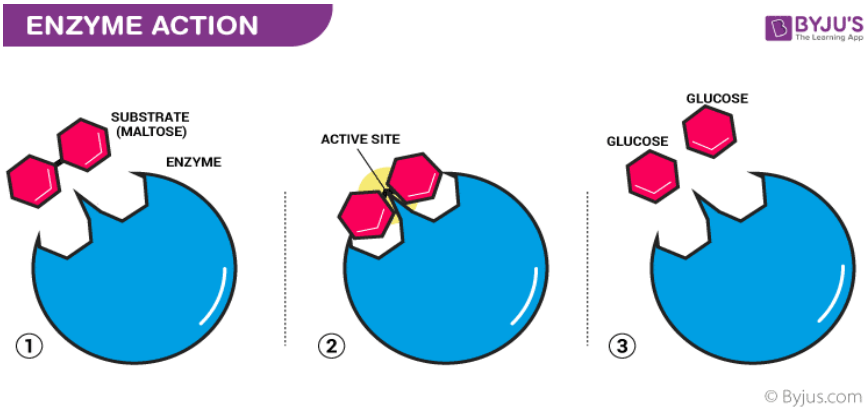
E+S → [ES]

Step 2: Disintegration of the complex molecule to give the product.

[ES]→E+P

Thus, the whole catalyst action of enzymes is summarized as:

E + S → [ES] → [EP] → E + P

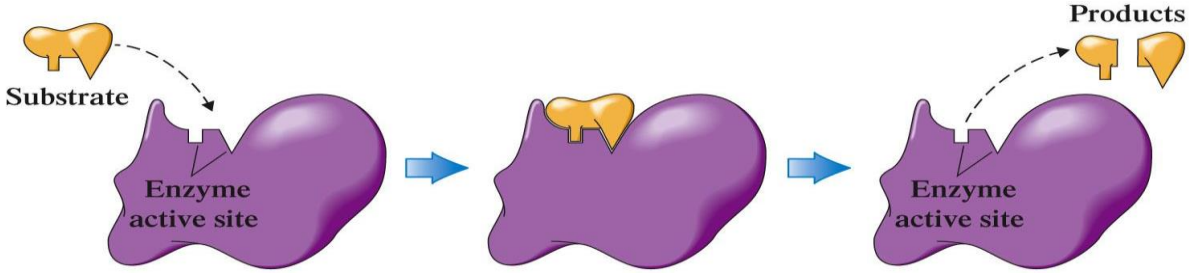


Lock & Key Model of Enzyme Action

- The active site is fixed, with a rigid shape (LOCK)
- The substrate (KEY) must fit exactly into the rigid enzyme (LOCK)
- Complementary shape & geometry between enzyme and substrate

– Key (substrate) fits into the lock (enzyme)

• Upon completion of the chemical reaction, the products are released from the active site, so the next substrate molecule can bind



Enzyme Specificity

• Absolute Specificity
- – An enzyme will catalyze a particular reaction for only one substrate
- – Most restrictive of all specificities

• Not common
- – Catalase has absolute specificity for hydrogen peroxide (H_2O_2)
- – Urease catalyzes only the hydrolysis of urea

• Group Specificity

– The enzyme will act only on similar substrates that have a specific functional group

• Carboxypeptidase cleaves amino acids one at a time from the carboxyl end of the peptide chain

• Hexokinase adds a phosphate group to hexoses

Enzyme Specificity

- Linkage Specificity
  - – The enzyme will act on a particular type of chemical bond, irrespective of the rest of the molecular structure
  - – The most general of the enzyme specificities
    - Phosphatases hydrolyze phosphate–ester bonds in all types of phosphate esters
    - Chymotrypsin catalyzes the hydrolysis of peptide bonds
- Stereochemical Specificity
  - – The enzyme can distinguish between stereoisomers
  - – Chirality is inherent in an active site (as amino acids are chiral compounds)

• L-Amino-acid oxidase catalyzes reactions of L-amino acids but not of D-amino acids

Factors Affecting Enzyme Activity

Enzyme activity

• Measure of the rate at which an enzyme converts substrate to products in a biochemical reaction

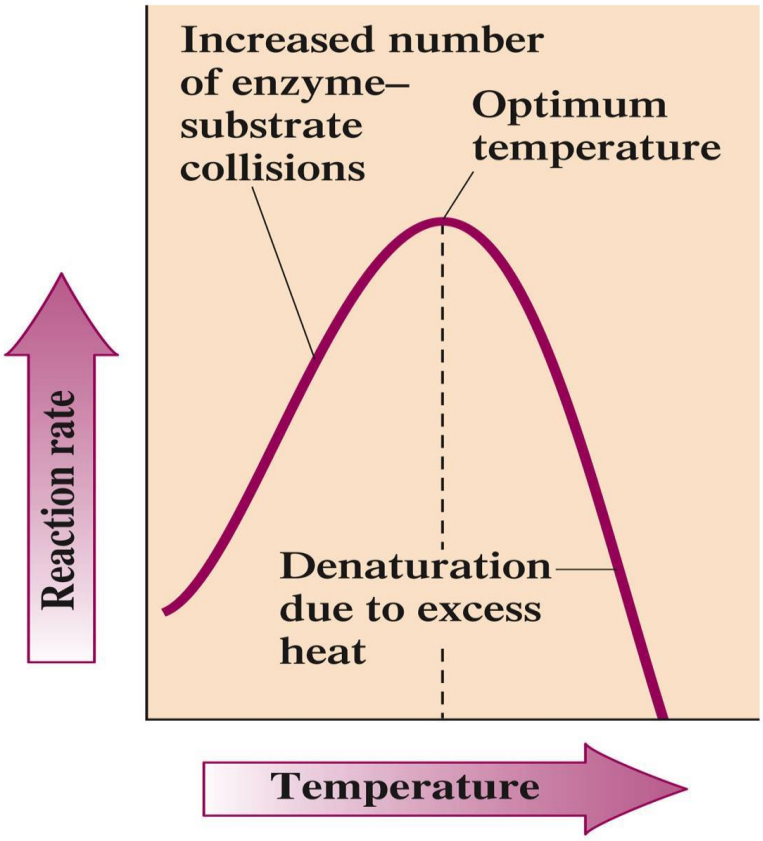
4 factors are affect on enzyme activity:
- Temperature
- pH
- Substrate concentration: [substrate]
- Enzyme concentration: [enzyme]

Temperature (t)
- With increased t the E_{KIN} increases
  - – More collisions
  - – Increased reaction rate
- Optimum temperature (t_{OPT}) is the t, at which the enzyme exhibits maximum activity

– The t_{OPT} for human enzymes = 37^0C • When the t increases beyond t_{OPT}

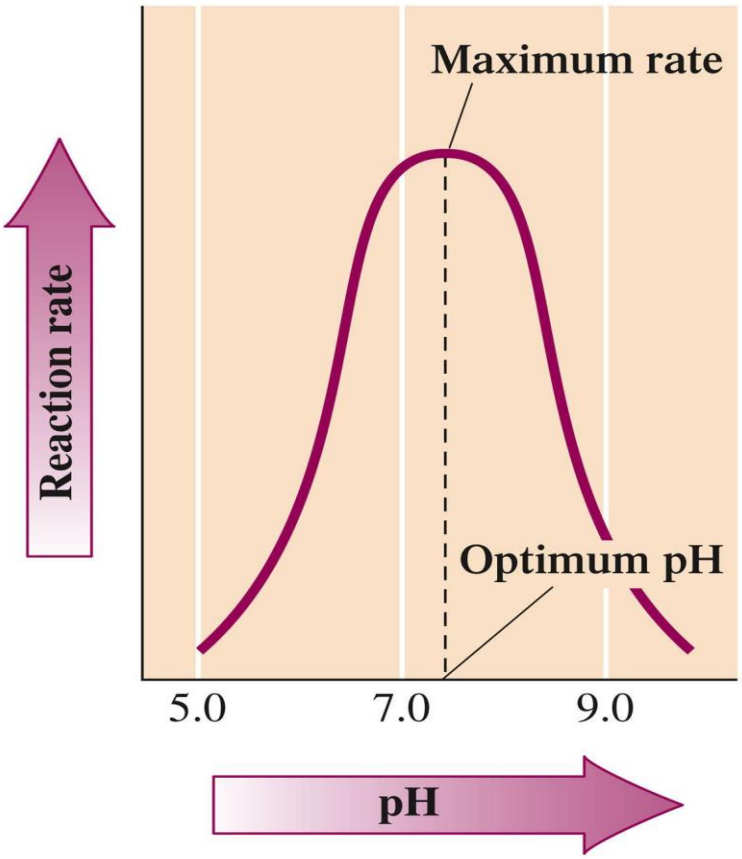
– Changes in the enzyme's tertiary structure occur, inactivating & denaturing it (e.g. fever)

• Little activity is observed at low t



pH

- Optimum pH (pH_{OPT}) is the pH, at which the enzyme exhibits maximum activity
- Most enzymes are active over a very narrow pH range
  - – Protein & amino acids are properly maintained
  - – Small changes in pH (low or high) can result in enzyme denaturation & loss of function
- Each enzyme has its characteristic pH_{OPT}, which usually falls within physiological pH range 7.0 - 7.5
- Digestive enzymes are exceptions:
  - – Pepsin (in stomach) – pH_{OPT} = 2.0
  - – Trypsin (in SI) – pH_{OPT} = 8.0



Substrate Concentration

If [enzyme] is kept constant & the [substrate] is increased

– The reaction rate increases until

a saturation point is met

• At saturation the reaction rate stays the same even if the [substrate] is increased

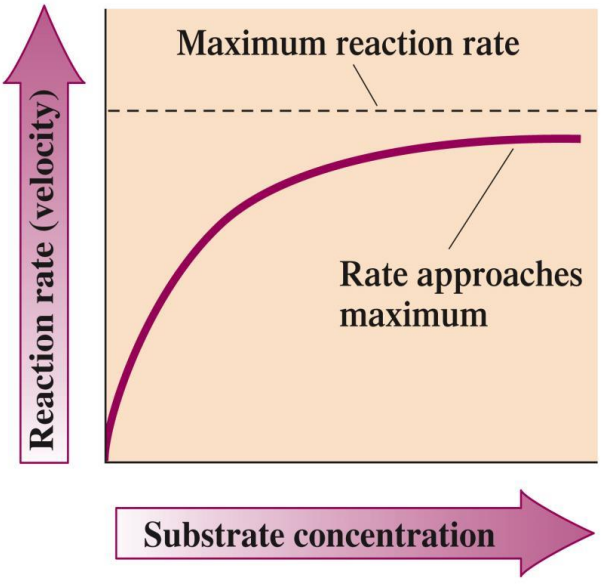
– At saturation point substrate molecules are bound to all available active sites of the enzyme molecules

Reaction takes place at the active site

– If they are all active sites are occupied the reaction is going at its maximum rate

• Each enzyme molecule is working at its maximum capacity

– The incoming substrate molecules must "wait their turn"



Enzyme Concentration

• If the [substrate] is kept constant & the [enzyme] is increased

- – The reaction rate increases
- –The greater the [enzyme], the greater the reaction rate

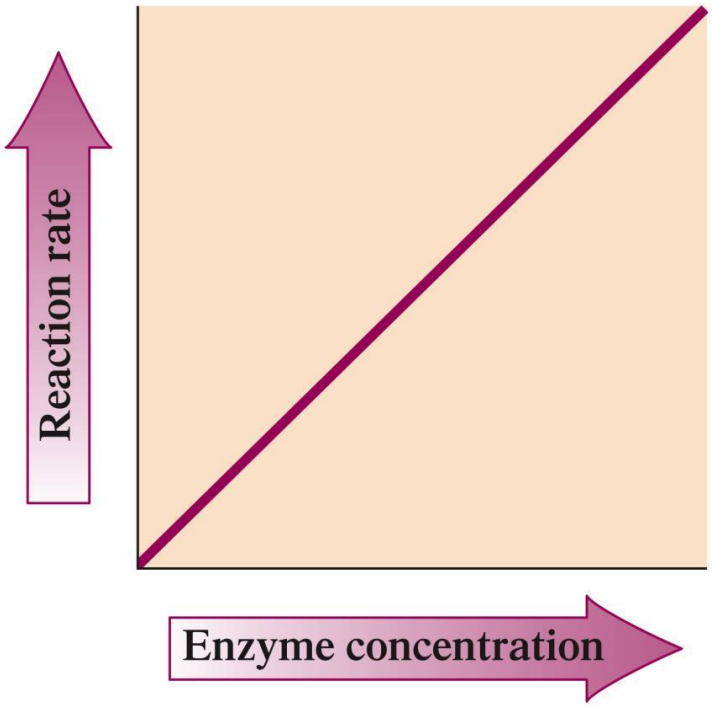
• RULE:

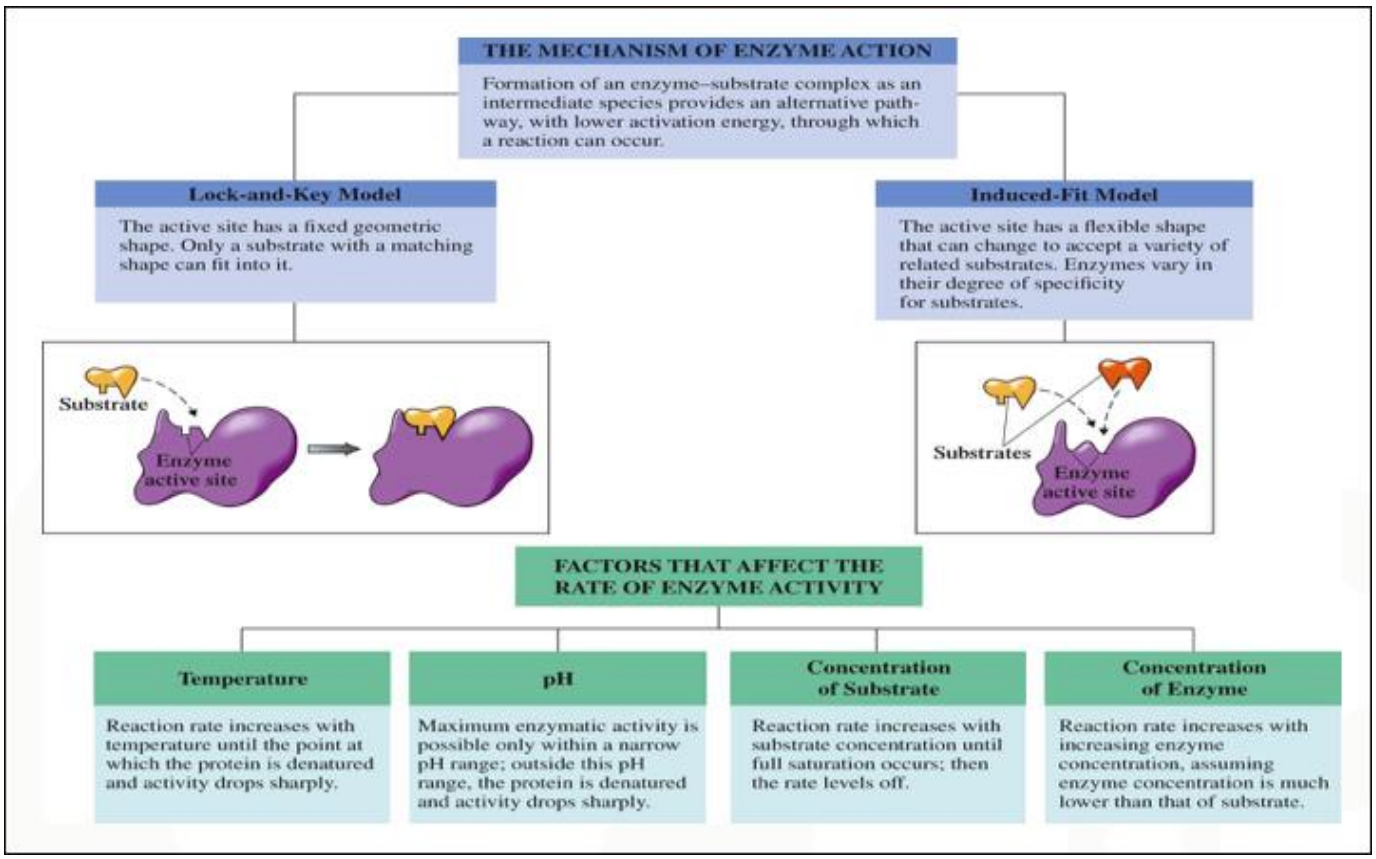
– The rate of an enzyme-catalyzed reaction is always directly proportional to the amount of the enzyme present

• In a living cell:

– The [substrate] is much higher than the [enzyme]

- Enzymes are not consumed in the reaction
- Enzymes can be reused many times

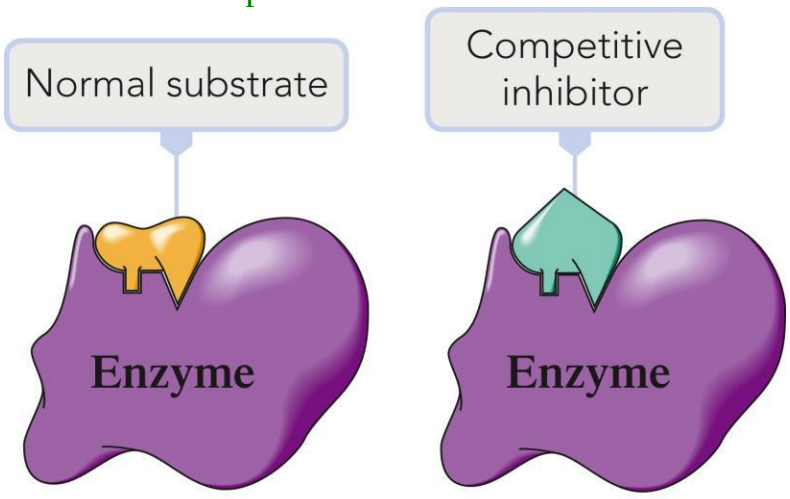




Enzyme Inhibition

- ENZYME INHIBITOR
  – Asubstancethatslowsdownorstopsthenormalcatalyticfunctionof an enzyme by binding to the enzyme
  Three types of inhibition:
- – Reversible competitive inhibition
  – Reversible non-competitive inhibition – Irreversible inhibition

Reversible Competitive Inhibition



• Competitive inhibitor resembles the substrate

- – Inhibitor competes with the substrate for binding to the active site of the enzyme
- – If an inhibitor is bound to the active site:

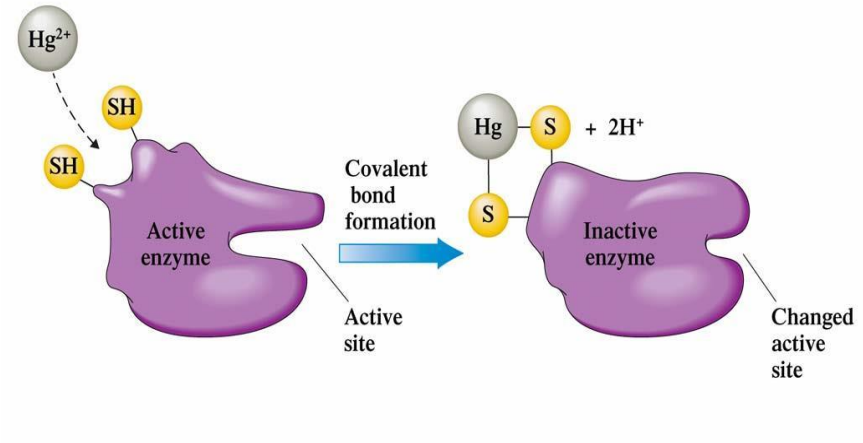
• Prevents the substrate molecules to access the active site

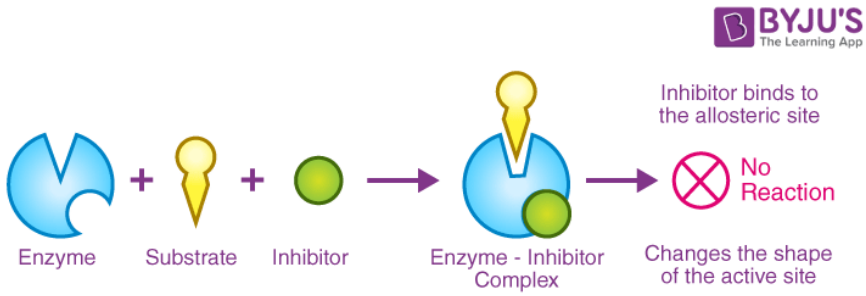
– Decreasing / stopping enzyme activity
• The binding of the competitive inhibitor to the active site is a reversible process
– Add much more substrate to outcompete the competitive inhibitor
• Many drugs are competitive inhibitors:
– Anti-histamines inhibit histidine decarboxylase, which converts histidine to histamine

Reversible Noncompetitive Inhibition

A non-competitive inhibitor decreases enzyme activity by binding to a site on the enzyme other than the active site

- – The non-competitive inhibitor alters the tertiary structure of the enzyme & the active site
  - Decreasing enzyme activity
  - Substrate cannot fit into active site
- – Process can be reversed only by lowering the [non-competitive inhibitor]





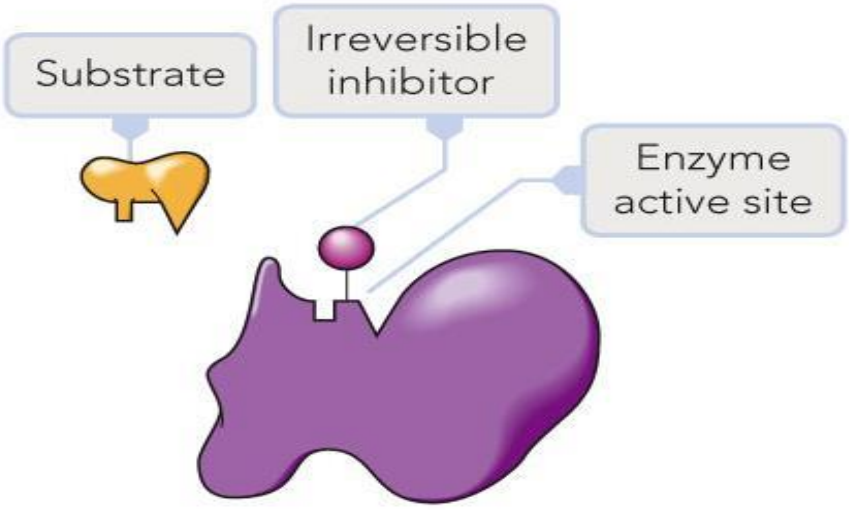
• Example:
– Heavy metals Pb^{2+} & Hg^{2+} bind to –SH of Cysteine, away from active site
• Disrupt the secondary & tertiary structure

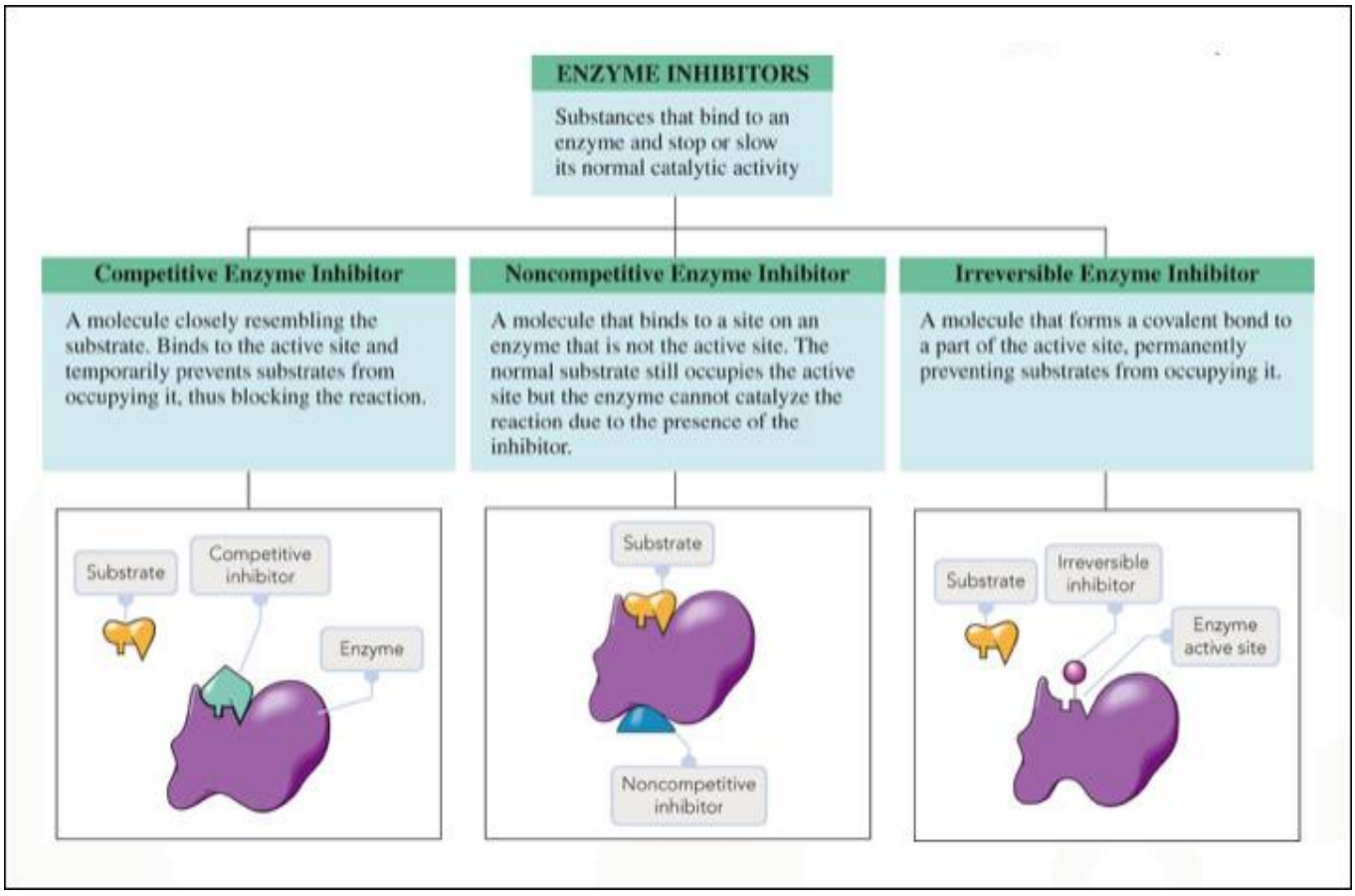
Irreversible Inhibition

- An irreversible inhibitor inactivates an enzyme by binding to its active site by a strong covalent bond
  - – Permanently deactivates the enzyme
  - –Irreversible inhibitors do not resemble substrates
- Addition of excess substrate doesn't reverse this process

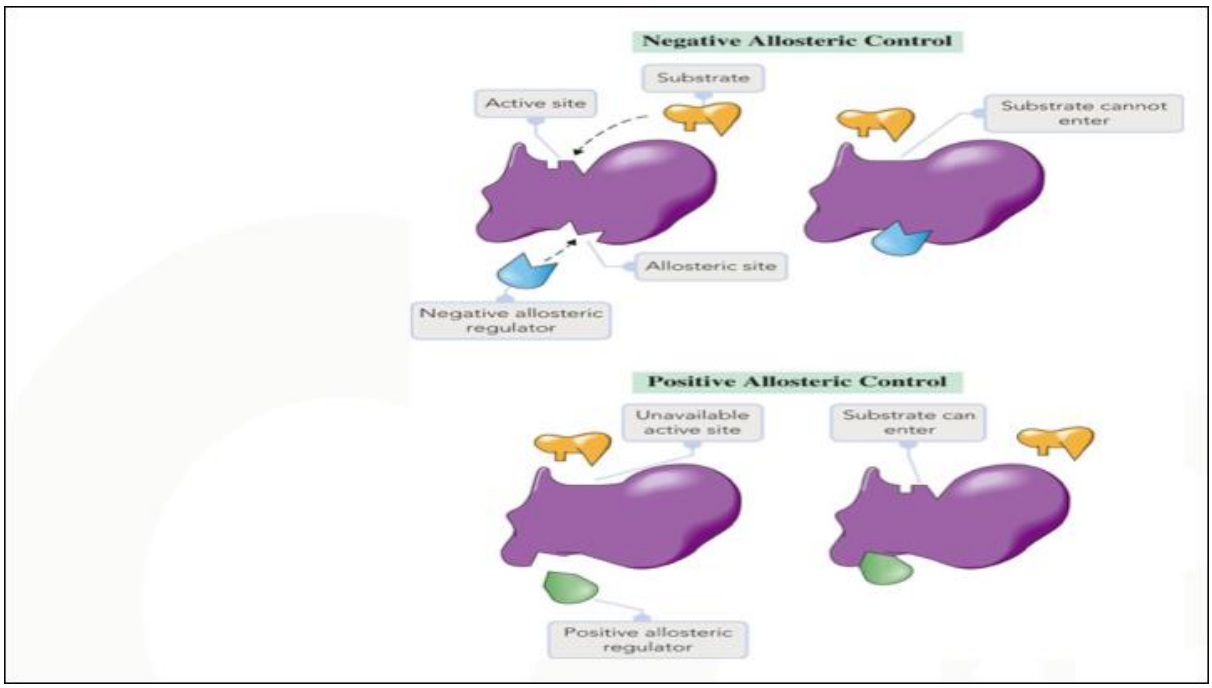
– Cannot be reversed

- Chemical warfare (nerve gases)
- Organophosphate insecticides





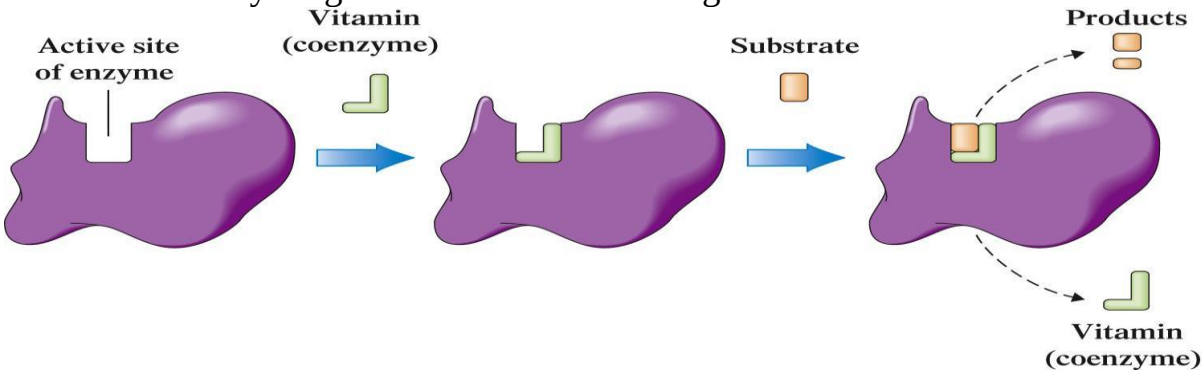
The different effects of
Positive & Negative regulators
on an Allosteric enzyme



Vitamins as Coenzymes

• Many enzymes require B vitamins as coenzymes – Allow the enzyme to function
• Coenzymes serve as temporary carriers of atoms or functional groups
- – Coenzymes provide chemical reactivity that the apoenzyme lacks
- – Important in metabolism reactions to release energy from foods

• E.g. redox reactions where they facilitate oxidation or reduction
• B vitamins don't remain permanently bonded to the apoenzyme
- – After the catalytic action the vitamin is released & can be repeatedly used by various enzymes
- – This recycling reduces the need for large amounts of B vitamins



Medical Uses of Enzymes

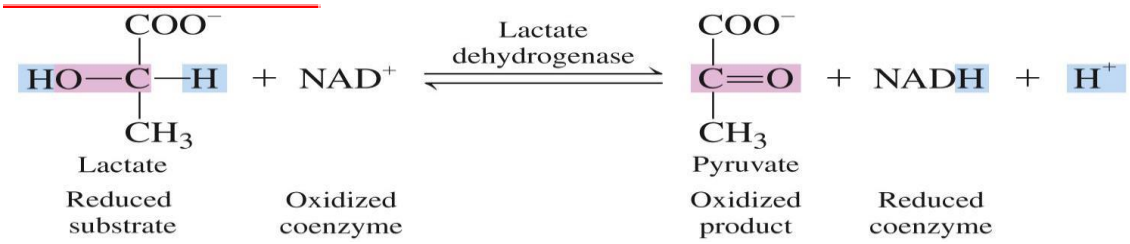
- Enzymes can be used in diagnosis & treatment of certain diseases
- Lactate dehydrogenase (LDH) is normally not found in high levels in blood, as it is produced in cells
  - – Increased levels of LDH in the blood indicate myocardial infarction (MI) (Heart attack)
  - – Tissue plasminogen activator (TPA) activates the enzyme plasminogen that dissolves blood clots

• Used in the treatment of MI

• There is no direct test to measure urea in the blood
– Urease converts urea into ammonia, which is easily measured & is used as urea indicator
• Blood Urea Nitrogen (BUN) is used to measure kidney function
– High urea levels in the blood indicate kidney malfunction
Isoenzymes
• Isoenzyme catalyze the same reaction in different tissues in the body
– e.g. lactate dehydrogenase (LDH) consists of 5 isoenzymes • Each isoenzyme of LDH has the same function
– Converts lactate to pyruvate

- LDH_1 isoenzyme is more prevalent in heart muscle
- LDH_5 form is found in skeletal muscle & liver

• Isoenzymes can be used to identify the damaged or diseased organ or tissue
• It is a marker for a particular location
• If LDH_1 isoenzyme was found in the blood >>> indicates heat muscle damage



General material and bulk-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 into biochemistry. Biochemical components of cells.

1. Biological chemistry as a science. Place of the biochemistry between other biological and medical disciplines.
2. Subjects and aims of biochemical studies. Leading role of biochemistry in discovery of molecular mechanisms of pathogenesis of human diseases.
3. Conjugation of biochemistry with other biomedical sciences. Medical biochemistry. Clinical biochemistry. Biochemical laboratory diagnostics.
4. History of biochemistry and the development of worldwide biochemical research.

Biochemical components of the cells, their chemical functions. Classes of biologic molecules, their origins.

Enzymes and coenzymes. Regulation of metabolism.

1. Chemical nature of enzymes. Composition of simple and complex enzymes. Cofactors and coenzymes. Role of coenzymes and apoenzyme in catalysis.
2. Cofactors and coenzymes. Structures and properties. Vitamins as precursors of coenzymes. Examples.
3. Active site of enzymes. Inhibitors and activators of the enzymes. Competitive and non-competitive inhibition. Medical application of enzyme inhibitors.
4. The mechanism of enzyme action. The kinetic of enzymatic catalysis. Dependence of the rate of reaction from concentration of substrate.
5. The types of activation and inhibition of enzymes. Phosphorylation and dephosphorylation of enzymes.
6. Allosteric regulation of enzymatic activity. Regulatory enzymes. Feedback regulation of their activity. Covalent modification of enzymes.
7. The systematic classification and nomenclature of enzymes.
8. Specificity of enzyme action, substrate specificity.
9. The kinetic of enzymatic reaction: dependence of the rate of reaction from concentration of enzyme, pH and temperature.
10. Isozymes. Biologic role. Diagnostic importance of isozyme determination.
11. Enzyme diagnostic and enzymotherapy in medicine.
12. Enzymotherapy. Principles of diagnostic and treatment.
13. Zymogens. Activation of zymogens, their role in the metabolism.
14. Cyclic nucleotides (c-AMP, c-GMP) as the regulators of enzyme catalysis and biological functions of cells. Action of the regulation of protein kinases.

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: Bioenergetics: general pathways of catabolism of carbohydrates, lipids, amino acids. Citric acid cycle. Biological oxidation and oxidative phosphorylation. Electron transport chain in mitochondria.

Topic relevance: Krebs cycle is an aerobic cycle of breaking down acetyl CoA. Each cycle yields a molecule of $FADH_2$ (flavin adenine dinucleotide$^+$ H_2), a molecule of GTP (guanosine triphosphate), three molecules of NADH (nicotinamide adenine dinucleotide$^+$ hydrogen) , and two molecules of carbon dioxide. The aerobic breakdown of pyruvate (final product of glycolysis), beta oxidation of fatty acids, and catabolism of amino acids results in the formation of acetyl CoA. Krebs cycle occurs inside the mitochondrial matrix of the eukaryotic cell and in the cytoplasm of prokaryotic cells. Energy, which can be defined as the ability to perform a work, can be of various types: potential, radiant, thermal, electrical. All organisms require energy to perform certain cellular functions essential for survival: transport of solutes across the cytoplasmic membrane, movement, biosynthesis.

Objective: Metabolic cycles are divided into two types: anabolic and catabolic. The anabolic (biosynthetic) ones consume energy to build cellular components, the catabolic ones are represented by sequences of chemical reactions whose function is to release free energy through the degradation or oxidation of organic molecules.

Basic concepts:

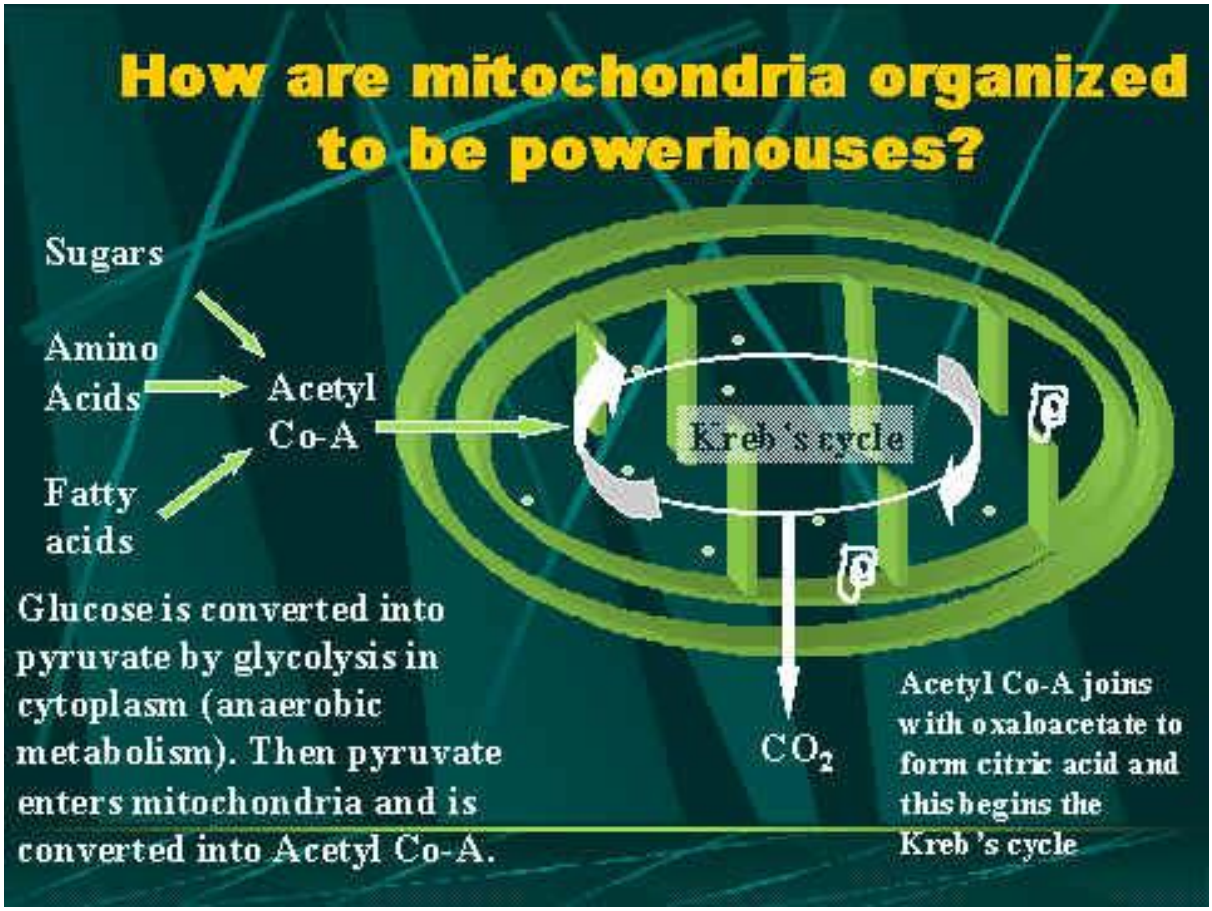
1. Anabolism, catabolism, metabolism.
2. Energy balance.
3. Anaplerotic reactions.
4. Amphibolic reactions.
5. Cytochrome P450.
6. Free radicals.
7. Antioxidant systems.

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.

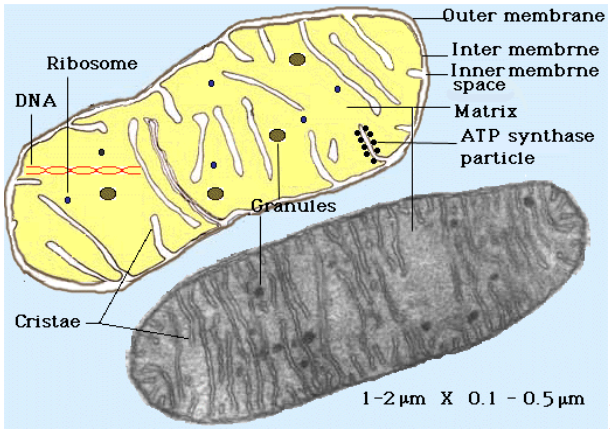
Content of the lecture material

GENERAL METABOLITIC WAYS

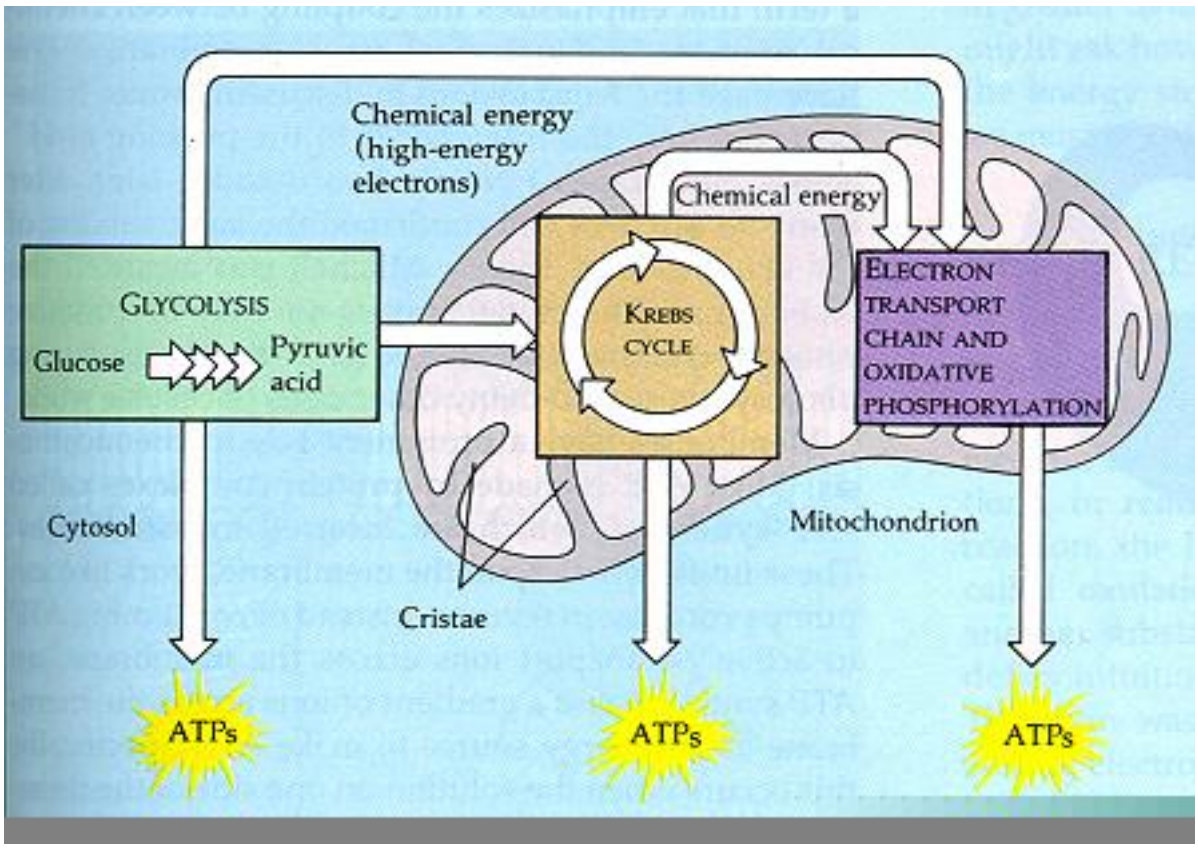


Shows how mitochondria is used in aerobic respiration

Mitochondria functions:

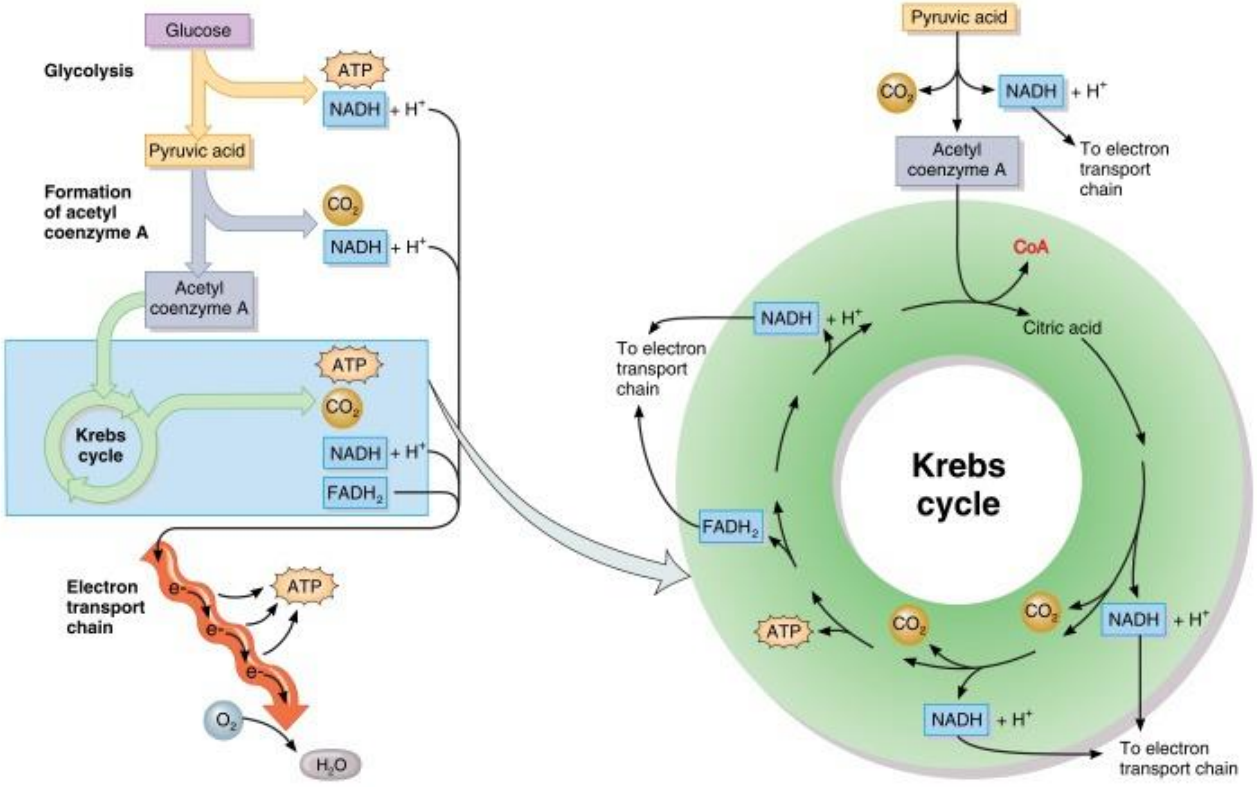


Inter and outer membrane of mitochondria

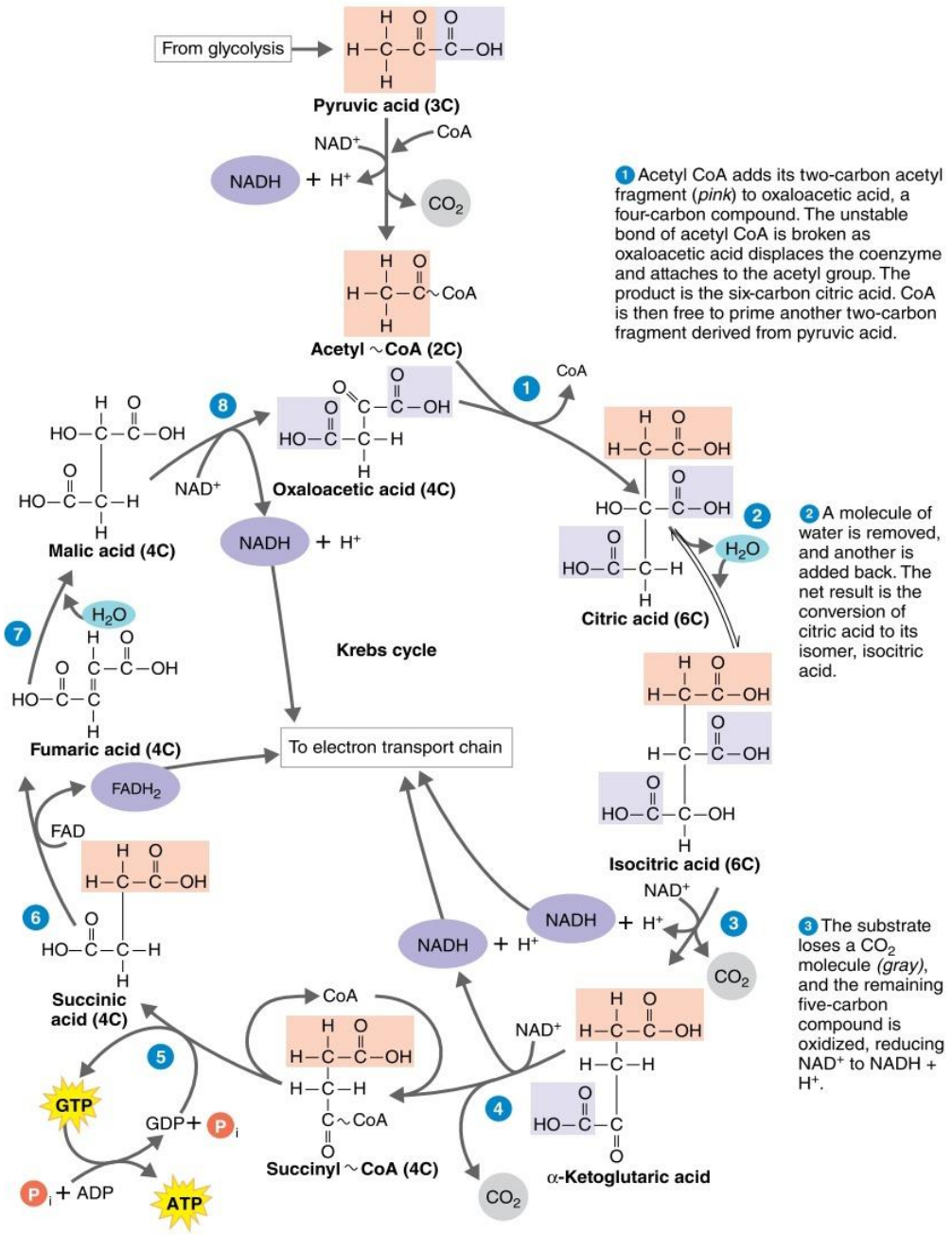


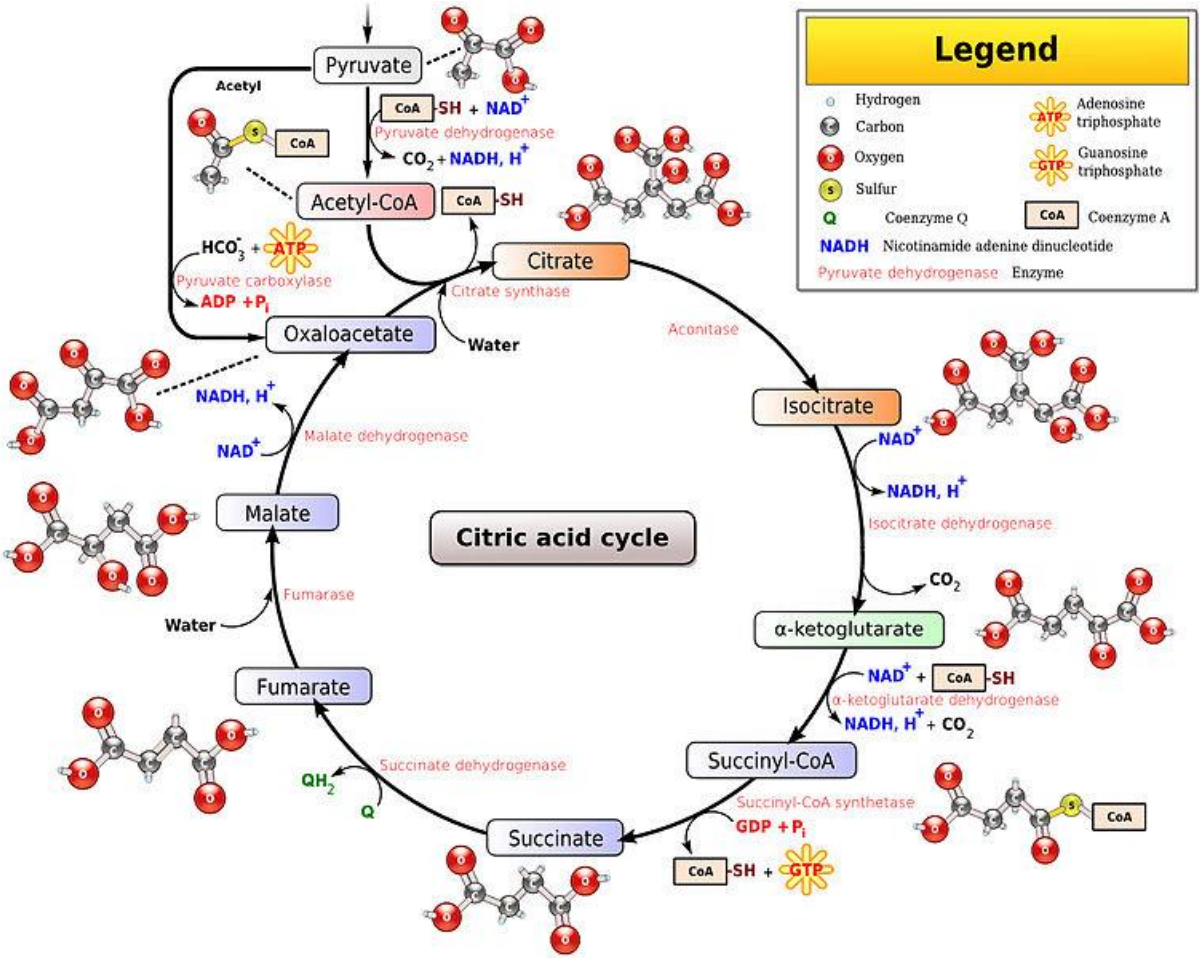
Energy cycles that take place in the cell

Citric acid cycle



5 The Krebs cycle.
5.13, page 124, for a
/ersion.



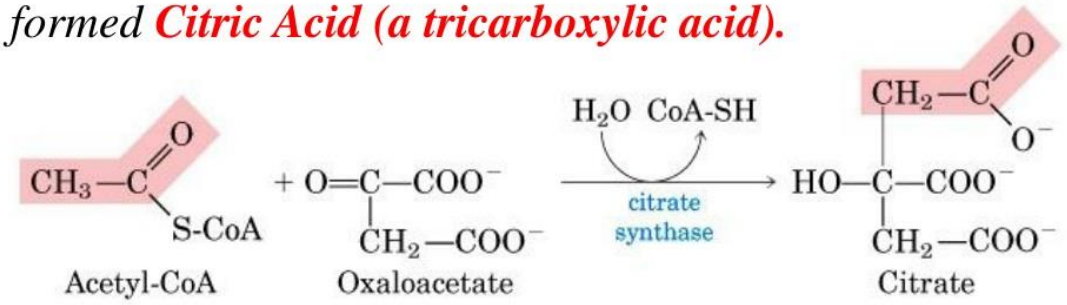


Citric Acid Cycle is also called as the tricarboxylic acid cycle or krebes cycle. It is a process of the oxidation of acetyl CoA, to CO_2 and H_2O

Reactions of Citric Acid Cycle

1. Citrate synthase:

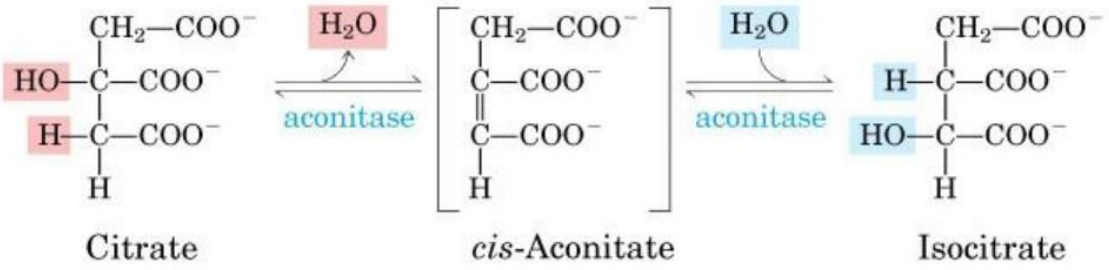
In the first step, acetyl CoA (formed from pyrovate under aerobic condition)combines with ***oxaloacetate*** *and formed* ***Citric Acid (a tricarboxylic acid).***



$\Delta G'^{\circ} = -32.2$ kJ/mol

2. Aconitase:

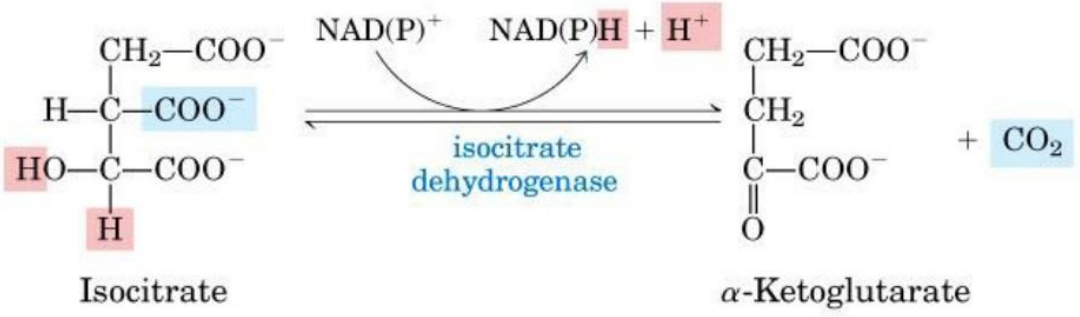
This enzyme catalyses the isomerization reaction by removing and then adding back the water (H and OH) to cis-aconitate in at different positions. Both the steps are carried out by Aconitase .Conversion of citrate to isocitrate is inhibited by fluoroacetate.



$\Delta G'^{\circ} = 13.3$ kJ/mol

3. Isocitrate dehydrogenase:

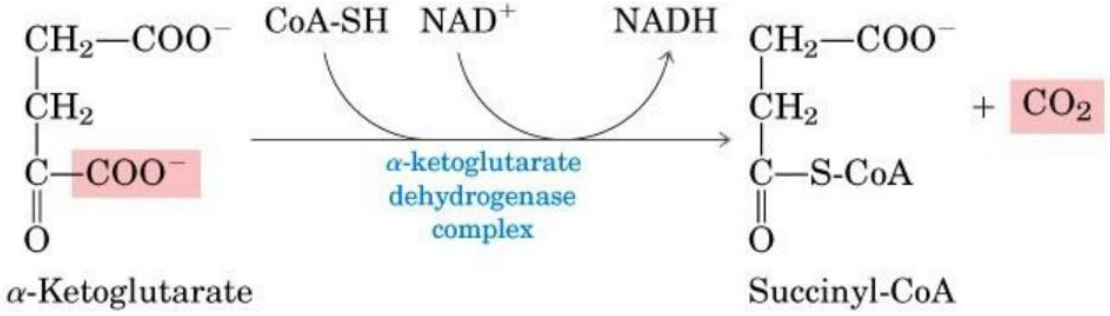
In the presence of Isocitratedehydrogenase, isocitrate is converted to oxalosuccinate which is later decarboxylated to form α-ketoglutarate



$\Delta G'^{\circ} = -20.9$ kJ/mol

4. α-Ketoglutarate dehydrogenase:

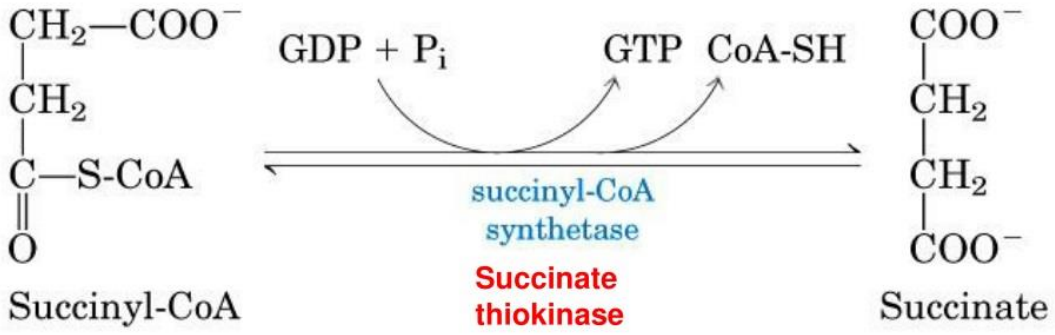
This is a complex of different enzymatic activities similar to the pyruvate dehydrogenase complex. It has the same mechanism of reaction with E1, E2 and E3 enzyme unites, and requires five coenzyme,i.e. TPP, NAD^+ ,FAD, coenzyme A and Lipoic acid.



$\Delta G'^{\circ} = -33.5$ kJ/mol

5. Succinate thiokinase:

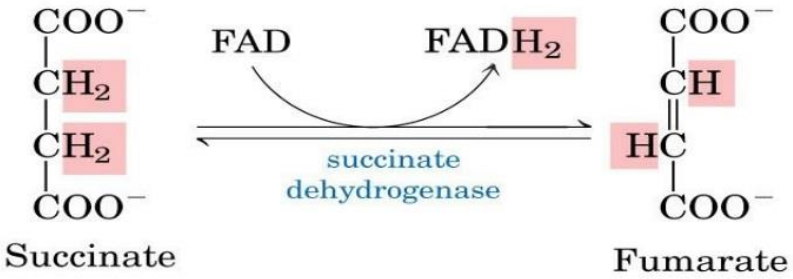
Sccinyl CoA is changed to succinate by succinate thiokinase. during this reaction, amolecule of GTP is formed. This in turn is equivalent to ATP and is known as substrate level phosphorylation since a high energy molecule is formed at the substrate level only.



$\Delta G'^{\circ} = -2.9$ kJ/mol

6. Succinate Dehydrogenase:

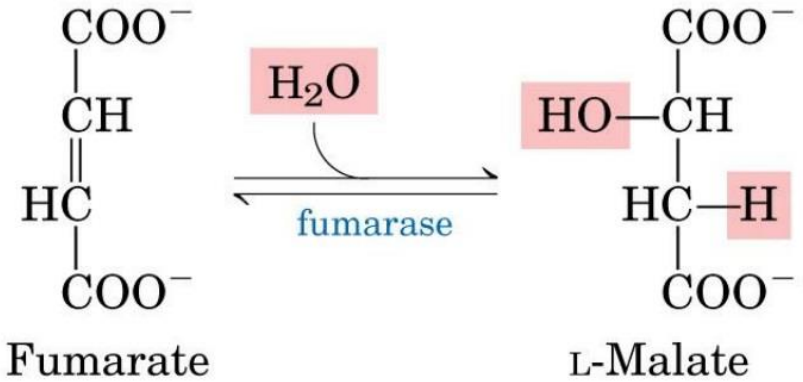
Oxidation of succinate to fumarate.This is the only citric acid cycle enzyme that is tightly bound to the inner mitochondrial membrane. It is an FAD dependent enzyme. Due to structural similarity between malonate and succinate, malonate inhibits succinate dehydrogenase, competitively.



$\Delta G'^{\circ} = 0$ kJ/mol

7. Fumarase:

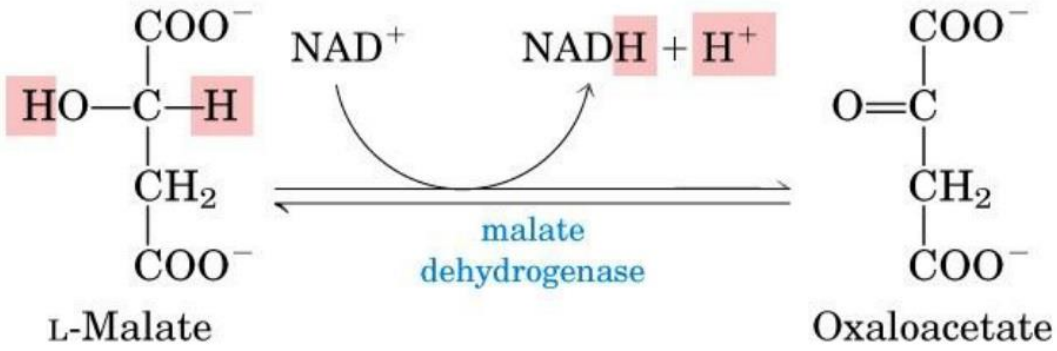
Hydration of Fumarate to malate: It is a highly stereospecific enzyme.



$\Delta G'^{\circ} = -3.8$ kJ/mol

8. L-Malate dehydrogenase:

Oxidation of malate to oxaloacetate: It is an NAD^+ dependent enzyme.



L-Malate Oxaloacetate

$\Delta G'^{\circ} = 29.7$ kJ/mol

Energy production during krebs cycle

As a result of the oxidation of one molecule of acetyl CoA in the krebs cycle, three molecules of NAD+ and one molecule of FAD are reduced.

Reducing equivalents(from $NADH+H^+$) enters the respiratory chain and results in the production of three molecules of ATP. Similarly , $FADH_2$ yields 2ATP. Besides, there is also a substrate level production of GTP. Total ATP yield, per molecule of acetyl CoA , is thus 12 ATP(Table 1.1)

Table(1.1) Energy production from Citric Acid Cycle

Isocitate ⟶ α-ketoglutarate	$NADH+H^+$	(3ATP)
α-ketoglutarate ⟶ Succinyl CoA	$NADH+H^+$	(3ATP)
Succinyl CoA ⟶ Succinate	-	(1ATP)
Succinate ⟶ Fumarate	$FADH_2$	(2ATP)
Malate ⟶ Oxaloacetate	$NADH+H^+$	(3ATP)
	total	(12ATP)

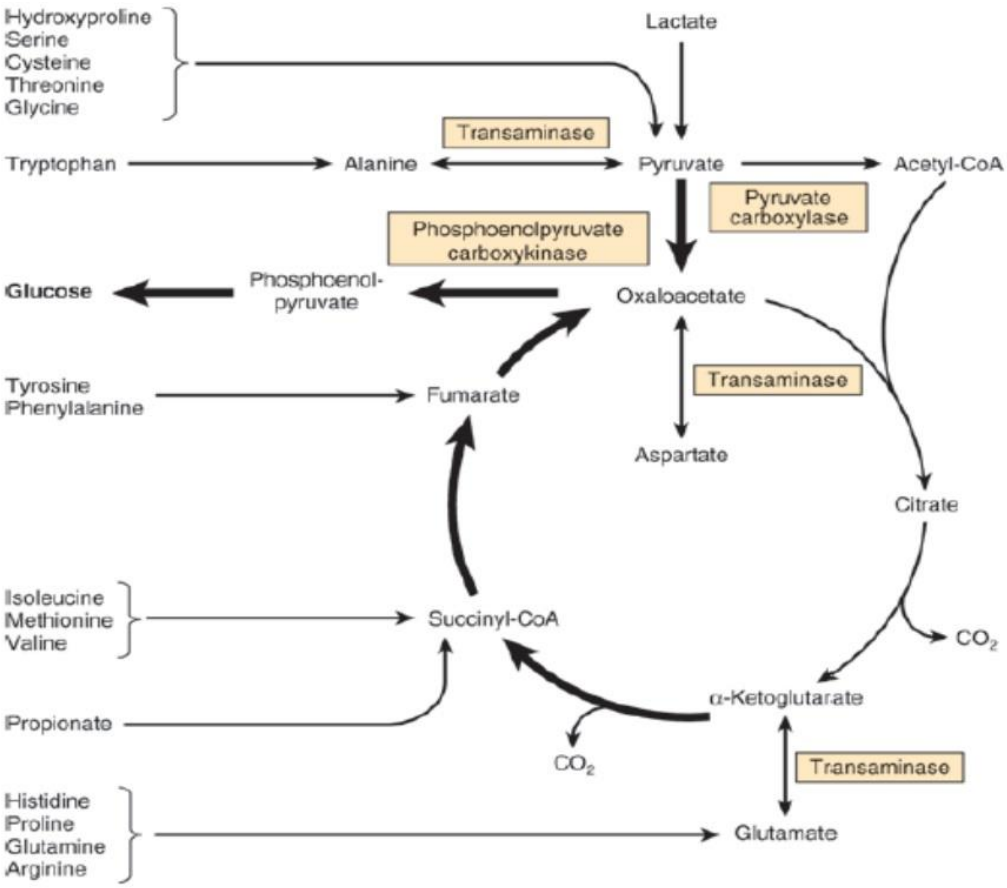
In addition, as mentioned above, conversion of pyruvate to acetyl CoA also generates $NADH+H^+$ and gives 3ATP

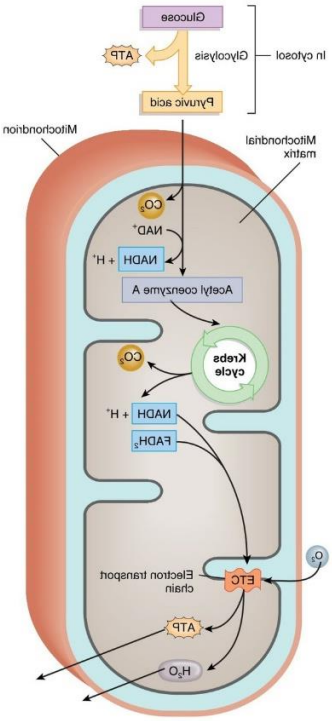
VITAMINS PLAY KEY ROLES IN THE CITRIC ACID CYCLE

Four of the B vitamins are essential in the citric acid cycle and hence energy-yielding metabolism:
(1)B2 (riboflavin), *in the form of flavin adenin dinucleotide (FAD), a cofactor for succinate dehydrogenase;* ***(2) niacin,*** *in the form of nicotinamide adenine dinucleotide (NAD), the electron acceptor for isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase;* ***(3) thiamin(vitamin B1),*** *as thiamin pyrophosphate(TPP), the coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction; and* ***(4) pantothenic acid,*** *as part of coenzyme A, the cofactor attached to "active" carboxylic acid residues such as acetyl-CoA and succinyl-CoA.*

THE CITRIC ACID CYCLE PLAYS A PIVOTAL ROLE IN METABOLISM

The citric acid cycle is not only a pathway for oxidation of two-carbon units, but is also a major pathway for interconversion of metabolites arising from ***transamination and deamination*** *of amino acids and providing the substrates for amino acid synthesis by* ***transamination*** *as well as for gluconeogenesis and fatty acid synthesis. Because it functions in both oxidative and synthetic processes, it is* ***amphibolic***.

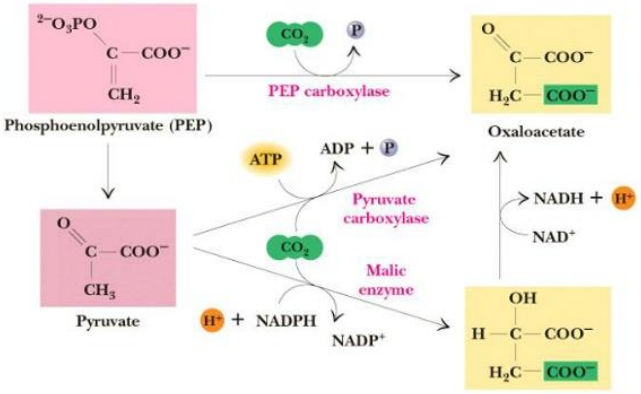


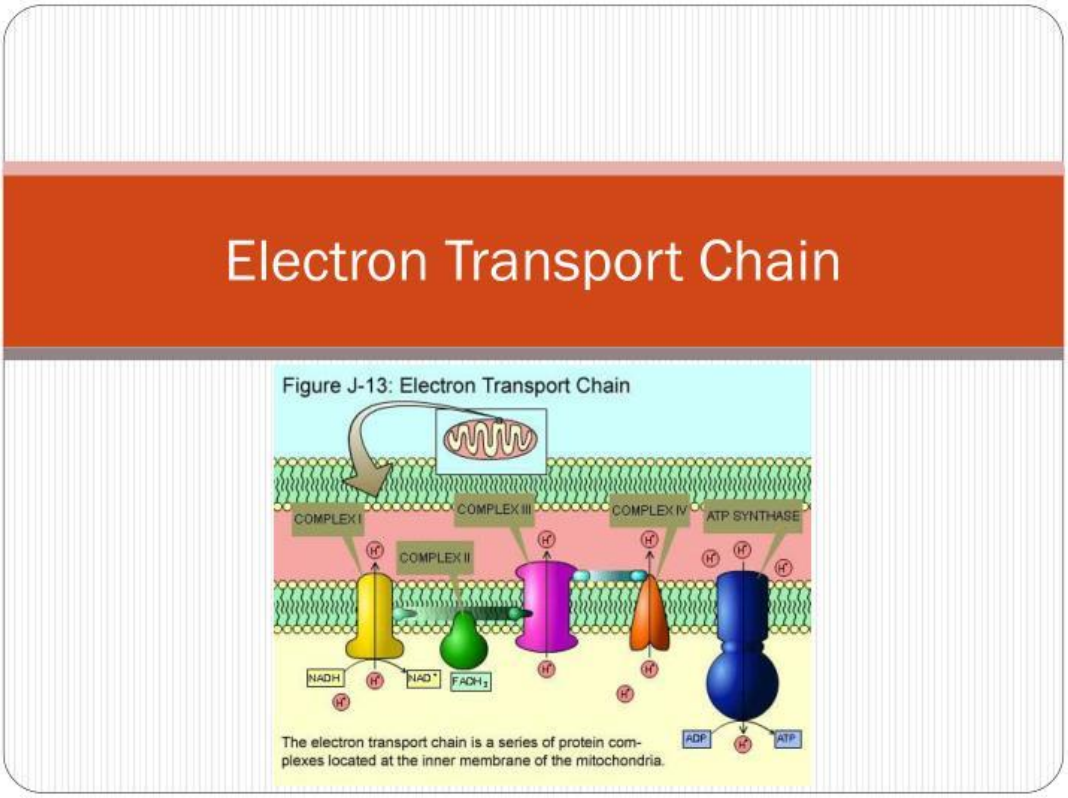


The Anaplerotic Reactions

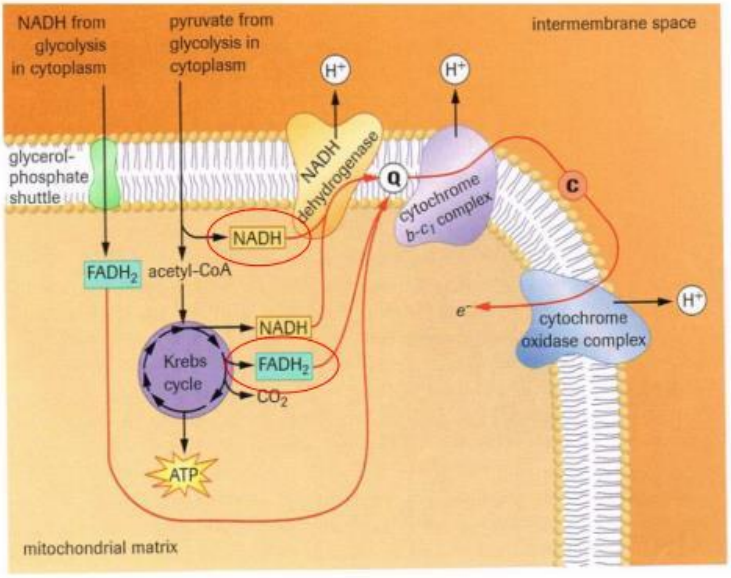
The "filling up" reactions

- PEP carboxylase - converts PEP to oxaloacetate
- Pyruvate carboxylase - converts pyruvate to oxaloacetate
- Malic enzyme converts pyruvate into malate



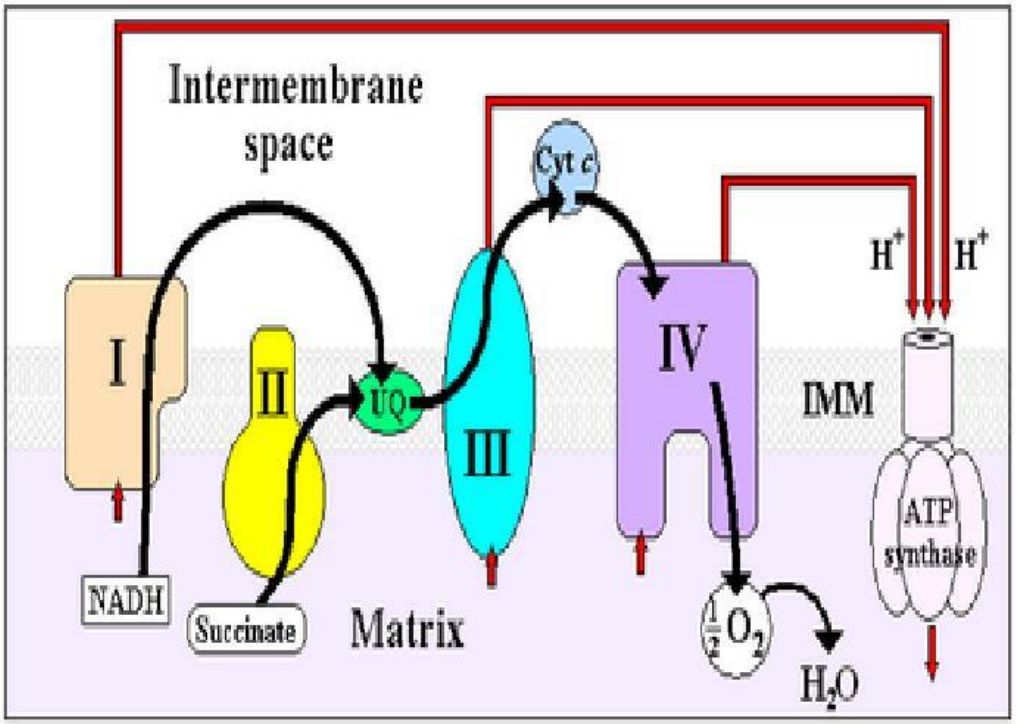


- NADH passes its electrons on to Complex I – NADH dehydrogenase
- $FADH_2$ passes its electrons to Q (shuttle)



- An **electron transport chain (ETC)** couples electron transfer between an electron donor (such as NADH) and an electron acceptor (such as O_2) with the transfer of H_+ ions (protons) across a membrane. The resulting electrochemical proton gradient is used to generate chemical energy in the form of adenosine triphosphate (*ATP*). Electron transport chains are the cellular mechanisms used for extracting energy from sunlight in photosynthesis and also from redox reactions, such as the oxidation of sugars (respiration).

- **Electron transport chains in mitochondria**
- Most eukaryotic cells contain mitochondria, which produce ATP from products of the Krebs cycle, fatty acid oxidation, and amino acid oxidation. At the mitochondrial inner membrane, electrons from NADH and succinate pass through the electron transport chain to oxygen, which is reduced to water



• Complex I

- In *Complex I* (NADH dehydrogenase, also called NADH:ubiquinone oxidoreductase; EC 1.6.5.3) two electrons are removed from NADH and transferred to a lipid-soluble carrier, *ubiquinone* (Q). The reduced product, *ubiquinol* (QH_2) freely diffuses within the membrane, and Complex I translocates four protons (H^+) across the membrane, thus producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of harmful superoxide.[3]

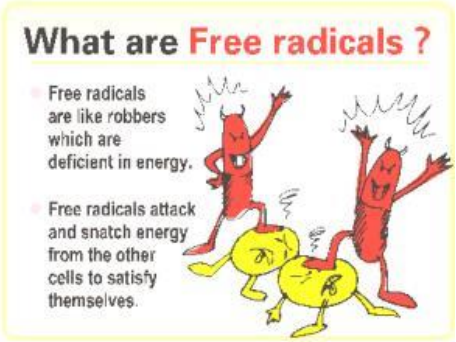
- **Complex II**
- In *Complex II* (succinate dehydrogenase; EC 1.3.5.1) additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q. Complex II consists of four protein subunits: SDHA, SDHB, SDHC, and SDHD. Other electron donors (e.g., fatty acids and glycerol 3-phosphate) also direct electrons into Q (via FAD).

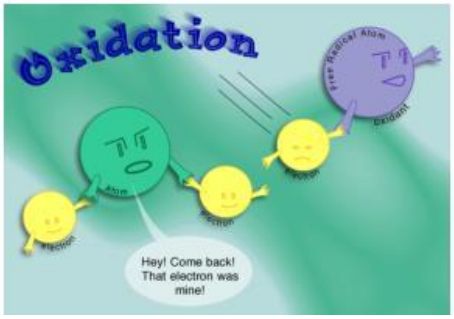
- **Complex III**
- In *Complex III* (cytochrome bc1 complex; EC 1.10.2.2), the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH_2 at the Q_O site and sequentially transferred to two molecules of cytochrome c, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Q_i site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by two quinol (4H+4e-) oxidations at the Q_o site to form one quinol (2H+2e-) at the Q_i site. (in total six protons are translocated: two protons reduce quinone to quinol and four protons are released from two ubiquinol molecules).
- When electron transfer is reduced (by a high membrane potential or respiratory inhibitors such as antimycin A), Complex III may leak electrons to molecular oxygen, resulting in superoxide formation

- **Complex IV**
- In *Complex IV* (cytochrome c oxidase; EC 1.9.3.1) four electrons are removed from four molecules of cytochrome c and transferred to molecular oxygen (O_2), producing two molecules of water. At the same time, four protons are translocated across the membrane, contributing to the proton gradient. The activity of cytochrome c is inhibited by cyanide

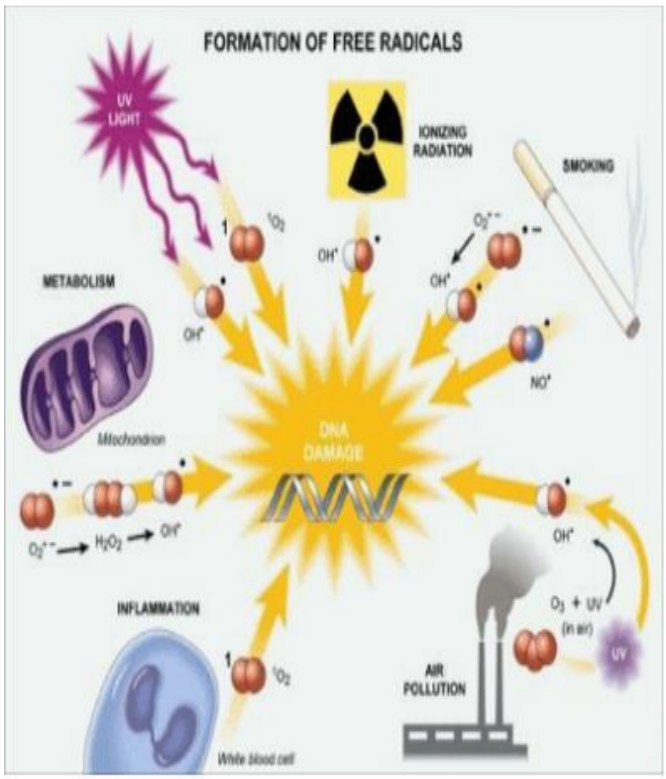
What are free radicals?

- Any molecule containing one or more *unpaired* electrons.
- These unpaired electrons readily form free radical molecules which are chemically reactive and highly unstable.





Types of free radicals



1. Superoxide, O_2^-
2. Hydrogen peroxide, H_2O_2
3. Hydroxyl radical, OH^-
4. Singlet oxygen, $^1O_2$
5. Hydroperoxy radical, HOO^-
6. Lipid peroxide radical, ROO^-
7. Nitric oxide, NO^-
8. peroxynitrite, $ONOO^-$

Properties of free radicals

1. Highly reactive
2. Very short half-life
3. Generate new radicals by chain reaction
4. Cause damage to biomolecules, cells and tissues

Most free radicals in biological systems are derivatives of oxygen (Reactive Oxygen Species, ROS), but there are also derivatives of nitrogen (Reactive Nitrogen Species, RNS), Reactive Metabolites or Intermediates.

The free radical diseases

- Cancer initiation and promotion is associated with chromosomal defects and oncogene activation. It is possible that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumour formation.

The free radical diseases

- **Atherosclerosis may be due to free radical reactions involving diet-derived lipids in the arterial wall and serum to yield peroxides and other substances. These compounds induce endothelial cell injury and produce changes in the arterial walls .**

Antioxidants

- vitamin E
- vitamin C
- carotenoids

vitamin E

- Vitamin E is a fat-soluble substance present in all cellular membranes and is mainly stored in adipose tissue, the liver and muscle. Vitamin E is a principal antioxidant in the body and protects polyunsaturated fatty acids in cell membranes from peroxidation.

Vitamin E and cancer

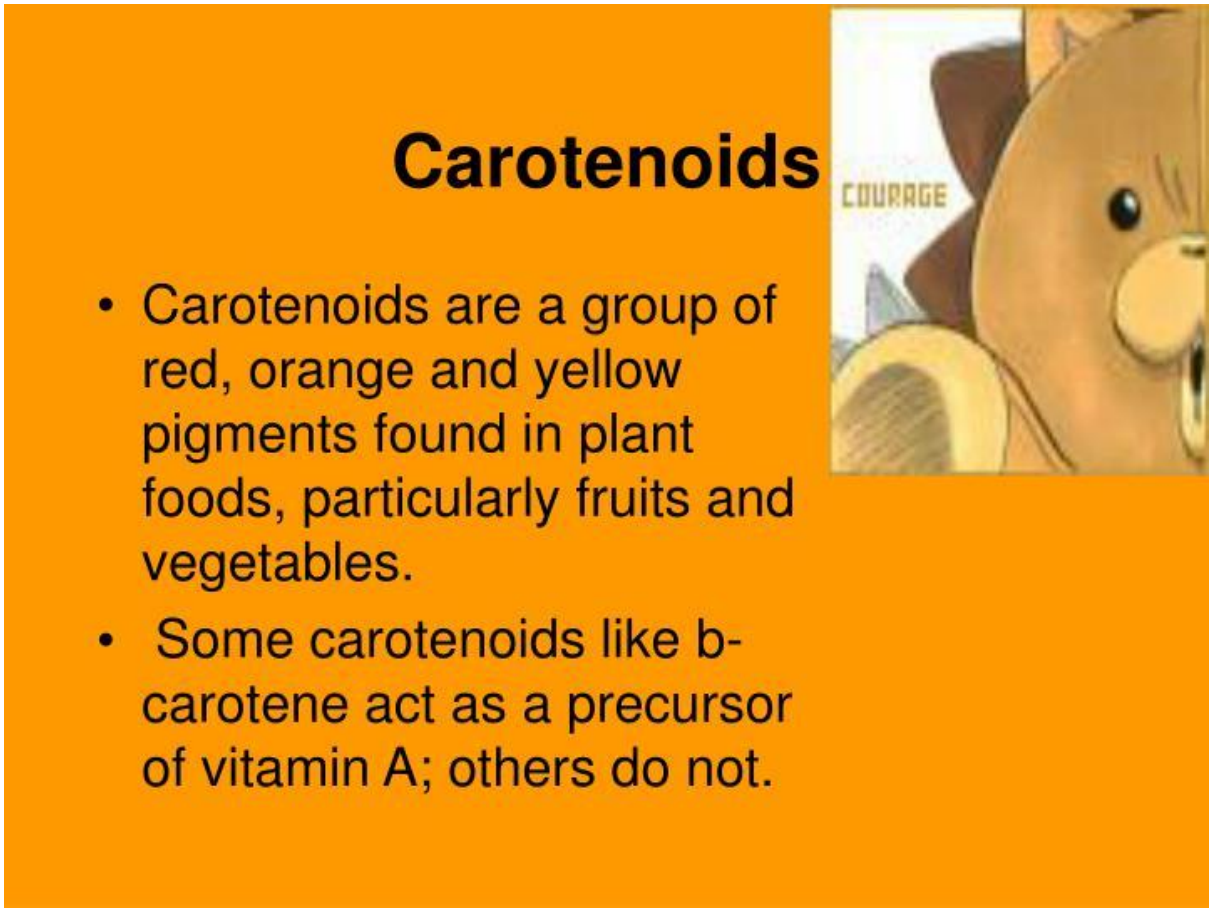
- Besides being a free radical scavenger, vitamin E at high intakes enhances the body's immune responses. Vitamin E also inhibits the conversion of nitrites in the stomach to nitrosamines, which are cancer promoters.

Vitamin C

- Vitamin C, or ascorbic acid, is a water-soluble vitamin. This vitamin is a free radical scavenger, it is considered to be one of the most important antioxidants in extra cellular fluids. Its protective effects extend to cancer, coronary artery disease, arthritis and aging.

Vitamin C and cancer

- Vitamin C is effective in protecting tissues against oxidative damage. It suppresses the formation of carcinogens. Numerous studies have reported the protective effect of fruit and vegetable consumption on incidence of cancer . This is mainly attributed to the protective effect of vitamin C against cancer.



General material and bulk-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:

Metabolism fundamentals. Citric acid cycle.

1. General characteristics of energy metabolism. Metabolism: catabolism, anabolism and amphibolic pathways.
2. General and specific pathways of catabolism. Stages of intercellular catabolism of biomolecules: proteins, carbohydrates, lipids.
3. The Citric Acid Cycle (CAC). Location in the cell. Enzymes of CAC. Importance of CAC.
4. Bioenergetics of Citric Acid Cycle. Physiologic importance of CAC.

Molecular basis of bioenergetics.

1. Reactions of biologic oxidation. Types of reactions: dehydrogenase, oxidase, oxygenase reactions and their biologic importance. Tissue respiration.

2. Chemical nature of dehydrogenases. Role of coenzymes in the functions of enzymes.
3. Flavoenzymes. Chemical nature and biochemical role.
4. Cytochromes. Cytochrome oxidase, its chemical nature, the biochemical role and mechanism of participation in the respiratory chain.
5. Synthesis of ATP from ADP. Oxidative phosphorylation, which is coupling with respiratory chain. Index P/O.
6. The chemiosmotic theory of oxidative phosphorylation. ATP synthase of mitochondria.
7. Phosphorylation at substrate level and its biological importance.
8. Mechanism of transport of reducing equivalents through the membrane of mitochondria. Shuttle mechanism.
9. High-energy compounds, their importance. Chemical nature of ATP and ADP, their role in metabolism.
10. Peroxide oxidation. Antioxidant systems and their biological role.
11. Microsomal oxidation. Cytochrome P450 and molecular organization of the electron transport chain. Detoxification of xenobiotics and formation of biological active compounds.

Methodological support:

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: Carbohydrate metabolism: glycolysis, glycogenolysis, oxidative decarboxylation of pyruvate, interconversion of monosaccharides, metabolism of fructose, galactose. Glycogen biosynthesis, pentose phosphate pathway, gluconeogenesis. Enzymopathies of carbohydrate metabolism (glycogen storage diseases). Diabetes mellitus.

Topic relevance: Carbohydrates are organic molecules composed of carbon, hydrogen, and oxygen atoms. The family of carbohydrates includes both simple and complex sugars. Glucose and fructose are examples of simple sugars, and starch, glycogen, and cellulose are all examples of complex sugars. During digestion, carbohydrates are broken down into simple, soluble sugars that can be transported across the intestinal wall into the circulatory system to be transported throughout the body. Carbohydrate digestion begins in the mouth with the action of salivary amylase on starches and ends with monosaccharides being absorbed across the epithelium of the small intestine. Once the absorbed monosaccharides are transported to the tissues, the process of cellular respiration begins.

Objective: study of the general patterns of carbohydrate metabolism as the main source of energy in the human body.

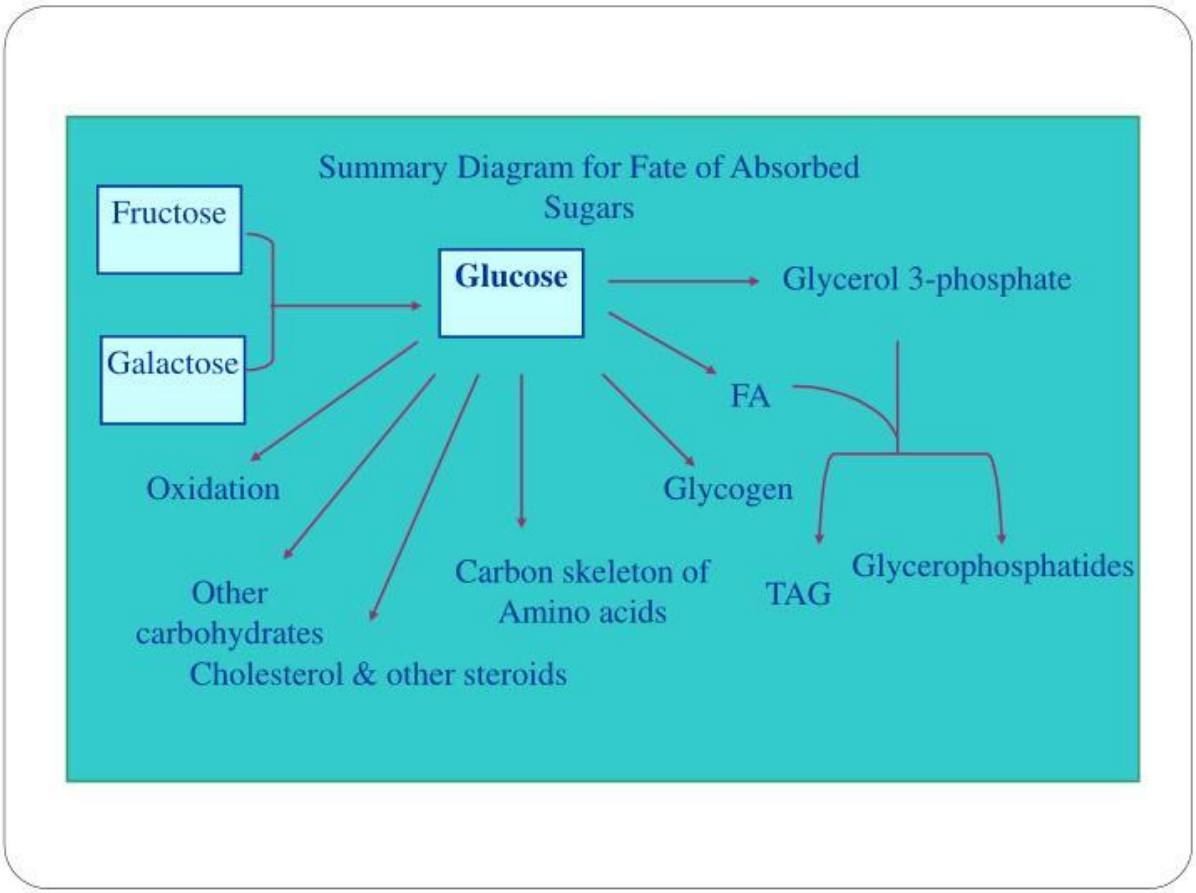
Basic concepts:

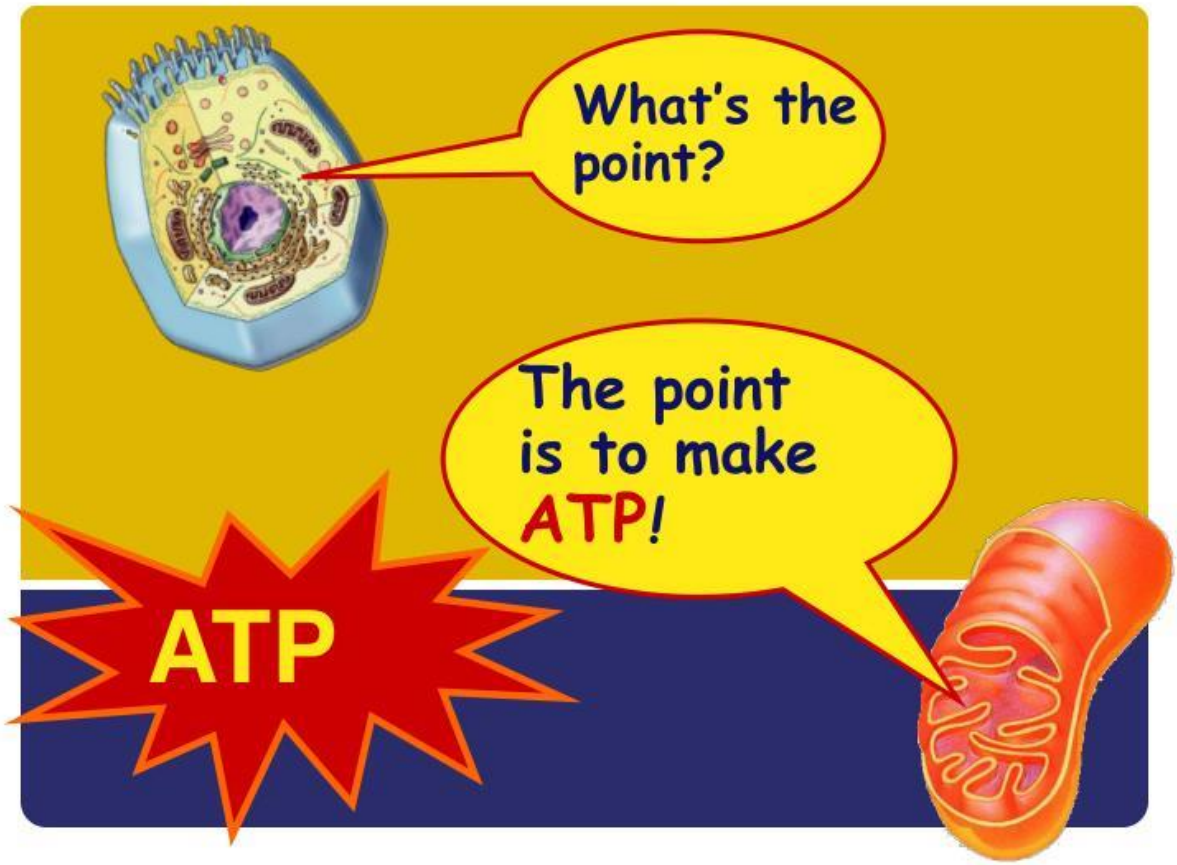
1. Glycolysis, glycogenolysis.
2. Regulation of glycolysis.
3. Shuttle mechanisms.
4. Multienzyme complex.

Plan and organizational structure of the lecture:

Pathways of glucose metabolism
General characteristics of anaerobic and aerobic oxidation of glucose.
Sequence of reactions and enzymes of glycolysis.
Glycolytic oxidoreduction.
Regulation of glycolysis.
Stages of aerobic oxidation of glucose.
Gluconeogenesis.
Synthesis of glycogen.
Catabolism of glycogen.
Regulation of glycogen metabolism.
Glucosemia
Hormonal regulation of glucose blood concentration.
Diabetes mellitus.

Content of the lecture material





Dietary sources of glucose

- Varied
  - Fruits, vegetables, cereals, grains
- All sources of dietary carbohydrate
  - Converted to glucose in liver
- Stored in cells as glycogen

How do we maintain blood glucose levels?

1) Diet
2) Liver glycogen
   - Glucose taken up from blood converted to glycogen

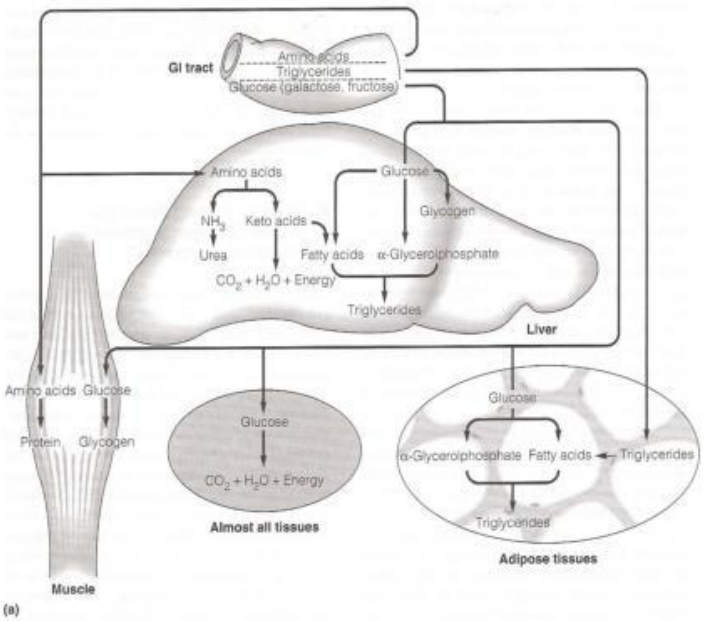
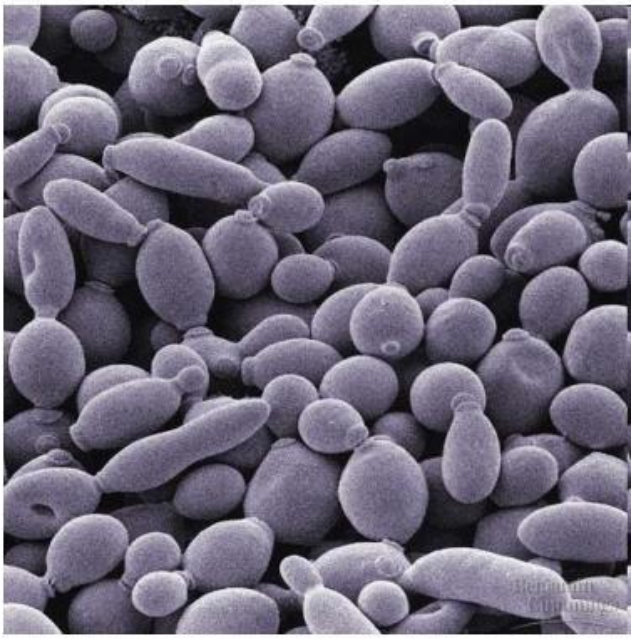


Figure 5-3 Flowchart of glucose and other metabolites in an individual (a) immediately after a meal and (b) during fasting. Modified from Vander, Sherman, and Luciano, 1980. Used with permission.

Glycolysis

- Term “anaerobic” refers to bacteria (like yeast)
  - In mammalian systems
    - Cells are never anaerobic
    - Lactate is produced in proportion to work rate, even when oxygen is present
    - Lactate is produced even at rest

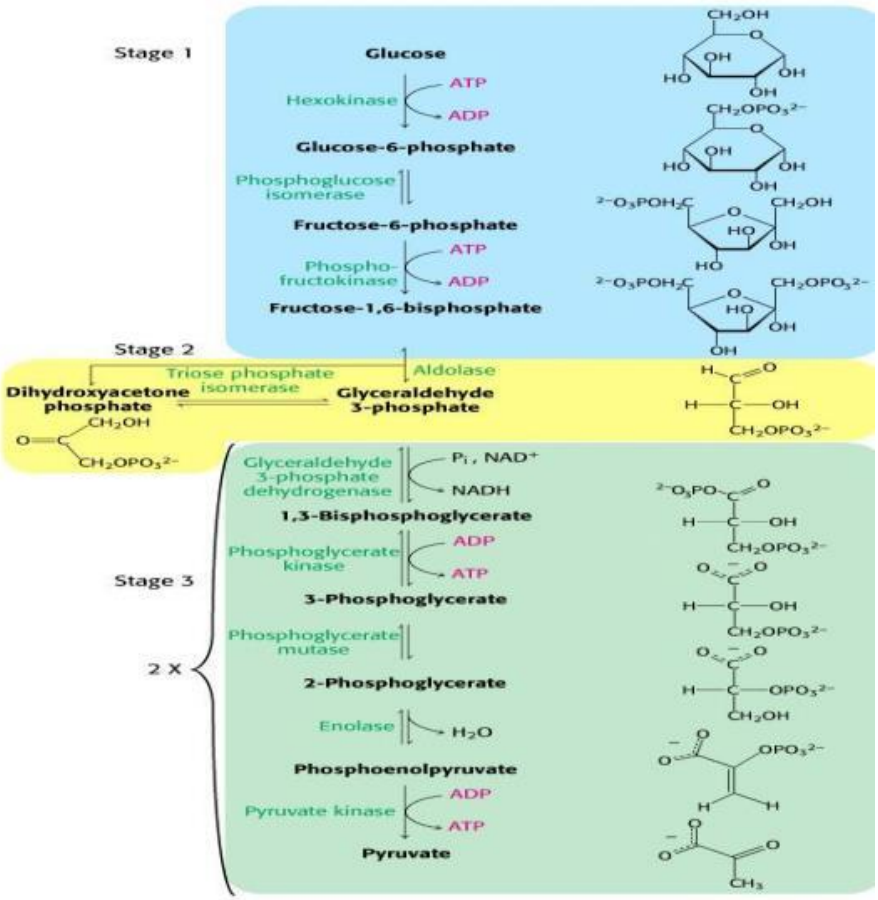


Glycolysis

- There are 2 main phases of glycolysis: Glycolysis I - activation phase, which uses ATP molecules
- Glycolysis II - oxidative and phosphorylation reactions, which not only reduce glucose to pyruvate but also produce ATP molecules
- $C_6H_{12}O_6 + 2ADP + 2P_i + 2\ NAD^+$ -----> 2 pyruvate + 2ATP + 2(NADH + H^+)

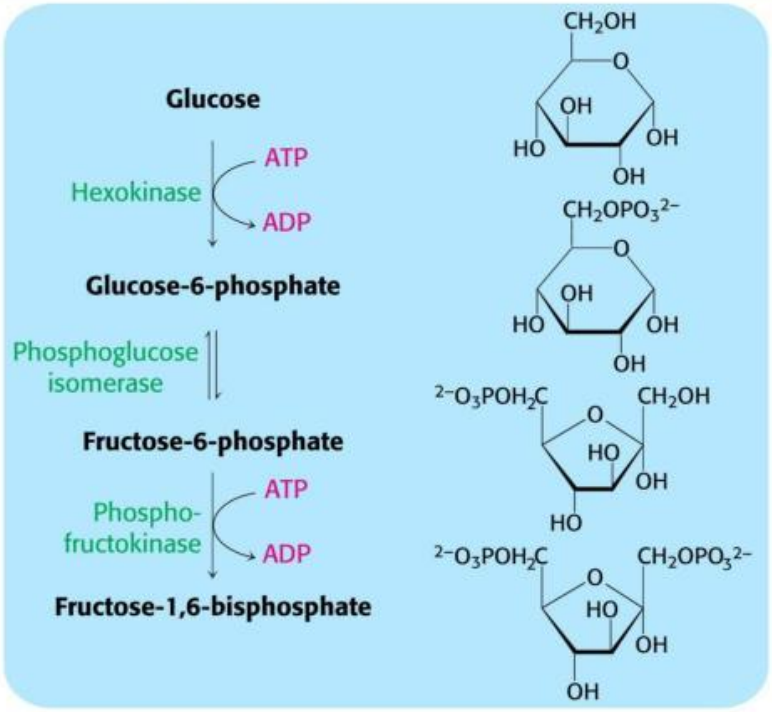
Glycolysis (10 reactions) can be divided into three stages

- In the 1st stage (hexose stage) **2 ATP** are ***consumed*** per glucose
- In the 3rd stage (triose stage) **4 ATP** are ***produced*** per glucose
- Net: **2 ATP** produced per glucose



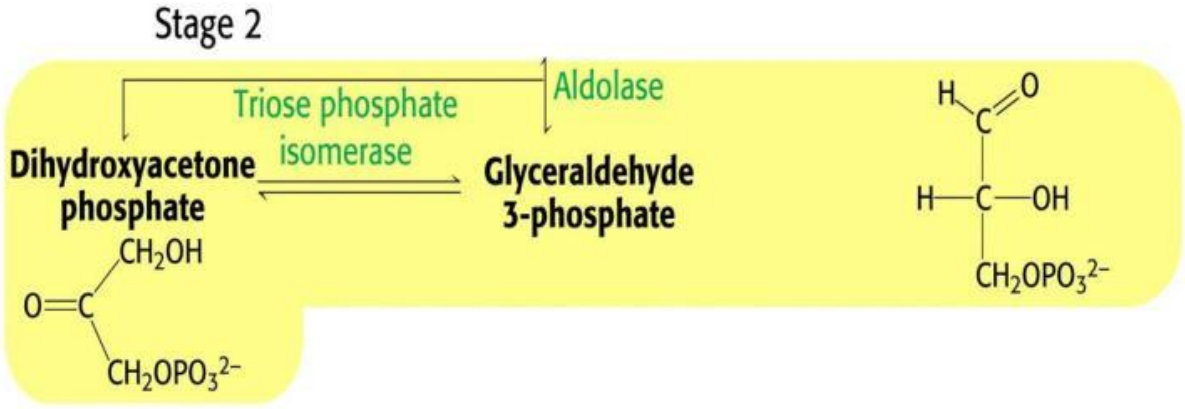
Stage 1, which is the conversion of **glucose** into **fructose 1,6-bisphosphate**, consists of three steps: a *phosphorylation*, an *isomerization*, and a second *phosphorylation* reaction.

The strategy of these initial steps in glycolysis is to trap the glucose in the cell and form a compound that can be readily cleaved into phospho-rylated three-carbon units.

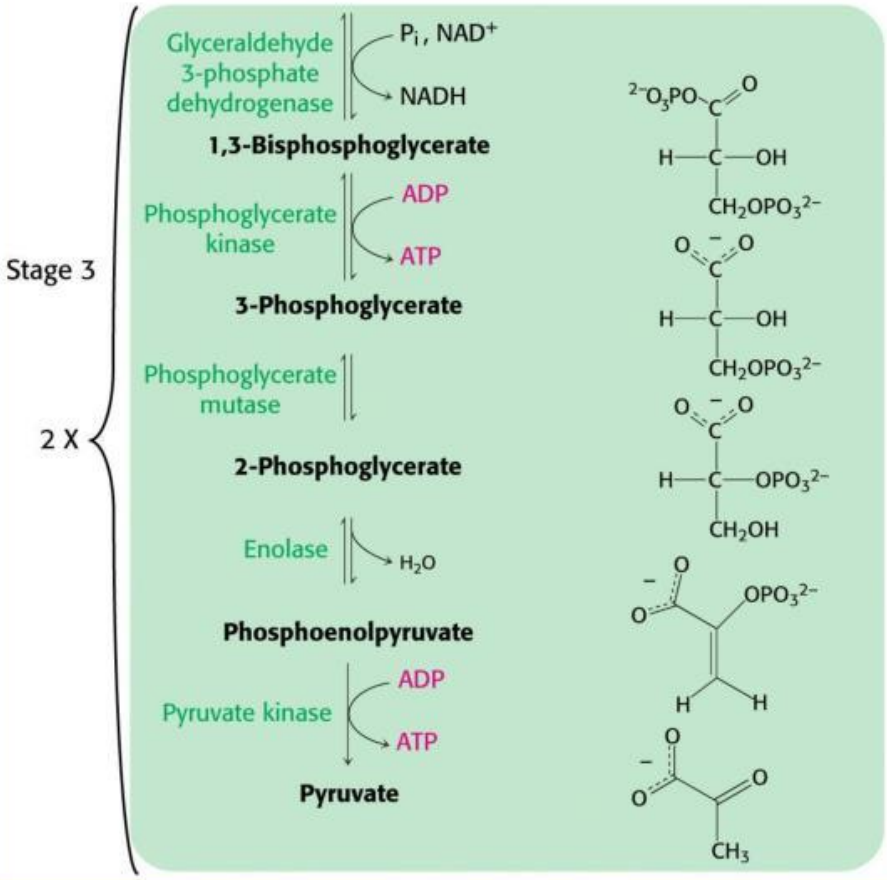


Stage 2 is the cleavage of the **fructose 1,6-bisphosphate** into two three-carbon fragments **dihydroxyacetone phosphate** and **glyceraldehyde 3-phosphate**.

Dihydroxyacetone phosphate and **glyceraldehyde 3-phosphate** are readily interconvertible.



In **stage 3**, ATP is harvested when the three-carbon fragments are oxidized to pyruvate.

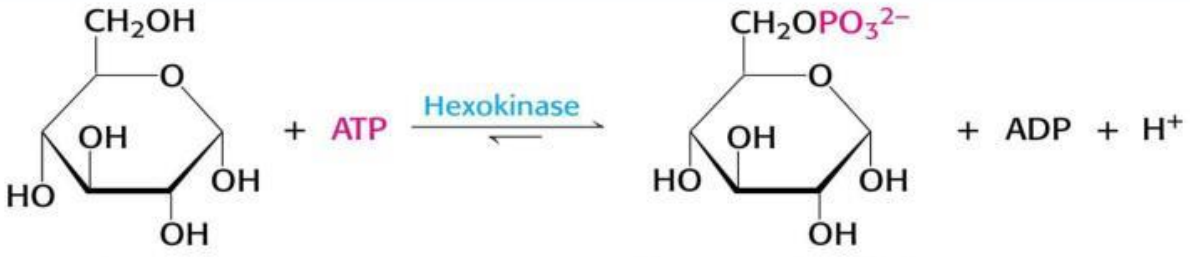


Glycolysis Has 10 Enzyme-Catalyzed Steps

- Each chemical reaction prepares a substrate for the next step in the process

1. Hexokinase

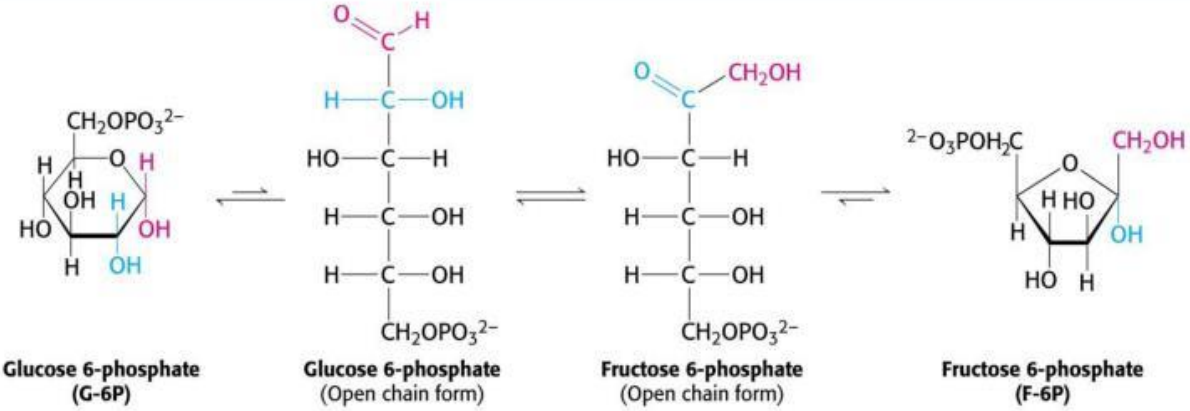
- Transfers the γ-phosphoryl of ATP to **glucose** C-6 oxygen to generate **glucose 6-phosphate** (**G6P**)
- Four kinases in glycolysis: steps 1,3,7, and 10
- All four kinases require Mg^{2+} and have a similar mechanism



Glucose | **Glucose 6-phosphate (G-6P)**

2. Glucose 6-Phosphate Isomerase

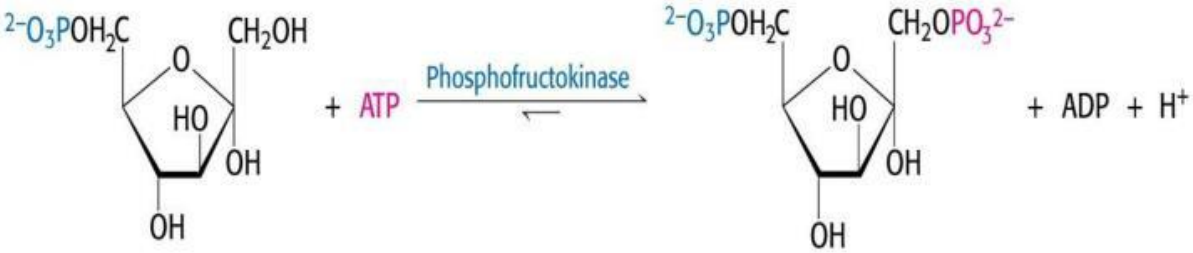
- Converts **glucose 6-phosphate** (**G6P**) (an aldose) to **fructose 6-phosphate** (**F6P**) (a ketose)
- Enzyme preferentially binds the a-anomer of G6P (converts to open chain form in the active site)
- Enzyme is highly stereospecific for G6P and F6P
- Isomerase reaction is near-equilibrium in cells



Glucose 6-phosphate (G-6P) | **Glucose 6-phosphate** (Open chain form) | **Fructose 6-phosphate** (Open chain form) | **Fructose 6-phosphate (F-6P)**

3. Phosphofructokinase-1 (PFK-1)

- Catalyzes transfer of a phosphoryl group from ATP to the C-1 hydroxyl group of **F6P** to form **fructose 1,6-*bis*phosphate (F1,6BP)**
- PFK-1 is **metabolically irreversible** and a critical **regulatory point** for glycolysis in most cells
- A second phosphofructokinase (PFK-2) synthesizes **fructose 2,6-*bis*phosphate (F2,6BP)**

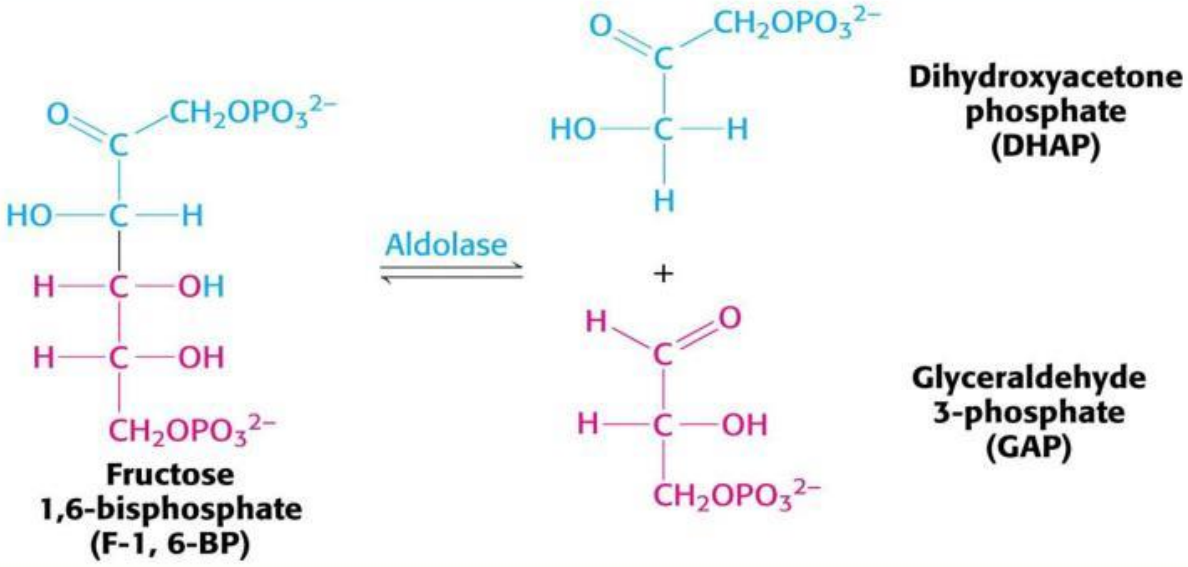


Fructose 6-phosphate (F-6P)

Fructose 1,6-bisphosphate (F-1, 6-BP)

4. Aldolase

- Aldolase cleaves the hexose F1,6BP into two triose phosphates: **glyceraldehyde 3-phosphate (GAP)** and **dihydroxyacetone phosphate (DHAP)**
- Reaction is near-equilibrium, not a control point



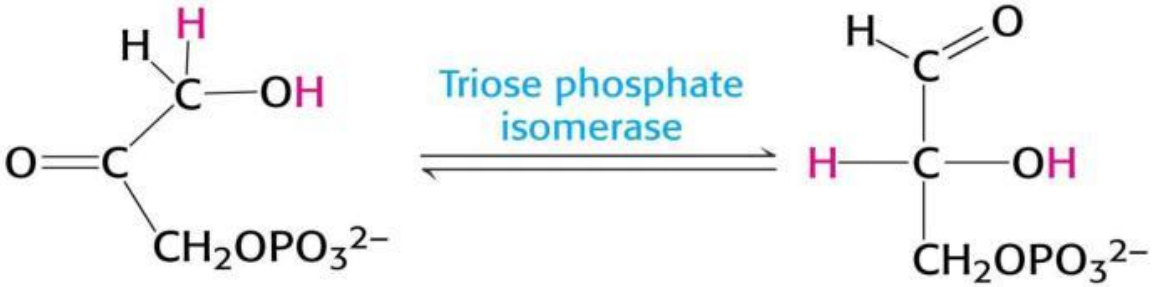
Fructose 1,6-bisphosphate (F-1, 6-BP)

Dihydroxyacetone phosphate (DHAP)

Glyceraldehyde 3-phosphate (GAP)

5. Triose Phosphate Isomerase (TPI)

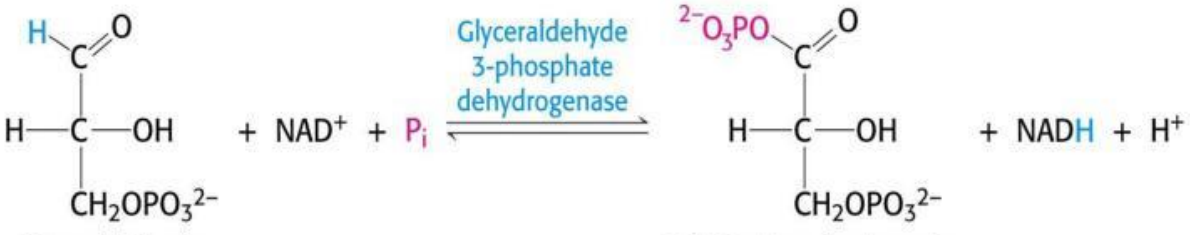
- Conversion of **DHAP** into ***GAP***
- Reaction is very fast, only the D-isomer of GAP is formed
- Reaction is reversible. At equilibrium, 96% of the triose phosphate is DHAP. However, the reaction proceeds readily from DHAP to GAP because the subsequent reactions of glycolysis remove this product.



Dihydroxyacetone phosphate ⇌ **Glyceraldehyde 3-phosphate**

6. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH)

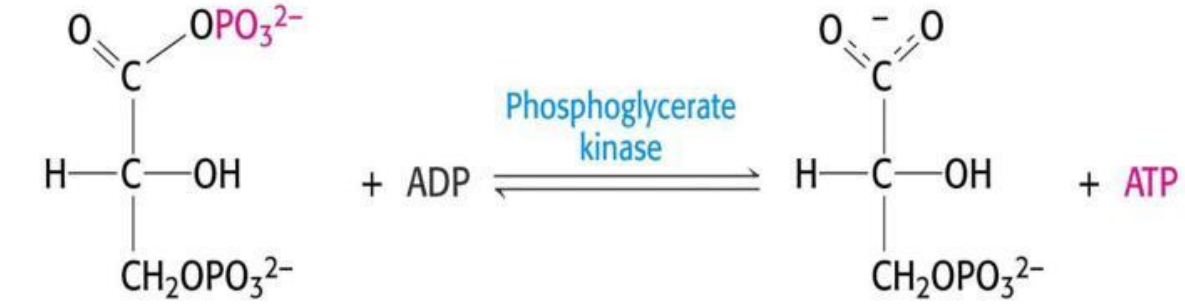
- Conversion of ***GAP*** to **1,3-*bis*phosphoglycerate** (**1,3BPG**)
- Molecule of NAD^+ is reduced to NADH
- Energy from oxidation of GAP is conserved in <u>acid-anhydride</u> linkage of 1,3BPG
- Next step of glycolysis uses the high-energy phosphate of 1,3BPG to form ATP from ADP



Glyceraldehyde 3-phosphate (GAP) ⇌ **1,3-Bisphosphoglycerate (1,3-BPG)**

7. Phosphoglycerate Kinase (PGK)

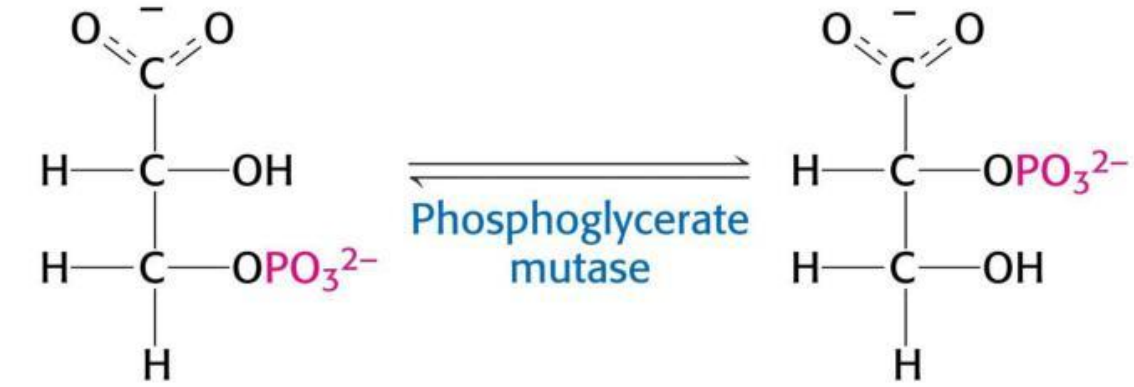
- Transfer of phosphoryl group from the energy-rich mixed anhydride 1,3BPG to ADP yields **ATP** and **3-phosphoglycerate (3PG)**
- **Substrate-level phosphorylation** - Steps 6 and 7 couple oxidation of an aldehyde to a carboxylic acid with the phosphorylation of ADP to ATP



1,3-Bisphosphoglycerate **3-Phosphoglycerate**

8. Phosphoglycerate Mutase

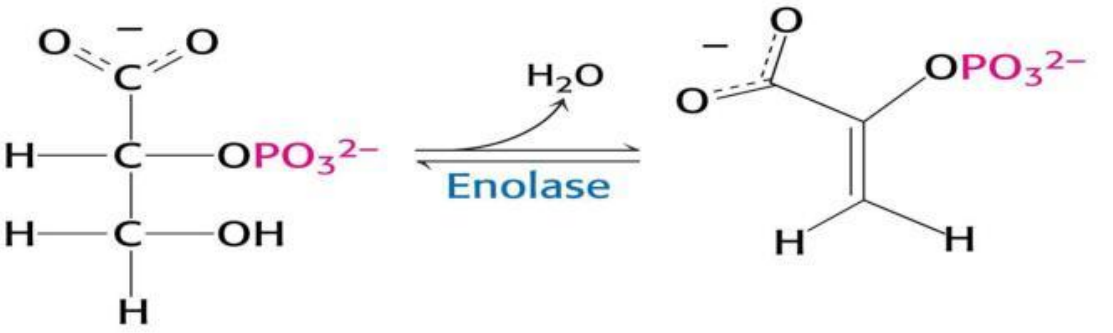
- Catalyzes transfer of a phosphoryl group from one part of a substrate molecule to another
- Reaction occurs without input of ATP energy



3-Phosphoglycerate **2-Phosphoglycerate**

9. Enolase: 2PG to PEP

- **2-Phosphoglycerate** (2PG) is dehydrated to **phosphoenolpyruvate** (PEP)
- Elimination of water from C-2 and C-3 yields the enol-phosphate PEP
- PEP has a very high phosphoryl group transfer potential because it exists in its unstable enol form

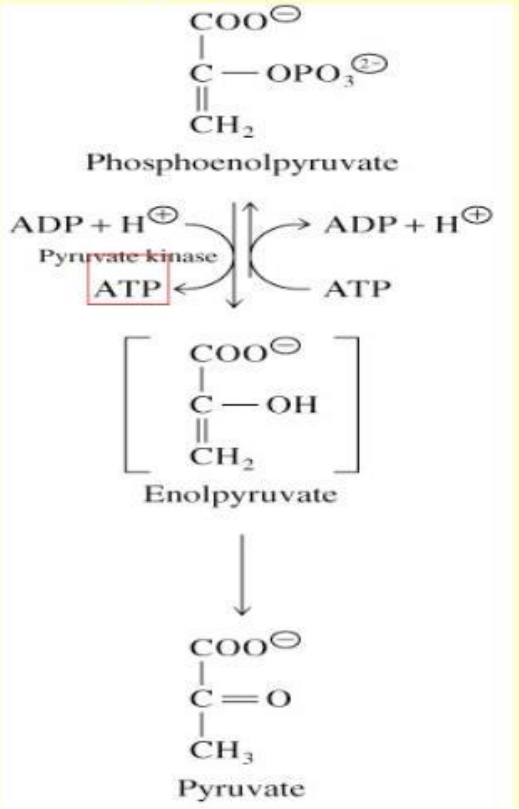


2-Phosphoglycerate **Phosphenolpyruvate**

10. Pyruvate Kinase (PK)

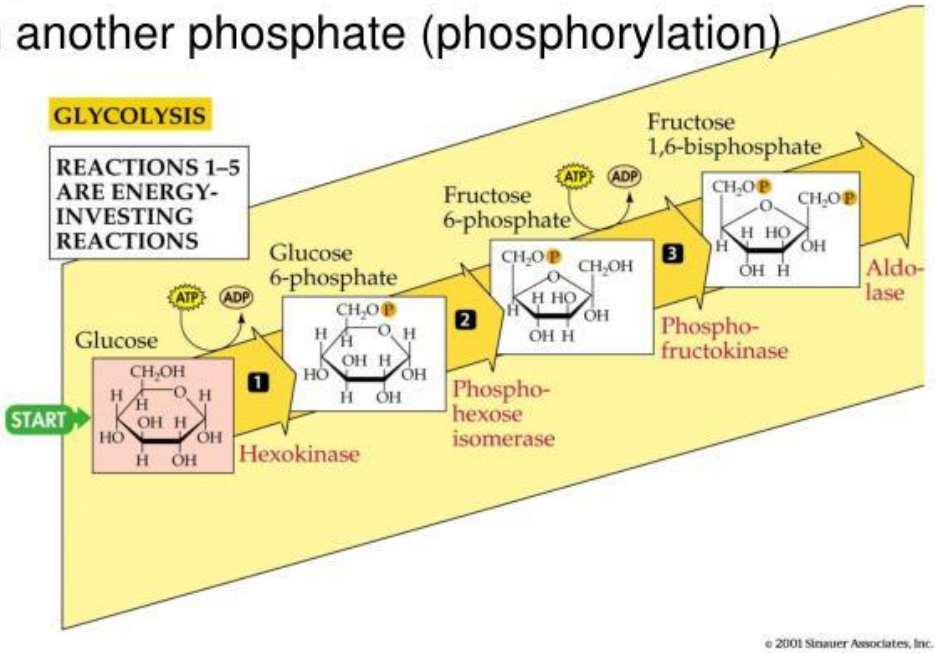
PEP + ADP → Pyruvate + ATP

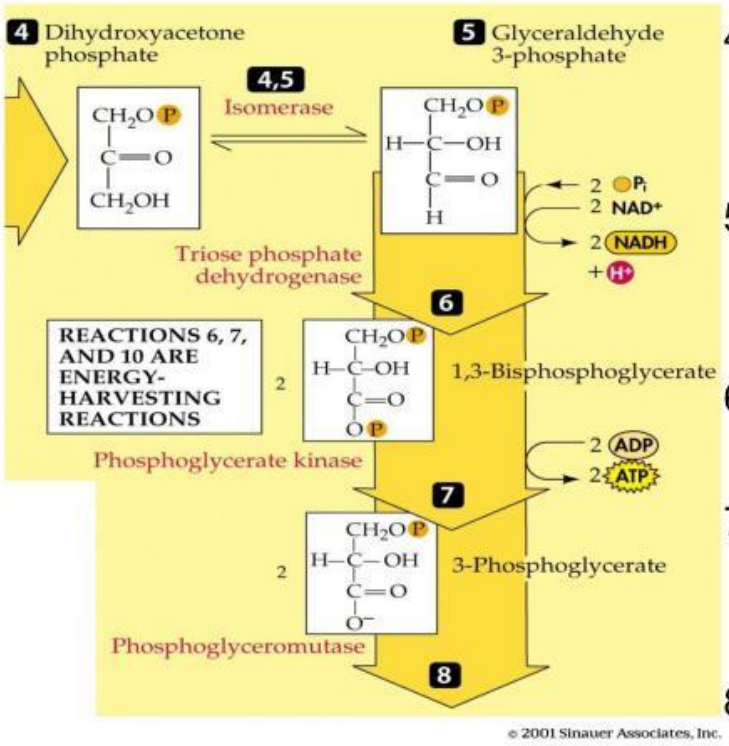
- Catalyzes a **substrate-level phosphorylation**
- Metabolically irreversible reaction
- Regulation both by allosteric modulators and by covalent modification
- Pyruvate kinase gene can be regulated by various hormones and nutrients



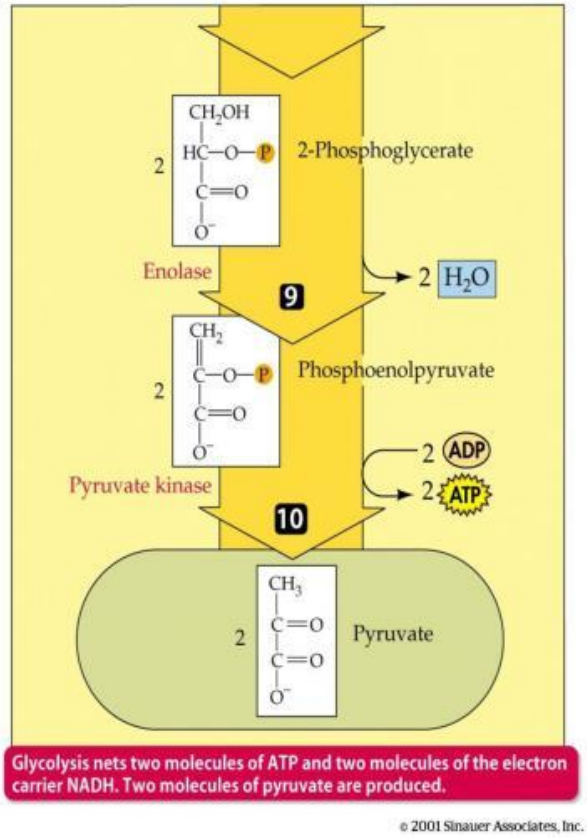
Glycolysis – Energy In

1. ATP phosphorylates glucose
2. Rearrangement
3. Stick on another phosphate (phosphorylation)



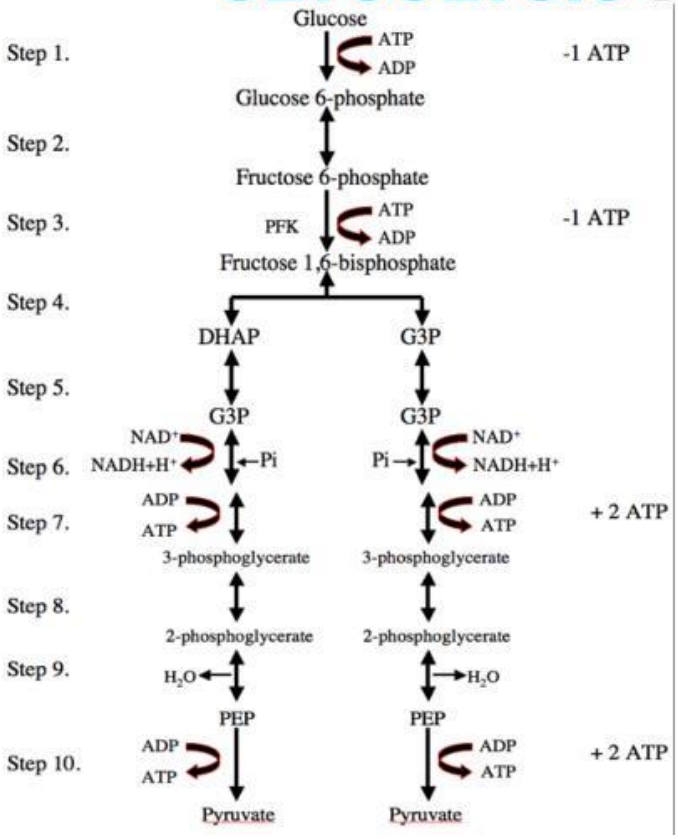


4. Split (F1,6BP) into two things, most important one G3P
5. Take 2nd thing and rearrange into G3P too!
6. Add a phosphate to get some NADH
7. Take off phosphate and put it on ADP (--> ATP)
8. Rearrange to get phosphate in diff place



9. Steal some H2O to convert from 2PG to PEP
10. Take that last phosphate off and the process makes ATP

GLYCOLYSIS PRODUCTS



- 2 **ATP**s are used in steps 1 & 3 to prepare glucose for splitting.
- F 1,6-BP splits into **DHAP** and **G3P**.
- DHAP converts to **G3P**.
- 2 **NADH** are formed in step 6.
- 2 **ATP** are formed by substrate-level phosphorylation in both steps 7 and 10.
- 2 **pyruvates** are produced in step 10.

Control of glycogenolysis

- Phosphorylase kinase
  - Adds Phosphate
  - Activates phosphorylase
  - Requires ATP
- Phosphorylase phosphatase
  - Removes phosphate
  - Inactivates phosphorylase
- Calcium
  - Released by sarcoplasmic reticulum during contractions
  - Speeds glycogenolysis

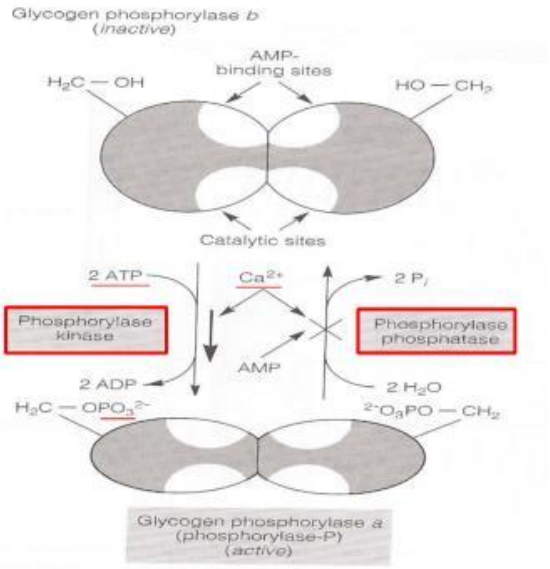


Figure 5-20 The conversion of phosphorylase *b* (the inactive form of the enzyme) to phosphorylase *a* (the active form) depends on the stimulation of phosphorylase kinase by Ca^{2+}. Calcium ions are released immediately when muscles contract, and this mechanism helps to link pathways of ATP supply with those of ATP utilization. During exercise, the levels of AMP increase; this helps to minimize the reconversion of phosphorylase *a* to *b* by inhibiting phosphorylase phosphatase. Modified from McGilvery, 1975.

Lactate shuttle

- Cell-to-cell
  - Lactate produced in glycolytic fibers oxidized in oxidative fibers
  - Lactate released into blood
    - Taken up by oxidative tissue and utilized

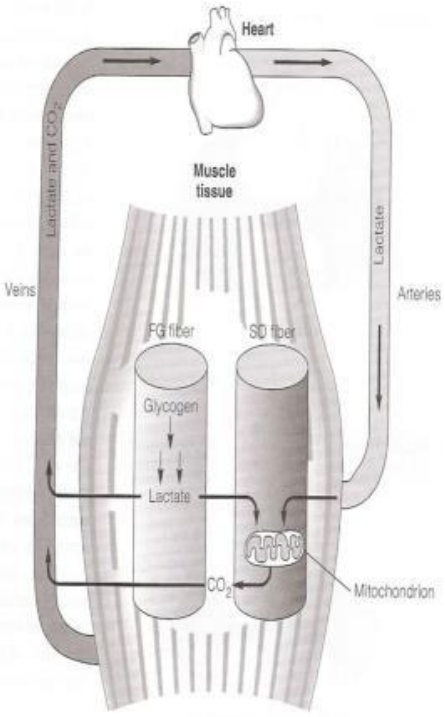


Figure 5-21 Diagram of the cell-cell lactate shuttle. Lactate produced in some cells (e.g., fast glycolytic (FG, type IIb) muscle cells) can shuttle to other cells (e.g., slow oxidative (SO, type I) fibers) and be oxidized. Also, lactate released into the venous blood can recirculate to the active muscle tissue bed and be oxidized. During exercise, the lactate shuttle can provide significant amounts of fuel. Muscle cell membrane lactate transport proteins (MCT1 and MCT4) facilitate lactate release and uptake. (See Brooks [1985] and Brooks et al. [1999] for additional information.)

Gluconeogenesis

- Making of new glucose
  - Mostly in liver
  - Lactate and pyruvate (usu. In the form of alanine) and glycerol can be used by the liver to produce glucose

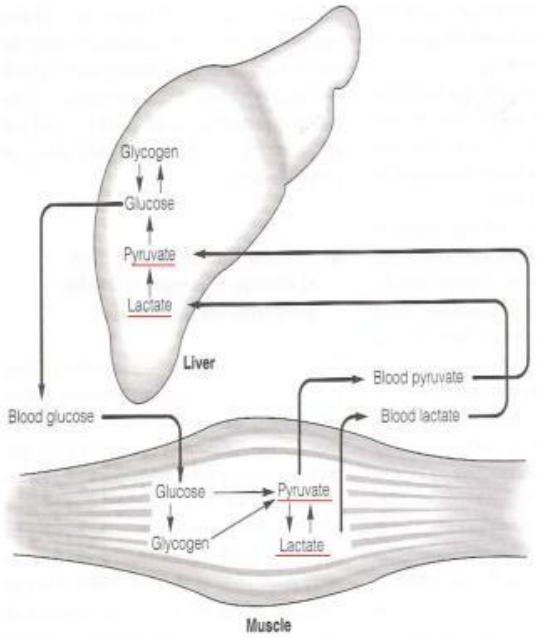


Figure 5-23 The Cori cycle, showing that pyruvate and lactate formed in muscle can circulate to liver and kidney. There, carboxylic acids can be synthesized to glucose. The glucose thus formed can then reenter the circulation. At rest, the lactate/pyruvate ratio (L/P) approximates 10, but rises an order of magnitude during exercise making lactate the more important gluconeogenic precursor.

Gluconeogenesis

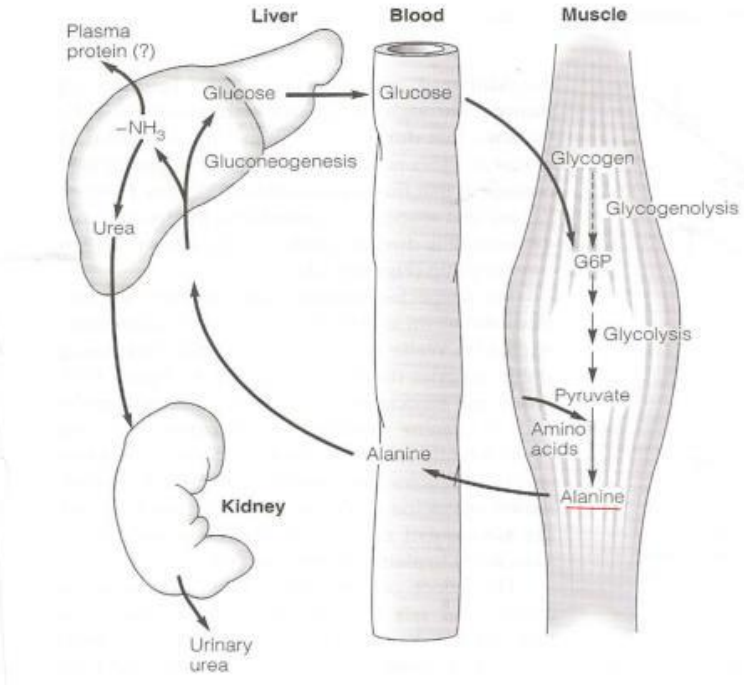
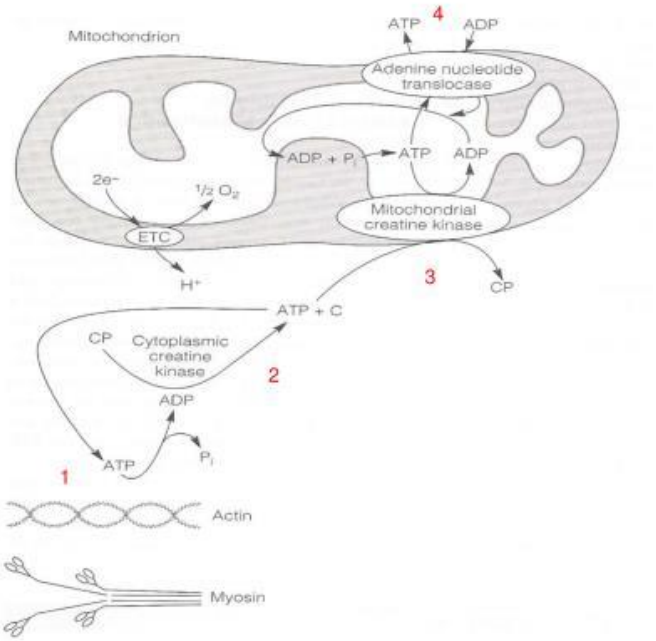


Figure 8-10 The glucose–alanine cycle as proposed by Cahill complements the Cori cycle and lactate shuttle and provides a means for transporting carbon atoms from skeletal muscle to the liver for gluconeogenesis. According to Odessey and Goldberg (1972), the branched-chain amino acids (mainly leucine) provide the nitrogen for amino acid formation in muscle. According to them, the carbon source for alanine formation is glycolytic.

Oxidative phosphorylation

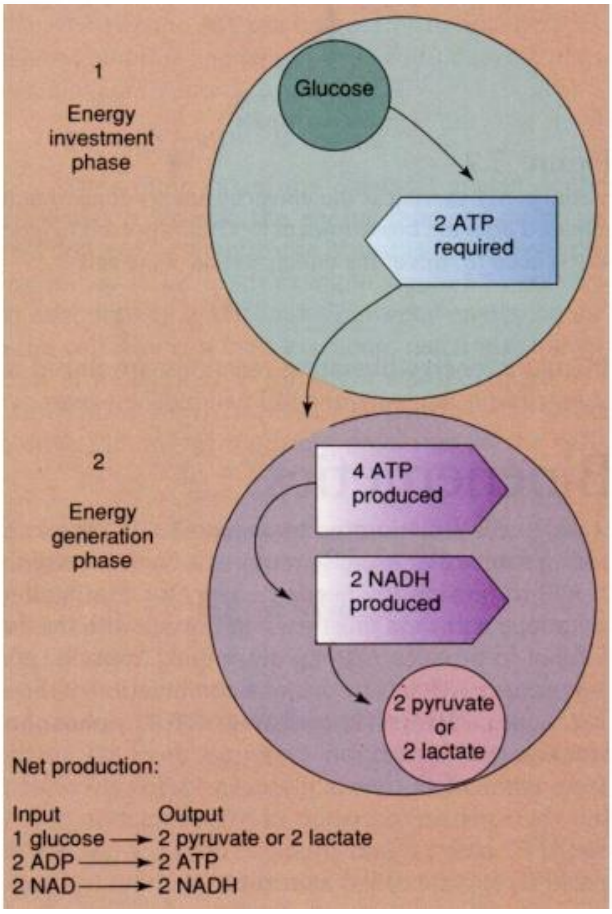
- Chemiosmotic theory of oxidative phosphorylation
  - Peter Mitchell (Nobel prize)
  - Chemical gradient created by proton pumping
  - Allows H^+ to come back in through the F_0-F_1 ATPase
  - This powers ATP formation
  - ETC control
    - ADP stimulates, ATP inhibits
    - Cr^- also stimulates

1) Muscle contraction and ATP breakdown
2) ATP resynthesized by CK (Myofibrillar)
3) Creatine phosphate resynthesized by CK (mito)
4) ATP synthesized by F1-ATPase



ATP yield of glycolysis

- Glycolysis itself
  - 4 ATP produced
  - 2 ATP required
    - Net yield: 2 ATP
  - Aerobic ATP yield from NADH oxidation
    - 6 ATP is malate-aspartate (2 NADH; 3 ATP per)
    - 4 ATP if glycerol-phosphate (2 $FADH_2$; 2 ATP per)
  - Thus, small ATP yield, but the energy conversion is rather efficient (30-50%)



Glycogen synthesis

- Glycogen can be synthesized from 3-C precursors
  - Lactate, amino acids, glycerol
  - Indirect glycogen synthesis
  - Allows for lactate production during exercise and reconversion following
  - Important in keeping liver glycogen and thus, blood glucose steady

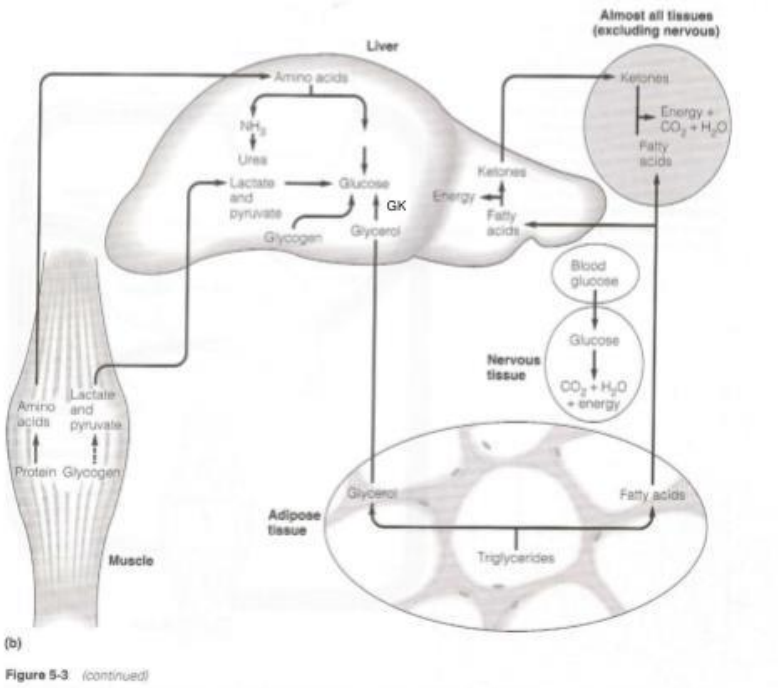


Figure 5-3 *(continued)*

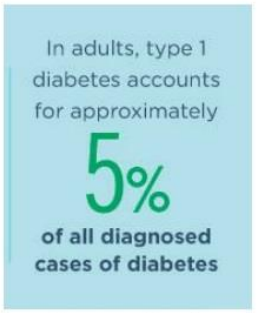
Importance of Blood Glucose

- Source of cellular energy
- Sources
  - Liver
  - Diet
- Use
  - Necessary for glycolysis
  - Necessary for liver/muscle glycogen stores

Types of diabetes:

- Type 1
- Type 2
- Gestational diabetes
- Prediabetes

Type 1 diabetes:

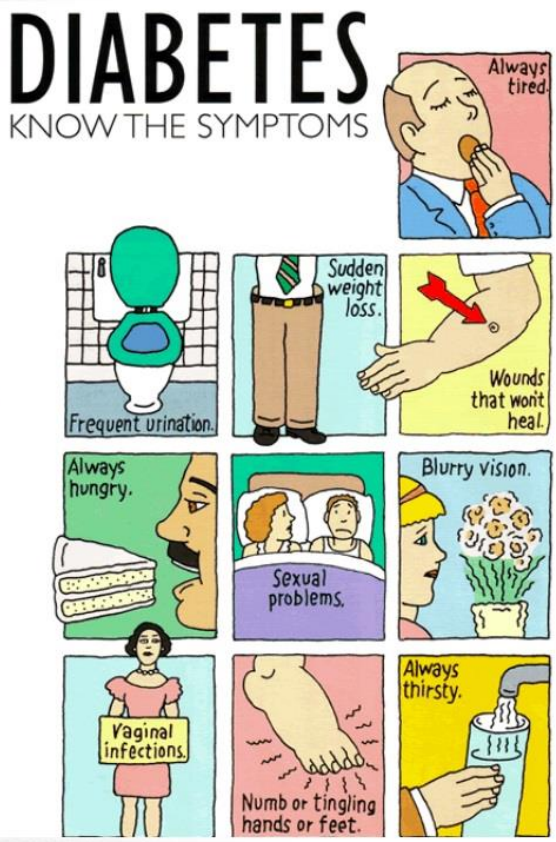


- Also known as juvenile diabetes
- Usually diagnosed in children and young adults
- When body's own immune system destroys the insulin producing cells of the pancreas – beta cells – which produce insulin
- Only 5% of people have this disease
- Body does not produce insulin
- Is not preventable
  - No primary intervention
- Causes?
  - Predisposition to diabetes – genetics - and something (i.e. weather, virus ... etc) in environment triggers the disease

Symptoms of Diabetes:

Common symptoms of diabetes include:

- Excessive thirst and appetite
- Increased urination (sometimes as often as every hour)
- Unusual weight loss or gain
- Fatigue
- Nausea, perhaps vomiting
- Blurred vision
- In women, frequent vaginal infections
- In men and women, yeast infections
- Dry mouth
- Slow-healing sores or cuts
- Itching skin, especially in the groin or vaginal area



Type 2 diabetes:

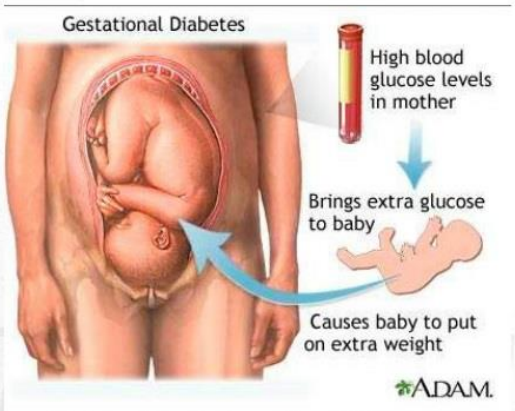
- Most common form of diabetes – about 90% of cases
- Used to be called adult onset, non insulin dependent diabetes
- Body produces insulin, but does not use it properly
  - glucose doesn't move into cells, they pile up in the bloodstream
- sx's when they do occur are often ignored because they may not seem serious

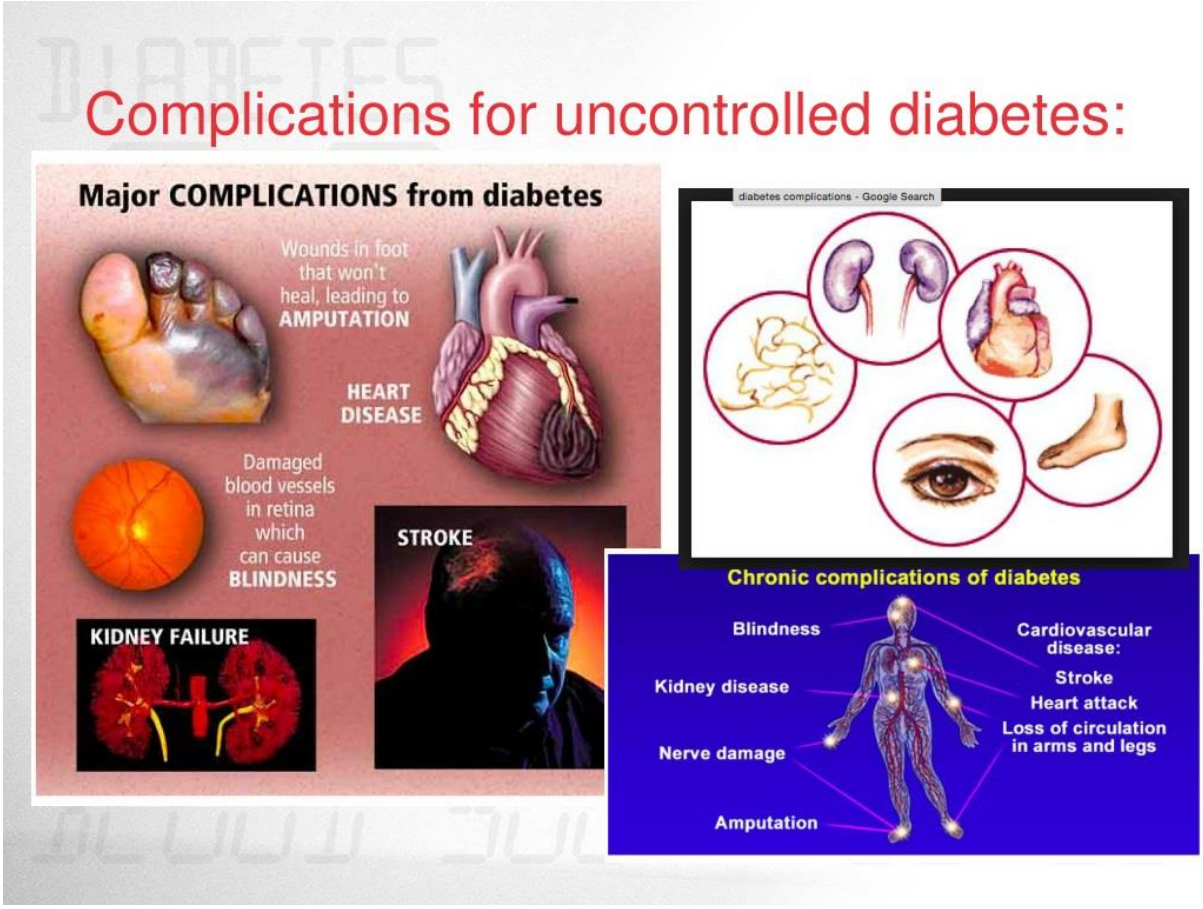
Risk factors:

- Genetics
- Family pmHx
- Polycystic ovary syndrome
  - Irregular menses
- Race
  - African Americans, Hispanics and Asians > whites
- Age
  - After age 45, but increases in younger adults and children
- Environmental factors
  - Inactivity
  - Weight gain

Gestational diabetes mellitus (GDM):

- Having diabetes during pregnancy
  - Family Hx of diabetes, overweight prior to pregnancy?
- Having gestational diabetes puts you at risk for diabetes type 2
- Giving birth to a baby >9 lbs also puts you at risk for type 2
- 18 out of every 100 pregnant females will develop GDM





General material and bulk-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:

Metabolism of carbohydrates.
Carbohydrate metabolism and its regulation.

1. Structure and functions of carbohydrates: monosaccharides, disaccharides, polysaccharides.
2. Glycosaminoglycans: hyaluronic acid, chondroitin sulfate, heparan sulfate. Proteoglycans, their role in the living organisms.
3. Digestion of carbohydrates in the digestive tract.
4. Anaerobic oxidation of carbohydrates. Glycolysis. Glycogenolysis.
5. Oxidative-reduction steps of anaerobic glycolysis, its importance. Shuttle mechanism for the transfer of reducing equivalents of NADH.
6. Aerobic oxidation of glucose. The sequence of reactions and enzymes of glycolysis.
7. Oxidative decarboxylation of pyruvate.

8. In born disturbances of glycogen metabolism: glycogenosis and other glycogen storage diseases.
9. Pentose phosphate pathway of carbohydrates metabolism. Oxidative and non-oxidative phases. Biological importance of specialities of functioning in various tissues.
10. Biosynthesis and catabolism of glycogen in the liver.
11. Mechanism of reciprocal regulation of glycogenolysis and glucogenesis by cAMP dependent cascade phosphorylation of enzymes.
12. Gluconeogenesis. Mechanism in the different tissues and organs. Corey's cycle and the glucose alanine cycle.
13. Hereditary pathology of carbohydrate metabolism: galactosemia, lactose intolerance, glycogen storage diseases.
14. Glucose lactate cycle (Corey's cycle) and glucose alanine cycle and their importance.
15. Pathways of the fructose and galactose metabolism.
16. The comparison of bioenergetics of aerobic and anaerobic oxidation of glucose and glycogen. Pasteur's effect.
17. Irreversible reactions of glycolysis. Their importance in the pathway.

Methodological support:

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/-
3. https://moodle.odmu.edu.ua/login/index.php

Lecture 9.

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

Topic relevance: 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.

Objective: study of the general patterns of lipid metabolism and their use as energy in the human body.

Basic concepts:

1. Monoacyl glycerol.
2. Triacyl glycerol.
3. Carnitine.
4. Ketone bodies.

Plan and organizational structure of the lecture:

1. Biological role of lipids.
2. Oxidation of triacylglycerols.
3. Oxidation of VHDK. Biological role.
4. Oxidation of glycerol.
5. The energy balance of the oxidation of VHL and glycerol.
6. Exchange of acetoacetic acid.
7. Formation of ketone bodies, their biological role.

Content of the lecture material

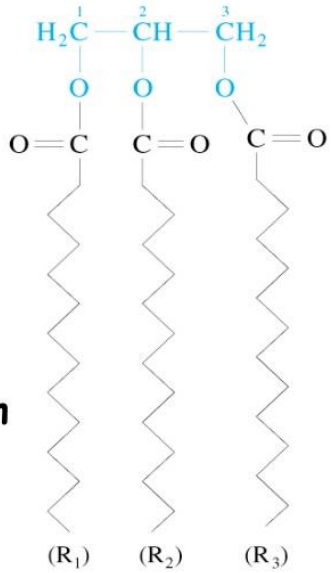
TRIACYLGLYCEROLS ARE HIGHLY CONCENTRATED ENERGY STORES

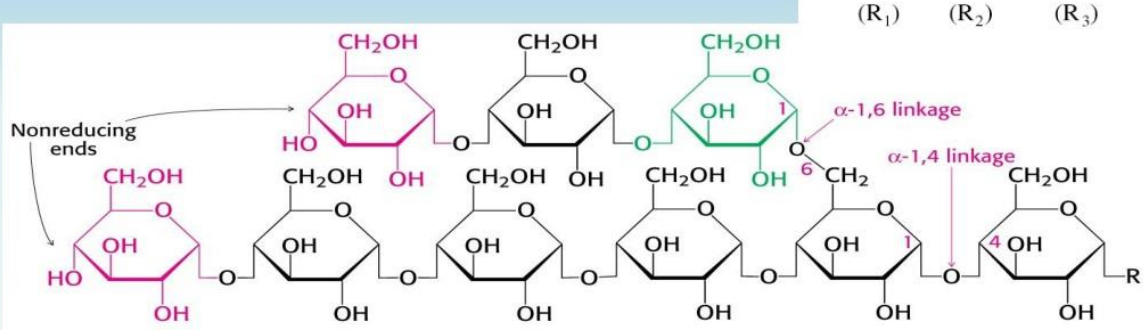
·Triacylglycerols (TGs) and glycogen - two major forms of stored energy

TGs which are more efficient energy stores because:

(1) They are stored in an anhydrous form

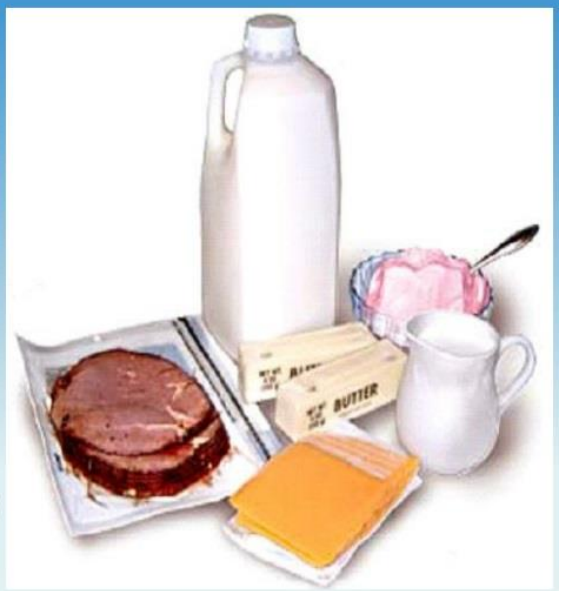
(2) Their fatty acids are more reduced than monosaccharides.





PHYSIOLOGICAL ROLE OF LIPIDS

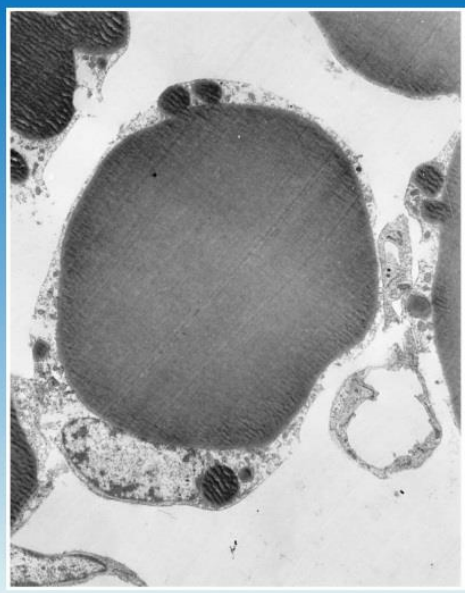
- *Energetic role* (fuel molecules)
- *Components of membranes* (structural role)
- *Precursors for many hormones* (steroids)
- *Signal molecules* (prostaglandins)
- *Protective role* (lipids surround important organs)
- *Enzyme cofactors* (vitamin K)
- *Electron carriers* (ubiquinone)
- *Insulation against temperature extremes*



- Fatty acids and glycerol - substances that are directly used as a fuel by mammalian organisms.
- Fatty acids (FA) and glycerol for metabolic fuels are obtained from triacylglycerols:

  (1) In the diet

  (2) Stored in adipocytes (fat storage cells)
- Free fatty acids occur only in trace amounts in cells

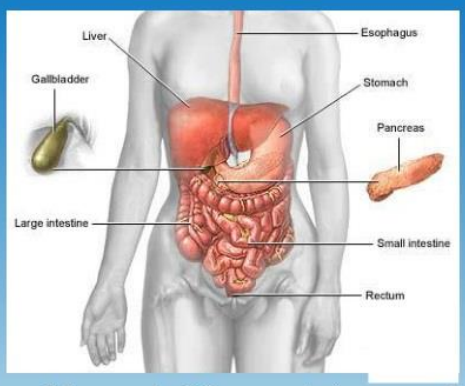


•For supplying of fatty acids as a fuel for organism, the **triacylglycerols have to be digested**

DIGESTION OF DIETARY LIPIDS

Lipids in diet:

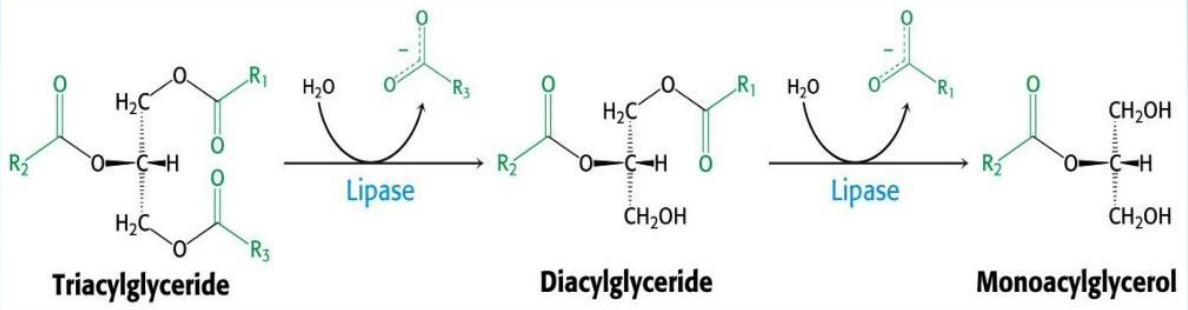
- ***triacylglycerols***
- ***phospholipids***
- ***cholesterol***



Digestion – in small intestine.

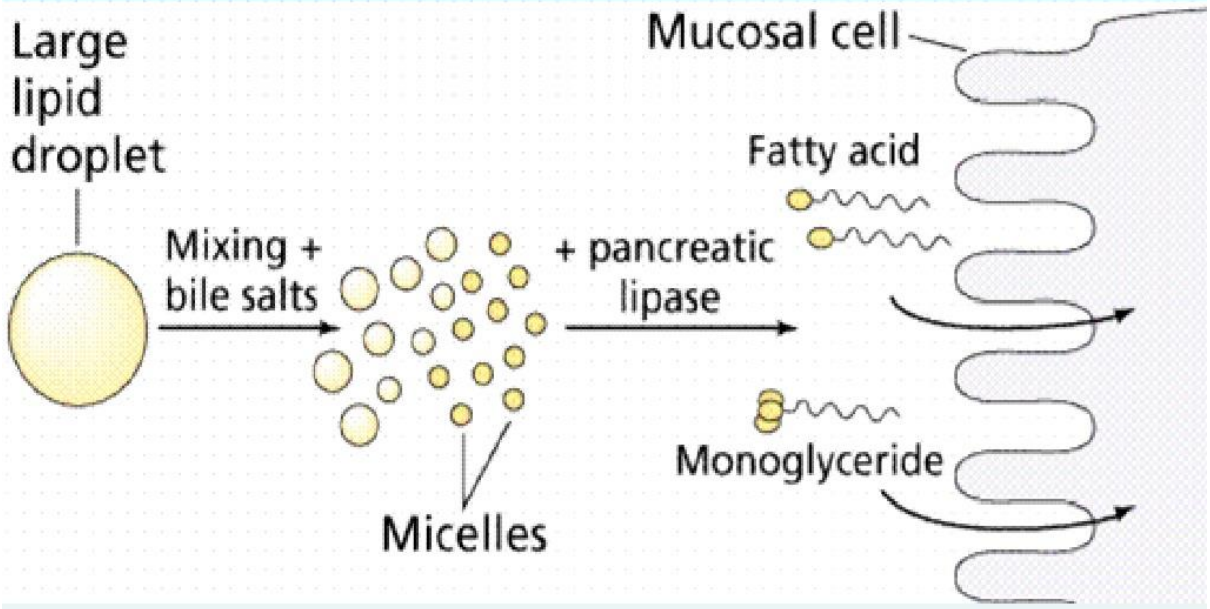
Enzyme – pancreatic ***lipase***.

Lipase catalyzes hydrolysis at the **C1** and **C3** positions of TGs producing ***free fatty acids*** and **2-monoacylglycerol.**



Bile salts also **activates the lipase**.

Inadequate production of bile salts results in ***steatorrhea***.

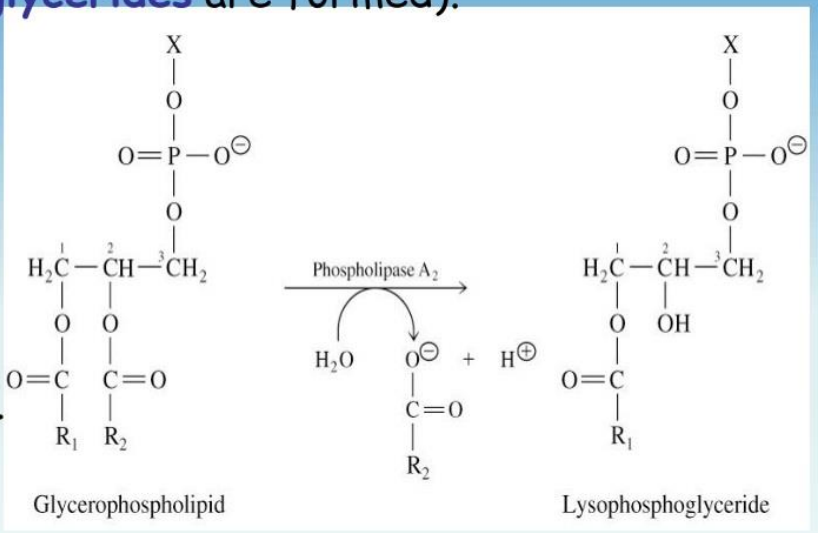


Dietary phospholipids are degraded by phospholipases

Phospholipases are synthesized in the **pancreas**.

Major phospholipase is ***phospholipase A_2*** (catalyses the hydrolysis of ester bond at **C2** of glycerophospholipids and **lysophosphoglycerides** are formed).

Lysophospho-glycerides are absorbed and in the intestinal cells are **reesterified back to glycero-phospholipids.**



Lysophosphoglycerides can act as **detergent** and therefore in high concentration can **disrupt cellular membranes.**

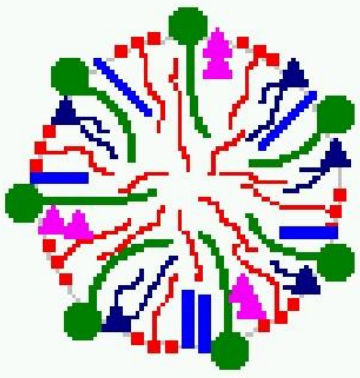
Lysophosphoglyceride is normally present in cells in low concentration.

Snake venom contain **phospholipase A_2** and causes **the lysis of erythrocytes** membranes.

ABSORPTION OF DIETARY LIPIDS

Lipid absorption – passive diffusion process.

2-monoacylglycerols, fatty acids, lysophosphoglycerides, free cholesterol form micelles with bile salts.

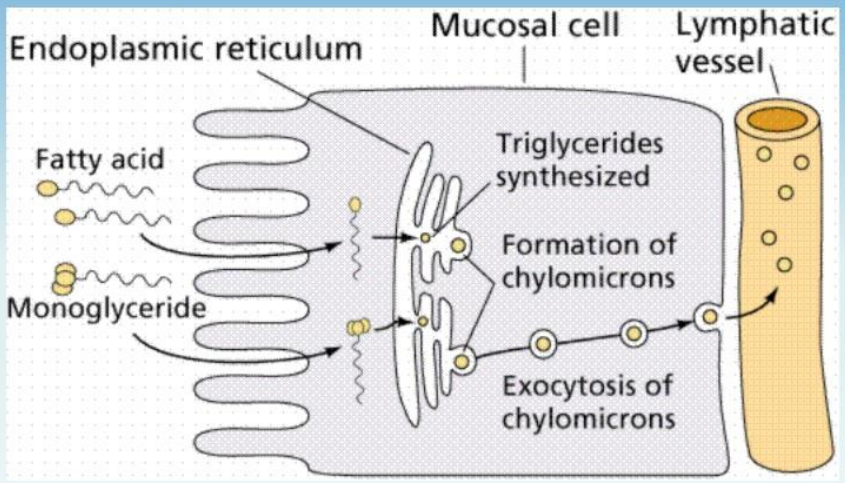


Bile salts
Monoglyceride
Fatty acids
Phospholipids
Cholesterol

Micelles migrate to the microvilli and **lipids diffuse** into the cells.

Bile acids are actively absorbed and transferred to the liver via portal vein.

Bile salts can **circulate through intestine and liver** several time per day.



In the intestinal cells the fatty acids are converted to **fatty acyl CoA molecules.**

Three of these molecules can combine with glycerol, or two with monoacylglycerol, to form a triacylglycerols.

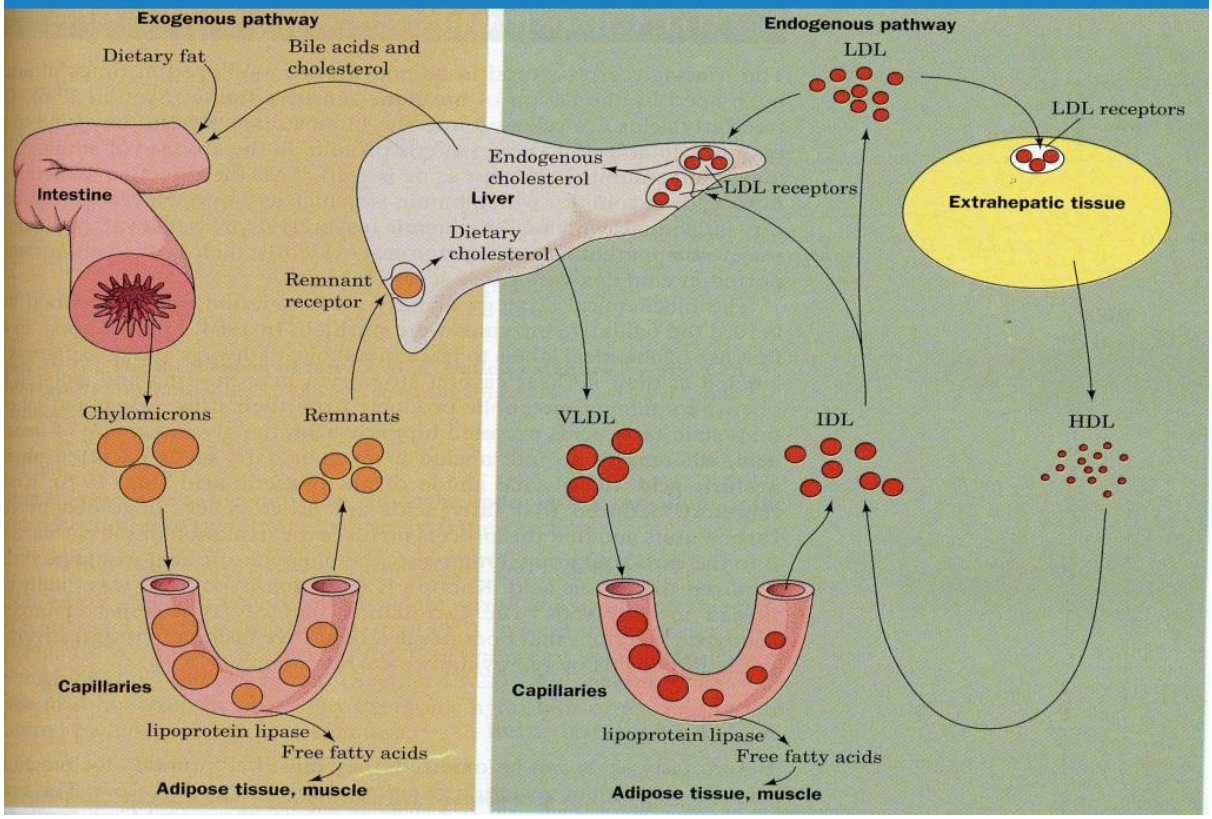
1. $CH_2(OH)-CH(O-C(=O)-R2)-CH_2(OH) + R1-CO-SCoA \rightarrow CH_2(O-C(=O)-R1)-CH(O-C(=O)-R2)-CH_2(OH) + HSCoA$

2. $CH_2(O-C(=O)-R1)-CH(O-C(=O)-R2)-CH_2(OH) + R3-CO-SCoA \rightarrow CH_2(O-C(=O)-R1)-CH(O-C(=O)-R2)-CH_2(O-C(=O)-R3) + HSCoA$

1-st reaction is catalyzed by ***monoacylglycerol acyltransferase***

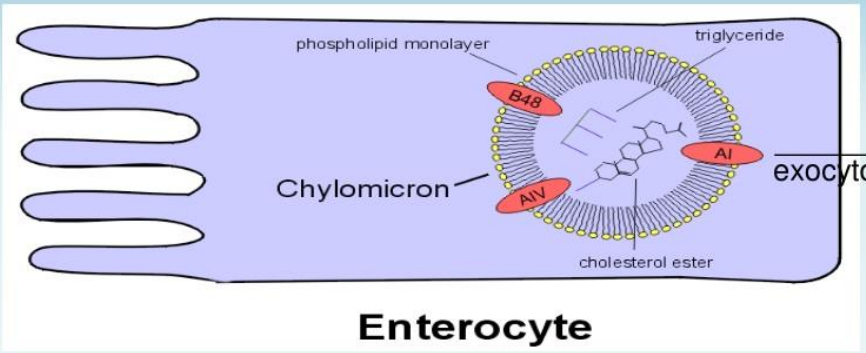
2-nd reaction is catalyzed by ***diacylglycerol acyltransferase***

Transport Forms of Lipids



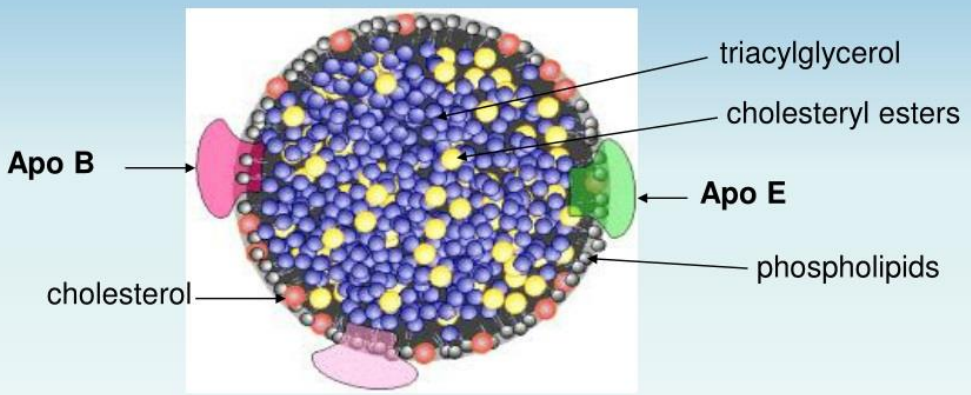
Chylomicrons

- are the largest lipoproteins (180 to 500 nm in diameter)
- are synthesized in the ER of intestinal cells
- contain 85 % of TGs (it is the main transport form of dietary TGs).
- apoprotein B-48 (apo B-48) is the main protein component
- deliver TGs from the intestine (via lymph and blood) to tissues (muscle for energy, adipose for storage).
- bind to membrane-bound *lipoprotein lipase* (at adipose tissue and muscle), where the triacylglycerols are again degraded into free fatty acids and monoacylglycerol for transport into the tissue
- are present in blood only after feeding



VLDL

- are formed in the liver
- contain 50 % of TGs and 22 % of cholesterol
- two lipoproteins — apo B-100 and apo E
- the main transport form of TGs synthesized in the organism (liver)
- deliver the TGs from liver to peripheral tissue (muscle for energy, adipose for storage)
- bind to membrane-bound *lipoprotein lipases* (triacylglycerols are again degraded into free fatty acids and monoacylglycerol)



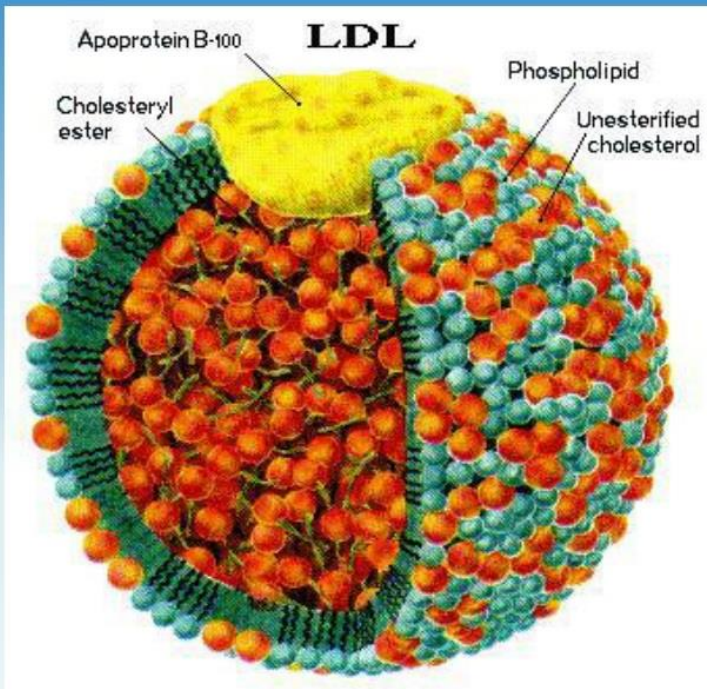
LDL

LDL are formed in the blood from IDL and in liver from IDL (enzyme – *liver lipase*)

LDL are enriched in cholesterol and cholesteryl esters (contain about 50 % of cholesterol)

Protein component - apo B-100

LDL is the major carrier of cholesterol (transport cholesterol to peripheral tissue)



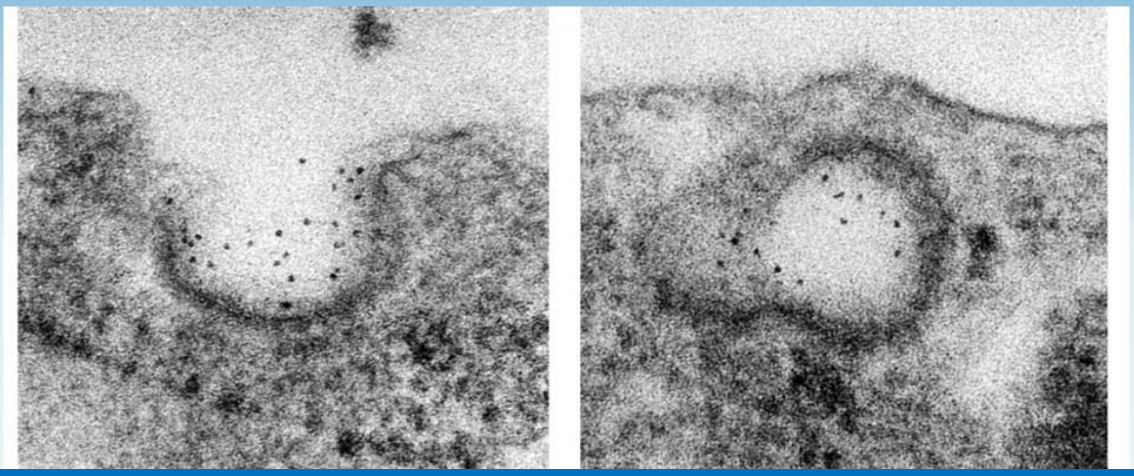
Cells of all organs have **LDL receptors**

Receptors for LDL are localized in specialized regions called ***coated pits***, which contain a specialized protein called ***clathrin***

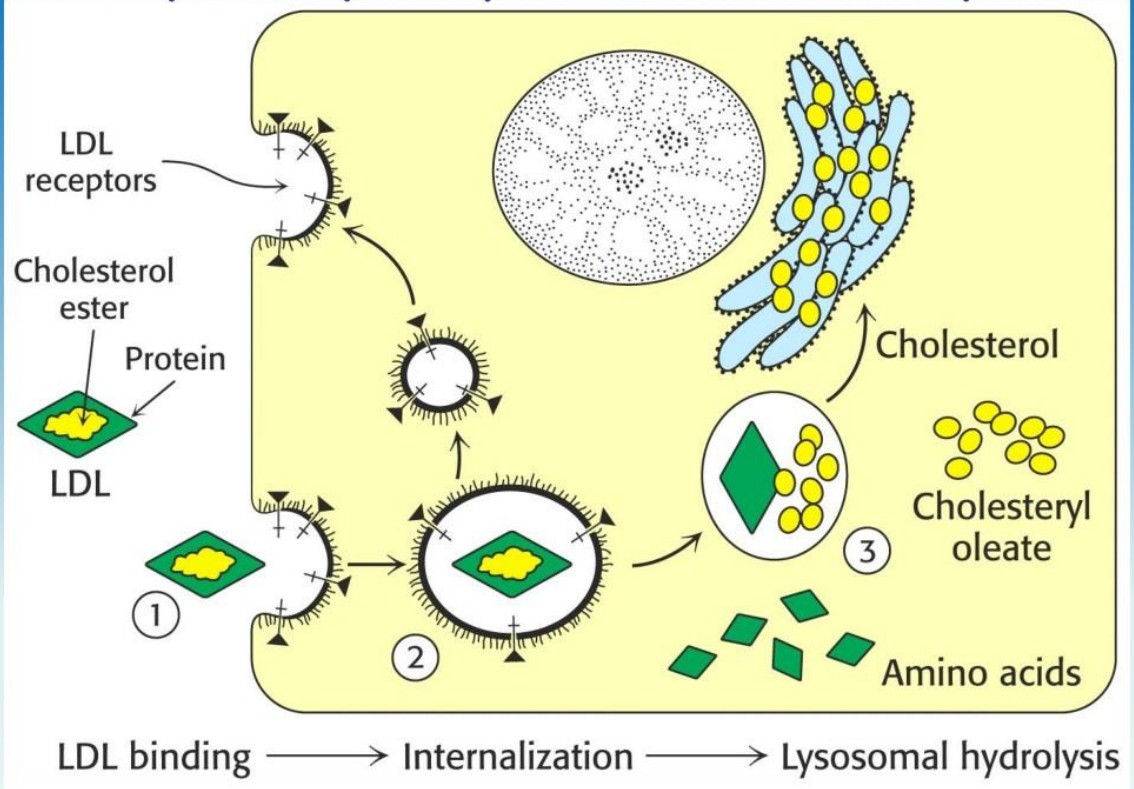
Apo B-100 on the surface of an LDL **binds to the receptor**

Receptor-LDL complex enters the cell by **endocytosis**.

Endocytic vesicle is formed

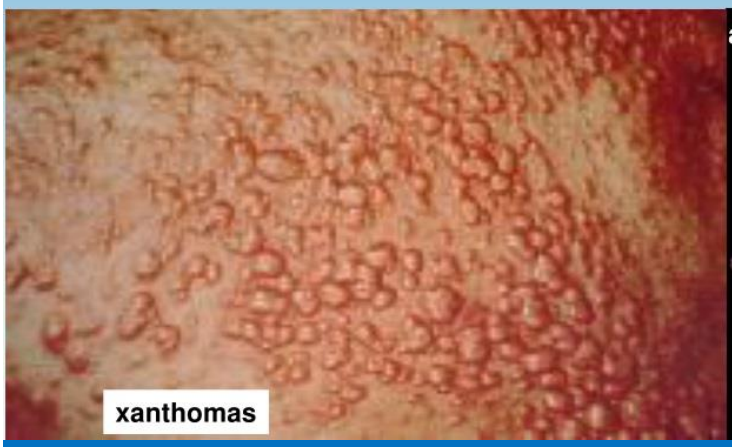


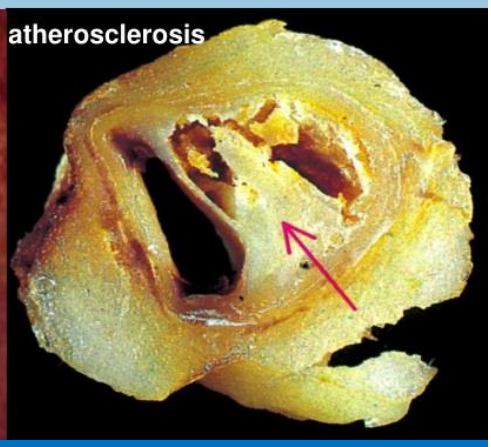
LDL uptake by *receptor-mediated endocytosis*



Familial hypercholesterolemia

- congenital disease when LDL receptor are not synthesized (mutation at a single autosomal locus)
- the concentration of cholesterol in blood markedly increases
- severe atherosclerosis is developed (deposition of cholesterol in arteries)
- nodules of cholesterol called xanthomas are prominent in skin and tendons
- most homozygotes die of coronary artery disease in childhood
- the disease in heterozygotes (1 in 500 people) has a milder and more variable clinical course

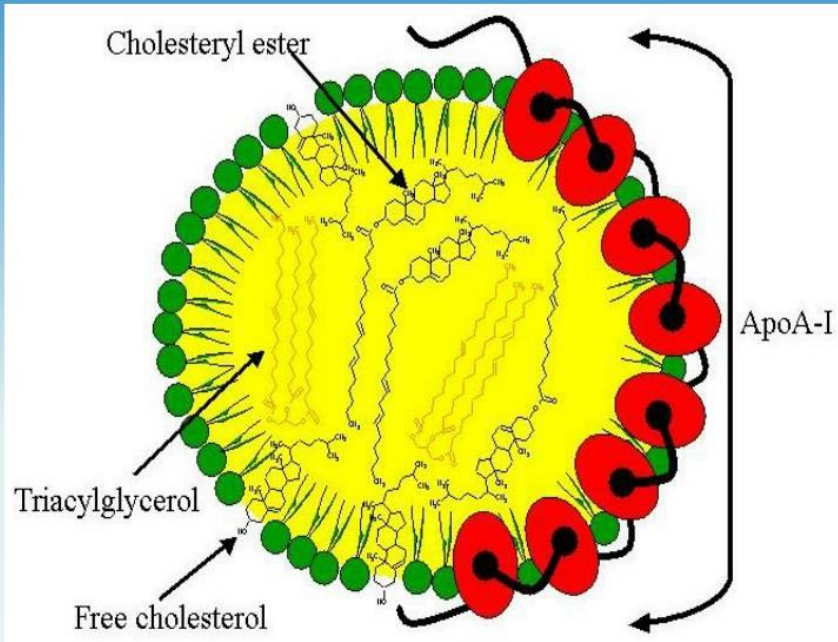


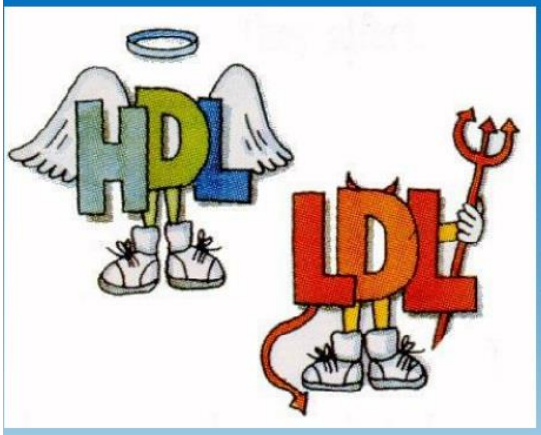


HDL

- are formed in the liver and partially in small intestine
- contain the great amount of proteins (about 40 %)

- pick up the cholesterol from peripheral tissue, chylomicrons and VLDL
- enzyme **acyltransferase** in HDL esterifies cholesterols, convert it to cholesterol esters and transport to the liver



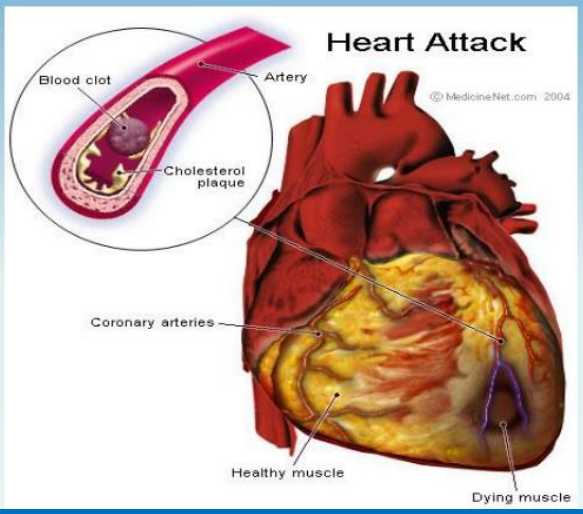


High serum levels of cholesterol cause disease and death by contributing to development of atherosclerosis

Cholesterol which is present in the form of the LDL is so-called "bad cholesterol."

Cholesterol in the form of HDL is referred to as "good cholesterol"

HDL functions as a shuttle that moves cholesterol throughout the body



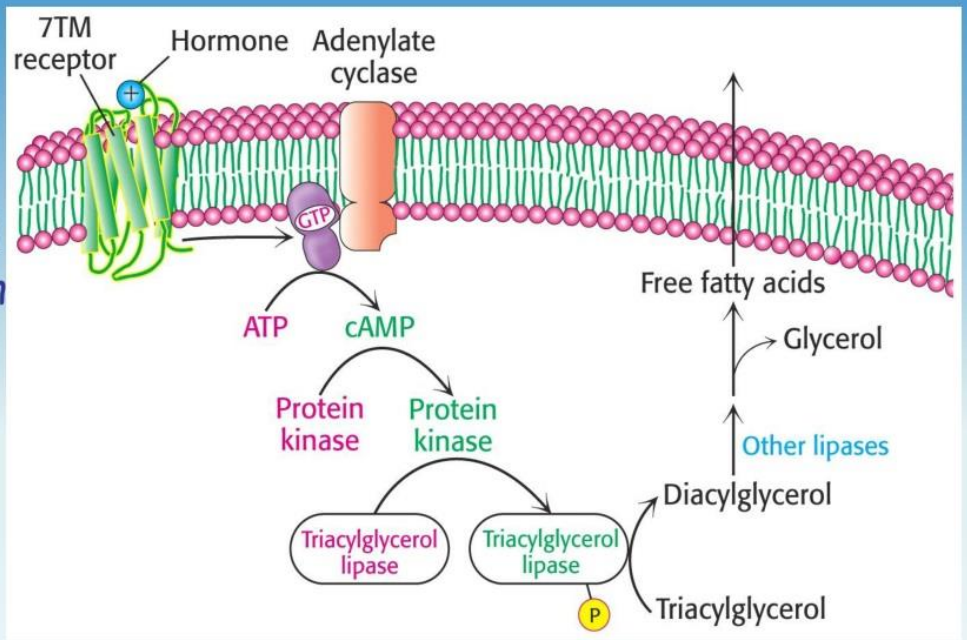
LDL/HDL Ratio

The ratio of cholesterol in the form of LDL to that in the form of HDL can be used to evaluate susceptibility to the development of atherosclerosis

For a healthy person, the LDL/HDL ratio is 3.5

At low carbohydrate and insulin concentrations (during fasting), TG hydrolysis is stimulated by *epinephrine, norepinephrine, glucagon,* and *adrenocorticotropic hormone.*

TG hydro-lysis is inhibited by *insulin* in fed state



Transport of Fatty Acids and Glycerol

- Fatty acids and glycerol diffuse through the adipocyte membrane and enter bloodstream.
- Glycerol is transported via the blood in free state and oxidized or converted to glucose in liver.
- Fatty acids are traveled bound to albumin.
- In heart, skeletal muscles and liver they are oxidized with energy release.

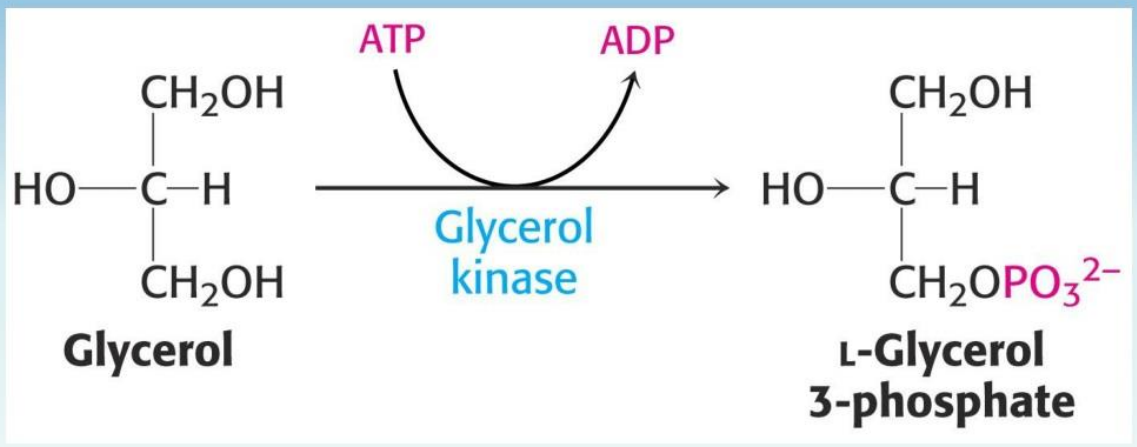
Oxidation of Glycerol

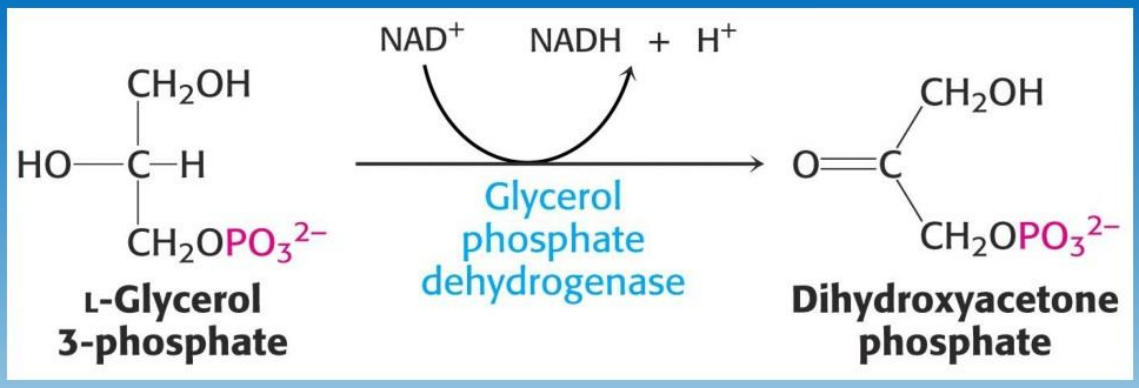
Glycerol is absorbed by the liver.

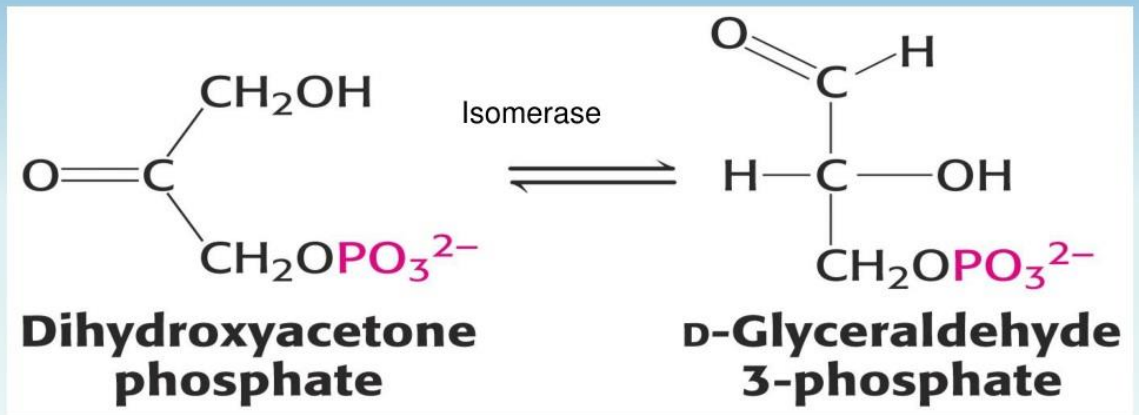
Steps: phosphorylation, oxidation and isomerisation.

Glyceraldehyde 3-phosphate is an intermediate in:

- ***glycolytic pathway***
- ***gluconeogenic pathways***







Stages of fatty acid oxidation

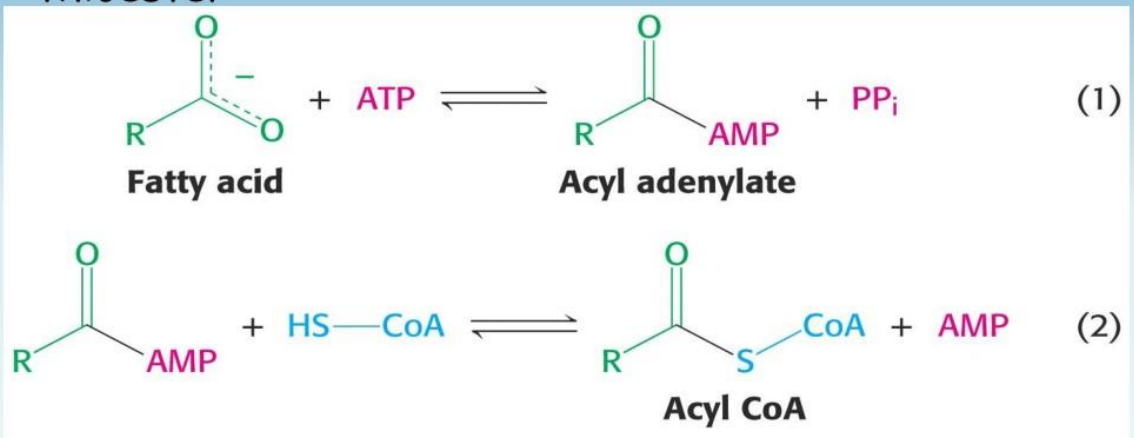
(1) **Activation** of fatty acids takes place **on the outer mitochondrial membrane**

(2) **Transport into the mitochondria**

(3) **Degradation** to two-carbon fragments (as acetyl CoA) **in the mitochondrial matrix** (b-oxidation pathway)

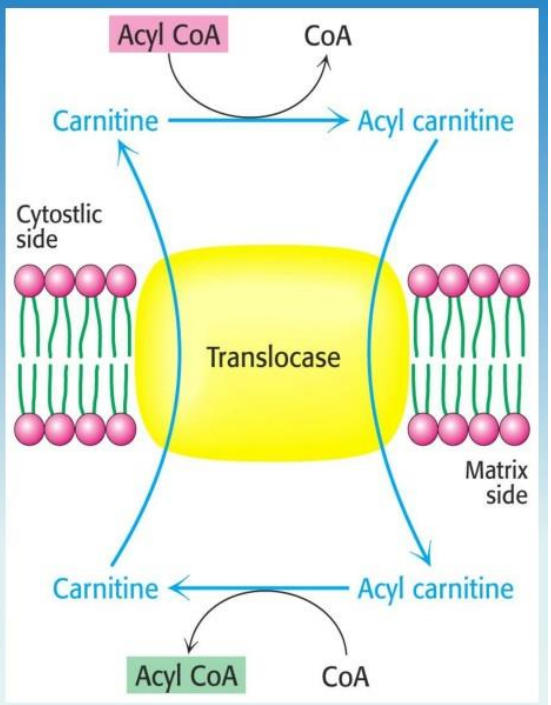
(1) Activation of Fatty Acids

- Fatty acids are converted to CoA thioesters by ***acyl-CoA synthetase*** (ATP dependent)
- The PP_i released is hydrolyzed by a ***pyrophosphatase*** to 2 P_i
- Two phosphoanhydride bonds (two ATP equivalents) are consumed to activate one fatty acid to a thioester

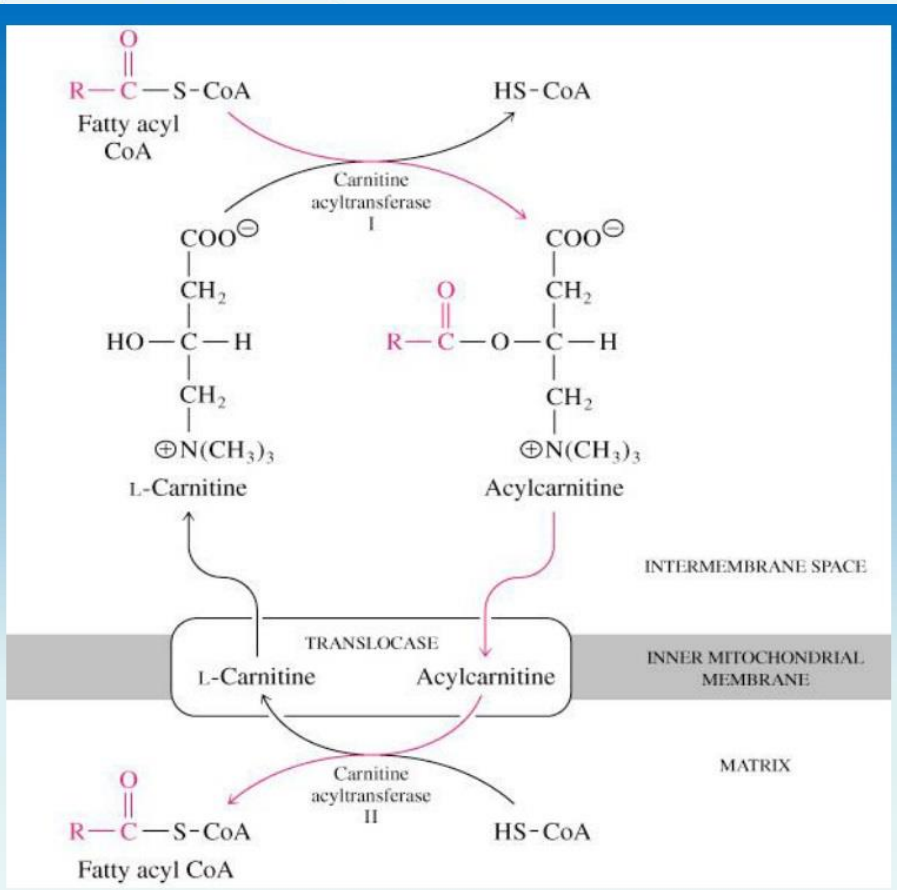


(2) Transport of Fatty Acyl CoA into Mitochondria

- The carnitine shuttle system.
- Fatty acyl CoA is first converted to acylcarnitine (enzyme *carnitine acyltransferase I* (bound to the outer mitochondrial membrane).
- Acylcarnitine enters the mitochondria by a *translocase.*
- The acyl group is transferred back to CoA (enzyme - *carnitine acyltransferase II*).

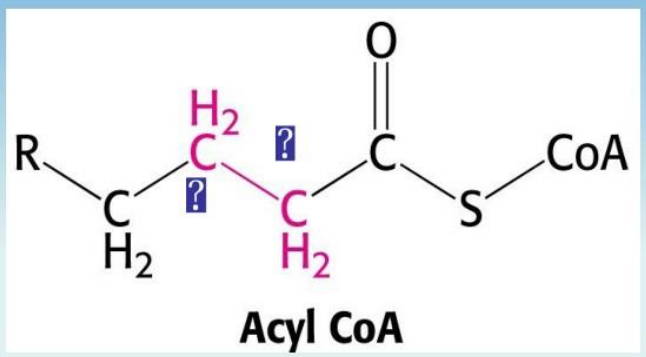


- **Carnitine shuttle system**
- Path of acyl group in red



(3) The Reactions of b oxidation

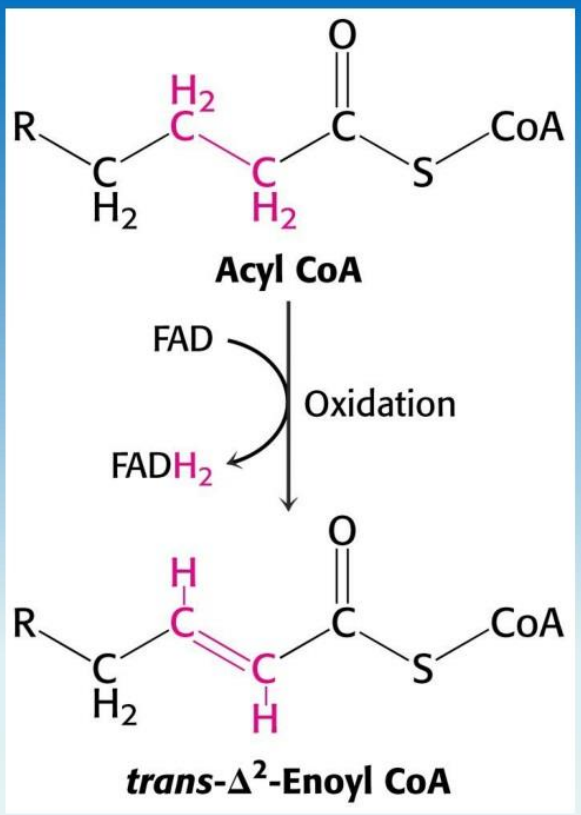
- The b-oxidation pathway (b-carbon atom (C3) is oxidized) degrades fatty acids two carbons at a time



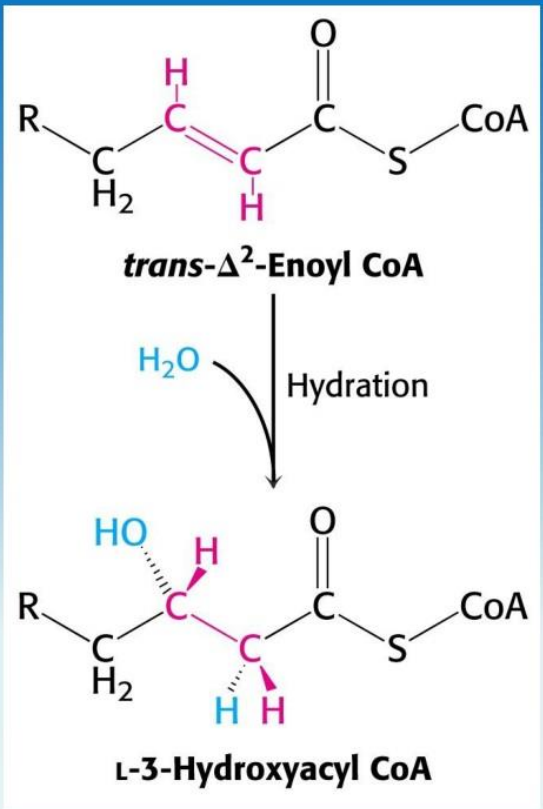
Acyl CoA

1. Oxidation of acyl CoA by an *acyl CoA dehydrogenase* to give an enoyl CoA

Coenzyme - FAD

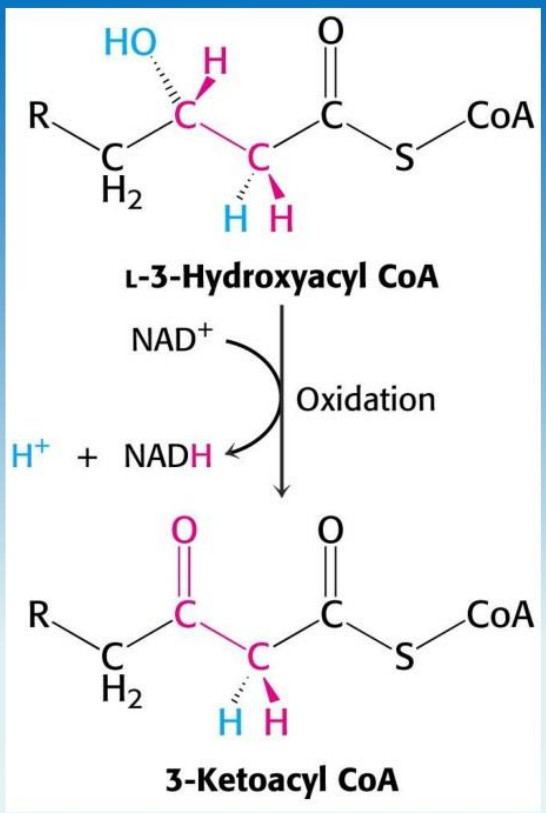


2. Hydration of the double bond between C-2 and C-3 by *enoyl CoA hydratase* with the 3-hydroxyacyl CoA (b-hydroxyacyl CoA) formation



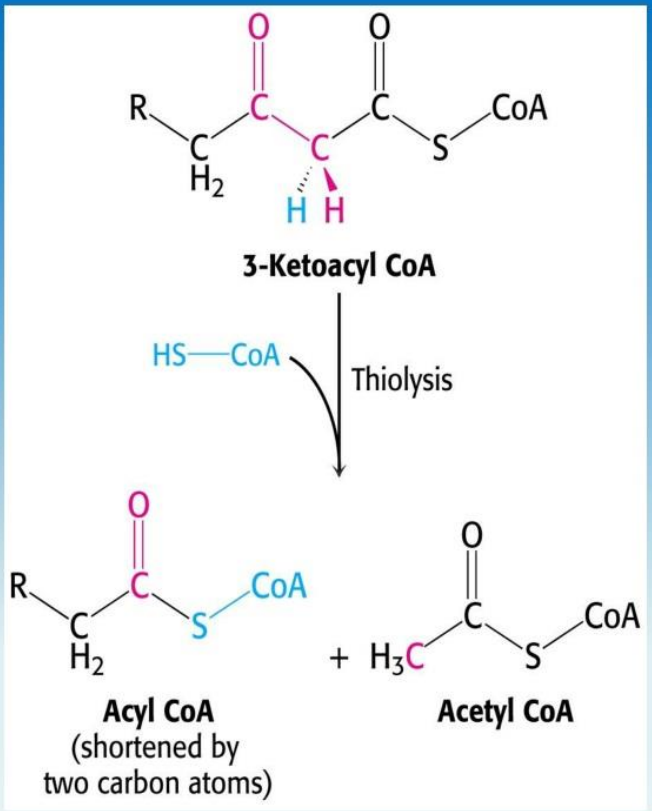
3. Oxidation of 3-hydroxyacyl CoA to 3-ketoacyl CoA by *3-hydroxyacyl CoA dehydrogenase*

Coenzyme – NAD^+

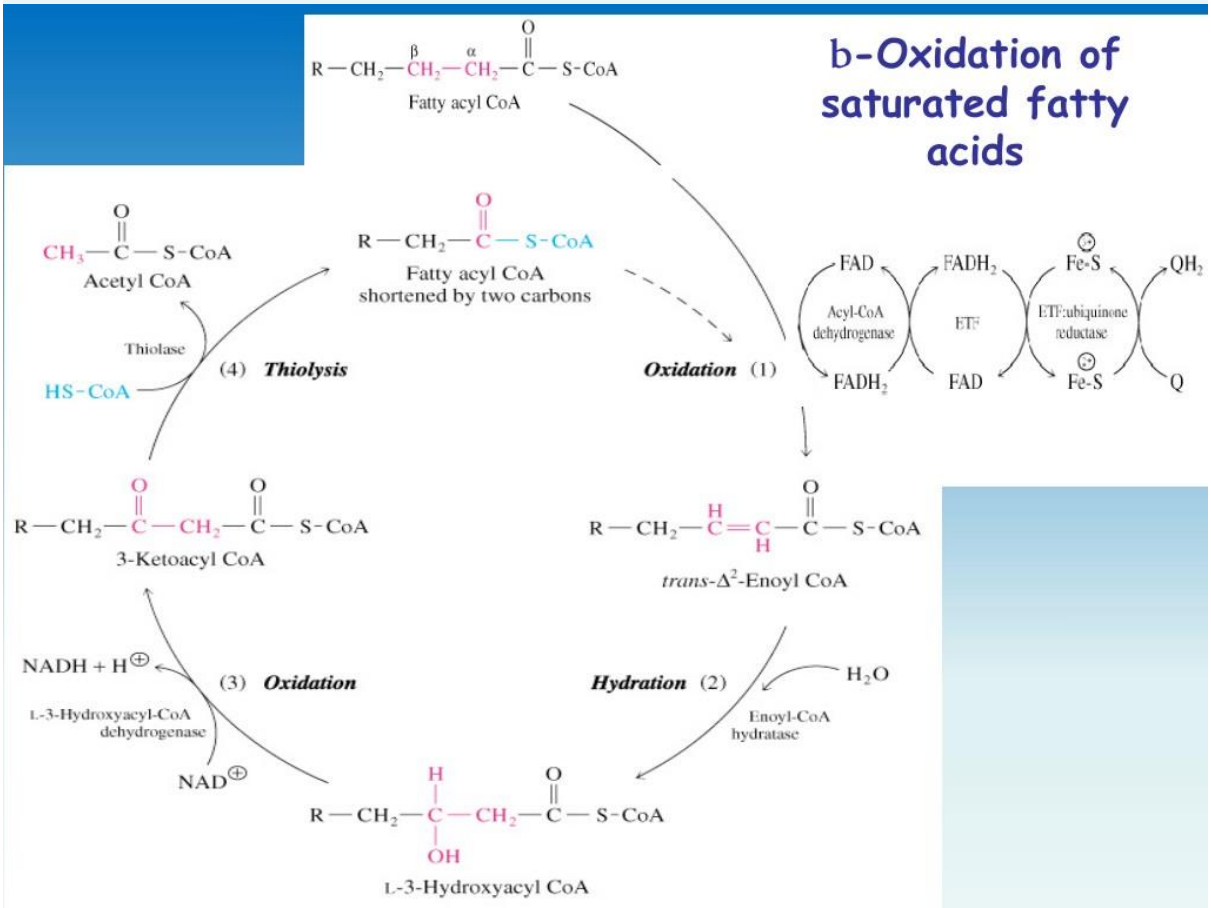


4. Cleavage of 3-ketoacyl CoA by the thiol group of a second molecule of CoA with the formation of acetyl CoA and an acyl CoA shortened by two carbon atoms.

Enzyme - *b-ketothiolase.*



b-Oxidation of saturated fatty acids



- One round of β oxidation: **4 enzyme steps** produce acetyl CoA from fatty acyl CoA
- **Each round** generates one molecule each of:
  $FADH_2$
  NADH
  Acetyl CoA
  Fatty acyl CoA (2 carbons shorter each round)

Fates of the products of β-oxidation:
- NADH and $FADH_2$ - are used in ETC
- acetyl CoA - enters the citric acid cycle
- acyl CoA – undergoes the next cycle of oxidation

ATP Generation from Fatty Acid Oxidation

Net yield of ATP per one oxidized palmitate

Palmitate ($C_{15}H_{31}COOH$) - 7 cycles – n/2-1

- The balanced equation for oxidizing one palmitoyl CoA by seven cycles of b oxidation

Palmitoyl CoA + 7 HS-CoA + 7 FAD^+ + 7 NAD^+ + 7 ~~H_2O~~ →
8 Acetyl CoA + 7$FADH_2$ + 7 NADH + 7 H^+

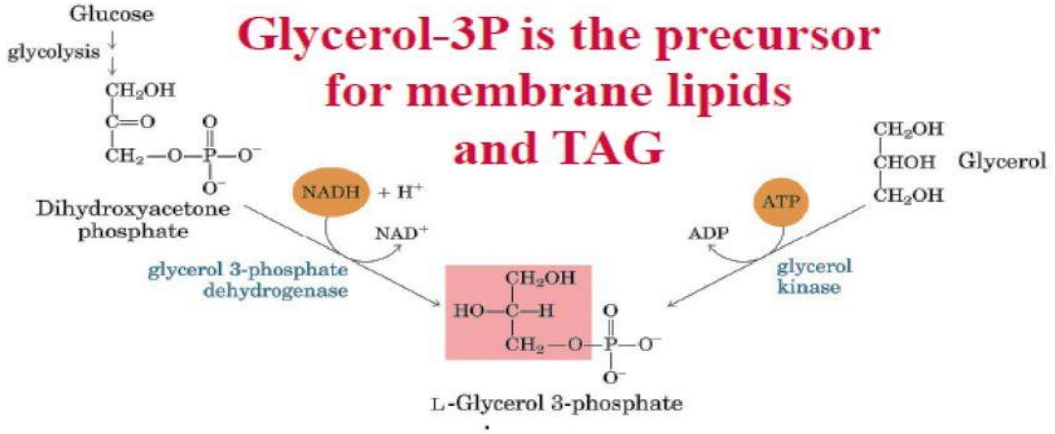
	ATP generated
8 acetyl CoA	10x8=80
7 $FADH_2$	7x1.5=10.5
7 NADH	7x2.5=17.5
	108 ATP
ATP expended to activate palmitate	-2
Net yield:	**106 ATP**

Biosynthesis of glycerol

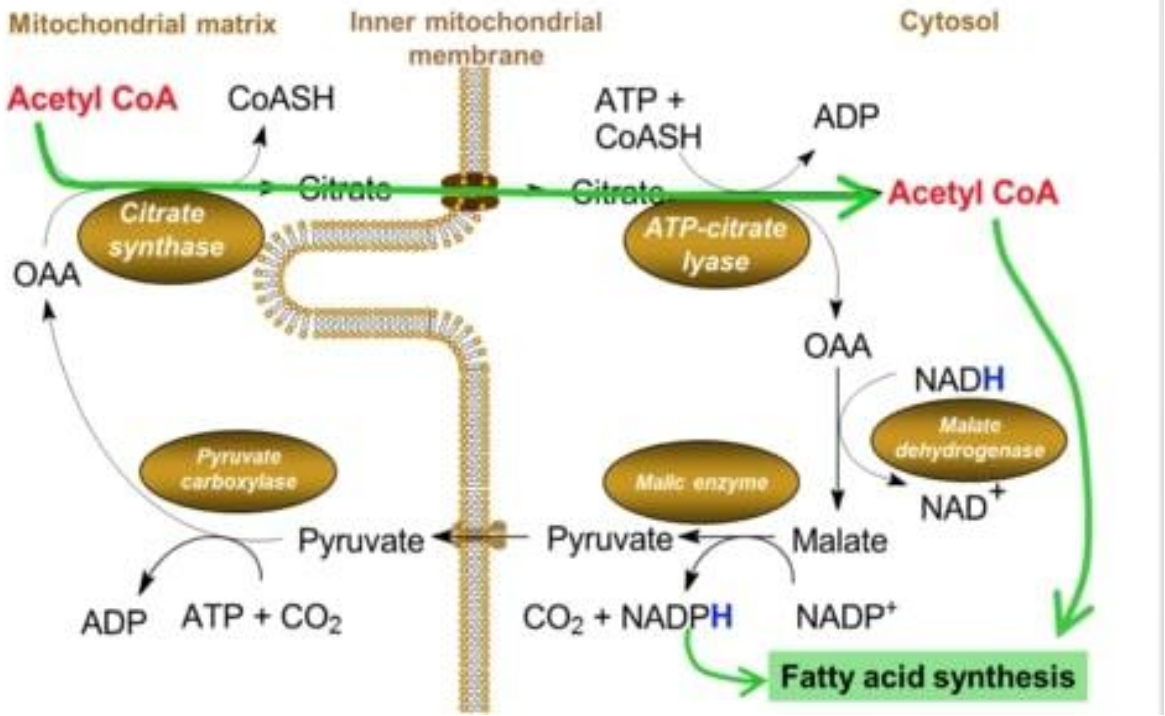
- Glucose is oxidized via glycolysis to **dihydroxy acetone phosphate**

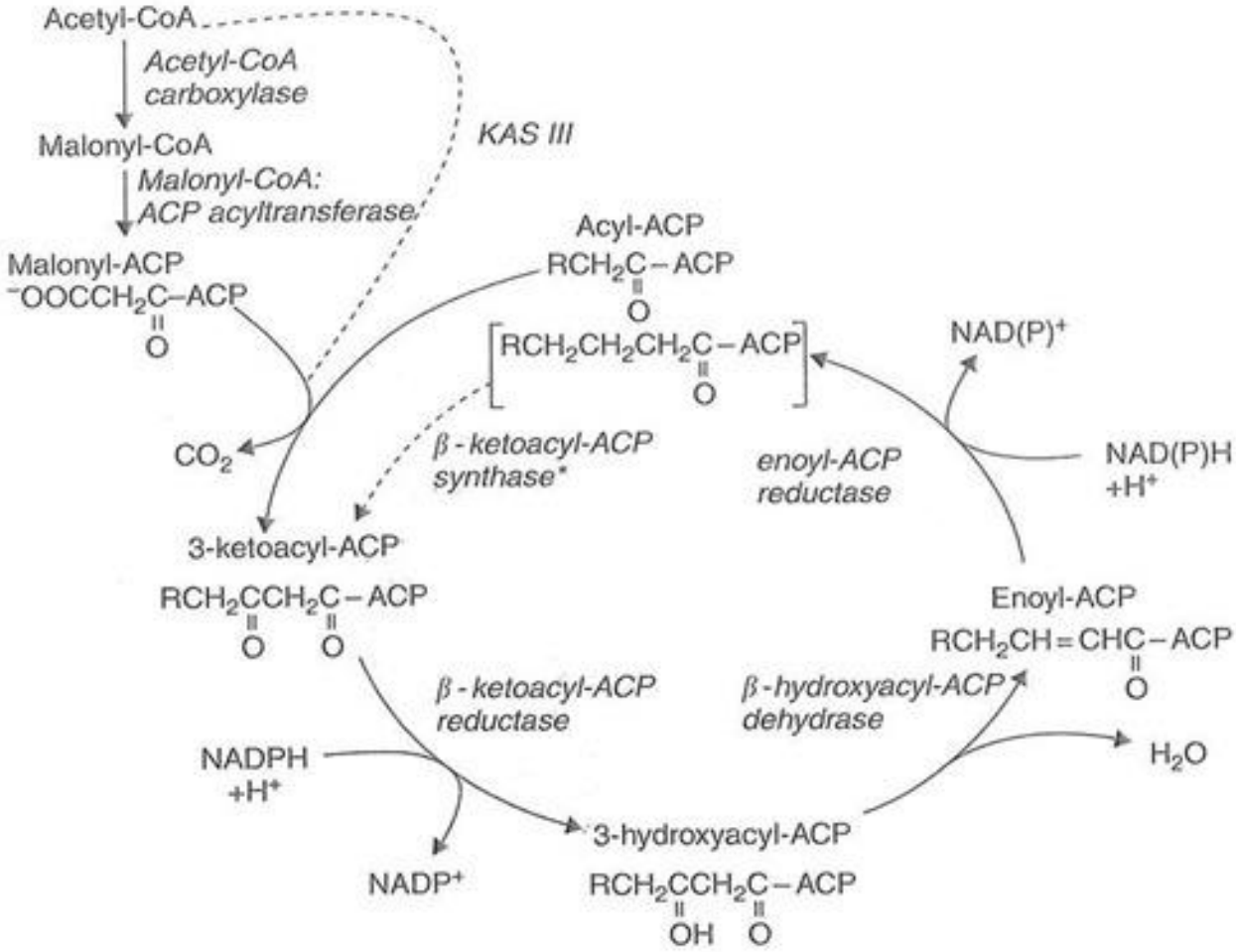
  ⬇

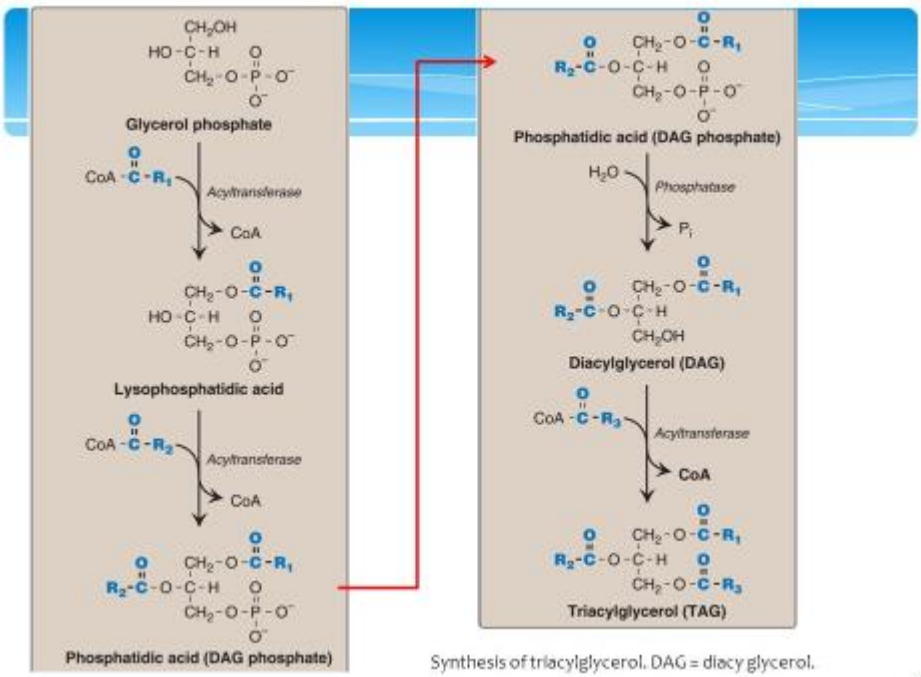
  reduced to **glycerol-3 phosphate** by the enzyme glycerol-3 phosphate dehydrogenase.



The citrate shuttle transfers acetyl CoA from mitochondria to cytosol

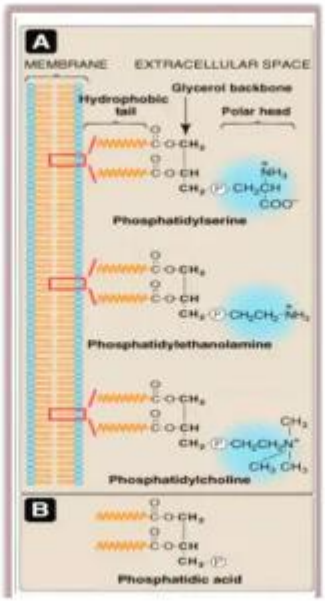


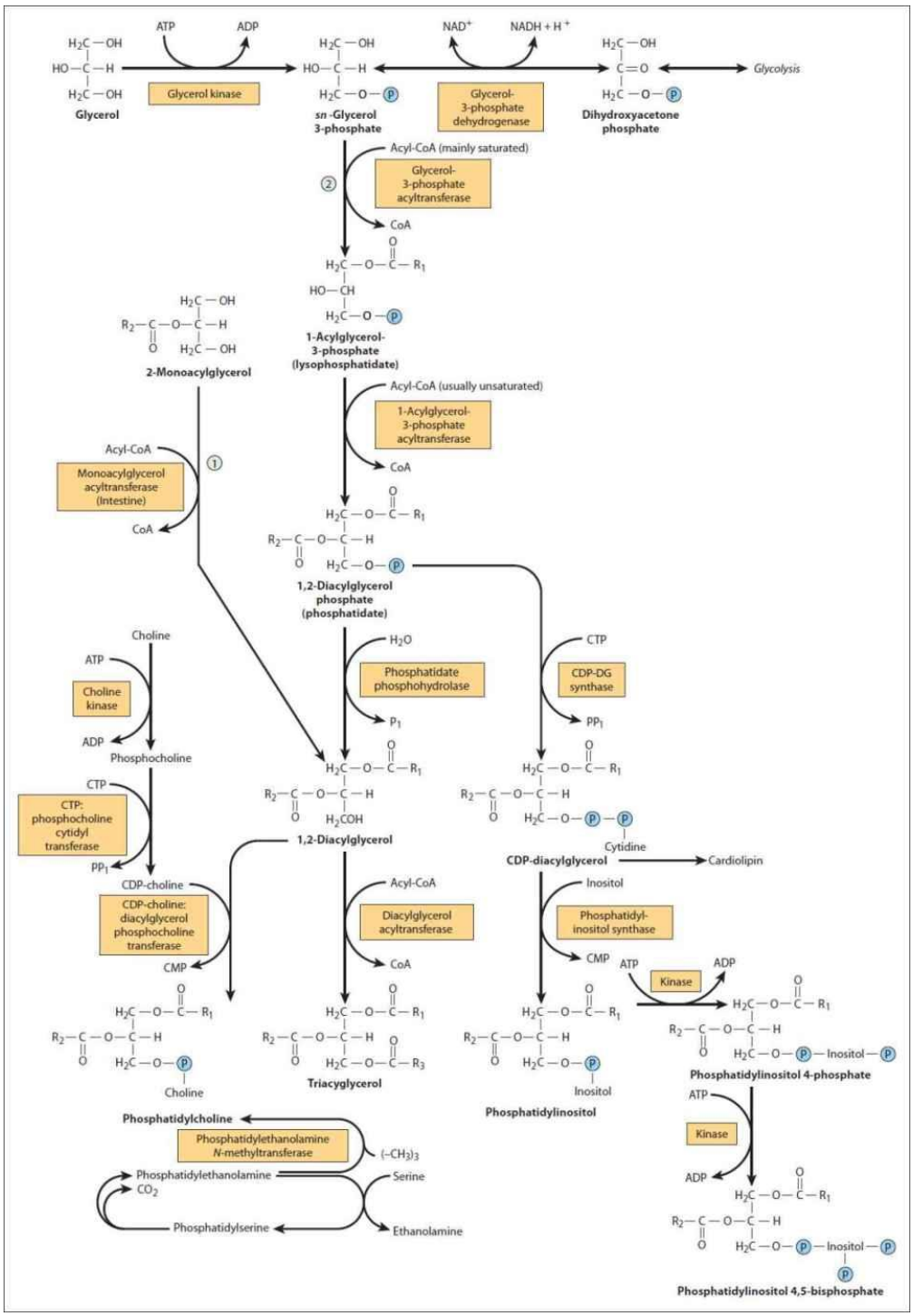




Synthesis of triacylglycerol. DAG = diacy glycerol.

Phosphilipids



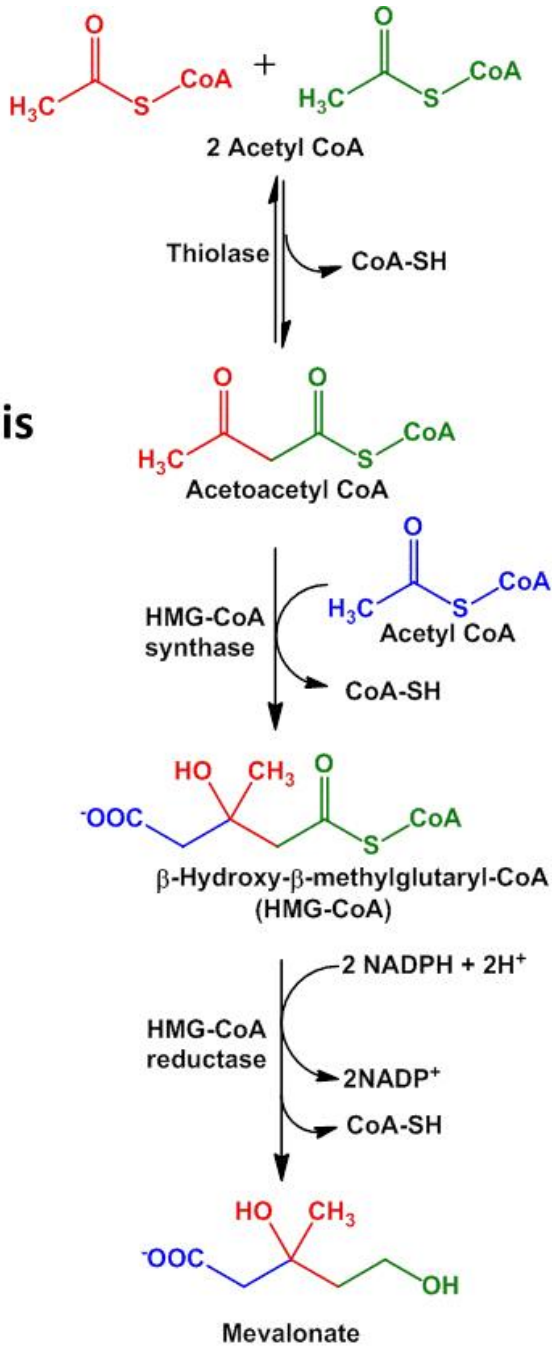

ATP
ADP
Glycerol kinase
Glycerol
sn -Glycerol 3-phosphate
NAD+
NADH + H+
Glycerol-3-phosphate dehydrogenase
Dihydroxyacetone phosphate
Glycolysis
Acyl-CoA (mainly saturated)
Glycerol-3-phosphate acyltransferase
CoA
1-Acylglycerol-3-phosphate (lysophosphatidate)
2-Monoacylglycerol
Acyl-CoA (usually unsaturated)
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
Choline
Triacylglycerol
Phosphatidylinositol
Phosphatidylinositol 4-phosphate
Phosphatidylinositol 4,5-bisphosphate
Phosphatidylcholine
Phosphatidylethanolamine N-methyltransferase
(–CH3)3
Phosphatidylethanolamine
CO2
Serine
Phosphatidylserine
Ethanolamine

FUNCTIONS OF CHOLESTEROL

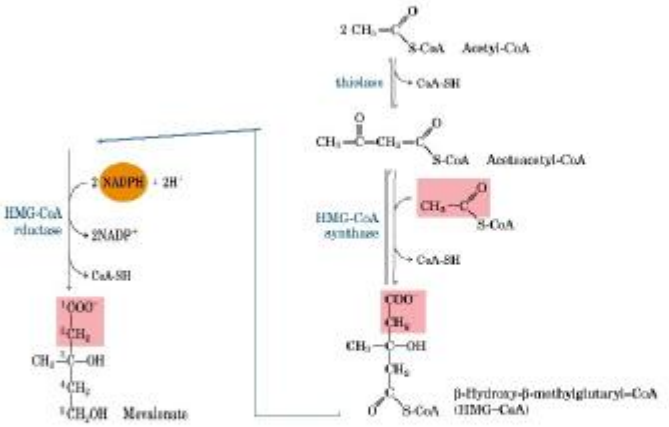
Cholesterol is the most abundant sterol in humans and performs a number of essential functions. For example-

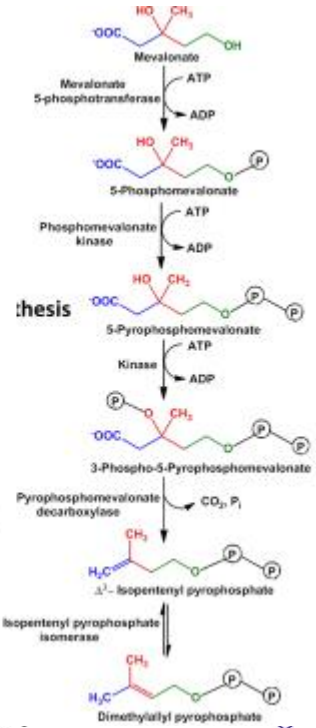
- It is a major constituent of the plasma membrane and of plasma lipoproteins.
- It is a precursor of bile salts,
- It is a precursor of steroid hormones that include adrenocortical hormones, sex hormones, placental hormones etc
- Also a precursor of vitamin D, cardiac glycosides, Sitosterol of the plant 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

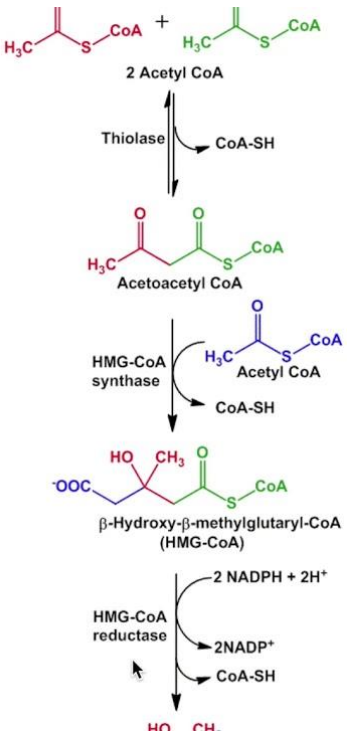


- Mevalonate formation is the first stage of cholesterol synthesis (summary so far)



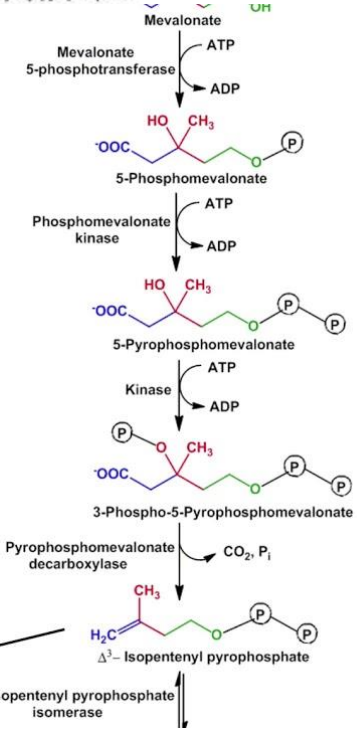


PharmaXChange

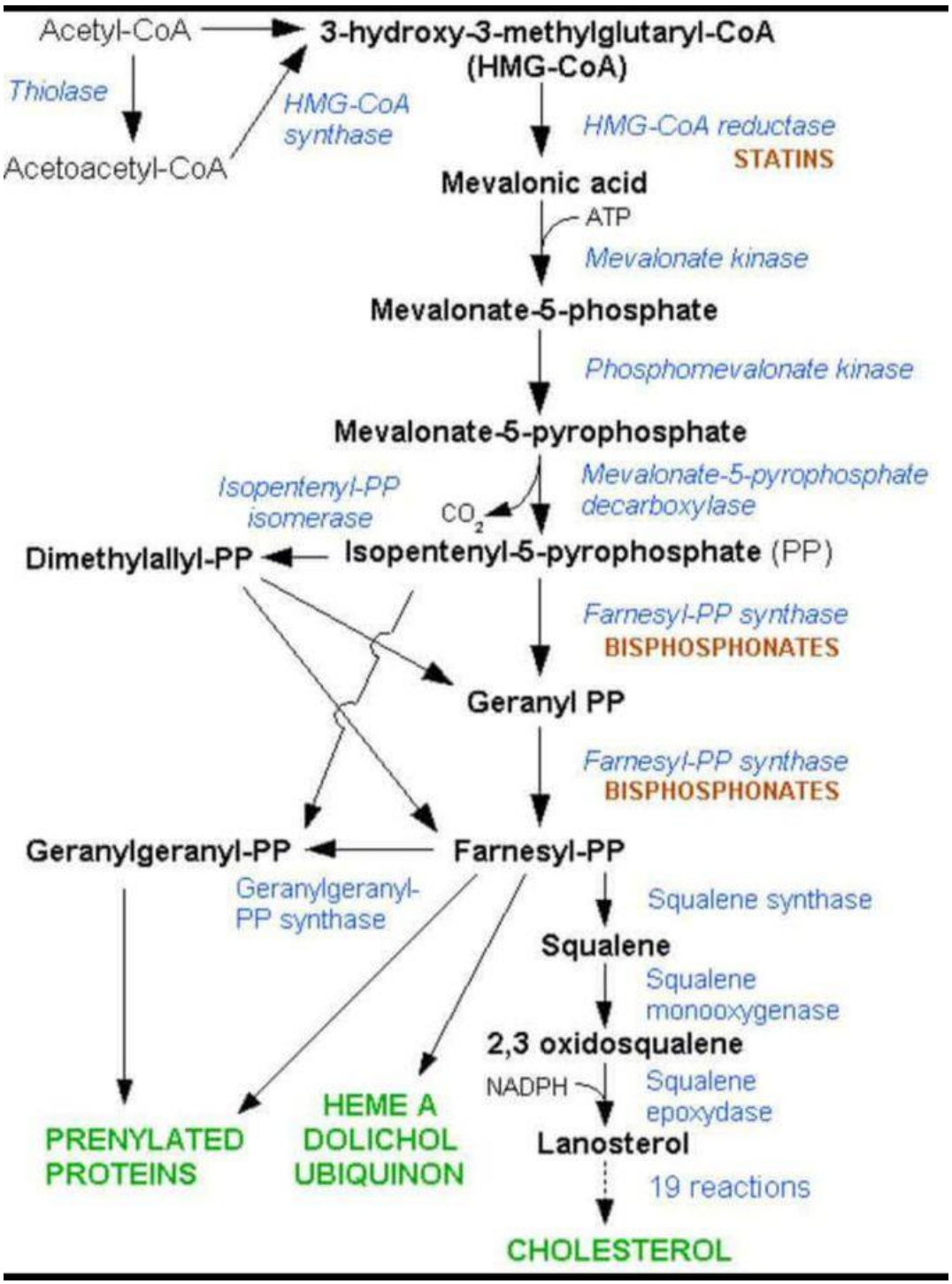


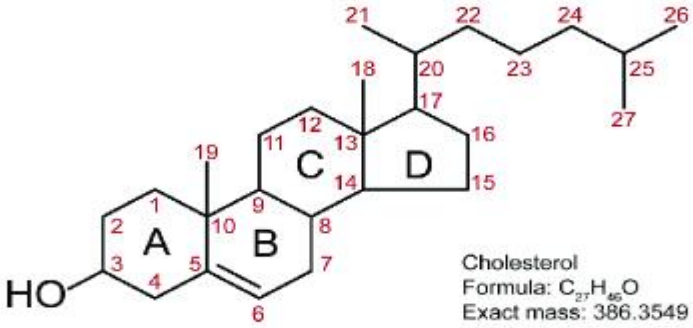
mevalonate (3 acetyl-CoA's per mevalonate).

- 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).



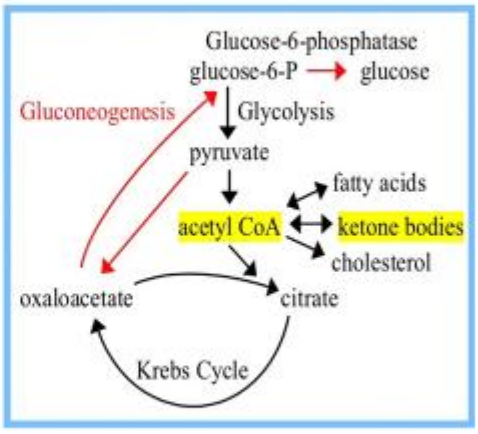
Larger Isoprenes
Squalene
(Next Video)

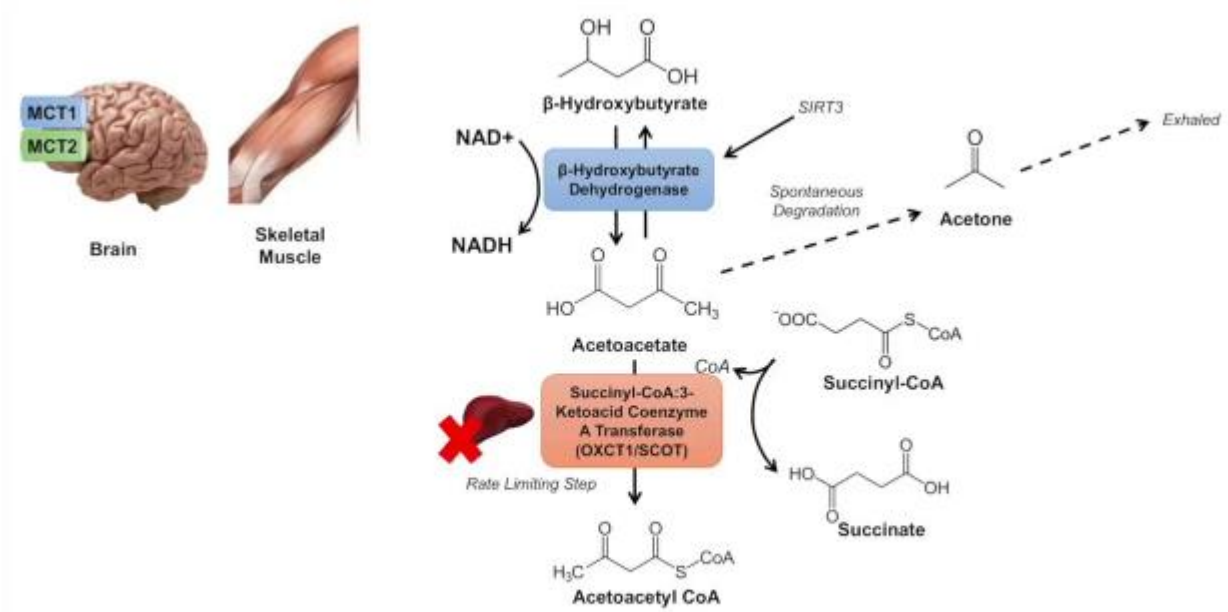

Acetyl-CoA
3-hydroxy-3-methylglutaryl-CoA
(HMG-CoA)
Thiolase
HMG-CoA
synthase
Acetoacetyl-CoA
HMG-CoA reductase
STATINS
Mevalonic acid
ATP
Mevalonate kinase
Mevalonate-5-phosphate
Phosphomevalonate kinase
Mevalonate-5-pyrophosphate
Isopentenyl-PP
isomerase
CO2
Mevalonate-5-pyrophosphate
decarboxylase
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
Lanosterol
19 reactions
CHOLESTEROL
PRENYLATED
PROTEINS
HEME A
DOLICHOL
UBIQUINON



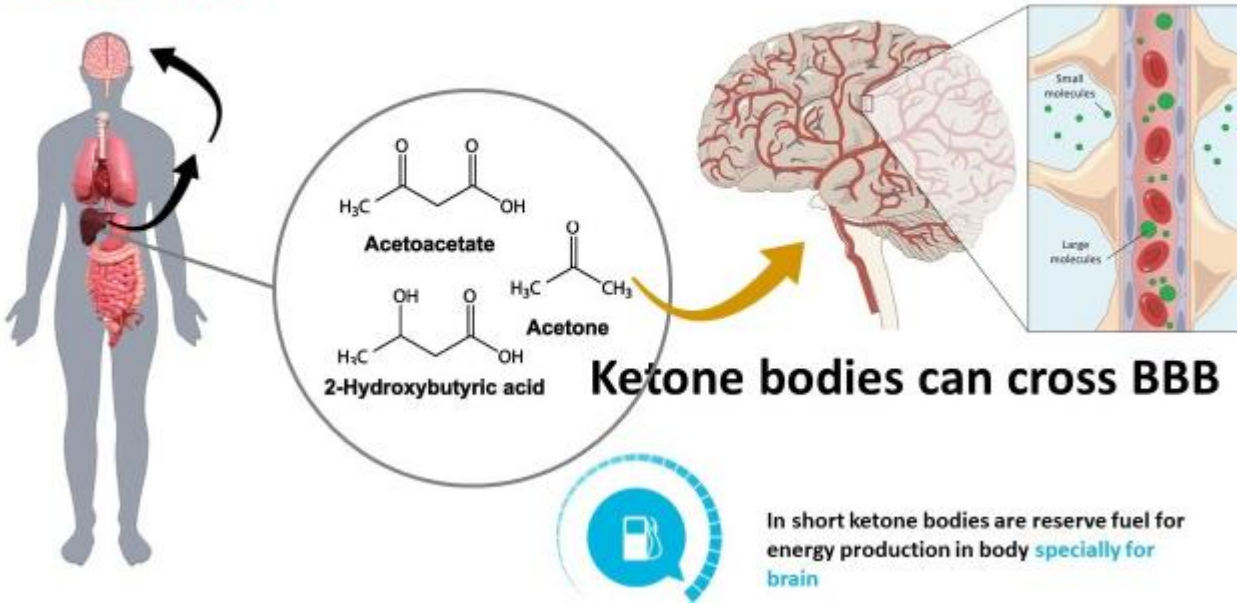
Ketone Bodies Metabolism

- 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.



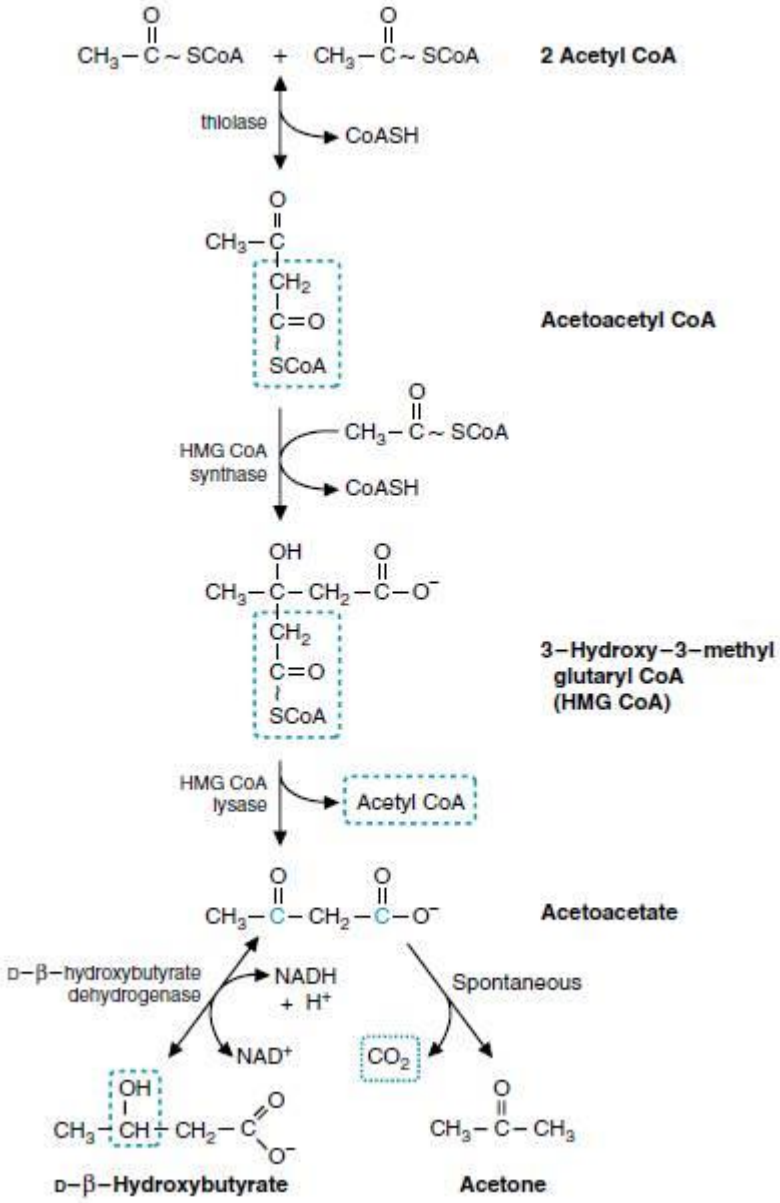


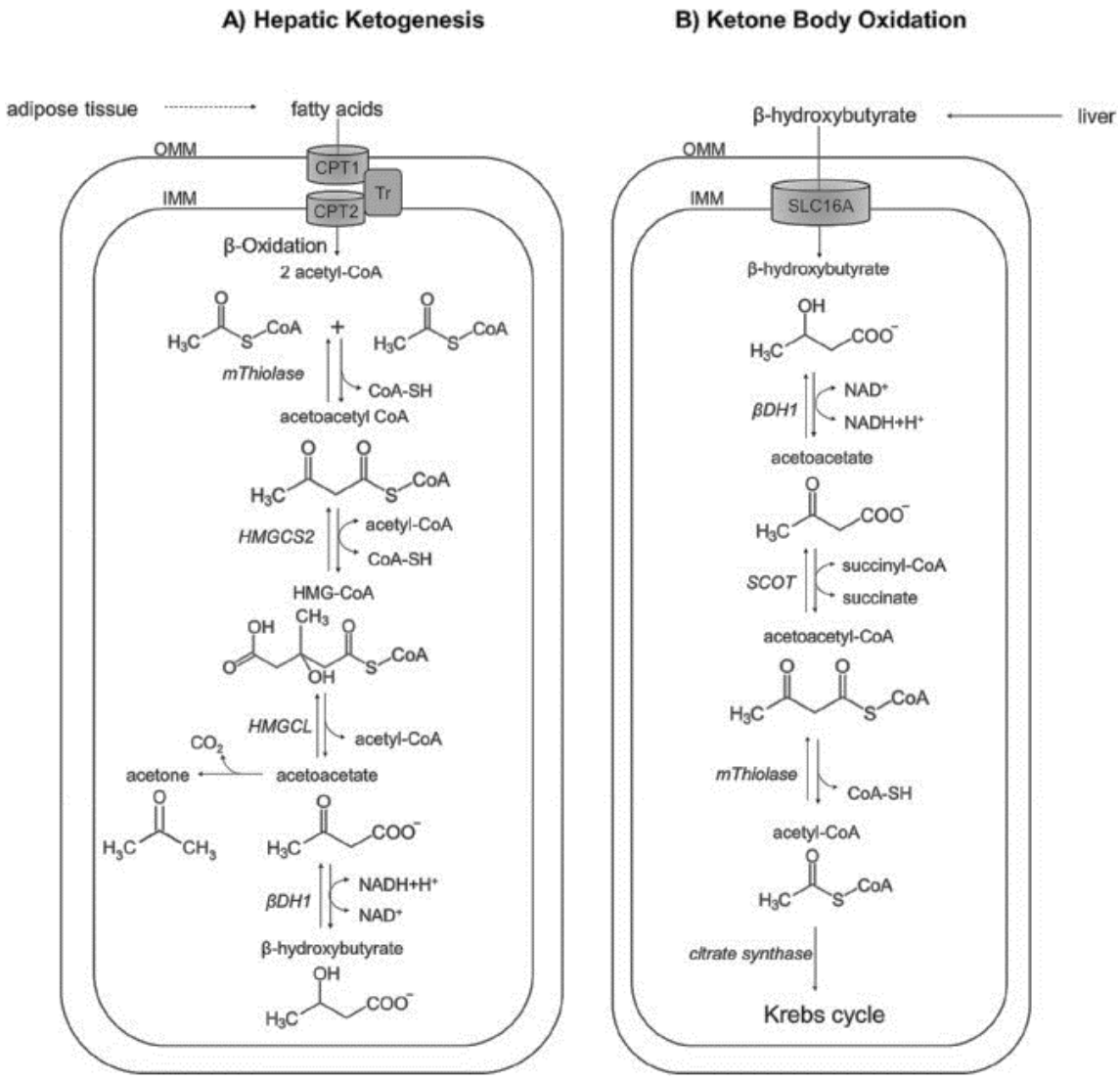
Ketone bodies and its metabolism



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


A) Hepatic Ketogenesis
B) Ketone Body Oxidation
adipose tissue
fatty acids
OMM
IMM
CPT1
CPT2
Tr
β-Oxidation
2 acetyl-CoA
mThiolase
CoA-SH
acetoacetyl CoA
HMGCS2
acetyl-CoA
CoA-SH
HMG-CoA
HMGCL
acetyl-CoA
CO2
acetone
acetoacetate
βDH1
NADH+H+
NAD+
β-hydroxybutyrate
β-hydroxybutyrate
liver
SLC16A
βDH1
NAD+
NADH+H+
acetoacetate
SCOT
succinyl-CoA
succinate
acetoacetyl-CoA
mThiolase
CoA-SH
acetyl-CoA
citrate synthase
Krebs cycle

General material and bulk-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:

Lipid metabolism and its regulation.

1. Classification of lipids. The main representatives, their biologic role.
2. Functions of lipids and general pathways of lipid metabolism.
3. Triacylglycerols. Chemical structure. Synthesis and catabolism.
4. Cholesterol, its role, biosynthesis and regulation of cholesterol biosynthesis. Atherosclerosis and obesity.
5. Phospholipids (phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine etc). Structure and biological role.
6. The chemical and biological role of membranes. Biomembrane lipids. Function of Na-K and Ca pumps.
7. Digestion and absorption of fat in the digestive tract. Causes of disorder of lipid absorption. Lipid transport and storage.
8. Peculiarities of digestion of the milk lipids in child organism.
9. Structure and functions of lipoproteins in the blood. Role of lipoproteins in the atherosclerosis development. Types of hyperlipoproteinemias.
10. Bioenergetics of fatty acids oxidation.
11. Bioenergetics of glycerol oxidation in the body.
12. Metabolism of glycerol (oxidation and biosynthesis).
13. Bile acids. Their role in digestion of lipids.
14. Ketone (acetone) bodies. Biosynthesis and utilization of the ketone bodies. Importance of diagnostic determination.
15. β-Oxidation of fatty acids. Role of carnitine in oxidation of fatty acids in mitochondria.
16. Biosynthesis of phospholipids in tissues.
17. Biosynthesis of fatty acids in the body. Structure and role of the fatty acid synthase multienzyme complex. Saturated fatty acid elongation.
18. Eicosanoids and their characteristics. Prostaglandins, prostacyclins, thromboxanes, leukotrienes. Biological activities of eicosanoids. Inhibitors of prostaglandin synthesis.

Methodological support:

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/

Lecture 10.

Topic: Amino acid metabolism. General pathways of amino acid transformation (deamination, transamination, decarboxylation). Ammonia metabolism: urea biosynthesis and its disorders. Special pathways of amino acid transformation; hereditary enzymopathies of amino acid metabolism.

Topic relevance: 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 - 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 hormonal effect.

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

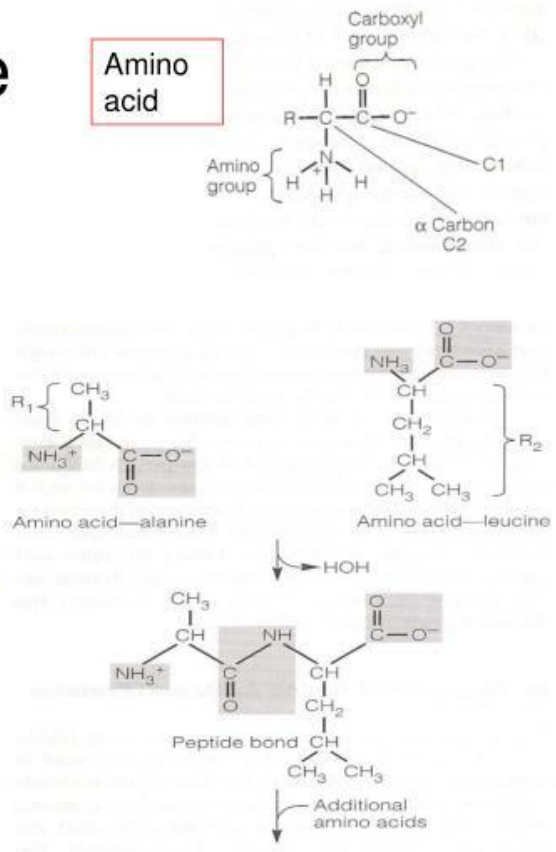
6. Violation of amino acid metabolism.
Content of the lecture material

proteins

- Only foodstuff that can form structures (tissues and enzymes)
- Made up of amino acids
- Protein synthesis, enzyme formation
- Can serve as fuel during long-term work
- 0.8 g/kg recommended for adults; probably too low for athletes

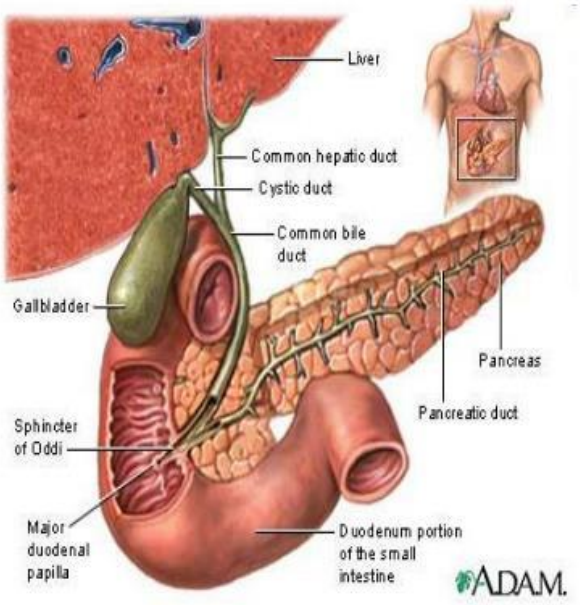
Protein structure

- Carboxyl and amino termini come together to from protein structures (peptides)

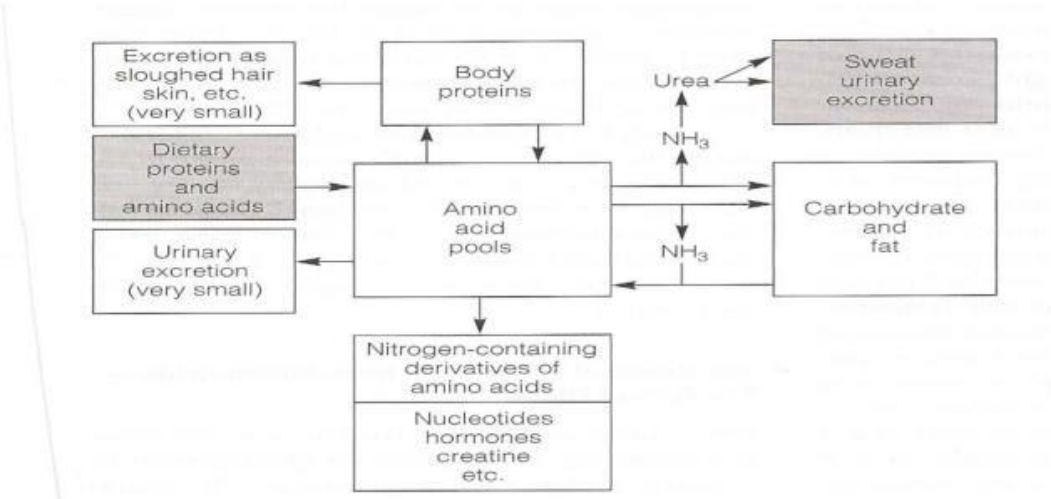


Proteins in the diet

- Digested in stomach and small intestine
  - Hydrocholoric acid (stomach)
  - Trypsin, chymotrypsin, carboxypeptidase (from pancreas)
  - Polypeptidases and dipeptidases in intestinal cells finish digestion



- The amino acid pool
  - Free amino acids in the liver, skeletal muscle, plasma, interstitial fluid and intracellular water
  - All interconnected in that metabolism in one affects the others
  - Continuous excretion of nitrogenous end-products
  - Necessitates constant input of new amino acids
  - So, CONSTANT Protein turnover



GENERAL WAYS OF AMINO ACIDS METABOLISM

The fates of amino acids:

1) for protein synthesis;

2) for synthesis of other nitrogen containing compounds (creatine, purines, choline, pyrimidine);

3) as the source of energy;

4) for the gluconeogenesis.

The general ways of amino acids degradation:

- **Deamination**
- **Transamination**
- **Decarboxilation**

The major site of amino acid degradation - the **liver**.

Deamination of amino acids

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

Four types of deamination:

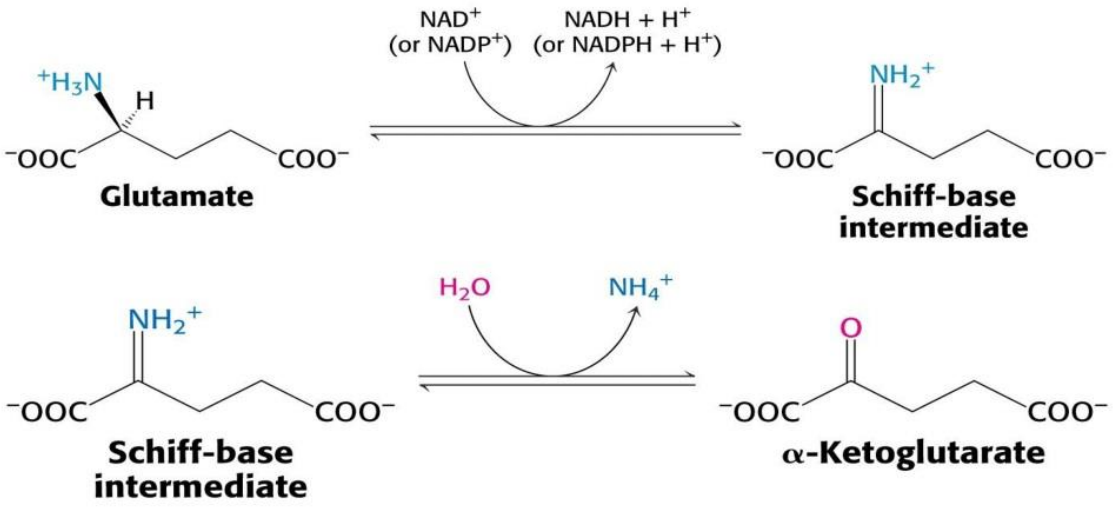
- ***oxidative*** (the most important for higher animals),
- ***reduction,***
- ***hydrolytic, and***
- ***intramolecular***

Oxidative deamination

L-Glutamate dehydrogenase plays a central role in amino acid deamination

In most organisms glutamate is the only amino acid that has active dehydrogenase

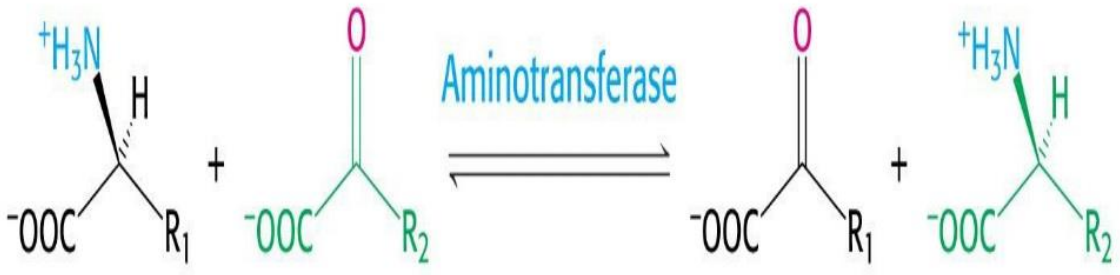
Present in both the cytosol and mitochondria of the liver



Transamination of amino acids

Transamination - *transfer of an amino group from an α-amino acid to an α-keto acid (usually to α-ketoglutarate)*

Enzymes: *aminotransferases (transaminases).*



α-amino acid α-keto acid *α-keto acid α-amino a*

There are different transaminases

The most common:

alanine aminotransferase

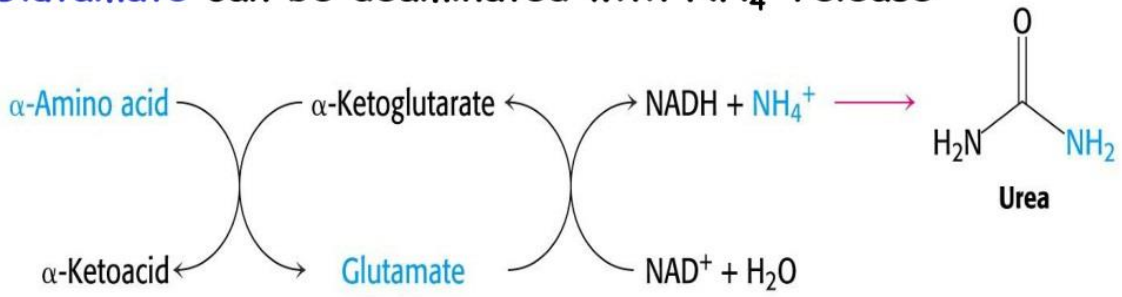
alanine + ✔-ketoglutarate ☂ pyruvate + glutamate

aspartate aminotransferase

aspartate + ✔-ketoglutarate ☂ oxaloacetate + glutamate

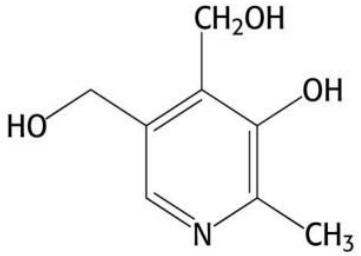
Aminotransferases **funnel ✔-amino groups from a variety of amino acids to ✔-ketoglutarate with glutamate formation**

Glutamate can be deaminated with NH_4^+ release



Mechanism of transamination

All aminotransferases require the prosthetic group ***pyridoxal phosphate (PLP),*** which is derived from ***pyridoxine (vitamin B_6).***

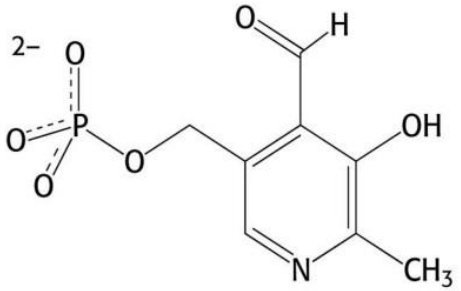


Pyridoxine (Vitamin B_6)

Ping-pong kinetic mechanism

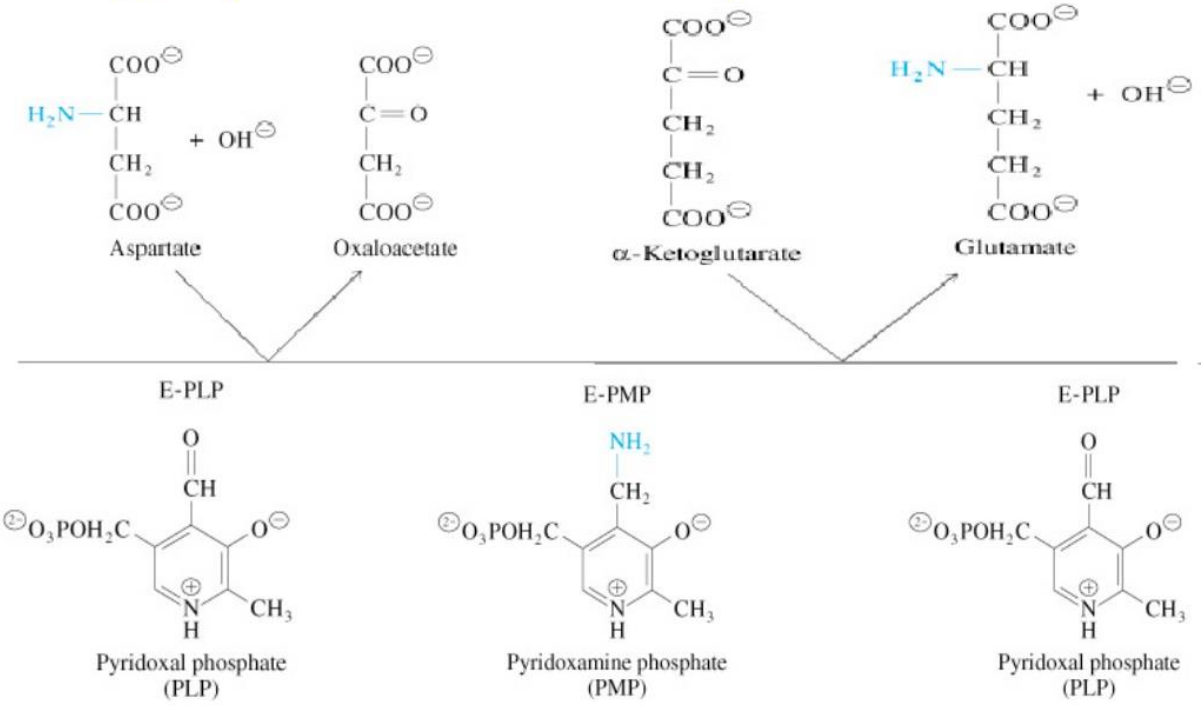
First step: the amino group of amino acid is transferred to pyridoxal phosphate, forming pyridoxamine phosphate and releasing ketoacid.

Second step: ✔-ketoglutarate reacts with pyridoxamine phosphate forming glutamate



Pyridoxal phosphate (PLP)

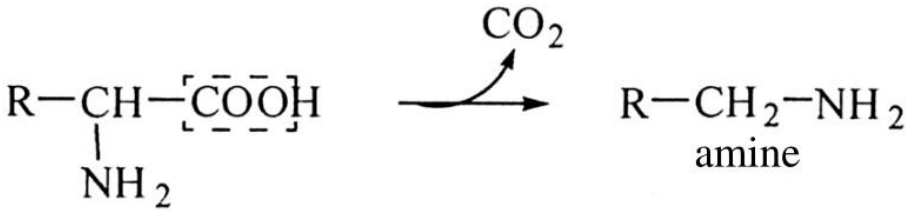
Ping-pong kinetic mechanism of aspartate transaminase



aspartate + α-ketoglutarate ⇄ oxaloacetate + glutamate

Decarboxylation of amino acids

Decarboxylation – removal of *carbon dioxide* from amino acid with formation of *amines*.

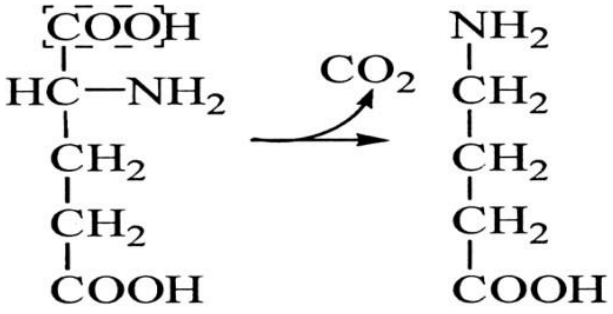


Usually amines have high physiological activity (hormones, neurotransmitters etc).

Enzyme: ***decarboxylases***
Coenzyme – **pyrydoxalphosphate**

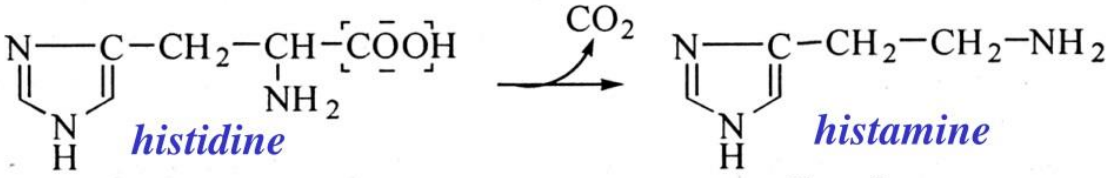
Significance of amino acid decarboxylation

1. Formation of physiologically active compounds



glutamate *gamma-aminobutyric acid (GABA)*

GABA – mediator of nervous system

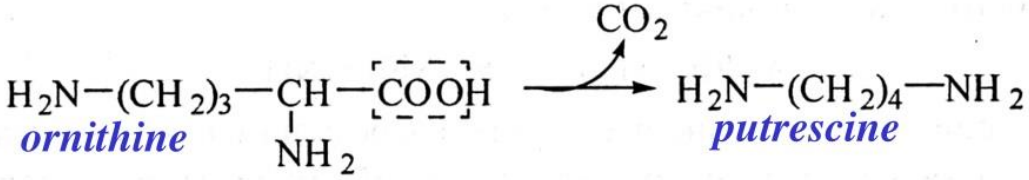


histidine *histamine*

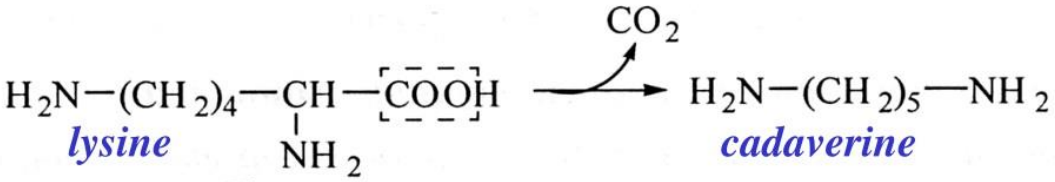
Histamine – mediator of inflammation, allergic reaction.

2. Catabolism of amino acids during the decay of proteins

Enzymes of microorganisms (in colon; dead organisms) decarboxylate amino acids with the formation of diamines.



ornithine *putrescine*



lysine *cadaverine*

Ammonia is a toxic substance to plants and animals (especially for brain)

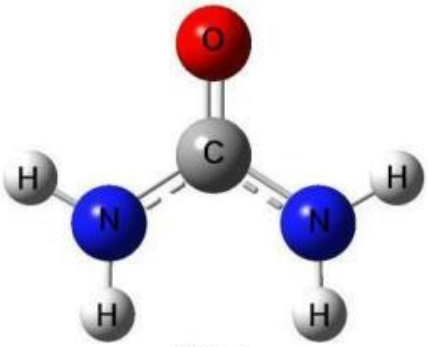
Normal concentration: **25-40 μmol/l** (0.4-0.7 mg/l)

Ammonia must be removed from the organism

Terrestrial vertebrates synthesize **urea** (excreted by the **kidneys**) - ureotelic organisms

Birds, reptiles synthesize **uric acid**

Urea formation takes place in the liver



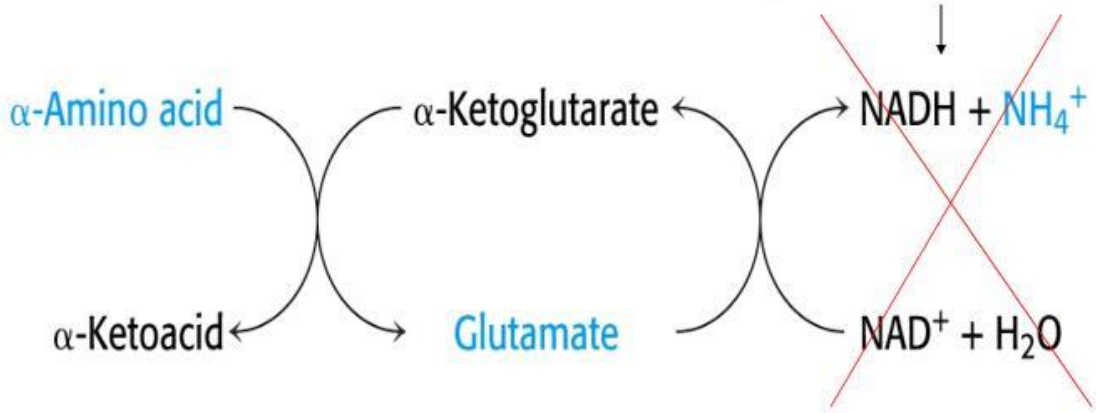
Urea

Peripheral Tissues Transport Nitrogen to the Liver

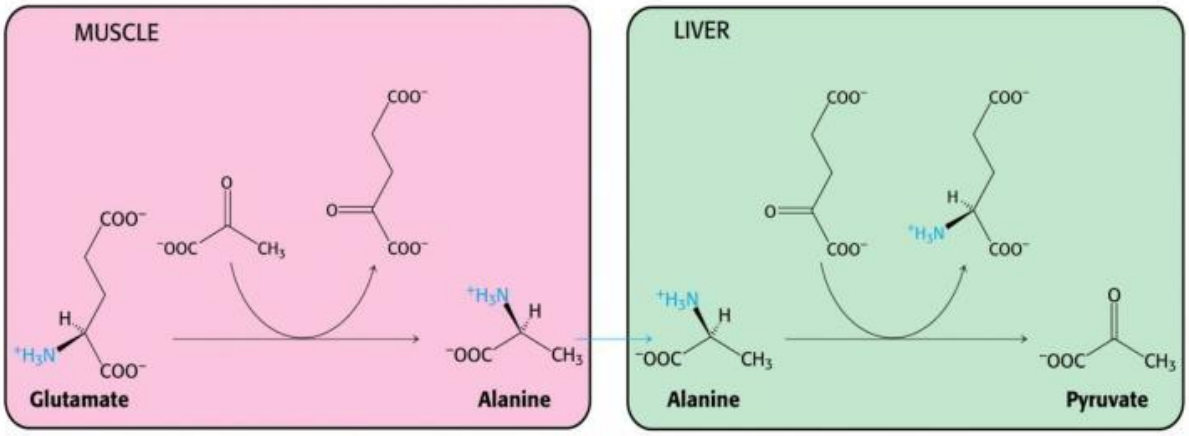
Two ways of nitrogen transport from peripheral tissues (muscle) to the liver:

1. Alanine cycle. Glutamate is formed by transamination reactions

Glutamate is not deaminated in peripheral tissues



Nitrogen is then transferred to **pyruvate** to form **alanine**, which is released into the blood.



The liver takes up the alanine and converts it back into pyruvate by transamination.

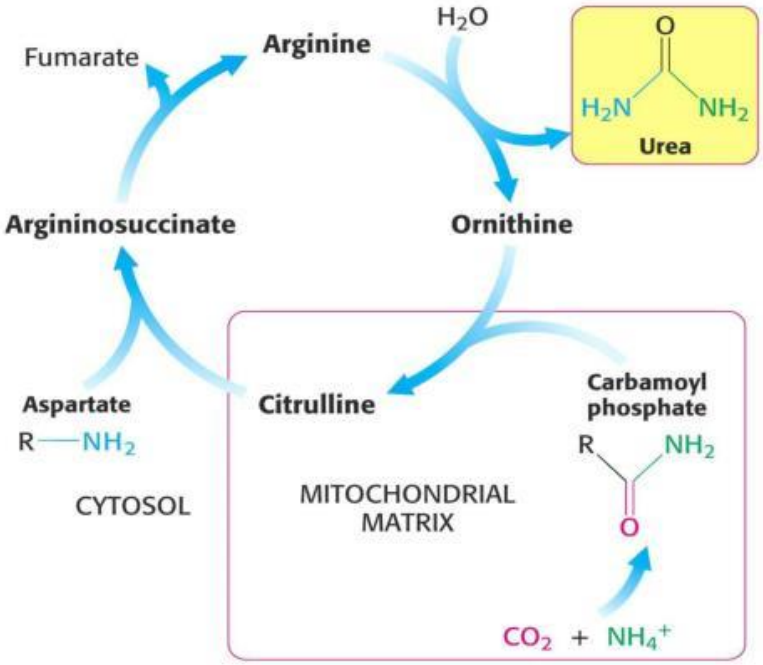
The glutamate formed in the liver is deaminated and ammonia is utilized in ***urea cycle***.

THE UREA CYCLE

Urea cycle - a cyclic pathway of urea synthesis first postulated by H.Krebs

The sources of nitrogen atoms in urea molecule:
- aspartate;
- NH_4^+.

Carbon atom comes from CO_2.

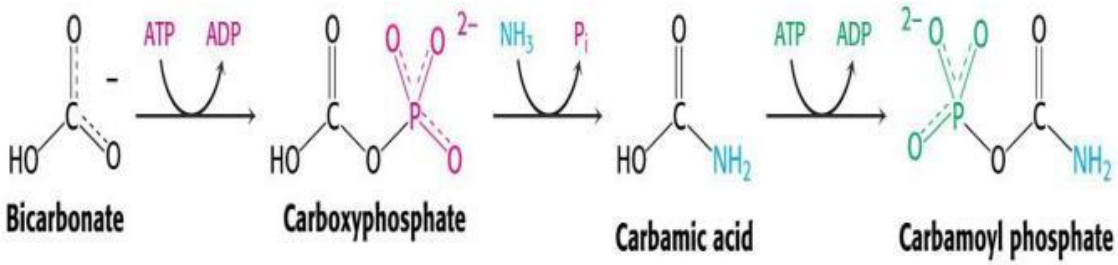


The free ammonia is coupling with carbon dioxide to form ***carbamoyl phosphate***

Two molecules of ATP are required

Reaction takes place in the matrix of liver mitochondria

Enzyme: ***carbamoyl phosphate synthetase*** (20 % of the protein of mitochondrial matrix)

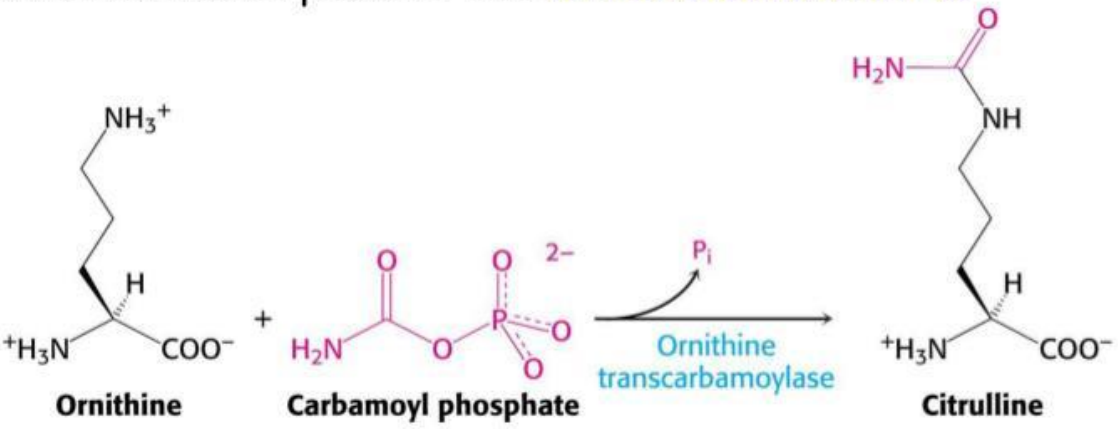


Carbamoyl phosphate donates carbamoyl group to **ornithine**

The product - **citruilline**

Enzyme: ***ornithine carbamoyltransferase***

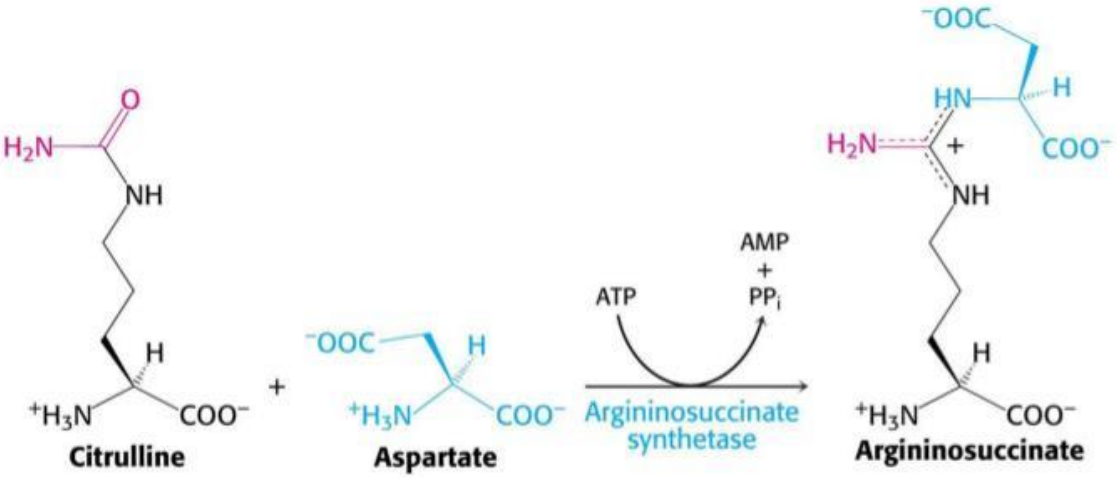
Reaction takes place in the mitochondrial matrix



Citrulline leaves the matrix and passes to the cytosol

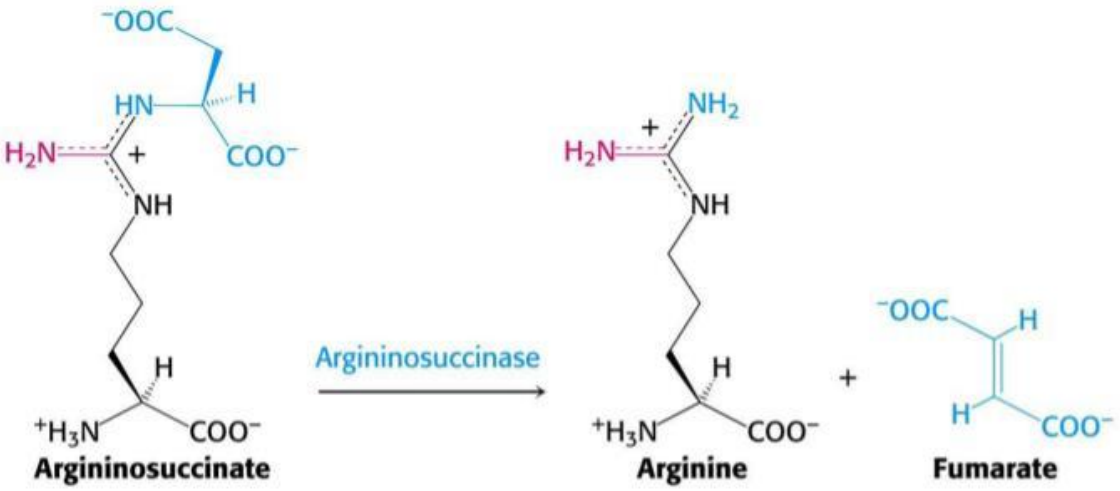
In the cytosol **citrulline** in the presence of ATP reacts with **aspartate** to form **argininosuccinate**

Enzyme: ***argininosuccinate synthetase***



Argininosuccinate is cleaved to free **arginine** and **fumarate**

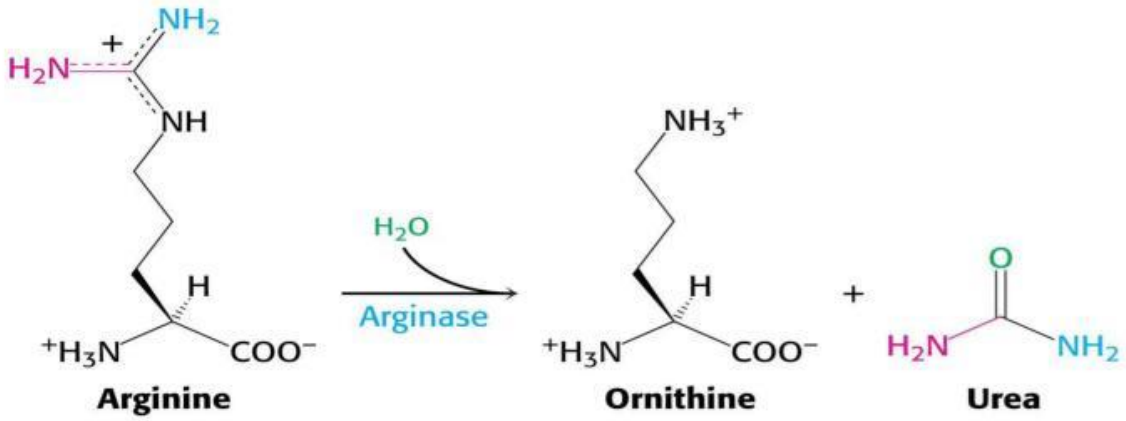
Enzyme: ***argininosuccinate lyase***



The **fumarate** enters the tricarboxylic acid cycle

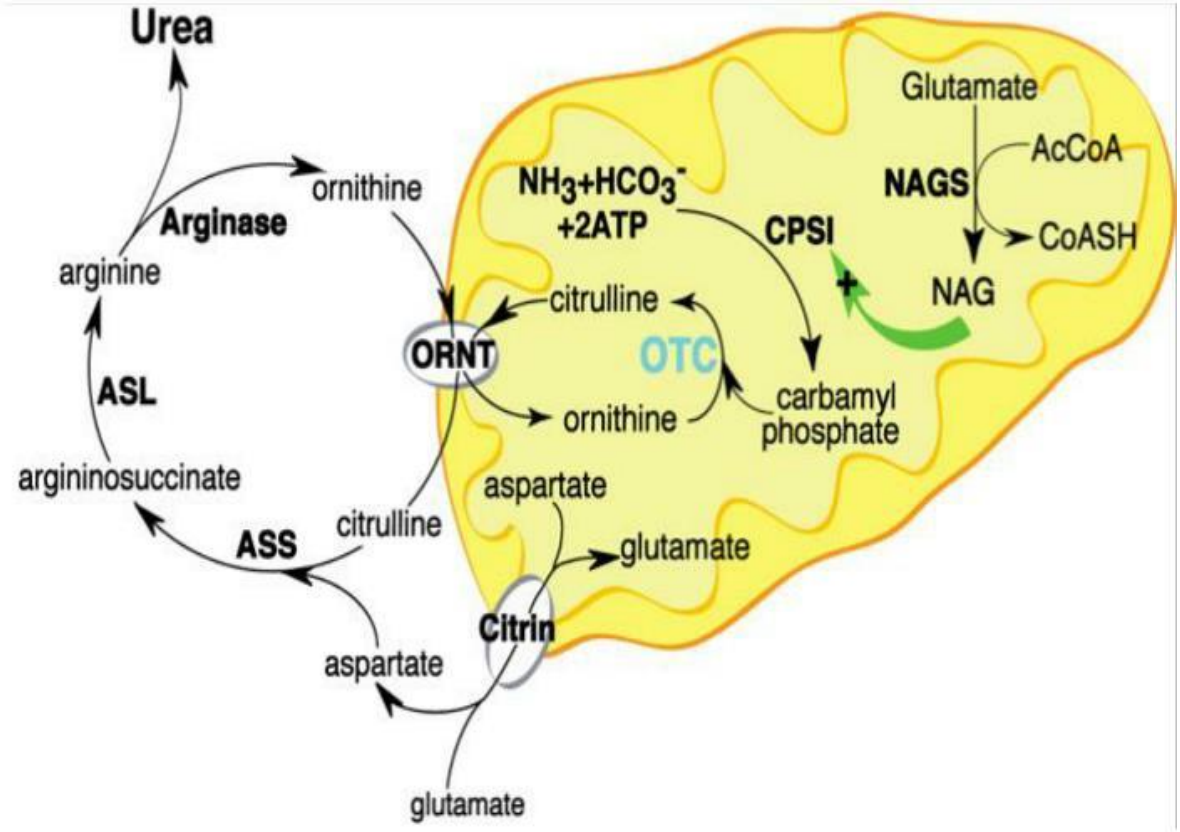
Arginine is hydrolyzed to generate **urea** and **ornithine**

Enzyme: ***arginase*** (present only in liver of ureotelic animals)



Ornithine is transported back into the mitochondrion to begin another cycle

Urea is excreted (about 40 g per day)

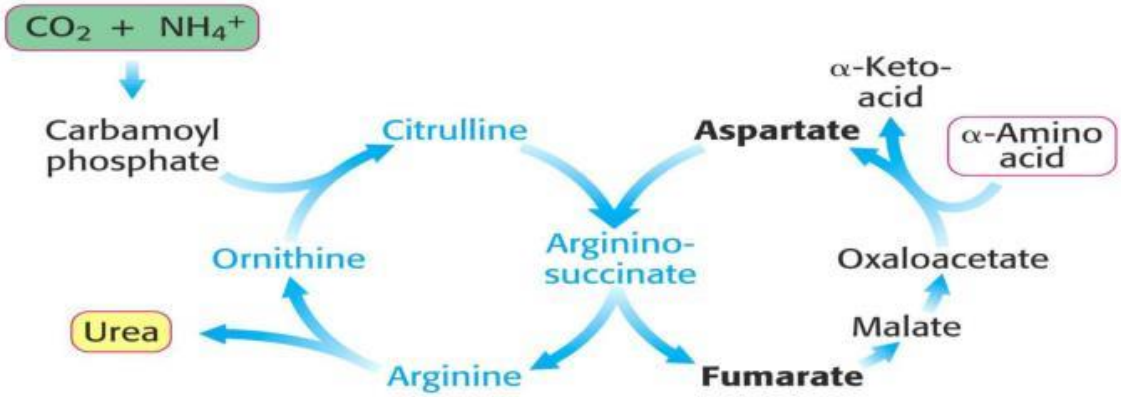


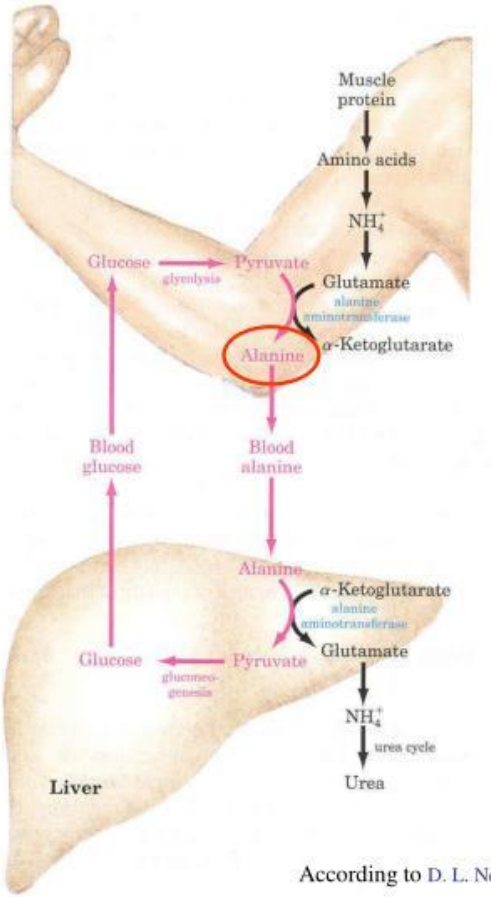
The Linkage between Urea Cycle, Citric Acid Cycle and Transamination of Oxaloacetate

Fumarate formed in urea cycle enters citric acid cycle and is converted to oxaloacetate.

Fates of oxaloacetate:

(1) transamination to aspartate,
(2) conversion into glucose,
(3) condensation with acetyl CoA to form citrate,
(4) conversion into pyruvate.





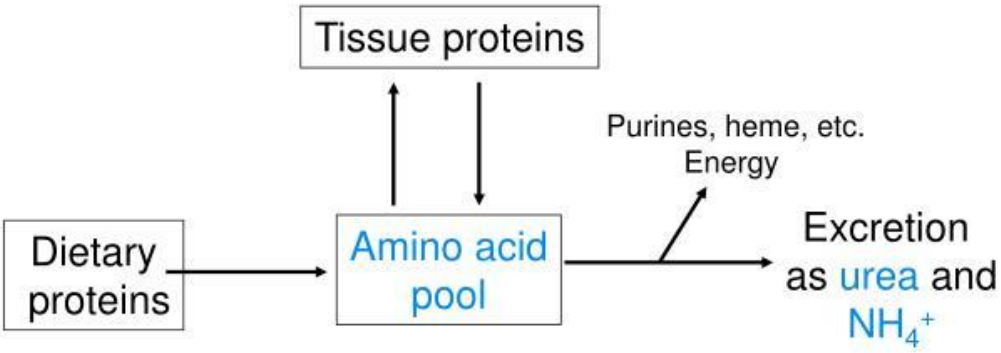
Glucose-alanine cycle

Alanine plays a special role in transporting amino groups to liver.

Ala is the carrier of ammonia and of the carbon skeleton of pyruvate from muscle to liver.
The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

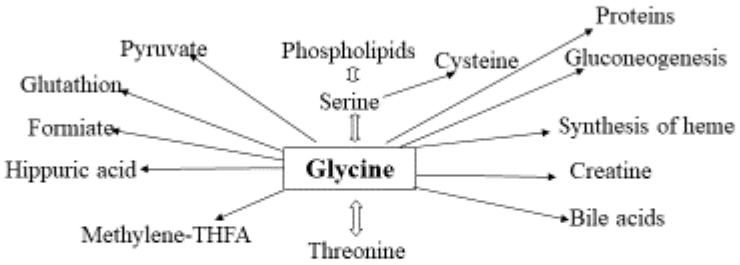
According to D. L. Nelson, M. M. Cox :LEHNINGER. PRINCIPLES OF BIOCHEMISTRY Fifth edition

Nitrogen balance

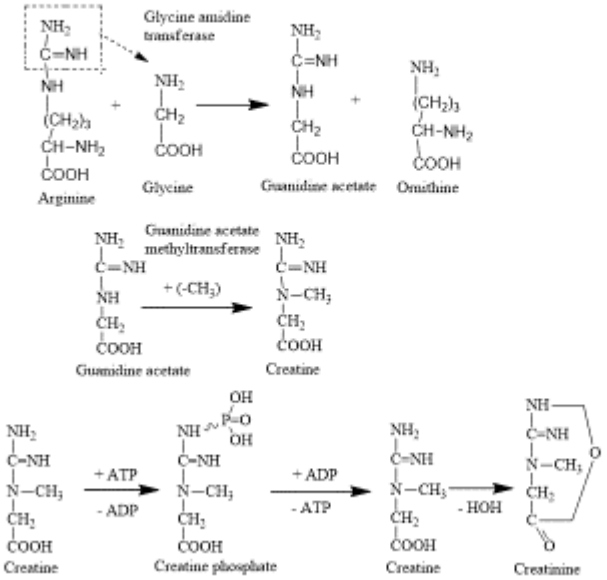


The amount of nitrogen ingested is balanced by the excretion of an equivalent amount of nitrogen. About 80% of excreted nitrogen is in the form of urea.

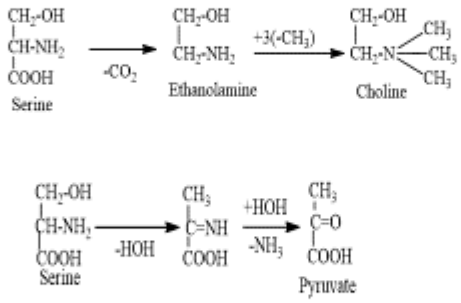
Metabolism of glycine



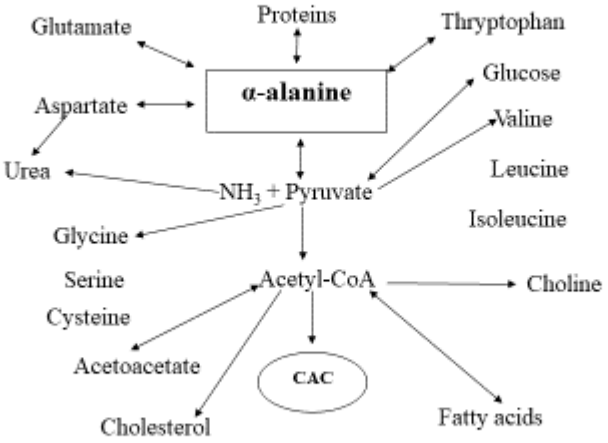
Synthesis of creatine



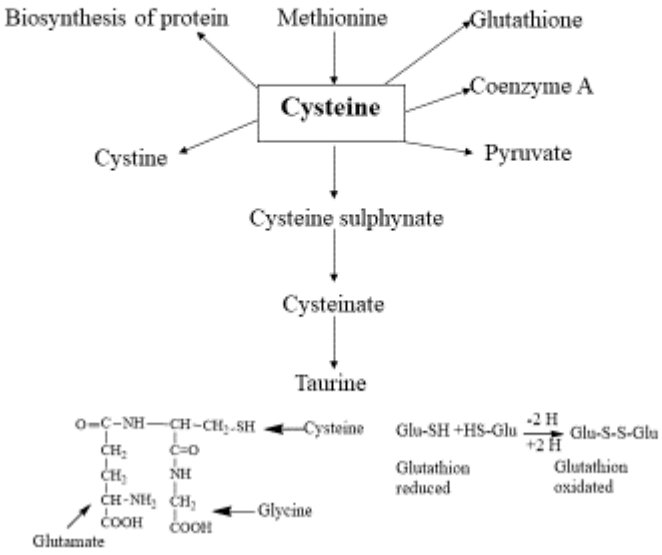
Metabolism of serine



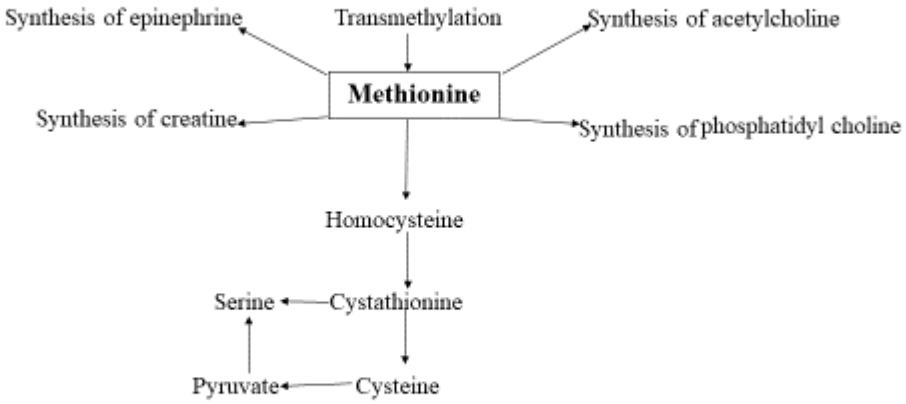
Metabolism of α-alanine



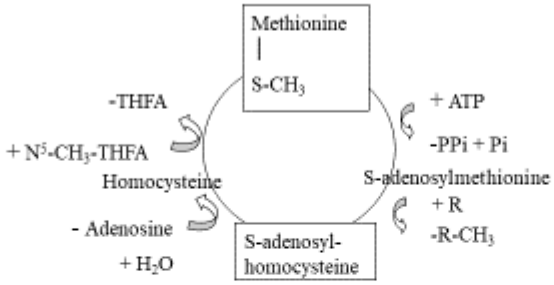
Metabolism of cysteine

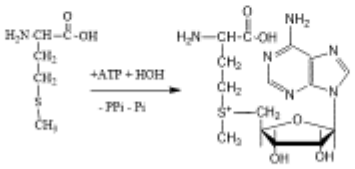


Metabolism of methionine

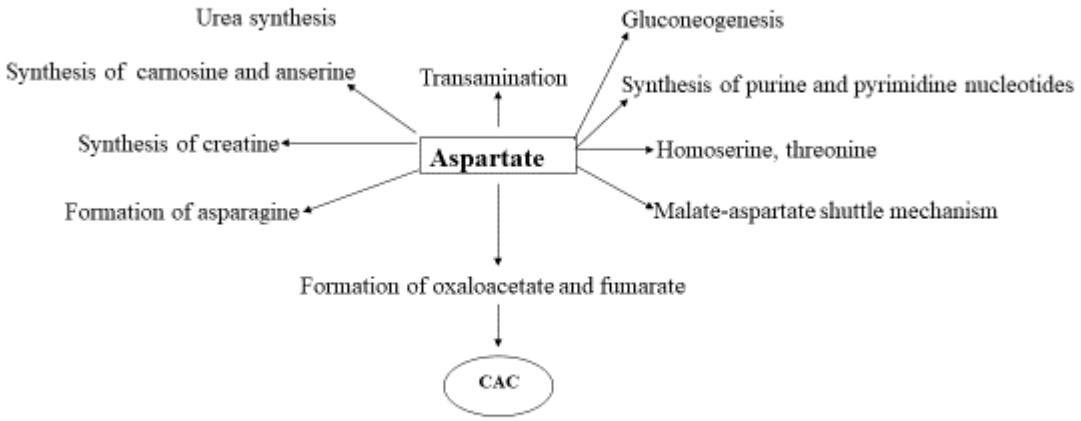


Scheme of S-adenosylmethionine synthesis

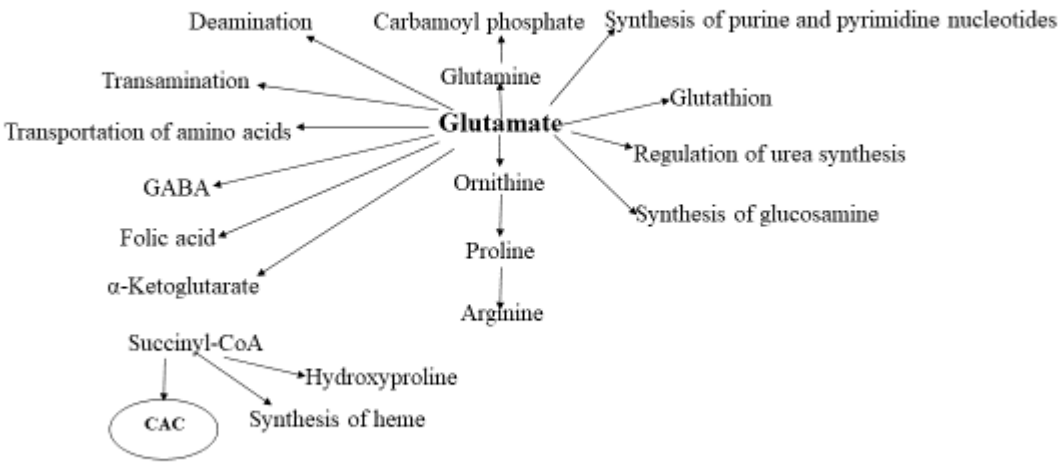




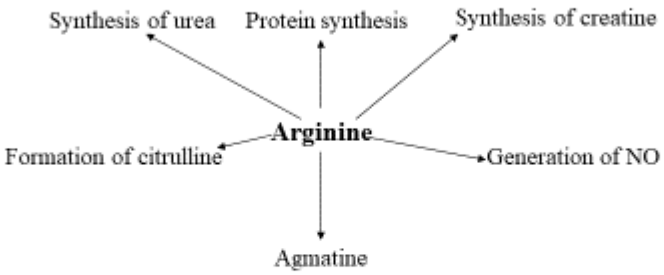
Metabolism of aspartate



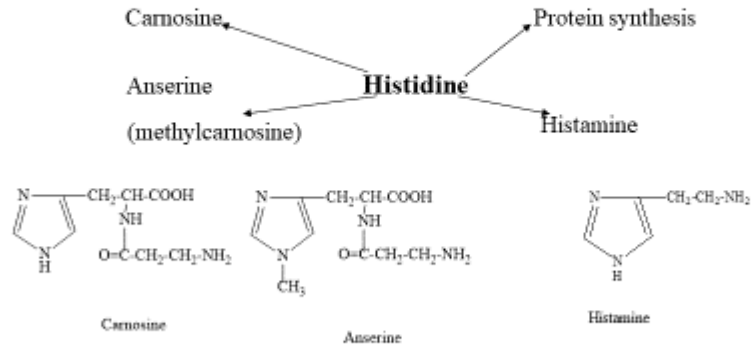
Metabolism of glutamate



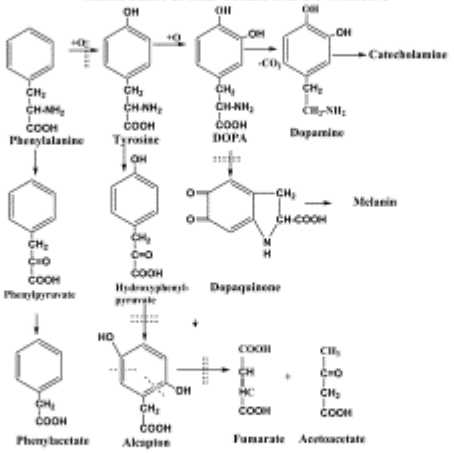
Metabolism of arginine



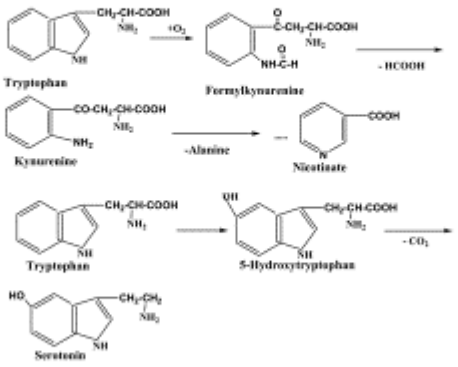
Metabolism of histidine



METABOLISM OF PHENYL ALANINE AND TYROSINE



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.

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

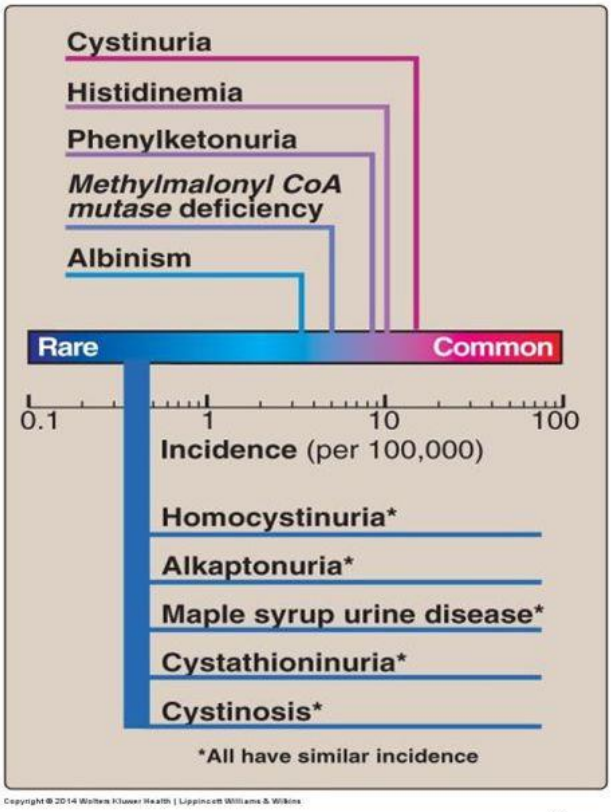
Table 18-2
Lehninger Principles of Biochemistry, Fifth Edition

Metabolic defects in amino acid metabolism

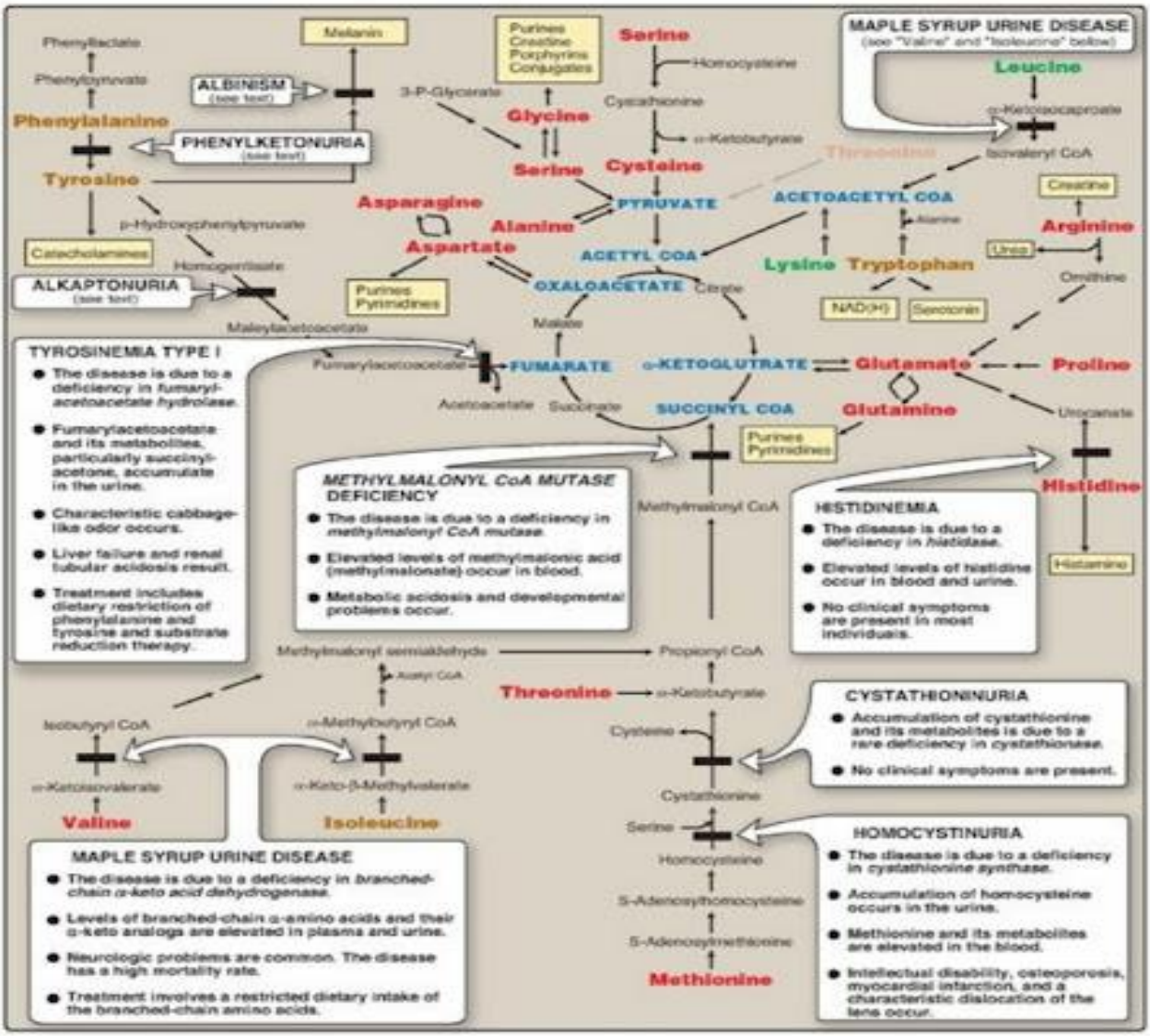
- Inborn errors of metabolism are commonly caused by mutant genes that generally result in abnormal proteins, most often enzymes. The inherited defects may be expressed as a total loss of enzyme activity or as a partial deficiency in activity.

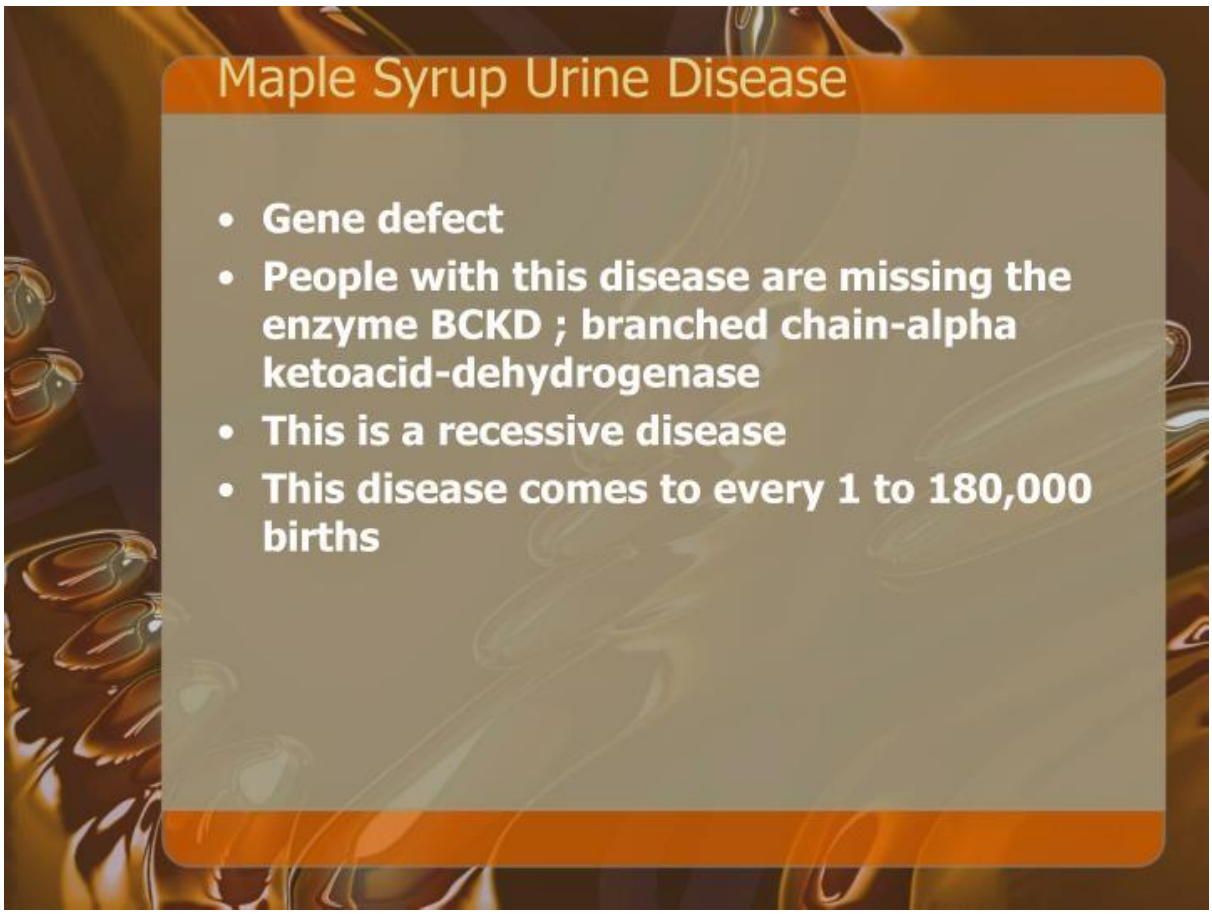
- Newborn screening and timely initiation of treatment are essential. By law, all states must screen for over 20 disorders. All states screen for PKU.

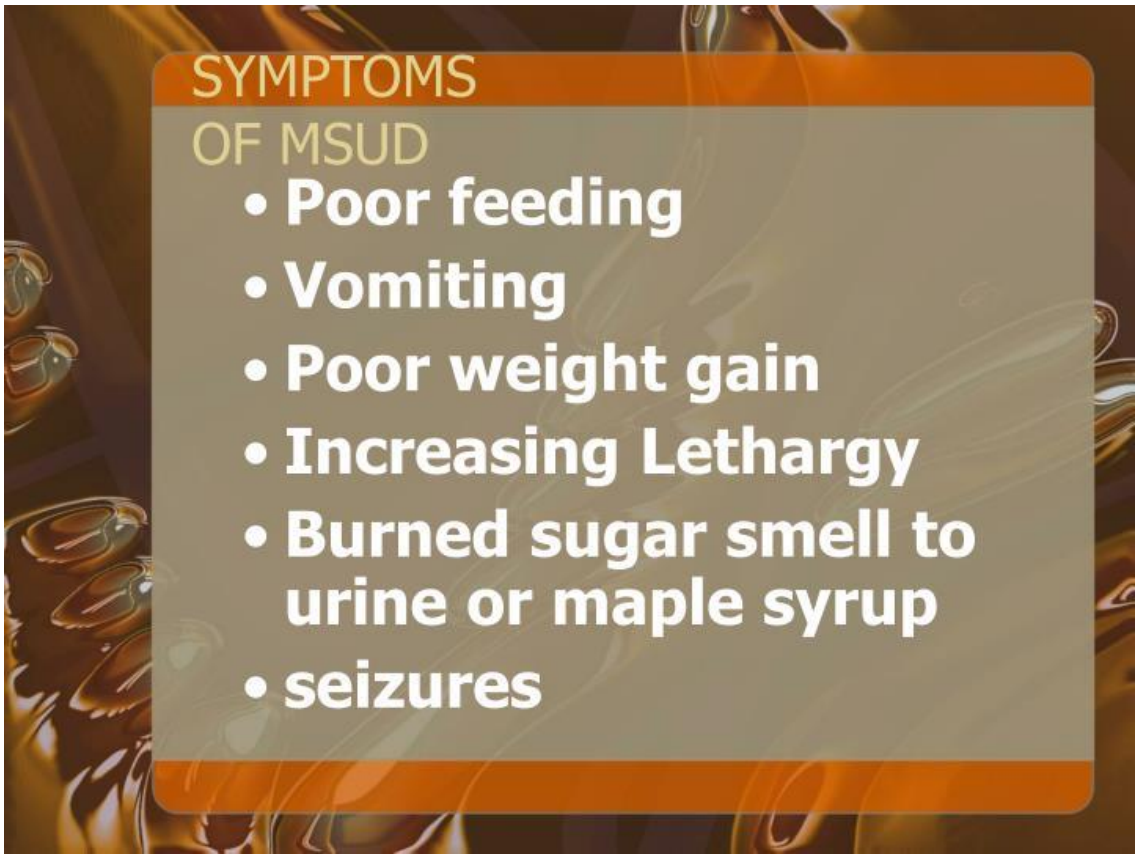
- Treatment: diets low in the amino acids whose catabolism is impaired.

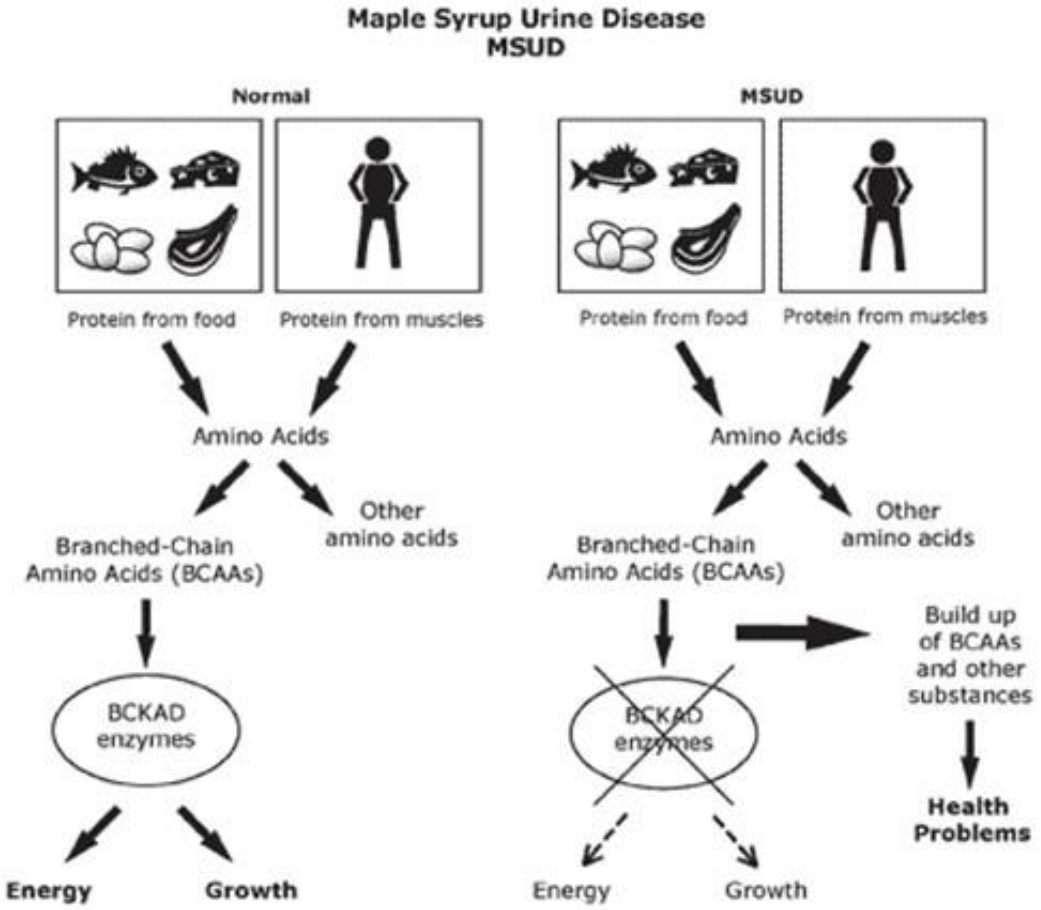


3


MAPLE SYRUP URINE DISEASE
(see "Valine" and "Isoleucine" below)
Leucine
Phenylalanine
Tyrosine
PHENYLKETONURIA
ALBINISM
ALKAPTONURIA
Melanin
Serine
Glycine
Cysteine
Asparagine
Alanine
Aspartate
PYRUVATE
ACETYL COA
ACETOACETYL COA
OXALOACETATE
Lysine
Tryptophan
Arginine
Proline
Glutamate
Glutamine
α-KETOGLUTRATE
FUMARATE
SUCCINYL COA
Histidine
Threonine
Valine
Isoleucine
Methionine
TYROSINEMIA TYPE I
The disease is due to a deficiency in fumarylacetoacetate hydrolase.
Fumarylacetoacetate and its metabolites, particularly succinylacetone, accumulate in the urine.
Characteristic cabbage-like odor occurs.
Liver failure and renal tubular acidosis result.
Treatment includes dietary restriction of phenylalanine and tyrosine and substrate reduction therapy.
METHYLMALONYL CoA MUTASE DEFICIENCY
The disease is due to a deficiency in methylmalonyl CoA mutase.
Elevated levels of methylmalonic acid (methylmalonate) occur in blood.
Metabolic acidosis and developmental problems occur.
HISTIDINEMIA
The disease is due to a deficiency in histidase.
Elevated levels of histidine occur in blood and urine.
No clinical symptoms are present in most individuals.
CYSTATHIONINURIA
Accumulation of cystathionine and its metabolites is due to a rare deficiency in cystathionase.
No clinical symptoms are present.
HOMOCYSTINURIA
The disease is due to a deficiency in cystathionine synthase.
Accumulation of homocysteine occurs in the urine.
Methionine and its metabolites are elevated in the blood.
Intellectual disability, osteoporosis, myocardial infarction, and a characteristic dislocation of the lens occur.
MAPLE SYRUP URINE DISEASE
The disease is due to a deficiency in branched-chain α-keto acid dehydrogenase.
Levels of branched-chain α-amino acids and their α-keto analogs are elevated in plasma and urine.
Neurologic problems are common. The disease has a high mortality rate.
Treatment involves a restricted dietary intake of the branched-chain amino acids.


Maple Syrup Urine Disease
• Gene defect
• People with this disease are missing the enzyme BCKD ; branched chain-alpha ketoacid-dehydrogenase
• This is a recessive disease
• This disease comes to every 1 to 180,000 births


SYMPTOMS
OF MSUD
• Poor feeding
• Vomiting
• Poor weight gain
• Increasing Lethargy
• Burned sugar smell to urine or maple syrup
• seizures



Signs and tests

- Plasma amino acid test
- Urine amino acid test
  - Newborn screening tests
  - Inborn errors of metabolism

PLASMA AMINO ACID CONCENTRATIONS

Isoleucine
- Children: 37 to 140
- Adults: 42 to 100

Leucine
- Children: 70 to 170
- Adults: 66 to 170

Valine
- Children: 160 to 350
- Adults: 150 to 310

General material and bulk-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:

Aminoacid metabolism. Enzymopathies of amino acid metabolism.

1. The pool of the amino acids in the body. Routes for transport and utilization of amino acids in tissues.
2. Transport of amino acids into the cells.
3. Transamination of amino acids. Mechanism of transamination reaction, transaminases and their biochemical importance.
4. Direct and indirect desamination of amino acids in tissues. Mechanism of oxidative deamination.
5. Decarboxylation of amino acids in human body. Physiologic role of obtained products. Oxidation of biogenic amines.
6. Pathways of formation and neutralization of ammonia in the body.
7. Biosynthesis of urea. The consequence of enzymatic reactions of urea biosynthesis and genetic abnormalities of urea cycle enzymes.
8. The common pathways of metabolism of carbon skeleton of amino acids in the human body. Glycogenic and ketogenic amino acids.
9. Biosynthesis and biologic role of creatine and creatine phosphate.
10. Metabolism of sulfur-containing amino acids cysteine and methionine.
11. Metabolism of arginine. The formation and biological role of nitric oxide, and NO-synthase.
12. Glutathione, its structure, biosynthesis and biologic function.
13. Specific pathways of metabolism of cyclic amino acids phenylalanine and tyrosine.
14. In born enzymopathies of metabolism of phenylalanine and tyrosine.
15. Metabolism of amino acid tryptophan and hereditary enzymopathies of tryptophan metabolism.

Methodological support:

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/

Lecture 11.

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

Topic relevance: 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

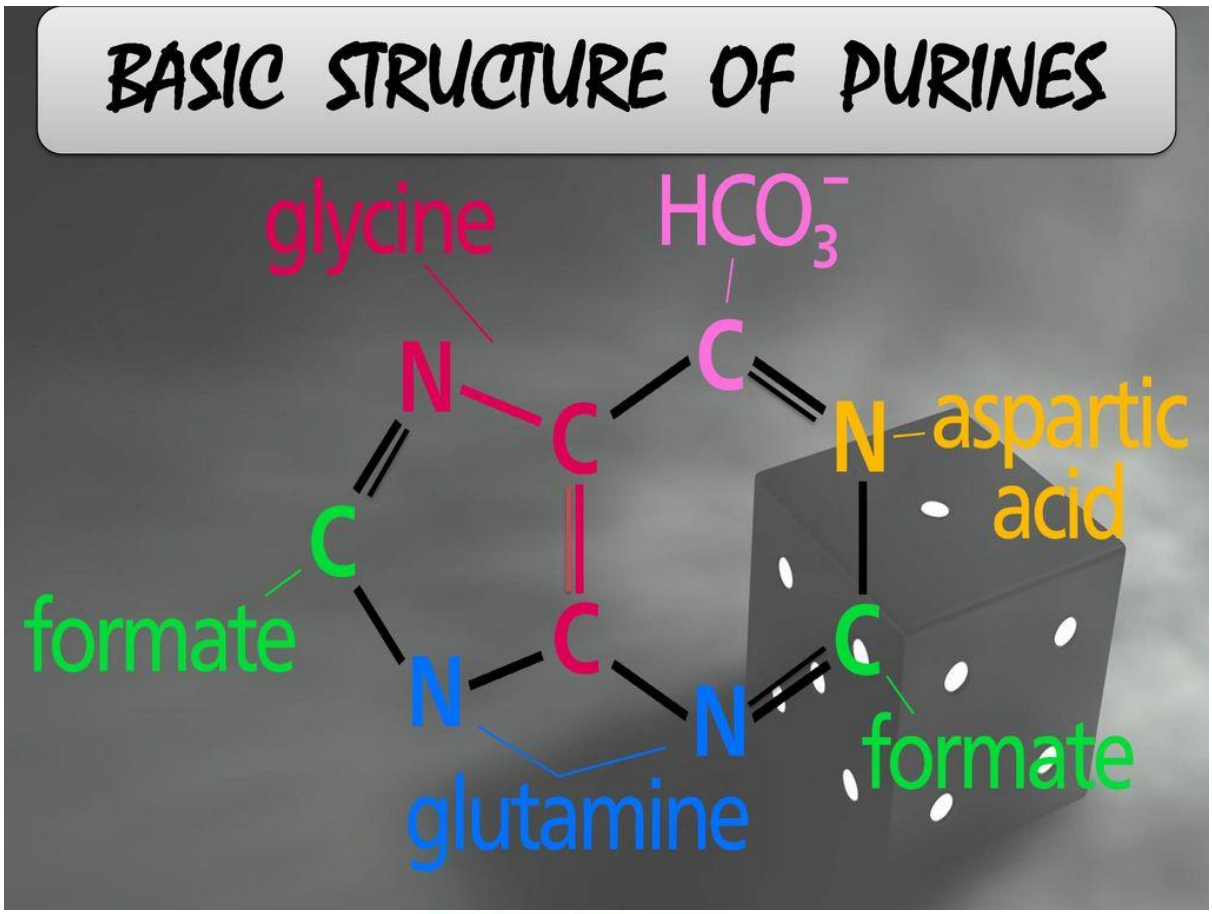
Objective: study the structure of the constituent parts of nucleic acids (DNA and RNA) - mononucleotides and know the formation of end products of purine and pyrimidine metabolism, including changes in pathology.

Basic concepts:

1. Nitrogen bases
2. Nucleoside.
3. Nucleotide.
4. Methotrexate.
5. Gout.
6. Lesch-Nyhan syndrome.
7. Orotaaciduria.

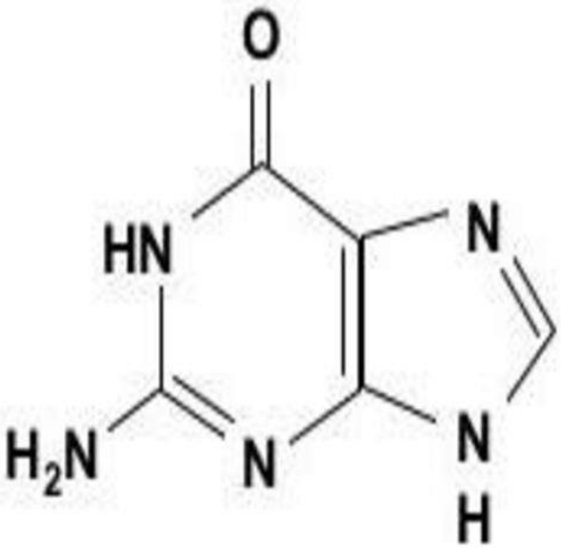
Plan and organizational structure of the lecture:

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.

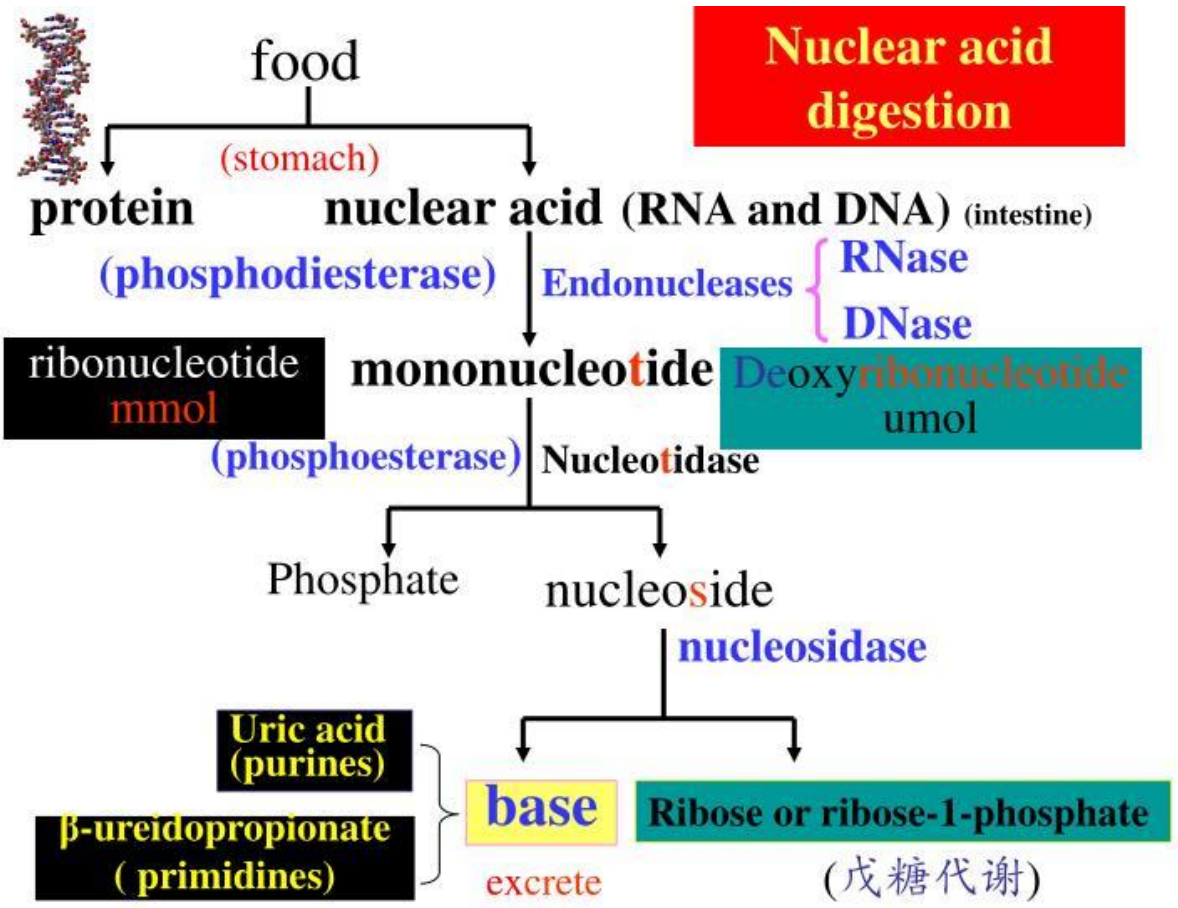


Purines

Adenine

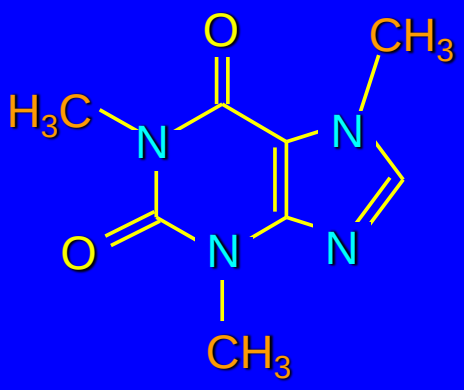


Guanine

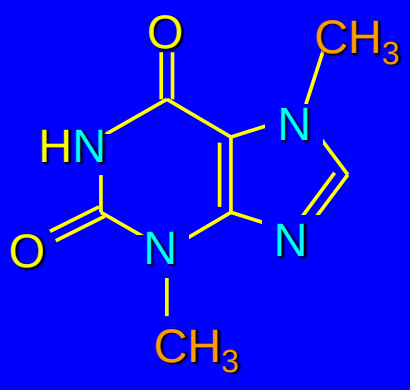


Caffeine and Theobromine

Caffeine (coffee) and theobromine (coffee and tea) are naturally occurring purines.



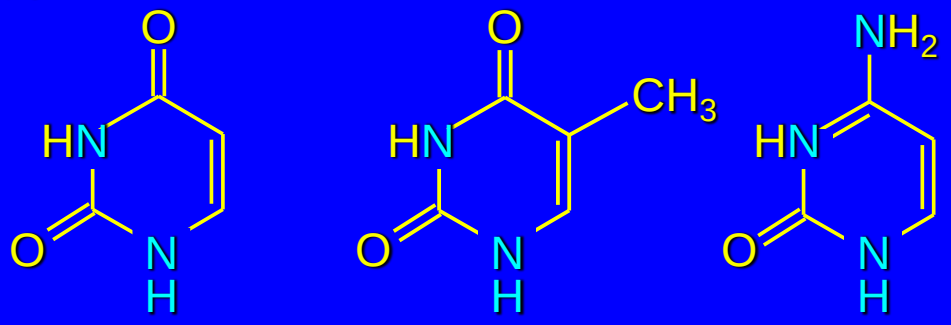
Caffeine



Theobromine

Important Pyrimidines

Pyrimidines that occur in DNA are cytosine and thymine. Cytosine and uracil are the pyrimidines in RNA.



Uracil Thymine Cytosine

Nucleosides

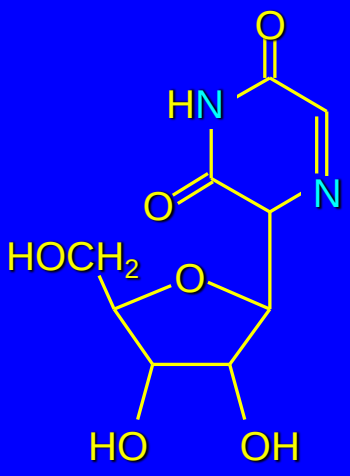
The classical structural definition is that a nucleoside is a pyrimidine or purine N-glycoside of D-ribofuranose or 2-deoxy-D-ribofuranose.

Informal use has extended this definition to apply to purine or pyrimidine N-glycosides of almost any carbohydrate.

The purine or pyrimidine part of a nucleoside is referred to as a *purine or pyrimidine base*.

Uridine and Adenosine

Uridine and adenosine are pyrimidine and purine nucleosides respectively of D-ribofuranose.

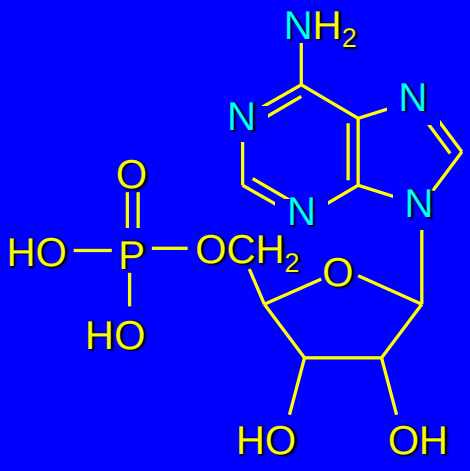


Uridine
(a pyrimidine nucleoside)

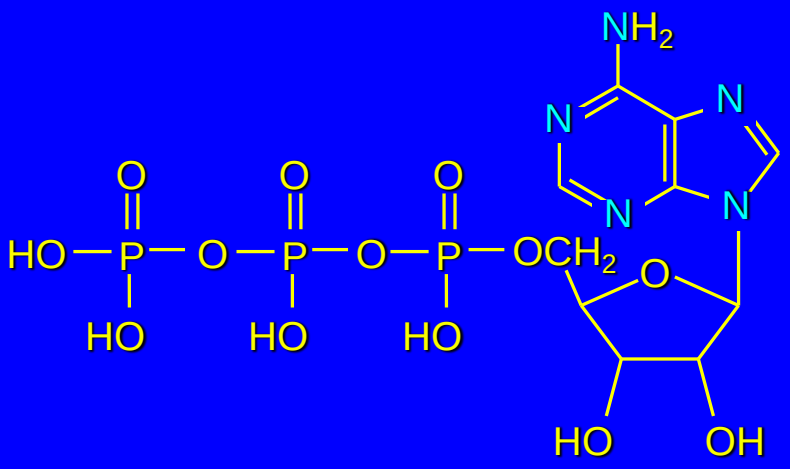
Adenosine
(a purine nucleoside)

Adenosine 5'-Monophosphate (AMP)

Adenosine 5'-monophosphate (AMP) is also called 5'-adenylic acid.

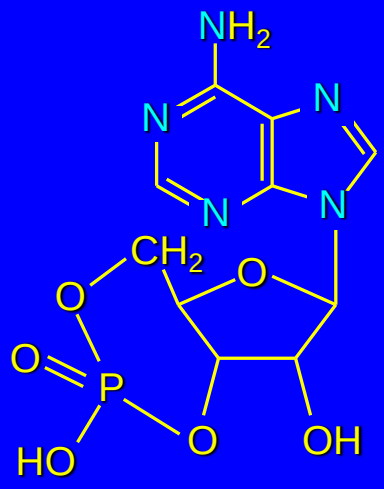


Adenosine Triphosphate (ATP)



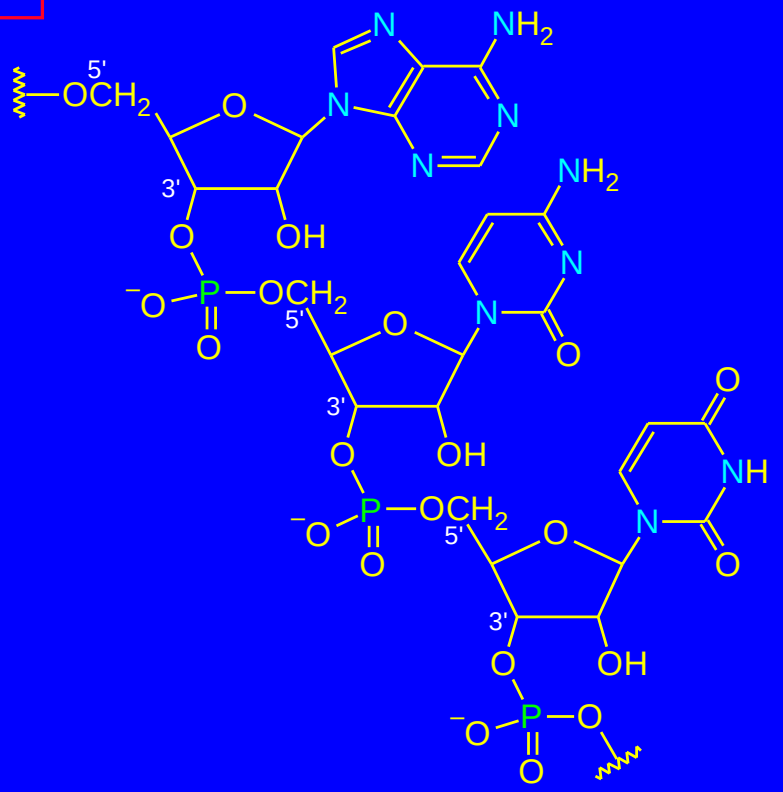
Adenosine 3'-5'-Cyclic Monophosphate (cAMP)

Cyclic AMP is an important regulator of many biological processes.



Fig

Section of polynucleotide chain.



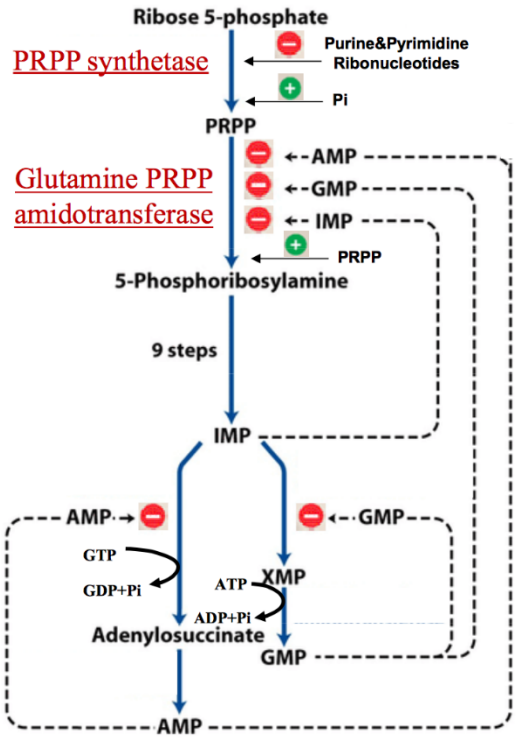
De novo synthesis of purine nucleotides

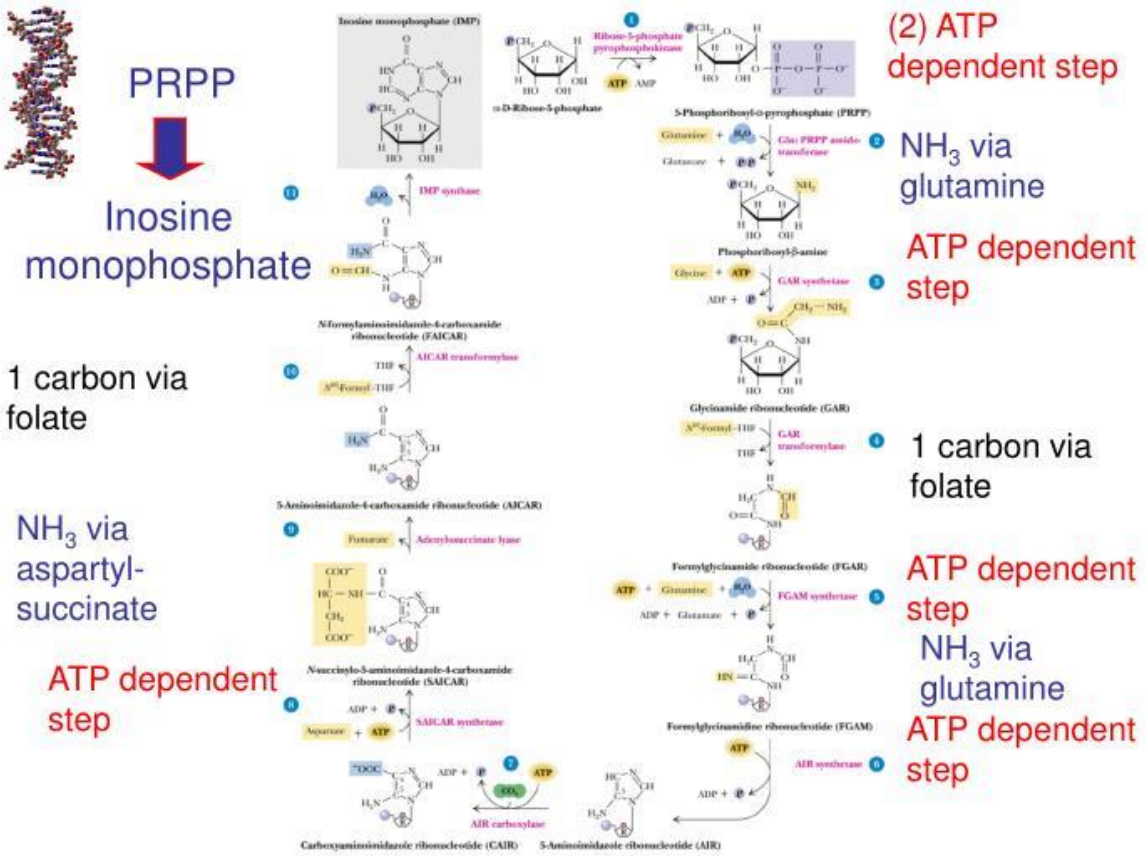
■ **PRPP synthetase is the rate-limiting step in the synthesis of both purines and pyrimidines.**

■ **Glutamine:PRPP amidotransferase catalyzes the first-committed step in purine synthesis.**

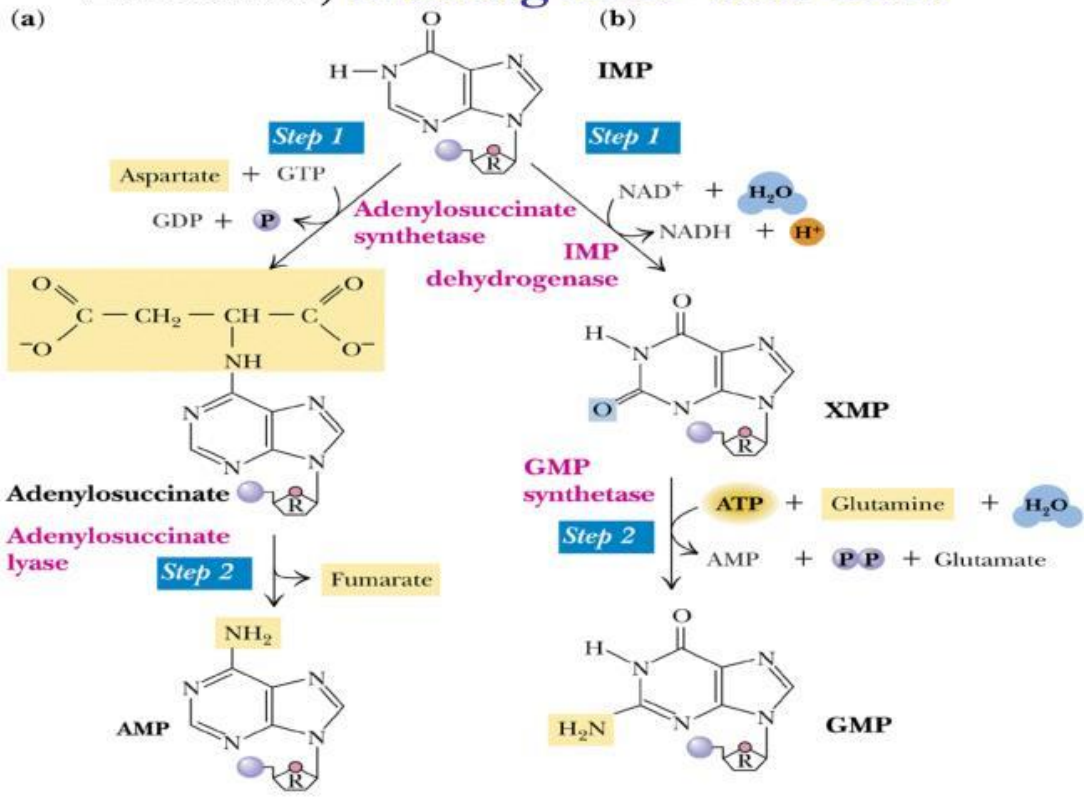
■ **IMP branch to AMP**
- **Inhibitor: AMP**
- **Need for GTP**

■ **IMP branch to GMP**
- **Inhibitor: GMP**
- **Need for ATP**

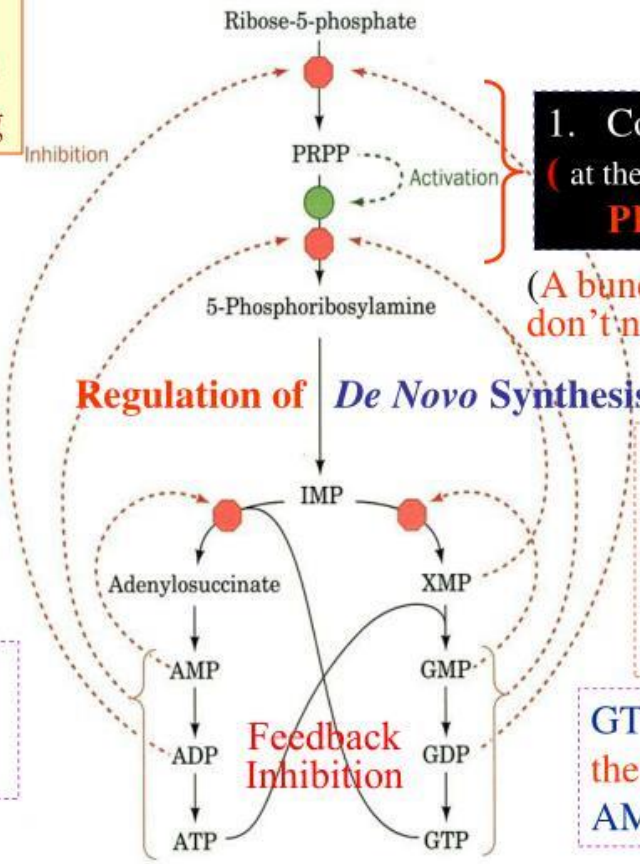




2.Second, Making AMP and GMP



Purines are synthesized on the Ribose ring



1. Committed Steps (at the first two steps): PRPP , PRA

(A bunch of steps you don't need to know)

2.End product inhibition and "feed forward" regulation

Regulation of *De Novo* Synthesis

3. "cross regulation" occurs from IMP to AMP and GMP

ATP provides the energy for GMP synthesis

GTP provides the energy for AMP synthesis

Xanthine Oxidase and Gout

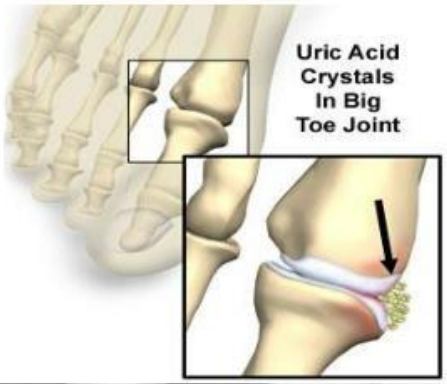
The scale of uric acid (normal value) :

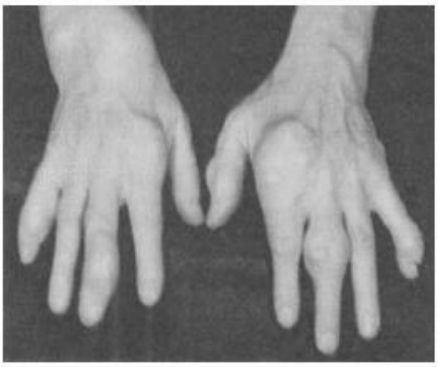
0.12～0.36mmol/L;

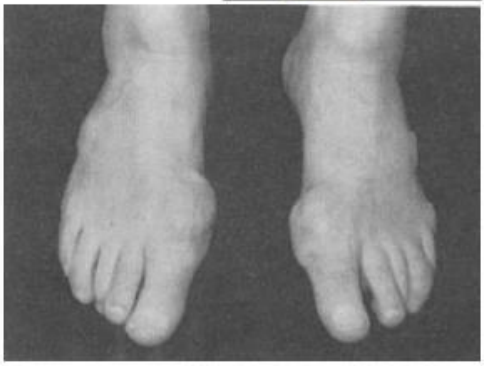
male, 0.27mmol/L;

formale, 0.21mmol/L

>0.48mmol/L(8mg%),







0.5-1 g of uric acid is formed daily in the organism

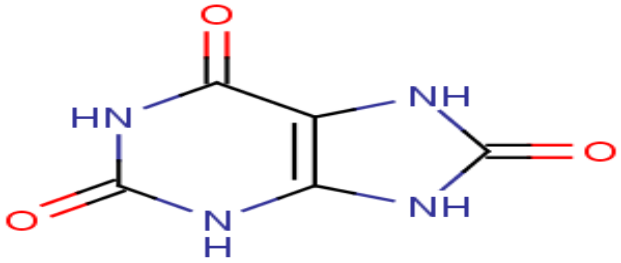
Normal concentration – 0.2-0.5 mmol/L

Uric acid – poorly soluble in water

Hyperuricemia:

-inherited (primary),

-gained (secondary).



Secondary: in radiatio
tumors, toxemia, kidney diseases, alimentary (hyperconsumption of meat, coffee, tea)

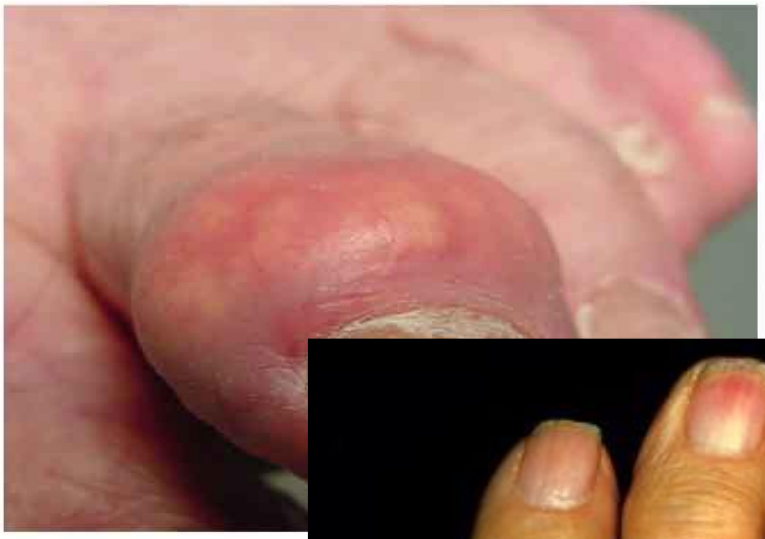
Gout – inherited disease accompanied with hyperuricemia and crystallization of uric acid and its salts in joints, cartilages and kidneys.

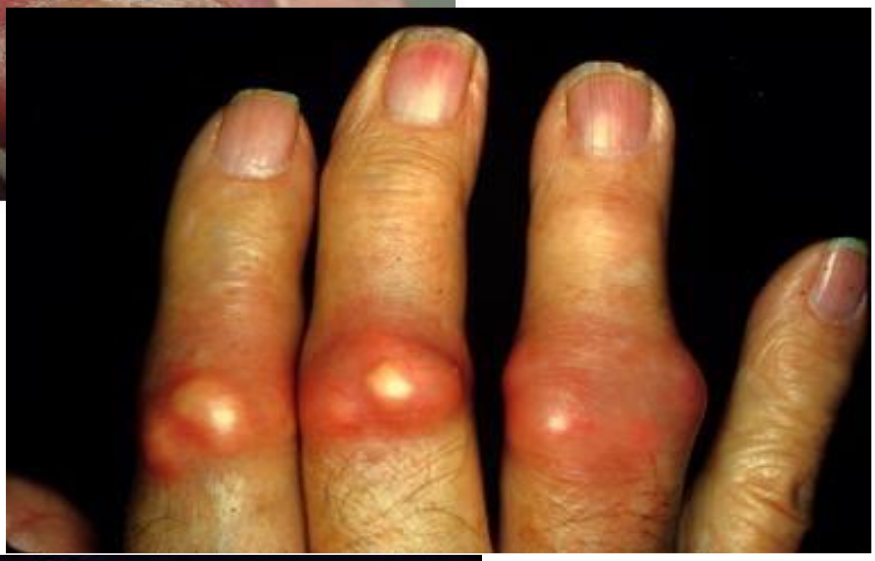
Symptoms:

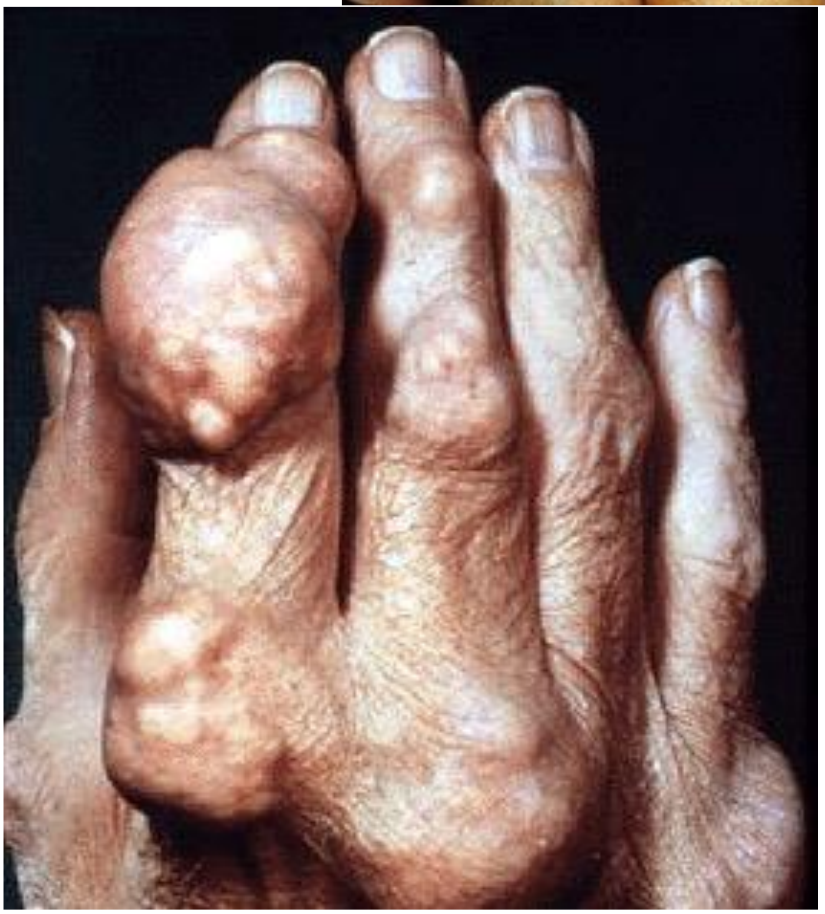
-joints inflammation, acute pain

-renal stones

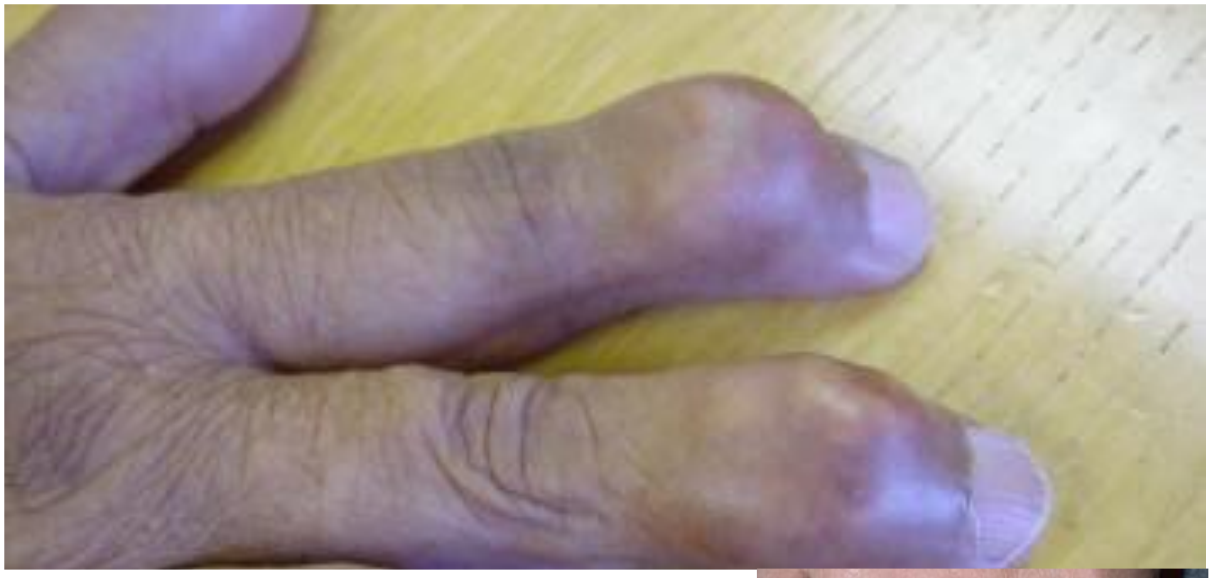
-tophuses.



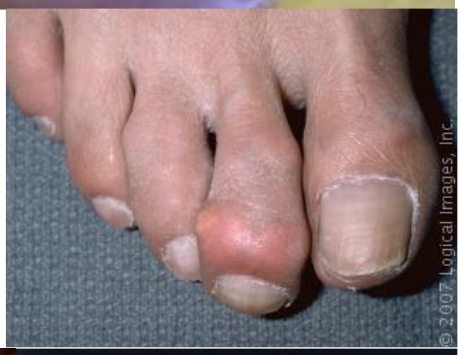


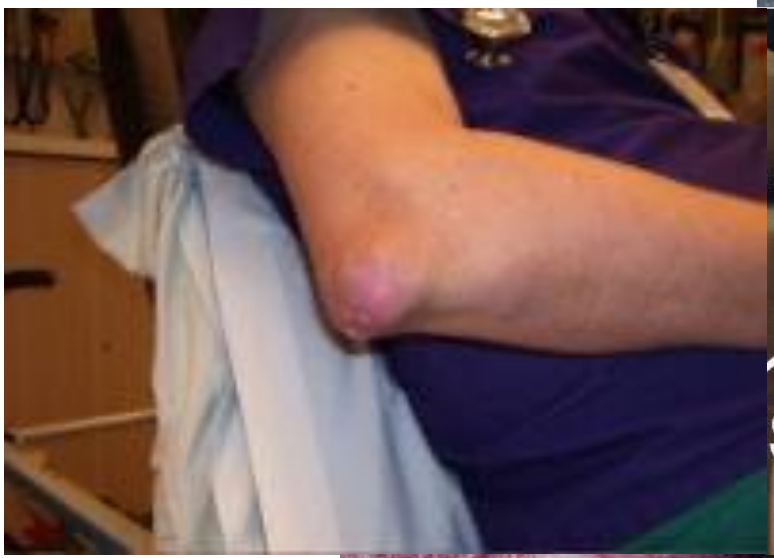


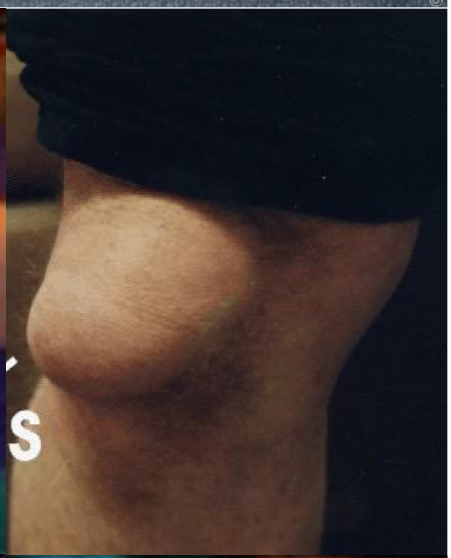
Gout: accumulation of uric acid salts in joints



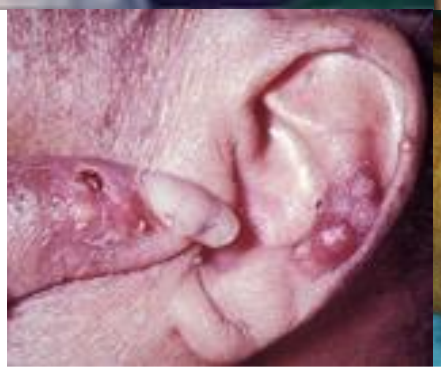
Gout: accumulation of uric acid salts in joints

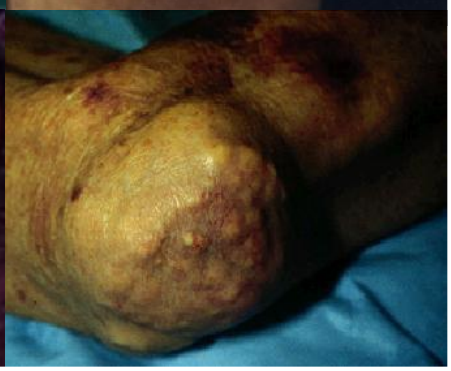


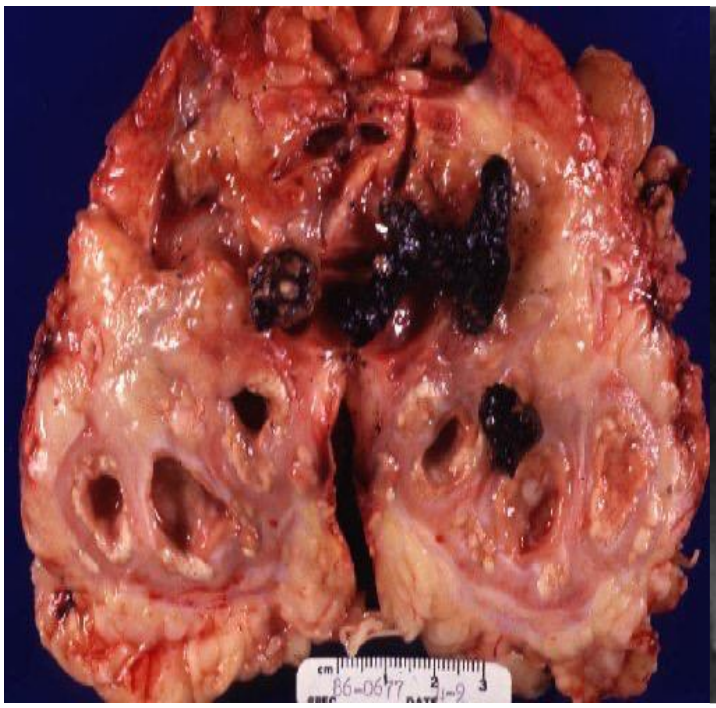


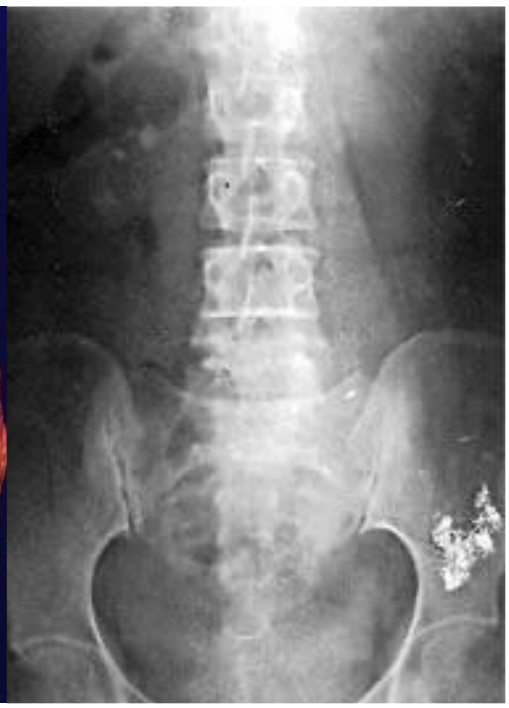


Gout: *tophuses* – accumulation of uric acid salts in cartilages, under skin.









Gout: kidney stones.

Lesch-Nyhan Syndrom: is a inherited disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase. LNS is present at birth in baby boys.

Hypoxanthine and guanine are not used in the salvage pathway of purine nucleotides synthesis.

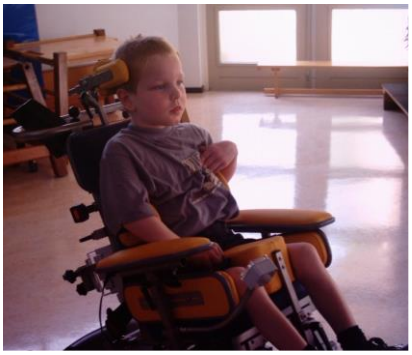
Hypoxanthine and guanine are not utilizied repeatedly but converted into uric acid.

Symptoms:

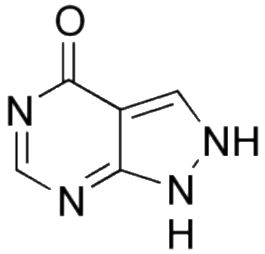
- severe gout

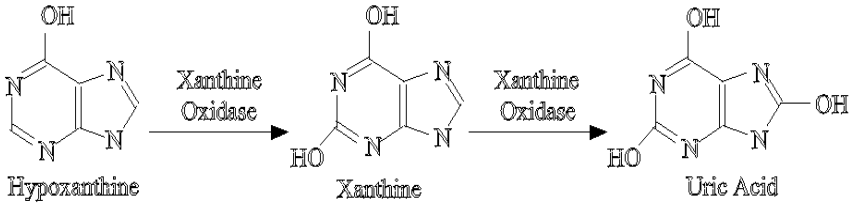
-severe mental and physical problems

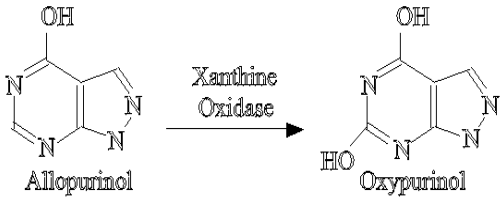
- self-mutilating behaviors



Treatment: *allopurinol* – competitive inhibitor of xanthine oxidase







OROTACIDURIA

inherited disorder of pyrimidine synthesis caused by a deficiency of the enzyme of *orotate-phosphoribosyltransferase* and *decarboxylase*.

Symptoms:

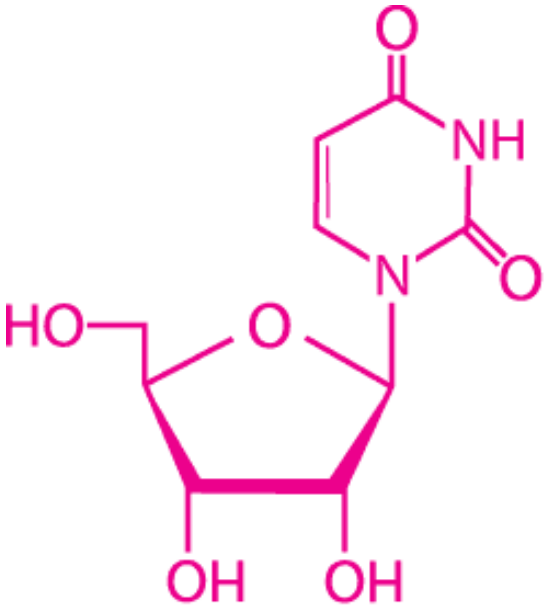
-excess of orotic acid and its excretion with urine (1.0-1.5 g)

-mental and physical retardation

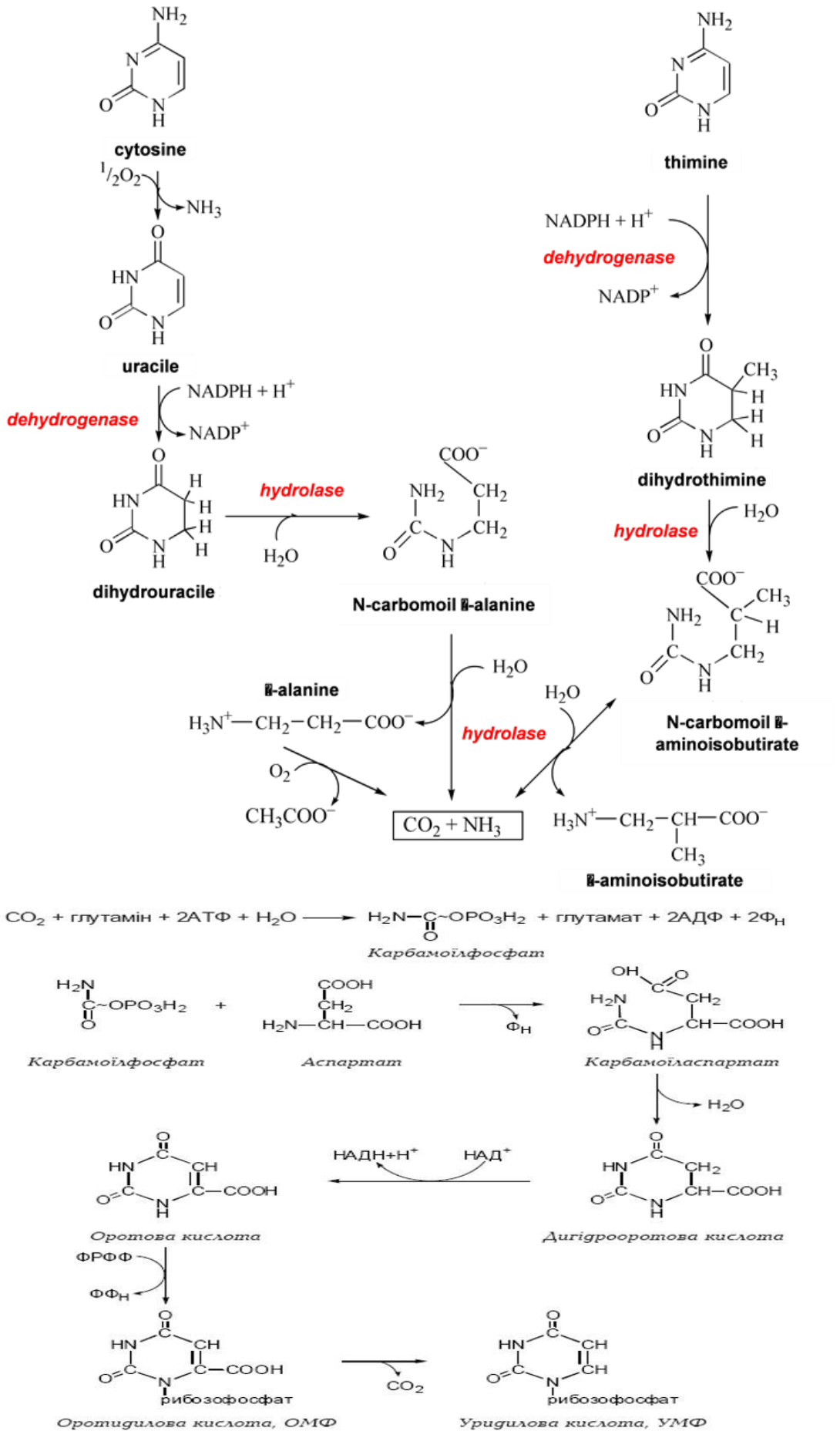
-megaloblastic anemia

TREATMENT OF OROTACIDURIA

Taking of uridin during the whole life



Uridine

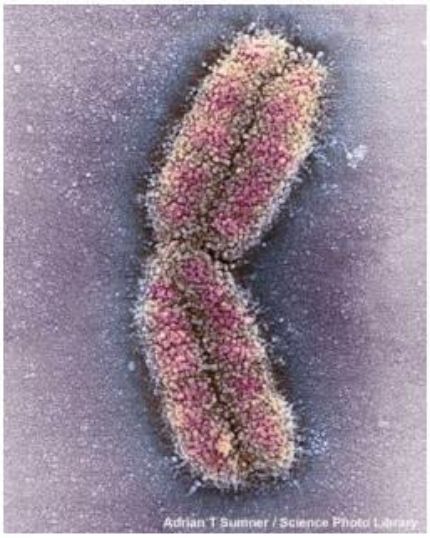

cytosine
thimine
uracile
dihydrothimine
dihydrouracile
N-carbomoil β-alanine
β-alanine
N-carbomoil β-aminoisobutirate
β-aminoisobutirate
dehydrogenase
hydrolase
NADPH + H+
NADP+
H2O
O2
NH3
CH3COO−
CO2 + NH3
CO2 + глутамін + 2АТФ + H2O
Карбамоїлфосфат
глутамат + 2АДФ + 2ФН
Аспартат
Карбамоїласпартат
Оротова кислота
Дигідрооротова кислота
НАДН+H+
НАД+
ФРФФ
ФФН
рибозофосфат
Оротидилова кислота, ОМФ
Уридилова кислота, УМФ

How is DNA packaged?

In eukaryotic cells, DNA is packaged as **chromosomes** in the nucleus.

There is around 2 m of DNA in a cell, so to fit it needs to be tightly coiled and folded.

Eukaryotic DNA is associated with proteins called **histones**. Together, these form **chromatin** – the substance from which chromosomes are made.



In prokaryotic cells, DNA is loose in the cytoplasm – there are no histones or chromosomes.

DNA and Protein Biosynthesis

According to Crick, the "central dogma" of molecular biology is:

"DNA makes RNA makes protein."

Three kinds of RNA are involved.

- messenger RNA (mRNA)
- transfer RNA (tRNA)
- ribosomal RNA (rRNA)

There are two main stages.

- transcription
- translation

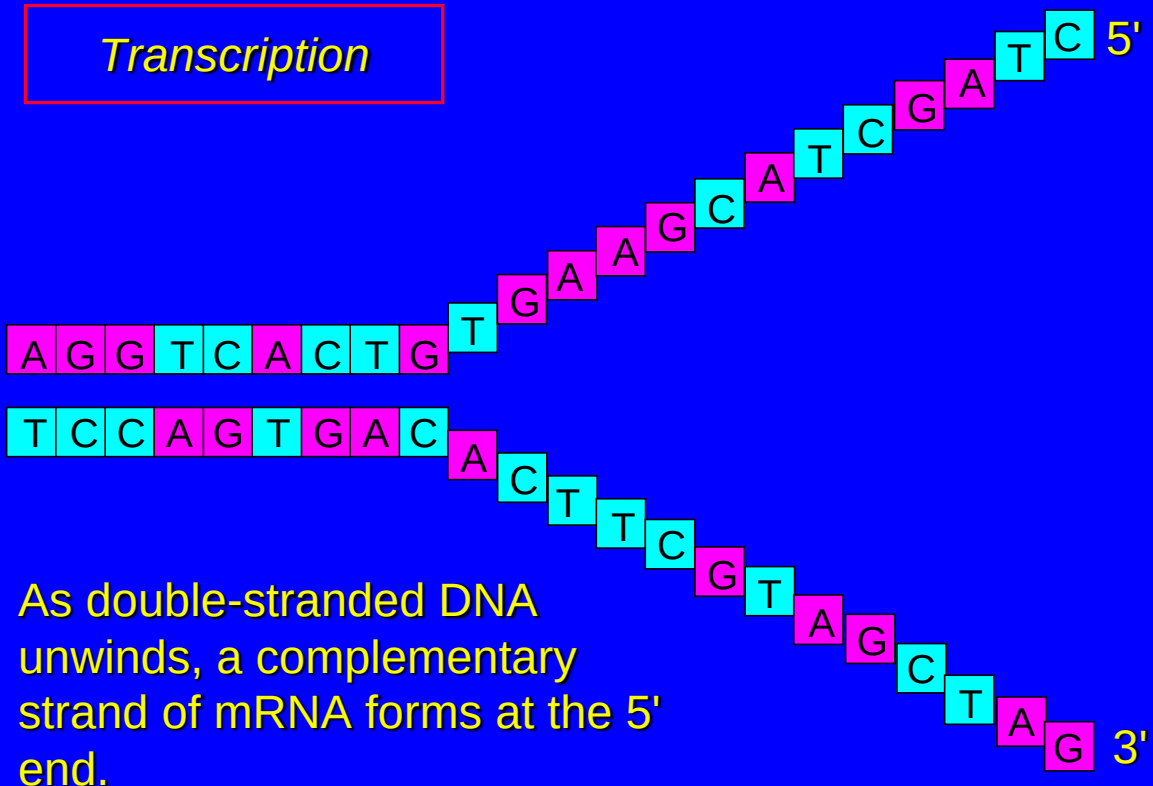
Transcription

Transcription is the formation of a strand of mRNA using one of the DNA strands as a template.

The nucleotide sequence of the mRNA is complementary to the nucleotide sequence of the DNA template.

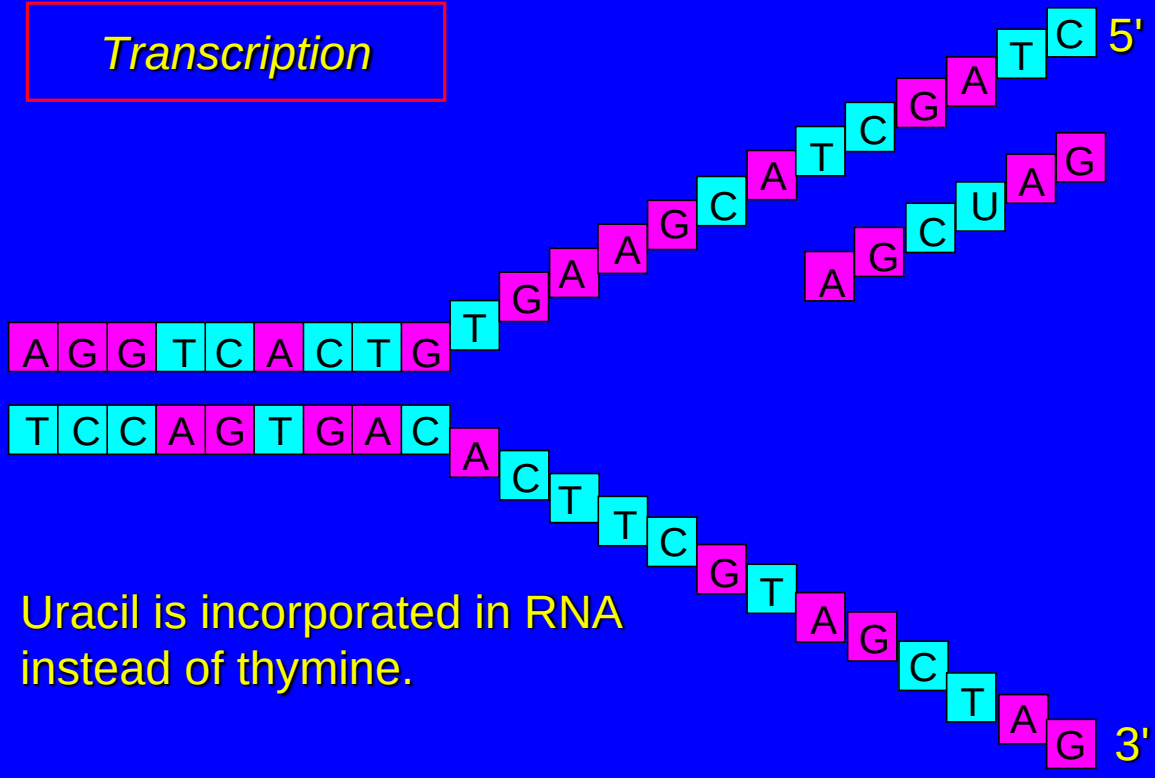
Transcription begins at the 5' end of DNA and is catalyzed by the enzyme *RNA polymerase*.

Transcription



As double-stranded DNA unwinds, a complementary strand of mRNA forms at the 5' end.

Transcription



Uracil is incorporated in RNA instead of thymine.

Translation

The nucleotide sequence of mRNA codes for the different amino acids found in proteins.

There are three nucleotides per codon.

There are 64 possible combinations of A, U, G, and C.

The genetic code is redundant. Some proteins are coded for by more than one codon.

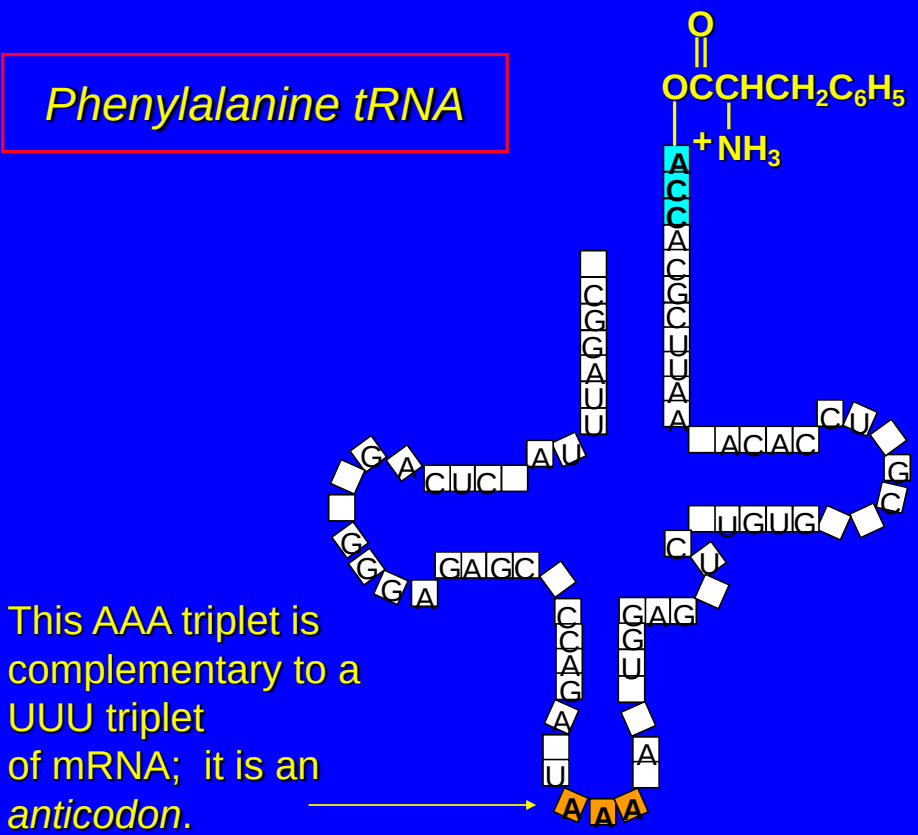
Transfer tRNA

There are 20 different tRNAs, one for each amino acid.

Each tRNA is single stranded with a CCA triplet at its 3' end.

A particular amino acid is attached to the tRNA by an ester linkage involving the carboxyl group of the amino acid and the 3' oxygen of the tRNA.

Phenylalanine tRNA

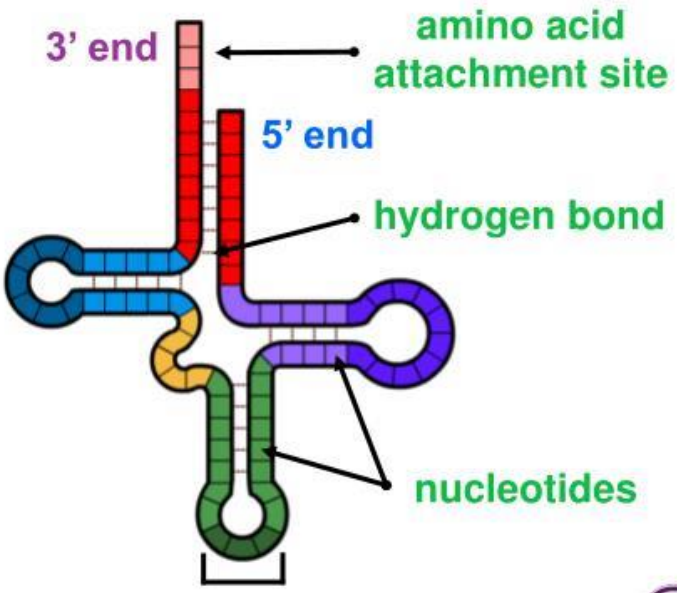


This AAA triplet is complementary to a UUU triplet of mRNA; it is an *anticodon*.

What is tRNA?

In the cytoplasm, amino acids become attached to **transfer RNA** (**tRNA**) molecules. Each tRNA is specific for one amino acid.

Each tRNA molecule has a sequence of three bases called an **anticodon**. These are complementary to codons on the mRNA molecule.



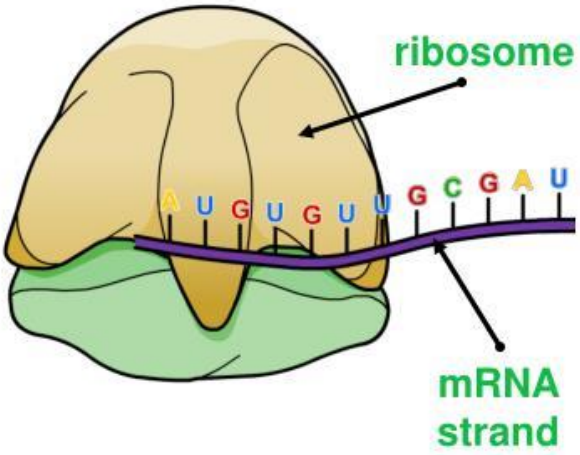
What is the anticodon for the codon A U G?

U A C

What is translation?

Once a molecule of mRNA has been transcribed, it moves out of the nucleus via a nuclear pore.

In the cytoplasm, the mRNA combines with a **ribosome** – the cellular structure on which the polypeptide chain will be built in a process called **translation**.



How are the correct amino acids transported to the ribosome, and how are they linked together in the correct order?

General material and bulk-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:

Molecular biology.

Fundamentals of Molecular Biology.

1. Nitrogen bases, nucleosides and nucleotides as the composite components of the nucleic acids. Minor nitrogen bases and nucleotides.
2. Free nucleotides: ATP, NAD+, NADP+, FAD, FMN, CTP, UTP, 3′,5′-cAMP, 3′,5′-cGMP, their biochemical functions.
3. Nucleic acids. General characteristics of DNA and RNA, their biological importance in the storage and the transfer of genetic information.
4. Features of DNA and RNA primary structure. Chemical bonds, which are responsible for the formation of nucleic acids primary structure.
5. Secondary structure of DNA, role of hydrogen bonds in its formation (Chargaff′s rules, Watson-Crick model), anti-parallelity of strands.
6. Tertiary structure of DNA. Physical and chemical properties of DNA, interaction with cation ligands, formation of nucleosomes.
7. Molecular organization of nuclear chromatin of eukaryotes; nucleosome organization, histone and non-histone proteins.
8. Structure, properties and biological functions of RNA. Types of RNA: m-RNA, t-RNA, r-RNA. Features of the different type of RNA structural organization.
9. Nucleoproteins: structure, biological functions.
10. Biosynthesis of purine nucleotides; scheme of IMP synthesis reactions.
11. Formation of AMP and GMP from IMP, mechanisms of regulation.
12. Biosynthesis of pyrimidine nucleotides; scheme of reactions, regulation of synthesis.
13. Biosynthesis of deoxyribonucleotides. Formation of the thymidine nucleotides. Inhibitors of TMP synthesis as anti-cancer medicines.
14. Catabolism of purine nucleotides, hereditary disturbances of the uric acid metabolism.
15. Scheme of the pyrimidine nucleotide catabolism.
16. Replication of DNA, its biological importance, and semiconservative mechanism of replication.
17. Sequence of the steps and DNA replication enzymes in prokaryotes and eukaryotes.

18. RNA transcription: prokaryotes and eukaryotes RNA-polymerases, signals of transcription: promoter, initiator and terminator fragments of genome.
19. Processing and post-translational modification of synthesized RNA.
20. Genetic (biologic) code, triplet structure and properties.
21. Transport RNA and transportation of amino acids. Amino acyl-tRNA-synthetases.
22. Steps and mechanism of translation (protein synthesis) in ribosomes: initiation, elongation and termination.

Principles of the molecular genetics.

1. Post-translational modification of peptide chains. Regulation of translation.
2. Inhibitors of transcription and translation in prokaryotes and eukaryotes. Antibiotics and interferons, they use in medicine. Diphtheria toxin.
3. Regulation of prokaryote gene expression: regulatory and structural fragments of lactose, Lac-operon, gene regulator, promoter, operator.
4. Amplification of genes. Examples. Polymerase chain reaction. Biological importance of gene amplification.
5. Mutations: genome, chromosome, gene. Mechanisms of mutagen activity; role of the induced mutations in the origin of the enzymopathology and hereditary human diseases.
6. Biological importance and mechanisms of DNA reparations. Reparation of UV-induced gene mutations: xeroderma pigmentosum.

Gene engineering: construction of recombinant DNA, gene cloning. Genetic engineering of enzymes, hormones, interferons, etc.

Methodological support:

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/

Lecture 12.

Biochemical and molecular biological mechanisms of hormone action; hierarchy of hormones. Hormones of protein-peptide nature.

Topic relevance: 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.

Objective: formation of concepts about 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.

Plan and organizational structure of the lecture:

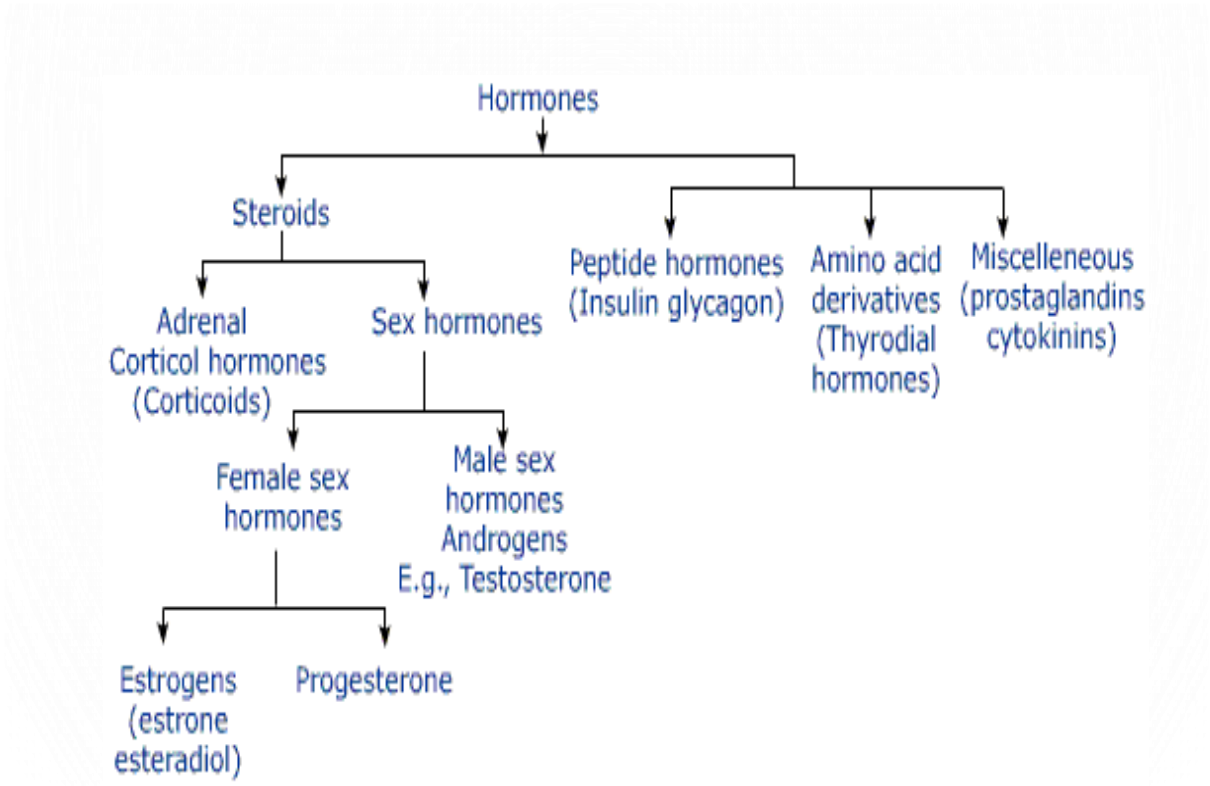
1. General laws of hormonal regulation.
2. Classification of hormones.
3. Mechanisms of hormone action on target cells.
4. Hormones of the hypothalamus.
5. Tropic hormones of the pituitary gland.
6. Thyroid hormones. Pathology.
7. Hormones of the parathyroid gland. Regulation of phosphorus-calcium metabolism.

Hormones – organic biologically active compounds of different chemical nature that are produced by the endocrine glands, enter directly into blood and accomplish humoral regulation of the metabolism of compounds and functions on the organism level.

Hormonoids (tissue hormones) – compounds that are produced not in glands but in different tissues and regulate metabolic processes on the local level, but some of them (serotonin, acetylcholine) enters blood and regulate processes on the organism level.

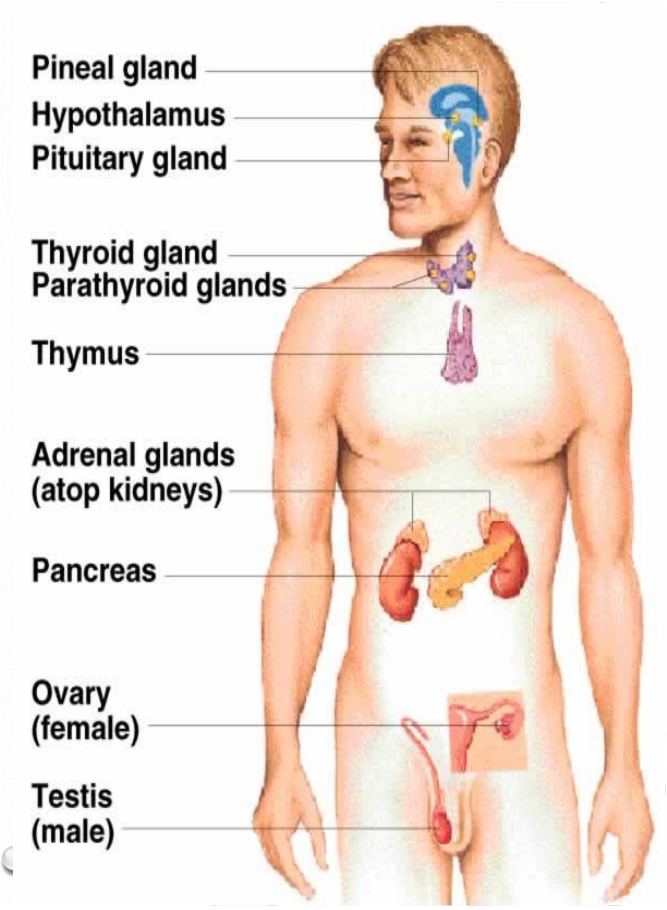
Specific stimulus for hormones secretion is:

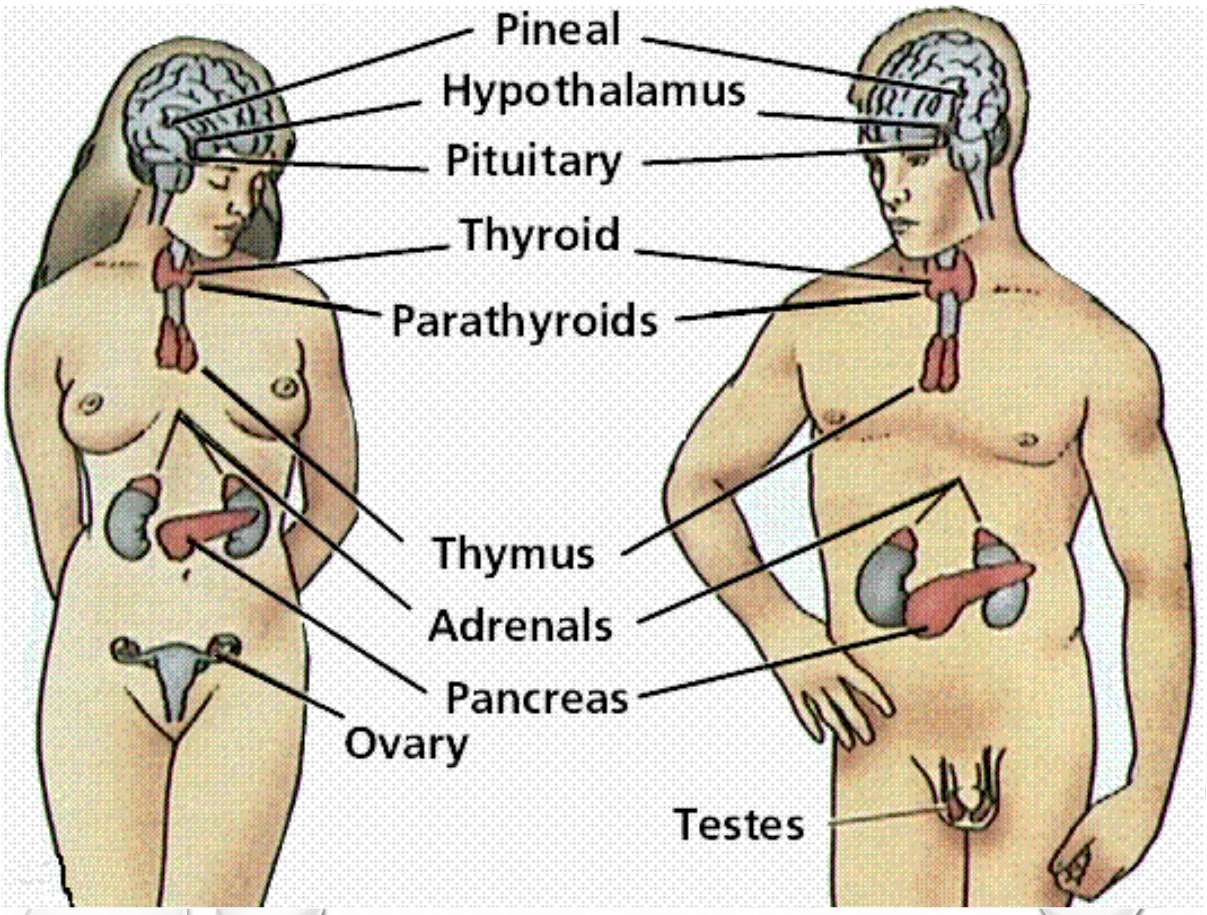
- nervous impulse
- concentration of the certain compound in blood passing through the endocrine gland



Endocrine glands:

1. Hypothalamus
2. Pituitary
3. Epiphysis
4. Thymus
5. Thyroid gland
6. Parathyroid glands
7. Langergans' islands of pancreas
8. Epinephrine glands
9. Sex glands





Classification of hormones according to chemical nature

1. *Proteins:* hormones of anterior pituitary (except ACTH), insulin, parathyroid hormone.

2. *Peptides:* acth, calcitonin, glucagon, vasopressin, oxytocin, hormones of hypothalamus (releasing factors and statins).

3. *Derivatives of amino acids:* catecholamins (epinephrine and norepinephrine), thyroxin, triodthyronin, hormones of epiphysis.

4. *Steroid (derivatives of cholesterol):* hormones of the cortex of epinephrine lands, sex hormones.

5. *Derivatives of polyunsaturated fatty (arachidonic) acids:* prostaglandins.

Introduction

- Hormones are *chemical* substances produced by a cell, a gland, or an organ in one part of the body that affects cells in nearby or other parts of the organism.
- Generally, only a *small amount* (picomol to nmol) of hormone is required to alter cell metabolism.
- In essence, it is a chemical *messenger* that transports a signal from one cell to another.
- Hormone action at the cellular level begins with the association of the hormone and its specific *receptor*.

Fate of hormones in the organism

- Are secreted directly into the blood
- Peptide and protein hormones are secreted by exocytosis
- Steroid (lipophilic) hormones continuously penetrate the membrane (they are not accumulated in cells, their concentration in blood is determined by the speed of synthesis)

Transport of hormones in blood

Protein and peptide nature – in free state

Steroid hormones and hormones of thyroid gland – bound with alpha-globulins or albumins

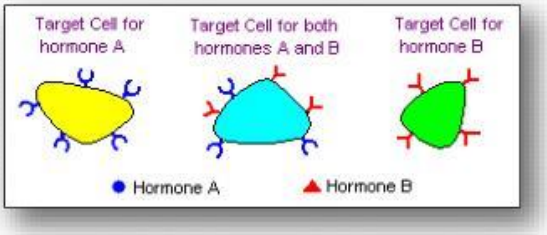
Catecholamines – in free state or bound with albumins, sulphates or glucuronic acid

Reach the target organs

Cells have the specific receptors to certain hormone

Target cell and receptor

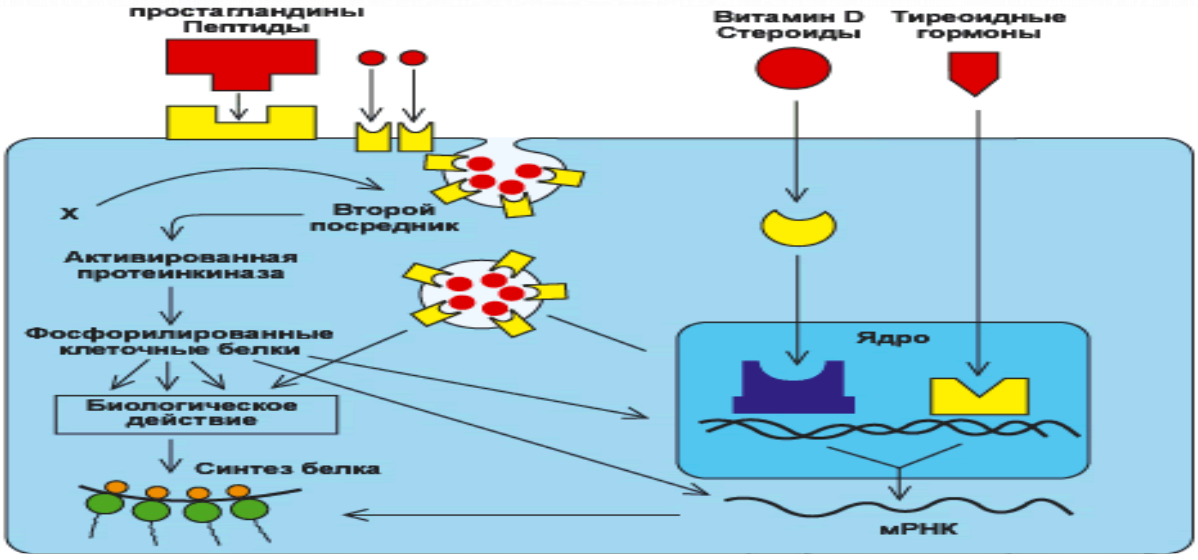
- Most hormones circulate in blood, coming into contact with essentially all cells. However, a given hormone usually affects only a limited number of cells, which are called *target cells*. A target cell responds to a hormone because it bears ***receptors*** for the hormone.
- Hormone receptors are found either exposed on the surface of the cell or within the cell, depending on the type of hormone.

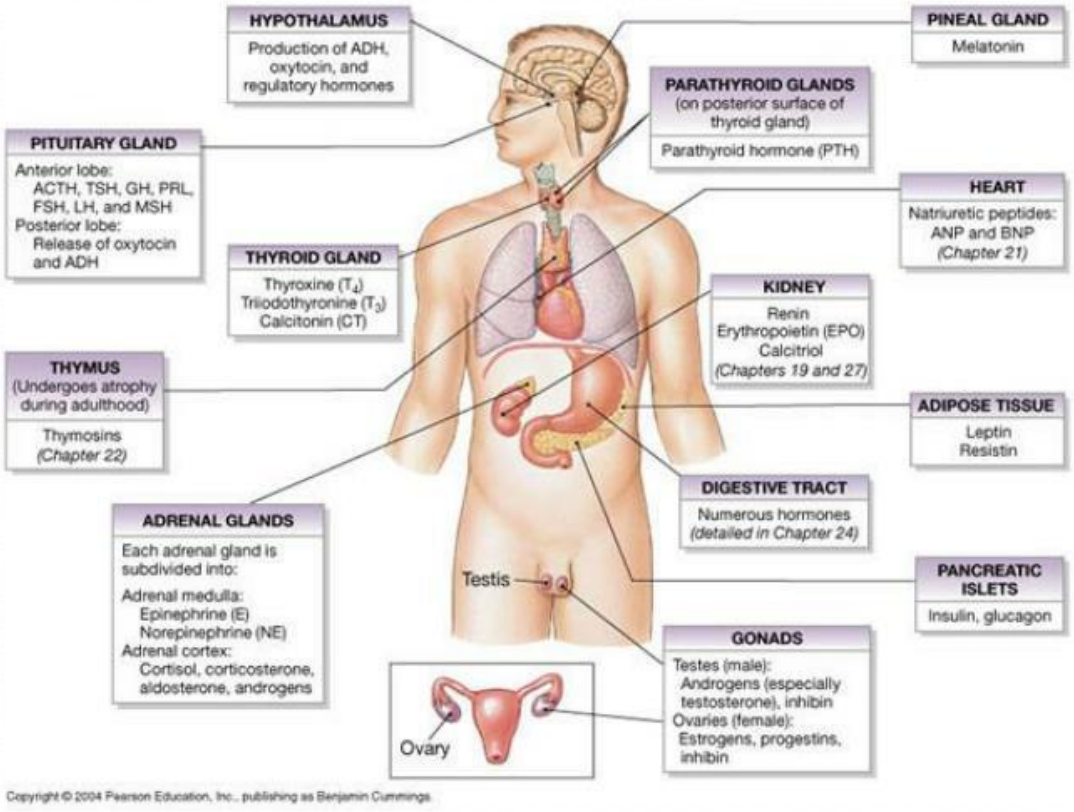


Receptors of hormones

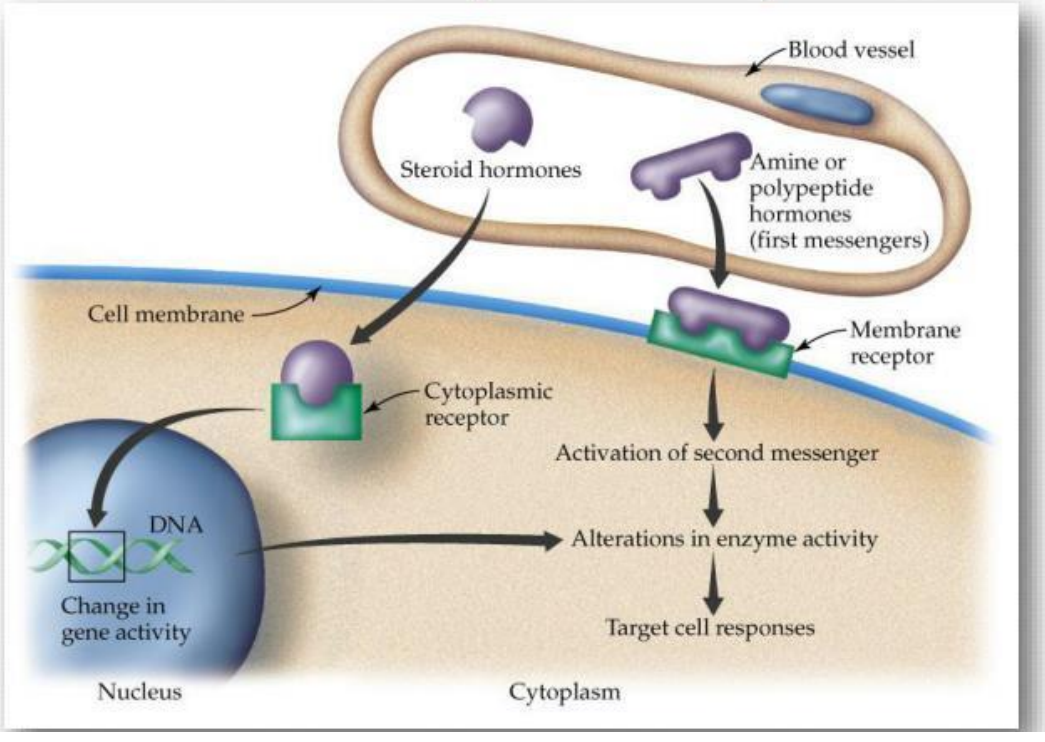
Two groups:

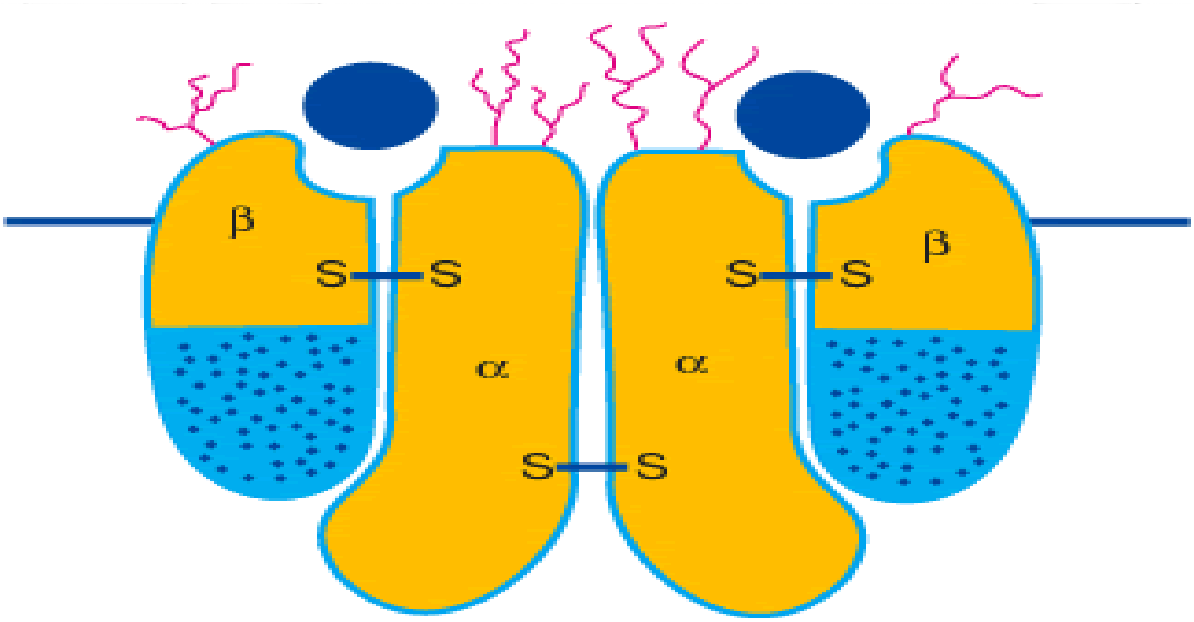
- Placed on the surface of membrane – peptide and protein hormones, prostaglandins;
- Placed inside the cells (cytoplasm, nucleus) – steroid and thyroid hormones





Hormone actions:
Hormone + Receptor complex





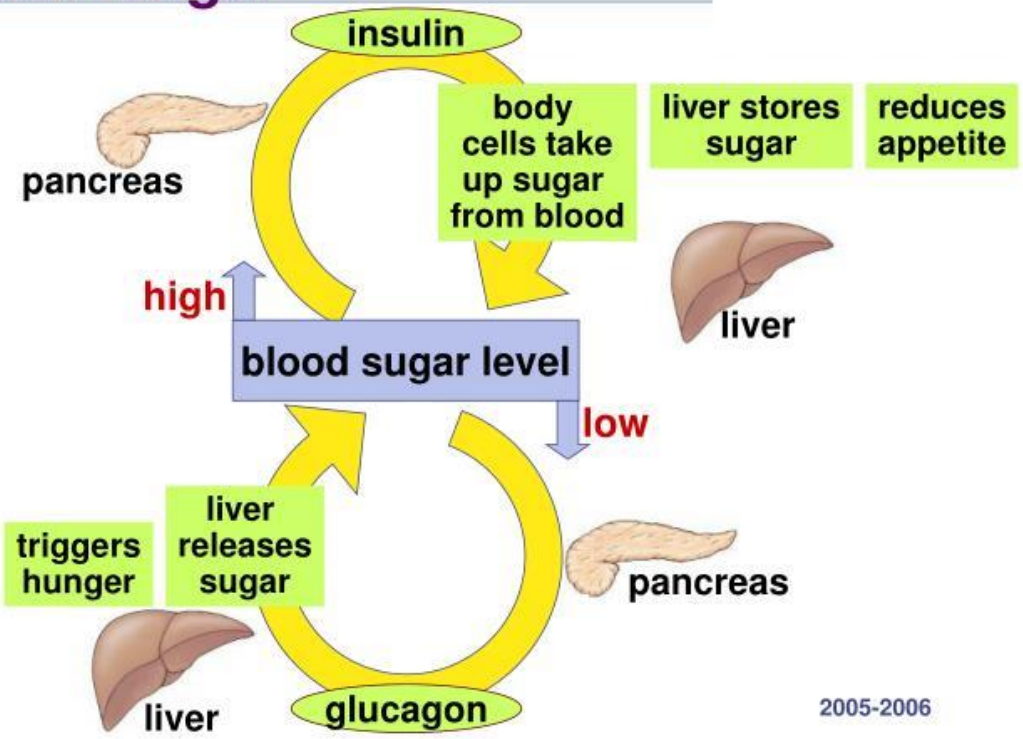
Model of the insulin receptor (Jacobs, Cautrecasas, 1982)

Glucagon

- Glucagon *raises blood glucose* levels by
  - stimulating the liver to metabolize glycogen into glucose molecules and
  - to release glucose into the blood.
  - stimulates adipose tissue to metabolize triglycerides into glucose and to *release glucose* into the blood.

Endocrine System Control Blood Sugar

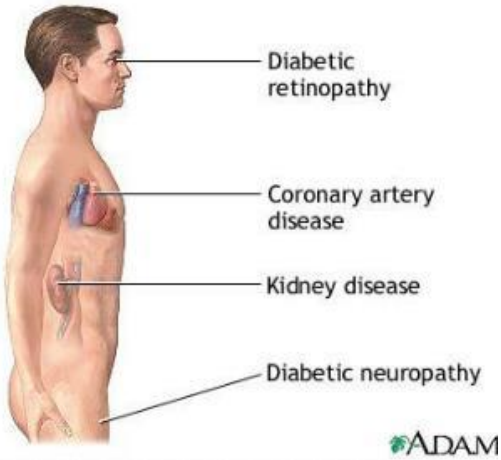
Feedback



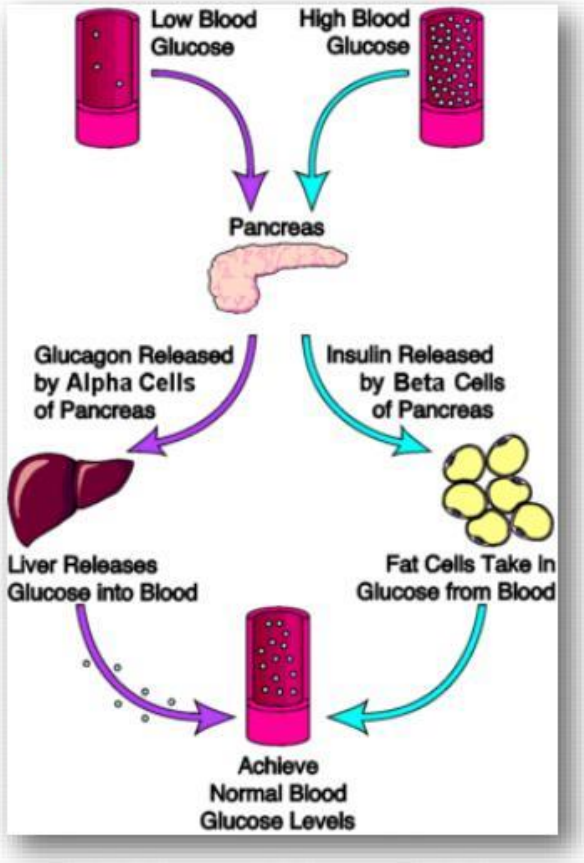
Diabetes mellitus and its complications

Two types of diabetes mellitus:

- Type 1 (Insulin-Dependent Diabetes Mellitus or IDDM). It is characterized by little (hypo) or no circulating *insulin*.
- Type 2: (Non Insulin-Dependent Diabetes Mellitus (NIDDM) and adult-onset diabetes). The problem appears to be a failure to express a sufficient number of *glucose transporters* in the plasma membrane of their skeletal muscles.



Insulin vs Glucagon



Inactivation of hormones

After biochemical effect hormones are released and metabolized

Hormones are inactivated mainly in liver

Inactive metabolites are excreted mainly with urine

Half-time life

- from several min to 20 min – for the majority of hormones
- till 1 h – for steroid hormones
- till 1 week – for thyroid hormones

The final effects of hormones action

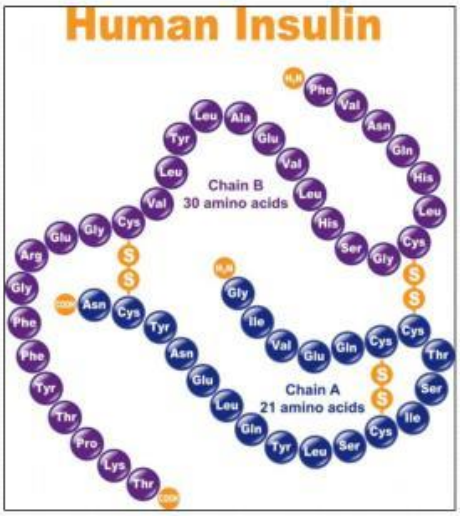
1. *Change the permeability of cell membrane*, accelerate the penetration of substrates, enzymes, coenzymes into the cell and out of cell.
2. *Acting on the allosteric centers affect the activity of enzymes* (hormones penetrating membranes).

3. *Affect the activity of enzymes through the messengers (cAMP)*. (Hormones that can not penetrate the membrane).

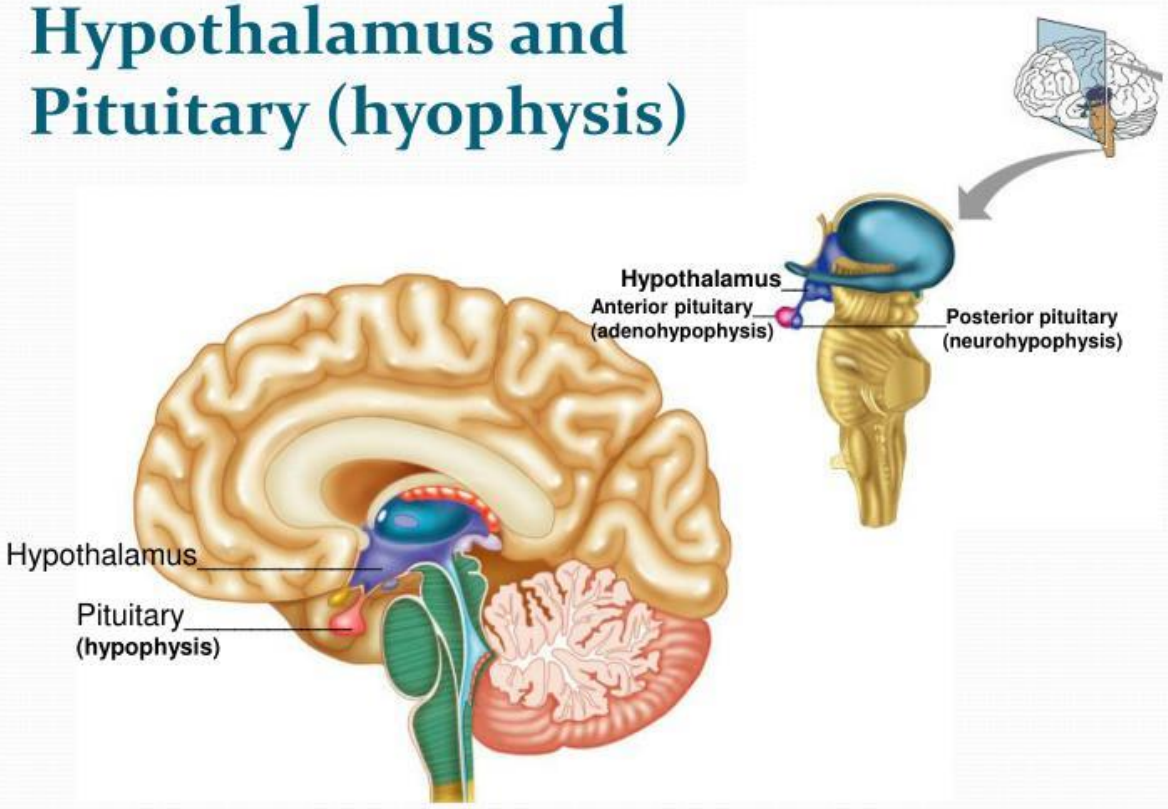
4. *Act on the genetic apparatus* of the cell (nucleus, DNA) and promote the synthesis of enzymes (Steroid and thyroid hormones).

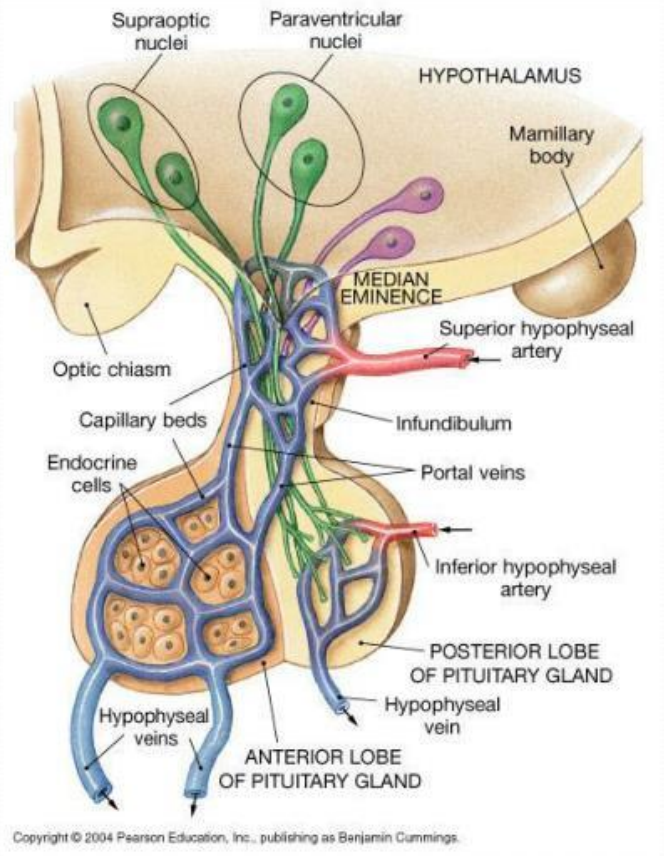
Protein/Peptide Hormones

- Hydrophilic
- Large
- Can't fit through membrane
- Second messenger mechanism of action
- Most hormones
- Example: Insulin



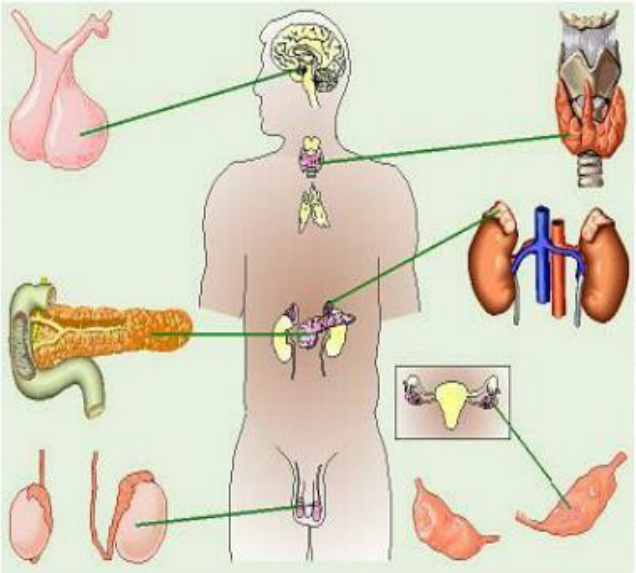
Hypothalamus and Pituitary (hyophysis)





Why is the Hypothalamus so Important?

- Secretes regulatory homones
  - RH (releasing hormones)
  - RIH (inhibitory hormones)
- "Directs" pituitary



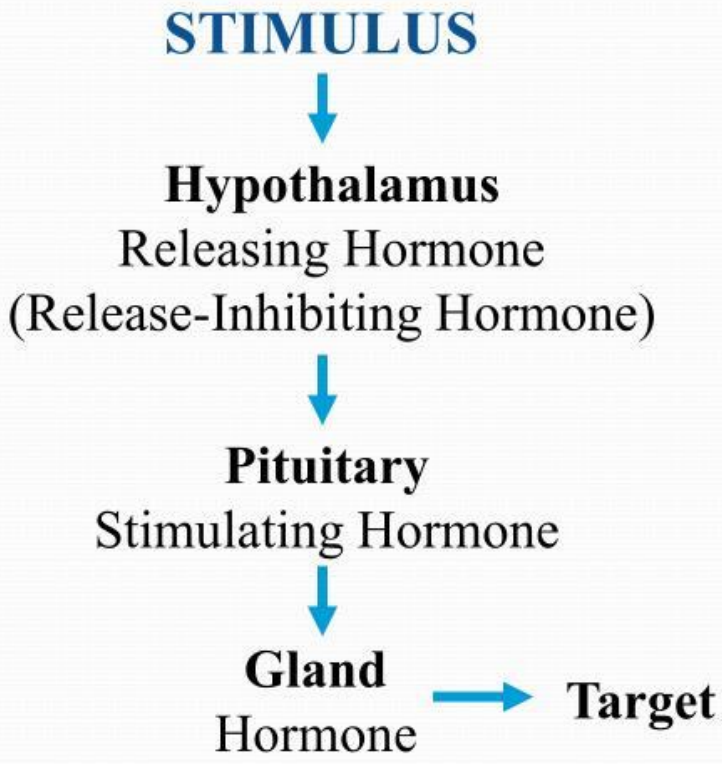
Hypothalamus controls anterior pituitary hormone release

- Releasing hormones (releasing factors) of **hypothalamus**
  Secreted like neurotransmitters from neuronal axons into capillaries and veins to anterior pituitary (adenohypophysis)
  TRH (thyroid releasing hormone) -----turns on* TSH
  CRH (corticotropin releasing hormone) -----turns on ACTH
  GnRH (gonadotropin releasing hormone) ---turns on FSH and LH
  PRF (prolactin releasing hormone) -----turns on PRL
  GHRH (growth hormone releasing hormone) ----turns on GH

- Inhibiting hormones of **hypothalamus**
  PIF (prolactin inhibiting factor) -----turns off PRL
  GH (growth hormone) inhibiting hormone ---turns off GH

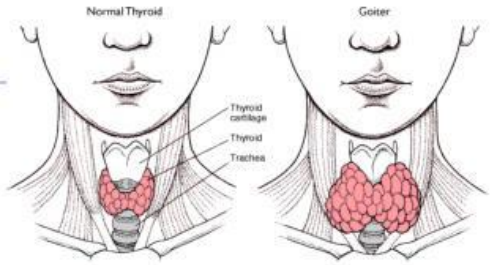
The hypothalamus controls secretion of hormones which in their turn control the secretion of hormones by the thyroid gland, the adrenal cortex and gonads: in this way the brain controls these endocrine glands

*Note: "turns on" means causes to be released



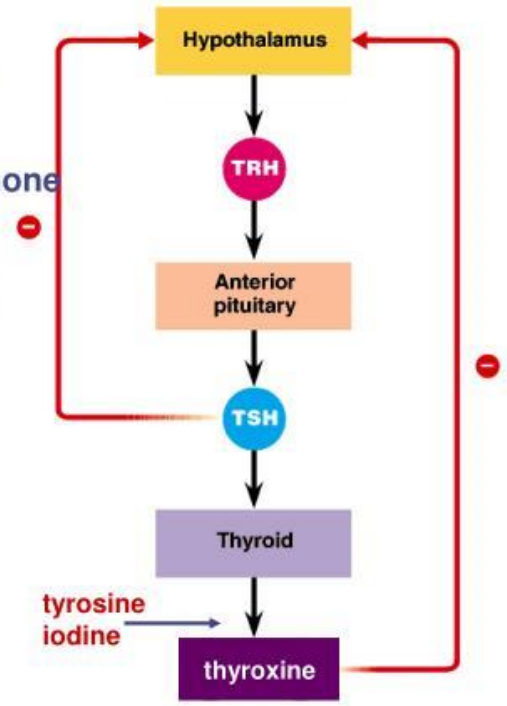
Goiter

Iodine deficiency causes thyroid to enlarge as it tries to produce thyroxine



Regulating metabolism

- **Hypothalamus**
  - TRH = TSH-releasing hormone
- **Anterior Pituitary**
  - TSH = thyroid stimulating hormone
- **Thyroid**
  - produces thyroxine hormones
  - metabolism & development
    - bone growth
    - mental development
    - metabolic use of energy
    - blood pressure & heart rate
    - muscle tone
    - digestion
    - reproduction



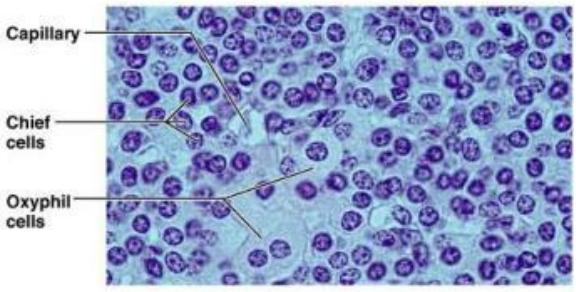
AP Biology

Some Effects of Thyroid Hormone (Thyroxine)

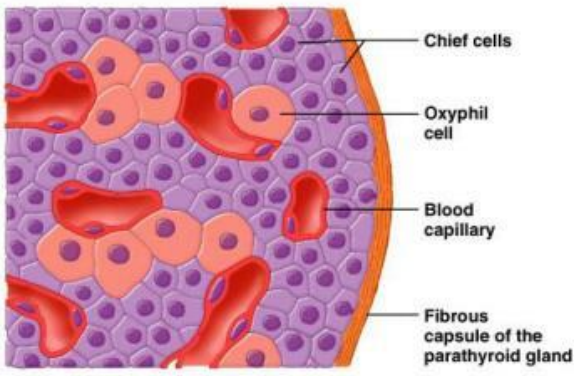
- Increases the *basal metabolic* rate
  - The rate at which the body uses oxygen to transform nutrients (carbohydrates, fats and proteins) into energy
- Affects many target cells throughout the body; some effects are
  - Protein synthesis
  - Bone *growth*
  - Neuronal *maturation*
  - Cell *differentiation*

Parathyroids
(two types of cells)

- Chief cells produce PTH
  - Parathyroid hormone (PTH) or parathormone
  - A small protein hormone



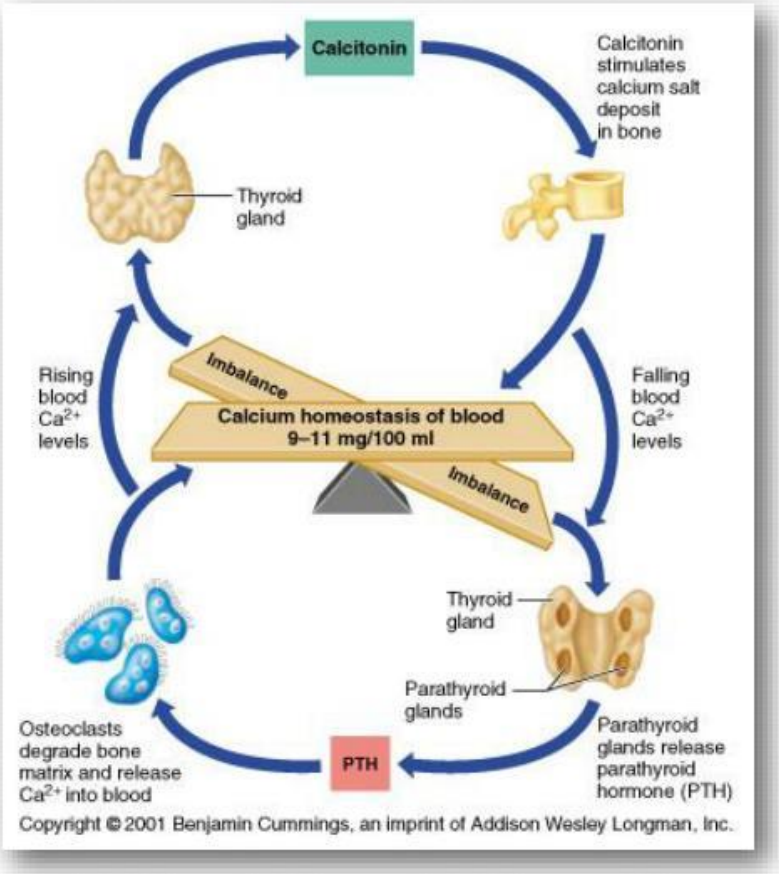
(b)



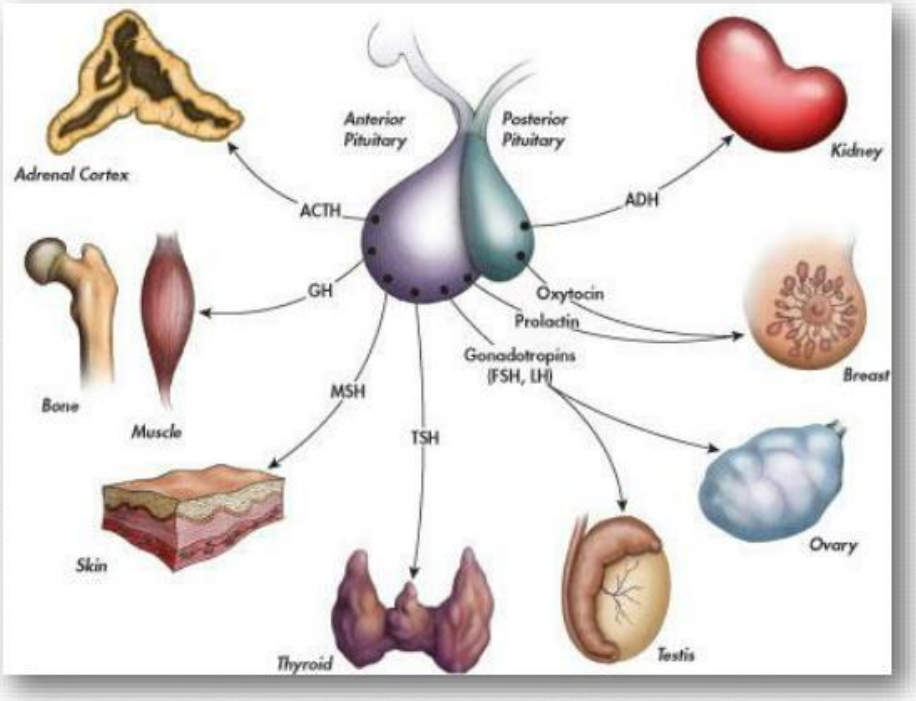
(c)

Function of PTH

- ***Increases* blood Ca^{++} (calcium) concentration when it gets too low**
- Mechanism of raising blood calcium
  1. Stimulates osteoclasts to release more Ca^{++} from bone
  2. *Decreases* secretion of Ca^{++} by kidney
  3. *Activates* vitamin D, which stimulates the uptake of Ca^{++} from the intestine
- Unwitting removal during thyroidectomy was lethal
- ***Has opposite effect on calcium as calcitonin (which lowers Ca^{++} levels)***



Target organs of hypophysis



The Effects of Calcitonin

- Secreted from thyroid parafollicular (C) cells when blood calcium levels are high
- Calcitonin *lowers* Ca^{++} by slowing the calcium-releasing activity of osteoclasts in bone and increasing calcium secretion by the kidney
- Acts mostly during childhood

Endocrine activity of the Thyroid Gland

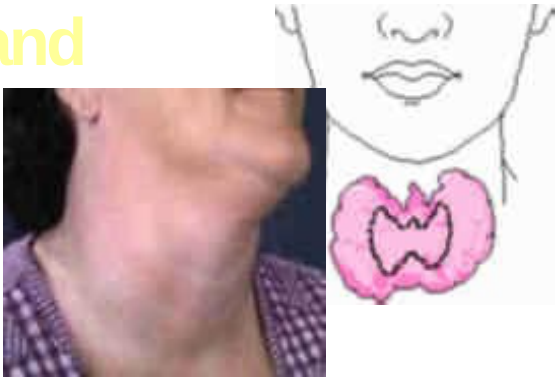
- **Hypothyroidism:**

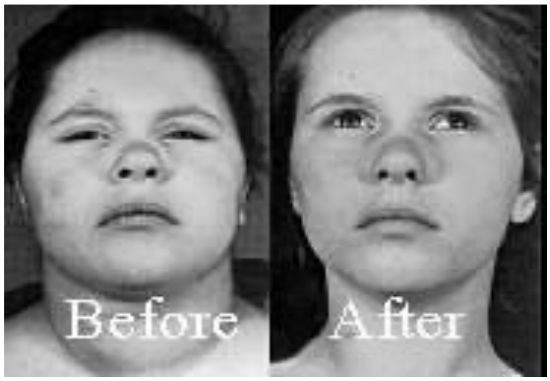
  endemic goiter: (due to I2 deficiency)

  Myxedema: bagginess under the eyes and swelling of the face.

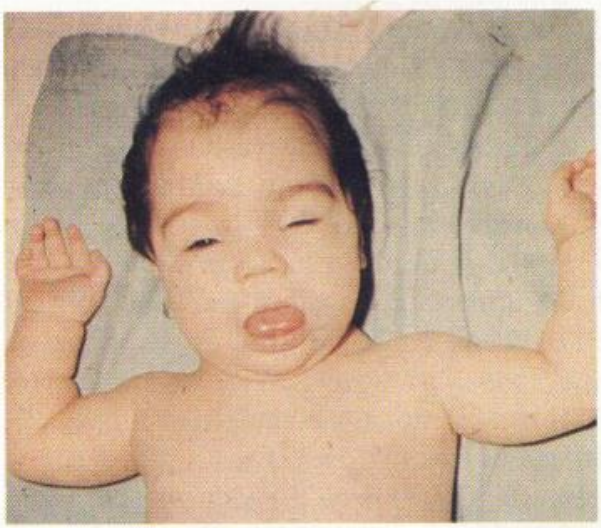
  Arteriosclerosis: due to increase in blood cholesterol

  Cretinism: extreme hypothyroidism during infancy and childhood





Hyposecretion of thyroid hormone



(a) Cretinism

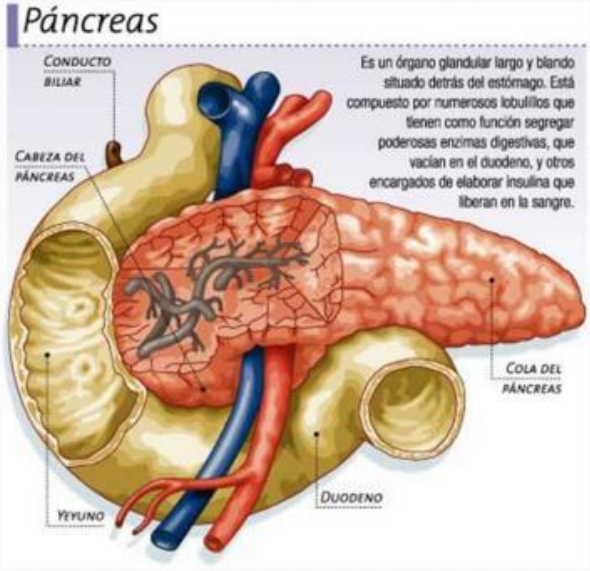
The Pancreas

Exocrine and endocrine cells

- Acinar cells (forming most of the pancreas)
  - *Exocrine* function
  - Secrete digestive enzymes
- Islet cells (of Langerhans)
  - *Endocrine* function

The pancreas secretes two hormones:

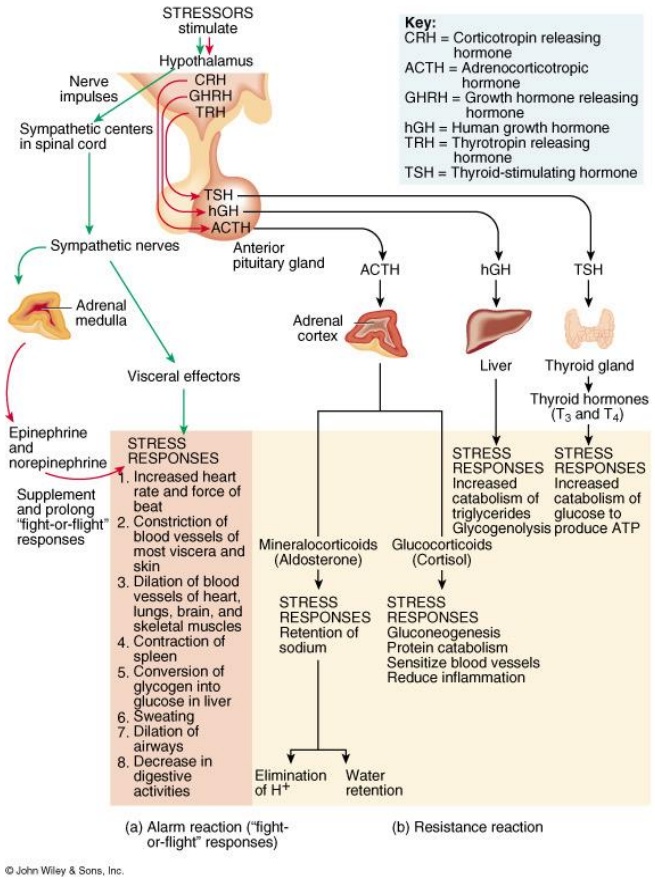
- Insulin
- Glucagon



Insulin

- *Insulin* is a small protein consisting of 51 amino acids.
- Beta cells secrete insulin in response to a rising level of circulating glucose ("blood sugar").
- Insulin affects many organs. It stimulates skeletal muscle fibers to
  - take up glucose and convert it into *glycogen*;
  - take up amino acids from the blood and convert them into *protein*.
- acts on liver cells
  - stimulating them to take up glucose from the blood and convert it into glycogen while
  - inhibiting "gluconeogenesis"; that is, the conversion of fats and proteins into glucose.
- *Drops* in the level of blood sugar

Overview of the interactions of hormones in response to stress



General material and bulk-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:

Biochemistry of intercellular communications.

Molecular mechanisms of hormone action on the target cells.

1. Hormones and their general characteristics. Role of hormones and other bioregulators in the system of the intracellular integration of the human organism functions.
2. Classification of hormones and bioregulators in correspondence of structure and mechanisms of hormone activity.
3. Endocrine, paracrine and autocrine mechanisms of the hormonal activity.
4. Reaction of the target cells on the hormone action. Membrane (ionotropic, metabotropic) and cytosol receptors.

5. Biochemical systems of the hormonal signals intracellular transfer: G-proteins, and secondary messengers cAMP, Ca^{2+}-calmodulin, inositol-3-phosphate, and diacylglycerol.
6. Molecular cell mechanisms of the steroid and thyroid hormone activity.

Biochemistry of hormonal regulation.

1. Neuropeptides of hypothalamus. Liberins and statins, their mechanisms of activity and biologic role.
2. Hormones of pituitary gland: melanotropin, thyrotropin, corticotropin, lutropin, somatotropin (growth hormone), prolactin. Their mechanisms of activity and biological role. Pathological processes related to the disturbances of hormone functions.
3. Hormones of posterior lobe of pituitary gland. Vasopressin and oxytocin: structure, biological functions. Pathological processes related to the disturbances of hormone functions.
4. Insulin: structure, biosynthesis and secretion.
5. Mechanism of insulin activity on the carbohydrate metabolism.
6. Mechanism of insulin activity on the lipid metabolism.
7. Mechanism of insulin activity on the protein and nucleotide metabolism.
8. Glucagon and its mechanisms of activity on the carbohydrate and lipid metabolism.
9. Thyroid hormones, their structures, biological effects of T3 and T4. Disturbances of metabolic processes due to hypo- and hyperthyreosis.
10. Epinephrine, norepinephrine, dopamine, their structure, biosynthesis, physiological effects, biochemical mechanisms of activity. Pathological processes related to the disturbances of hormone functions.
11. Steroid hormones of the suprarenal glands (C_{21}-steroids), glucocorticoids and mineralocorticoids, their structures and properties.
12. Mechanisms of glucocorticoids activity on the carbohydrate and lipid metabolism.
13. Mechanisms of glucocorticoids activity on the protein and nucleotide metabolism.
14. Anti-inflammatory mechanism of glucocorticoids activity.
15. Glucocorticoids as hormones of adaptation.
16. Mechanisms of mineralocorticoids activity. Pathological processes related to the disturbances of hormone functions.
17. Mechanism of the hyperpigmentation due to the Addison desease.
18. Mechanism of the fat accumulation due to the Itsenko-Cushing syndrome.
19. Hormones, which leads to hyperglycemia.
20. Hormones, which regulates of water-mineral metabolism.
21. Female sex hormones estrogens, progesterone. Physiological and biochemical effects, related to the ovulation cycle phases.
22. Male sex hormones (C_{19}-steroids). Physiological and biochemical effects of androgens, regulation of synthesis and secretion.

23. Hormonal regulation of calcium homeostasis in the human organism. Parathormone, calcitonin, calcitriol.
24. Kallikrein-kinin and renin-angiotensin systems, their physiological and biochemical effects.
25. Inhibitors of renin-angiotensin system.
26. Prostaglandins, prostacyclins, thromboxanes. Mechanism of the formation. Physiological and biochemical effects. Anti-inflammatory medicines as inhibitors of prostaglandin synthesis.
27. Leukotrienes. Mechanism of the formation. Physiological and biochemical effects. Inhibitors of leukotriene synthesis.

Methodological support:

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/

Lecture 13.

Hormones and bioregulators - derivatives of amino acids; hormones and physiologically active compounds of lipid nature. Local hormones.

Topic relevance: The interaction of the nervous and endocrine systems allows us to speak of 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.

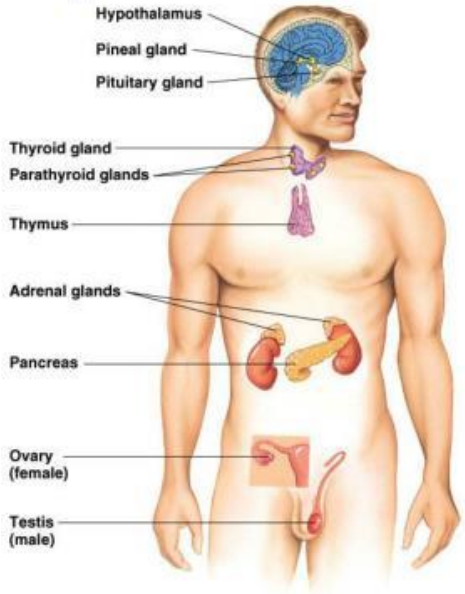
Objective: study of the effect of hormones of endocrine glands and tissue hormones on proliferation and metabolism in the tissues of the human body. Study of pathological conditions associated with hormonal dysregulation.

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:

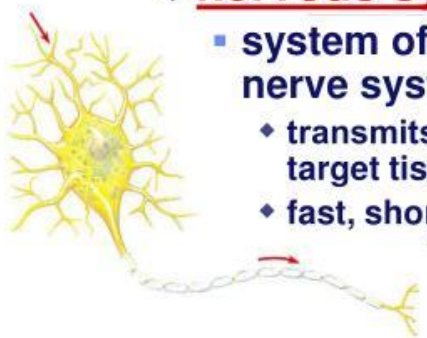
1. Insulin. Synthesis, forms of insulin in the blood, mechanisms of action. Pathology.
2. Glucagon. Mechanisms of action.
3. Hormones, derivatives of amino acids.
4. Steroid hormones. Mechanisms of action. Pathology.
5. Eicosanoids. Mechanisms of action. Pathology.

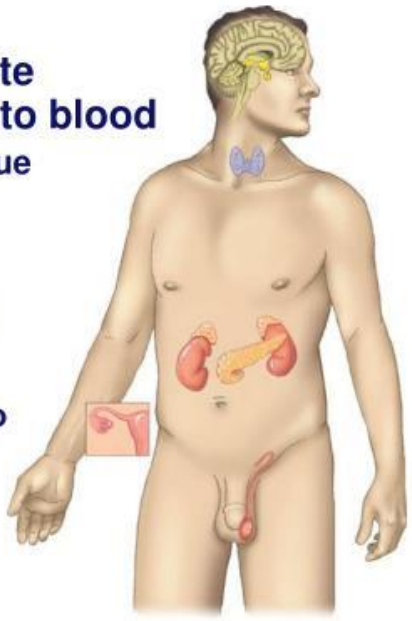


Endocrine System Hormones

Regulation & Communication

- Animals rely on 2 systems for regulation
  - endocrine system
    - ductless gland which secrete chemical signals directly into blood
      - chemical travels to target tissue
      - slow, long-lasting response
  - nervous system
    - system of neurons, central nerve system
      - transmits "electrical" signal to target tissue
      - fast, short-lasting response



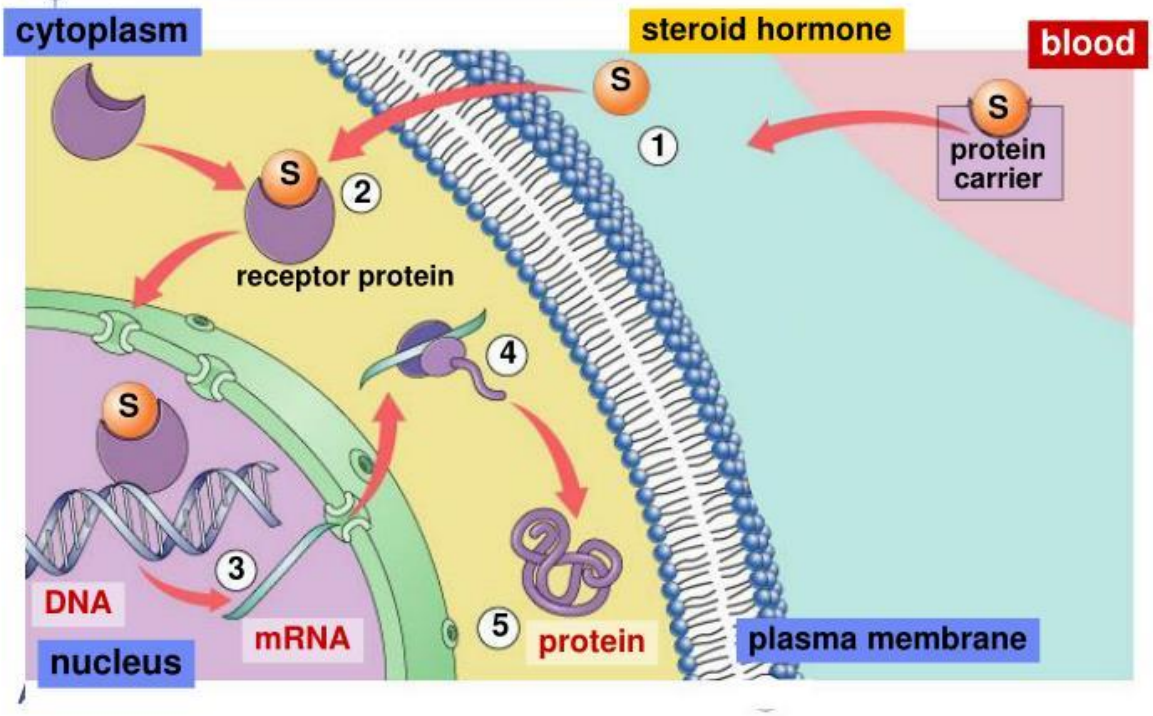


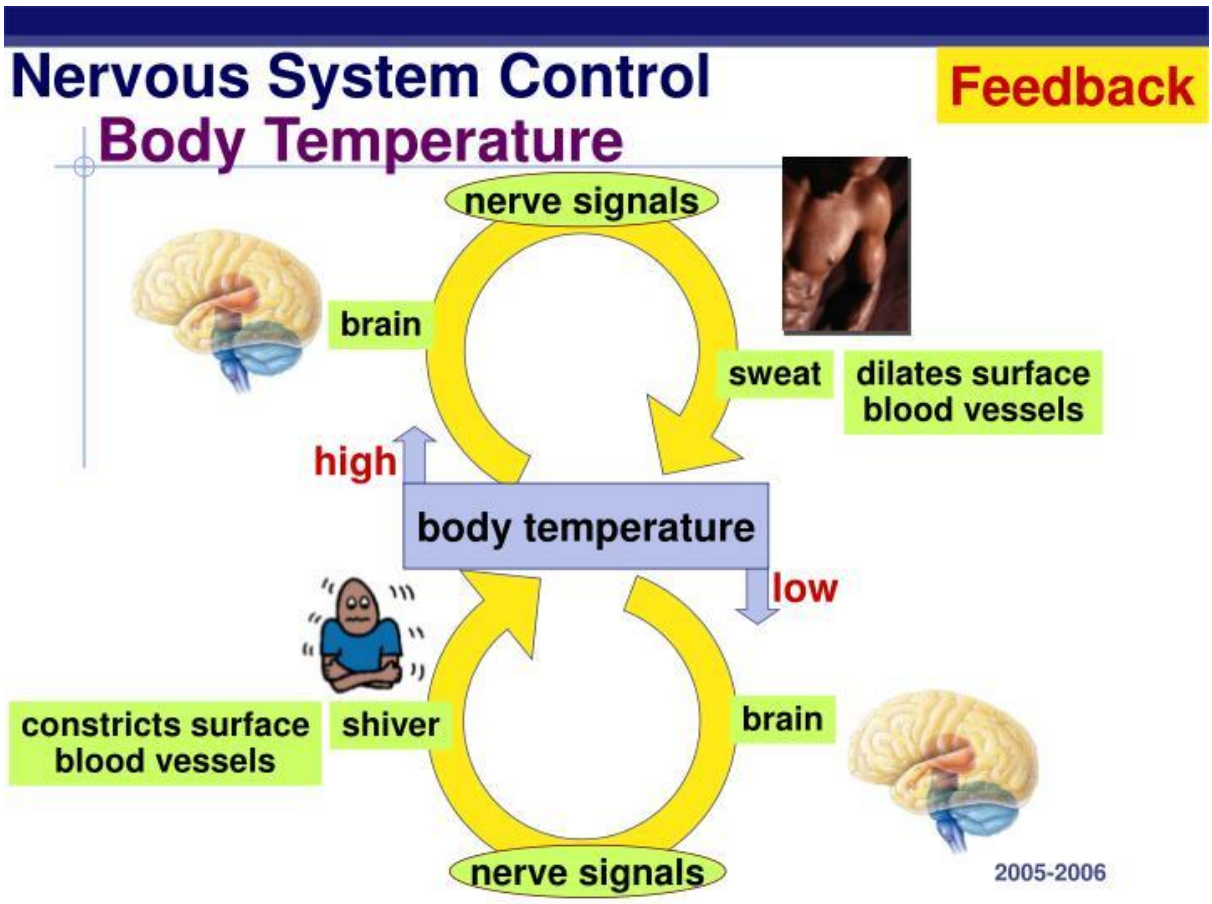
How do hormones act on target cells

- **Lipid-based hormones**
  - hydrophobic & lipid-soluble
    - diffuse across membrane & enter cells
    - bind to receptor proteins in cytoplasm & nucleus
    - bind to DNA as transcription factors
- **Protein-based hormones**
  - hydrophilic & not lipid soluble
    - can't diffuse across membrane
    - trigger secondary messenger pathway
    - activate cellular response
      - enzyme action, uptake or secretion of molecules…

AP Biology

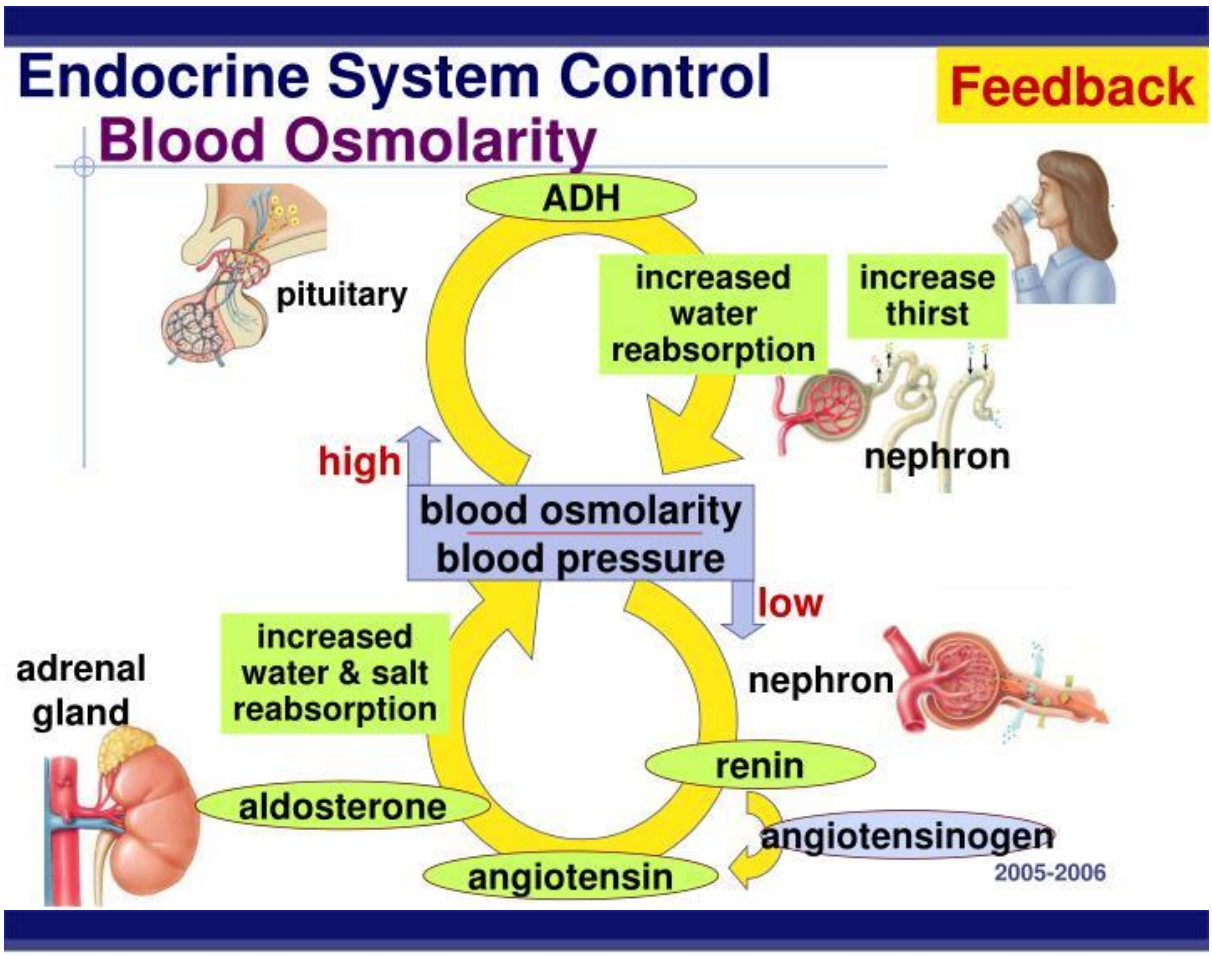
Action of lipid (steroid) hormones

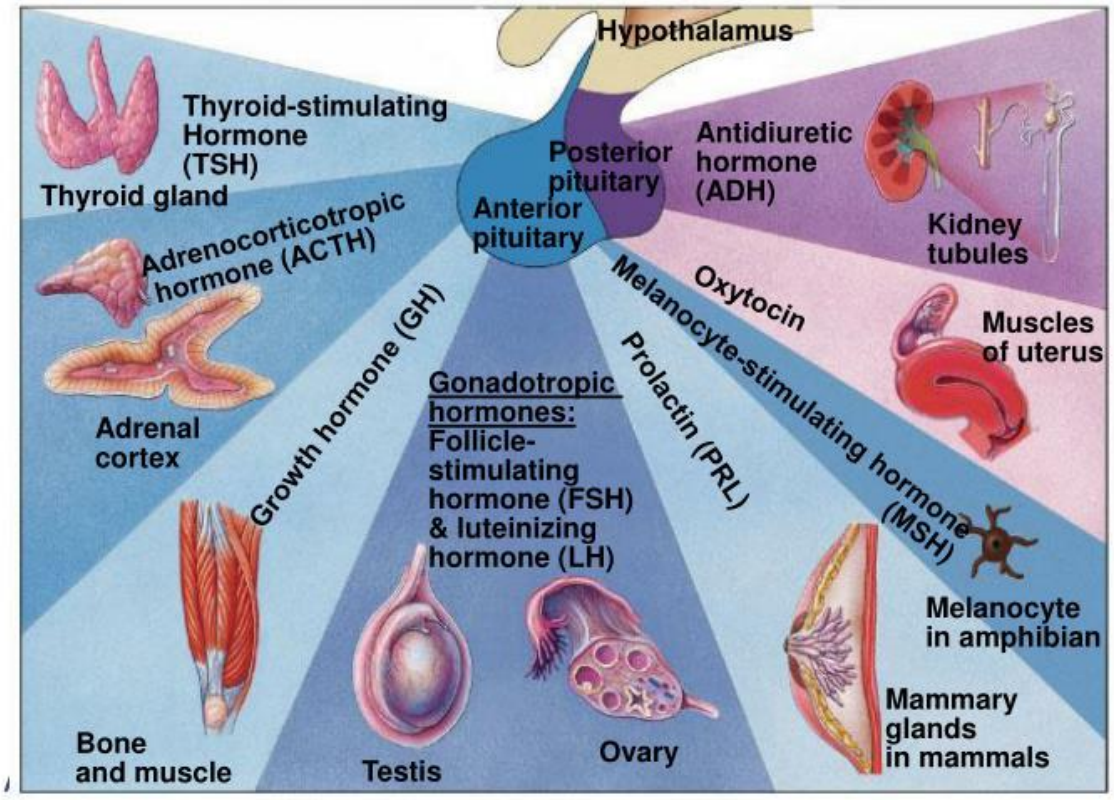




Anterior hypophysis hormones

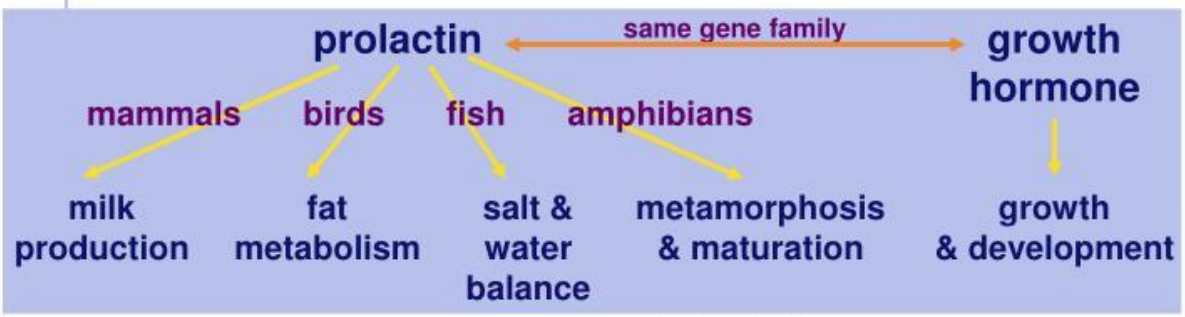
HORMONE	TARGET	FUNCTION
Thyroid (TSH) Stimulating	Thyroid gland	TH synthesis & release
Growth (GH)	Many tissues	growth
Adrenocortico-Tropin (ACTH)	Adrenal cortex	Cortisol release (androgens)
Prolactin (Prl)	Breast	Milk production
Follicle (FSH)	Gonads	Egg/sperm prod.
Luteinizing (LH)	Gonads	Sex hormones

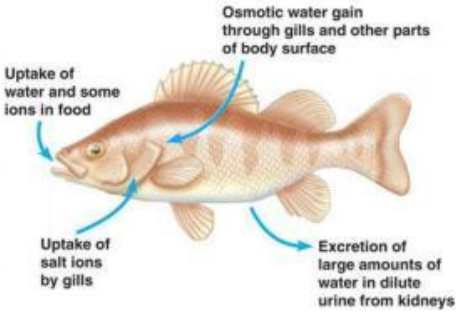

Endocrine System Control
Blood Osmolarity
Feedback
ADH
pituitary
increased water reabsorption
increase thirst
nephron
high
blood osmolarity
blood pressure
low
increased water & salt reabsorption
adrenal gland
nephron
renin
aldosterone
angiotensinogen
angiotensin
2005-2006


Hypothalamus
Thyroid-stimulating Hormone (TSH)
Thyroid gland
Posterior pituitary
Antidiuretic hormone (ADH)
Anterior pituitary
Kidney tubules
Adrenocorticotropic hormone (ACTH)
Oxytocin
Muscles of uterus
Growth hormone (GH)
Gonadotropic hormones:
Follicle-stimulating hormone (FSH) & luteinizing hormone (LH)
Prolactin (PRL)
Melanocyte-stimulating hormone (MSH)
Adrenal cortex
Melanocyte in amphibian
Mammary glands in mammals
Bone and muscle
Testis
Ovary

Homology in hormones

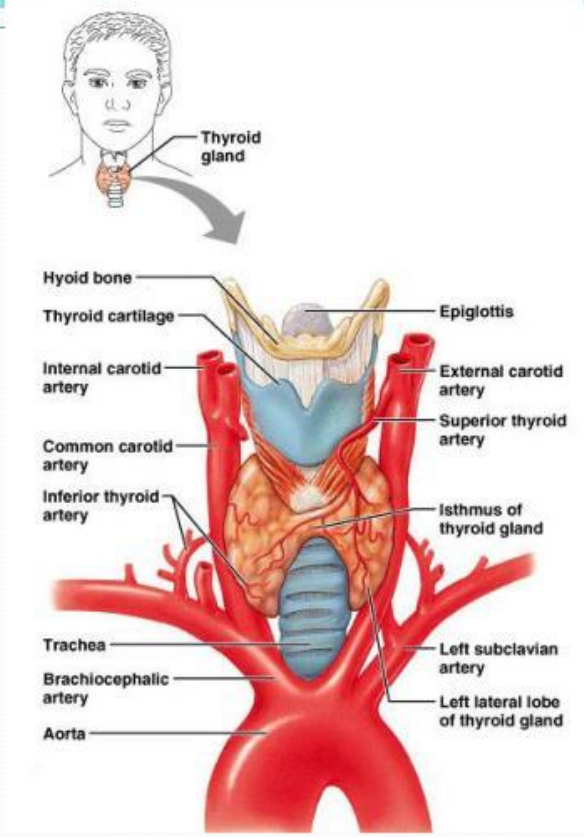
What does this tell you about these hormones?



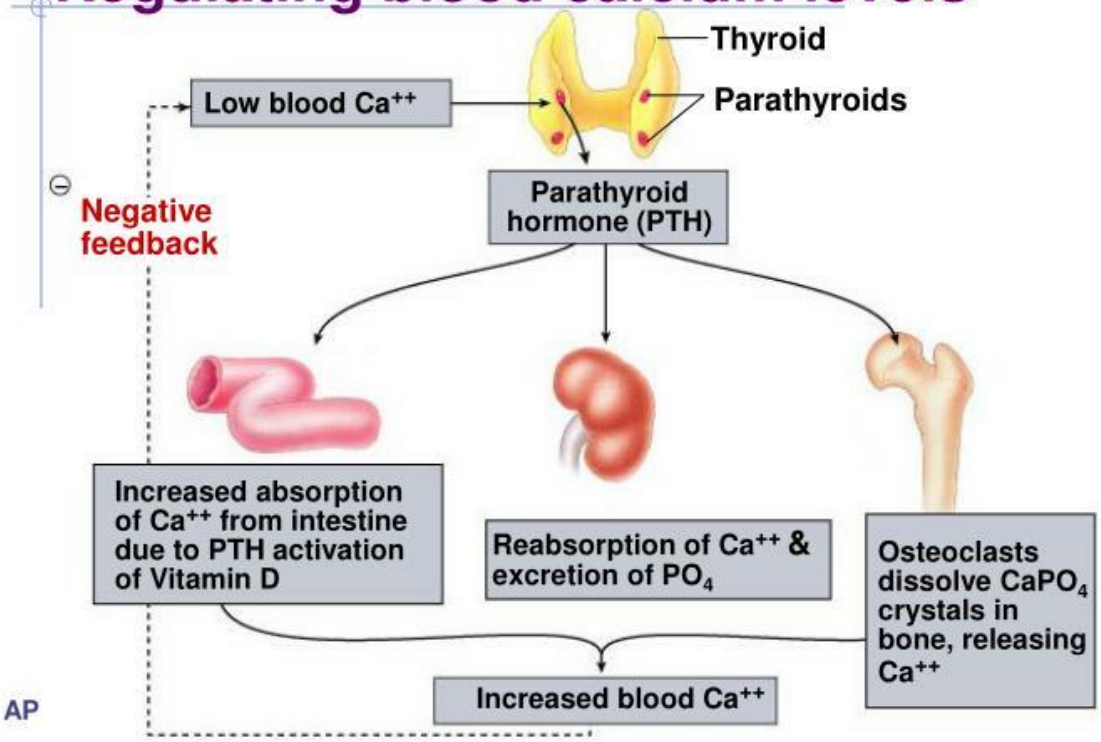


The Thyroid Gland

- Anterior neck on trachea just inferior to larynx
- Two lateral lobes and an isthmus
- Produces hormones
  - Thyroid hormone: tyrosine based with 3 or 4 iodine molecules
    - T4 (thyroxine) and T3



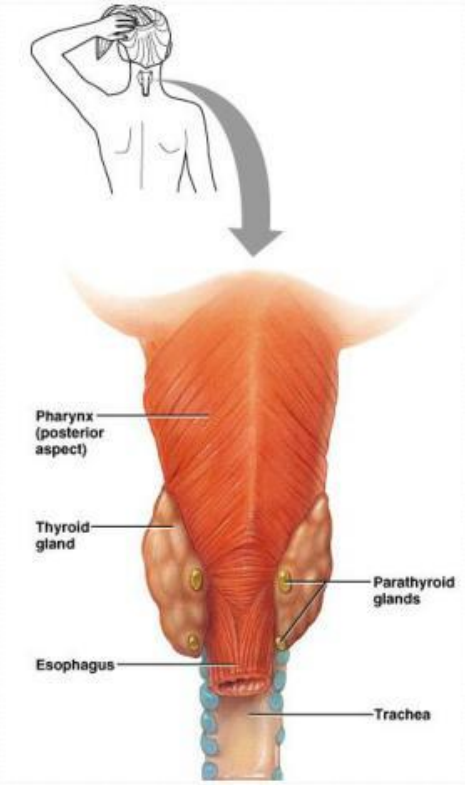
Regulating blood calcium levels

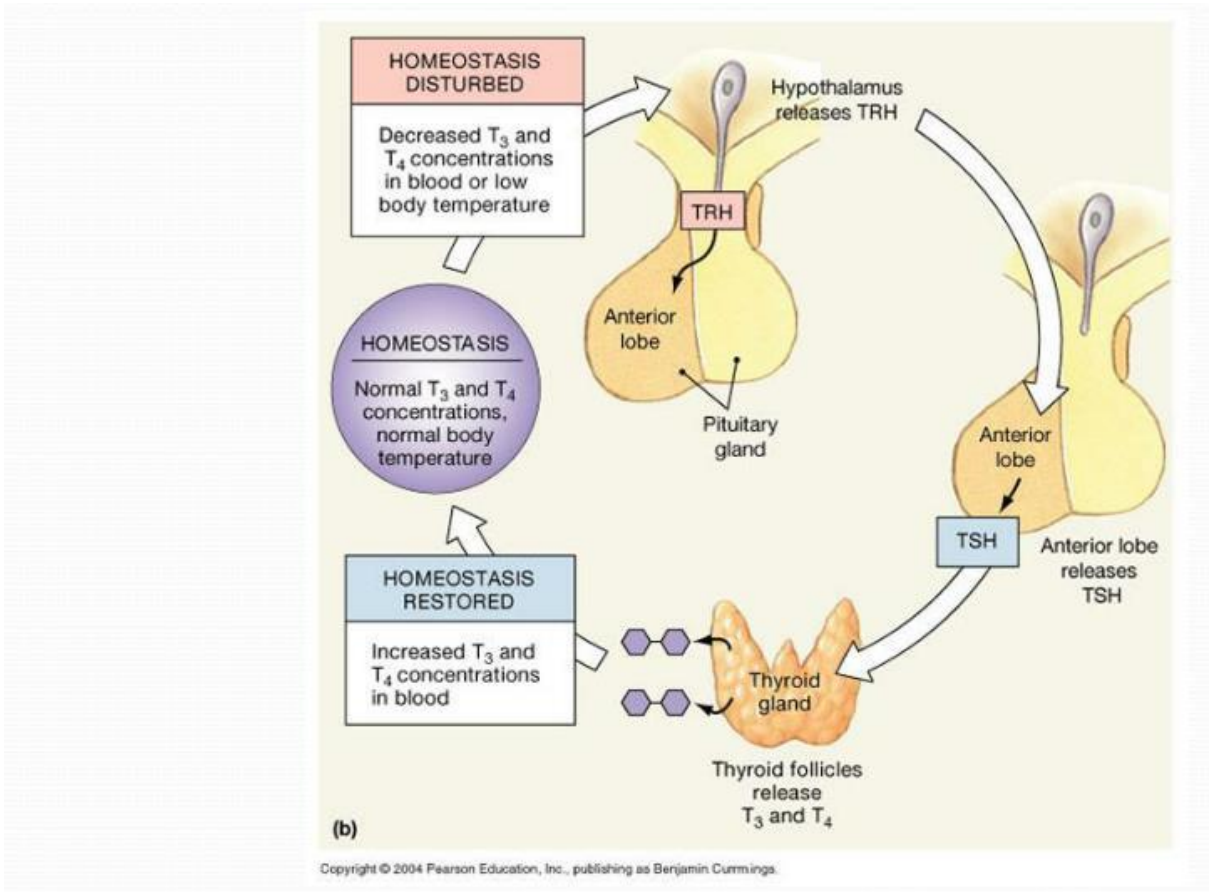


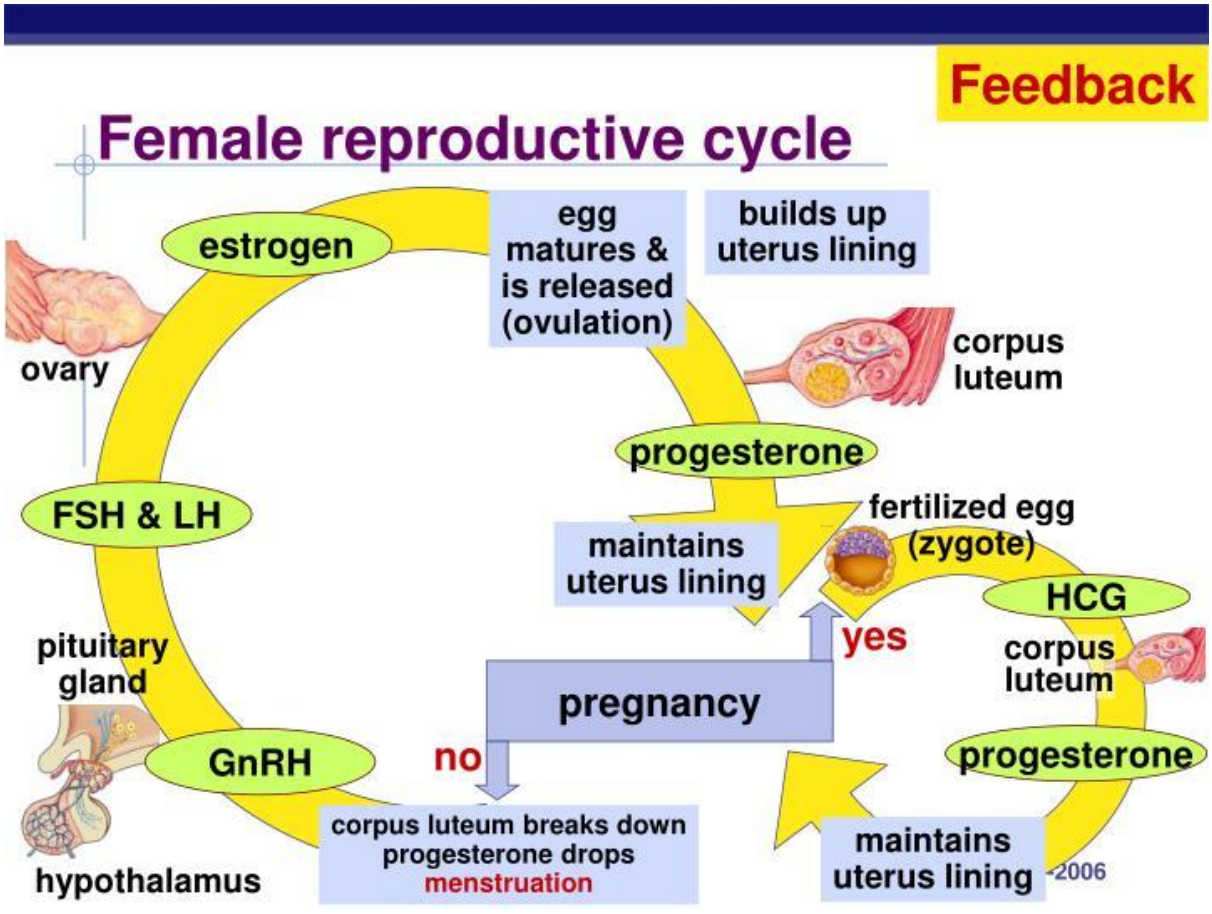
The Parathyroid Glands

- Most people have four
- On posterior surface of thyroid gland

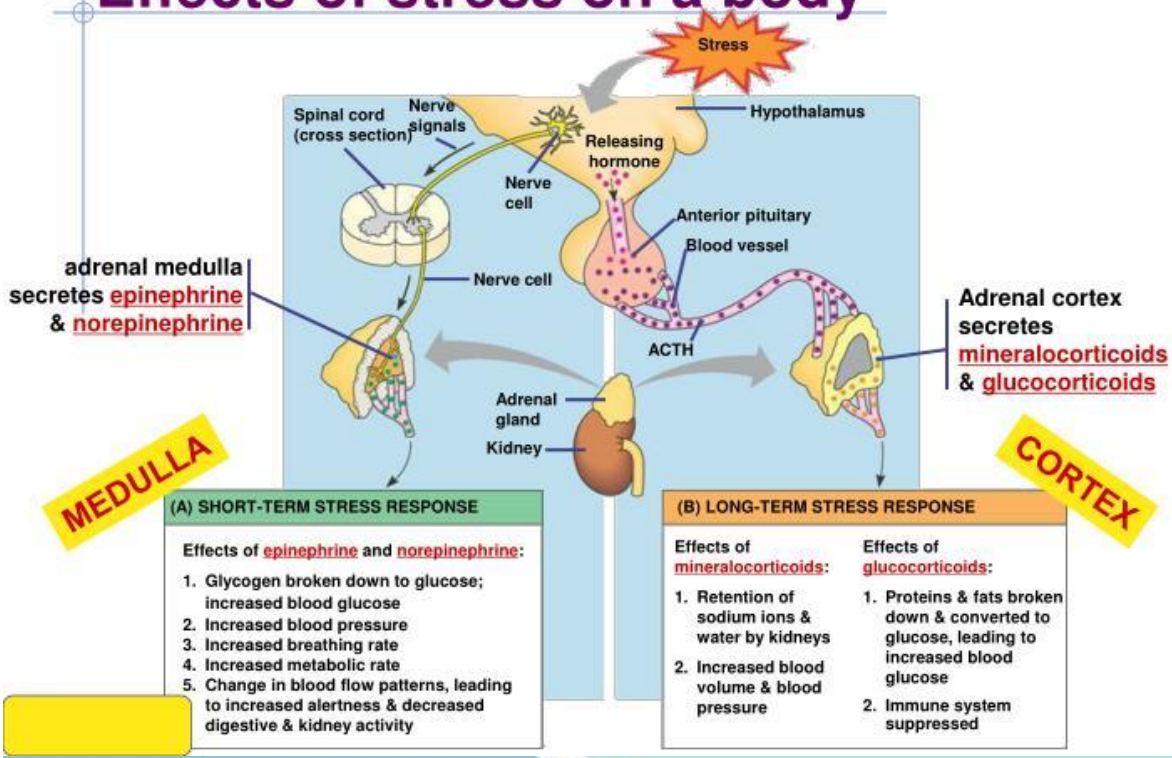
(sometimes embedded)



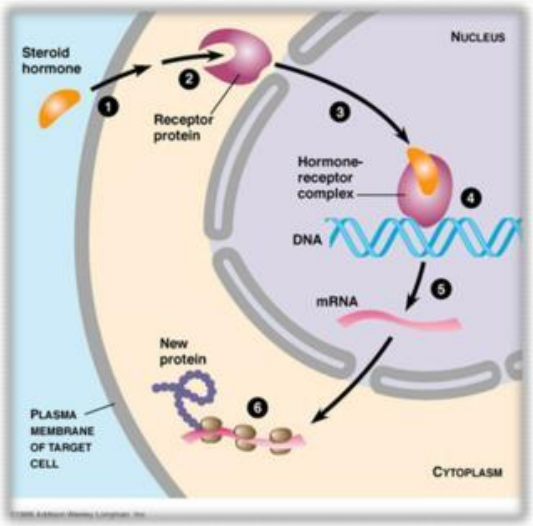

HOMEOSTASIS DISTURBED
Decreased T_3 and T_4 concentrations in blood or low body temperature
Hypothalamus releases TRH
TRH
Anterior lobe
Pituitary gland
HOMEOSTASIS
Normal T_3 and T_4 concentrations, normal body temperature
Anterior lobe
TSH
Anterior lobe releases TSH
HOMEOSTASIS RESTORED
Increased T_3 and T_4 concentrations in blood
Thyroid gland
Thyroid follicles release T_3 and T_4
(b)
Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.

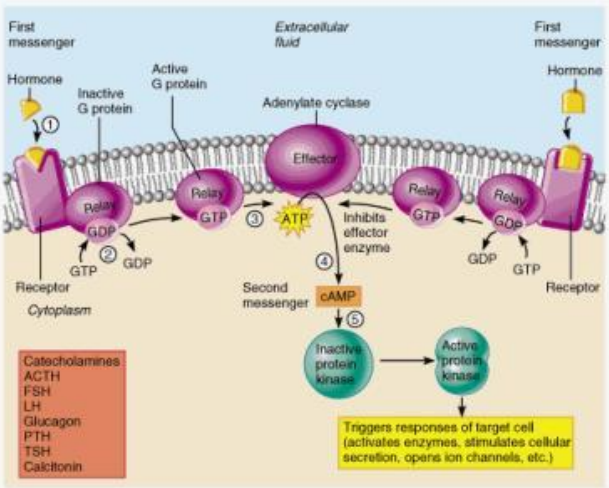

Feedback
Female reproductive cycle
estrogen
egg matures & is released (ovulation)
builds up uterus lining
ovary
corpus luteum
progesterone
FSH & LH
maintains uterus lining
fertilized egg (zygote)
HCG
pituitary gland
yes
corpus luteum
pregnancy
GnRH
no
progesterone
corpus luteum breaks down
progesterone drops
menstruation
maintains uterus lining
hypothalamus
2006

Effects of stress on a body



Hormone-receptor interaction

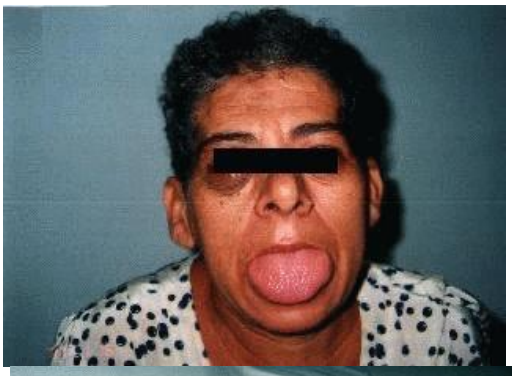




(a)

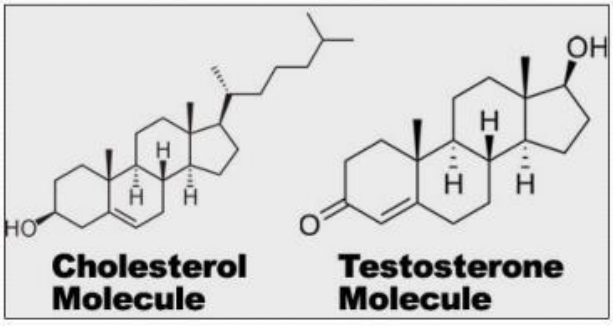
Hormones Released from the Anterior Pituitary or Adenohypophysis

- **Hypo-secretion:**
  During childhood causes Dwarfism
- **Hyper-secretion:**
  During childhood causes
  Gigantism (up to 8 – 9 ft.)
  During Adulthood causes
  Acromegaly:
  Enlargement of the small bones of the hand and feet
  Enlargement of the cranium, nose, and lower jaw
  Tongue, liver, and kidneys become enlarged



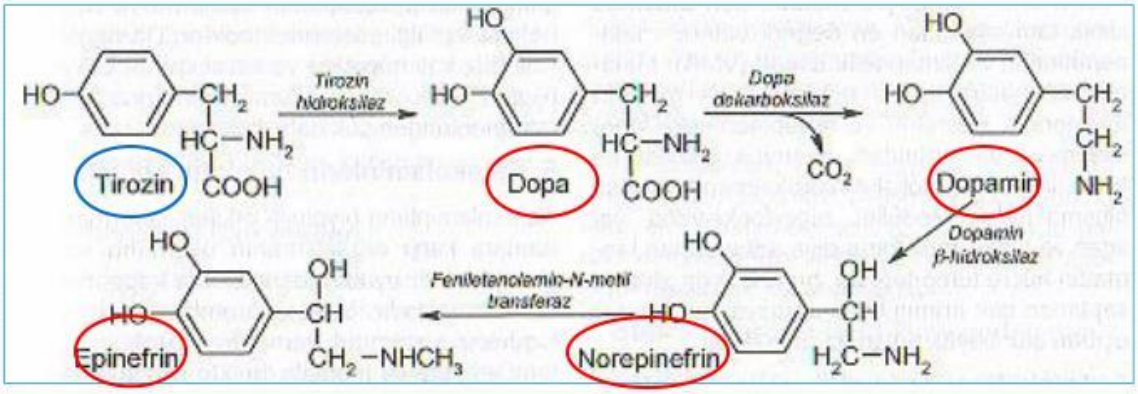
Steroid Hormones

- Small
- Lipophilic (or hydrophobic)
- Travel in blood with carrier.
- Change protein synthesis.
- Cytoplasmic or nuclear receptors.
- Synthesized from cholesterol.
- Example: testosterone



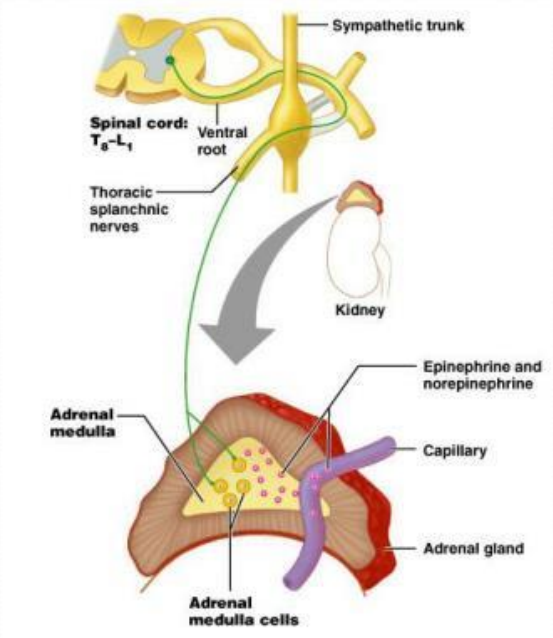
Cholesterol Molecule **Testosterone Molecule**

Synthesis of adrenal medulla hormones



Adrenal medulla hormones

- Part of autonomic nervous system
- Spherical chromaffin cells are modified postganglionic sympathetic neurons
  - Secrete ***epinephrine*** and ***norepinephrine***
  - Amine hormones
  - *Fight, flight, fright*
- Vesicles store the hormones



Epinephrine and norepinephrine

- Also called **adrenaline** and **noradrenaline**, two separate but related hormones secreted by the medulla of the adrenal glands.
- They are also produced at the ends of sympathetic nerve fibres, where they serve as chemical mediators for conveying the nerve impulses to effector organs.
- Chemically, the two compounds differ only slightly; and they exert similar pharmacological actions, which resemble the effects of stimulation of the sympathetic nervous system.

Functions

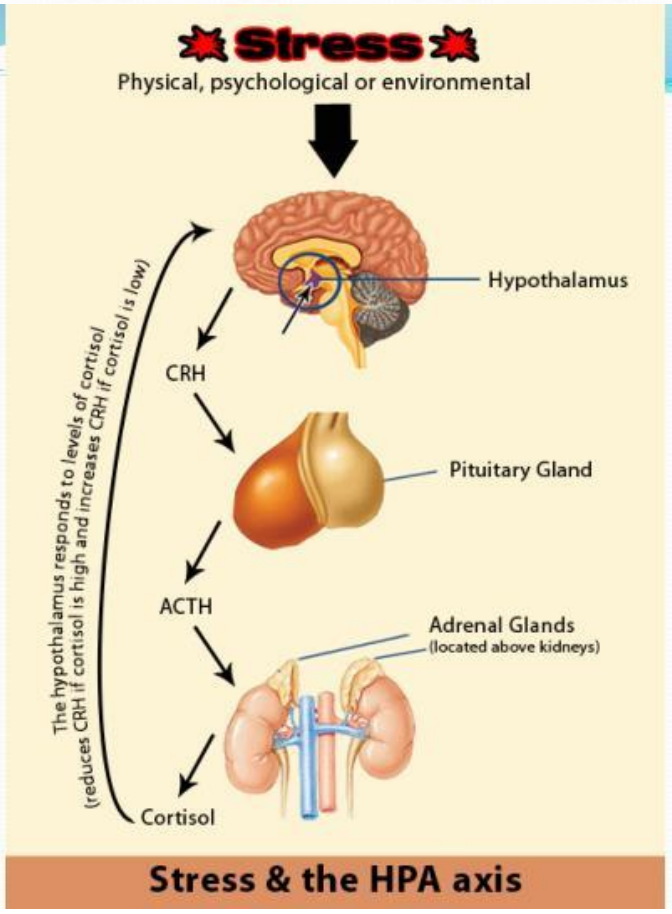
- **Epinephrine** - stimulates pulses and *increases blood* pressure, *stimulates* the metabolism in emergencies, decreases insulin secretion.

- **Norepinephrine** (generally excites smooth muscle) - stimulates the functions of the *circulatory* and *respiratory* systems: attention, consciousness, control of body temperature etc.

Cortisol, the most important *glucocorticoid*

- It is essential for life
- Helps the body deal with *stressful* situations within minutes
  - Physical: trauma, surgery, exercise
  - Psychological: anxiety, depression, crowding
  - Physiological: fasting, hypoglycemia, fever, infection
- *Regulates* or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions including water balance

People with adrenal insufficiency: these stresses can cause hypotension, shock and death: must give glucocorticoids, eg for surgery or if have infection, etc.



Stress & the HPA axis

Endocrine activity of the Adrenal Cortex

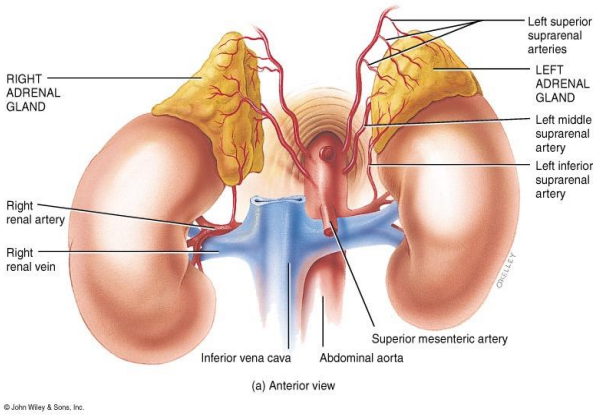
- Hyper-secretion:

  Aldosteronism:

  Hypokalemia, increase in extracellular fluid and blood volume,and hypertension, may also have period of muscular paralysis
- Hypo-secretion:

  Addison's disease

  Mineralocorticoids deficiency, death occurs in four days to two weeks if untreated



Endocrine activity of the Adrenal Cortex

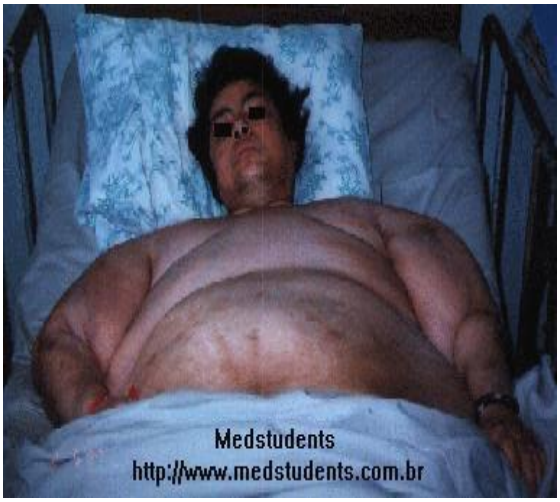
- Hypo-secretion

  Addison's disease - glucocorticoid deficiency

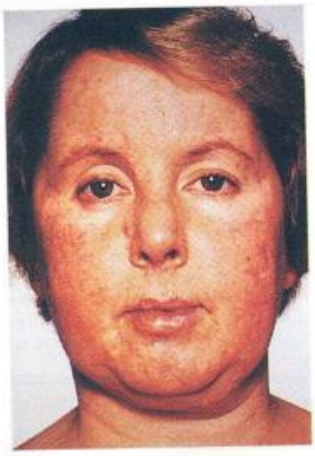
  person becomes highly susceptible to disease and deteriorating effects of stress
- Hyper-secretion:

  Cushing's Syndrome

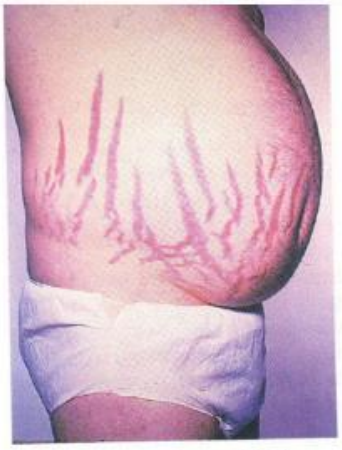
  mobilization of fat from lower body to the thoracic and upper abdominal regions giving raise to "Buffalo Torso"



What Would the Feedback Loop Look Like for Cushing's Syndrome?



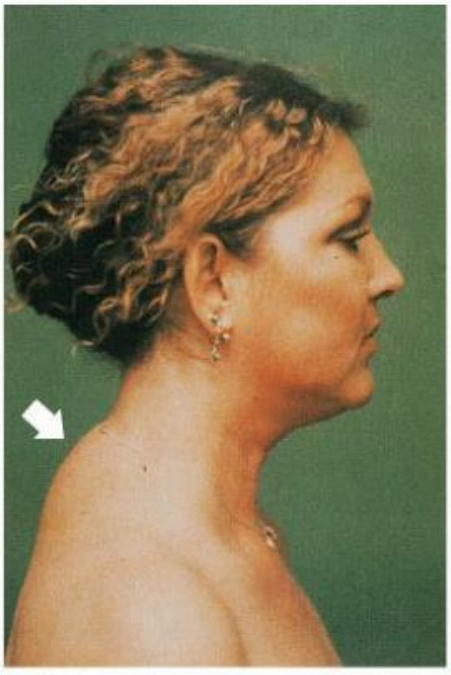
(a) Facial features



(b) Pendulous abdomen with striae

Before and after onset of Cushing's disease

(a) Before



(b) After

Aldosterone, the main *mineralocorticoid*

- Secreted by adrenal *cortex* in response to a decline in either blood volume or blood pressure (e.g. severe hemorrhage)
  - Is terminal hormone in renin-angiotensin mechanism
- Prompts distal and collecting tubules in kidney to *reabsorb* more *sodium*
  - Water passively follows
  - Blood volume thus *increases*

The Gonads (testes and ovaries)

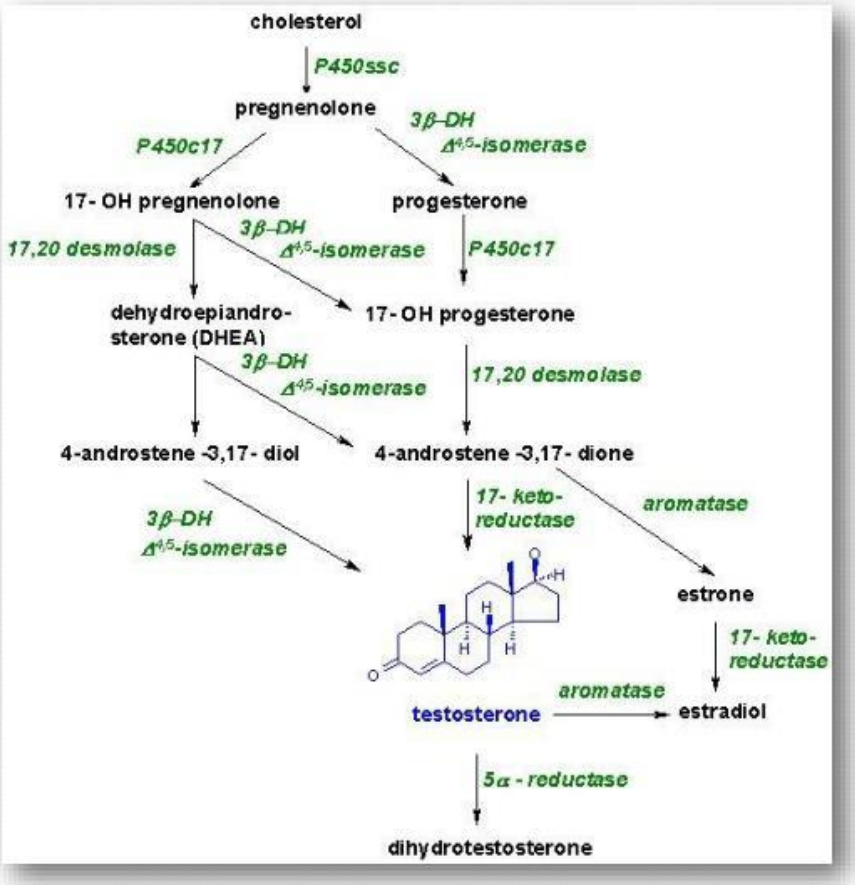
- The gonads produce germ cells and the sex hormones.
- Testes
  - Interstitial cells secrete *androgens* (testosterone)
  - Primary androgen is testosterone
    - Maintains secondary sex characteristics
    - Helps promote sperm formation
- Ovaries
  - Androgens secreted by thecal folliculi
    - Directly converted to estrogens by follicular granulosa cells
  - Granulosa cells also produce *progesterone*
  - Corpus luteum also secretes *estrogen* and progesterone

Actions of Testosterone

- pubertal transformation:
  - enlargement of testes, penis and scrotum
  - pubic and axillary hair
  - bone growth
  - red cell mass increase
  - skeletal muscle mass increase
  - larynx enlarges - deepening of the voice
  - increase in sebaceous glands - often cause of acne
  - beard development

Actions of estrogens

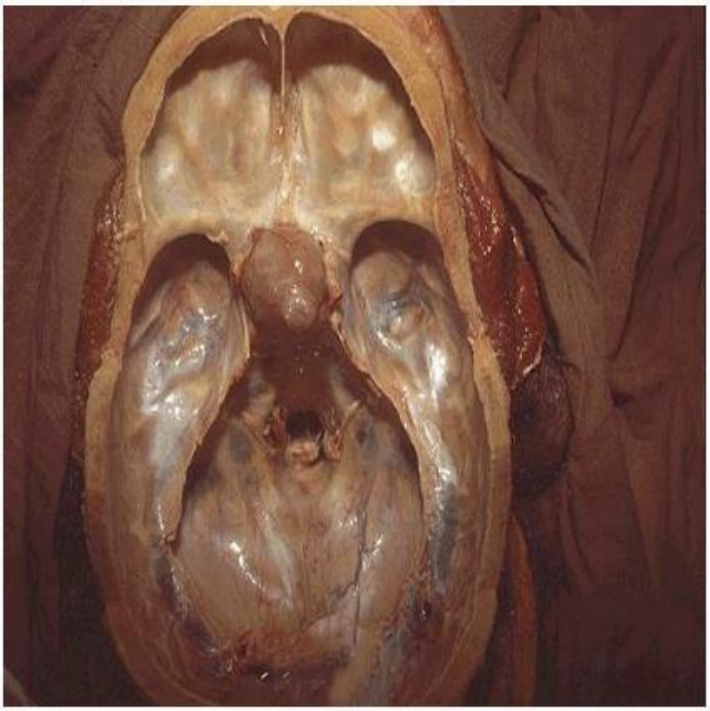
- on sexual organs (primary and secondary sexual characteristics)
  - ovaries : stimulate *follicular* growth; small doses cause an increase in weight of ovary; large doses cause atrophy
  - uterus: endometrial growth
  - development and *maintenance of internal* (fallopian tubes, uterus, vagina), and external genitalia
  - skin: increase in vascularization, development of soft, textured and smooth skin
  - bone: increase osteoblastic activity
  - electrolytes: retention of Na+, Cl- and water by the kidney
  - cholesterol: *hypocholesterolemic* effect



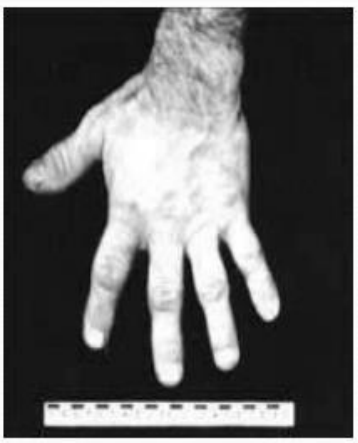
Growth Hormone

- Stimulus = Tissue growth/ repair
- Hypothalamus releases GHRH
- Anterior Pituitary releases GH
- ↑ Protein synthesis, growth, etc.
- ↑GH and release of somatostatin shuts off GHRH and GH release

What happens with excess GH?



↑GH as an Adult



↓GH = pituitary dwarfism

General material and bulk-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:

Biochemistry of hormonal regulation.

1. 1. Neuropeptides of hypothalamus. Liberins and statins, their mechanisms of activity and biologic role.
2. Hormones of pituitary gland: melanotropin, thyrotropin, corticotropin, lutropin, somatotropin (growth hormone), prolactin. Their mechanisms of activity and biological role. Pathological processes related to the disturbances of hormone functions.
3. Hormones of posterior lobe of pituitary gland. Vasopressin and oxytocin: structure, biological functions. Pathological processes related to the disturbances of hormone functions.
4. Insulin: structure, biosynthesis and secretion.
5. Mechanism of insulin activity on the carbohydrate metabolism.
6. Mechanism of insulin activity on the lipid metabolism.
7. Mechanism of insulin activity on the protein and nucleotide metabolism.
8. Glucagon and its mechanisms of activity on the carbohydrate and lipid metabolism.
9. Thyroid hormones, their structures, biological effects of T3 and T4. Disturbances of metabolic processes due to hypo- and hyperthyreosis.
10. Epinephrine, norepinephrine, dopamine, their structure, biosynthesis, physiological effects, biochemical mechanisms of activity. Pathological processes related to the disturbances of hormone functions.
11. Steroid hormones of the suprarenal glands (C_{21}-steroids), glucocorticoids and mineralocorticoids, their structures and properties.
12. Mechanisms of glucocorticoids activity on the carbohydrate and lipid metabolism.
13. Mechanisms of glucocorticoids activity on the protein and nucleotide metabolism.
14. Anti-inflammatory mechanism of glucocorticoids activity.
15. Glucocorticoids as hormones of adaptation.
16. Mechanisms of mineralocorticoids activity. Pathological processes related to the disturbances of hormone functions.
17. Mechanism of the hyperpigmentation due to the Addison desease.
18. Mechanism of the fat accumulation due to the Itsenko-Cushing syndrome.
19. Hormones, which leads to hyperglycemia.

20. Hormones, which regulates of water-mineral metabolism.
21. Female sex hormones estrogens, progesterone. Physiological and biochemical effects, related to the ovulation cycle phases.
22. Male sex hormones (C_{19}-steroids). Physiological and biochemical effects of androgens, regulation of synthesis and secretion.
23. Hormonal regulation of calcium homeostasis in the human organism. Parathormone, calcitonin, calcitriol.
24. Kallikrein-kinin and renin-angiotensin systems, their physiological and biochemical effects.
25. Inhibitors of renin-angiotensin system.
26. Prostaglandins, prostacyclins, thromboxanes. Mechanism of the formation. Physiological and biochemical effects. Anti-inflammatory medicines as inhibitors of prostaglandin synthesis.
27. Leukotrienes. Mechanism of the formation. Physiological and biochemical effects. Inhibitors of leukotriene synthesis.

Methodological support:

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/

Lecture 14.

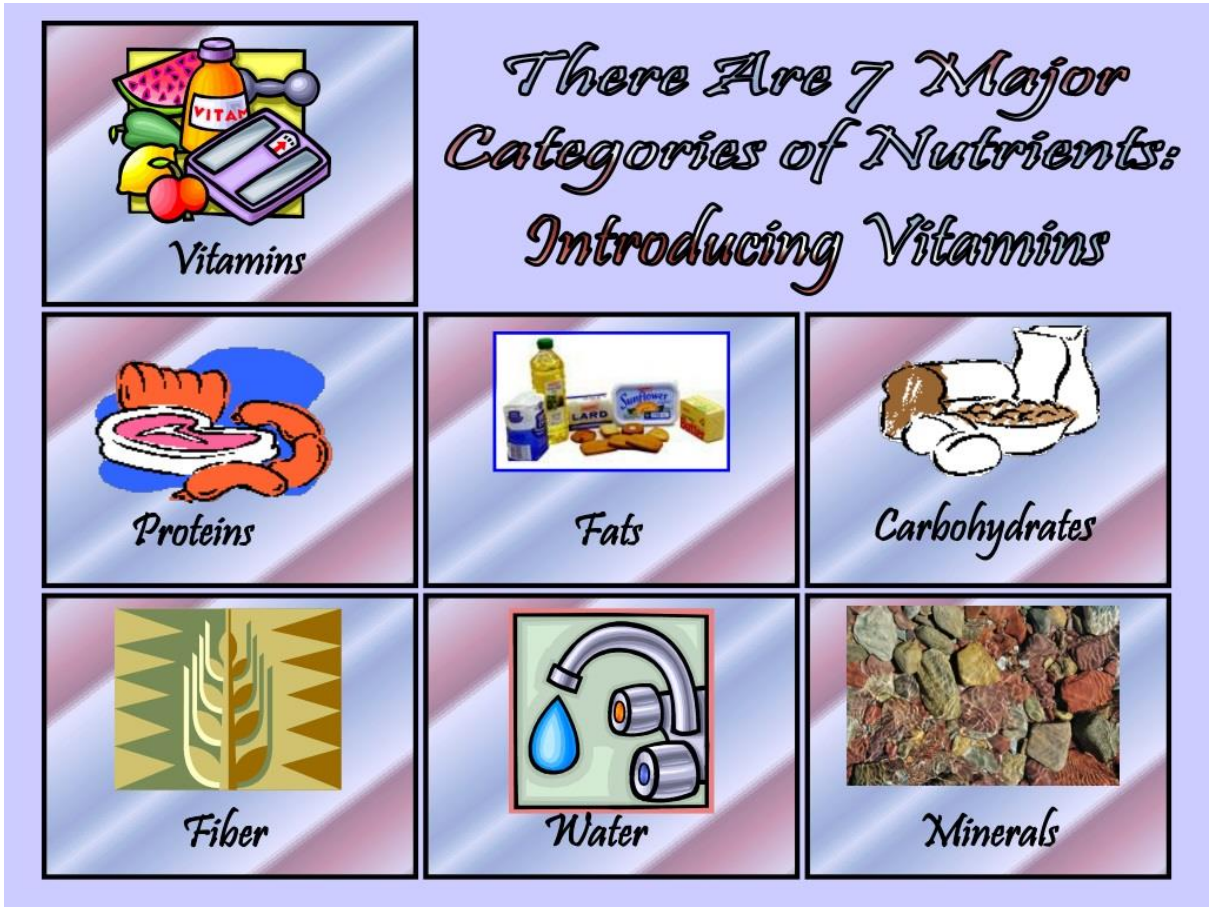
Topic: Biochemistry of human nutrition. Vitamins and trace elements as components of human nutrition. Water-soluble vitamins.

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.

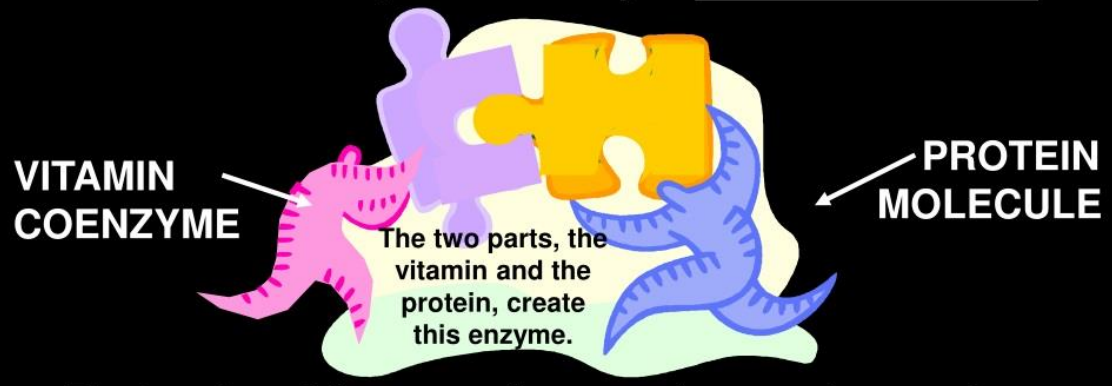


Controversy: Vitamin Supplements: Do the Benefits Outweigh the Risks?

Which is the best source?

What are vitamins?...

Vitamins do NOT provide energy. They do NOT build the body physically. They do perform very specific functions by working in compounds called 'enzymes'. Enzymes have two parts: the protein molecule and its helper…the coenzyme made out of a vitamin.



The function of the enzyme is to speed up reactions, such as digestion, metabolism and cellular reproduction. A deficiency of a vitamin does not stop the work of the enzyme, but the enzyme will function must less efficiently. Long-term deficiencies can result in cellular death, causing body damage difficult or impossible to repair.

Two classifications of vitamins...

The two types of vitamins are classified by the materials in which they will dissolve.

Fat-soluble vitamins -- vitamins A, D, E and K -- dissolve in fat before they are absorbed in the blood stream to carry out their functions. Excesses of these vitamins are stored in the liver. Because they are stored, they are not needed every day in the diet.

By contrast, water-soluble vitamins dissolve in water and are not stored; they are eliminated in urine. We need a continuous supply of them in our diets. The water-soluble vitamins are the B-complex group and vitamin C.

Water soluble vitamins are unstable...

Water-soluble vitamins are easily destroyed or washed out during food storage or preparation. Proper storage and preparation of food can minimize vitamin loss. To reduce vitamin loss:

1. refrigerate fresh produce; eat raw if possible
2. keep milk and grains away from strong light
3. use as little water as possible when cooking vegetables
4. use a lid when cooking to prevent evaporation of cooking liquids
5. and use the cooking water from vegetables to prepare soups
6. avoid alkalinity during cooking such as the use of baking soda
7. use whole grains, with as little processing as possible

Names of water soluble vitamins...

Eight of the water-soluble vitamins are known as the B-complex group: thiamin (vitamin B1), riboflavin (vitamin B2), niacin, vitamin B6, folate, vitamin B12, biotin and pantothenic acid. Note, some of the B vitamins are known by number, others by name, and still others by name or number.

Vitamin C is the only other water-soluble vitamin.

This label from a ready-to-eat cereal shows that several B vitamins and Vitamin C are either naturally or artificially added. →

Nutrition Facts

Serving Size 3/4 cup (30g/1.1 oz)
Servings Per Package 11

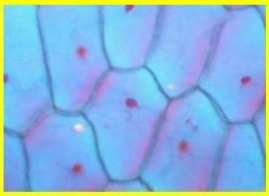
Amount Per Serving	Cereal	Cereal with 1/2 cup Vitamins A&D skim milk
Calories	120	160
Calories from Fat	15	15
	% Daily Value**	
Total Fat 2g*	3%	3%
Saturated Fat 1g	5%	5%
Cholesterol 0mg	0%	0%
Sodium 210mg	9%	11%
Potassium 45mg	1%	7%
Total Carbohydrate 24g	8%	10%
Dietary Fiber 1g	4%	4%
Sugars 9g		
Protein 2g		
Vitamin A	15%	20%
Vitamin C	25%	25%
Calcium	0%	15%
Iron	25%	25%
Vitamin D	10%	25%
Thiamin	25%	30%
Riboflavin	25%	35%
Niacin	25%	25%
Vitamin B_6	25%	25%
Folate	25%	25%
Phosphorus	2%	15%

Vitamin C...

The main sources of Vitamin C are citrus fruits and vegetables: oranges, lemons, limes, grapefruit, tomatoes, etc., in addition to broccoli strawberries, melon, dark green vegetables, and potatoes.

Also known as ascorbic acid, the functions of vitamin C are:

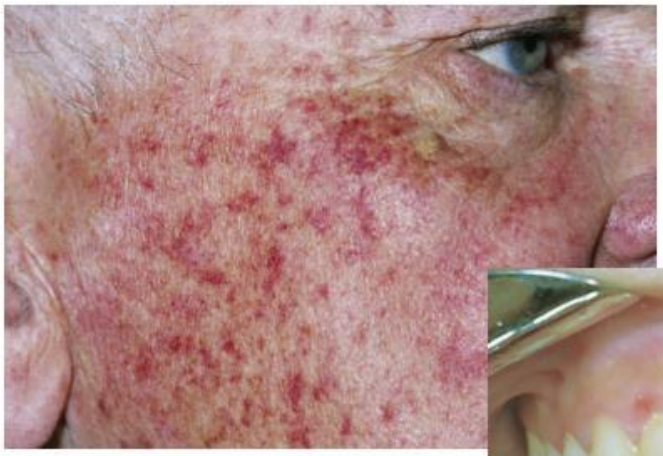
1. helps hold body cells together

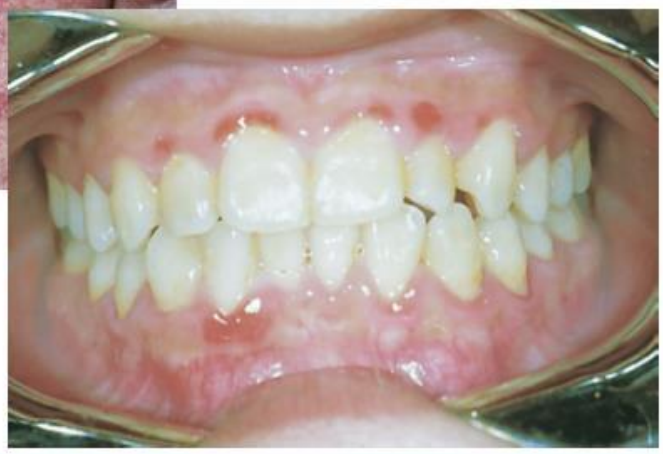


Cell cement

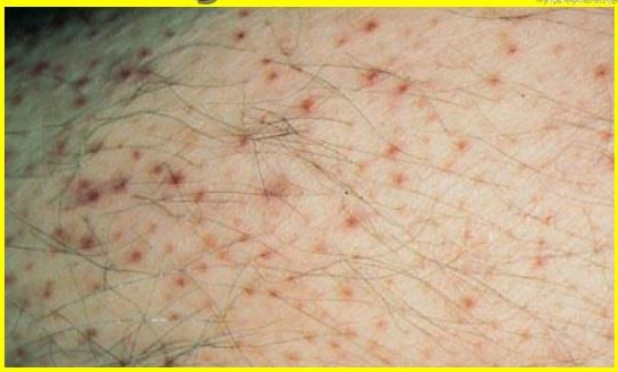
2. aids in wound healing
3. assists in bone and tooth formation
4. strengthens the blood vessel walls
5. is vital for the function of the immune system
6. improves absorption and utilization of iron

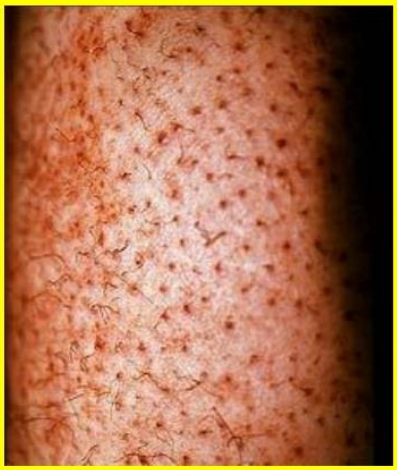
Deficiency Symptoms





Scurvy... C





Petechial (pə-tē'kē-al) hemorrhages (small, pin-point bleeding under the skin) and corkscrew hairs are symptomatic of scurvy, the disease that can result from a deficiency of Vitamin C.

In the past, scurvy was common among sailors and other people deprived of fresh fruits and vegetables for long periods of time. To prevent the disease on long voyages, sailors took along a supply of limes, because they didn't spoil as quickly as other citrus fruits. For that reason, sailors were often called 'limeys'.

Consumer Corner: Vitamin C and the Common Cold

- In drug-like doses, vitamin C may act like a weak antihistamine.

Can vitamin C ease the suffering of a person with a cold?

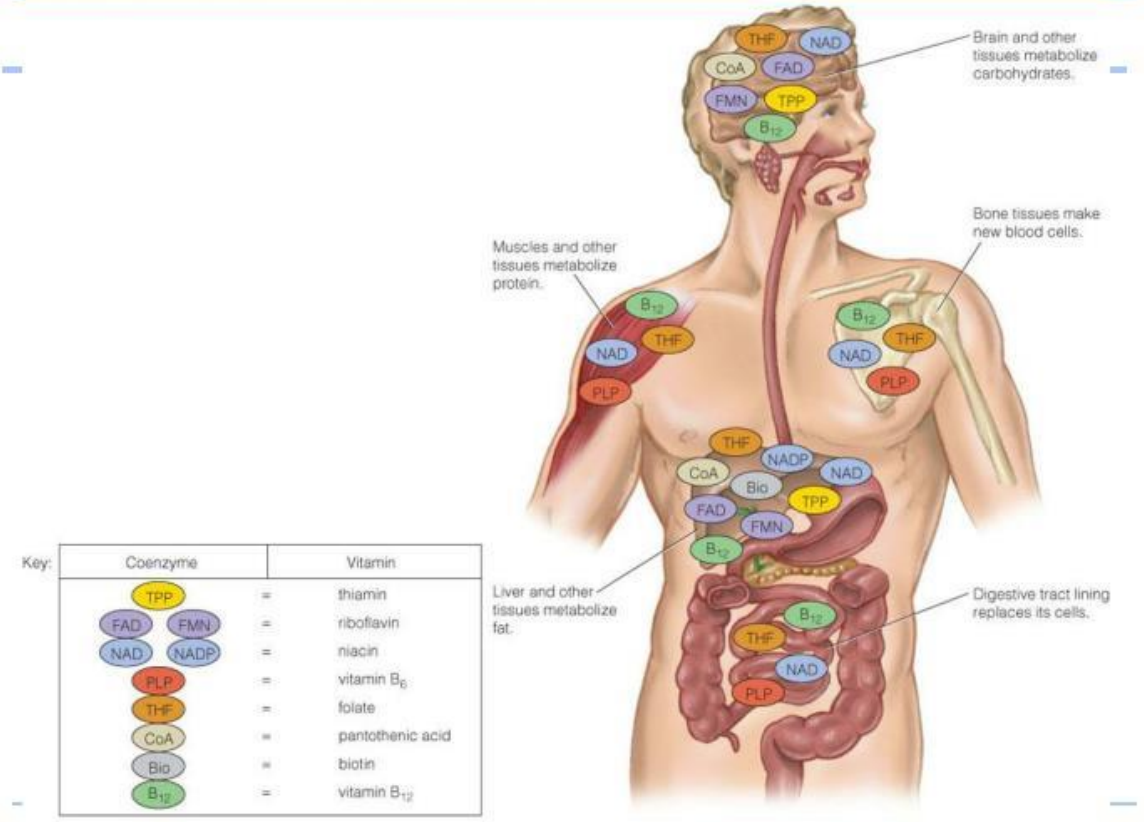
From the B-complex group: Thiamin (Vitamin B1)

Thiamin (thī'ə-mĭn, -mēn') is also spelled thiamine.

Functions: Necessary for normal carbohydrate metabolism (releasing energy from food); promotes normal appetite; important in function of nervous system by transmitting high-speed impulses.

The main sources of thiamin are pork, liver, whole grains, enriched grain products, peas, meat, legumes, watermelon, and sunflower seeds..

B Vitamin Roles in Metabolism

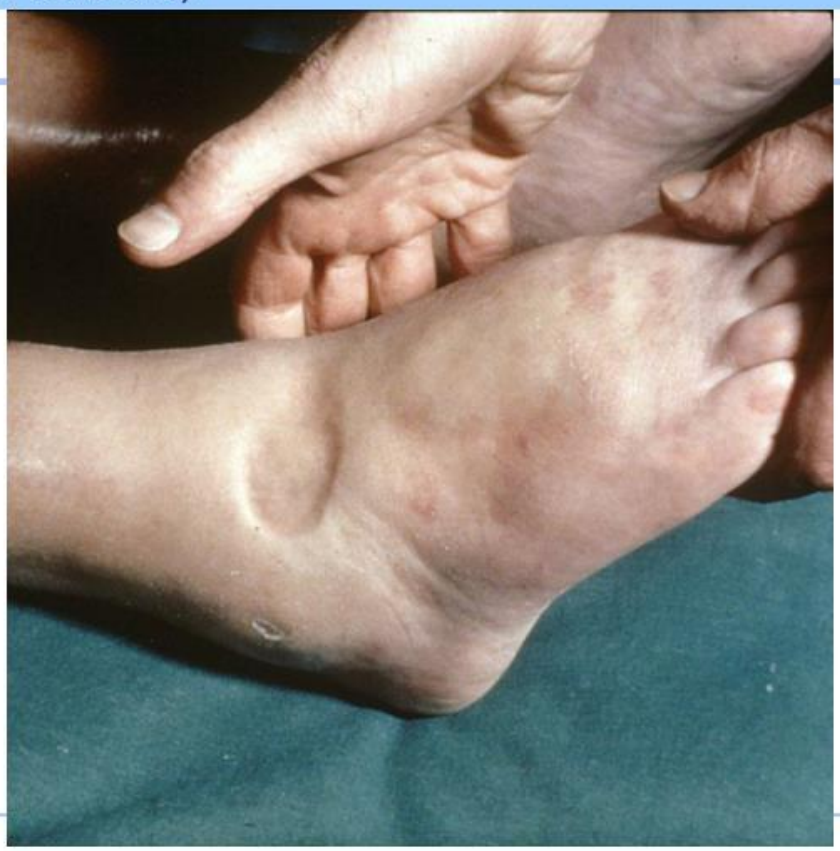


Normal (left) and thiamine deficient rat (right). A very marked effect on growth as well as a rough hair coat and weakness on the legs are apparent.

Thiamin Deficiency

- Beriberi
  - First observed in East Asia, where rice provided 80 to 90 percent of the total calories most people consumed.
  - Polished rice became widespread, and beriberi became epidemic.

Thiamin Deficiency



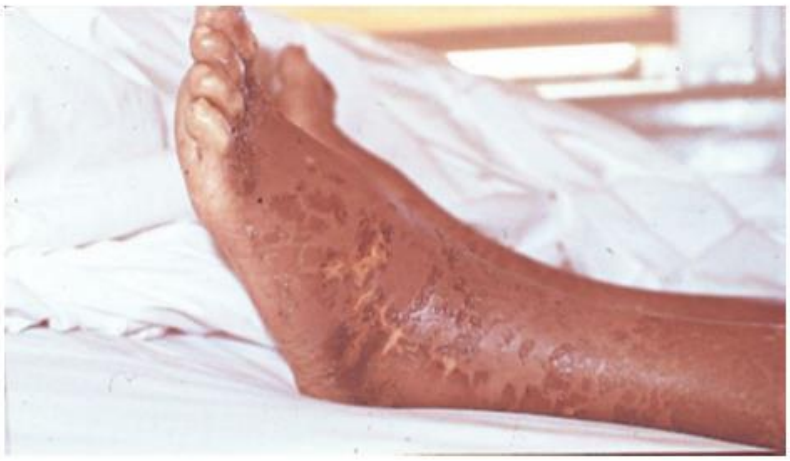
From the B-complex group: Riboflavin (Vitamin B2)

Functions: Important for growth; Necessary for normal protein and carbohydrate metabolism and tissue repair; promotes good vision, healthy skin.

The main sources of riboflavin are liver, milk, dark green vegetables, whole and enriched grain products, eggs.

Niacin

- Pellagra symptoms: 4 "D's"
  - Diarrhea
  - Dermatitis
  - Dementia
  - Death



Niacin

- Pellagra is still common in parts of Africa and Asia.
- Pellagra still occurs in the U.S. among poorly nourished people, especially those with alcohol addiction.

From the B-complex group: Niacin (Vitamin B3)

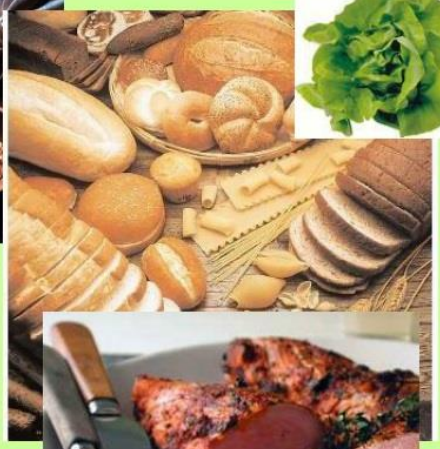
Niacin is also known as 'nicotinamide' or 'nicotinic acid'.

Functions: Necessary for normal carbohydrate metabolism (releasing energy from food); aids digestion, promotes normal appetite; romotes healthy skin, nerves.

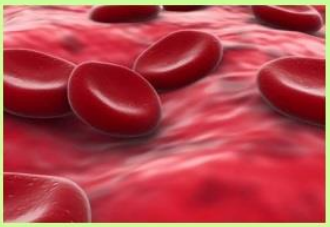
The main sources of niacin are liver, fish, poultry, meat, peanuts, whole and enriched grain products.

From the B-complex group: Pyridoxine(Vitamin B6)

Pyridoxine (pĭr'ĭ-dŏk'sēn, -sĭn) is also known as Vitamin B6.



Functions: Aids in protein and carbohydrate absorption and metabolism; aids in red blood cell formation; helps the body use fats

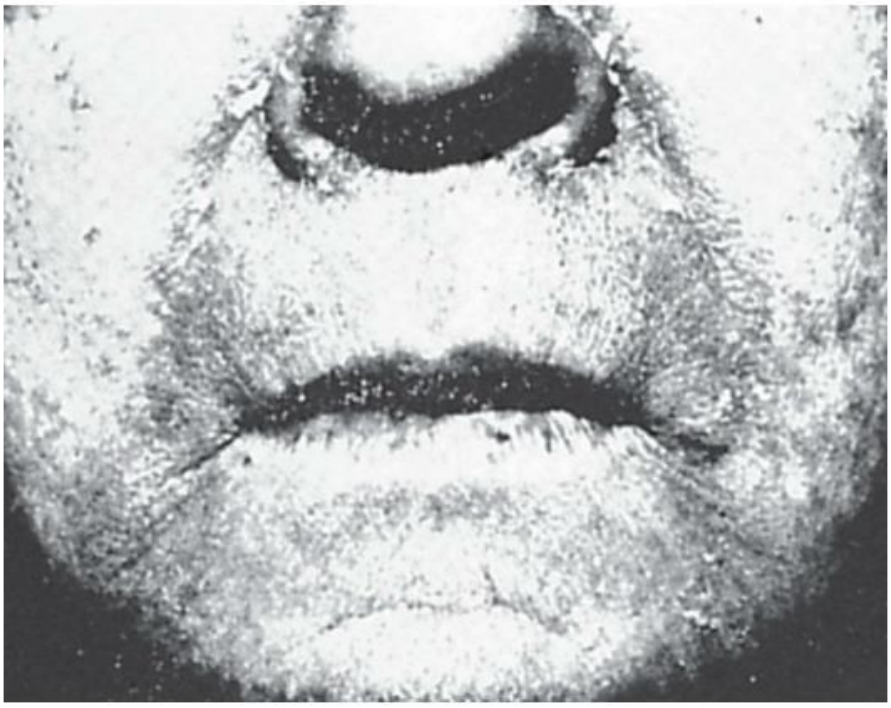


The main sources of pyridoxine are pork, meats, whole grains and cereals, legumes, green, leafy vegetables.

Vitamin B_6

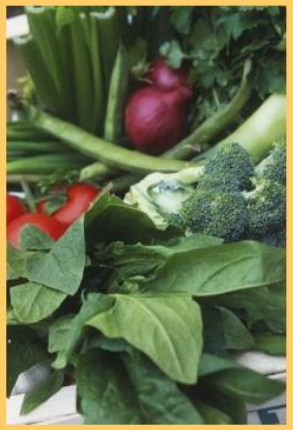
- Vitamin B_6 participates in more than 100 reactions in body tissues.
  - Needed to convert one amino acid to another amino acid that is lacking
  - Aids in conversion of tryptophan to niacin
  - Plays important roles in the synthesis of hemoglobin and neurotransmitters
  - Assists in releasing glucose from glycogen
  - Has roles in immune function and steroid hormone activity
  - Critical to fetal nervous system development

Vitamin B_6



From the B-complex group: Folic Acid

Folic Acid is also known as folate, folacin, or Vitamin B9.



Functions: Aids in protein metabolism; promotes red blood cell formation; prevents birth defects of spine and brain; some evidence that it lowers coronary heart disease risk.

Sources: Folic acid is found in leafy green vegetables, beans, peas and lentils, liver, beets, brussel sprouts, poultry, nutritional yeast, tuna, wheat germ, mushrooms, oranges, asparagus, broccoli, spinach, bananas, strawberries, and cantaloupes. In 1998, the U.S. Food and Drug Administration (FDA) required food manufacturers to add folic acid to enriched bread and grain products to boost intake and to help prevent neural tube defects.

The deficient chick (on the left) is severely stunted and anemic. The control chick on the right was fed the same food ration plus 100 micrograms of folic acid per 100 grams of diet.

From the B-complex group: Vitamin B12

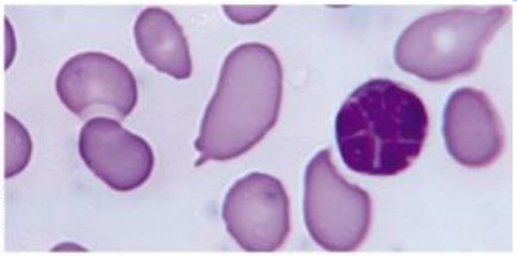
Vitamin B12 is also known as cobalamin (kō-băl'ə-mĭn).

Functions: Aids in building of genetic material; aids in development of normal red blood cells; maintenance of nervous system.

Sources: Vitamin B12 is found only in animal foods such as meats, liver, kidney, fish, eggs, milk and milk products, oysters, and shellfish. Normally, ingested vitamin B12 combines with 'intrinsic factor', which is produced by cells in the stomach. Intrinsic factor is necessary for vitamin B12 to be absorbed in the small intestine.

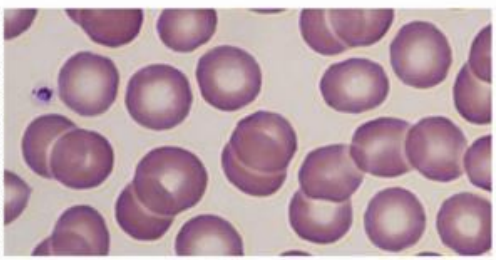
Vitamin B_{12}

The anemia of folate deficiency is indistinguishable from that of vitamin B_{12} deficiency.



Carolina Biological/Visuals Unlimited

Blood cells of pericious anemia. The cells are larger than normal and irregular in shape.



Ed Reschke/Peter Arnold, Inc.

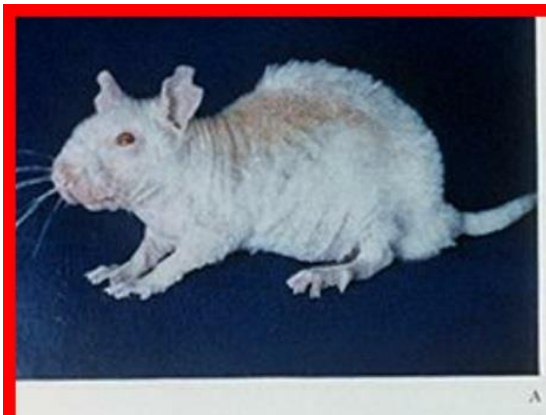
Normal blood cells. The size, shape, and color of these red blood cells show that they are normal.

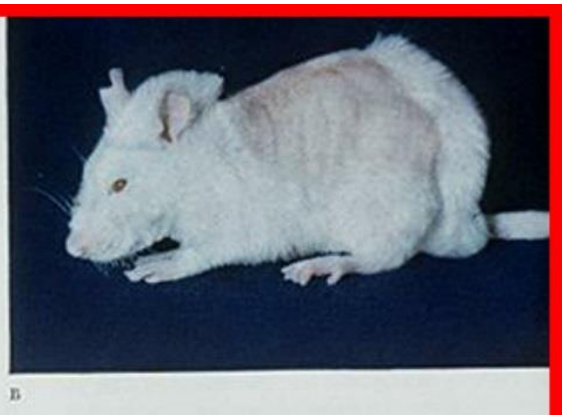
From the B-complex group: Biotin

Biotin (bī'ə-tĭn) has no numerical designation, but is also known as Vitamin H.

Functions: Necessary for normal carbohydrate and fatty acid metabolism (releasing energy from food; healthy skin, hair, and nails.

Sources: Liver, kidney, egg yolk, milk, and most fresh vegetables. It can also be made by intestinal bacteria, but it is unknown how much can be absorbed from that process.





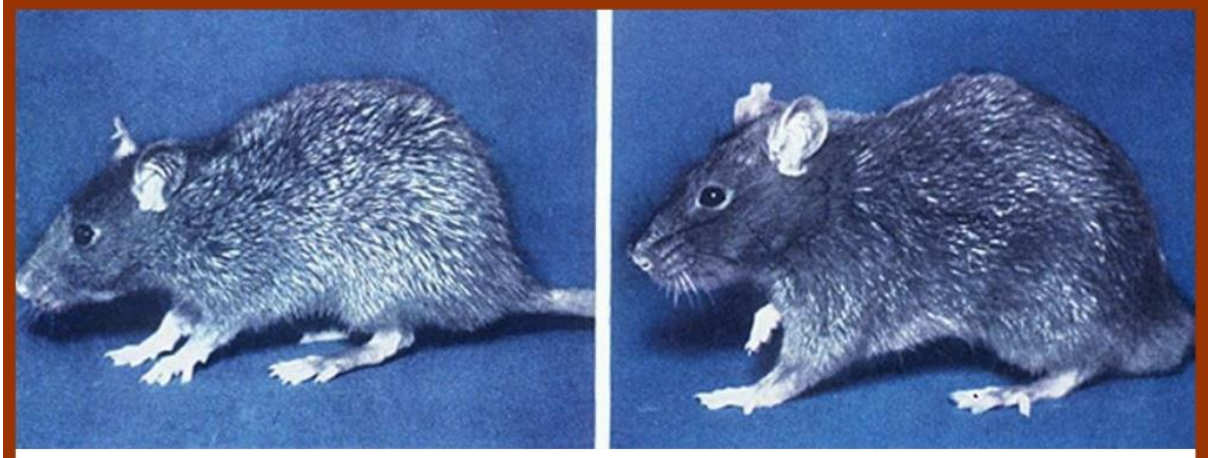
A biotin deficiency in a rat, shown in the upper left picture, results in dermatitis (skin rash) and resulting alopecia (hair loss) producing a characteristic "spectacle eye" appearance. Upper right-same rat after three weeks of biotin therapy. Lower right-same rat after three months of therapy.

From the B-complex group: Pantothenic Acid

Pantothenic acid (păn'tə-thĕn'ĭk) is also known as Vitamin B5.

Functions: Necessary for normal carbohydrate and fatty acid metabolism (releasing energy from food); aids in formation of hormones; healthy skin.

Sources: Liver, kidney, meats, egg yolk, whole grains, legumes; most fruits and vegetables, and like biotin, it is also made by intestinal bacteria. About half of pantothenic acid is lost in the milling of grains and heavily refined foods.



The rat on the left shows a pantothenic acid deficiency. It results in the loss or absence of pigment in hair. It may be complete or patchy, affect the length of the fiber or be in well-defined bands or speckled. Similar hair effects are found in copper deficiencies. The animal with this deficiency also walks with a 'goose-stepping'. The rat on the right has undergone nutritional therapy.

The benefits of treating graying hair with pantothenic acid to reduce hair loss or hair color loss have not been evidenced in humans.

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 № 15

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.

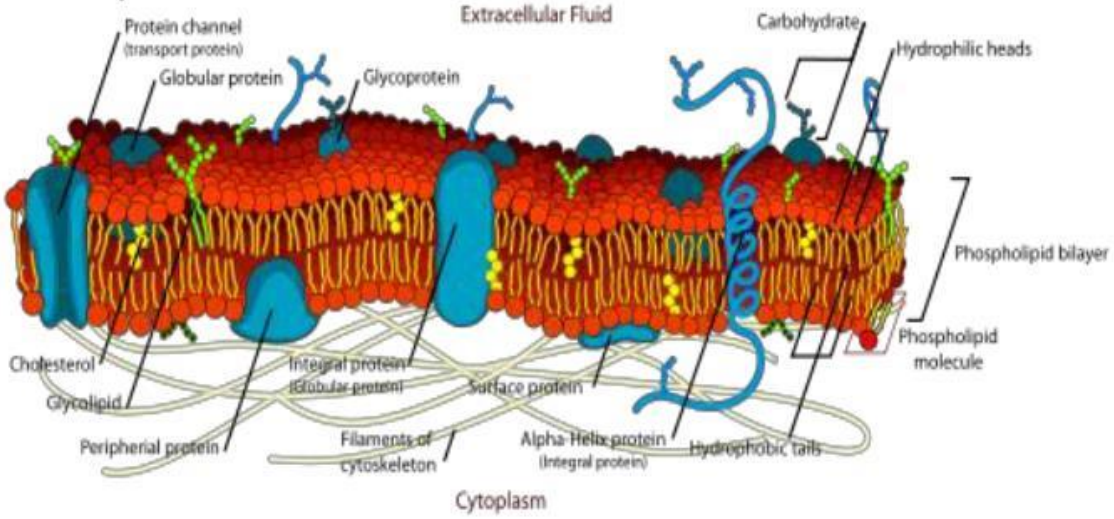
Basic concepts:
1. Chicken blindness.
2. Xerophthalmos.
3. Keratomalacia.
4. Rickets.

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.

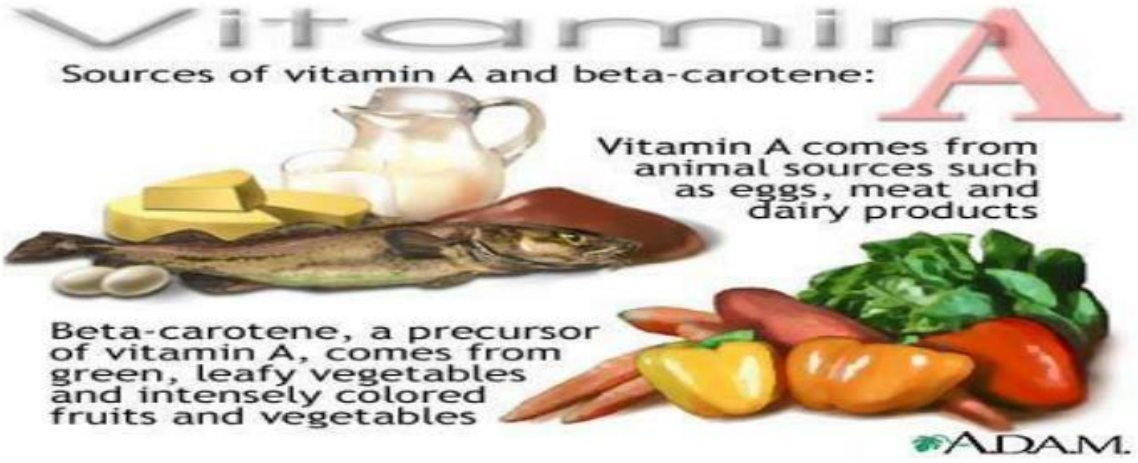
Vitamin A D E K

- These fat soluble vitamins work as a family
- They reside in membranes



Vitamin A

- First identified as an essential growth factor found in animal fats and fish oils
- Plant carotenoids also have vitamin a activity

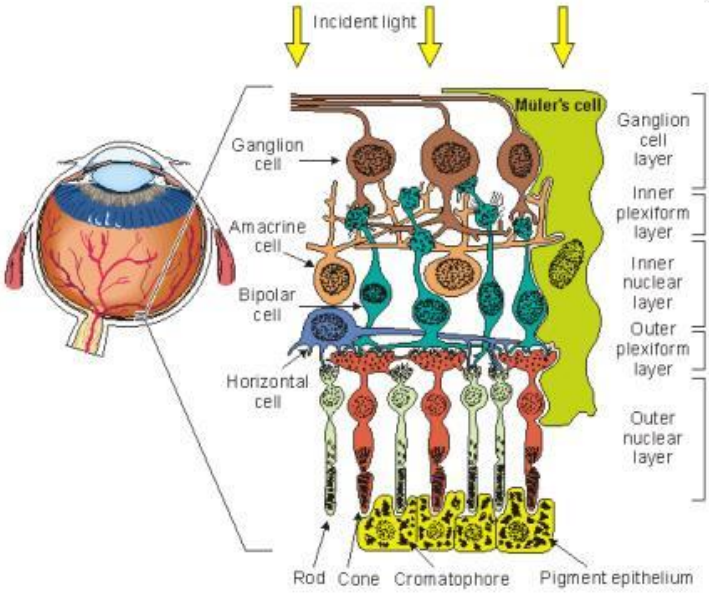


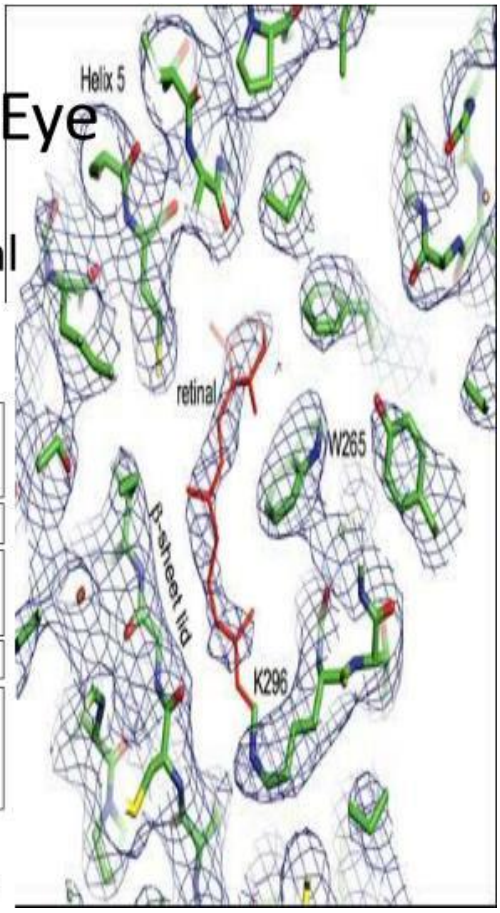
Vitamin A in the Body

- Vitamin A is used in two forms.
- Retinal in the eye.
- Retinoic acid in other tissues.
- Retinyl Palmitate as a vitamin A storage form.
- Beta Carotene from vegetables and fruits consists of two vitamin A molecules joined at the tail.
- Enzymes allow symmetric cleavage to yield two molecules of vitamin A.

Vitamin A Eye

- Eye uses vitamin a as retinal





Eyesight

- The vitamin then reunites with the pigment, but a little vitamin A is destroyed each time this reaction takes place, and fresh vitamin A must replenish the supply.
- If the vitamin A supply runs low, **night blindness** can result – a lag before the eye can see again after a flash of bright light at night.

© David Farr/Image Smythe

In dim light, you can make out the details in this room.

A flash of bright light momentarily blinds you as the pigment in the retina is bleached.

You quickly recover and can see the details again in a few seconds.

With inadequate vitamin A, you do not recover but remain blind for many seconds; this is night blindness.

Skin And Body Linings

- The skin in vitamin A deficiency

Vitamin A Deficiencies

- Fetal abnormalities of eyes, lungs, gut, and immune.
- Night blindness, clouding and malformation of cornea, corneal degradation, and dry eyes.
- Weak immunity, direct and dysfunctional mucus membranes.
- Keratinization of epithelial tissues, eyes, lungs, urogenital, and GI.
- Skin disorders, acne, eczema, psoriasis.
- Infertility
- Diabetes, hormone imbalances of thyroid, adrenal, sex hormones.
- Weak teeth and bones

Toxicity of Vitamin A in Arctic Animals

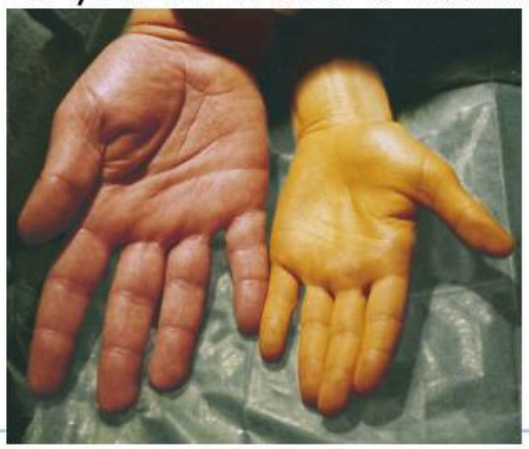
- Their Livers are Loaded with Toxic Levels of Vitamin A

Food Sources of Vitamin A

Colorful foods are often rich in vitamins

Beta-Carotene And Carotenoids

- Beta-carotene from food is not converted to retinol efficiently enough to cause vitamin A toxicity. Excess beta-carotene is stored the fat under the skin, imparting a yellow cast.
- Do you think this is harmful?

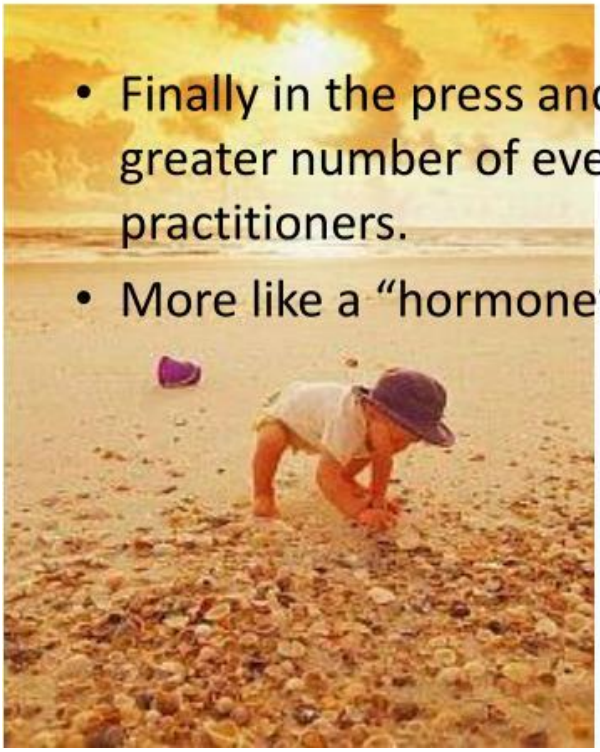


Answer: NO

Vitamin A Levels

- Quest Diagnostic Labs levels
- 38-98 mcg/L

Vitamin D



- Finally in the press and on the minds of a greater number of even conventional practitioners.
- More like a “hormone” than a vitamin.

Forms of Vitamin D

- D2 ergocalciferol produced by invertebrates, yeast, fungus, plants.
- D3 cholecalciferol produced in the skin of vertebrates via ultraviolet B (UVB). Small amounts occur naturally in fatty fish, eggs, and meats.
- Free

Vitamin D

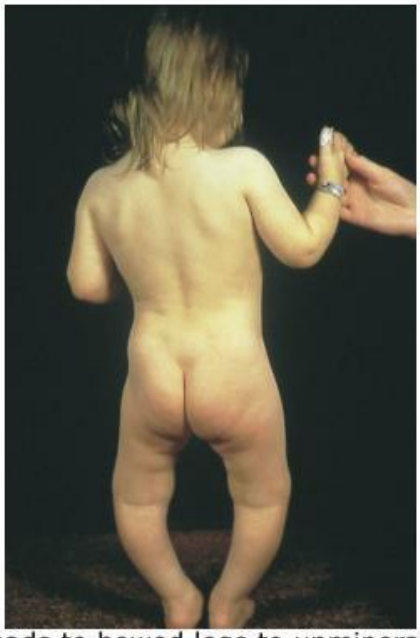
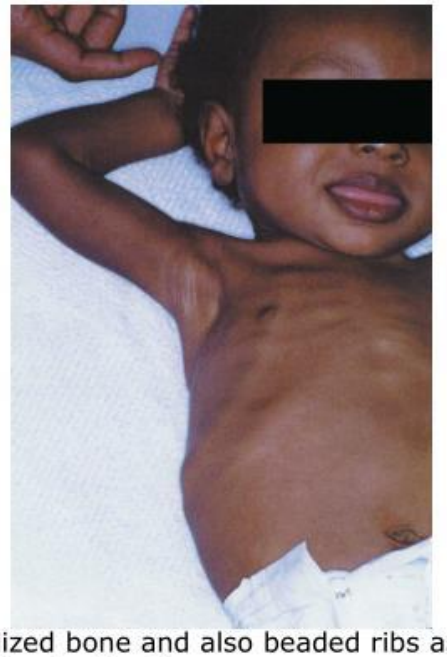
- The sunshine Vitamin: D

Vitamin D Production

- Provitamin D3 is a precursor of cholesterol found in the skin.
- UVB converts it to vitamin D3.
- D3 is transferred from blood to liver where it is transformed to 25 hydroxy D3 (calcidiol).
- 25 hydroxy D3 is transferred to the kidneys for transformation to 1,25 hydroxy D3 (calcitriol) the active form of vitamin D.
- 1,25 hydroxy D3 is also produced by the Immune system for immune activation and modulation.

Vitamin D Function

- Vitamin D has receptors throughout the body.
- Brain
- Genes
- Immune System
- Membranes
- Bones
- Skin
- Heart and vasculature
- Lungs
- Intestines
- Liver
- Hormone sensitive tissues, breast, ovaries, prostate
- antioxidant

Rickets leads to bowed legs to unmineralized bone and also beaded ribs as calcium is deposited on the ribs, rather than in the ribs.

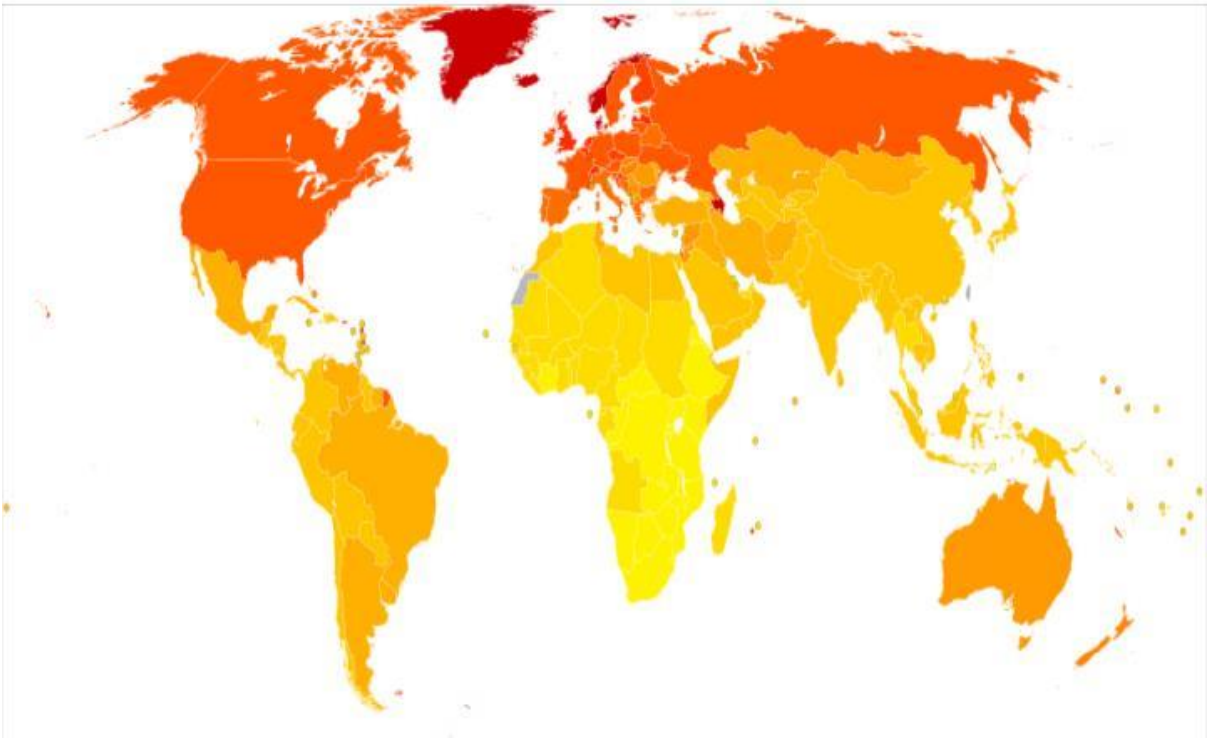
Vitamin D Calcium Metabolism

- Vitamin D helps orchestrate calcium/phosphorus levels and ratios along with vitamin A and K, and parathyroid hormone.
- Calcium flux across a membrane is more than bone metabolism.
- Calcium flux is also an integral part of muscle function, cardiovascular function, clotting, energy production and cell signaling.

Vitamin D Deficiency

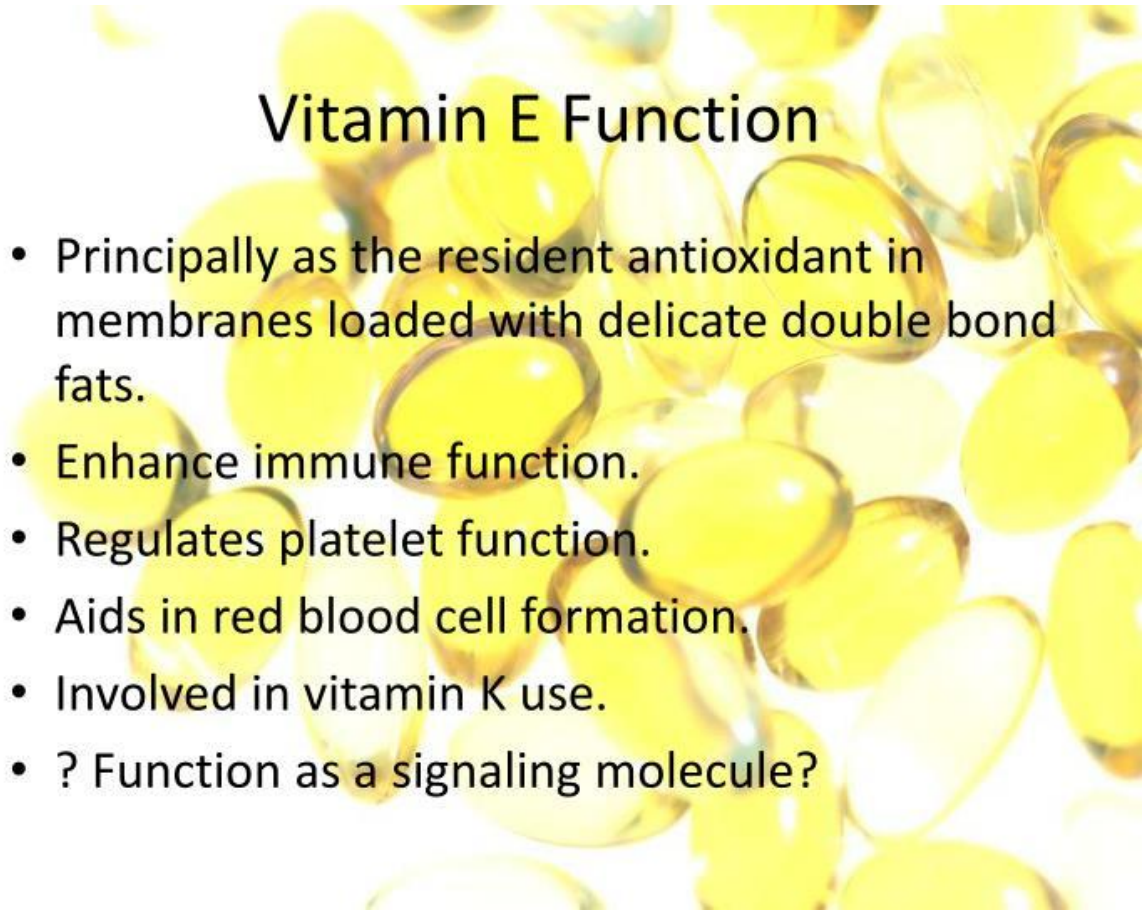
- Bones, rickets, osteomalacia, osteoporosis, bad teeth
- Auto immunity, rheumatoid arthritis, jra, lupus, MS, parkinsons, dementias including Alzheimer, autoimmune thyroid (graves, hashimotos).
- Cancers breast, prostate, lung, colon, pancreas
- Cardiovascular disease, hypertension.
- Insulin resistance/diabetes.
- Infections , flu, pneumonias, colds.
- Mood disorders, depression, seasonal affect.
- Hair and skin health.

Multiple Sclerosis



Vitamin E

- Vitamin E is a generic name given to a family of essential fat soluble antioxidants 4 tocopherols (alpha, beta, gamma, and delta), and 4 groups of tocotrienols.
- D Alpha tocopherol has the greatest activity.
- They reside in membranes where they work to protect against oxidation of these delicate fats by free radicals produced by the energy producing machinery of our cells.



Vitamin E Function

- Principally as the resident antioxidant in membranes loaded with delicate double bond fats.
- Enhance immune function.
- Regulates platelet function.
- Aids in red blood cell formation.
- Involved in vitamin K use.
- ? Function as a signaling molecule?

Vitamin E Sources

Vitamin E Deficiencies

- Cardiovascular disease (majority of vitamin E found in LDL fraction of cholesterol)
- Cancer
- Alzheimers
- Cataracts

Food Sources of Vitamin E

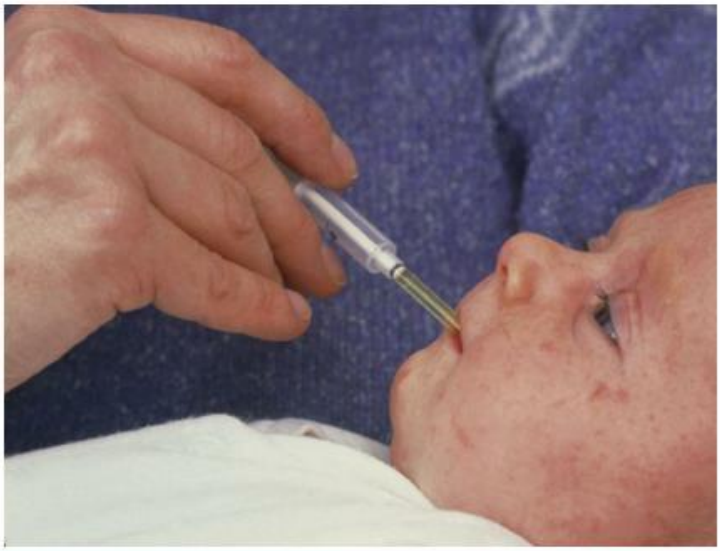
- Raw vegetable oils contain substantial vitamin E, but high temperatures destroy it

Vitamin E Levels and Dosing

- QDL 5.7-19.9 mg/L
- AI 22.5 iu/day
- Average intake 11.4iu/day females
  15iu/day males
- Optimum 100-400iu/day

Sources of Vitamin K

- Vitamin K can be made by intestinal bacteria.
- Newborns are given a dose of vitamin K at birth.



Overdoses and Toxicity of Vitamin E

- Doses greater than 800-1500iu/day may increase mortality due to all causes.
- Possible mechanisms, altered beta oxidation reducing the ability to burn fat for energy, or inability to burn dangerous fats?
- Bleeding may be a possibility especially in those deficient in vitamin K.

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 16.

Topic: Chemical composition and functions of blood. Transport of gases by blood. Biochemistry and pathobiochemistry of hemoglobins. Biosynthesis of porphyrins, heme catabolism. Metabolism 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:

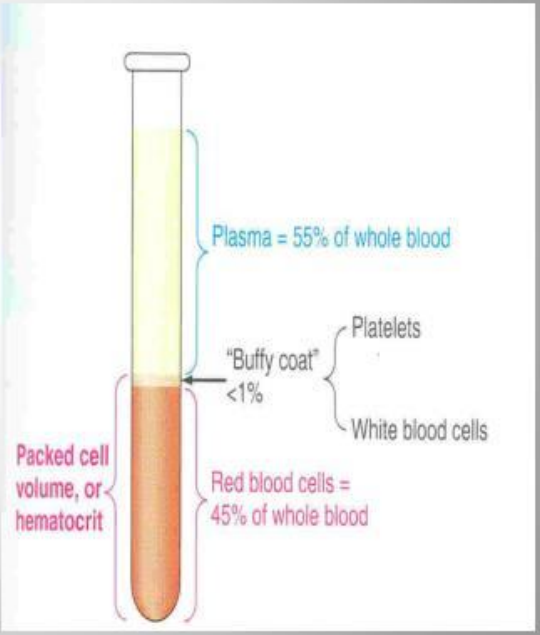
1. The cooperative effect of Oxygen.
2. Bohr effect.
3. Azotemia.
4. Dysproteinemia.
5. Paraproteinemia.

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.

Composition of blood

- Blood consists of formed elements that are suspended and carried in a fluid called plasma



Plasma

- Straw colored, nonliving part of blood.
- 90% Water
- Helps to regulate body temperature
- Contains Electrolytes
- Plasma transports blood cells, products of digestion and hormones throughout the body.

General Function of the Blood

1- Transportation:

A) Gases: O_2 , CO_2 , ...

B) Nutrient and metabolic Wastes: Glucose, amino acids, ...

C) Hormones and Enzymes

D) Antibodies

E) Electrolytes and Ions

General Function of the Blood Cont.

2- Regulation:

A) Temperature regulation

B) pH regulation: By buffering systems found in the blood that maintain the pH between 7.35 to 7.45

C) Electrolytes regulation (Na, K, Cl,.....)

D) Blood pressure regulation: by increasing or decreasing blood flow to the kidneys

General Function of the Blood Cont.

3- Protection:

A) Defense mechanism: By white blood cells

B) Clotting mechanism: Blood contains materials that stop bleeding when vessels are damaged (Hemostasis)

Plasma

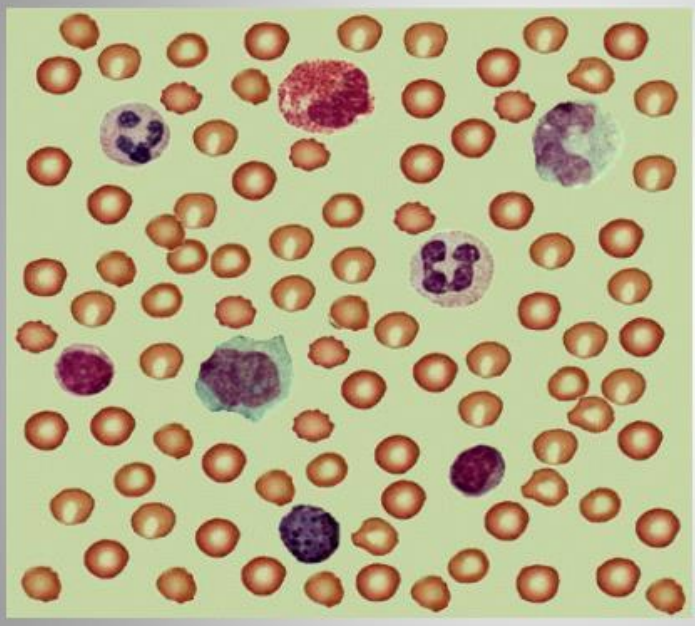
- Straw colored fluid made of water (~92%), other contents include:
- Proteins make the bulk of the solutes: manufactured in the liver

  - **Albumins** (60%), are the most abundant type of plasma proteins, maintain the plasma volume by osmotic pressure. (↓No⟶ edema).
  - **Globulins** (35%), alpha and beta Globulins transport lipids and certain minerals through the bloodstream. Gamma Globulins are antibodies.
  - **Fibrinogen** (4%) for blood clotting

Plasma, cont.

- Nutrients: glucose, amino acids, lipids, cholesterol
- Electrolytes: Na^+, K^+, Ca^{++}, Mg^{++}, H^+, Cl^-, HCO_3^-, PO_4^{--}, SO_4^{--}
- Waste: urea, creatinine, uric acid, bilirubin
- Gases: O_2 , CO_2
- Protein bound hormones
- Plasma without clotting factors is called "serum"

Blood Film



Formed Elements

BLOOD COMPOSITION

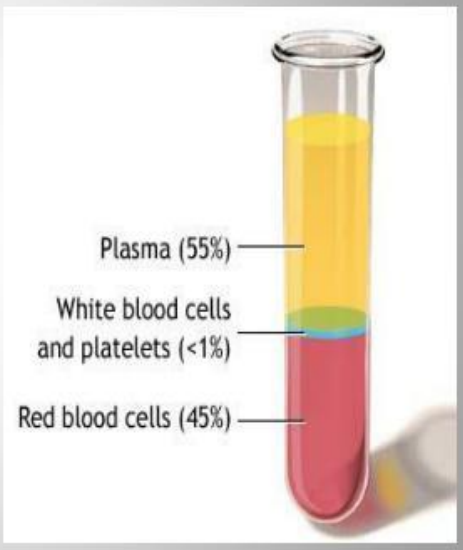
1. Cellular components
   - Red Blood Cells, RBCs (Erythrocytes)
   - White Blood Cells ,WBCs (Leukocytes)
   - Platelets (Thrombocytes)

2. Plasma
   - 92% water, ions, plasma proteins (Albumin, globulin, Fibrinogen)
   - Same ionic composition as interstitial fluid

15

Blood Volume

5 liter in adult
- 45% is packed cells volume (PCV)
- 55% is plasma volume



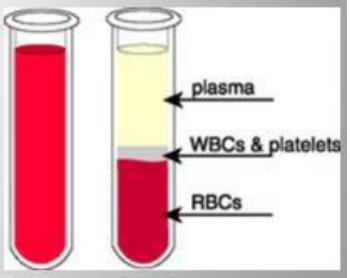
16

Blood Cells

- **Each type of blood cell performs a different function**
- Red blood cells (Erythrocytes)

- White blood cells (Leukocytes)

- Platelets (Thrombocytes)



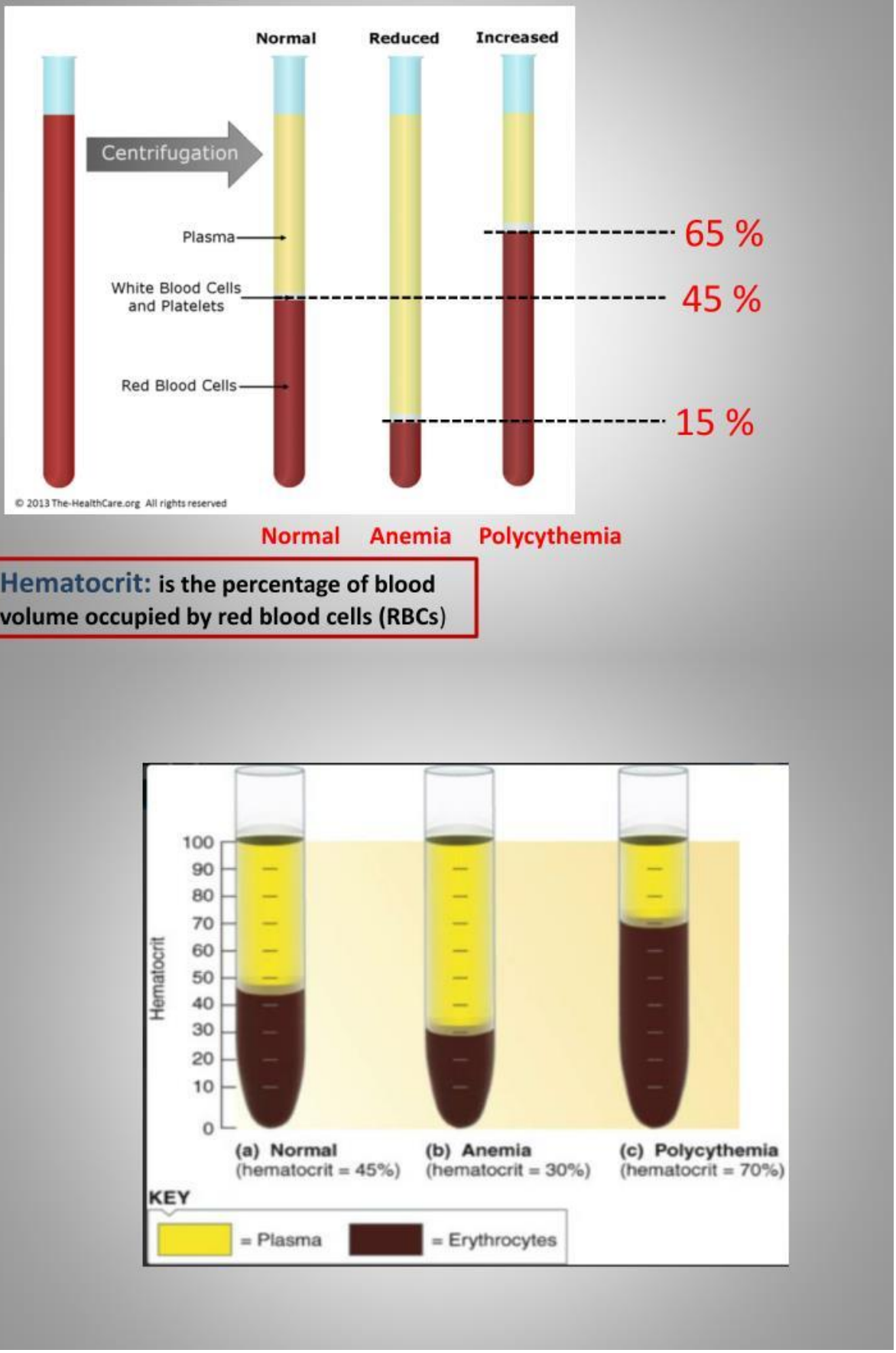
Hematopoiesis

• Is a formation of blood cells from stem cells in the red bone marrow (myeloid stem cell) & lymphatic tissue (lymphoid stem cell)

• **Erythropoiesis** is formation of RBCs – Stimulated by erythropoietin (EPO) from kidney

• **Leukopoiesis** is formation of WBCs – Stimulated by variety of cytokines

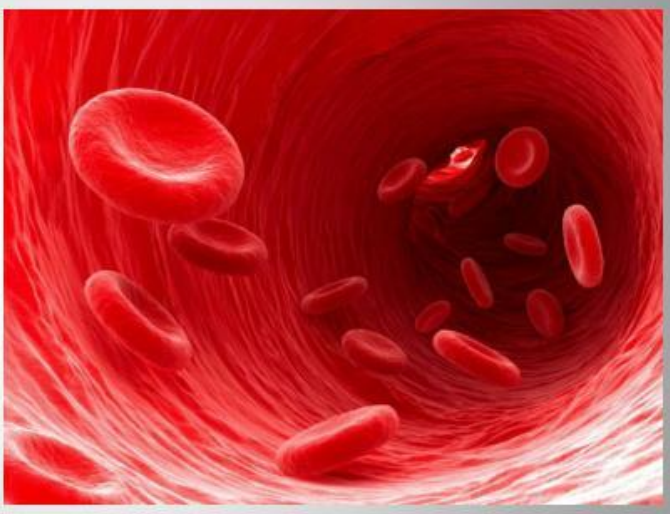
• **Thrombopoiesis** is formation of platelets



Hematocrit: is the percentage of blood volume occupied by red blood cells (RBCs)

Red Blood Cells

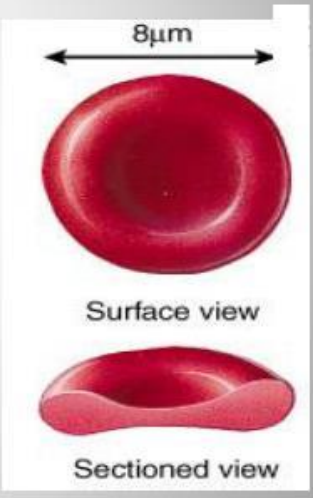
- Function
  - O_2 transport
  - CO_2 transport
  - Buffer



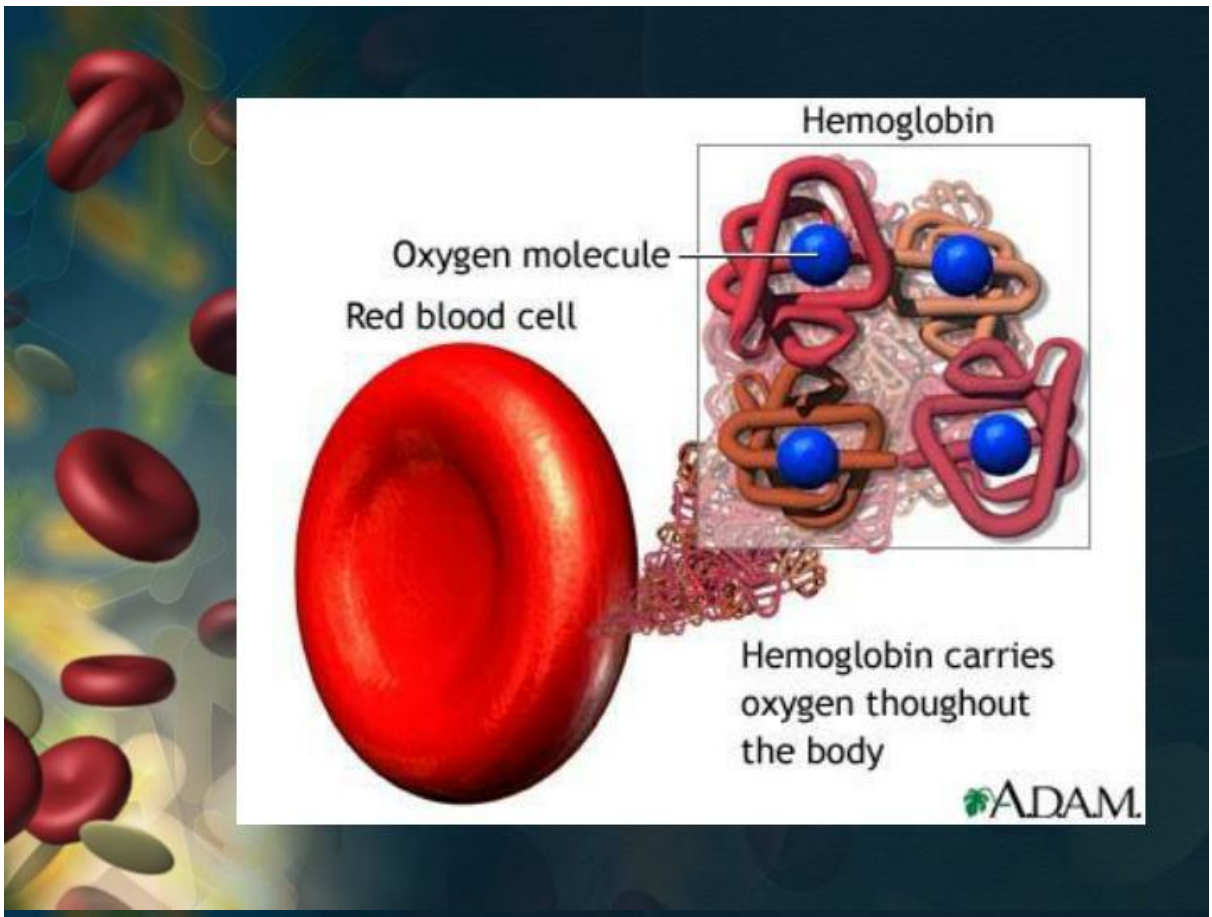
19

Red Blood Cells (Erythrocytes)

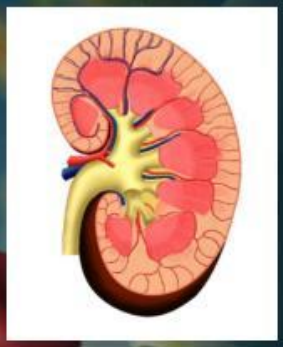
- Shape & size
  - Flattened Biconcave Disc
  - Lack nuclei and mitochondria
  - Diameter 7-8 μm
  - Flexible
  - Life span- 120 days
  - Number =4.7-5.2 million/ mm^3



20



Erythropoiesis

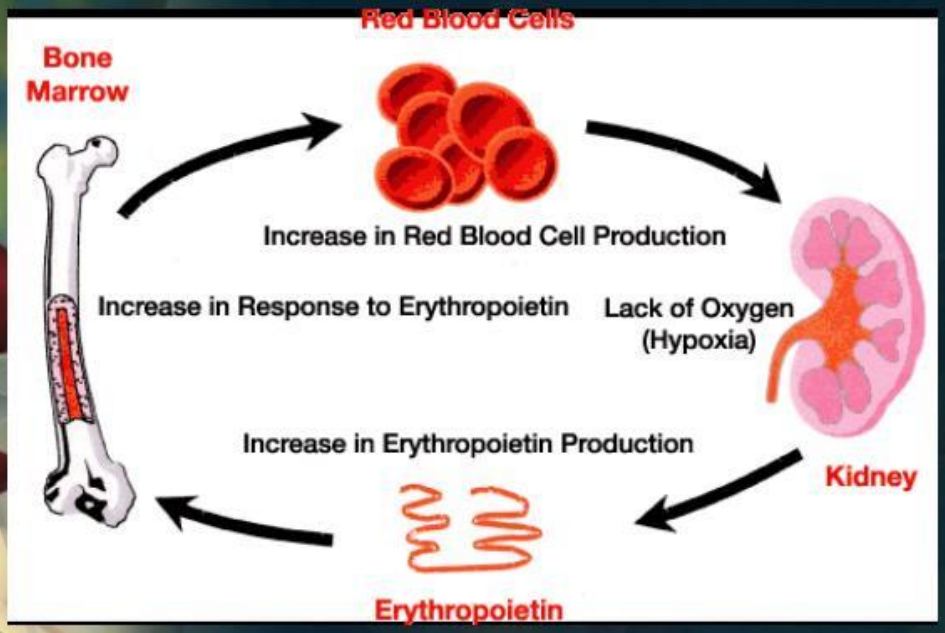


1. Pluripotent stem cells (PPSC) exist in bone marrow
2. Erythropoietin (EPO) *(a hormone)* is released by kidneys in response to blood hypoxia

(kidney dysfunction can affect RBC production)

3. PPSC stimulated by EPO will begin to undergo mitiotic division and develop into myeloid stem cells.

Erythropoiesis



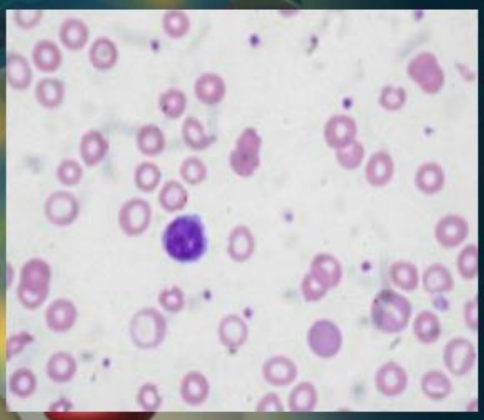
Lifespan and Destruction of RBCs

- Lifespan of RBC = related to metabolism and weight of animal
- Process of aging is called **senescence**.
- As RBC becomes more senescent, enzyme activity decreases
  - Glycolytic enzyme unable to break down glucose
  - Cell becomes more round
  - Volume decreases
  - 90% of destruction of senescent RBCs occurs by extravascular hemolysis.

Normal Removal of Aged RBCs

- Surface membrane alterations (oxidative damage) or damaged cells are recognized by macrophages.
- Macrophages found all over the body (mostly of the spleen) phagocytize (engulf and “eat up”) old RBCs.
- Components of RBC breakdown are either removed by the body as waste or recycled.

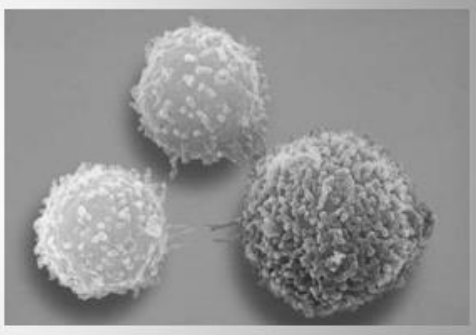
Anemia – *Not enough RBCs*



- Pathological condition that results in decreased oxygen-carrying capacity of the blood.
- Regenerative
  - Caused by blood loss or hemolysis
- Non-regenerative
  - Caused by reduced or defective erythropoiesis
    - Defective hemoglobin production

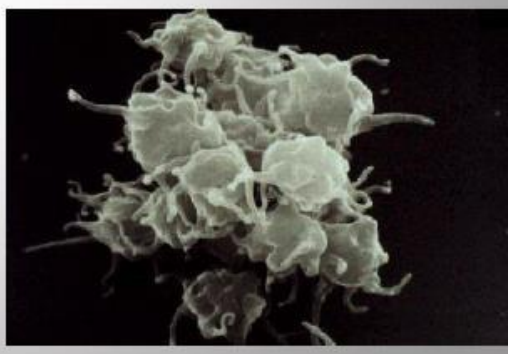
White Blood Cells (Leukocytes)

- **Shape & size**
  - Have nucleus and mitochondria
  - Two types: granular and non-granular, Amoeboid
  - Diapedesis - can "slip between" capillary wall
  - Number =4,000-11,000 / mm^3

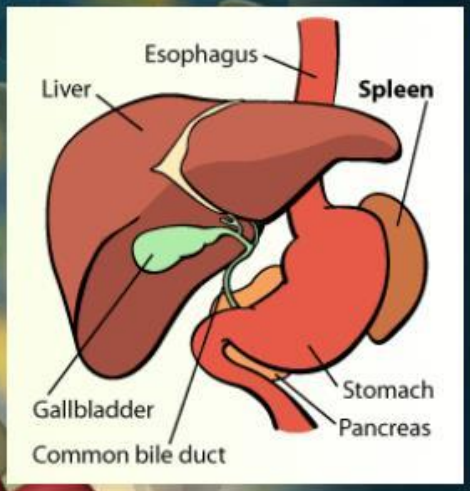


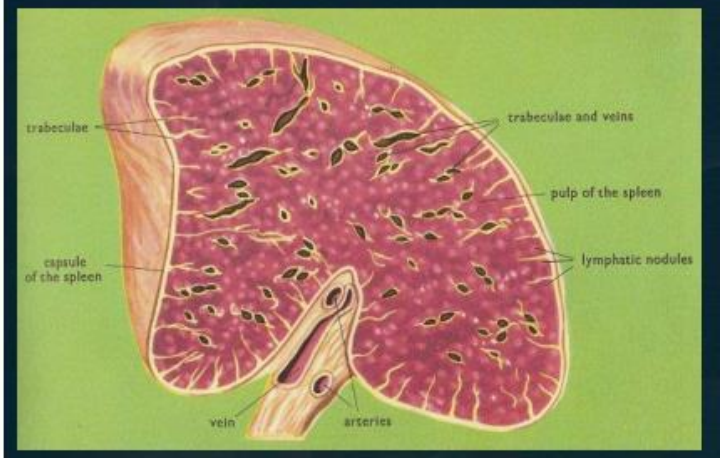
Platelets (Thrombocytes)

- **Shape & size**
  - Are smallest of formed elements.
  - Lack nucleus
  - Irregularly shaped fragments of megakaryocytes, amoeboid.
  - Diameter: 2-3 µm
  - Life span- from 5 to 10 days
  - Essential for clotting
  - Number =250,000-500,000/ mm^3



Blood Storage

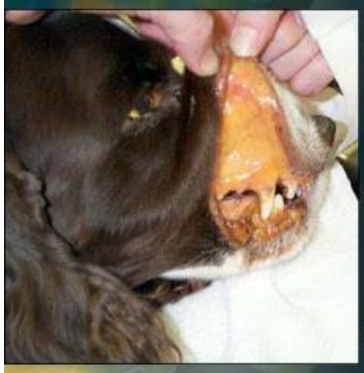




Bilirubin

- In the liver, bilirubin is ***conjugated*** – joined to glucuronic acid -- and becomes water soluble.
- Conjugated bilirubin is excreted as a bile pigment into the intestines.
- In the intestines, it is converted into urobilinogen by bacteria.
- Some urobilinogen is reabsorbed by kidneys and eliminated in the urine.
- Some urobilinogen is converted to stercobilinogen and excreted in the stool.
- These compounds are responsible for the color of urine and feces.

Jaundice/Icterus



- Excessive RBC breakdown = **hyperbilirubinemia** (excess unconjugated bilirubin in plasma).
- If there is too much bilirubin in the blood for the liver to be able to break down, it is deposited in the tissues.
- Bilirubin in the tissues = **jaundice**; clinically seen as a yellowish color in the MM and sclera of the eyes
- In liver disease, the liver is unable to break down a normal amount of bilirubin from normal RBC destruction and jaundice results.

RESPIRATORY GASES; How are they transported in the blood???

OXYGEN, O_2

The only way for Oxygen to be carried around the body is by the HEAMOGLOBIN, in red blood cells.

CARBONDIOXIDE, CO_2

Carbondioxide is transported by the blood in three ways;

A)As sodium hydrogencarbonate in plasma

B)As carbomino compounds in red cells

C) In simple solution, dissolved in the plasma

Transport of O_2 by the Heamoglobin

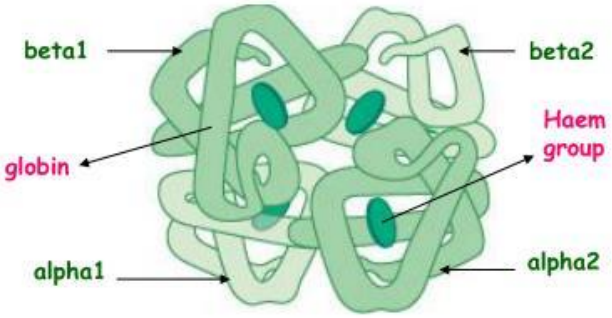
Inside red blood cells, there are many heamoglobin molecules which are large globular proteins, each consisting of two alpha subunits and two beta subunits.

Each subunit has a haem group and a polypeptide globin, This globin molecule coils around the haem groups which contain iron.

The iron atom can reversibly bond with one molecule of oxygen. Since it has four subunits, a heamoblobin molecule can combine reversibly with up to four oxygen molecules forming oxyheamoglobin molecules.

$$Hb + 4O_2 \longrightarrow HbO_8$$

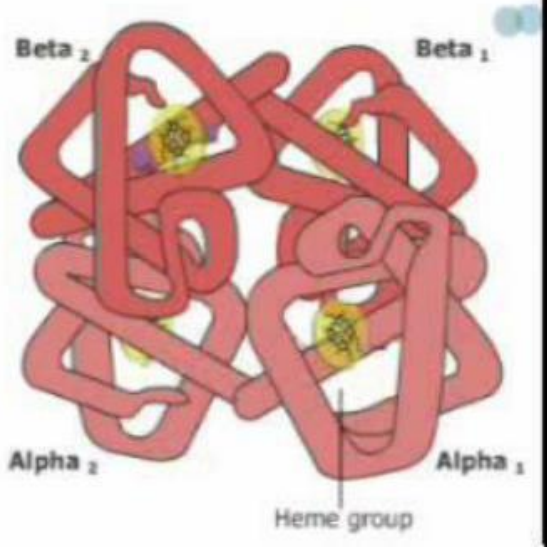
Red Blood Cell



Transport of O_2 by the Heamoglobin

When an heamoglobin molecule is not bonded to oxygen molecule, deoxyheamoglobin (heamoglobin without O_2 molecules) stays in tensed state.

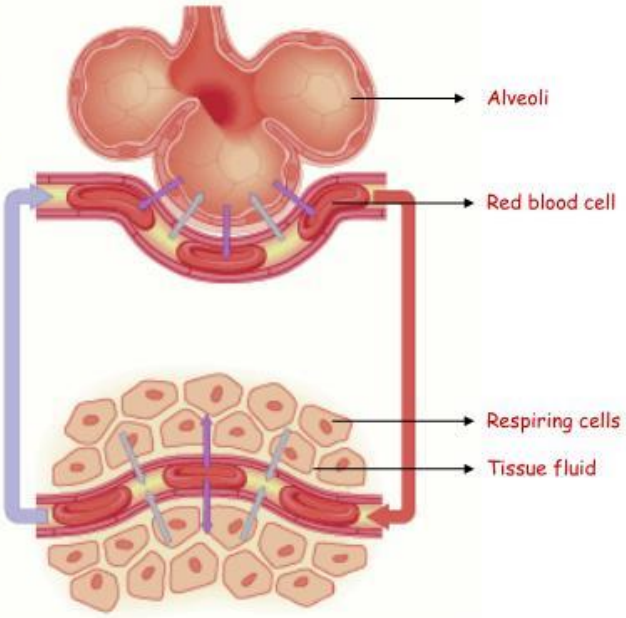
When the first molecule of oxygen combines with a heamoglobin, oxyheamoglobin shifts to relaxed state in which the shape of heamoglobin changes hence it becomes easier for other three oxygen molecules to bind to the other haems.



Transport of O_2 by the Heamoglobin

Because of the conditions mentioned before, red blood cells hence heamoglobins are very efficient in transporting oxygen from alveoli in the lungs to the respiring cells in the tissues.

The tissue fluid surrounds the cells and supplies them with oxygen. As the cells respire, they produce carbondioxide so the tissue fluid needs to be replaced continually with the fresh one. In order to supply cells with oxygen, the red blood cells move to the lungs where there is high pO_2 and increased alkalinity. They combine with the oxygen molecules and move to the respiring cells where there is high pCO_2 and acidity. As a result, they give up their oxygens and oxygen molecules reach respiring cells via tissue fluid.



Transport of Carbondioxide in the Blood

A)As sodium hydrogencarbonate in plasma

- Carbondioxide, which is produced in respiration, diffuses from body tissues into the blood where most of it, is taken by the red blood cells.

- In red blood cells,carbondioxide combines with water to form carbonic acid, H_2CO_3. This reaction is catalysed an enyzme called carbonic anhydrase.

- Then carbonic acid dissociates into hydrogencarbonate and hydrogen ions.

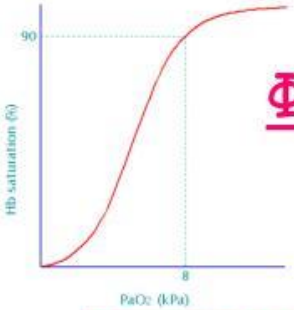
- Hydrogen ions causes oxyhaemoglobin to dissociate. Therefore oxygen diffuses into the cells for respiration.

Transport of Carbondioxide in the Blood

‡ The hydrogen ions combine with haemoglobin and form haemoglobinic acid. This meas that haemoglobin molecules act as buffers mopping up hydrogen ions and preventing changes in pH.

ξ Hydrogencarbonate ions are pumped out of the red blood cells and enter the plasma where they combine with sodium so becoming sodium hydrogencarbonate.

Ξ To make sure that red blood cells remain electrically neutral, chloride ions move into the red blood cells by a process known as Chloride Shift!



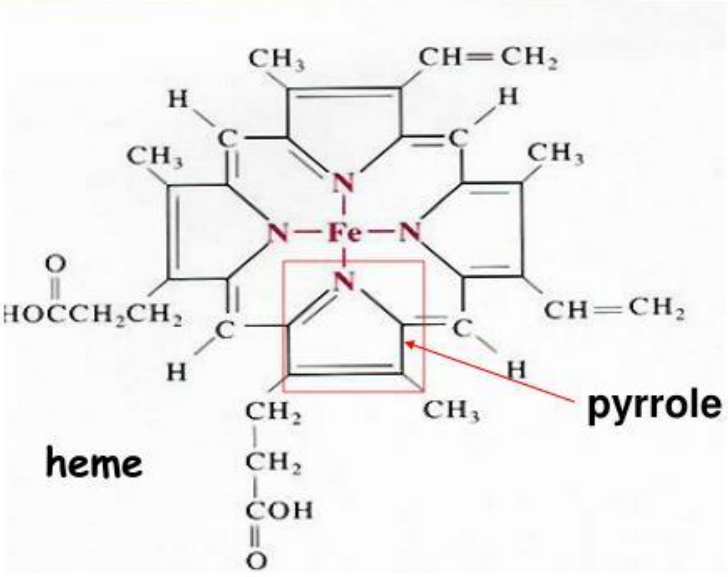
Φ BOHR EFFECT Φ

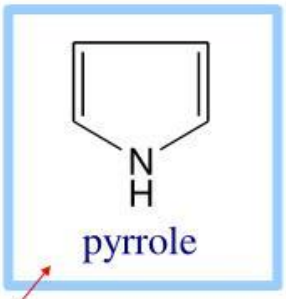
The exact shape of the dissociation curve depends on a number of factors such as

- Partial pressure of carbondioxide (pCO_2)
- Temperature
- pH

Because of the Bohr Effect, an increase in partial pessure of carbon dioxide, a decrease in pH or increased temperature causes hemoglobin to bind to oxygen with less affinity.

Bohr Effect is very important in respiring tissues since pCO_2 increases in tissue fluid. This causes oxyhaemoglobin to release its oxygens readily causing more oxygen to be delivered to the tissues needed for respiration.





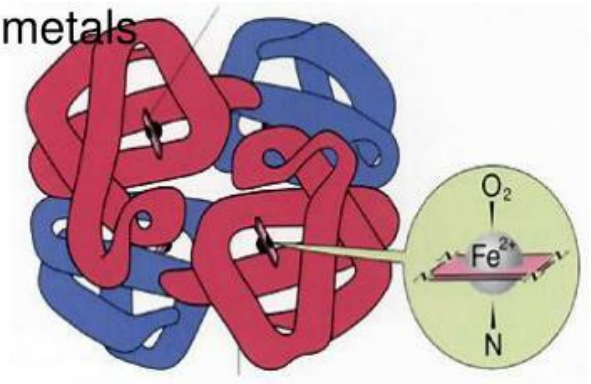
Heme is a derivative of the porphyrin. Porphyrins are cyclic compounds formed by fusion of 4 pyrrole rings linked by methenyl bridges.

Heme is the prosthetic group of hemoglobin, myoglobin, & cytochromes and so on.

- most common porphyrin in humans is heme
  - one ferrous goup in tetrapyrole ring

- heme proteins (hemoproteins) are rapidly synthsized and degraded
  - 6 to 7 g per day hemoglobin turned over

- cyclic compounds that bind metals
  - usually iron
    - Fe^{+2} = ferrous
    - Fe^{+3} = ferric



Synthesis of heme

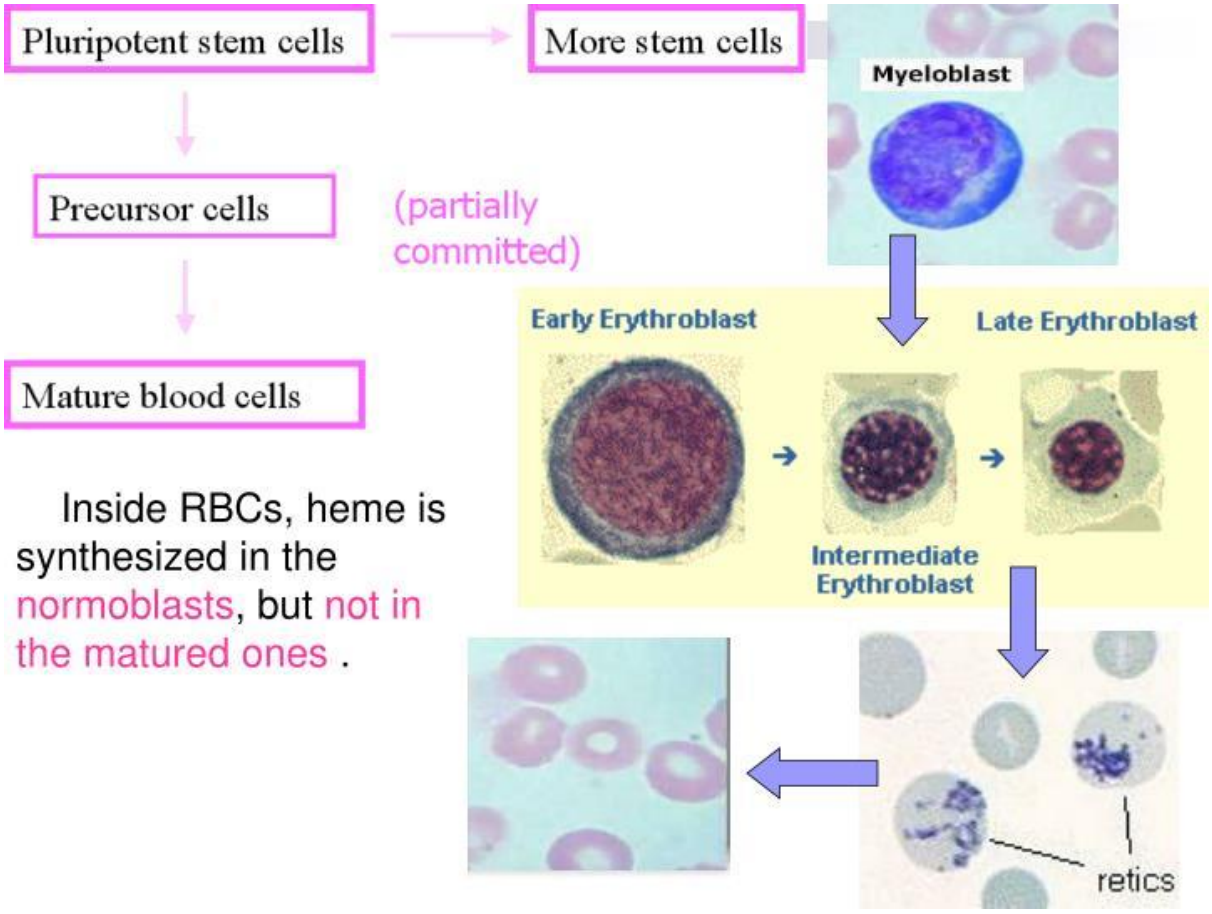
★ The substrates mainly include succinyl-CoA, glycine, Fe^{2+} .

★ Heme can be synthesized by almost all the tissues in the body which require hemoproteins.

★major sites of synthes is liver and bone marrow (erythroblasts: reticulocyte, prorubricyte)

- cytochrome p450 in liver
- hemoglobin in bone marrow
- heme production equal to globin synthesis in marrow
- variable in liver dependent on heme pool balance

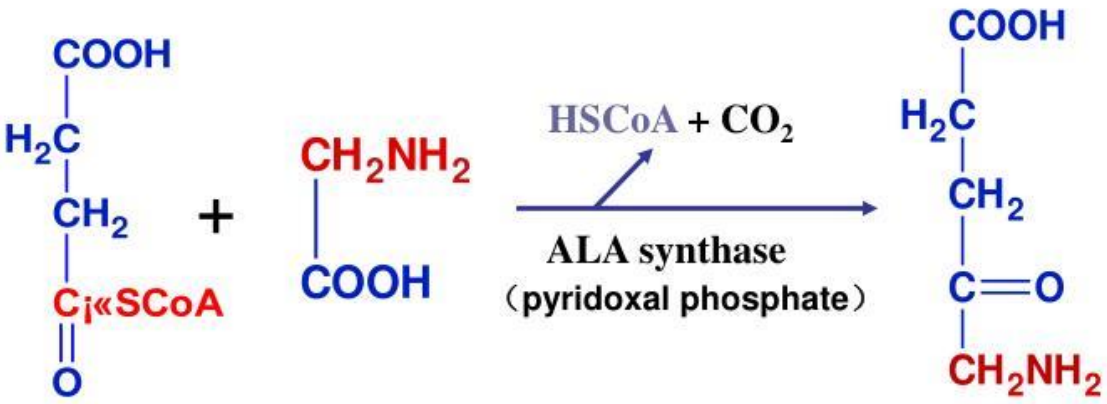
Inside RBCs, heme is synthesized in the normoblasts, but not in the matured ones .



Inside RBCs, heme is synthesized in the normoblasts, but not in the matured ones .

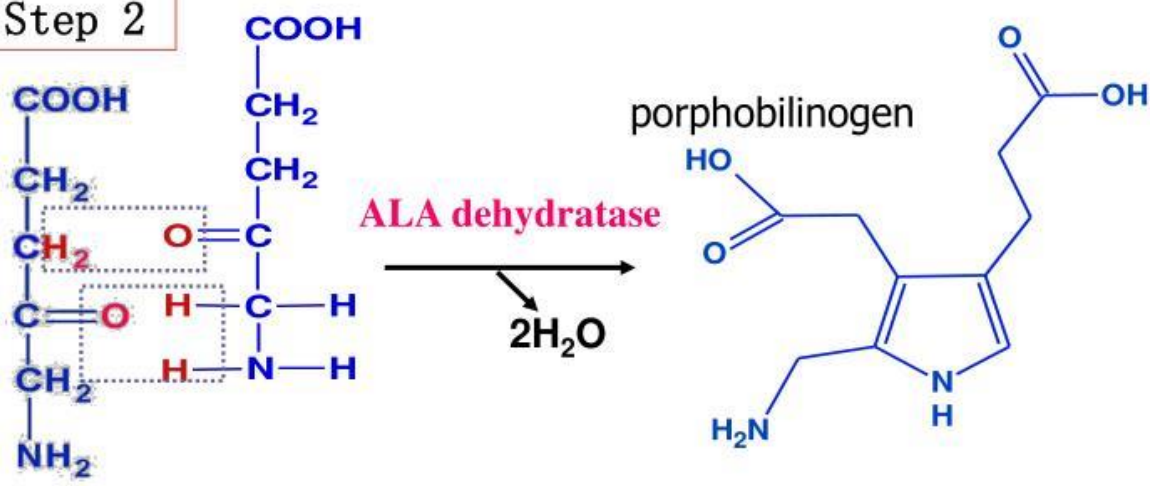
Step 1

In mitochondrion



① Heme synthesis begins with condensation of glycine & succinyl-CoA, with decarboxylation, to form **δ-aminolevulinic acid** (**ALA**).

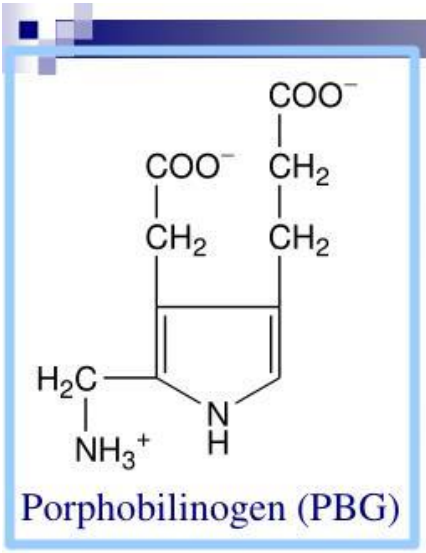
Step 2



★ The succeeding few reactions occur in the cytoplasm.

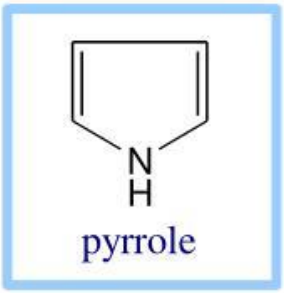
one ALA condenses with another molecule of ALA to form porphobilinogen(PBG).

★ the condensation involves removal of 2 molecules of water and the enzyme is ALA dehydratase .



Porphobilinogen (PBG)

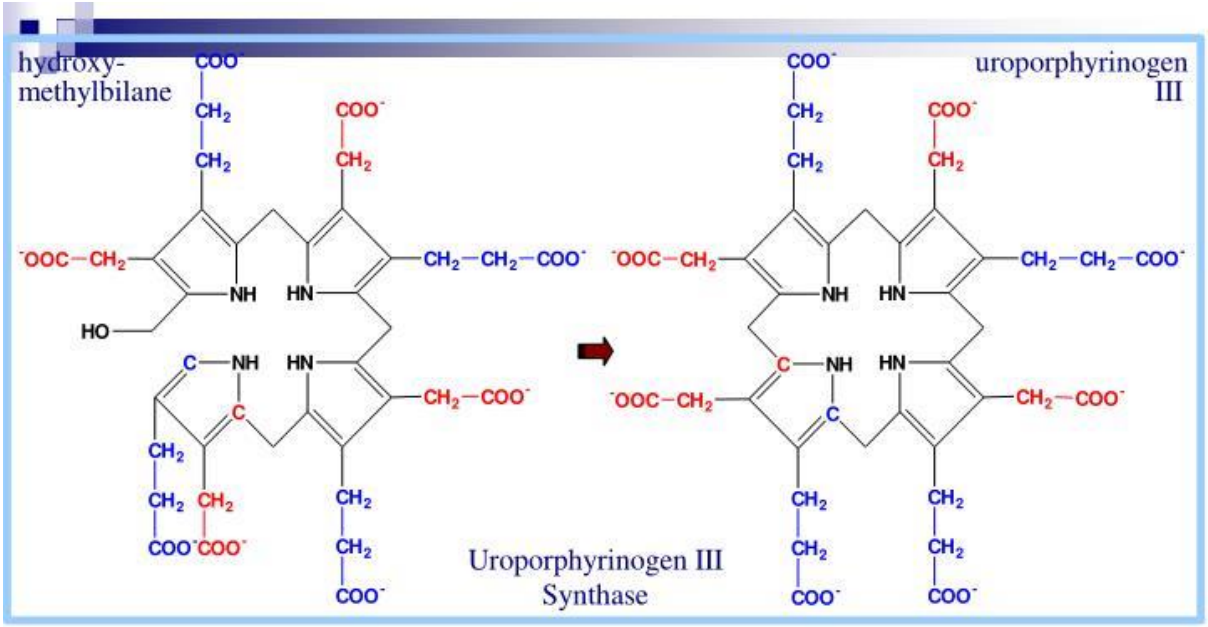
Porphobilinogen (PBG) is the first pathway intermediate that includes a **pyrrole** ring.



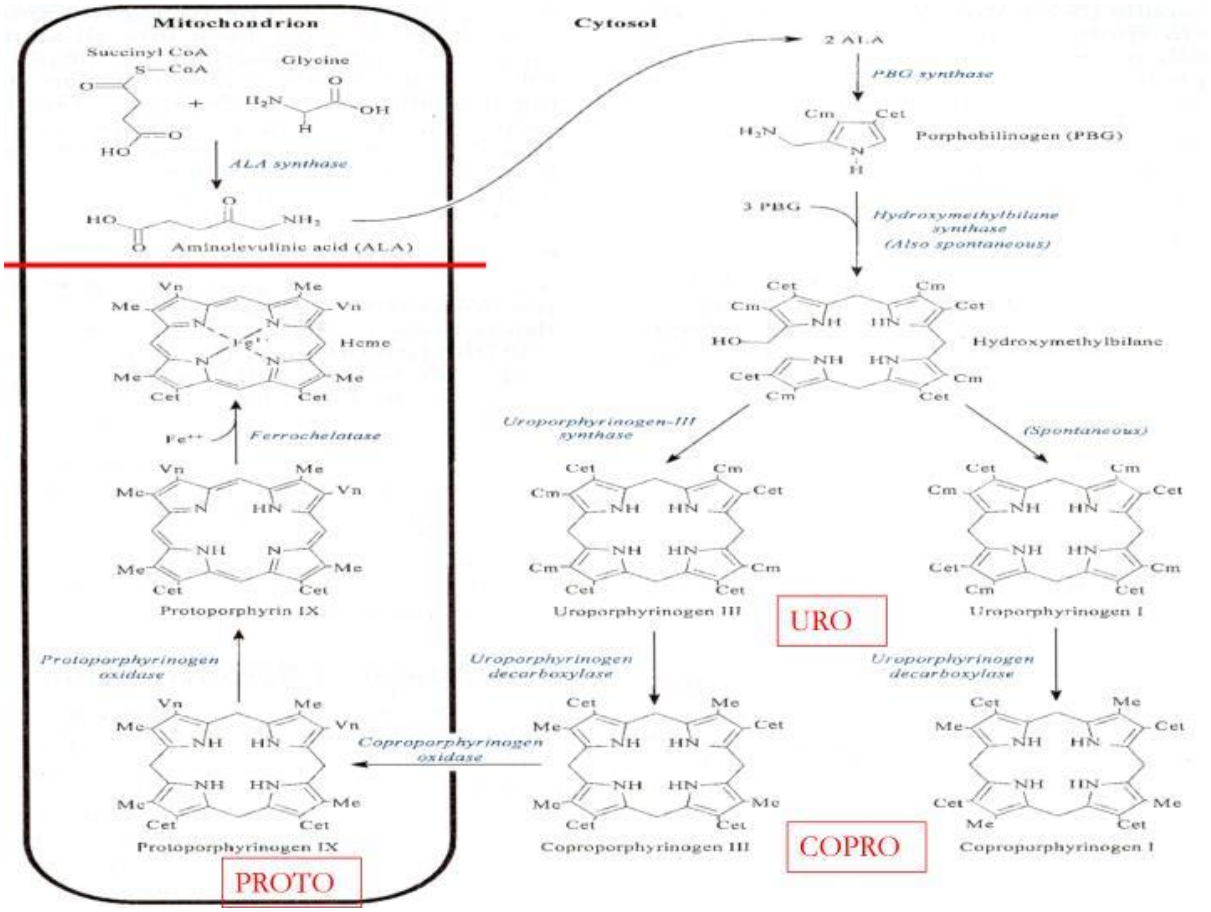
pyrrole

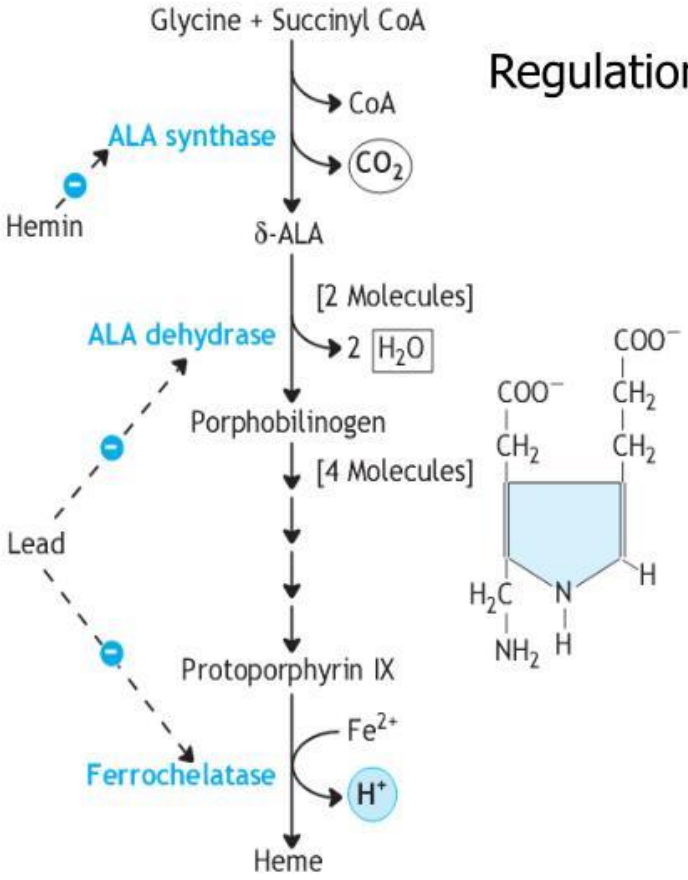
The **porphyrin** ring is formed by condensation of **4** molecules of **porphobilinogen**.

Porphobilinogen Deaminase catalyzes successive PBG **condensations**, initiated in each case by **elimination** of the **amino group**.



Uroporphyrinogen III Synthase converts the linear tetrapyrrole hydroxymethylbilane to the macrocyclic uroporphyrinogen III.





Regulation of heme synthesis

Figure 9–7. Pathway for heme biosynthesis. Regulation by hemin at the key aminolevulinic acid (ALA) synthase-catalyzed step and by lead at two steps is indicated.

Porphyrias

Porphyrias are genetic diseases in which activity of one of the enzymes involved in heme synthesis is decreased (e.g., PBG Synthase, Porphobilinogen Deaminase, etc…).

Symptoms vary depending on

- ★ the enzyme
- ★ the severity of the deficiency
- ★ whether heme synthesis is affected primarily in liver or in developing erythrocytes.

common symptom of Porphyrias

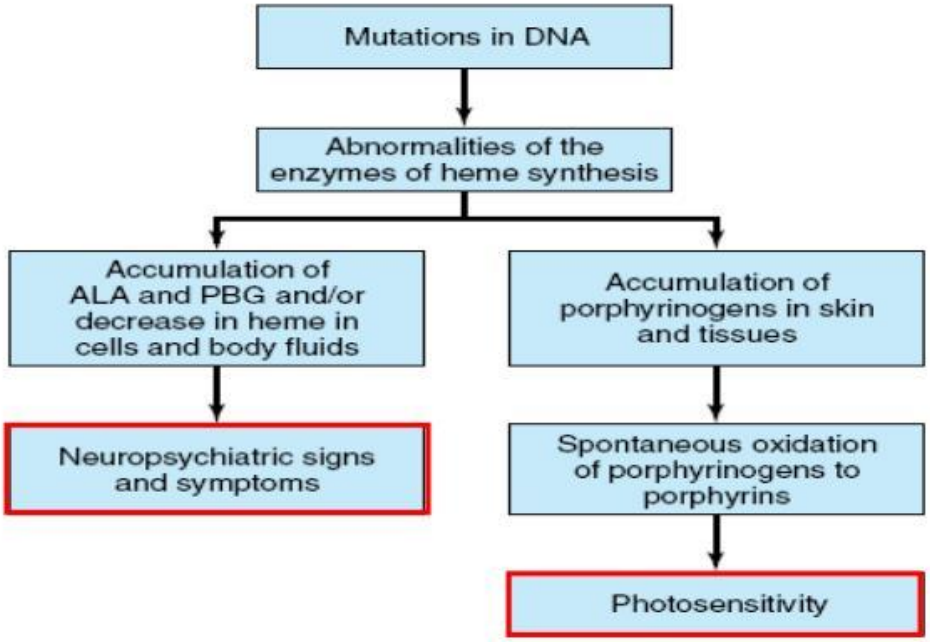


Figure 32–11. Biochemical causes of the major signs and symptoms of the porphyrias.

common symptom of Porphyrias

1. Occasional episodes of severe neurological symptoms are associated with some porphyrias.

- **had acute bouts of abdominal pain and mental confusion**

Permanent nerve damage and even death can result, if not treated promptly.

Elevated δ-aminolevulinic acid (ALA), arising from derepression of ALA Synthase gene transcription, is considered responsible for the neurological symptoms.

2. Photosensitivity is another common symptom.

- formation of superoxide radicals.
- **Skin damage** may result from exposure to light. This is attributable to elevated levels of **light-absorbing pathway intermediates** and their degradation products.

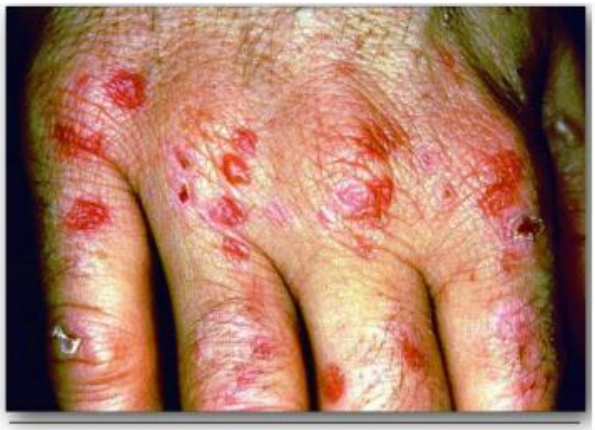


Figure 21.5
Skin eruptions in a patient with porphyria cutanea tarda.

3. porphyrins build up in the body and are excreted in the urine and stool in excessive amounts. When present in very high levels, they cause the urine to have a spectacular port wine color.

Figure 21.6
Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).

King George III - Mad King George

had acute bouts of abdominal pain and mental confusion
- may have been porphyria sufferer
- complicated by all the drugs his doctors gave him

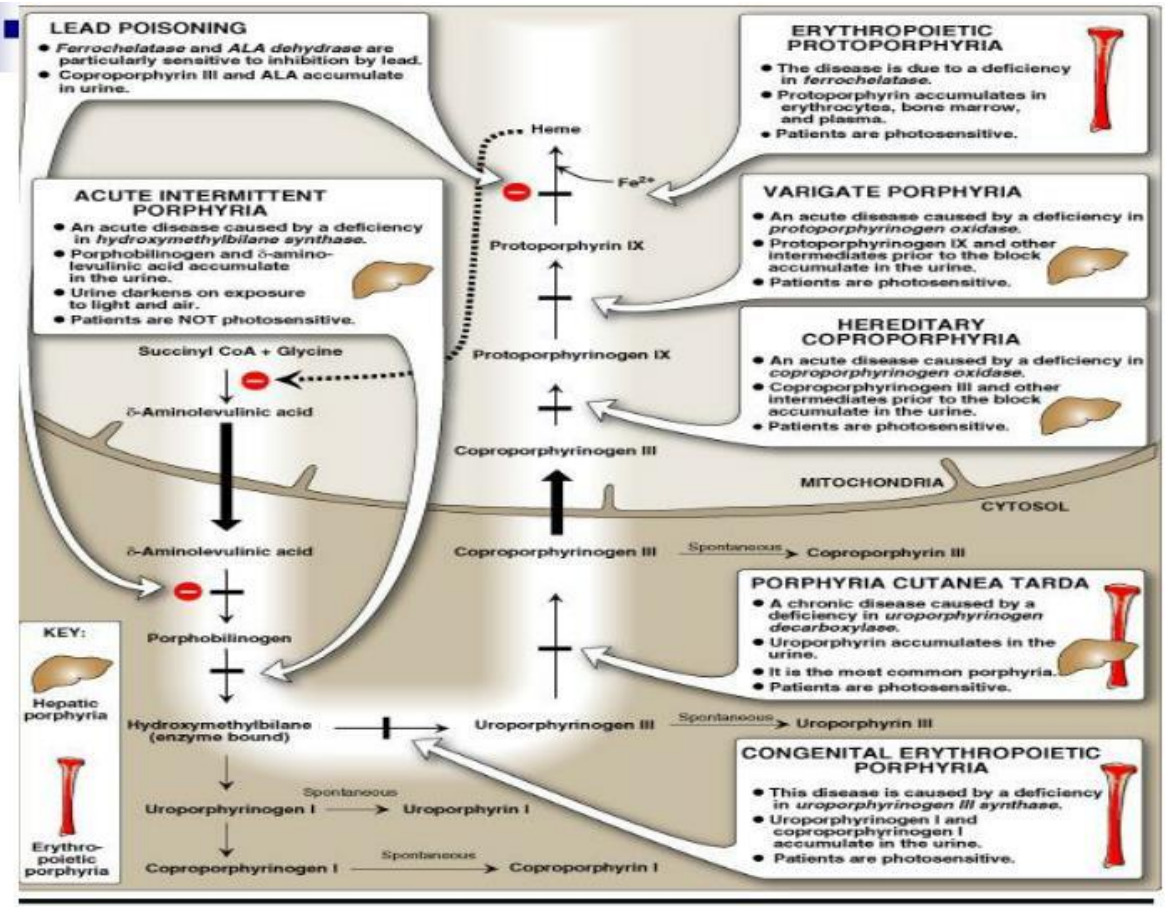
vampires and wherewolves?
- some have put forth that porphyrias misinterpreted in Middle Ages
- consider photosensitivity, red blood (even teeth) hypertrichosis

Acute hepatic porphyrias

Each acute hepatic porphyria is a result of a deficiency of one of the enzymes in the heme biosynthesis pathway. These deficiencies result in an accumulation of the precursors of porphyrins in the liver (delta-aminolevulinic acid, ALA and porphobilinogen, PBG) and also, in the case of variagate porphyria and hereditary coproporphyria, an accumulation of porphyrins resulting in cutaneous manifestations.

- similar symptoms - acute attacks of gastrointestinal pain, neurologic / psychologic, cardiovascular.

When an acute attack is confirmed, urgent treatment with an injection of human hemin and/or perfusion of carbohydrates is required. Management includes the prevention of attacks (by avoiding causal factors) and the protection of skin from the light in cases of cutaneous manifestations.

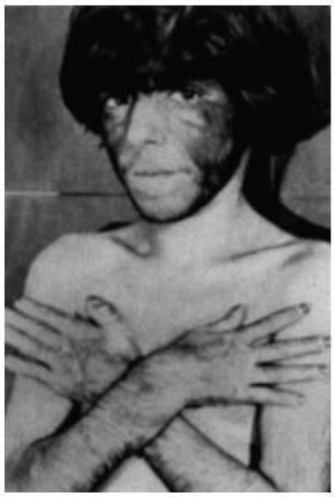


Acquired Porphyrias

- hexochlorobenzene used as a fungicide in Turkey in 1950s
  - thousands of children ate bread from treated wheat

- they acquired porphyria cutanea tarda due to inhibition of uroporphyrinogen decarboxylase

- due to hypertrichosis - referred to locally as the “monkey children”



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 № 17

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

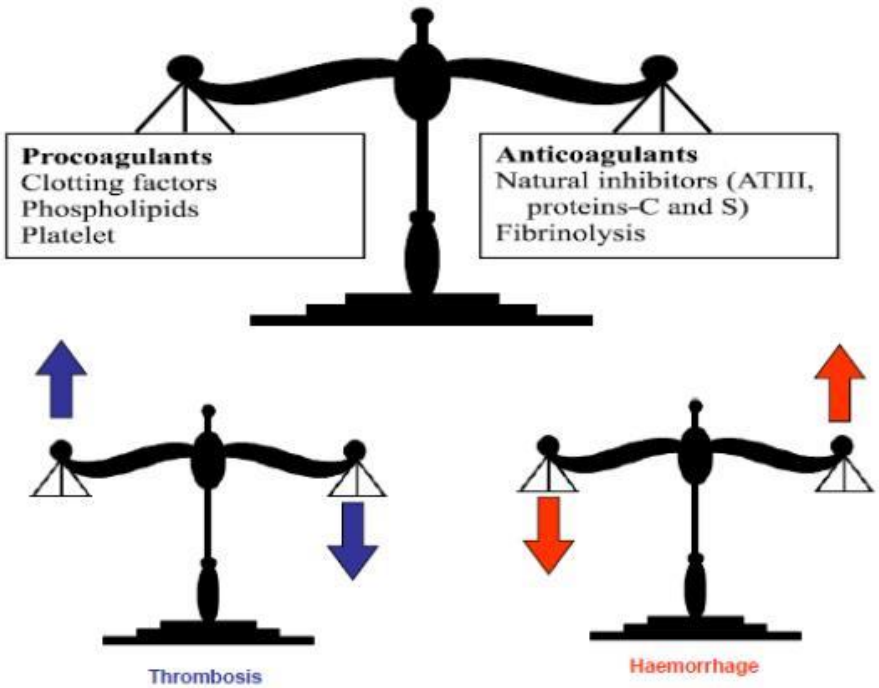
Content of the lecture material

Mechanism of Blood Coagulation

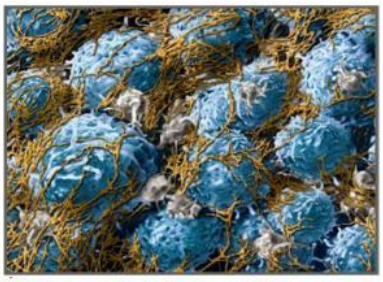
- **A crucial physiological *balance* exists between factors promoting coagulation (procoagulants) and factors inhibiting coagulation (anticoagulants).**

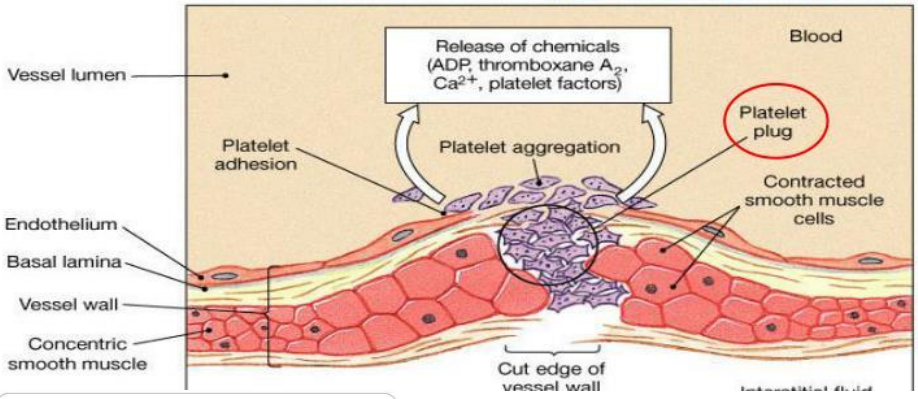
- **Coagulation of blood depends on the *balance* between these two factors.**

- **Disturbances in this *balance* could lead to thrombosis or bleeding**



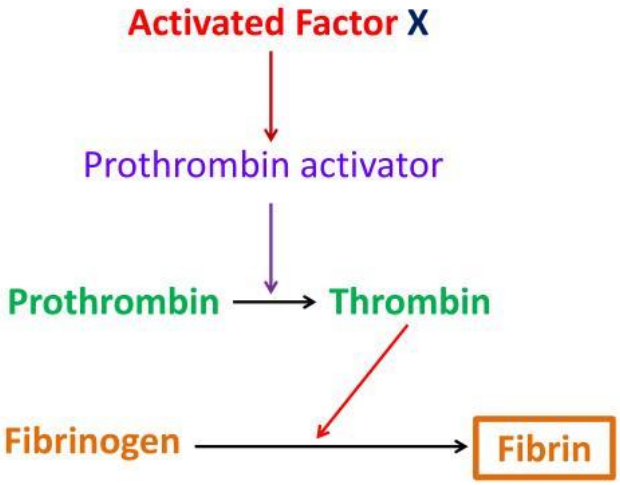
Coagulation: Formation of fibrin meshwork (Threads) to form a CLOT

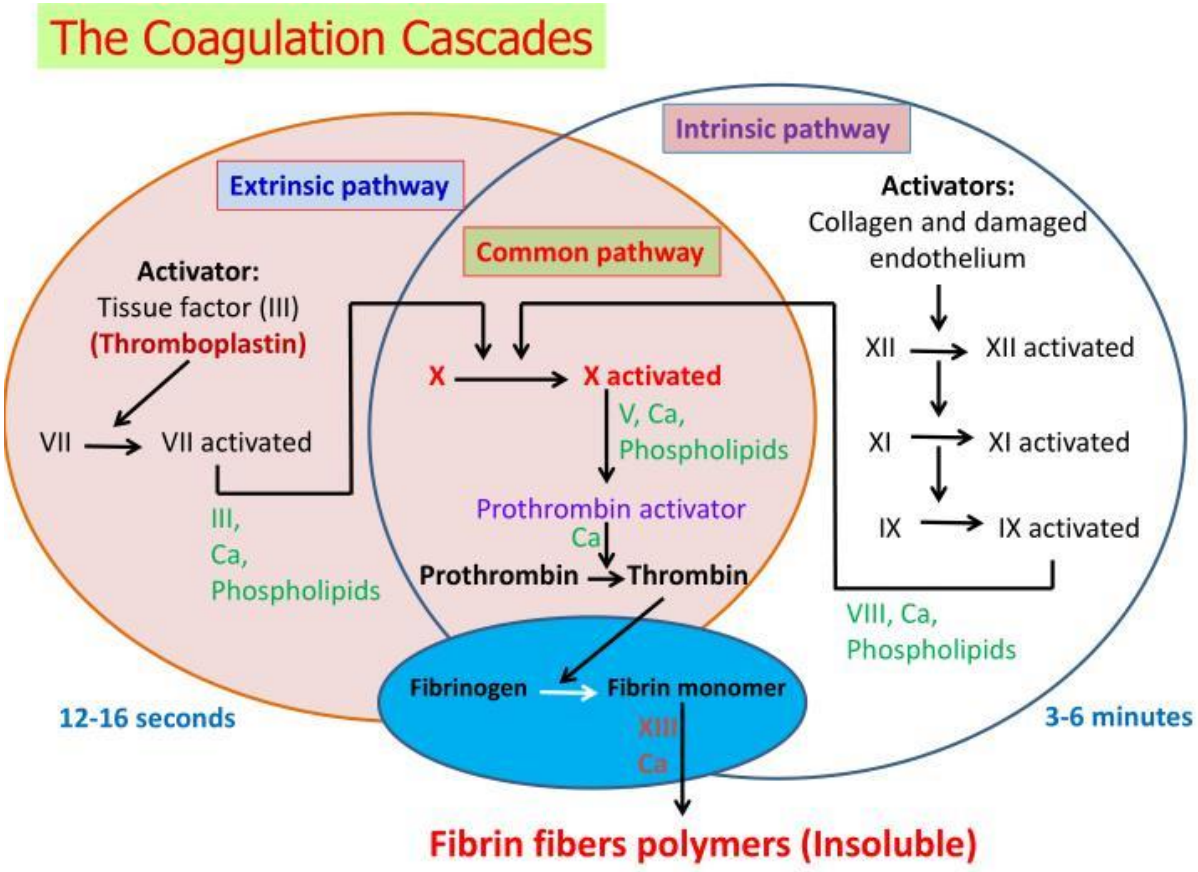




Clotting Factors

Factors	Names
I	Fibrinogen
II	Prothrombin
III	Thromboplastin (tissue factor)
IV	Calcium
V	Labile factor
VII	Stable factor
VIII	Antihemophilic factor A
IX	Antihemophilic factor B
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin stablizing factors


Activated Factor X
Prothrombin activator
Prothrombin
Thrombin
Fibrinogen
Fibrin


The Coagulation Cascades
Intrinsic pathway
Extrinsic pathway
Activators:
Collagen and damaged endothelium
Common pathway
Activator:
Tissue factor (III)
(Thromboplastin)
X
X activated
V, Ca, Phospholipids
XII
XII activated
VII
VII activated
XI
XI activated
III, Ca, Phospholipids
Prothrombin activator
Ca
IX
IX activated
Prothrombin
Thrombin
VIII, Ca, Phospholipids
Fibrinogen
Fibrin monomer
XIII
Ca
12-16 seconds
3-6 minutes
Fibrin fibers polymers (Insoluble)

Thrombin

- Thrombin changes fibrinogen to fibrin
- Thrombin is essential in platelet morphological changes to form primary plug
- Thrombin stimulates platelets to release ADP & thromboxane A2; both stimulate further platelets aggregation
- Activates factor V, VIII, XIII

Blood coagulation
(clot formation)

- A series of biochemical reactions leading to the formation of a blood clot within few seconds after injury
- Prothrombin (inactive thrombin) is activated by a long intrinsic or short extrinsic pathways
- This reaction leads to the activation of thrombin enzyme from inactive form prothrombin
- Thrombin will change fibrinogen (plasma protein) into fibrin (insoluble protein)

Intrinsic pathway

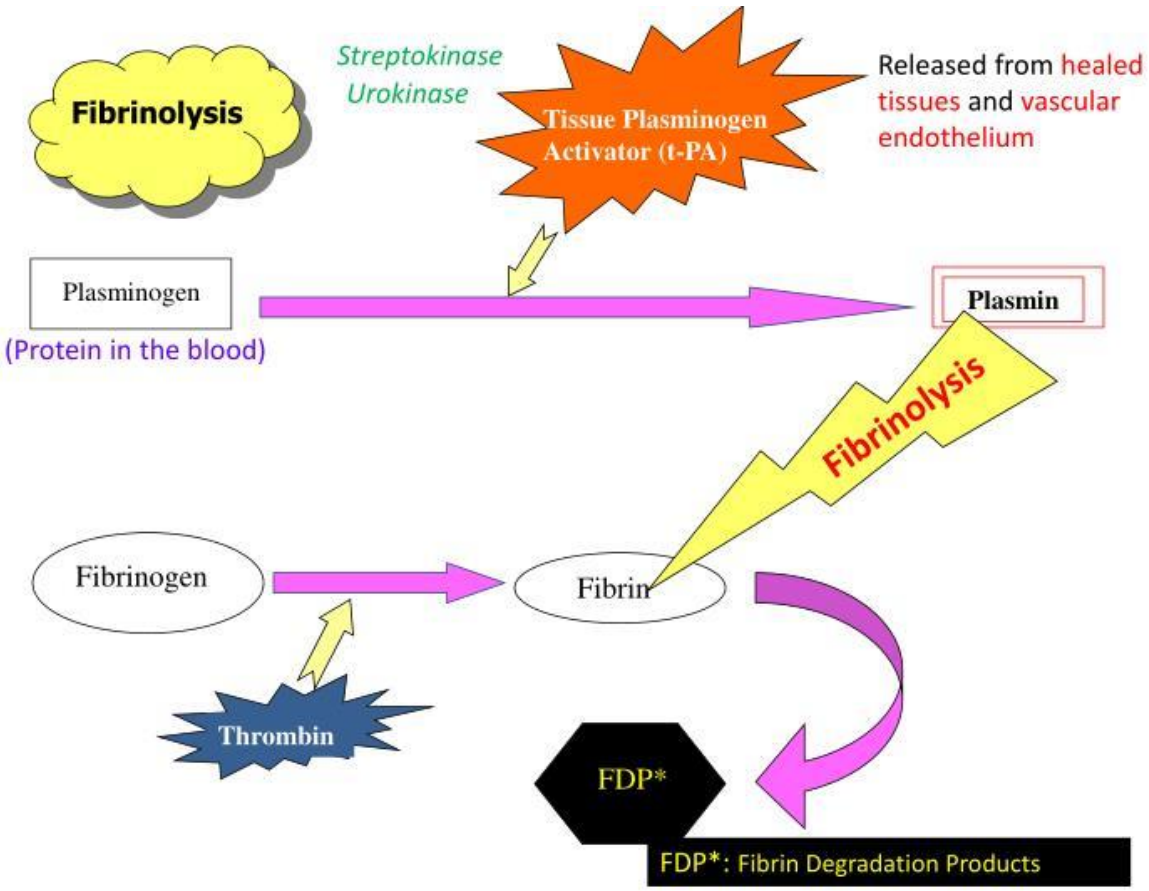
- The trigger is the activation of factor XII by contact with foreign surface, injured blood vessel, and glass.
- Activated factor XII will activate factor XI
- Activated factor Xl will activate IX
- Activated factor IX + factor VIII + platelet phospholipid factor (PF3)+ Ca activate factor X
- Following this step the pathway is common for both intrinsic and extrinsic

Extrinsic pathway

- Triggered by material released from damaged tissues (tissue thromboplastin)
- Tissue thromboplastin + VII + Ca → activate X

Common pathway

- Activated factor X + factor V +PF3 + Ca activate prothrombin activator; a proteolytic enzyme which activates prothrombin.
- Activated prothrombin activates thrombin
- Thrombin acts on fibrinogen and change it into insoluble thread like fibrin.
- Factor XIII + Ca → strong fibrin (strong clot)



Anticoagulants

Anticoagulants for clinical use:

- **Heparin**
  - Commercial, extracted from animals
- **Coumarins**
  - Warfarin, competitive with vitamin K
  - decrease Factors II, VII, IX, X

Prevention of blood coagulation outside the body:

(decrease calcium ion concentration)

- **Oxalate** (precipitation, toxic)
- **Citrate** (deionizer)
- **EDTA** (Chelating agent)

Conditions that cause excessive bleeding

- **Vitamin K Deficiency**
  - Factor II, Factor VII, Factor IX, Factor X require vitamin K for their synthesis
  - Hepatitis, Cirrhosis, acute yellow atrophy AND GI disease

- **Hemophilia**
  - ↑ bleeding tendency.
  - Affects males.
  - 85% due to Factor VIII deficiency (hemophilia A), and 15% due to Factor IX deficiency (hemophilia B).

- **Thrombocytopenia**
  - Very low number of platelets in blood (< 50,000/µl)
  - ***Thrombocytopenia purpura***, hemorrhages throughout all the body tissues
  - ***Idiopathic Thrombocytopenia,*** unknown cause.

Secondary Coagulation Disorders cont'd

- **Thrombocytopenia refers to a decreased number of platelets and is the most common coagulation disorder seen in small animal veterinary practice.**
- The causes are often unknown.
- Infection with certain bacterial, viral, and parasitic agents can result in thrombocytopenia

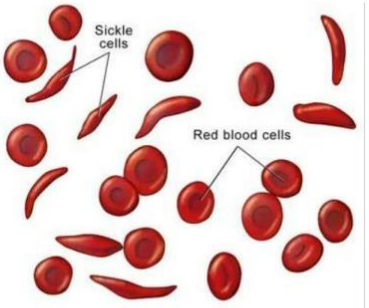
- Thrombocytopenia can also occur as a result of bone marrow depression, which reduces the production of platelets or autoimmune disease that increases the rate of platelet destruction.
- The liver is the site of production of most coagulation factors, any condition that affects liver function can result in a coagulation disorder.

ANEMIA

- Iron-Deficiency Anemia (most common)
- Aplastic Anemia – bone marrow does not produce enough RBC
- Hemorrhagic anemia – due to extreme blood loss
- Pernicious anemia – B12 deficiency
- Sickle Cell Anemia (genetic) - blood cells abnormally shaped

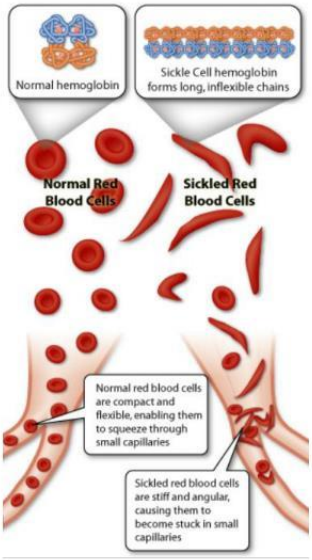
SICKLE CELL ANEMIA

- Genetic Disorder
- Abnormally shaped blood cells
- Parents can be carriers (asymptomatic)



Complications

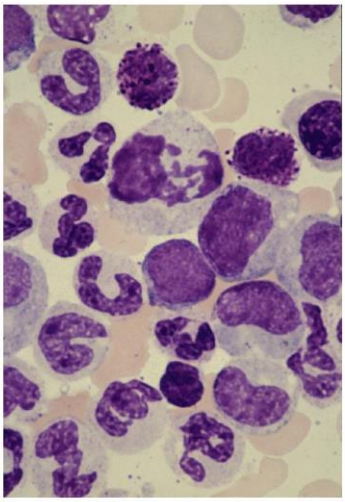
1. Pain
2. Lethargy
3. Lifelong anemia (low red blood count)
4. Organ failure
5. Stroke

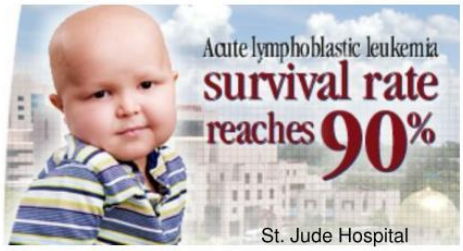


Leukemia

- Type of cancer
- Overproduction of immature white blood cells
- They take the place of RBCs
- Treatable with bone marrow transplants, chemothemotherapy, radiation

Blood Smear of a patient with Leukemia





Leukemia is one of the most common childhood cancers. It occurs when large numbers of abnormal white blood cells fill the bone marrow and sometimes enter the bloodstream.

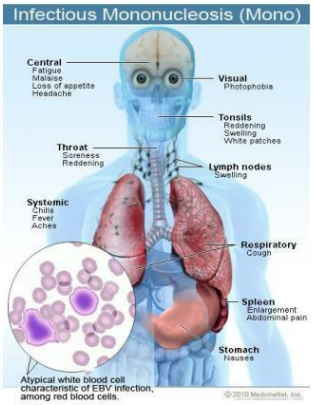
Because these abnormal blood cells are defective, they don't help protect the body against infection the way normal white blood cells do. And because they grow uncontrollably, they take over the bone marrow and interfere with the body's production of other important types of cells in the bloodstream, like **red blood cells** (which carry oxygen) and **platelets** (which help blood to clot).

Infectious mononucleosis

sometimes called "mono" or "the kissing disease," is an infection usually caused by the Epstein-Barr virus (EBV).

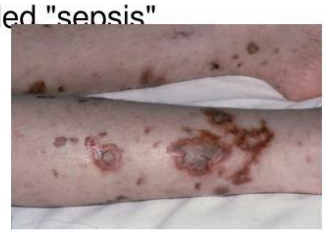
The designation "mononucleosis" refers to an increase in one type of white blood cells (lymphocytes) in the bloodstream
EBV is very common, and many people have been exposed to the virus at some time in childhood.

Article at Medicinenet



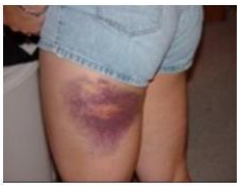
Blood poisoning - Septicemia

- An infection enters the blood stream
- Can be deadly
- Treated with antibiotics
- Also called "sepsis"



Thrombocytopenia

- Low production of Platelets
- Causing bleeding or bruising



A bruise is caused when tiny blood vessels are damaged or broken as the result of a blow to the skin (be it bumping against something or hitting yourself with a hammer).

The raised area of a bump or bruise results from blood leaking from these injured blood vessels into the tissues as well as from the body's response to the injury.

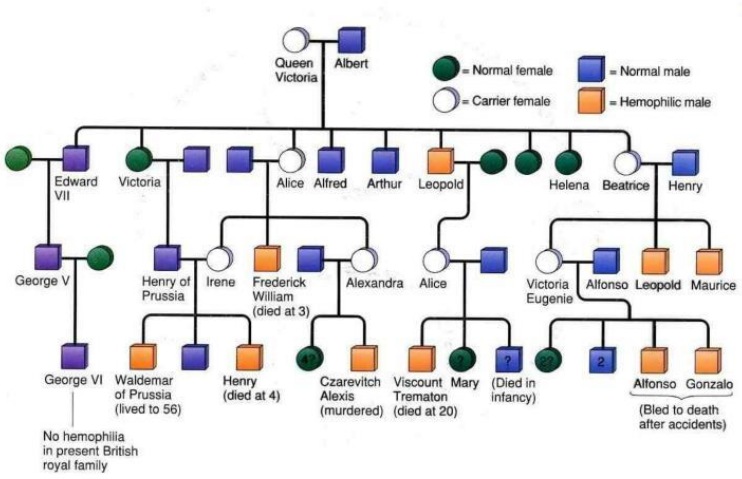
HEMOPHILIA

This disorder causes a failure of the blood to clot

Patients can be treated with blood transfusions that include clotting agents.

Queen Victoria

Carrier for Hemophilia



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