MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV

# BIOLOGICAL AND BIOORGANIC CHEMISTRY

## Workbook for foreign students of specialization "Medicine"

## Part 2: General and Functional Biochemistry

КИЇВСЬКИЙ ВУЗ УНІВЕРСИТЕТ 2019

Compliers:
T. Halenova, O. Savchuk, L. Ostapchenko

Reviewers:
Dr. Sc, professor T. M. Falalyeyeva,
Dr. Sc, K. O. Dvorshchenko

*Approved by Academic Council*
*of Educational Scientific Center "Institute of Biology and Medicine"*
*(protocol № 9 from March 11, 2019)*

"Biological and bioorganic chemistry". Workbook for foreign students of specialization "Medicine". Part 2: : General and functional biochemistry / Compliers: T. Halenova, O. Savchuk, L. Ostapchenko – К : Kyiv University Publishing and Printing Centre, 2019. – 115 p.

# CONTENTS

# Lesson 1

The main topic
**"THE STRUCTURE AND FUNCTIONS OF BIOLOGICAL MOLECULES: checking prior knowledge"**

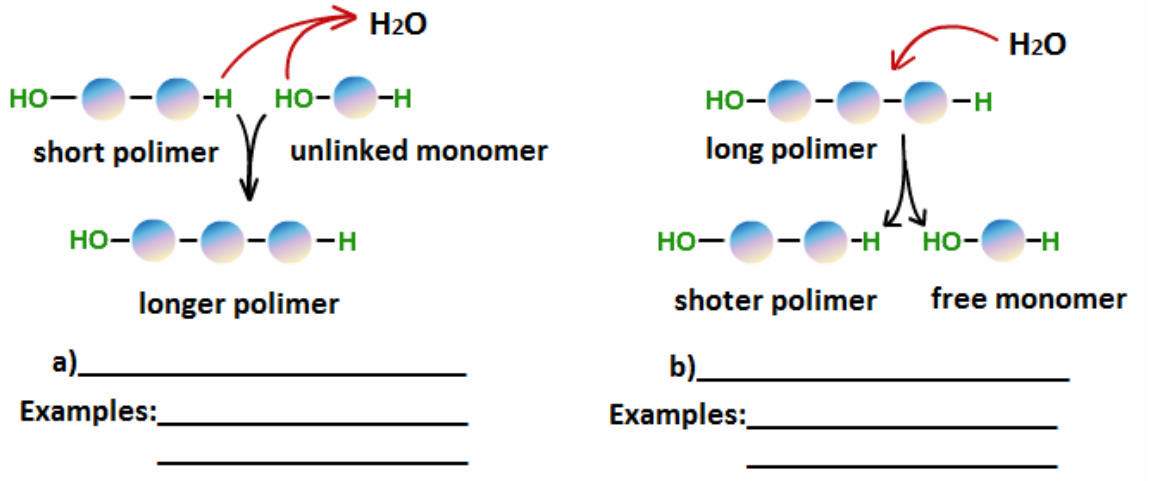
## 1. Introduction to macromolecules

✎ ***Fill in the gaps:***

The most common elements in living organisms are _____, _____, _____, and _____. These four elements constitute about ____% of your body weight. All compounds can be classified in two broad categories: 1)_____ and 2)_____ compounds. Organic compounds are all based on _____ atoms. Carbon can form single bonds with another atom and also bond to other carbon molecules forming double and triple bonds. This allows carbon based molecules to form single and double rings, chains, and branching chains.

Each small organic molecule can be a unit of a large organic molecule called a _____. The smaller single subunits that make up macromolecules are known as _____ and the large organic molecule they form is called a _____. When monomers are joined together the reaction is called _____ _____ or _____ as water is produced when the monomers are bonded together. To break the polymers down again the reaction is called _____.

✎ ***Define the types of following reactions on the picture below and give your own examples for each type:***




✎ ***Define the following terms:***

1. Metabolism: _____
_____
2. Catabolism: _____
_____
3. Anabolism: _____
_____

✎ ***Complete the table of common functional groups in biomolecules:***

| Name of group | General structural formula | Category | Found in |
|---|---|---|---|
| ***hydroxyl*** | | alcohols | |
| ***ether*** | | ethers | |
| ***carbonyl*** | | aldehydes | |
| | | ketones | |
| ***carboxyl*** | | carboxylic acids | |
| | | esters | |
| ***amino*** | | amines | |
| ***amido*** | | amids | |
| ***sulfhydryl*** | | thiols | |
| ***phosphoryl*** *(phosphate)* | | organic phosphates | |

✎ ***Describe the structure a single water molecule and how this leads to its (polar) nature:***

_____
_____
_____
_____
_____

✎ ***In the space below, draw two water molecules attracted to one another by hydrogen bonding. Include labels to show the polarity of the molecules and the hydrogen bond:***

✎ ***Define the following terms:***

1. Hydrophilic compound: _____
_____

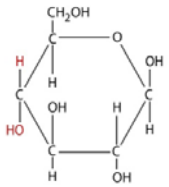
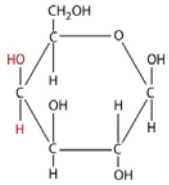
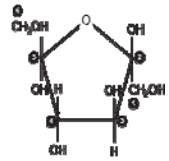
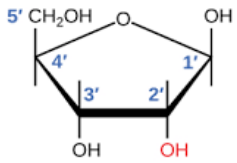
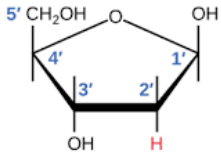
2. Hydrophobic compound: _____
_____

## 2. Carbohydrates

✎ ***State the main functions of carbohydrates in our body:***

1. _____
2. _____
3. _____

✎ ***Complete the table of common monosaccharides:***

| Name | Structural formula | Chemical formula | Class of sugar | Properties and functions |
|---|---|---|---|---|
| ***Glucose*** |  | | | |
| ***Galactose*** |  | | | |
| ***Fructose*** |  | | | |
| ***Ribose*** |  | | | |
| ***Deoxyribose*** |  | | | |

✎ ***Define the following terms:***

1. Isomers: _____

_____

2. Enantiomers: _____

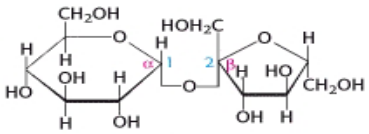
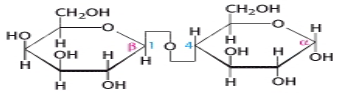
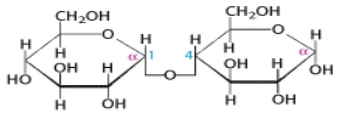
_____

3. Diasteriomers: _____

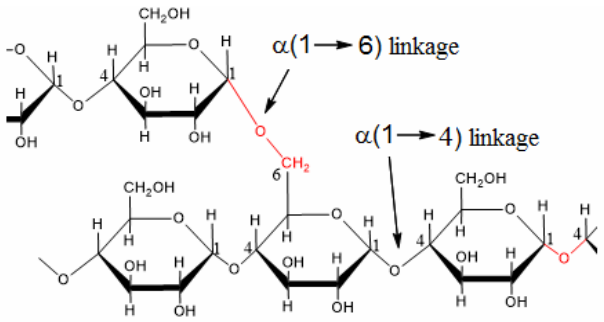
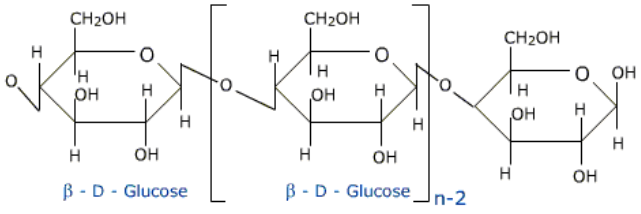
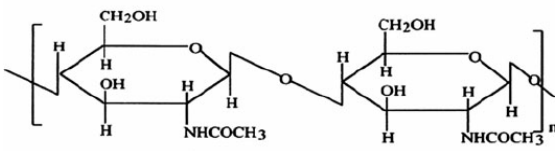
_____

4. Epimers: _____

✎ ***Complete the table of common disaccharides:***

| Name | Structural formula | Monomers | Name of linkage |
|---|---|---|---|
| ***Sucrose*** |  | | |
| ***Lactose*** |  | | |
| ***Maltose*** |  | | |

✎ ***Complete the table of common polysaccharides:***

| Name | Structure | Properties and functions |
|---|---|---|
| ***Glycogen*** |  | |
| ***Starch*** | Its structure is similar to the structure of glycogen (but not so branched):<br>*Amilose:*_____<br>_____<br>*Amilopectine*:_____<br>_____ | |
| ***Cellulose*** |  | |
| ***Chitin*** |  β 1-4, N-acetyl-glucosamine | |

## 3. Lipids

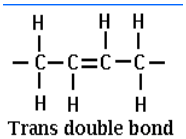
✎ ***State the main functions of lipids in our body:***

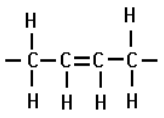
1. _____
2. _____
3. _____
4. _____

✎ ***Complete the table of structure and properties of fatty acids:***

| Type of FAs | | Definition | Structure and properties (fluidity) | Example |
|---|---|---|---|---|
| ***Saturated*** | | | | |
| ***Unsaturated*** | ***Monoun-saturated*** | | | |
| | ***Polyun-saturated*** | | | |

✎ ***Compare the structure and biological significance of cis- and trans-fatty acids:***


Trans double bond


Cis double bond

✎ ***Draw this reaction in the space below to show how the fatty acid(s) molecule attaches to the glycerol backbone:***

✎ ***Complete the table of structure and biological significance of lipids:***

| Type of FAs | Structure | Biological significance | Example |
|---|---|---|---|
| ***Triacylglycerols*** | | | |
| ***Glicerophospholipids*** | | | |
| ***Sphingolipids*** | | | |
| ***Steroids*** | | | |

## 4. Proteins

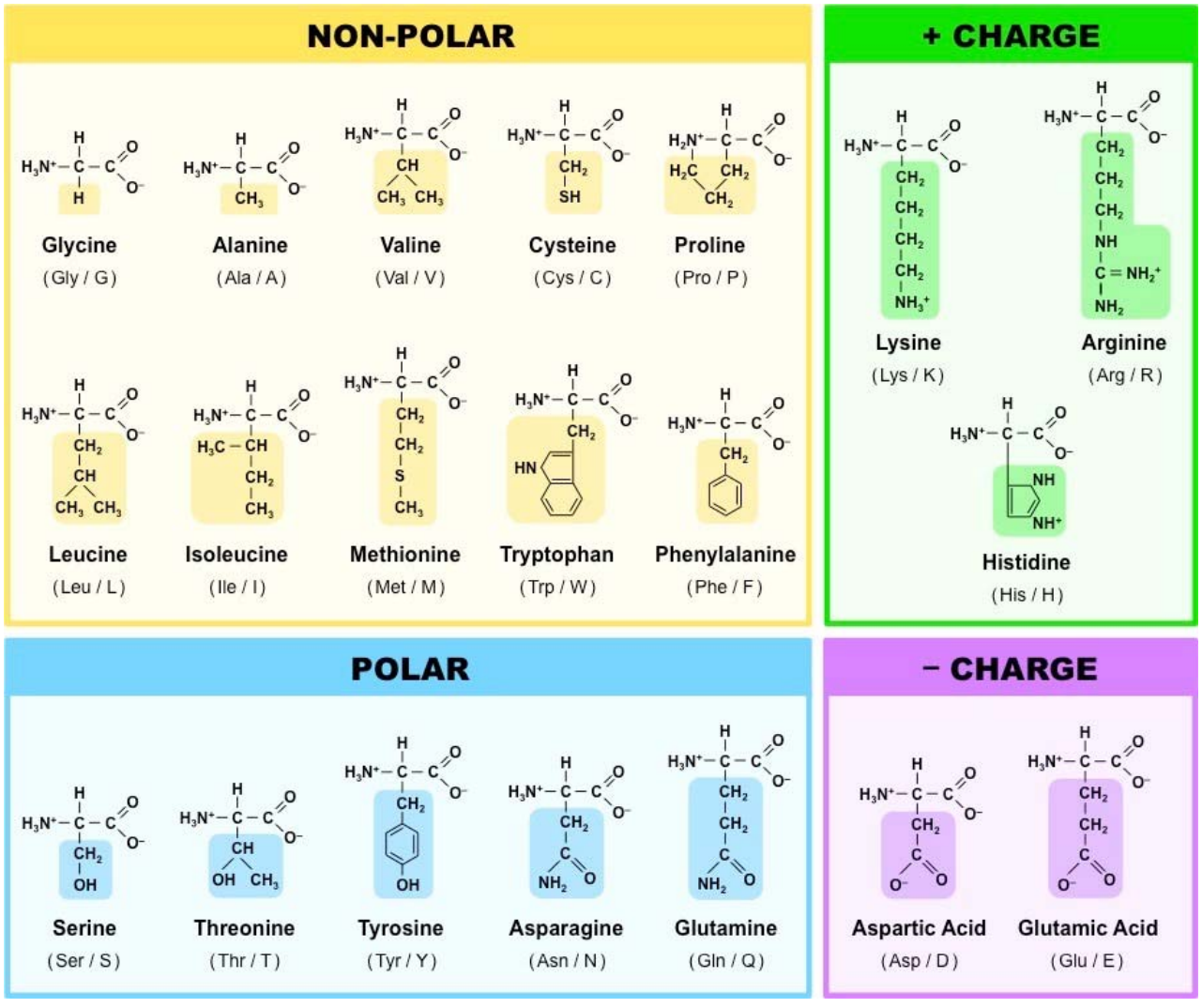
✎ ***State the main functions of proteins in our body (with the examples):***

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____

✎ ***Draw the basic structure of amino acid in the space below. Label the different groups:***

***1.*** _____
***2.*** _____
***3.*** _____
***4.*** _____

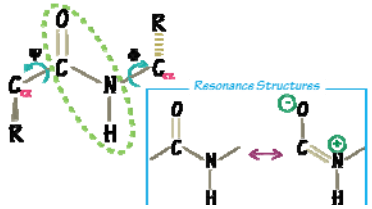
**The structure of naturally occur amino acids**



✎ *Write the reaction for the formation of peptide bond between:*

| *1) alanine and serine* | *2) alanine and proline* |
|---|---|
| | |

✎ *Describe the properties of peptide bond between:*




✎ *Explain the four levels of protein structure:*

| | *Primary* | *Secondary* | | *Tertiary* | *Quaternary* |
|---|---|---|---|---|---|
| *What is it?* | | | | | |
| *Simple drawing* | | *alpha(α)-helix* | *beta(β)-pleated sheet* | | |
| *Which type of bonds stabilize its structure?* | | | | | |

✎ *Define the term* **"DENATURATION":**

## 5. Nucleic Acids

✎ ***State the main functions of nucleic acids in our body (with the examples):***

1._____
2._____
3._____
4._____
5._____

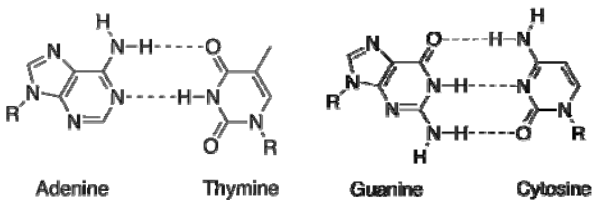
✎ ***Draw the basic structure of a monomer of nucleic acid in the space below. Label the different parts:***

***1.*** _____
***2.*** _____
***3.*** _____

✎ ***Explain the biological significance of some free nucleotides:***

✎ ***Write the reaction between two nucleotides. Label the phosphodiester bond and 3'/5'-ends:***

✎ ***Define the term* "COMPLEMENTARY BASE PAIRS":**



✎ ***Compare the structure and functions of different types of nucleic acids:***

| | ***DNA*** | ***RNA*** | | |
|---|---|---|---|---|
| ***Sugar*** | | | | |
| ***Complemen-tary base*** | | | | |
| ***Secondary structure*** | | | | |
| ***Functions*** | | ***mRNA*** | ***rRNA*** | ***tRNA*** |
| | | | | |

✎ ***Explain the Chromosome Structure:***

✎ ***Define the following terms:***

1. Replication: _____
_____

1. Gene: _____
_____

2. Introns/Exons: _____
_____

5. Transcription: _____
_____

3. Splicing: _____
_____

6. Translation: _____
_____

4. Codon: _____
_____

6. Anticodon: _____

✎ ***Draw the main steps of protein synthesis:***

## Lesson 2

The main topic
**“ENZYMES: STRUCTURE AND FUNCTIONS”**

📖 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Composition and structure of enzymes;
⇨ Mechanism of enzyme action;
⇨ Factors that affect the rate of enzyme action;
⇨ Enzyme specificity;
⇨ Enzyme kinetics ( Km & Vmax );
⇨ Regulation of enzyme activity;
⇨ Enzyme inhibition;
⇨ Classes of enzymes;
⇨ Clinical uses of enzymes in diagnosis and prognosis of different diseases.

✎ ***Summarize in the following table similarities and differences between Enzymes and Catalysts:***

| *Similarities* | *Differences* |
|---|---|
| | |

✎ ***Describe the structure of enzymes:***

| *Simple enzymes* | *Conjugated enzymes* |
|---|---|
| | |

✎ ***Compare two different natures of cofactors:***

| *Coenzyme* | *Prosthetic group* |
|---|---|
| | |

✎ *Define the term* **"ACTIVE SITE":**

_____

_____

***Catalytic site:*** _____

***Binding site:*** _____

✎ ***State the function of polar regions of amino acids on the active site of the enzyme:***

_____

✎ ***Complete the table of enzyme specificity:***

| *Type* | *Definition (reaction type)* | *Example* |
|---|---|---|
| ***Absolute*** | | |
| ***Group*** | | |
| ***Linkage*** | | |
| ***Optical or stereo*** | | |

✎ ***Compare two different theories of enzyme substrate binding:***

| *The lock and key hypothesis* | *The induced-fit hypothesis* |
|---|---|
| | |

✎ ***Completing the picture below, describe the main four steps of enzyme reaction (label the components of enzyme reaction 1-5):***

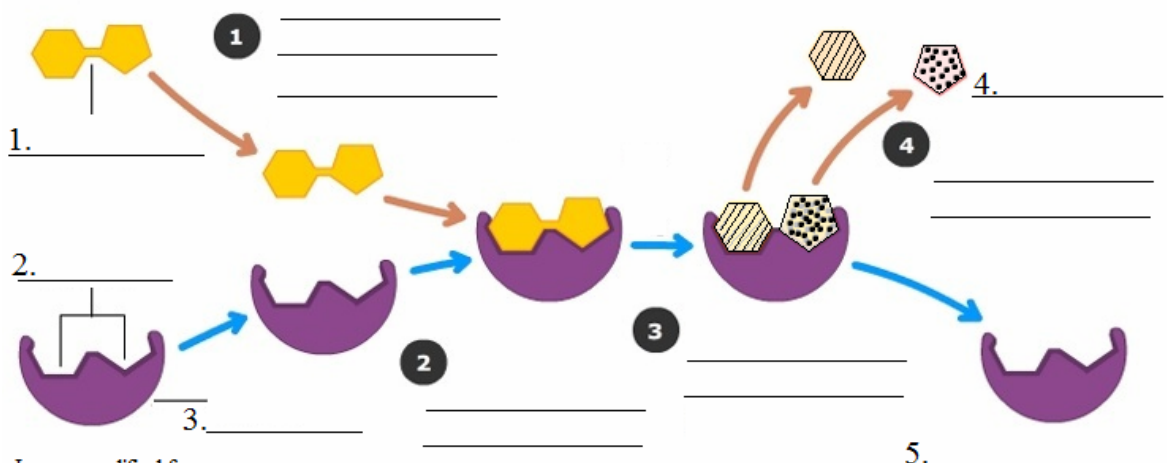
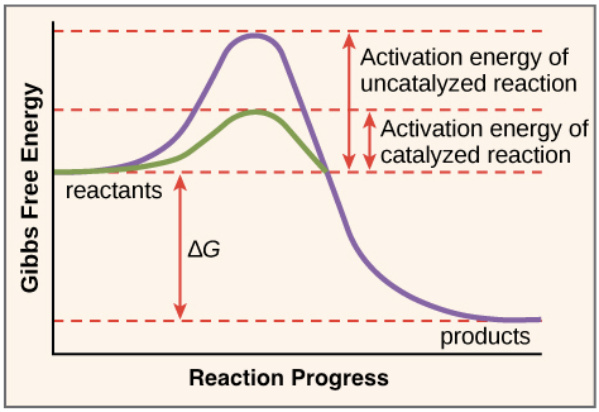
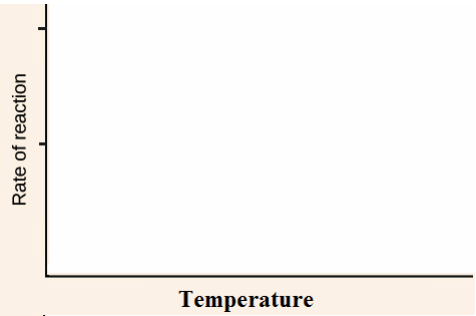



Image modified from:

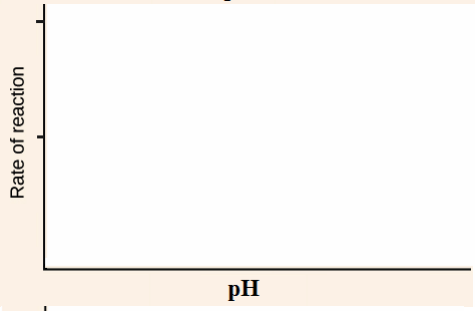
✎ ***Explain the mechanism of enzyme action:***



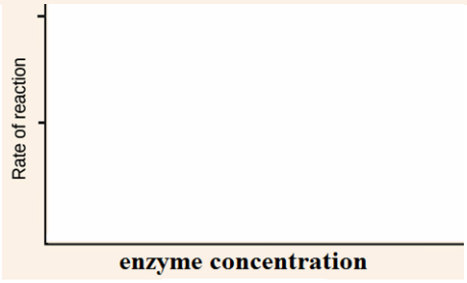

✎ ***Explain the effects of temperature, pH as well as enzyme and substrate concentration on the rate of an enzyme-controlled reaction. Draw a sample graph in the space on the left and then explain/describe on the right:***



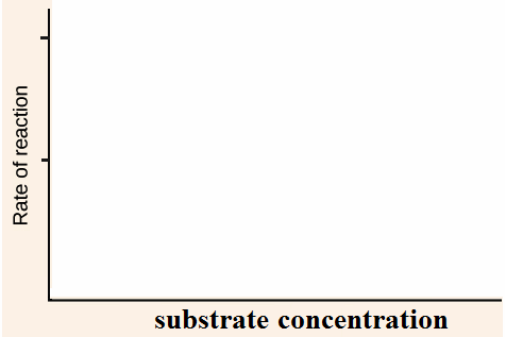

***Temperature***




***pH***




***Enzyme concentration***




***Substrate concentration***

✎ ***State the different ways of regulation of enzyme activity:***

1._____
2._____
3._____
4._____

✎ ***Define the term* "ALOSTERIC SITE":**

_____

✎ ***Compare the different types of enzyme inhibitors:***

| ***Irreversible inhibitors*** | ***Reversible inhibitors*** |
|---|---|
| | *1. Competitive reversible inhibitors*<br><br>*2. Non-competitive reversible inhibitors*<br><br>*3. Uncompetitive reversible inhibitors* |

✎ ***Complete the table of enzyme classification:***

| ***Class*** | ***The simple schema of reaction*** | ***Common examples*** |
|---|---|---|
| ***1.*** | | |
| ***2.*** | | |
| ***3.*** | | |
| ***4.*** | | |
| ***5.*** | | |
| ***6.*** | | |

## Lesson 3

The main topic
**“VITAMINS: STRUCTURE AND BIOLOGICAL SIGNIFICANCE”**

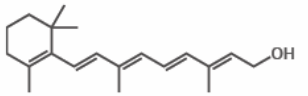
📖 ***Follow this plan at home to prepare for classroom discussion:***

⇨ The general information about vitamins;
⇨ Classification of the vitamins;
⇨ The role of water-soluble vitamins in the metabolism of humans;
⇨ The biological significance of fat-soluble vitamins;
⇨ Vitamin-like substances;
⇨ Health effects of vitamins and antivitamins.

✎ ***Complete the table of coenzyme forms of water-soluble vitamins:***

| *Vitamin* | *Coenzyme (abbreviation)* | *Group transferred* | *Enzyme where used as prosthetic group* |
|---|---|---|---|
| ***B1 Thiamine*** | | | |
| ***B2 Riboflavin*** | | | |
| ***B3 (PP) Niacin*** | | | |
| ***B5 Pantothenic acid*** | | | |
| ***B6 Pyridoxine*** | | | |
| ***B7 (H) Biotin*** | | | |
| ***B9 Folic acid*** | | | |
| ***B12 Cobalamin*** | | | |

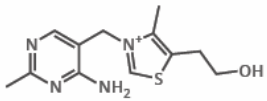
## VITAMIN A



**RETINOL**
*active form in mammalian tissues*

Important for eyesight. Also strengthens immune system and keeps skin and linings of parts of the body healthy.

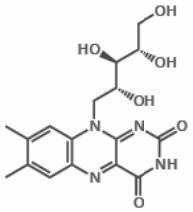
## VITAMIN B1



**THIAMIN**
*can also occur in pyrophosphate ester form*

Used to keep nerves & muscle tissue healthy. Also important for processing of carbohydrates and some proteins.

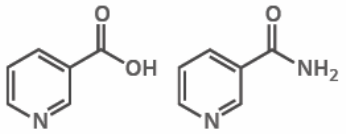
## VITAMIN B2



**RIBOFLAVIN**
*excess turns urine bright yellow*

Important for body growth, red blood cell production, and keeping the eyes healthy. Also helps processing of carbohydrates.

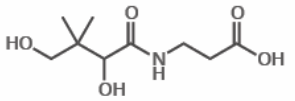
## VITAMIN B3



**NICOTINIC ACID** **NICOTINEAMIDE**
*niacin is collective name for these compounds*

Helps with digestion and digestive system health. Also helps with the processing of carbohydrates.

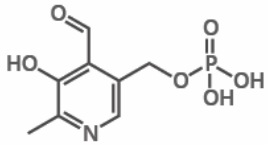
## VITAMIN B5



**PANTOTHENIC ACID**
*also occurs in pyrophosphate ester form*

Important for manufacturing red blood cells and maintaining a healthy digestive system. Also helps process carbohydrates.

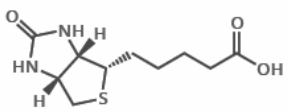
## VITAMIN B6



**PYRIDOXAL PHOSPHATE**
*active form in mammalian tissues*

Helps make some brain chemicals; needed for normal brain function. Also helps make red blood cells and immune system cells.

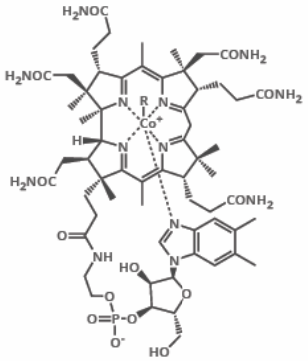
## VITAMIN B7



**BIOTIN**
*produced by intestinal bacteria*

Needed for metabolism of various compounds. Often recommended for strengthening hair, but evidence is variable.

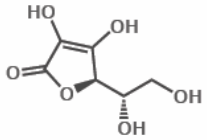
## VITAMIN B12



**COBALAMIN**
*usually contains CN as the R group*

Important for the nervous system, for making red blood cells, and helps in the production of DNA and RNA.

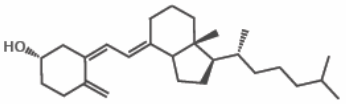
## VITAMIN C



**ASCORBIC ACID**
*deficiency can cause scurvy*

Important for a healthy immune system; helps produce collagen, used to make skin and other tissues. Also helps wound healing.

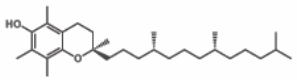
## VITAMIN D



**CHOLECALCIFEROL**
*natural form; different form used in supplements*

Important for bone health and maintaining the immune system function. May also have a preventative role in cancers.

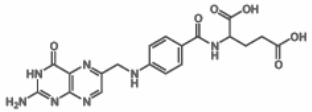
## VITAMIN E



**ALPHA-TOCOPHEROL**
*group includes tocopherols & tocotrienols*

An antioxidant that helps prevent damage to cells and may have a preventative role in cancer. Also helps make red blood cells.

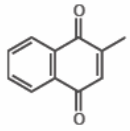
## VITAMIN B9



**FOLIC ACID**
*found as tetrahydrofolate in food*

Important for brain function & mental health. Aids production of DNA & RNA. Important when tissues are growing quickly.

## VITAMIN K



**MENADIONE**
*all K vitamins are menadione or derivatives*

Helps blood clot properly, & plays a key role in bone health. Newborns receive vitamin K injections to prevent bleeding.

## Key

**Vitamins can be divided broadly into two classes.**

**WATER-SOLUBLE VITAMINS**
These vitamins are not stored in the body. As such, generally, they are required more frequently than the fat-soluble vitamins.

**FAT-SOLUBLE VITAMINS**
These vitamins are stored in the liver and fatty tissues until required. As such, they can be harmful if too much is taken in.

✎ ***Complete the table of vitamin functions and manifestations of hypo- and avitaminoses:***

| *Vitamin* | *Function* | *Deficiency* | *Signs and symptoms* |
|---|---|---|---|
| ***B1 Thiamine*** | | | |
| ***B2 Riboflavin*** | | | |
| ***B3 (PP) Niacin*** | | | |
| ***B5 Pantothenic acid*** | | | |
| ***B6 Pyridoxine*** | | | |
| ***B7 (H) Biotin*** | | | |

| *Vitamin* | *Function* | *Deficiency* | *Signs and symptoms* |
|---|---|---|---|
| ***B9 Folic acid*** | | | |
| ***B12 Cobalamin*** | | | |
| ***C ascorbic acid*** | | | |
| ***A Retinol Retinal Retinoic acid*** | | | |
| ***D Cholecalciferol Ergocalciferol*** | | | |
| ***K Phylloquinin*** | | | |
| ***E б-Tocopherol*** | | | |

**Questions from KROK-1**

1. A 10-year-old girl has a history of repeated acute respiratory viral infection. After recovering she presents with multiple petechial hemorrhages on the sites of friction from clothing rubbing the skin. What kind of hypovitaminosis has this girl?

A. *C*
B. $B_6$
C. $B_1$
D. *A*
E. $B_2$

2. A doctor recommends a patient with duodenal ulcer to drink cabbage and potato juice after the therapy course. Which substances contained in these vegetables help to heal and prevent the ulcers?

A. Vitamin *U*
B. Pantothenic acid
C. Vitamin *C*
D. Vitamin $B_1$
E. Vitamin *K*

3. A patient has an increased pyruvate concentration in blood, most of it is excreted with the urine. What kind of avitaminosis has this patient?

A. $B_1$
*B.* *E*
C. $B_3$
D. $B_6$
E. $B_2$

4. Vitamin $B_1$ deficiency causes disturbance of oxidative decarboxylation of $\alpha$-ketoglutaric acid. This leads to the impaired synthesis of the following coenzyme:

A. Thiamine pyrophosphate
B. Nicotinamide adenine dinucleotide
C. Flavine adenine dinucleotide
D. Lipoic acid
E. Coenzyme A

5. Examination of a child who hasn't got fresh fruit and vegetables during winter revealed numerous subcutaneous hemorrhages, gingivitis, carious cavities in teeth. What vitamin combination should be prescribed in this case?

A. Ascorbic acid and rutin
B. Thiamine and pyridoxine
C. Folic acid and cobalamin
D. Riboflavin and nicotinamide
E. Calciferol and ascorbic acid

6. Vitamin A together with specific cytoreceptors penetrates through the nuclear membranes, induces transcription processes that stimulate growth and differentiation of cells. This biological function is realized by the following form of vitamin A:

A. Trans-retinoic acid
B. Trans-retinal
C. Cis-retinal
D. Retinol
E. Carotin

7. Blood test of a patient suffering from atrophic gastritis gave the following results: RBCs - 2*,* 0 · $10^{12}$*/l*, Hb- 87 g/l, colour index - 1,3, WBCs - 4*,* 0 · $10^9$*/l*, thrombocytes - 180 $10^9$*/l*. Anaemia migh have been caused by the following substance deficiency:

A. Vitamin $B_{12}$
*B.* Vitamin *A*
*C.* Vitamin *K*
D. Iron
E. Zinc

8. A 64 year old woman has impairment of twilight vision (hemeralopy). What vitamin should be recommended in the first place?

A. *A*
B. $B_2$
C. *E*
D. *C*
E. $B_6$

9. A patient diagnosed with focal tuberculosis of the upper lobe of the right lung had been taking isoniazid as a part of combination therapy. After some time, the patient reported of muscle weakness, decreased skin sensitivity, blurred vision, impaired motor coordination. Which vitamin preparation should be used to address these phenomena?

A. Vitamin $B_6$
*B.* Vitamin *A*
*C.* Vitamin *D*
D. Vitamin $B_{12}$
*E.* Vitamin *C*

10. A number of diseases can be diagnosed by evaluating activity of blood transaminases. What vitamin is one of cofactors of these enzymes?

A. $B_6$
B. $B_2$
C. $B_1$
D. $B_8$
E. $B_5$

11. A 20-year-old male patient complains of general weakness, rapid fatigability, irritability, decreased performance, bleeding gums, petechiae

on the skin. What vitamin deficiency may be a cause of these changes?

A. Ascorbic acid
B. Riboflavin
C. Thiamine
D. Retinol
E. Folic acid

12. Malaria is treated with structural analogs of vitamin $B_2$ (riboflavin). These drugs disrupt the synthesis of the following enzymes in plasmodium:

A. FAD-dependent dehydrogenase
B. Cytochrome oxidase
C. Peptidase
D. NAD-dependent dehydrogenase
E. Aminotransferase

13. It has been found out that one of pesticide components is sodium arsenate that blocks lipoic acid. Which enzyme activity is impaired by this pesticide?

A. Pyruvate dehydrogenase complex
B. Microsomal oxidation
C. Methemoglobin reductase
D. Glutathione peroxidase
E. Glutathione reductase

14. Steatosis is caused by the accumulation of triacylglycerols in hepatocytes. One of the mechanisms of this disease development is a decrease in the utilization of VLDL neutral fat. What lipotropics prevent the development of steatosis?

A. Methionine, $B_6$, $B_{12}$
B. Arginine, $B_2$, $B_3$
C. Alanine, $B_1$, $PP$
D. Valine, $B_3$, $B_2$
E. Isoleucine, $B_1$, $B_2$

15. A patient complains of photoreception disorder and frequent acute viral diseases. He has been prescribed a vitamin that affects photoreception processes by producing rhodopsin, the photosensitive pigment. What vitamin is it?

A. Retinol acetate
B. Tocopherol acetate
C. Pyridoxine hydrochloride
D. Cyanocobalamin
E. Thiamine

16. During regular check-up a child is detected with interrupted mineralization of bones. What vitamin deficiency can be the cause?

A. Calciferol
B. Cobalamin
C. TocopherolI
D. Folic acid
E. Riboflavin

17. A patient, who has been suffering for a long time from intestine disbacteriosis, has increased hemorrhaging caused by disruption of posttranslational modification of blood-coagulation factors II, VII, IX, and X in the liver. What vitamin deficiency is the cause of this condition?

A. K
B. P
C. $B_{12}$
D. C
E. $B_9$

18. After an extended treatment with sulfonamides a patient has developed macrocytic anemia. Production of active forms of the following vitamin is disrupted in such a condition:

A. Folic acid
B. Pyridoxine
C. Thiamine
D. Cyanocobalamin
E. Riboflavin

19. Coenzyme A participates in numerous important metabolic reactions. It is a derivative of the following vitamin:

A. Pantothenic acid
B. Calciferol
C. Thiamine
D. Niacin
E. Ubiquinone

20. An infant, who was on synthetic formula feeding, developed signs of vitamin *B*1 deficiency. What reactions does this vitamin take part in?

A. Keto acid oxidative decarboxylation
B. Amino acids transamination
C. Amino acids decarboxylation
D. Proline hydroxylation
E. Redox reactions

# Lesson 4

The main topics

## "ANABOLIC AND CATABOLIC PROCESSES IN HUMANS. KREBS CYCLE. GENERAL BASES OF BIOENERGETICS"

📖 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Common stages of intracellular catabolism of biomolecules: proteins, carbohydrates, and lipids;
⇨ Structure and role of ATP;
⇨ TCA cycle: localization, sequence of enzymatic reactions, importance in metabolism;
⇨ Energy balance of TCA cycle;
⇨ The modern theory of biological oxidation;
⇨ Molecular complexes of mitochondrial inner membranes;
⇨ Oxidative phosphorylation: mitochondrial ATP synthase;
⇨ Formation of final products of metabolism - water and carbon dioxide;
⇨ Inhibitors of tissue respiration;
⇨ Uncouplers of oxidative phosphorylation and tissue respiration.

✎ ***Define the following terms:***

1. Homeostasis:

_____
_____

2. Metabolic pathways:

_____
_____

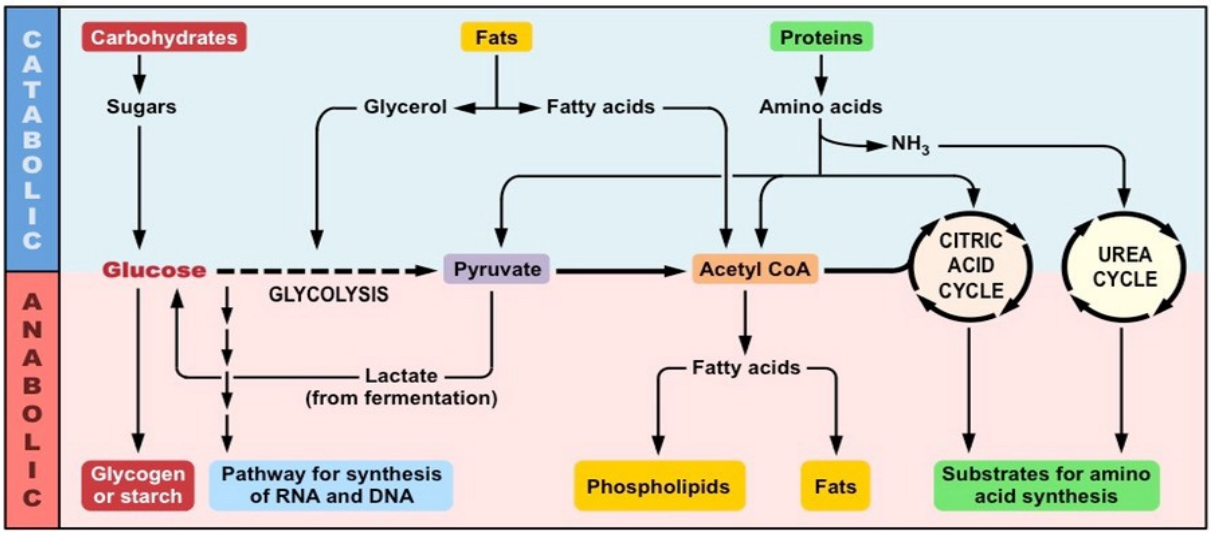
Anabolic pathways: _____

Catabolic pathway: _____

3. Metabolites:

_____
_____

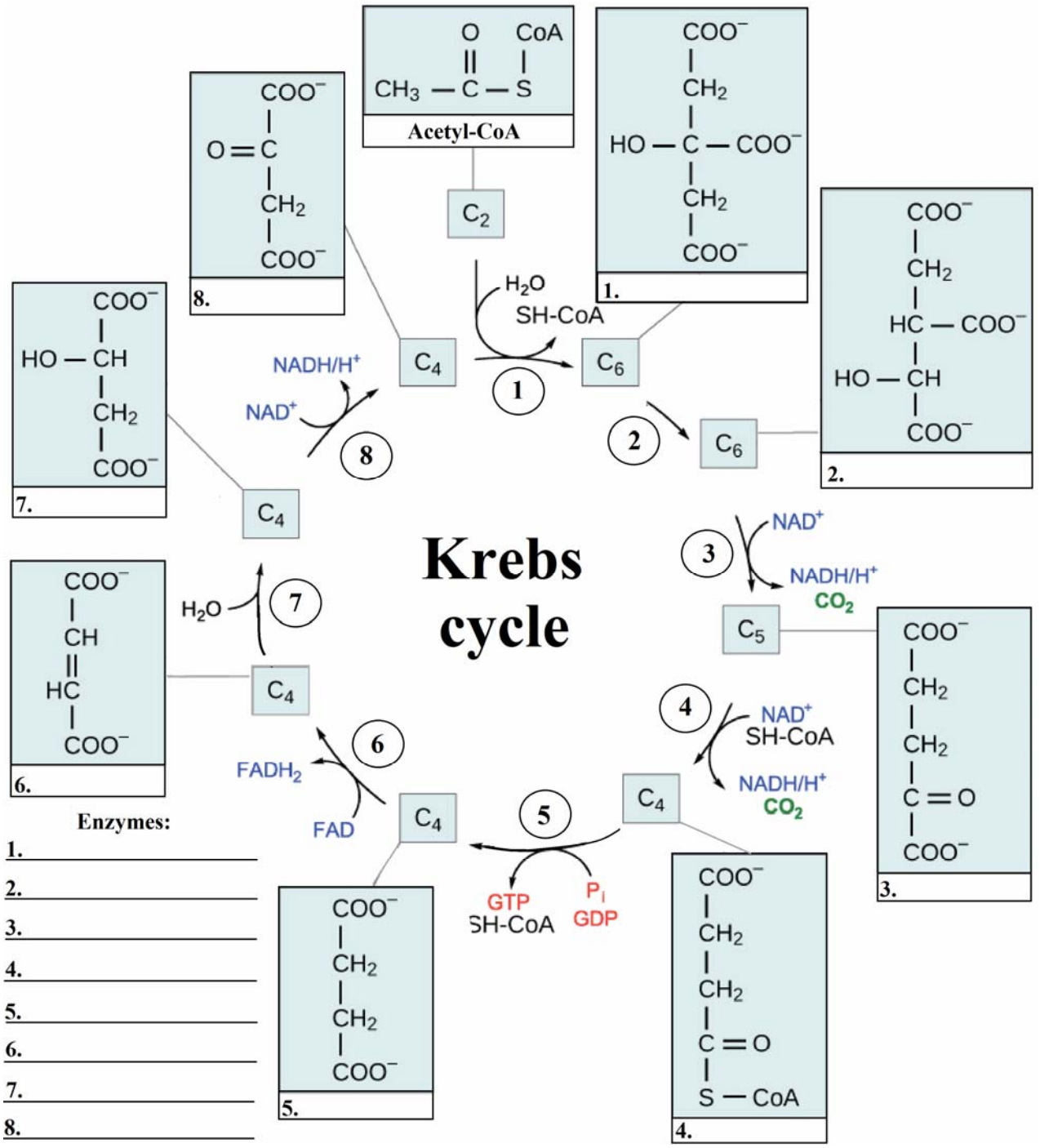
✎ ***Describe the relationship between anabolic and catabolic pathways:***



✎ ***Describe the structure of ATP molecule (draw it in the free space below):***

_____
_____
_____
_____
_____
_____
_____

✎ ***Complete the scheme of TCA below with the name of the appropriate products and enzymes (1-8):***



✎ ***What is the overall net reaction of Citric acid cycle?***

_____

_____

✎ ***State the most important factors needed for the duration of Citric acid cycle with a high rate:***

1._____

2._____

3._____

4._____

✎ ***Describe the activators and inhibitors for Citric acid cycle (include the name of enzymes):***

| *Activators of CAC* | *Inhibitors of CAC* |
|---|---|
| | |

✎ ***Explain the main role of Citric acid cycle:***

_____

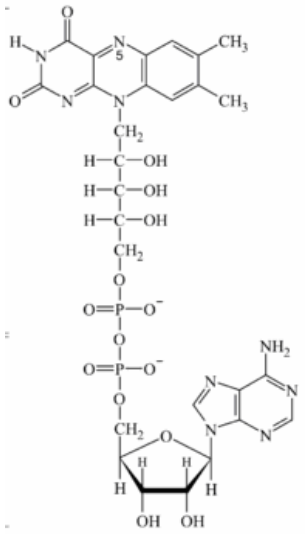
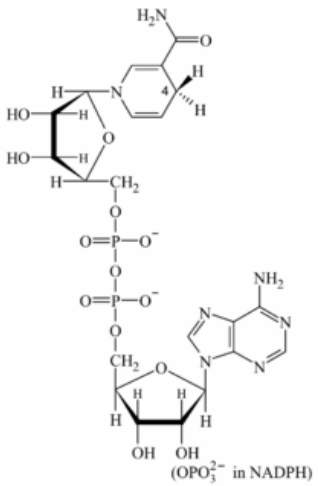
_____

_____

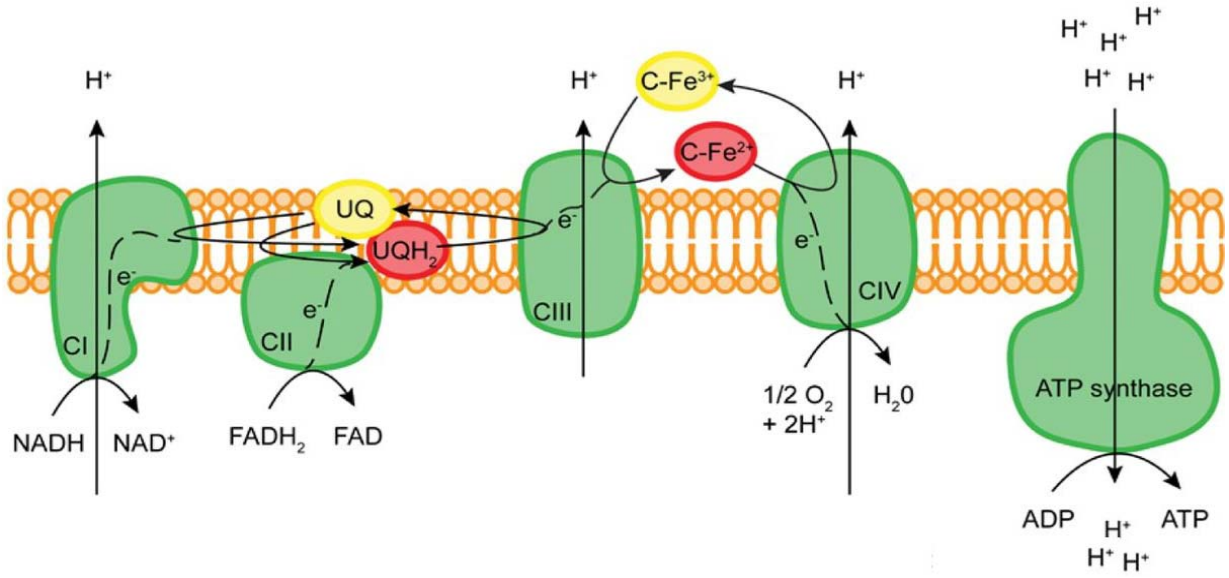
_____

_____

✎ ***Describe the structure of FADH and NAD(P)H molecules:***

| *FAD* | *NAD(P)* |
|---|---|
|  |  |

✎ ***Describe the structure and functions of molecular complexes (C1-CIV) in the mitochondrial inner membranes:***




| # | *Name* | *Compositions* | *Functions* |
|---|---|---|---|
| ***CI*** | | | |
| ***CII*** | | | |
| ***CIII*** | | | |
| ***CIV*** | | | |
| ***CV*** | | | |

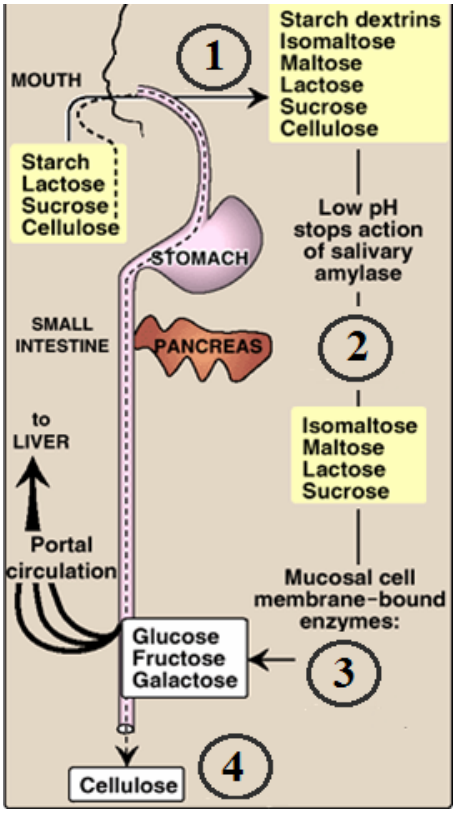
# Lesson 5

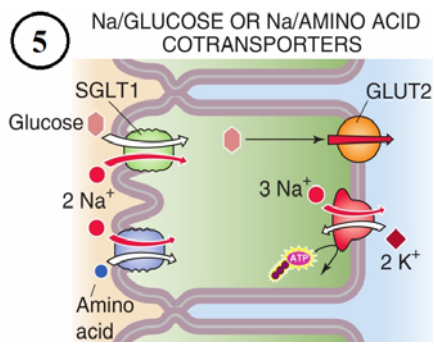
The main topics
**“ANAEROBIC OXIDATION OF GLUCOSE – GLYCOLYSIS. SYNTHESIS OF GLUCOSE – GLUCONEOGENESIS”**

📖 ***Follow this plan at home to prepare for classroom discussion:***

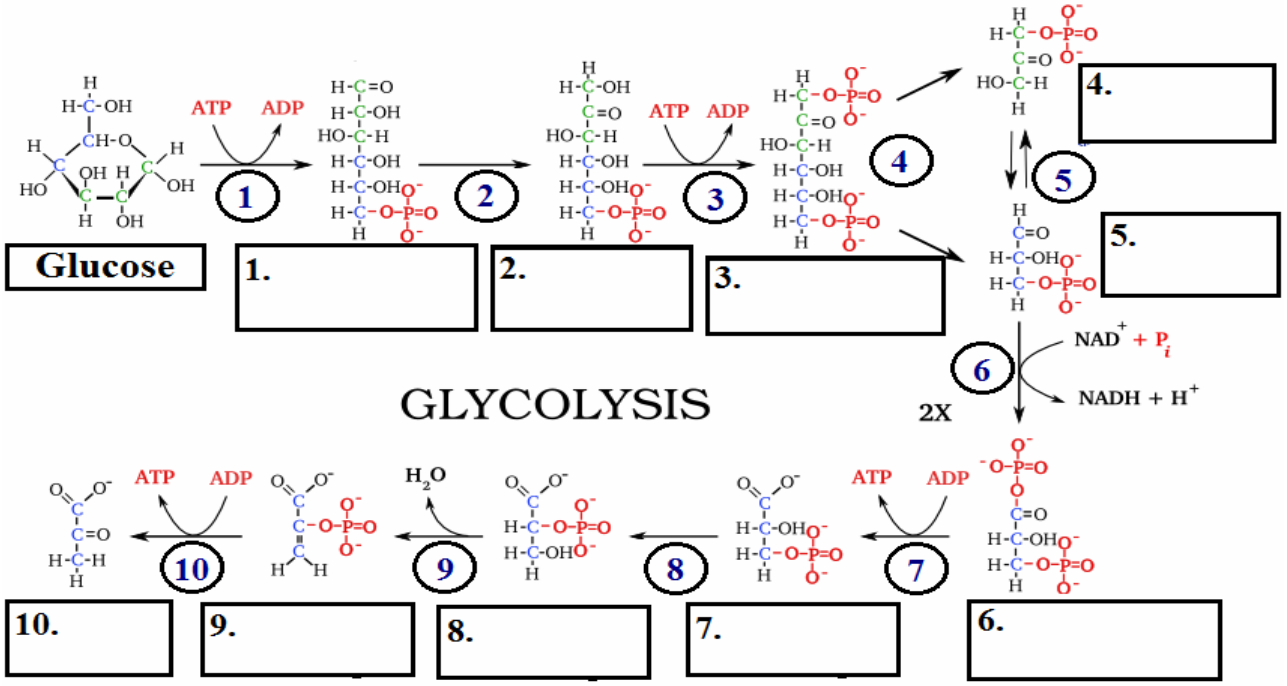
⇨ Digestion and absorption of the main dietary carbohydrates in the gastrointestinal tract;
⇨ Anaerobic glucose oxidation: localization, general scheme of reactions, role, regulation;
⇨ Aerobic glucose oxidation: localization, general scheme of reactions, role, regulation;
⇨ Supply and demand of glycolytic intermediates;
⇨ Oxidative decarboxylation of pyruvate. Enzymes, coenzymes, and sequence of reactions in the multi-enzyme complex;
⇨ Glucose biosynthesis (gluconeogenesis).

✎ ***Describe the main stage of carbohydrate digestion in different part of gastrointestinal tract:***
Include in your description the names of the most important enzymes in this process!!



✎ *Complete the scheme of glycolysis below with the name of appropriate products and enzymes (1-10):*




**Enzymes:**

1
2
3
4
5
6
7
8
9
10

✎ *What does glycolysis accomplish for the cell?*

1. _____
2. _____

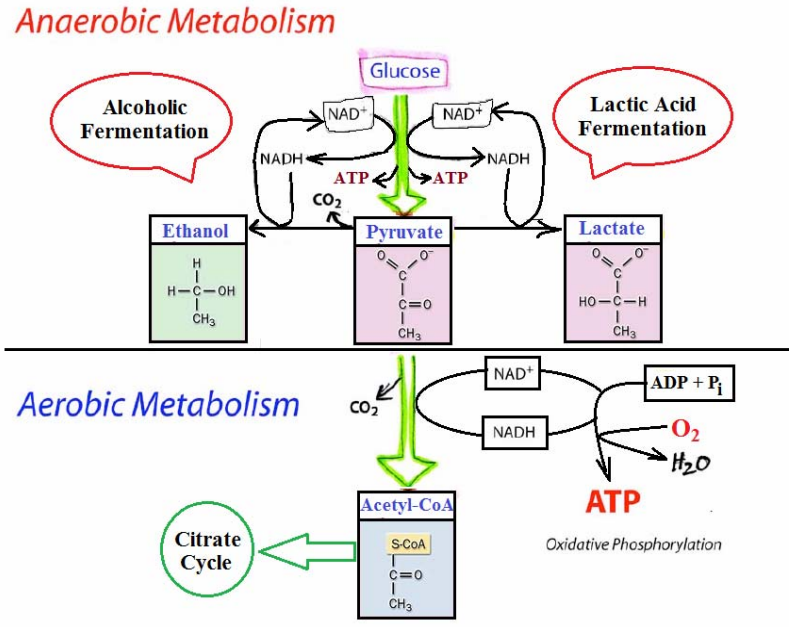
✎ *What are the key enzymes in glycolysis?*

1. _____
2. _____
3. _____

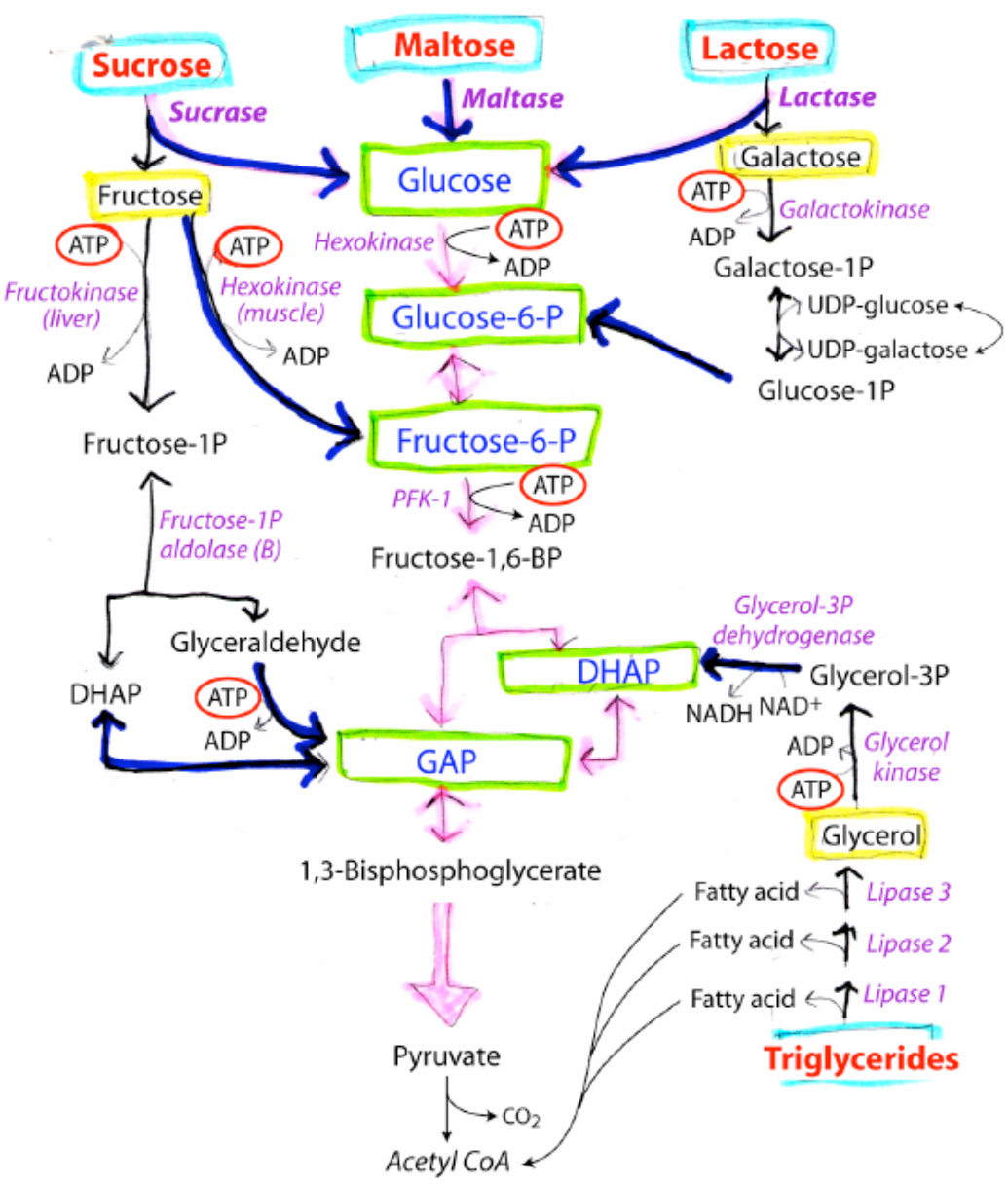
✎ *What is the overall net reaction of glycolysis?*

---

🕮 *Using the figure below describe the three metabolic ways for pyruvate:*



✎ ***Using the figure below explain how fructose, galactose and glycerol can enter the glycolytic pathway:***



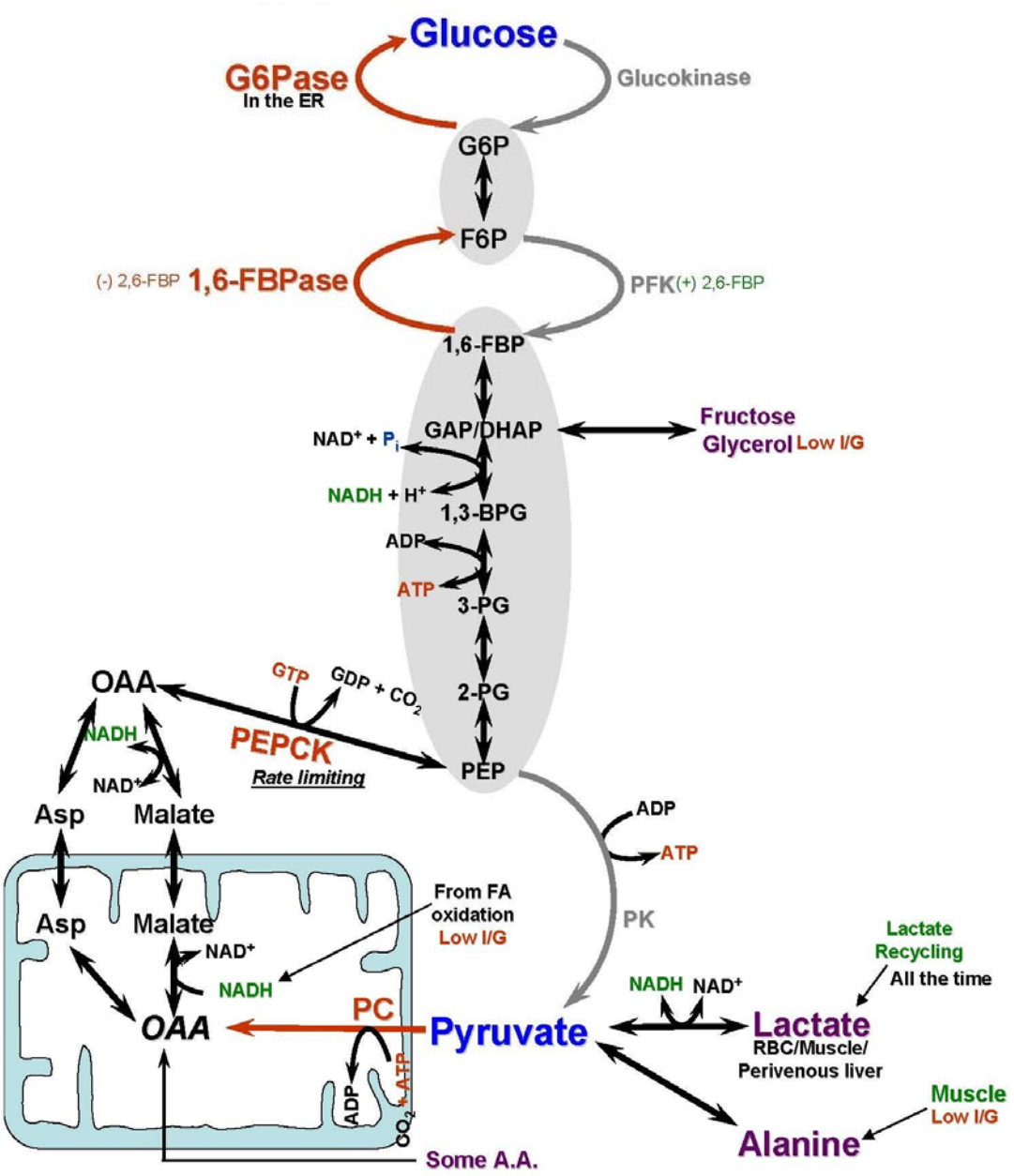

✎ ***What do you know about such pathological conditions like "lactose-intolerant (or lactose-sensitive)" and "fructose intolerance":***

✎ *Define the term* **"GLUCONEOGENESIS":**

_____

_____

✎ *Using the figure below describe the gluconeogenesis:*




✎ *What does gluconeogenesis accomplish for the organism?*

_____

_____

✎ *What is the overall net reaction of gluconeogenesis?*

_____

✎ *What are the key enzymes in gluconeogenesis?*

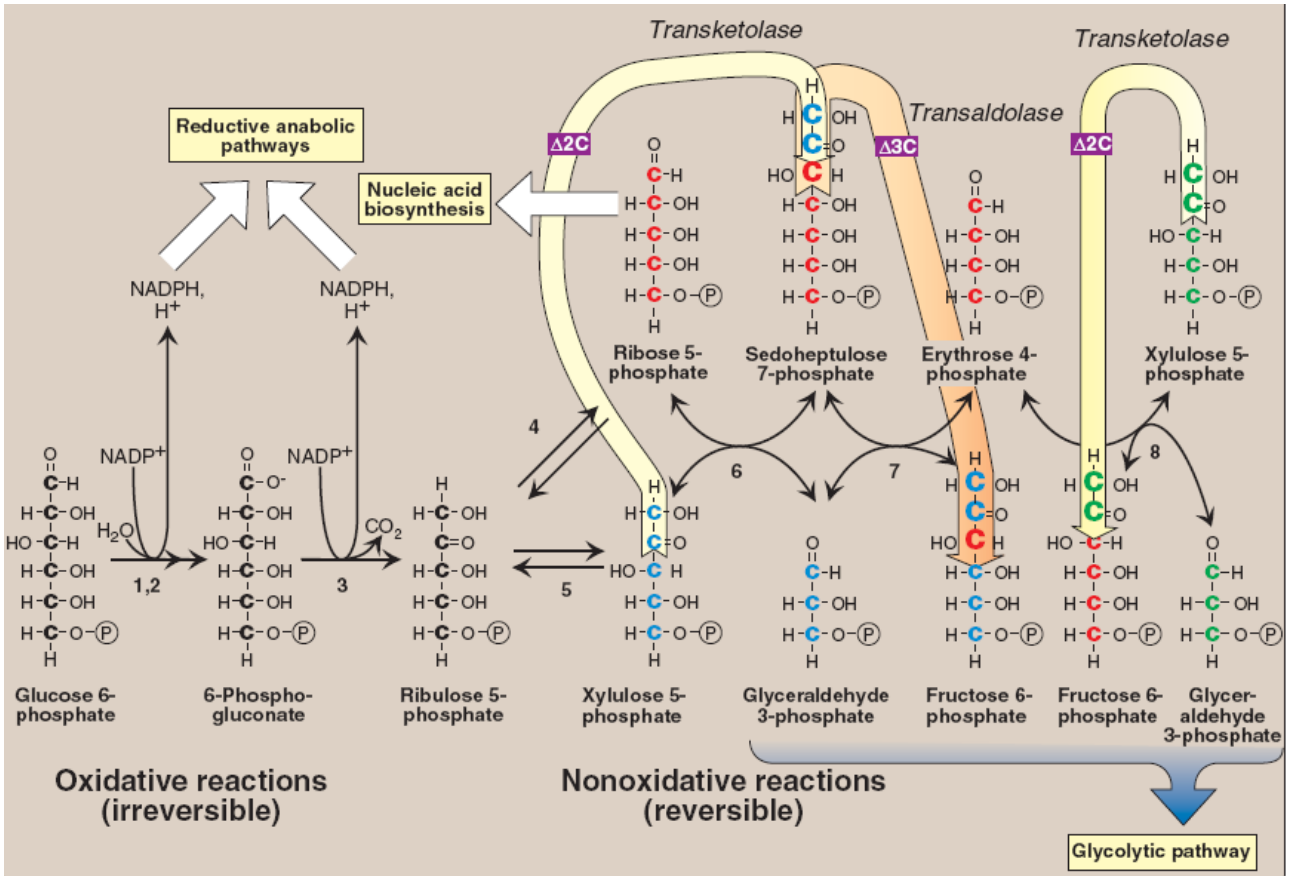
*1.* _____

*2.* _____

*3.* _____

*4.* _____

✎ *Using the figure below describe the* **PENTOSE PHOSPHATE PATHWAY:**




✎ *What does the pentose phosphate pathway accomplish for the cell?*

*1.*_____

_____

*2.*_____

_____

✎ *What is the overall net reaction of the pentose phosphate pathway when it is utilized to generate the maximum amount of NADPH?*

_____

✎ *What do you know about such pathological condition like "Glucose-6P dehydrogenase deficiency" in humans?*

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

# *PRACTICAL WORK 1*

## DETERMINATION OF α-AMYLASE ACTIVITY IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

AMYLASE belongs to the family of glycoside hydrolase enzymes that break down starch into glucose molecules by acting on α-1,4- glycosidic bonds. The α-amylases (EC 3.2.1.1) cleave at random locations on the starch chain and ultimately yield maltotriose and maltose, glucose and "limit dextrin" from amylose and amylopectin. In mammals, α-amylase is a major digestive enzyme.

**Diagnostic significance**:
Amylase is measured for the diagnosis of acute pancreatitis when its levels in serum are increased. In acute pancreatitis α-amylase starts rising approximately four hours after the pain started, it reaches peak in 24 hours and remains elevated for 3 to 7 days. The high levels of amylase are also associated with other disorders, like biliary tract diseases, severe glomerular dysfunction and salivary gland disorders.

**Normal reference values**:
Serum: 3.3 – 8.9 mg/(s·L)
Urine: less than 44 mg/(s·L)

### ASSAY PRINCIPLE:

This method is based on the color development that results from the binding of iodine to starch polymers. In the presence of α-amylase starch hydrolyze to smaller products which do not bind to iodine. The change of color intensity is proportional to α-amylase activity.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 640 nm;
2. Water bath or heating block capable of maintaining temperature at 37 ± 1 °C;
3. Pipettes capable of accurately dispensing volumes from 0.1 to 1.0 mL;
4. Test tubes, 10 mL.

### REAGENTS:

***1. Substrate-Buffer solution***
This solution contains starch – α-amylase substrate. This solution has been prepared by mixing of starch solution (with concentration of starch 10 mg/mL) and 0.2 M phosphate-buffered saline (pH 7.0) in ratio 1:24.

***2. Iodine solution (0.1 N)***
To prepare this solution 3 g of potassium iodide and 1.27 g of iodine have been dissolved in 100 mL of distilled water.

***3. Inhibitor solution***

**PROCEDURE:**

| *Pipette the following solutions into the appropriately marked test tubes (B and T):* | | |
|---|---|---|
| **Reagent** | **Tube** | |
| | ***Blank (B)*** | ***Test (T)*** |
| ***1. Substrate-Buffer solution*** | 0.5 mL | 0.5 mL |
| *Place all test tubes into water bath (37 ± 1 °C) exactly for 5 minutes.* | | |
| ***2. Sample (serum)*** | - | 0.01 mL |
| *Carefully mix each tube thoroughly.*<br>*Place all test tubes into water bath (37 ± 1 °C) exactly for 5 minutes.*<br>*Add to the test tubes:* | | |
| ***3. Inhibitor solution***<br>***4. Sample***<br>***5. Iodine solution*** | 4.5 mL<br>0.01 mL<br>0.05 mL | 4.5 mL<br>-<br>0.05 mL |
| *Set wavelength of spectrophotometer at 640nm and zero the instrument with the distilled water.*<br>*Read the absorbance of all test tubes.*<br>**NOTE:** The final color is stable for at least 10 minutes.<br><br>*Record the absorbance of the test tubes:*<br>$E_B$ _____<br>$E_T$ _____ | | |

**CALCULATION of α-amylase activity (A):**

$$\textbf{A, mg/(s·L)} = \frac{E_B - E_T}{E_B} \times 66.6$$

**Your calculation: _____**

**CONCLUSION:**

# Lesson 6

The main topic

## "BIOCHEMISTRY AND REGULATION OF GLYCOGEN METABOLISM"

📖 ***Follow this plan at home to prepare for classroom discussion:***

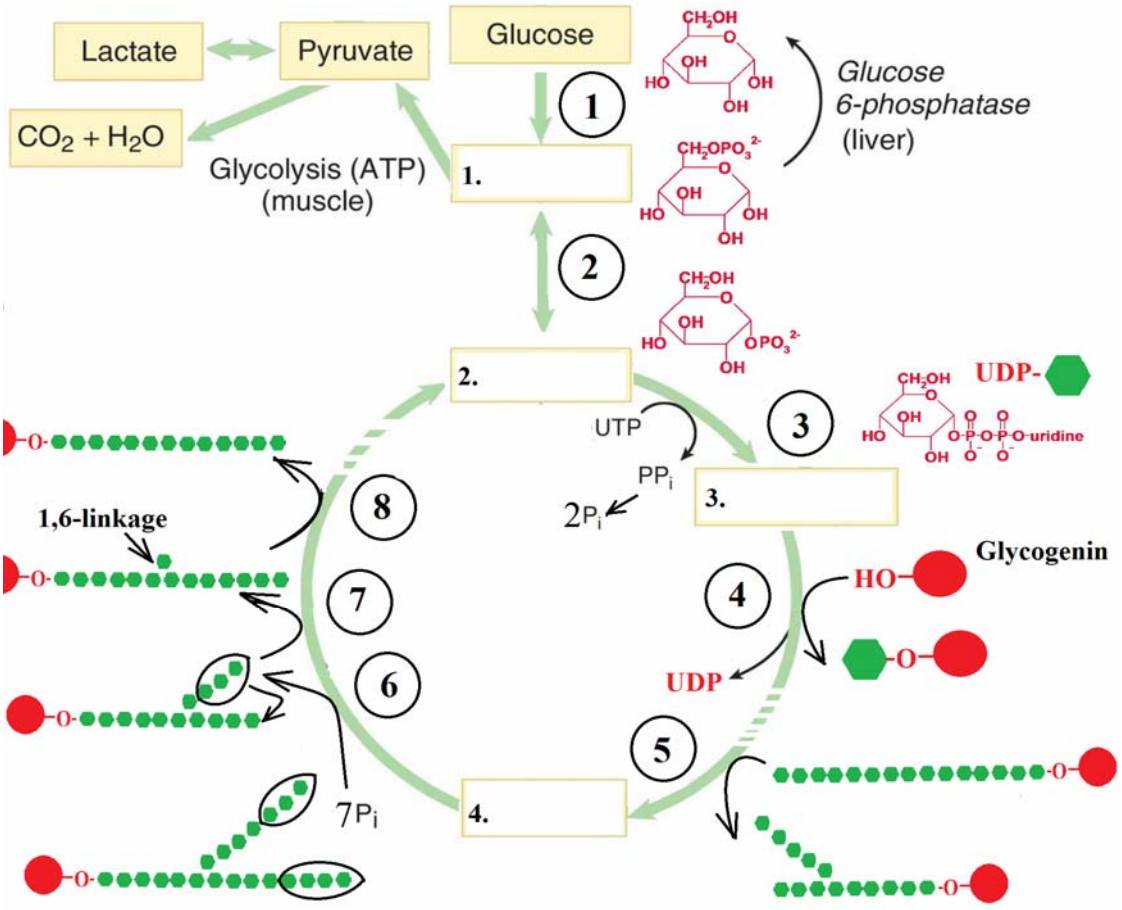
⇨ Biosynthesis of glycogen: enzymatic reactions and physiological significance;
⇨ Phophorolytic pathway of glycogen breakdown in the liver and muscle;
⇨ Hormonal regulation of glycogen metabolism in the muscle and liver;
⇨ Hormonal regulation of blood glucose concentration and glucose metabolism;
⇨ Blood glucose (glycemia): normoglycemia, hypo- and hyperglycemia, glucosuria;
⇨ Disorders of carbohydrate metabolism.

✎ ***Define the term* "GLYCOGENESIS":**

_____

_____

✎ ***Complete the scheme of glycogen metabolism with the name of appropriate products and enzymes (1-10):***




**Enzymes:**

**1.**_____ **6.**_____
**2.**_____ **7.**_____
**3.** _____ **8.** _____
**4.** _____
**5.** _____

✎ ***Define the term "GLYCOGENOLYSIS":***

_____

_____

✎ ***What is the net reaction of glycogen synthesis?***

_____

✎ ***What is the overall net reaction of glycogenolysis?***
*for liver, kidney, small intestine:*

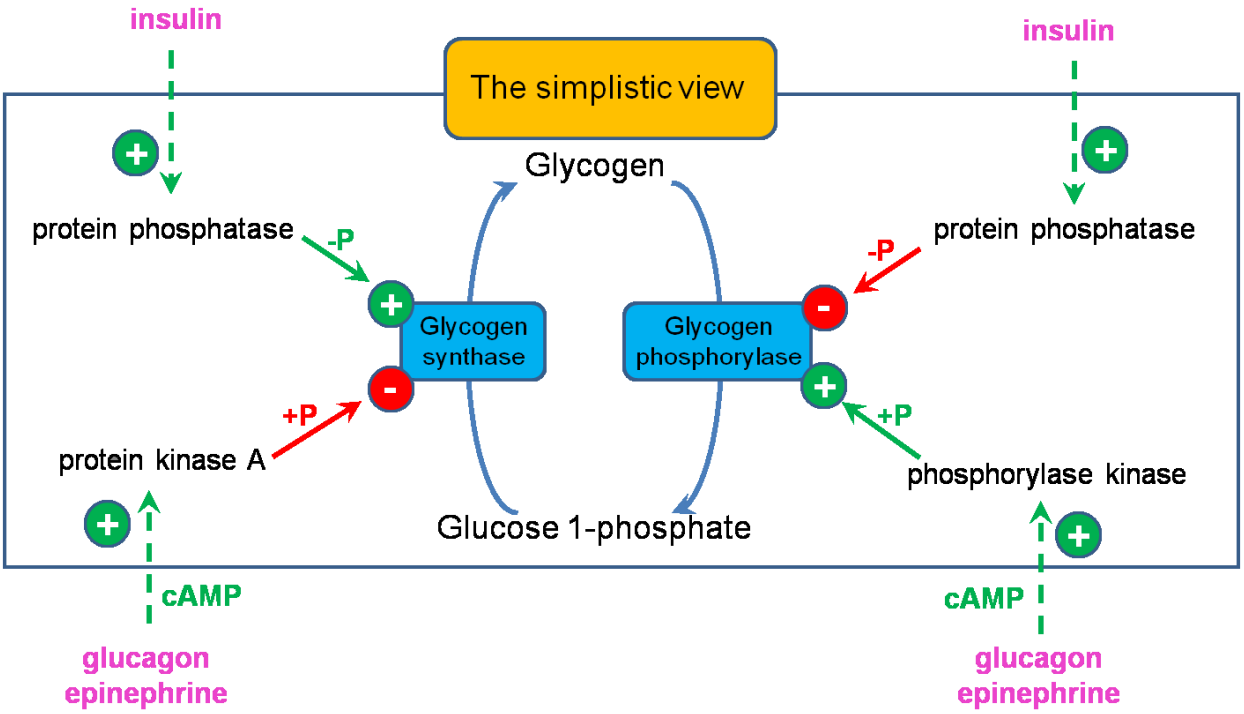
_____

*for other tissues:*

_____

✎ ***What are the key enzymes in glycogen metabolism?***
1._____
2._____

✎ ***Using the figure below, describe the process of regulation of glycogen metabolism:***




_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

## Questions from KROK-1

1. The central intermediate which is common for the catabolic pathways of proteins,
carbohydrates and lipids is:
A. Succinyl-CoA
B. Acetyl-CoA
C. Oxaloacetate
D. Lactate
E. Citrate

2. During the necropsy of a 20-year old girl a pathologist concluded that the death of the patient had resulted from poisoning by cyanides. The activity of what enzyme is mostly inhibited by cyanides?
A. Malate dehydrogenase
B. Cytochrome oxidase
C. Heme synthase
D. Aspartate aminotransferase
E. Carbamoyl phosphate synthetase

3. Galactosemia is revealed in the child. Concentration of glucose in the blood is not considerably changed. Deficiency of what enzyme caused this illness?
A Galactose-1-phosphate uridyltransferase
B Amylo-1,6-glucosidase
C Phosphoglucomutase
D Galactokinase
E Hexokinase

4. Characteristic sign of glycogenosis is muscle pain during physical work. Blood examination reveals usually hypoglycemia. This pathology is caused by congenital deficiency of the following enzyme:
A. Glycogen phosphorylase
B. Glucose 6-phosphate dehydrogenase
C. Alpha amylase
D. Gamma amylase
E. Lysosomal glycosidase

5. A 34-year-old patient's resistance to heavy physical load is reduced while the skeletal muscles glycogen level is increased. By decreasing of the activity of what enzyme can this phenomenon be explained?
A. Phosphofructokinase
B. Glucose-6-phosphate dehydrogenase
C. Glycogen phosphorylase
D. Glycogen synthetase
E. Glucose-6-phosphatase

6. A patient is ill with diabetes mellitus that is accompanied by hyperglycemia of over 7,2 millimole/l on an empty stomach. The level of what blood plasma protein allows to estimate the glycemia rate retrospectively (4-8 weeks before examination)?
A. Glycated hemoglobin
B. Albumin
C. Fibrinogen
D. C-reactive protein
E. Ceruloplasmin

7. A 62-year-old female patient has developed a cataract (lenticular opacity) secondary to the diabetes mellitus. What type of protein modification is observed in case of diabetic cataract?
A. Glycosylation
B. Phosphorylation
C. ADP-ribosylation
D. Methylation
E. Limited proteolysis

8. The B cells of endocrine portion of pancreas are selectively damaged by alloxan poisoning. How will it be reflected in blood plasma?
A. The content of sugar increases
B. The content of fibrinogen decrease
C. The level of sugar decreases
D. The content of globulins decreases
E. The content of albumins decreases

9. Untrained people often have muscle pain after sprints as a result of lactate accumulation. This might be caused by intensification of the following biochemical process:
A. Glycolysis
B. Gluconeogenesis
C. Pentose phosphate pathway
D. Lipogenesis
E. Glycogenesis

10. A patient was delivered to the hospital by an emergency team. Objectively: grave condition, unconscious, adynamy. Cutaneous surfaces are dry, eyes are sunken, face is cyanotic. There is tachycardia and smell of acetone from the mouth. Analysis results: blood glucose - 20,1 micromole/l, urine glucose - 3,5% . What is the most probable diagnosis?
A. Hyperglycemic coma
B. Hypoglycemic coma
C. Acute heart failure
D. Acute alcoholic intoxication
E. Anaphylactic shock

11. Patient with diabetes mellitus experienced loss of consciousness and convulsions after injection of insulin. What is the result of biochemical blood analysis for concentration of the sugar?
A. 1,5 mmol/L
B. 8,0 mmol/L
C. 10,0 mmol/L
D. 3,3 mmol/L
E. 5,5 mmol/L

12. On the empty stomach in the patients blood glucose level was 5,65 mmol/L, in an hour after usage of sugar it was 8,55 mmol/L, in a 2 hours - 4,95 mmol/L. Such indicators are typical for:

A. Healthy person
B. Patient with hidden diabetes mellitus
C. Patient with insulin-dependent diabetes mellitus
D. Patient with non-insulin dependent diabetes mellitus
E. Patient with thyrotoxicosis

13. A child is languid, apathetic. Liver is enlarged and liver biopsy revealed a significant excess of glycogene. Glucose concentration in the blood stream is below normal. What is the cause of low glucose concentration?

A. Low (absent) activity of glycogene phosphorylase in liver
B. Low (absent) activity of hexokinase
C. High activity of glycogen synthetase
D. Low (absent) activity of alfa-1,4- glucosidase
E. Deficit of glucose 1-phosphaturidine transferase

14. After a sprint an untrained person develops muscle hypoxia. This leads to the accumulation of the following metabolite in muscles:

A. Lactate
B. Ketone bodies
C. Acetyl CoA
D. Glucose 6-phosphate
E. Oxaloacetate

15. Myocyte cytoplasm contains a big number of dissolved metabolites of glucose oxidation. Name one of them that turns directly into a lactate:

A. Pyruvate
B. Oxaloacetate
C. Glycerophosphate
D. Glucose 6-phosphate
E. Fructose 6-phosphate

16. A child's blood presents high content of galactose, glucose concentration is low. There are such presentations as cataract, mental deficiency, adipose degeneration of liver. What disease is it?

A. Galactosemia
B. Diabetes mellitus
C. Lactosemia
D. Steroid diabetes
E. Fructosemia

17. A 45 y.o. woman suffers from Cushing's syndrome - steroid diabetes. Biochemical examination revealed: hyperglycemia, hypochloremia. Which of the under-mentioned processes is the first to be activated?

A. Gluconeogenesis
B. Glycogenolysis
C. Glucose reabsorption
D. Glucose transport to the cell
E. Glycolysis

18. The patient with complaints of permanent thirst applied to the doctor. Hyperglycemia, polyuria and increased concentration of 17-ketosteroids in the urine were revealed. What disease is the most likely?

A. Steroid diabetes
B. Insulin-dependent diabetes mellitus
C. Myxoedema
D. Type I glycogenosis
E. Addison's disease

19. When blood circulation in the damaged tissue is restored, then lactate accumulation comes to a stop and glucose consumption decelerates. These metabolic changes are caused by activation of the following process:

A. Aerobic glycolysis
B. Anaerobic glycolysis
C. Lipolysis
D. Gluconeogenesis
E. Glycogen biosynthesis

20. During starvation muscle proteins break up into free amino acids. These compounds will be the most probably involved into the following process:

A. Cori cycle
B. Gluconeogenesis in muscles
C. Synthesis of higher fatty acids
D. Glycogenolysis
E. Decarboxylation

21. A newborn develops dyspepsia after the milk feeding. When the milk is substituted by the glucose solution the dyspepsia symptoms disappear. The newborn has the subnormal activity of the following enzyme:

A. Lactase
B. Invertase
C. Maltase
D. Amylase
E. Isomaltase

22. After taking sulfonamides and aspirin by a 38-year-old patient, hemolysis of erythrocytes caused by the insufficiency of glucose-6-phosphate dehydrogenase developed. The disturbance of what coenzyme formation does this pathology result from?

A. Ubiquinone
B. FADH2
C. Pyridoxal phosphate
D. FMNH2
E. NADPH

23. A child with point mutation has the absence of glucose- 6- phosphate body tissues, hypoglycemia and hepatomegaly detected. Define the type of pathology which these symptoms are characteristic of:

A. Girke's disease
B. Measles
C. Addison's disease
D. Parkinson's disease
E. McArdle's disease

24. The concentration of glucose in the blood plasma of a healthy man varies within the following limits:

A. 2.0-4.0 mM/L
B. 3.3-5.5 mM/L
C. 10.0-25.0 mM/L
D. 6.0-9.5 mM/L
E. 1.0-2.0 mM/L

25. Some hours after an intensive physical training a sportsman showed activated gluconeogenesis. Which of the following is the basic substrate of gluconeogenesis?

A. Serine
B. Aspartate
C. Glutamate
D. a-Ketoglutarate
E. Lactate

26. A newborn child had dyspepsia phenomena (diarrhea, vomiting) detected after feeding with milk. After additional feeding with glucose the morbid symptoms disappeared. The insufficient activity of what enzyme that takes part in the carbohydrates breakdown causes the indicated disorders?

A. Saccharase
B. Amylase
C. Lactase
D.lsomaltase
E. Maltase

27. A 2-year-old boy has the increase of liver and spleen sizes detected and eye cataract present. The total sugar level in blood is increased, but glucose tolerance is within the normal range. The inherited disturbance of the metabolism of what substance is the cause of the indicated state?

A. Glucose
B. Fructose
C. Galactose
D. Maltose
E. Saccharose

28. A 57-year-old patient, suffering from insulin dependent diabetes mellitus, showed the development of ketoacidosis. The biochemical mechanism of the development of this pathology is decreasing of acetyl-CoA utilization due to the deficiency of:

A. 2-Oxoglutarate
B. Oxaloacetate
C. Glutamate
D. Aspartate
E. Succinate

29. A 38-year-old man is receiving treatment for schizophrenia in hospital. The initial levels of glucose, ketone bodies and urea in the blood are within the normal range. Shock therapy put into practice by regular insulin injections resulted in the development of the comatose state which improved the clinical status of the patient. What is the most probable cause of insulin coma?

A. Hyperglycemia
B. Dehydratation of tissues
C. Metabolic acidosis
D. Ketonemia
E. Hypoglycemia

29. A 7-year-old girl manifests obvious signs of anemia. Laboratory tests showed the deficiency of pyruvate kinase activity in erythrocytes. The disorder of what biochemical process is a major factor in the development of anemia?

A. Deamination of amino acid
B. Oxidative phosphorylation
C. Tissue respiration
D. Breaking up of peroxides
E. Anaerobic glycolysis

30. A 45-year-old woman does not have any symptoms of insulin dependent diabetes mell itus but testing on an empty stomach showed the increase of the blood glucose level (7.5 mM/l). What additional laboratory test needs to be done to substantiate the diagnosis?

A. Determination of tolerance to glucose
B. Determination of ketone bodies concentration in the urine
C. Determination of rest nitrogen level in the blood
D. Determination of tolerance to glucose on an empty stomach
E. Determination of glycosylated hemoglobin level

31. What biochemical process is stimulated in the liver and kidneys of a patient exhausted by starvation?

A. Synthesis of bilirubin
B. Synthesis of urea
C. Gluconeogenesis
D.Formation of hippuric acid
E. Synthesis of uric acid

*PRACTICAL WORK 2*

## DETERMINATION OF GLUCOSE CONSENTRATION IN SERUM BY GLUCOSE OXIDASE/PEROXIDASE METHOD

### 🕮 BACKGROUND

GLUCOSE ($C_6H_{12}O_6$) is a ubiquitous fuel molecule in biology. It is oxidized through a series of enzyme-catalyzed reactions to form carbon dioxide and water, yielding the universal energy molecule ATP.

**Diagnostic significance**:
Due to its importance in metabolism, glucose level is a key diagnostic parameter for many metabolic disorders. The increased glucose levels have been associated with diabetes mellitus and with hyperactivity of thyroid, pituitary and adrenal glands. The decreased levels are found in insulin secreting tumors, myxedema, hypopituitarism and hypoadrenalism.

**Normal reference values**:
Serum: adults 12-60 y.o.: 4.1 – 5.9 mmol/L
adults 60-90 y.o.: 4.6 – 6.4 mmol/L
children less than 12 y.o.: 3.3 – 5.6 mmol/L
Urine: 0.1-0.8 mmol/L

### ASSAY PRINCIPLE

Glucose oxidase catalyses the oxidation of β-D-glucose to D-glucono-δ-lactone with the concurrent release of hydrogen peroxide. In the presence of peroxidase (POD) this hydrogen peroxide ($H_2O_2$) enters into a second reaction involving *p*-hydroxybenzoic acid and 4-aminoantipyrine (also called 4-aminophenazone) with the quantitative formation of a quinoneimine dye complex (pink colored) which is measured at 510 nm.

*The reactions involved are:*

$$\beta\text{-D-Glucose} + O2 + H2O \xrightarrow{(GOX)} \text{D-glucono-}\delta\text{-lactone} + H_2O_2$$

$$2H_2O_2 + p\text{-hydroxybenzoic acid} + \text{4-aminoantipyrine} \xrightarrow{(POD)} \text{quinoneimine dye} + 4H_2O$$

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 510 nm;
2. Water bath or heating block capable of maintaining temperature at 37 ± 1 °C;
3. Pipettes capable of accurately dispensing volumes from 0.02 to 1.0 mL;
4. Test tubes, 10 mL.

### REAGENTS:

***1. Enzyme Mixture***
This solution contains an enzyme mixture: glucose oxidase (18000 U/L) and peroxidase (2200 U/L) and 4-aminophenazone (110 mg/L);

***2. Glucose Standard***
This standard solution contains 1800 mg/L of glucose (or 10 mmol/L of glucose).

**PROCEDURE:**

| *Pipette the following solutions into the appropriately marked dry clean test tubes (B, S, T):* | | | |
|---|---|---|---|
| **Reagent** | **Tube** | | |
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (T)*** |
| ***1. NaCl 0.9%*** | 0.02 mL | - | - |
| ***2. Glucose standard*** | - | 0.02 mL | - |
| ***3. Sample (serum)*** | - | - | 0.02 mL |
| ***4. Enzyme Mixture*** | 2 mL | 2 mL | 2 mL |

*Carefully mix each tube thoroughly.*
*Lat the test tubes stand for 30 minutes at room temperature or 12 min at 37 °C.*
*Set wavelength of spectrophotometer at 640nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 60 minutes.

*Record the absorbance of the test tubes:*

$E_S$ _____

$E_T$ _____

**CALCULATION of glucose concentration (C):**

**C, mmol/L** $= \frac{E_T}{Es} \times 10,$ where 10 is concentration of glucose in standard solution

**Your calculation: _____**

**CONCLUSION:**

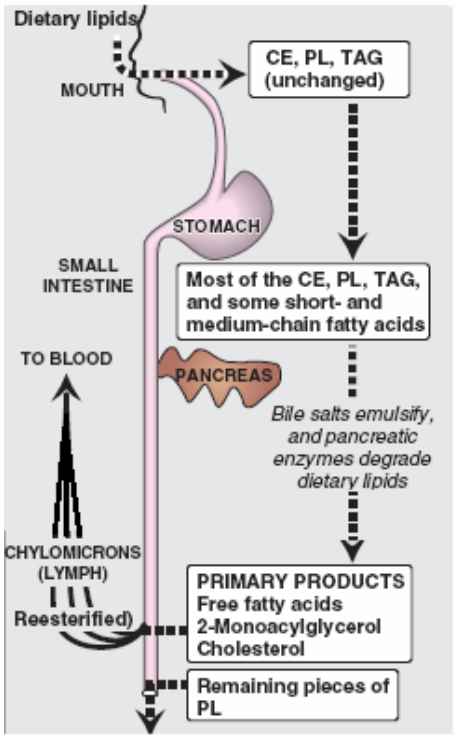
# Lesson 7

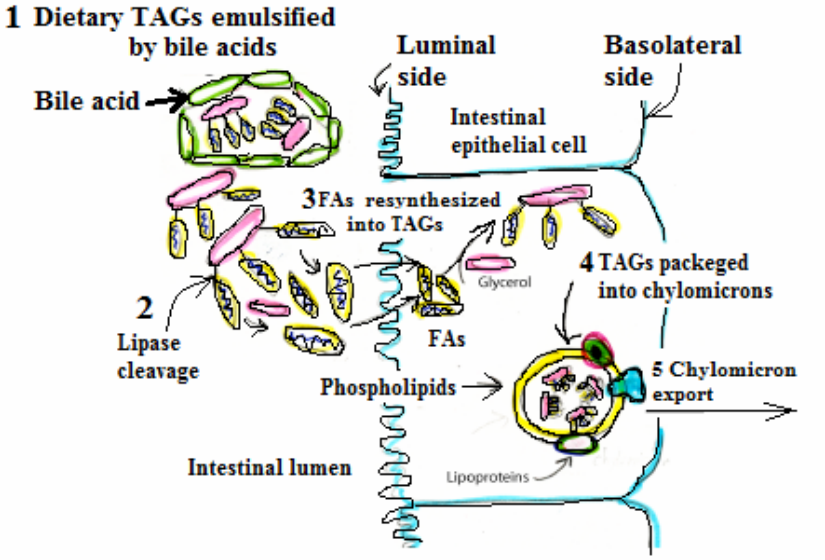
The main topic
**"LIPID METABOLISM AND ITS REGULATION (part 1)"**

📖 ***Follow this plan at home to prepare for classroom discussion:***

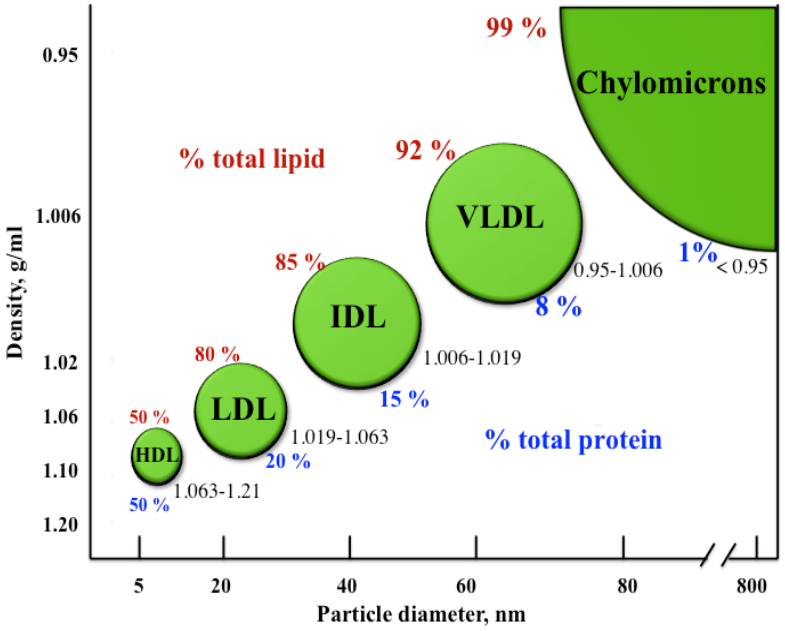
⇨ Digestion and absorption of lipids in the gastrointestinal tract;
⇨ Bile acids and their role in digestion and absorption of lipids;
⇨ Catabolism of triacylglycerols in adipocytes of adipose tissue: role, sequence of reactions. Neurohormonal regulation of lipolysis by adrenaline, noradrenaline, glucagon, and insulin;
⇨ Oxidation of fatty acids (β-oxidation): localization, scheme of reactions. Energy value of β-oxidation of fatty acids in cells;
⇨ Ketogenesis and ketolysis: localization, scheme of reactions, regulation;
⇨ Glycerol metabolism: localization, general scheme of reactions, role;
⇨ Biosynthesis of fatty acids: localization, scheme of reactions, regulation;
⇨ Biosynthesis of triacylglycerols and phosphoglycerols: scheme of reactions, role, regulation.
⇨ Metabolism of sphingolipids. Genetic abnormalities of sphingolipid metabolism - sphingolipidoses.

✎ ***Describe the main stage of lipid digestion in different part of gastrointestinal tract:***
Include in your description the names of the most important enzymes in this process!!

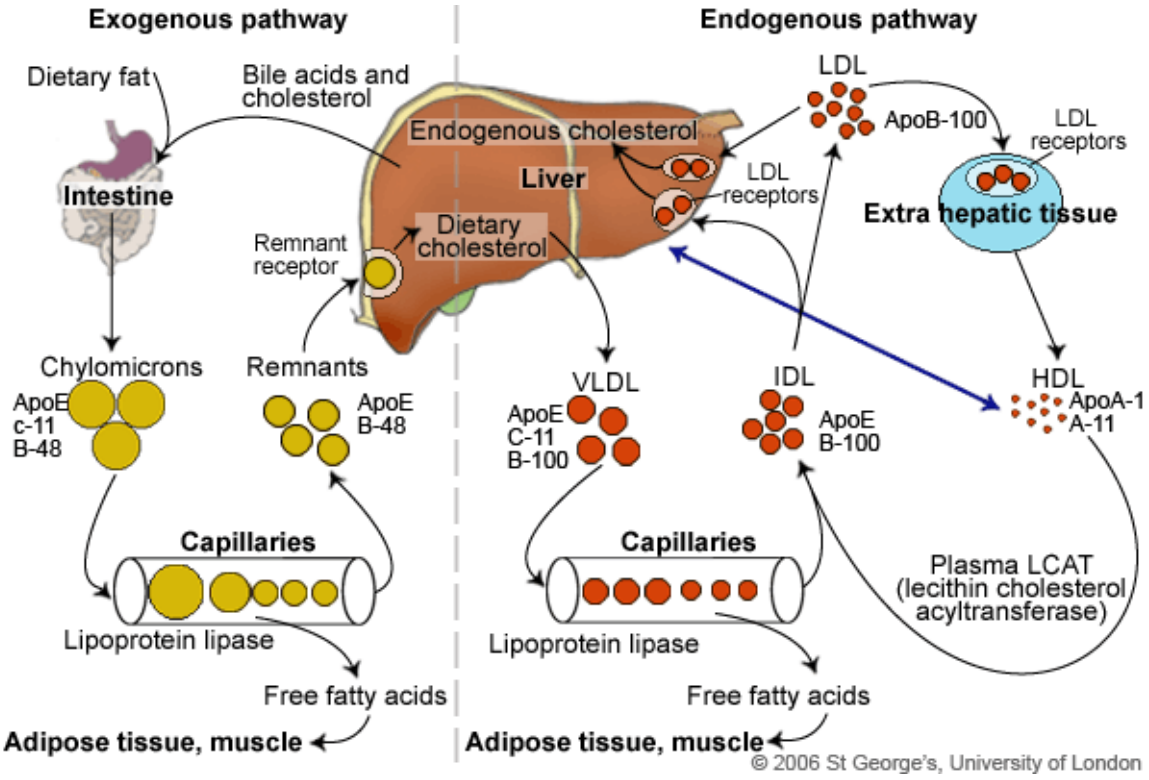


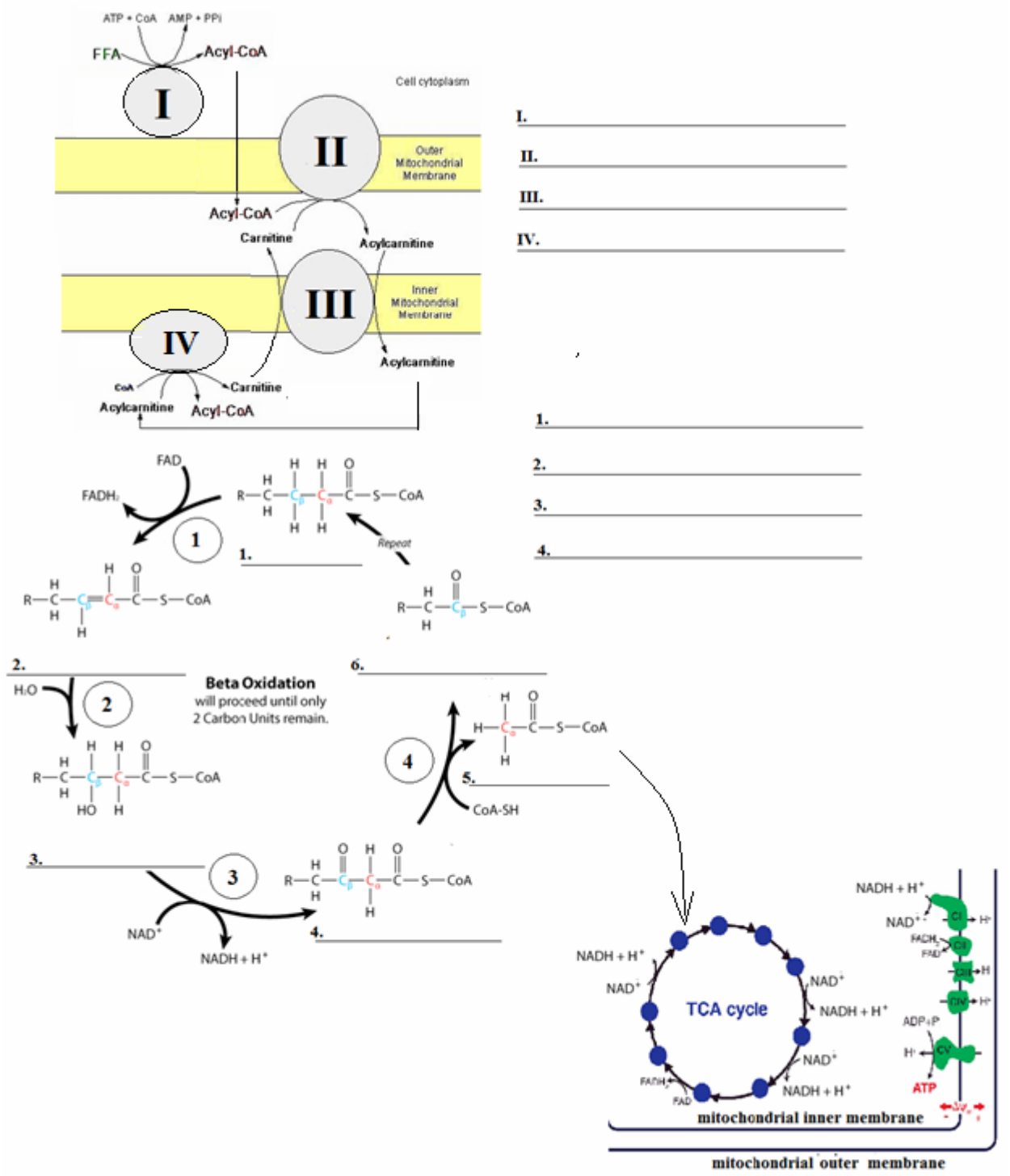
✎ *Using the figure below describe the types of lipoproteins:*




✎ *Using the figure below describe the process of lipoprotein metabolism:*



✎ ***Complete the scheme of FAs metabolism with the name of appropriate products and enzymes (I-IV and 1-4):***




✎ ***What are the net reactions of the complete oxidation of palmitoyl-CoA (C16):***

_____

✎ ***Calculate the net ATP yield from cashing in the redox potential of palmitate using the β oxidation pathway:***

_____

_____

_____

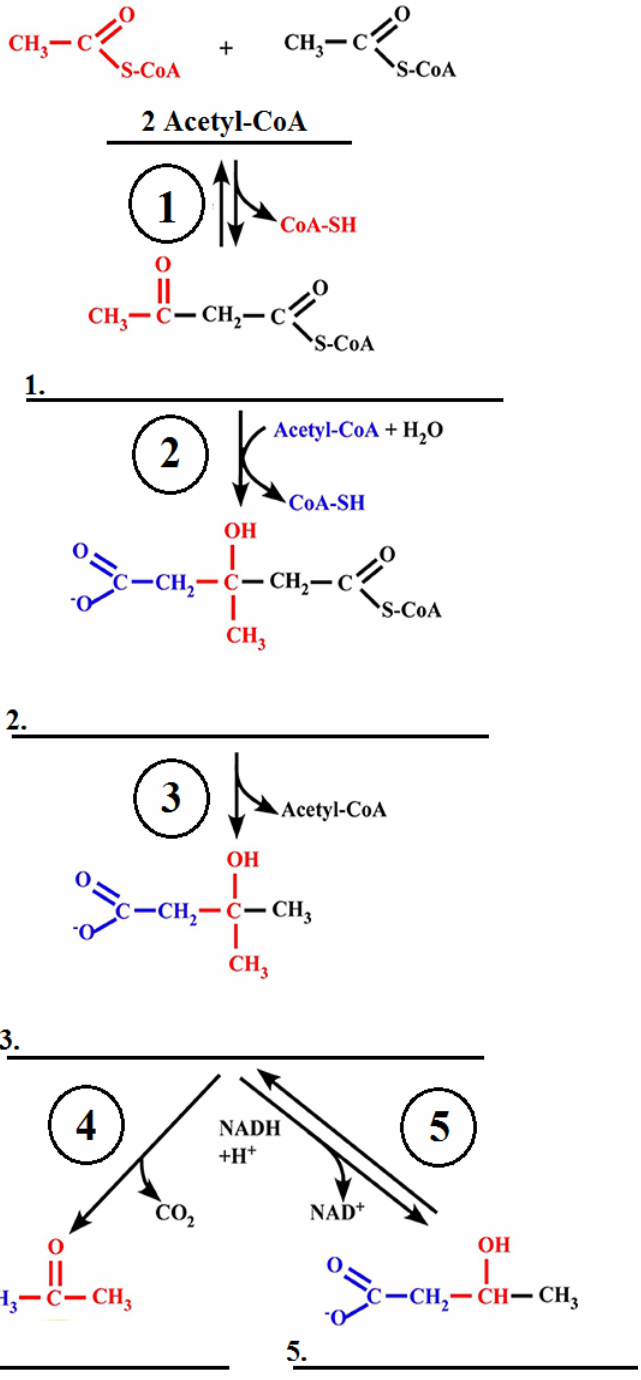
_____

✎ *Define the term* **"KETOGENESIS":**

_____

_____

✎ *Complete the scheme of keton bodies synthesis with the name of appropriate products and enzymes (1-5):*




*ENZYMES:*

*1.* _____

*2.* _____

*3.* _____

*4.* _____

*5.* _____

✎ *Which ones are keton bodies?*

*1.* _____

*2.* _____

*3.* _____

✎ *What are the functions of keton bodies?*

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

✎ *Define the terms*:

**Ketonemia**_____

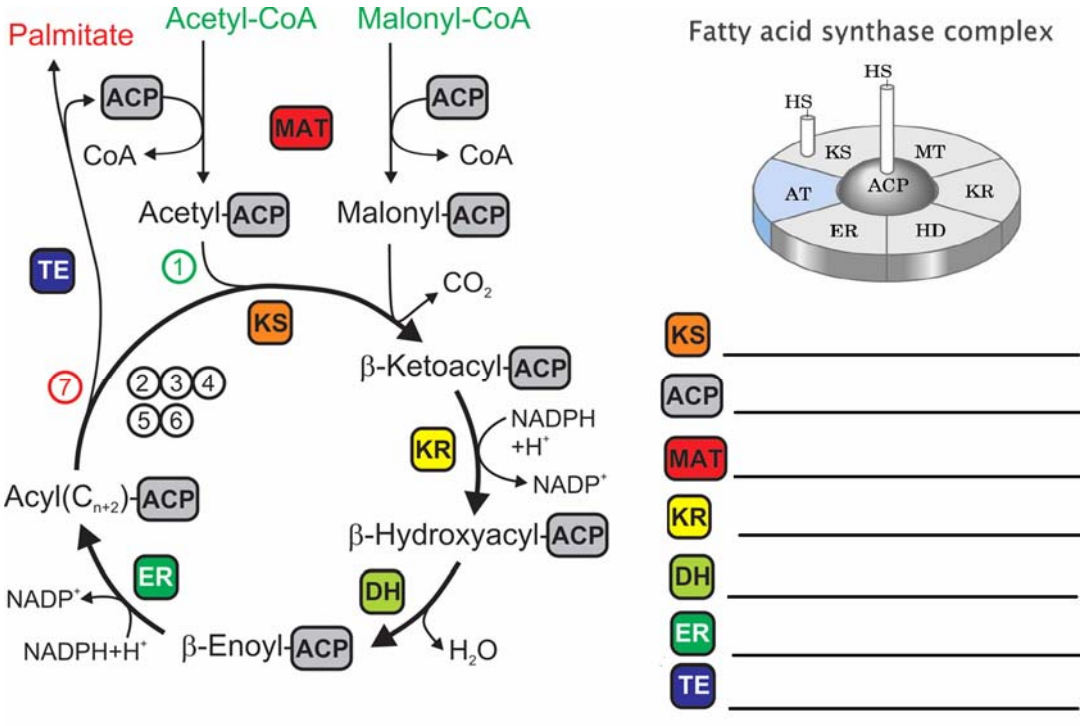
**Ketonuria**_____

**Ketosis**_____

✎ ***Complete the table of types of Diabetes mellitus:***

| | *Type 1* | *Type 2* |
|---|---|---|
| ***Synonym*** | | |
| ***Age of onset*** | | |
| ***Prevalence*** | | |
| ***Genetic predisposition*** | | |
| ***Defect or deficiency*** | | |
| ***Ketosis*** | | |
| ***Plasma insulin*** | | |
| ***Acute complications*** | | |
| ***Oral hypoglycemia drugs*** | | |
| ***Treatment with insulin*** | | |

✎ ***Using the figure below describe the process of FAs synthesis. Label the part of FA synthase complex:***




✎ ***What is the net fatty acid synthesis reaction for palmitate (C16:0)?***

_____

_____

_____

# *PRACTICAL WORK 3*

## THE COLORIMETRIC DETERMINATION OF THE SERUM TOTAL LIPIDS BY THE SULFO-PHOSPHO-VANILLIN METHOD

### 🕮 BACKGROUND

LIPIDS are a diverse group of molecules that include monoglycerides, diglycerides, trigylcerides, fats, sterols and others. Lipids are not only define and preserve cellular membrane integrity, but they are also involved in cellular processes such as membrane trafficking, signal transduction, apoptosis and energy storage.

**Diagnostic significance**:

Perturbation in the metabolism of lipids has been linked to many diseases such as cancer, diabetes, Alzheimer's disease, and coronary heart disease.

**Normal reference values**:

Serum: 4-8 g/L

### ASSAY PRINCIPLE:

Lipids react with sulfuric acid to form carbonium ions, which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 530 nm;
2. Water bath capable of maintaining temperature at 100 ± 2 °C;
3. Pipettes capable of accurately dispensing volumes of 0.01 and 1.0 mL;
4. Test tubes, 10 mL.

### REAGENTS:

***1. Color reagent***

This solution contains 10 mmol/L of vanillin and 11 mol/L of phosphoric acid

***2. Total lipid standard***

The concentration of lipids in this standard solution is 8 g/L

***3. Acidic reagent***

**NOTE:** Acidic reagent causes severe burns. Exercise extreme care in handling this chemical.

Do not pipette this solution with mouth!!

### PROCEDURE

| *Pipette the following solutions into the appropriately marked test tubes (B, S and T):* | | | |
|---|---|---|---|
| **Reagent** | **Tube** | | |
| | ***Blank (B)*** | ***Standard (S)*** | ***Test T)*** |
| ***1. Sample (serum)*** | - | - | 0.01 mL |
| ***2. Total lipid standard*** | - | 0.01mL | - |
| ***3. NaCl, 0.9%*** | 0.01mL | - | - |
| ***4. Acidic reagent*** | 1.0 mL | 1.0 mL | 1.0 mL |
| *Carefully mix each tube thoroughly.*<br>*Place all test tubes into water bath (100 ± 2 °C) exactly for 10 minutes.*<br>*Remove all test tubes and place them in cold water for 5 minutes.* | | | |
| ***5.Color reagent*** | 2.0 mL | 2.0 mL | 2.0 mL |
| *Carefully mix each tube by swirling.* | | | |

**NOTE:** Mixing acidic reagent with a color reagent might generate some amount of heat, therefore exercise appropriate care.)
*Let all test tubes stand at room temperature (20-25 °C) for 25 minutes.*
*Set wavelength of spectrophotometer at 530 nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 25 minutes.

*Record the absorbance of the test tubes:*

$E_S$ _____

$E_T$ _____

**CALCULATION of total lipid concentration (C):**

**C, g/L** = $\frac{E_T}{Es}$ **× 8,** where 8 is the concentration of lipids in standard solution

**Your calculation: _____**

**CONCLUSION:**

# Lesson 8

The main topic
**"LIPID METABOLISM AND ITS REGULATION (part 2)"**

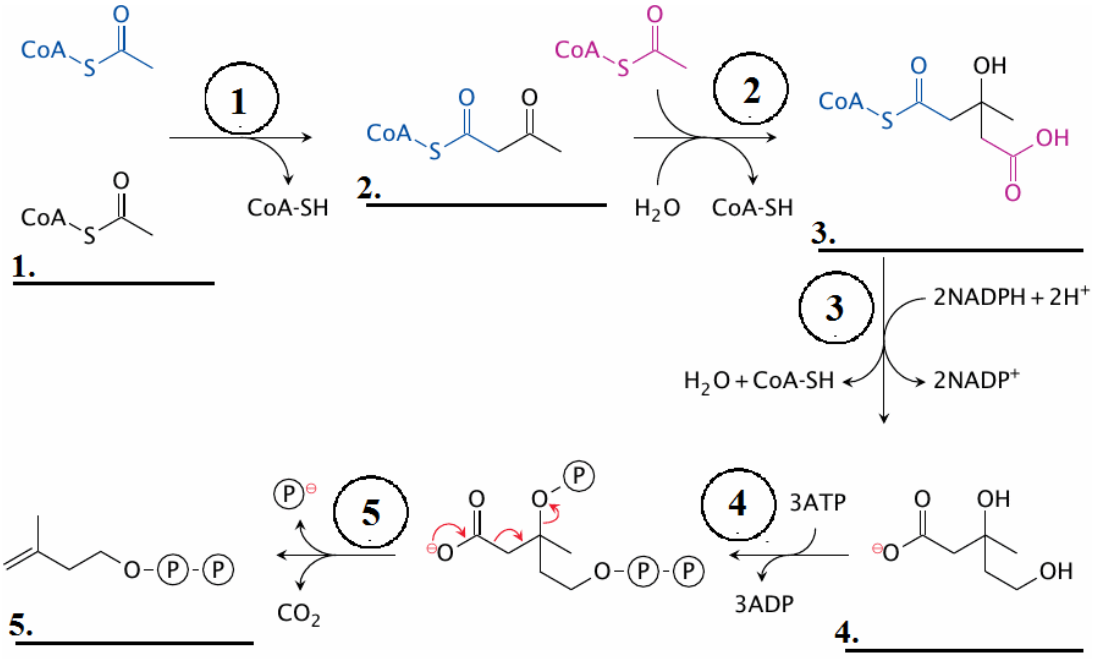
📖 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Cholesterol biosynthesis: localization, scheme of reactions, regulation;
⇨ Pathways of cholesterol biotransformation: etherification; formation of bile acids, steroid hormones, vitamin $D_3$;
⇨ Disorders of cholesterol metabolism;
⇨ Plasma lipoproteins: lipid and protein (apoproteins) composition. Hyperlipoproteinemia;
⇨ Pathologies of lipid metabolism: atherosclerosis, obesity, diabetes mellitus, steatorrhea.
⇨ Disorders of metabolism of ketone bodies under pathological conditions;

✎ ***Describe the structure of cholesterol molecule (draw it in the free space below):***

_____
_____
_____
_____
_____
_____

✎ ***Complete the scheme of the first step of cholesterol synthesis with the name of appropriate products (1-5) and enzymes (1-5):***



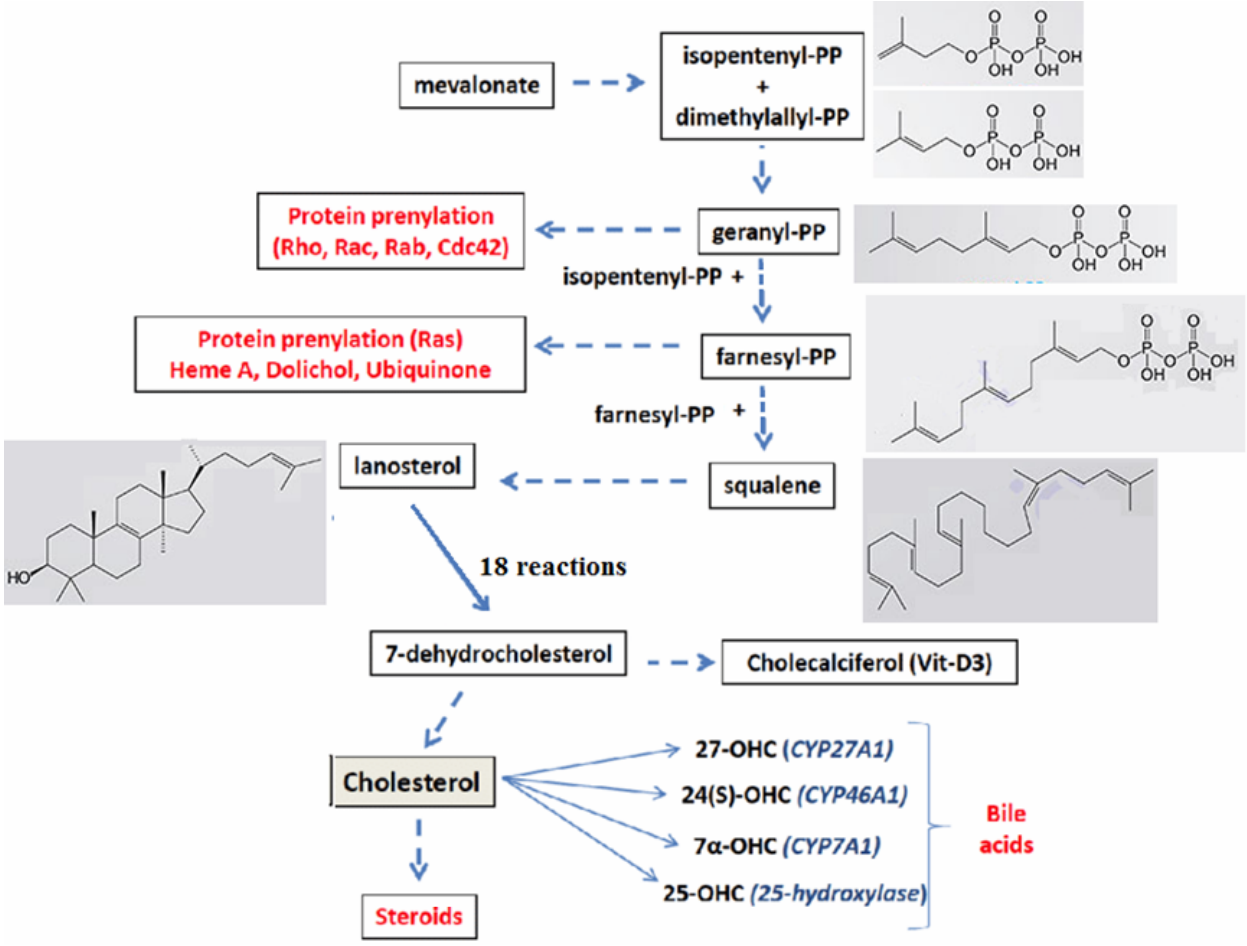

***ENZYMES:***

***1.***_____ ***4.***_____

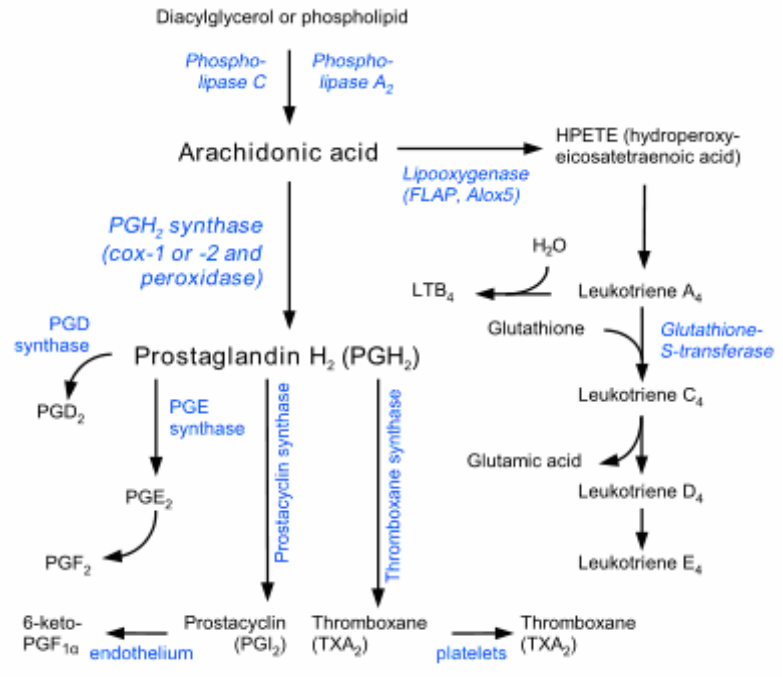
***2.***_____ ***5.***_____

***3.***_____

✎ ***Using the figure below, describe cholesterol pathway:***

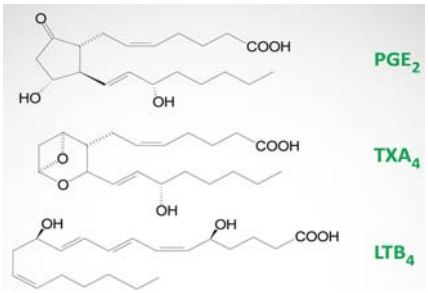



✎ ***Using the figure below, describe eicosanoid biosynthesis:***




✎ ***Draw the structure of arachidonic acid in the free space below:***

***Some example of eicosanoids:***



## Questions from KROK-1

1. After consumption of rich food a patient has nausea and heartburn, steatorrhea. This condition might be caused by:
A Bile acid deficiency
B Increased lipase secretion
C Disturbed tripsin synthesis
D Amylase deficiency
E Disturbed phospholipase synthesis

2. Fatty of phospholipids is disordered due to fat infiltration of the liver. Indicate which of the presented substances can enhance the process of methylation during phospholipids synthesis?
A Methionine
B Ascorbic acid
C Glucose
D Glycerin
E Citrate

3. Increased amount of free fatty acids is observed in the blood of the patients with diabetes mellitus. It can be caused by:
A Increased activity of triglyceridelipase adipocytes
B Storage of palmitatoil-CoA
C Activation of the ketone bodies utilization
D Activation of the synthesis of the apolipoproteins
E Decreased activity of phosphatidylcholine-cholesterol-acyltransferase blood plasma

4. A patient with high rate of obesity was advised to use carnitine as a food additive in order to enhance "fat burning". What is the role of carnitine in the process of fat oxidation?
A Transport of FFA (free fatty acids) from cytosol to the mitochondria
B Transport of FFA from fat depots to the tissues
C It takes part in one of reactions of FFA beta-oxidation
D FFA activation
E Activation of intracellular lipolysis

5. An experimantal animal that was kept on protein-free diet developed fatty liver infiltration, in particular as a result of deficiency of methylating agents. This is caused by disturbed generation of the following metabolite:
A Choline
B DOPA
C Cholesterol
D Acetoacetate
E Linoleic acid

6. Carnitine including drug was recomended to the sportsman for improving results. What process is activated most of all with help of carnitine?
A Transport of fatty acids to the mitochondria
B Synthesis of steroid hormones
C Synthesis of ketone bodies
D Synthesis of lipids
E Tissue respiration

7. After intake of rich food a patient feels nausea and sluggishness; with time there appeared signs of steatorrhea. Blood cholesterine concentration is 9,2 micromole/l. This condition was caused by lack of:
A Bile acids
B Triglycerides
C Fatty acids
D Phospholipids
E Chylomicrons

8. Examination of a man who hadn't been consuming fats but had been getting enough carbohydrates and proteins for a long time revealed dermatitis, poor wound healing, vision impairment. What is the probable cause of metabolic disorder?
A Lack of linoleic acid, vitamins A, D, E, K
B Lack of palmitic acid
C Lack of vitamins PP, H
D Low caloric value of diet
E Lack of butiric acid

9. An experimental animal has been given excessive amount of carbon-labeled glucose for a week. What compound can the label be found in?
A Palmitic acid
B Methionine
C Vitamin A
D Choline
E Arachidonic acid

10. A sportsman was recommended to take a medication that contains carnitine in order to improve his results. What process is activated by carnitine the most?
A Fatty acids transport to mitochondrions
B Synthesis of steroid hormones
C Synthesis of ketone bodies
D Synyhesis of lipids
E Tissue respiration

11. Examination of a patient suffering from chronic hepatitis revealed a significant decrease in the synthesis and secretion of bile acids. What process will be mainly disturbed in the patient’s bowels?
A Fats emulsification
B Protein digestion
C Carbohydrate digestion
D Glycerin absorption
E Amino acid absorption

12. A 6 year old child was delivered to a hospital. Examination revealed that the child couldn't
fix his eyes, didn't keep his eyes on toys, eye ground had the cherry-red spot sign. Laboratory analyses showed that brain, liver and spleen had high rate of ganglioside glycometide. What congenital disease is the child ill with?
A Tay-Sachs disease
B Wilson's syndrome
C Turner's syndrome
D Niemann-Pick disease
E MacArdle disease

13. NSAID blockade the utilization of arachidonic acid via cyclooxigenase pathway, which results in formation of some bioactive substances. Name them:

A Prostaglandins
B Thyroxine
C Biogenic amins
D Somatomedins
E Insulin-like growth factors

14. Arachidonic acid, an essential component of a human diet, acts as a precursor of the vitally important physiologically active biomolecules. Which substances are synthesized via cyclooxigenase pathway from arachidonic acid?

A. Ethanolamine
B. Choline
C. Noradrenaline
D. Prostaglandins
E. Triiodothyronine

15. A 1-year-old child with symptoms of muscle involvement was admitted to the hospital. Examination revealed carnitine deficiency in his muscles. What process disturbance is the biochemical basis of this pathology?

A Transporting of fatty acids to mitochodrions
B Regulation of Ca2+ level in mitochondrions
C Substrate phosphorylation
D Lactic acid utilization
E Actin and myosin synthesis

16. Laboratory investigation of the patient's blood plasma, which was performed 4 hours after a consumption of a fat diet, displayed a marked increase of plasma turbidity. The most credible cause of this phenomenon is the increase of in the plasma.

A. HDL
B. Chylomicrons
C. LDL
D. Cholesterol
E. Phospholipids

17. Patients who suffer from severe diabetes and don't receive insulin have metabolic acidosis. This is caused by increased concentration of the following metabolites:

A Ketone bodies
B Fatty acids
C Unsaturated fatty acids
D Triacylglycerols
E Cholesterol

18. In a human body the adipose tissue is the basic location of triacylglycerols (TAG) deposit. At the same time their synthesis takes place in hepatocytes. In the form of what molecular complex are TAG transported from the liver into the adipose tissue?

A. Chylomicrons
B. VLDL
C. LDL
D. HDL
E. Complexes with albumin

19. Laboratory investigation of a patient revealed a high level of plasma LDL. What disease can be diagnosed?

A. Gastritis
B. Nephropathy
C. Acute pancreatitis
D.Atherosclerosis
E. Pneumonia

20. Aerobic oxidation of substrates is typical for cardiac myocytes. Which of the following is the major oxidation substrate of cardiac muscles?

A. Fatty acids
B. Triacylglycerols
C. Glycerol
D. Glucose
E. Amino acids

21. Which of the following enzymes accelerates the lipolysis under the action of epinephrine in stress situations?

A. Triacylglycerol lipase
B. Lypoprotein lipase
C. Phospholipase A2
D. Phospholi pase C
E. Cholesterol esterase

22. Clinical signs and laboratory testing of a patient allow make the assumption of gall-bladder inflammation, colloid properties of bile disorder and occurrence of gall-stones. Which substances can underlie the formation of gall-stones?

A. Oxalates
B. Urates
C. Cholesterol
D. Chlorides
E. Phosphates

23. Emotional stress causes activation of hormon-sensitive triglyceride lipase in the adipocytes. What secondary mediator takes part in this process?

A Cyclic adenosine monophosphate
B Cyclic guanosine monophosphate
C Adenosine monophosphate
D Diacylglycerol
E Ions of $Ca2^{+}$

24. The insufficient secretion of what enzyme is the cause of incomplete fats degradation in the digestive tract and appearance of great quantity of neutral fats in feces?

A. Pepsin
B. Phospholipase
C. Enterokinase
D.Amylase
E. Pancreatic lipase

# *PRACTICAL WORK 4*

## DETERMINATION OF TOTAL CHOLESTEROL IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

CHOLESTEROL is a lipid sterol that is produced in and transported throughout the bloodstream in eukaryotes. Cholesterol is a critical compound used in the structure of cell membranes, hormones, and cell signaling. It is an essential component of animal cell structure in order to maintain permeability and fluidity. Cholesterol is a precursor for steroid hormones including the adrenal gland hormones cortisol and aldosterone, sex hormones progesterone, estrogens, and testosterone, and bile acids and vitamin D. Cholesterol is transported throughout the body within lipoproteins, which have cell-specific signals that direct the lipids they transport to certain tissues. For this reason, lipoproteins exist in different forms within the blood based on their density. These include chylomicrons, very-low density lipoproteins (VLDLs), low-density lipoproteins (LDLs), intermediate-density lipoproteins (IDLs), and high-density lipoproteins (HDLs). The higher the lipid content within a lipoprotein, the lower its density. Cholesterol exists within a lipoprotein as a free alcohol and as a fatty cholesteryl ester, which is the predominant form of cholesterol transport and storage.

**Diagnostic significance**: The measurement of serum cholesterol levels can be an indicator of liver function, biliary function, intestinal absorption, propensity toward coronary artery disease, and thyroid function. Cholesterol levels are important in the diagnosis and classification of hyperlipoproteinemias. Stress, age, gender, hormonal balance, and pregnancy affect normal cholesterol levels. All adults 20 years of age and over should have a fasting lipoprotein profile (cholesterol, LDL, HDL) once every 5 years to screen for coronary heart disease risk.

**Normal reference values**:

Serum: children 0-12 y.o.: 2.95 – 5.30 mmol/L
adults 12-40 y.o.: 3.08 – 6.94 mmol/L
adults 40-70 y.o.: 3.81 – 7.85 mmol/L

### ASSAY PRINCIPLE:

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide is measured quantitatively in a peroxidase catalyzed reaction that produces color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration.

*The reaction sequence is as follows:*

$$\text{Cholesteryl ester} + H_2O \xrightarrow[\textit{cholesteryl ester hydrolase}]{} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\textit{cholesterol oxidase}]{} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow[\textit{peroxidase}]{} \text{4-(p-benzoquinonemonoimino)-phenazone} + 4\,H_2O$$

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 500 nm;
2. Water bath or heating block capable of maintaining temperature at 37 ± 1 °C;
3. Pipettes capable of accurately dispensing volumes of 0.02 and 1.0 mL;

4. Test tubes, 10 mL

**REAGENTS:**

***1. Enzyme Mixture***
This solution contains enzyme mixture (cholesterol esterase > 150 U/L; cholesterol oxidase > 100 U/L; peroxidase > 5 kU/L and 0.3 mM 4-aminophenazone, 30 mM phenol, 30 mM Tris buffer. **NOTE:** Protect this solution from light;

***2. Cholesterol Standard***
The concentration of cholesterol in this standard solution is 2 mg/mL or 5.17 mmol/L

**PROCEDURE**

*Pipette the following solutions into the appropriately marked test tubes (B, S and T):*

| **Reagent** | **Tube** | | |
|---|---|---|---|
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (T)*** |
| ***1. Enzyme mixture*** | 2.0 mL | 2.0 mL | 2.0 mL |
| ***2. NaCl, 0.9%*** | 0.02 mL | - | - |
| ***3. Cholesterol standard*** | - | 0.02 mL | - |
| ***4. Sample (serum)*** | - | - | 0.02 mL |

*Carefully mix each tube thoroughly.*
*Incubate the test tubes for 10 minutes at 37ºC or 20 minutes at room temperature.*
**NOTE:** Protect the reaction mixture from light.
*Set wavelength of spectrophotometer at 500 nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 60 minutes.

*Record the absorbance of the test tubes:*

$E_S$ _____

$E_T$ _____

**CALCULATION of total cholesterol concentration (C):**

$$\textbf{C, mmol/L} = \frac{E_T}{Es} \times 5.17,$$

where 5.17 is the concentration of cholesterol in the standard solution

**Your calculation: _____**

**CONCLUSION:**

_____

_____

_____

_____

_____

# *PRACTICAL WORK 5*

## DETERMINATION OF LDL-CHOLESTEROL IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

LOW DENSITY LIPOPROTEINS (LDL) are synthesized in the liver by the action of various lipolytic enzymes on triglyceride-rich Very Low Density Lipoproteins (VLDLs). Specific LDL receptors exist to facilitate the elimination of LDL from plasma by liver parenchymal cells. LDL carries cholesterol to the peripheral tissues where it can be deposited and increase the risk of arteriosclerotic heart and peripherial vascular disease. It has been shown that most of the cholesterol stored in atherosclerotic plaques originates from LDL. Hence high levels of LDL are atherogenic. For this reason the LDL-Cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis.

**Diagnostic significance**:

The accurate measurement of LDL-Cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture. High blood levels of LDLs are associated with health problems and cardiovascular disease. For this reason, LDL is often referred to as the "bad cholesterol." LDL particles that accumulate within arteries can form plaques over time, which can increase chances of a stroke or vascular disease.

**Normal reference values**:

Serum: less than 2.59

### ASSAY PRINCIPLE:

When a sample is mixed with Reagent 1 the protecting reagent binds to LDL and protects LDL from enzyme reactions. Cholesterol esterase and cholesterol oxidase react with non-LDL lipoprotein, very low density lipoprotein (VLDL) and HDL. Hydrogen peroxide produced by the enzyme reactions with non-LDL reactions with non-LDL cholesterol is decomposed by catalase in Reagent 1. When Reagent 2 is added, the protecting reagent is removed from LDL and catalase is inactivated by sodium azide. In the second process, cholesterol esterase and cholesterol oxidase react only with LDL-Cholesterol. Hydrogen peroxide produced by the enzyme reactions with LDL-Cholesterol yields a color complex upon oxidase condensation with phenol and 4-aminoantipyrene in the presence of peroxidase. By measuring the absorbance of the blue color complex product, at approximately 600 nm, the LDL- Cholesterol concentration in the sample can be calculated when compared with the absorbance of the LDL-calibrator.

*The reactions involved are:*

**Step 1**

Cholesteryl ester
Cholesterol
HDL + **LDL** + VLDL

***Reagent 1***
*cholesteryl esterase, cholesterol oxidase*
***protector of LDL***
$\longrightarrow$

Fatty acid + $H_2O_2$
**protected LDL-Cholesterol**

*peroxidase*
$2H_2O_2 \longrightarrow O_2 + 2\ H_2O$

**Step 2**

***Reagent 2***
*detergent, sodium azide*
*cholesteryl esterase, cholesterol oxidase*

complex
**protector-(LDL-Cholesterol)** $\longrightarrow$ cholest-4-en-3-one + $H_2O_2$

*peroxidase*
$2H_2O_2$ + 4-aminophenazone + phenol $\longrightarrow$ colored product + 4 $H_2O$

**APPARATUS:**

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 600 nm;
2. Water bath or heating block capable of maintaining temperature at 37 ± 1 °C;
3. Pipettes capable of accurately dispensing volumes of 0.024, 0.2 and 1 mL;
5. Test tubes, 10 mL

**REAGENTS:**

***1. Reagent 1***

This solution contains enzyme mixture (cholesterol esterase ≈ 5000 U/L; cholesterol oxidase ≈ 5000 U/L; peroxidase ≈ 10 kU/L and protector for LDL, 25 mM Tris buffer.
**NOTE:** Protect this solution from light;

***2. Reagent 2***

This solution contains 3.4 mM 4-aminophenazone, detergent for LDL-protector complex, peroxidase ≈ 10 kU/L, chromogen, 25 mM Tris buffer.
**NOTE:** Protect this solution from light;

***3. Cholesterol Standard***

The concentration of cholesterol in this standard solution is 5.17 mmol/L;

**PROCEDURE:**

*Pipette the following solutions into the appropriately marked test tubes (B, S and T):*

| **Reagent** | **Tube** | | |
|---|---|---|---|
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (D)*** |
| ***1. Sample (serum)*** | - | - | 0.024 mL |
| ***2. Reagent 1*** | 2.4 mL | 2.4 mL | 2.4 mL |

*Carefully mix each tube thoroughly.*
*Incubate the test tubes for 5 minutes at 37ºC.*
**NOTE:** Protect the reaction from light.
*Set wavelength of spectrophotometer at 600 nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*

*Record the absorbance of the test tubes:*

$E_{S1}$ _____

$E_{T1}$ _____

*Add into the test tube:*

| | | | |
|---|---|---|---|
| ***3. Reagent 2*** | 0.6 mL | 0.6 mL | 0.6 mL |
| ***4. Cholesterol Standard*** | - | 0.024 mL | - |

*Carefully mix each tube thoroughly.*
*Incubate the test tubes for 5 minutes at 37ºC.*
**NOTE:** Protect the reaction from light.
*Set wavelength of spectrophotometer at 600 nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 5 minutes.

*Record the absorbance of the test tubes:*

$E_{S2}$ _____

$E_{T2}$ _____

**CALCULATION of LDL-cholesterol concentration (C):**

**1)** $\Delta Es = E_{S2} - E_{S1}$

**2)** $\Delta E_T = E_{T2} - E_{T1}$

**3) C, mmol/L** $= \dfrac{\Delta E_T}{\Delta Es} \times 5.17,$

where 5.17 is concentration of cholesterol in standard solution

**Your calculation:**

**1)** _____

**2)** _____

**3)** _____

**CONCLUSION:**

# *PRACTICAL WORK 6*

## DETERMINATION OF HDL-CHOLESTEROL IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

HIGH DENSITY LIPOPROTEIN (HDL) is the fraction of plasma lipoprotein with a hydrated density of 1.063 to 1.21 g/mL. It is composed of 50 per cent protein and 50 per cent lipids. HDL cholesterol functions as a transporter of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed into bile acids and excreted through the intestine via the biliary tract. The cholesterol carried within HDL particles is sometimes called "good cholesterol." Monitoring circulatory levels of different lipoproteins is critical to the diagnosis of lipid transport disorders such as atherosclerosis.

**Diagnostic significance**:

Low HDL-cholesterol levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease. Thus, the determination of serum HDL-cholesterol has been recognized as a useful tool in identifying high risk patients.

**Normal reference values**:

Serum (man): less than 1.42 mmol/L

(woman): less than 1.68 mmol/L

### ASSAY PRINCIPLE:

The Direct HDL-cholesterol assay is a homogeneous method for direct measurement of serum HDL-cholesterol levels without the need for any off-line pretreatment or centrifugation steps. The method is in a two-reagent format. The first reagent stabilizes LDL, VLDL, and chylomicrons. Thus, the enzymes of second reagent selectively react with the cholesterol present in the HDL particles. Consequently, only the HDL cholesterol is subject to cholesterol measurement.

*The reaction sequence is as follows:*

$$\text{HDL Cholesterol} \xrightarrow{\textit{cholesteryl ester hydrolase}} \text{cholesterol + fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\textit{cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\textit{peroxidase}} \text{4-(p-benzoquinonemonoimino)-phenazone} + 4\ H_2O$$

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 600 nm;
2. Water bath or heating block capable of maintaining temperature at 37 ± 1 °C;
3. Pipettes capable of accurately dispensing volumes of 0.024, 0.2, and 1.0 mL;
4. Test tubes, 10 mL

### REAGENTS:

***1. Reagent 1***

This solution contains peroxidase ≈ 5 kU/L, 0.9 mM 4-aminophenazone and protector for LDL, 30 mM Tris buffer. **NOTE:** Protect this solution from light;

***2. Reagent 2***

This solution contains enzyme mixture: cholesterol esterase ≈ 4000 U/L; cholesterol oxidase ≈ 10000 U/L; chromogen, 30 mM Tris buffer. **NOTE:** Protect this solution from light;

***3. Cholesterol Standard***

The concentration of cholesterol in this standard solution is 5.17 mmol/L

**PROCEDURE**

| *Pipette the following solutions into the appropriately marked test tubes (B, S and T):* | | | |
|---|---|---|---|
| **Reagent** | **Tube** | | |
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (D)*** |
| ***1. NaCl 0.9%*** | 0.024 mL | 0.012 mL | - |
| ***2. Sample (serum)*** | - | - | 0.024 mL |
| ***3. Reagent 1*** | 2.4 mL | 2.4 mL | 2.4 mL |
| ***4. Cholesterol Standard*** | - | 0.012 mL | - |
| *Carefully mix each tube thoroughly.*<br>*Incubate the test tubes for 5 minutes at 37ºC.*<br>**NOTE:** Protect the reaction from light.<br>*Set wavelength of spectrophotometer at 600 nm and zero the instrument with the "Blank".*<br>*Read the absorbance of all test tubes.*<br>*Record the absorbance of the test tubes:*<br>$E_{S1}$ _____<br>$E_{T1}$ _____<br>*Add into the test tube:* | | | |
| ***3. Reagent 2*** | 0.6 mL | 0.6 mL | 0.6 mL |
| *Carefully mix each tube thoroughly.*<br>*Incubate the test tubes for 5 minutes at 37ºC.*<br>**NOTE:** Protect the reaction from light.<br>*Set wavelength of spectrophotometer at 600 nm and zero the instrument with the "Blank".*<br>*Read the absorbance of all test tubes.*<br>**NOTE:** The final color is stable for at least 5 minutes.<br>*Record the absorbance of the test tubes:*<br>$E_{S2}$ _____<br>$E_{T2}$ _____ | | | |

"

**CALCULATION of HDL-cholesterol concentration (C):**

**1)** $\Delta Es = E_{S2} - E_{S1}$

**2)** $\Delta E_T = E_{T2} - E_{T1}$

**3) C, mmol/L** $= \dfrac{\Delta E_T}{\Delta Es} \times 2.587,$

where 2.587 is the concentration of cholesterol in the standard solution with considering of the dilution degree of initial standard solution (x2)

**Your calculation:**

**1)** _____

**2)** _____

**3)** _____

**CONCLUSION:**

# Lesson 9

The main topic
**“PROTEIN METABOLISM AND ITS REGULATION”**

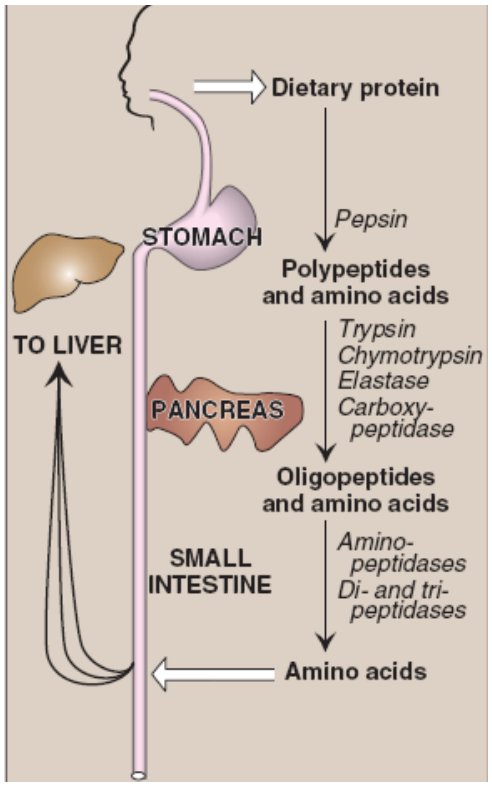
🕮 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Essential, non-essential, conditionally non-essential amino acids;
⇨ Enzymes involved in the digestion of proteins. Mechanisms of their activation;
⇨ Chemical composition of gastric juice. Role of HCl;
⇨ Mechanisms of absorption of amino acids in the intestine;
⇨ Abnormal protein digestion in the gastrointestinal tract;
⇨ The basic pathways of replenishment and use of the amino acid pool;
⇨ Decarboxylation of amino acids: enzymes, physiological significance. Biogenic amines: reactions of their formation, role;
⇨ Direct and indirect deamination of L-amino acids;
⇨ Transamination. Mechanism of action of aminotransferases, their role in metabolism of amino acids, clinical significance of their determination in the blood;
⇨ Pathways of ammonia formation;
⇨ Transport of ammonia from tissues to the liver and kidney. Reactions of glutamine and asparagine formation and their role. Role of alanine in ammonia transport;
⇨ Ornithine cycle of urea formation in the liver: enzymatic reactions, role. Genetic defects of enzymes of urea cycle (enzymopathies);
⇨ Hyperammonemia: its causes, manifestations, consequences;
⇨ Metabolism of carbon skeletons of amino acids in the body, relation to Krebs cycle;
⇨ Glucogenic and ketogenic amino acids.

✎ ***Complete the table of amino acid types:***

| | ***Essential*** | ***Non-essential*** | ***Conditionally non-essential*** |
|---|---|---|---|
| ***Definition*** | | | |
| ***Example*** | | | |

✎ ***Describe the main stage of protein digestion in different part of gastrointestinal tract:***
Include in your description the names of the most important enzymes in this process!!




✎ ***Name the main components of gastric secretion:***

_____

_____

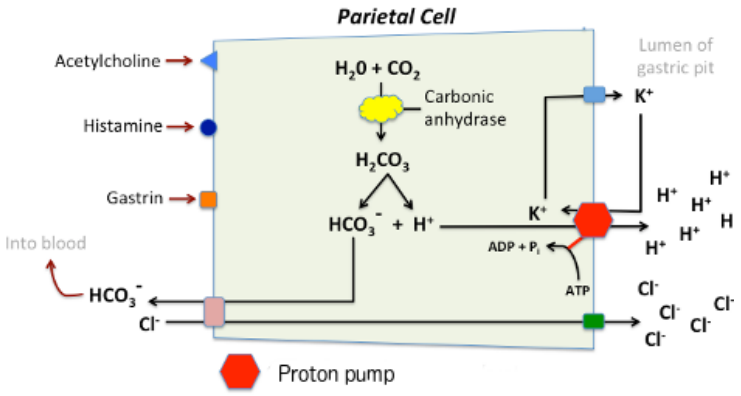
✎ ***State the function of hydrochloric acid:***

*1.* _____

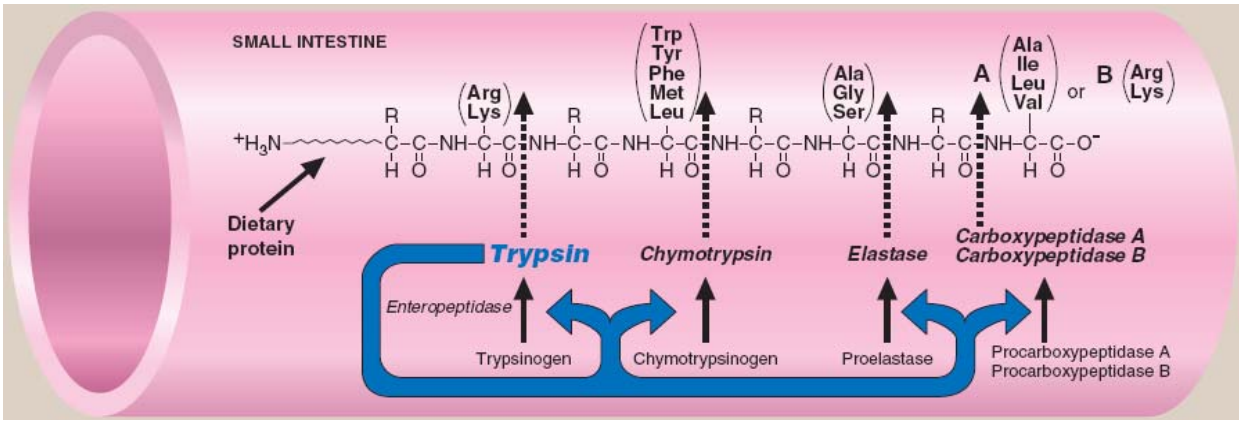
*2.* _____

*3.* _____

✎ ***Describe the synthesis of hydrochloric acid:***




✎ ***Describe the differences in action of the main proteases from the pancreas:***




_____

_____

_____

_____

_____

_____

✎ ***What celiac disease (celiac sprue) is?***

_____

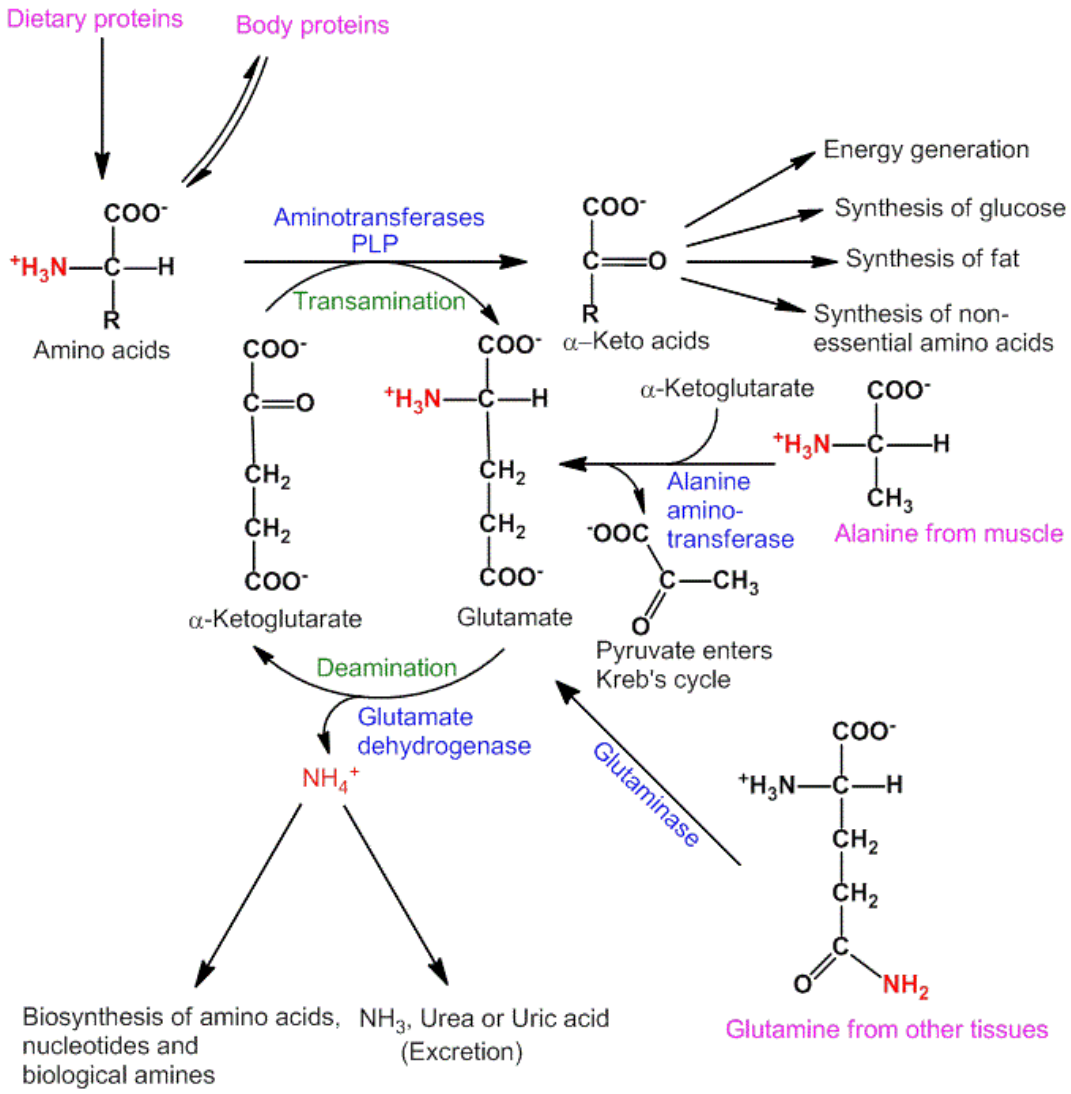
_____

_____

_____

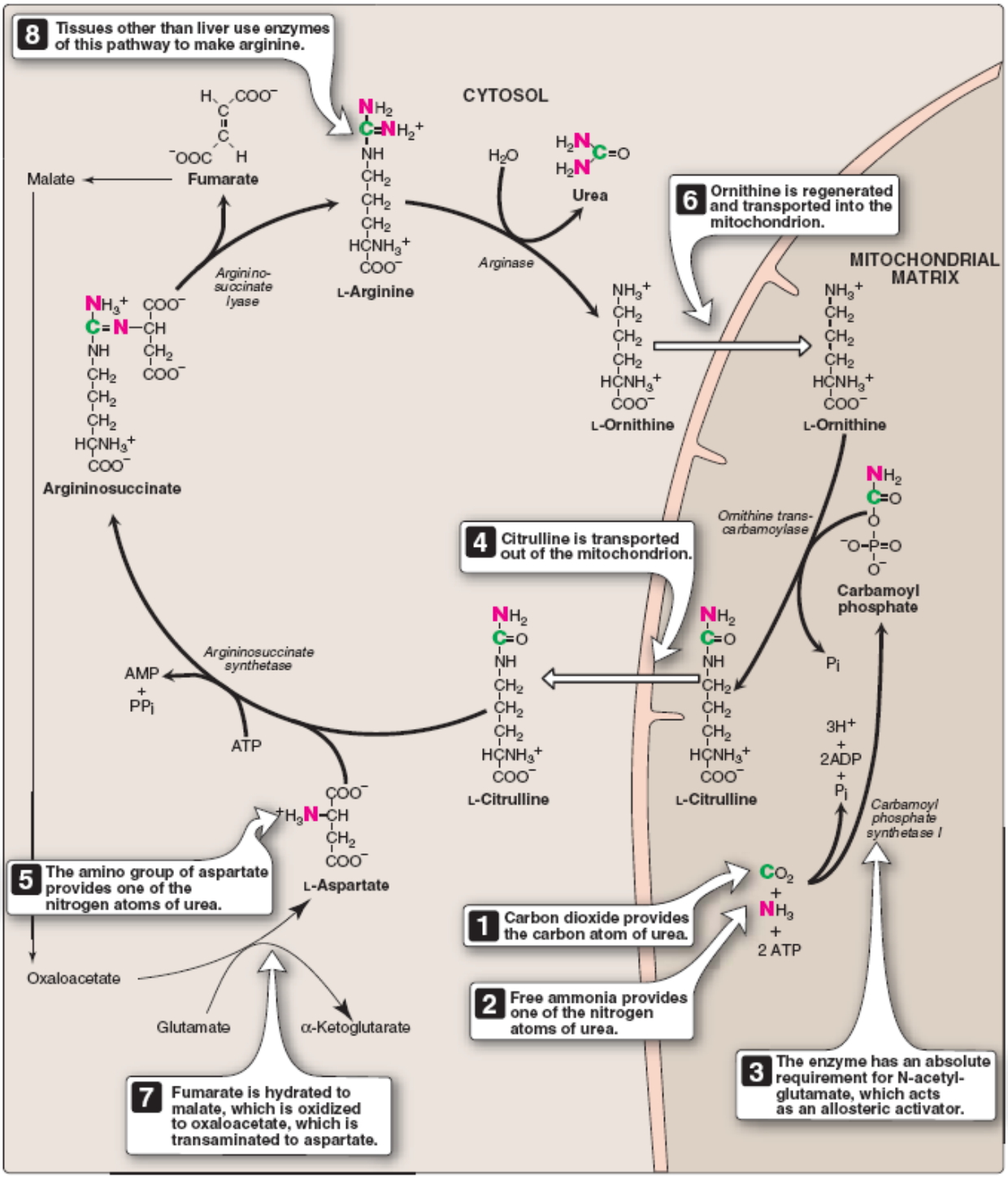
_____

✎ *Describe the main ways of* **REMOVAL OF NITROGEN FROM AMINO ACIDS**




| Oxidative deamination of amino acids | Non-oxidative deamination |
|---|---|
| It is the removal of amino group as free ammonia coupled with oxidation. In the mitochondria, ***glutamate dehydrogenase*** promotes oxidative deamination to convert glutamate to α-ketoglutarate and liberate ammonia through the formation of an intermediate α-iminoglutarate. Glutamate dehyrogenase is the only enzyme which can use either $NAD^+$ or $NADP^+$. The α-ketoglutarate formed is recycled to the Citric acid cycle (Kreb's cycle). ***L-amino acid oxidase and D-amino oxidase*** possessing FMN and FAD are also present to help promote the conversion (oxidation) of glutamate to α-ketoglutarate and ammonia and thus in process the oxygen gets reduced to $H_2O_2$. | Certain amino acids (such as serine, threonine, homoserine, cysteine and histidine) without oxidation get deaminated to liberate ammonia in presence of specific enzymes (dehydratases for -OH containing amino acids, desulfhydrases for sulfur containing amino acids, and histidases for histidine). |

✎ *Describe the reactions of the urea cycle*




✎ *What is the net reaction of the urea cycle?*

_____

✎ *Define the term* **"HYPERAMMONEMIA":**

_____

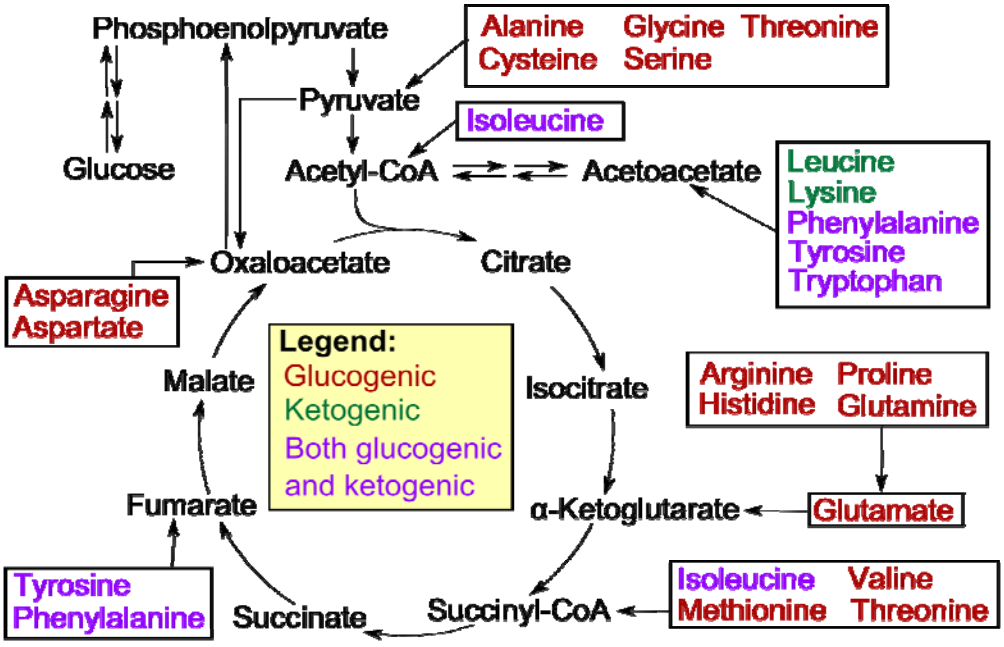
_____

_____

_____

_____

✎ *Describe the* **CATABOLISM OF THE CARBON SKELETONS OF AMINO ACIDS**




✎ ***Define the term "DECARBOXYLATION OF AMINO ACIDS"*:**

_____

_____

_____

Enzyme:_____

Coenzyme _____

✎ ***Complete the table of significant products of amino acid decarboxylation1:***

| *Amino acid* | *Amine* | *Amine function* |
|---|---|---|
| ***Cysteine*** | | |
| ***Glutamate*** | | |
| ***Histidine*** | | |
| ***Tyrosine*** | | |
| ***5-hydroxytryptophan*** | | |

**Questions from KROK-1**

1. Patient with encephalopathy was admitted to the neurological in-patient department. Correlation of increasing of encephalopathy and substances absorbed by the bloodstream from the intestines was revealed. What substances that are created in the intestines can cause endotoxemia?

A Indole
B Butyrate
C Acetacetate
D Biotin
E Ornithine

2. Examination of a patient suffering from cancer of urinary bladder revealed high rate of serotonin and hydroxyanthranilic acid. It is caused by excess of the following amino acid in the organism:

A Tryptophan
B Alanine
C Histidine
D Methionine
E Tyrosine

3. A 4 y.o. child with signs of durative protein starvation was admitted to the hospital. The signs were as follows: growth inhibition, anemia, edema, mental deficiency. Choose a cause of edema development:

A Reduced synthesis of albumins
B Reduced synthesis of globulins
C Reduced synthesis of hemoglobin
D Reduced synthesis of lipoproteins
E Reduced synthesis of glycoproteins

4. The concentration of albumins in human blood sample is lower than normal. This leads to edema of tissues. What blood function is damaged?

A Maintaining the oncotic blood pressure
B Maintaining the Ph level
C Maintaining the body temperature
D Maintaining the blood sedimentation system
E All answers are correct

5. Ammonia is a very toxic substance, especially for nervous system. What substance takes the most active part in ammonia detoxication in brain tissues?

A Glutamic acid
B Lysine
C Proline
D Histidine
E Alanine

6. A patient has pellagra. Interrogation revealed that he had lived mostly on maize for a long time and eaten little meat. This disease had been caused by the deficit of the following substance in the maize:

A Tryptophan
B Tyrosine
C Proline
D Alanine
E Histidine

7. A patient with serious damage of muscular tissue was admitted to the traumatological department. What biochemical urine index will be increased in this case?

A Creatinine
B Common lipids
C Glucose
D Mineral salts
E Uric acid

8. Nappies of a newborn have dark spots that witness of formation of homogentisic acid. Metabolic imbalance of which substance is it connected with?

A Thyrosine
B Galactose
C Methionine
D Cholesterine
E Tryptophane

9. A 1,5-year-old child presents with both mental and physical lag, decolorizing of skin and hair, decrease in catecholamine concentration in blood. When a few drops of 5% solution of trichloroacetic iron had been added to the child's urine it turned olive green. Such alteration are typical for the following pathology of the amino acid metabolism:

A Phenylketonuria
B Alkaptonuria
C Tyrosinosis
D Albinism
E Xanthinuria

10. The greater amount of nitrogen is excreted from the organism in form of urea. Inhibition of urea synthesis and accumulation of ammonia in blood and tissues are induced by the decreased activity of the following liver enzyme:

A Carbamoyl phosphate synthetase
B Aspartate aminotransferase
C Urease
D Amylase
E Pepsin

11. After a serious viral infection a 3-year-old child has repeated vomiting, loss of consciousness, convulsions. Examination revealed hyperammoniemia. What may have caused changes of biochemical blood indices of this child?

A Disorder of ammonia neutralization in ornithinic cycle
B Activated processes of aminoacids decarboxylation
C Disorder of biogenic amines neutralization
D Increased purtefaction of proteins in intestines
E Inhibited activity of transamination enzymes

12. Albinos can't stand sun impact - they don't aquire sun-tan but get sunburns. Disturbed metabolism of what aminoacid underlies this phenomenon?

A Phenilalanine

B Methionine
C Tryptophan
D Glutamic acid
E Histidine

13. Glutamate decarboxylation results in formation of inhibitory transmitter in CNS. Name it:
A GABA
B Glutathione
C Histamine
D Serotonin
E Asparagine

14. In course of histidine catabolism a biogenic amin is formed that has powerful vasodilatating effect. Name it:
A Histamine
B Serotonin
C Dioxyphenylalanine
D Noradrenalin
E Dopamine

15. A patient diagnosed with carcinoid of bowels was admitted to the hospital. Analysis revealed high production of serotonin. It is known that this substance is formed of tryptophane aminooacid. What biochemical mechanism underlies this process?
A Decarboxylation
B Desamination
C Microsomal oxydation
D Transamination
E Formation of paired compounds

16. During hypersensitivity test a patient got subcutaneous injection of an antigen which caused reddening of skin, edema, pain as a result of histamine action. This biogenic amine is generated as a result of transformation of the following histidine amino acid:
A Decarboxylation
B Methylation
C Phosphorylation
D Isomerization
E Deaminization

17. A patient complained about dizziness, memory impairment, periodical convulsions. It was revealed that these changes were caused by a product of decarboxylation of glutamic acid. Name this product:
A GABA
B Pyridoxal phosphate
C TDP
D ATP
E THFA

18. Laboratory examination of a child revealed increased concentration of leucine, valine, isoleucine and their ketoderivatives in blood and urine. Urine smelt of maple syrup. This disease is characterized by the deficit of the following enzyme:
A Dehydrogenase of branched amino acids
B Aminotransferase
C Glucose-6-phosphatase
D Phosphofructokinase
E Phosphofructomutase

19. A newborn child was found to have reduced intensity of sucking, frequent vomiting, hypotonia. In urine and blood exhibit increased concentration of citrulline. What metabolic process is disturbed?
A Ornithinic cycle
B Tricarboxylic acid cycle
C Glycolysis
D Glyconeogenesis
E Cori cycle

20. Plasmic factors of blood coagulation are exposed to post-translational modification with the participation of vitamin K. It is necessary as a cofactor in the enzyme system of gammacarboxylation of protein factors of blood coagulation due to the increased affinity of their molecules with calcium ions. What amino acid is carboxylated in these proteins?
A Glutamate
B Valine
C Serine
D Phenylalanine
E Arginine

21. Pharmacological effects of antidepressants are connected with inhibition of an enzyme catalyzing biogenic amines noradrenaline and serotonine in the mitochondrions of cerebral neurons. What enzyme participates in this process?
A Monoamine oxidase
B Transaminase
C Decarboxylase
D Peptidase
E Lyase

22. A child manifests epileptic seizures caused by vitamin B6 deficiency. This is conditioned by the decrease of the gamma-aminobutyrate level in the nervous tissue which acts as an inhibiting neurotransmitter. The activity of which enzyme is decreased in this case?
A. Pyridoxal kinase
B. Alanine aminotransferase
C. Glutamate dehydrogenase
D. Glutamate decarboxylase
E. Glutamate synthetase

# *PRACTICAL WORK 7*

## DETERMINATION OF UREA IN SERUM BYCOLORIMETRIC METHOD

### 🕮 BACKGROUND

UREA is primarily produced in the liver and secreted by the kidneys. Urea is the major end product of protein catabolism in animals. It is the primary vehicle for removal of toxic ammonia from the body.

**Diagnostic significance:**

The determination of urea is very useful for the medical clinician to assess kidney function of patients. In general, increased urea levels are associated with nephritis, renal ischemia, urinary tract obstruction, and certain extrarenal diseases, e.g., congestive heart failure, liver diseases and diabetes. The decreased levels indicate acute hepatic insufficiency or may result from over-vigorous parenteral fluid therapy.

**Normal reference values**:

Serum: 1.7 – 8.3 mmol/L

Urine: 333 – 583 mmol/24 h

### ASSAY PRINCIPLE:

Urea in the presence of $Fe^{3+}$ and tiosemicarbaside in acidic medium condenses with diacetyl monoxime at 100°C to form a red coloured complex. The intensity of the formed color is directly proportional to the amount of urea present in the sample. Absorbance is measured at 540-560 nm.

$$\text{Urea + Diacetyl monoxime} \xrightarrow{100°C} \text{Red Coloured Complex}$$

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 546 nm;
2. Water bath capable of maintaining temperature at 100 ± 2 °C;
3. Low-speed centrifuge (3 rpm required);
4. Pipettes capable of accurately dispensing volumes of 0.1 and 1 mL;
5. Test tubes, 10 mL

### REAGENTS:

***1. Diacetyl monoxime solution;***

***2. Tiosemicarbaside solution;***

***2. Urea Standard***

The concentration of urea in this standard solution is 10 mmol/L;

***3. Acid reagent***

This solution contains 5% trichloroacetic acid (TCA)

**NOTE:** Be very careful using this solution. Do not pipette this solution with mouth!!

### PROCEDURE

**Sample preparation:**

- 0.1 mL of ***serum*** mix with 0.9 mL of ***acid reagent*** and centrifuge mixer at 2500 rpm for 5 min.
- 0.1 mL of ***urea standard*** mix with 0.9 mL of ***acid reagent*** and centrifuge mixer at 2500 rpm for 5 min.

In the further step use 0.1 mL of ***deproteinated serum*** and ***urea standard after centrifugation.***

***NOTE:*** The final results multiply by coefficient of dilution (x10).

*Pipette the following solutions into the appropriately marked dry clean test tubes (B, S, T):*

| Reagent | Tube | | |
|---|---|---|---|
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (T)*** |
| ***1. Sample*** (deproteinated serum) | - | - | 0.01 mL |
| ***2. Urea standard*** (after centrifugation) | - | 0.01 mL | - |
| ***3. NaCl 0.9%*** | 0.01 mL | - | - |
| ***4. Tiosemicarbaside solution*** | 1 mL | 1 mL | 1 mL |
| ***5. Diacetyl monoxime solution*** | 1 mL | 1 mL | 1 mL |

*Close test tubes with stoppers.*
*Carefully mix each tube thoroughly.*
*Place all test tubes into the boiling water bath (100 ± 2 °C) exactly for 10 minutes.*
*Remove all test tubes and cool them under running tap water for 5 minutes.*
*Set wavelength of spectrophotometer at 546 nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 15 minutes.

*Record the absorbance of the test tubes:*

$E_S$ _____

$E_T$ _____

**CALCULATION of urea concentration (C):**

$$\textbf{C, mmol/L} = \frac{E_T}{Es} \times 10 \times 10,$$

where 10 is the concentration of urea in the standard solution (10 mmol/L);
10 is the coefficient of dilution

**Your calculation: _____**

**CONCLUSION:**

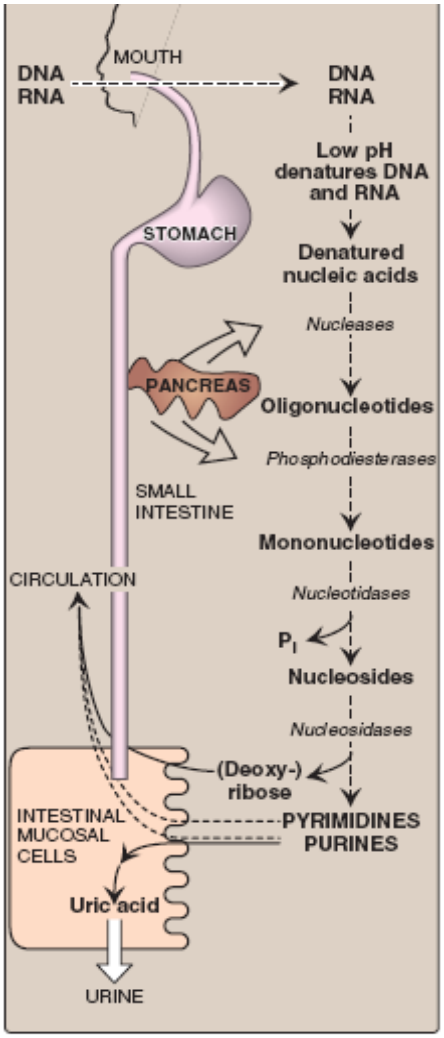
## Lesson 10

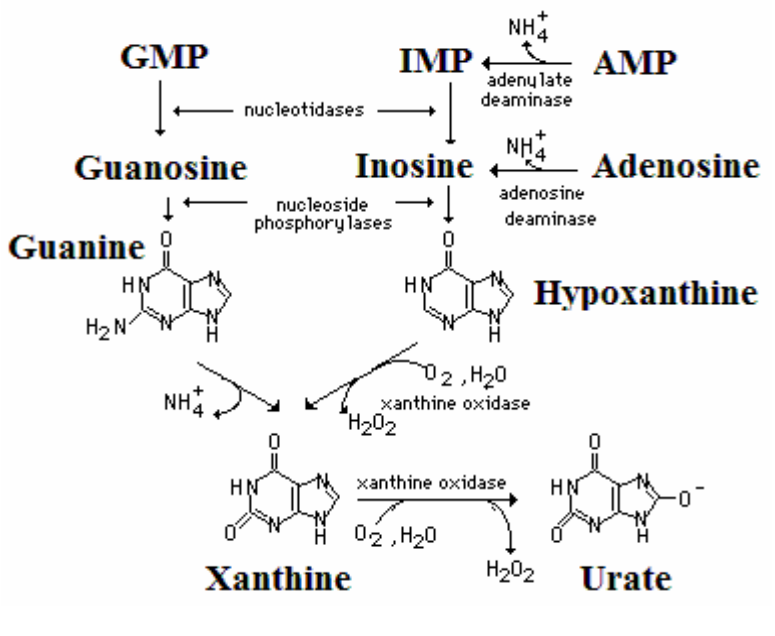
The main topic
**“NUCLEOTIDES METHABOLISM AND ITS REGULATION”**

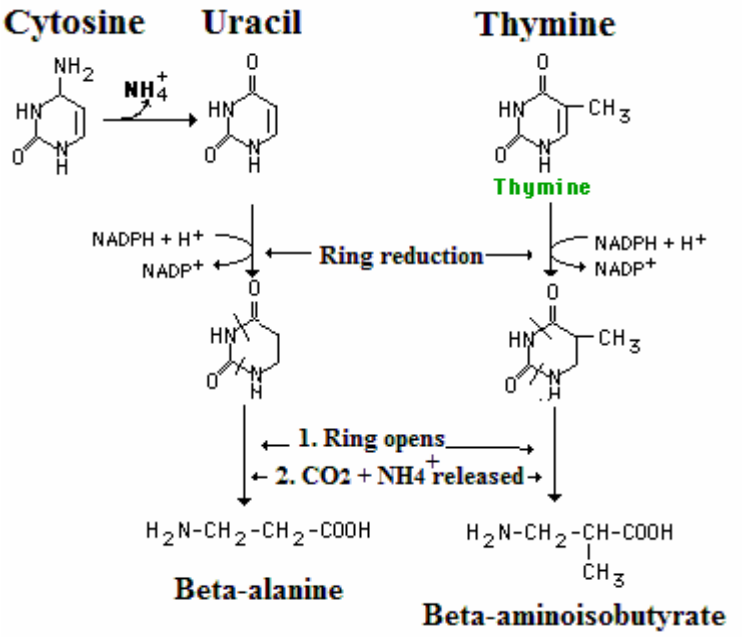
🕮 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Structure and role of nucleotides;
⇨ Breakdown of purine and pyrimidine nucleotides;
⇨ Synthesis of purine and pyrimidine nucleotides. Regulation. Disorders.

✎ ***Describe the main stage of nucleic acids digestion in different part of gastrointestinal tract:***
Include in your description the names of the most important enzymes in this process!!



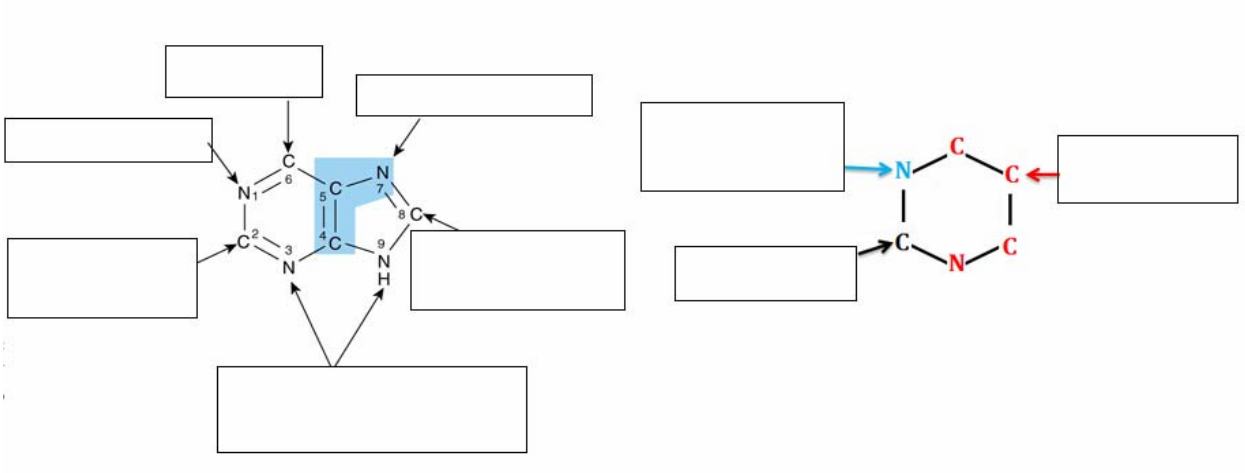




✎ ***Define the term “HYPERURICEMIA”*:**

✎ ***Describe the symptoms of "Gout Diseases":***

✎ ***Label the sources of the individual atoms in the purine and pyrimidine rings:***



# *PRACTICAL WORK 8*

## DETERMINATION OF URIC ACID IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

URIC ACID is the major product of the catabolism of purine nucleosides (adenosine and guanosine) from the purine metabolism pathway. Nearly half of the uric acid is eliminated and replaced daily by urinary excretion and through microbial degradation in the intestinal tract.

**Diagnostic significance:**

An abnormal increase in the level of uric acid in the circulation above 7.0 mg/dL (0.42 mmol/L) is referred to as hyperuricemia. The gout is the major form of the ailment resulting in the deposition of urates in the soft tissues, especially in the joint areas. The increased levels may be also associated with leukemia, toxemia of pregnancy and severe renal impairment. Less common are the cases of hypouricemia where the concentration of uric acid is below 2.0 mg/dL (0.12 mmol/L). These cases are usually secondary to cases of hepatocellular disease, renal reabsorption defect, or overtreatment with uricosuric drugs used in the treatment of hyperuricemia.

**Normal reference values**:

Serum: children less than 12 y.o.: 119 – 327 µmol/L
adults 12-60 y.o.: 246 – 452 µmol/L (man)
137 – 393 µmol/L (woman)
adults 60-90 y.o.: 250 – 476 µmol/L (man)
208 – 434 µmol/L (woman)
Urine: 1.48-4.43 mmol/24 h

### ASSAY PRINCIPLE:

Uric acid in basic medium reduce phosphotungstic reagent to form blue colored product. The depth of blue color is proportional to the concentration of uric acid.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 650 nm;
2. Low-speed centrifuge (3000 rpm required);
3. Pipettes capable of accurately dispensing volumes of 0.05, 0.2, and 1 mL;
4. Test tubes, 10 mL.

### REAGENTS:

***1. Phosphotungstic reagent***

This solution contains 0.12 mol/L of $Na_2WO_4$, 0.43 mol/L of $H_3PO_4$ and 0.29 mol/L of $Li_2SO_4$;

***2. Catalyst;***

***3. Sodium tungstate***

The concentration of $Na_2WO_4$ in this solution is 0.3 mol/L;

***4. Uric acid standard***

The concentration of uric acid in this standard solution is 300 µmol/L;

***5. Sodium carbonate***

The concentration of $Na_2CO_3$ in this solution is 10%;

**PROCEDURE**

| *Pipette the following solutions into the appropriately marked dry clean test tubes (B, S, T):* | | | |
|---|---|---|---|
| **Reagent** | **Tube** | | |
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (T)*** |
| ***1. Distillate water*** | 4.5 mL | 4 mL | 4 mL |
| ***2. Sample (serum)*** | - | - | 0.5 mL |
| ***3. Uric acid standard*** | - | 0.5 mL | - |
| ***4. Catalyst*** | 0.25 mL | 0.25 mL | 0.25 mL |
| ***5. Sodium tungstate*** | 0.25 mL | 0.25 mL | 0.25 mL |
| *Carefully mix each tube thoroughly.*<br>*Lat the test tubes stand for 10 minutes at room temperature.*<br>*Centrifuge at 3000 rpm for 5 min, take the supernatant for further step of experiment* | | | |
| ***6. Supernatant*** | 2 mL | 2 mL | 2 mL |
| ***7. Sodium carbonate*** | 1 mL | 1 mL | 1 mL |
| ***8. Phosphotungstic reagent*** | 0.6 mL | 0.6 mL | 0.6 mL |
| *Carefully mix each tube thoroughly.*<br>*Let the test tubes stand for 30 minutes at room temperature.*<br>*Set wavelength of spectrophotometer at 650nm and zero the instrument with the "Blank".*<br>*Read the absorbance of all test tubes.*<br>**NOTE:** The final color is stable for at least 30 minutes.<br><br>*Record the absorbance of the test tubes:*<br>$E_S$ _____<br>$E_T$ _____ | | | |

**CALCULATION of uric acid concentration (C):**

$$\textbf{C, µmol/L} = \frac{E_T}{Es} \times 300,$$

where 300 is the concentration of uric acid in the standard solution (µmol/L)

**Your calculation: _____**

**CONCLUSION:**

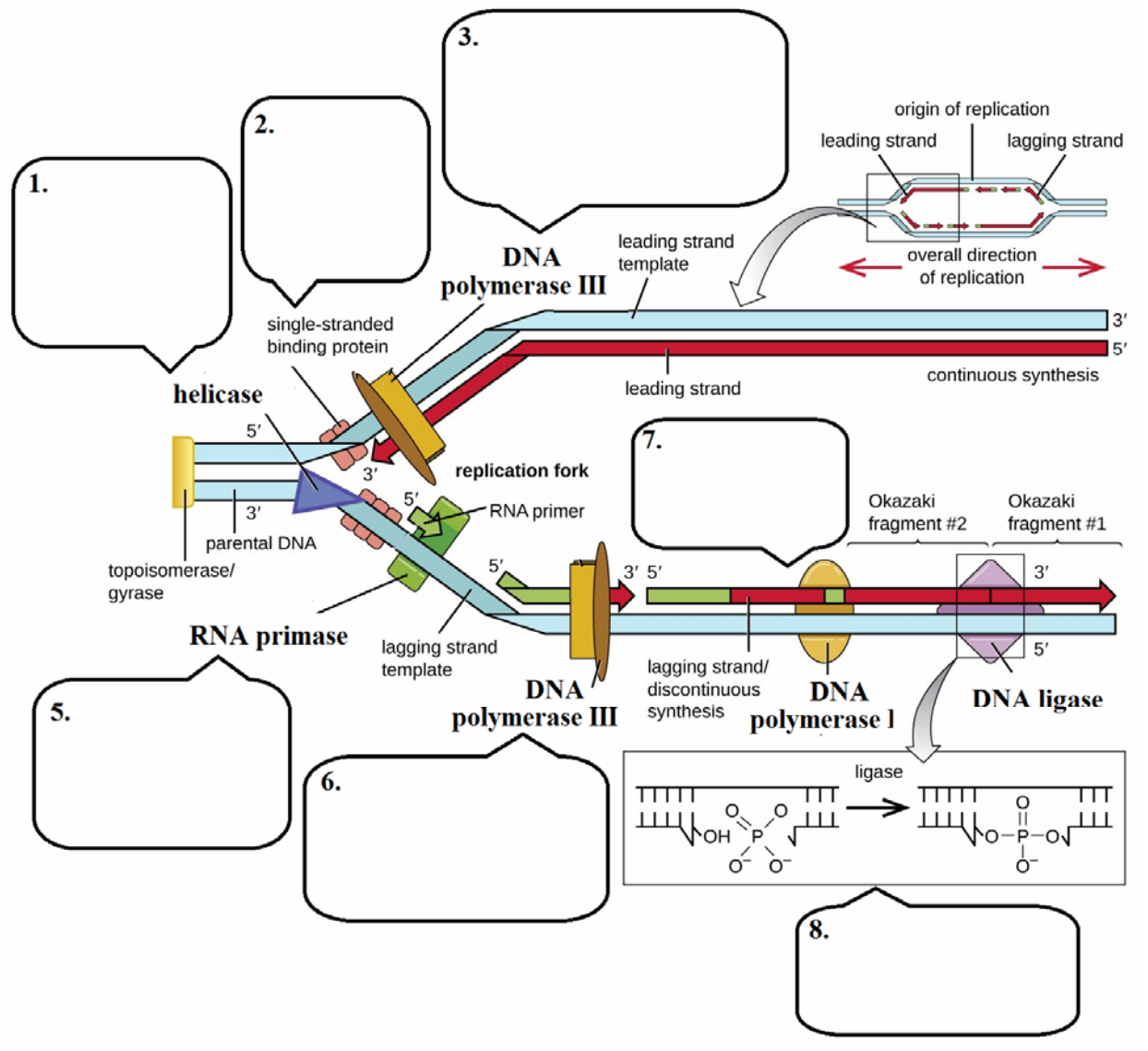
# Lesson 11

The main topic
**“SYNTHESIS OF NUCLEIC ACIDS AND PROTEINS”**

📖 ***Follow this plan at home to prepare for classroom discussion:***

⇨ DNA replication; biological significance; semi-conservative mechanism of replication;
⇨ Biological significance and mechanisms of DNA repair;
⇨ Mutations: genomic, chromosomal, gene;
⇨ Molecular mechanisms of RNA transcription;
⇨ RNA posttranscriptional modification. Antibiotics that are inhibitors of transcription;
⇨ Ribosomal protein-synthesizing system. Structure of eukaryotic ribosomes;
⇨ tRNA and activation of amino acids. Aminoacyl-tRNA synthetases;
⇨ Stages and mechanisms of translation. Initiating and terminating codons of mRNA;
⇨ Posttranslational modification of peptide chains;
⇨ Regulation of translation. Antibiotics that are inhibitors of translation in prokaryotes and eukaryotes, their use in medicine;

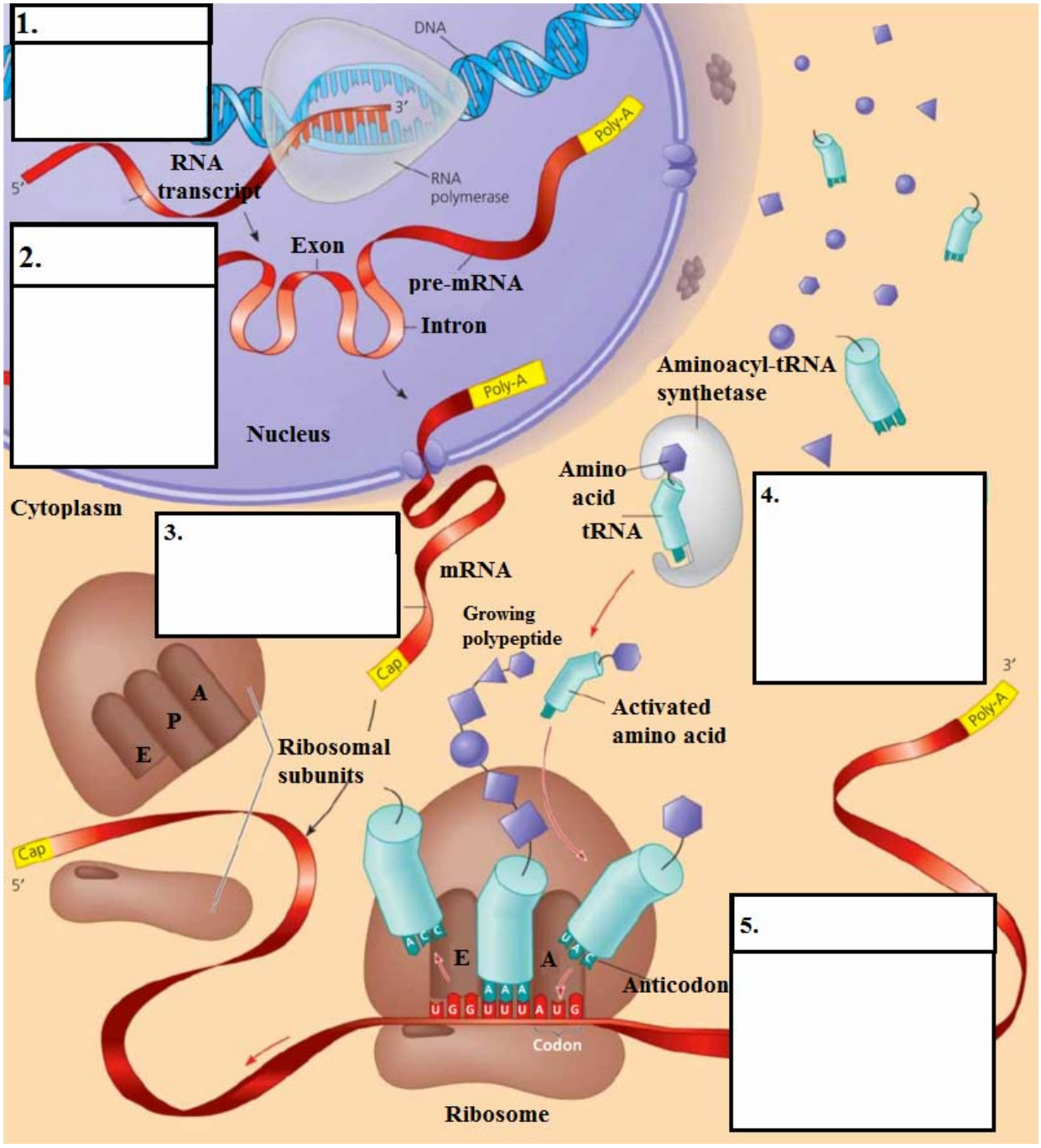
✎ ***Describe the main stage of DNA replication:***



✎ ***Complete the table of the types of mutation:***

| Type | | Definition | Example |
|---|---|---|---|
| ***point mutation*** | ***silent*** | | *DNA level* TTC TT**T**<br>*RNA level* AAG ⇨ AA**A**<br>*Protein level* Lys **Lys** |
| | ***missense*** | | *DNA level* TTC T**G**C<br>*RNA level* AAG ⇨ A**C**G<br>*Protein level* Lys **Thr** |
| | ***nonsense*** | | *DNA level* TTC **A**TC<br>*RNA level* AAG ⇨ **U**AG<br>*Protein level* Lys **STOP** |
| ***Frame shift mutation*** | ***additions*** | | *DNA* GGT-CTC-CTC GGT-**G**CT-CCT-C<br>*RNA* CCA-GAG-GAG ⇨ CCA-**C**GA-GGA-G<br>*AAs* Pro-Glu-Glu Pro-**Arg-Gly…** |
| | ***deletion*** | | *DNA* GGT-CTC-CTC GGT-**C..C**-CTC<br>*RNA* CCA-GAG-GAG ⇨ CCA-GGG-AG..<br>*AAs* Pro-Glu-Glu Pro- **Gly…** |
| ***Chromosome mutation*** | ***deletion*** | | ABCDEF ⇨ AB..DEF |
| | ***duplication*** | | ABCDEF ⇨ AB**B**CDEF |
| | ***inversion*** | | AB**CDE**F ⇨ AB**ECD**F |
| | ***translocation*** | | ABCDEF ABC**def**<br>abcdef ⇨ abc**DEF** |
| ***Genome mutation*** | ***aneuploidy*** | | ***2n*** ⇨ 2n+1 or 2n-1 |
| | ***polyploidy*** | | ***2n*** ⇨ 3n |
| | ***autopolyploidy*** | | |
| | ***allopolyploidy*** | | |

✎ *Describe the main stage of protein synthesis:*



## Questions from KROK-1

1. Methotrexate (competitive inhibitor of the dihydrofolatreductase) is prescribed for treatment of the tumour.On which level does methotrexate inhibit synthesis of the nucleic acids?
A Mononucleotide synthesis
B Replication
C Transcription
D Reparation
E Processing

2. Blood of a 12 year old boy presents low concentration of uric acid and accumulation of xanthine and hypoxanthine. This child has genetic defect of the following enzyme:
A Xanthine oxidase
B Arginase
C Urease
D Ornithine carbamoyltransferase
E Glycerylkinase

3.An experiment proved that UV-radiated cells of patients with xeroderma pigmentosum restore the native DNA structure slower than cells of healthy individuals as a result of reparation enzyme defect. What enzyme helps this process?
A Endonuclease
B RNA ligase
C Primase
D DNA polymerase III
E DNA gyirase

4. A 20 year old patient complains of general weakness, dizziness, quick fatigability. Blood analysis results: Hb- 80 g/l. Microscopical examination results: erythrocytes are of modified form. This condition might be caused by:
A Sickle-cell anemia
B Hepatocellular jaundice
C Acute intermittent porphyria
D Obturative jaundice
E Addison's disease

5. A 48 year old patient complained about intense pain, slight swelling and reddening of skin over the joints, temperature rise up to 38oC. Blood analysis revealed high concentration of urates. This condition might be caused by disturbed metabolism of:
A Purines
B Collagen
C Cholesterol
D Pyrimidines
E Carbohydrates

6. A patient has yellow skin colour, dark urine, achromatic feces. What substance will have strengthened concentration in the blood serum?
A Unconjugated bilirubin
B Conjugated bilirubin
C Mesobilirubin
D Verdoglobin
E Biliverdin

7. A 46 year old woman suffering from chololithiasis developed jaundice. Her urine became dark-yellow and feces became colourless. Blood serum will have the highest concentration of the following substance:
A Conjugated bilirubin
B Unconjugated bilirubin
C Biliverdin
D Mesobilirubin
E Urobilinogen

8. A 46 year old patient applied to a doctor complaining about joint pain that becomes stronger the day before weather changes. Blood examination revealed strengthened concentration of uric acid. The most probable cause of the disease is the intensified disintegration of the following substance:
A Adenosine monophosphate
B Cytidine monophosphate
C Uridine triphosphate
D Uridine monophosphate
E Thymidine monophosphate

9. A 42-year man suffering from gout has increased level of urinary acid in the blood. Allopurinol was prescribed to decrease the level of urinary acid. Competitive inhibitor of what enzyme is allopurinol?
A Xanthinoxidase
B Adenosinedeaminase
C Adeninephosphoribosiltransferase
D Hypoxantinphosphoribosiltransferase
E Guaninedeaminase

10. Patient experienced increased susceptibility of the skin to the sunlight. His urine after some time became dark-red. What is the most likely cause of this?
A Porphyria
B Hemolytic jaundice
C Albinism
D Pellagra
E Alkaptonuria

11. A 65 year old man suffering from gout complains of kidney pain. Ultrasound examination revealed renal calculi. The most probable cause of calculi formation is the strengthened concentration of the following substance:
A Uric acid
B Cholesterol
C Bilirubin
D Urea
E Cystine

12. It was found out that some compounds, for instance fungi toxins and some antibiotics can inhibit activity of RNA-polymerase. What process will be disturbed in a cell in case of inhibition of this enzyme?
A Transcription
B Processing
C Replication
D Translation
E Reparation

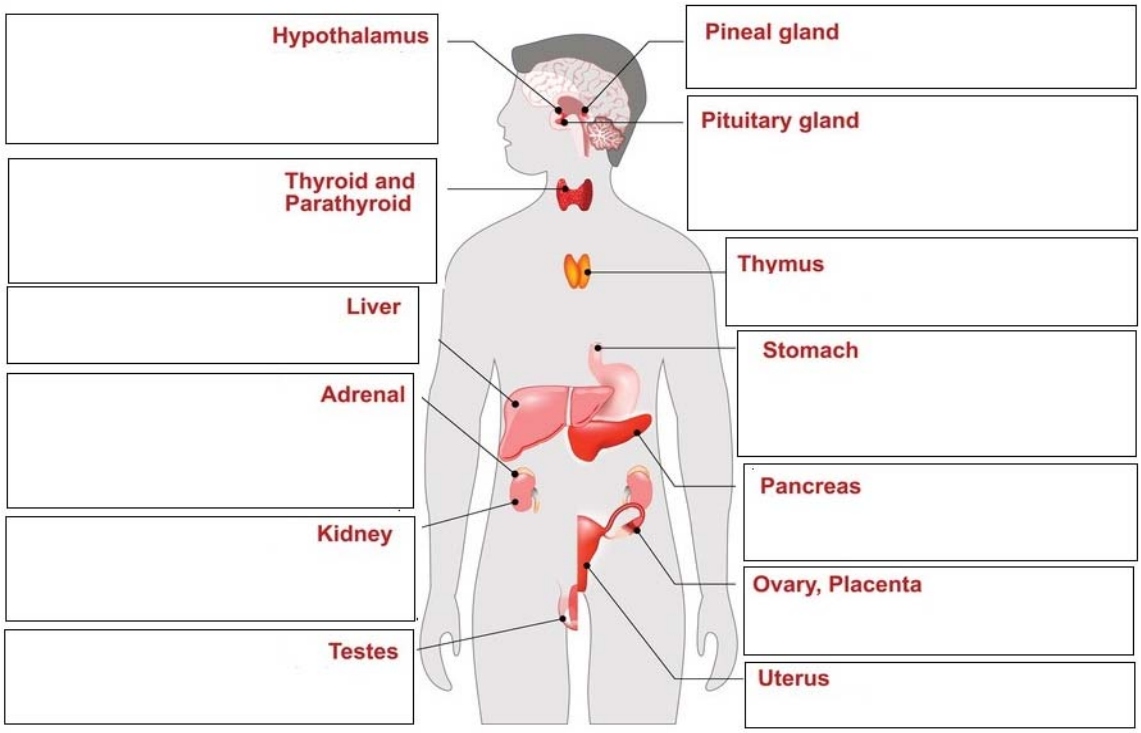
# Lesson 12

The main topic
**“HORMONES: STRUCTURE, BIOLOGICAL SIGNIFICANCE AND MECHANISM OF THEIR ACTION”**

📖 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Hormones: general description, classification, role;
⇨ Molecular and cellular mechanisms of action for steroid and thyroid hormones;
⇨ Mechanisms of action of protein-peptide hormones and hormones that are amino acid derivatives. Biochemical systems of intracellular transduction of hormonal signals;
⇨ Hormones of hypothalamus: structure, role, mechanism of action, changes in production;
⇨ Hormones of the anterior pituitary. Pathological processes associated with dysfunction of these hormones;
⇨ Vasopressin and oxytocin: structure, biological functions, abnormal synthesis and secretion;
⇨ Insulin: structure, biosynthesis, secretion; effects on metabolism of carbohydrate, lipids, and proteins. Growth-stimulating effects of insulin. Disorders of synthesis and secretion;
⇨ Glucagon: structure, role in metabolism, secretion disorders;
⇨ Thyroid hormones: structure, biological effects, mechanism of action;
⇨ Catecholamines: structure, physiological effects, biochemical mechanisms of action;
⇨ Steroid hormones of the adrenal cortex (C21-steroids) - glucocorticoids and mineralocorticoids; structure, properties, mechanism of action;
⇨ Male and female sex hormones. Physiological and biochemical effects; regulation of synthesis and secretion;
⇨ General characteristics of intestinal hormones;
⇨ Structure and role of melatonin, synthesis site, mechanism of action, disorders;
⇨ Eicosanoids: structure, biological and pharmacological properties. Aspirin and other nonsteroidal anti-inflammatory drugs as inhibitors of prostaglandin synthesis.

✎ ***Complete the figure with the name of appropriate hormones:***




✎ ***Complete the table of structure and biological significance of main hormones:***

| Gland | Main hormone released | Structure | Effect |
|---|---|---|---|
| ***Hypotha-lamus*** | | | |
| ***Pituitary Gland (Master Gland)*** | ***Anterior*** | | |
| | ***Posterior*** | | |

| | | | |
|---|---|---|---|
| ***Pineal Gland*** | | | |
| ***Thyroid Gland*** | | | |
| ***Parathyroid Gland*** | | | |
| ***Pancreas*** | | | |
| ***Adrenal Gland*** | ***Adrenal Medulla*** | | |

| | | | |
|---|---|---|---|
| | ***Adrenal Cortex*** | | |
| ***Ovaries (female)*** <br> ***Placenta*** | | | |
| ***Testes (male)*** | | | |

✎ ***Describe two mechanism of hormone action (draw the scheme of general mechanism):***

| **Hormones with Cell Surface Receptors** | **Hormones with Intracellular Receptors** |
|---|---|

| | |
|---|---|
| | |

# *PRACTICAL WORK 9*

## DETERMINATION OF THE INORGANIC PHOSPHORUS IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

PHOSPHATE is one of the most important of the inorganic ions in biological systems. It functions in a variety of roles. One of the most important roles is as a molecular switch, turning enzyme activity on and off through the mediation of the various protein kinases and phosphatases in biological systems. Phosphate is also of great importance in mineralization processes.

**Diagnostic significance**:

The increased levels are found in hypoparathyroidism, renal failure, bone metastasis and liver diseases. The decreased levels are found in hyperparathyroidism, rickets and Vitamin D deficiency.

**Normal reference values**:

Serum: adults 12-60 y.o.: 0.87 – 1.45 mmol/L
children 2-12 y.o.: 1.45 – 1.78 mmol/L
Urine: 29 – 48 mmol/24 hours (or 0.9 - 1.5 g/24 hours)

### ASSAY PRINCIPLE:

Phosphate ions in an acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex in the presence of different reducing agents can be reduced to a molybdinum blue complex. The intensity of the formed molybdinum blue complex is directly proportional to the amount of inorganic phosphorus present in the sample.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 620 nm;
2. Pipettes capable of accurately dispensing volumes of 0.05 and 1.0 mL;
3. Test tubes, 10 mL

### REAGENTS:

***1. Color Reagent***

This solution contains ammonium molybdate (40 mmol/L) prepared on 1.1 N sulfuric acid;

***2. Reducing agents***

This solution contains triethanolamine in concentration of 3.42 mol/L;

***3. Phosphorus Standard***

The concentration of phosphorus in this standard solution is 1.615 mmol/L (or 5 mg/dl).

### PROCEDURE

| *Pipette the following solutions into the appropriately marked dry clean test tubes (B, S and T):* | | | |
|---|---|---|---|
| **Reagent** | **Tube** | | |
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (T)*** |
| ***1. Sample (serum)*** | - | - | 0.05 mL |
| ***2. Phosphorus Standard*** | - | 0.05 mL | - |
| ***3. Distilled water*** | 0.05 mL | - | - |
| ***4. Color Reagent*** | 1 mL | 1 mL | 1 mL |
| *Carefully mix each tube thoroughly.*<br>*Let the test tubes stand for 15 minutes at room temperature.*<br>*Add into the test tubes:* | | | |

| | | | |
|---|---|---|---|
| ***5. Reducing agents*** | 1 mL | 1 mL | 1 mL |

*Carefully mix each tube thoroughly.*
*Set wavelength of spectrophotometer at 620nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 15 minutes.

*Record the absorbance of the test tubes:*

$E_S$ _____

$E_T$ _____

**CALCULATION of inorganic phosphorus concentration (C):**

$$\textbf{C, mmol/L} = \frac{E_T}{Es} \times 1.615,$$

where 1.615 is the concentration of inorganic phosphorus in the standard solution (mmol/L)

**Your calculation: _____**

**CONCLUSION:**

# Lesson 13

The main topic
**"BIOCHEMISTRY OF BLOOD (part 1)"**

📖 ***Follow this plan at home to prepare for classroom discussion:***

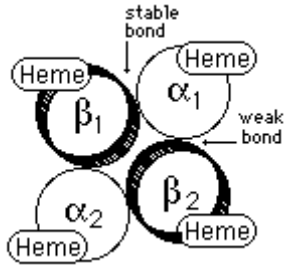
⇨ Functions of blood in maintenance of vital processes;
⇨ Physical and chemical properties of blood, serum, lymph, pH, osmotic and oncotic pressure, relative density, viscosity;
⇨ Inorganic compounds of blood: their content and role;
⇨ Acid-base balance of blood, its regulation, disorders. Blood buffer systems. Their role in maintenance of acid-base balance. Disorders: acidosis, alkalosis;
⇨ Structure, role, and properties of hemoglobin. Types of hemoglobin. Mechanisms of hemoglobin participation in the transport of $O_2$ and $CO_2$. Pathological forms of hemoglobin;
⇨ Hemoglobin synthesis. Regulation of this process. Porphyrias: causes, types;
⇨ Hemoglobin breakdown. Bile pigments, their transformation, significance of their determination. Pathobiochemistry of jaundices;
⇨ Hemoglobinopathies and thalassemias.

✎ ***Complete the table of normal blood composition:***

<table>
<tr><th colspan="2">Plasma<br>55-60% v/v</th><th colspan="2">Cellular components and their functions</th></tr>
<tr><td>Water</td><td>91%</td><td rowspan="2">RBCs<br>normal value –</td><td rowspan="2"></td></tr>
<tr><td rowspan="3">Solid<br>9%</td><td>Inorganic components:<br>Na –<br>K –<br>Ca –<br>Mg –<br>Cl –<br>$HCO_3^-$ –<br>$HPO_4^{2-}$ –</td></tr>
<tr><td rowspan="2">Organic components:<br>- Glucose –<br>- Lactate –<br>- Pyruvate –<br>- Urea –<br>- Uric acid –<br>- Creatinin –<br>- Ammonia –<br>- Protein –<br>-Hemoglobin –<br>- Bilirubin –<br>- Lipids (total) –<br>- Triacylglycerols –<br>- Cholesterol –<br>- LDL –<br>- HDL –<br>- ALT –<br>- ASL –<br>- LDH –<br>- alpha-amilase –</td><td>WBCs<br>Neu –<br>Lym –<br>Eos –<br>Mon –<br>Bas –</td><td></td></tr>
<tr><td>Platelets<br>normal value –</td><td></td></tr>
</table>

✎ ***Explain the difference between PLASMA and SERUM:***

✎ ***Describe HEMOGLOBIN STRUCTURE (Hb A):***




**Minor hemoglobins:**

*Fetal hemoglobin (Hb F)*

*Hemoglobin A2 (Hb A2):*

*Hemoglobin A1c (HbA1c):*

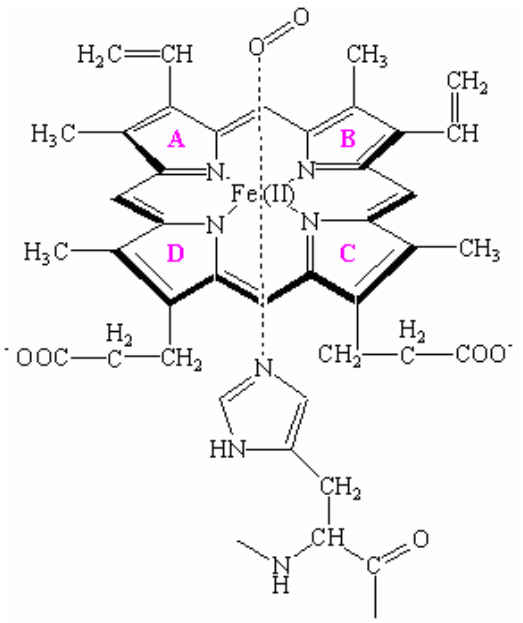
✎ ***Define the following terms:***

*oxyhemoglobin:*

*deoxyhemoglobin:*

*carboxyhemoglobin:*

*methemoglobin:*




✎ ***Describe the main* HEMOGLOBINOPATHIES:**

**Sickle cell anemia (hemoglobin S disease)**

**Hemoglobin C disease**

**Hemoglobin SC disease**

**Methemoglobinemias**

**Thalassemias**

✎ ***Describe the main* stage *of hemoglobin catabolism*:**

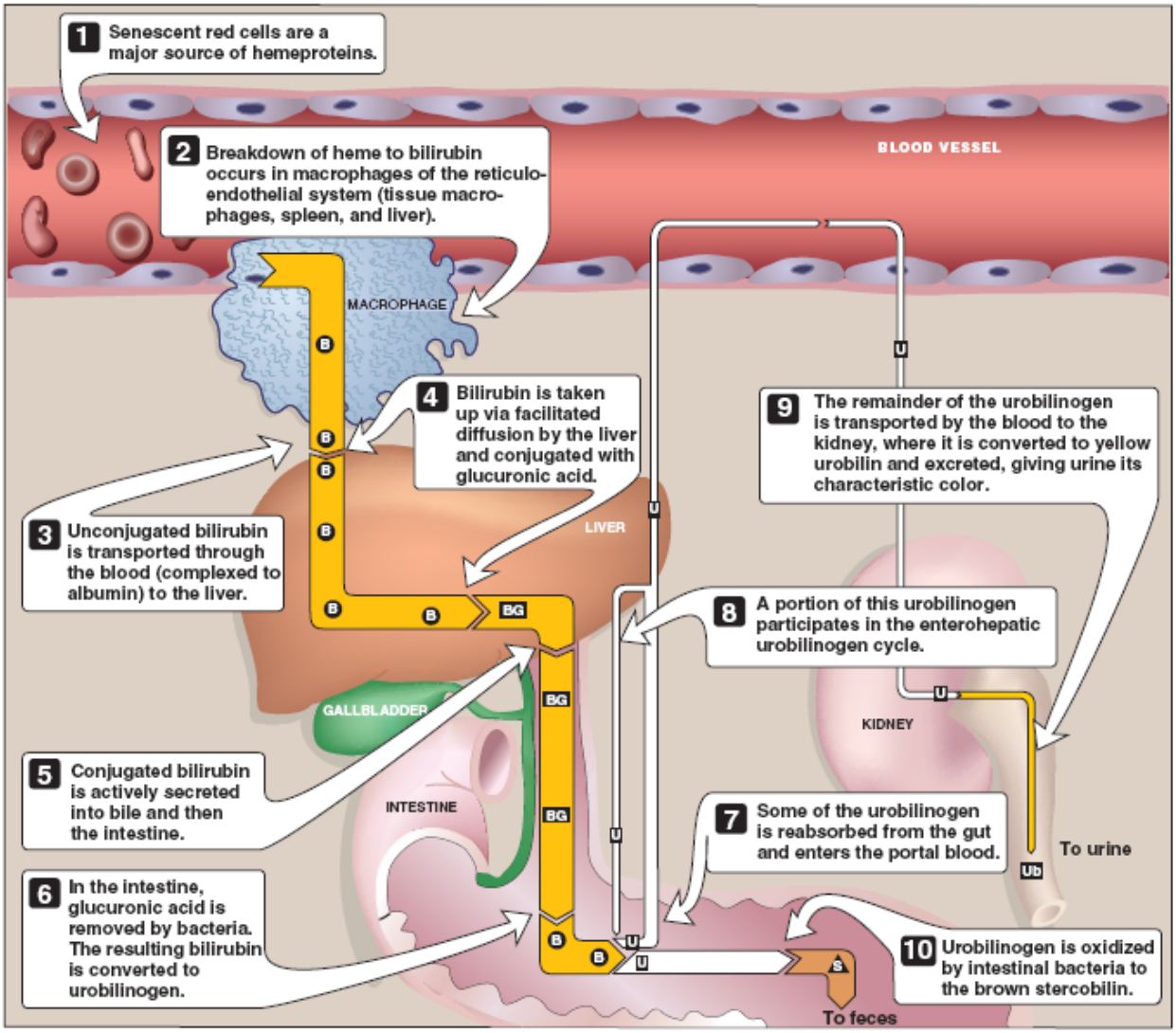



**Figure 21.10**
Catabolism of heme B = bilirubin; BG = bilirubin diglucuronide; U = urobilinogen; Ub = urobilin; S = stercobilin.

✎ **Describe the main types of JAUNDICE:**

| *Hemolytic jaundice* | *Hepatocellular jaundice* | *Obstructive jaundice* | *Jaundice in newborns* |
|---|---|---|---|
| | | | |

*PRACTICAL WORK 10*

## DETECTION OF HEMOGLOBIN IN BLOOD BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

HEMOGLOBIN (Hb) is an iron containing globular metalloprotein found primarily in red blood cells. It carries oxygen from the lungs to the rest of the body as oxyhemoglobin and then returns back to the lungs as deoxyhemoglobin. In most vertebrates, hemoglobin is in tetrameric form comprising two α-globin chains and two β-globin chains ($\alpha_2\beta_2$). Each chain is associated with a non-protein heme group. Heme binds and releases oxygen or carbon dioxide in response to slight changes in local gas tension. Free oxygen or carbon dioxide bound by one heme group facilitates subsequent binding by the other heme groups in a given hemoglobin molecule. Subtle changes in pH also regulate hemoglobin affinity for free gases, resulting in a high level of hemostatic control. Aside from oxygen transport, hemoglobin can bind and transport other molecules like nitric oxide (NO) and carbon monoxide (CO). CO binds competitively to the heme group and will block oxygen binding, leading to hypoxia and, in severe cases, death. NO binds to the thiol groups of the globin protein structure to form an S-nitrosothiol which dissociates into free NO and thiol again, as the hemoglobin releases oxygen from its heme site. Hemoglobin also plays an important role in maintaining the shape of the red blood cells. Abnormal hemoglobin structure can disrupt the shape of red blood cells and impede their function and flow through blood vessels. This is the underling cause of sickle-cell anemia.

**Diagnostic significance:**

Hemoglobin values are associated with a variety of conditions ranging from anemias (low Hgb), erythrocytosis (high Hgb), thalassemias (aberrant chain synthesis), and sickling disorders (abnormal complex shape).

**Normal reference values**:

Serum: children less than 12 y.o.: 110 – 150 g/L
adults 12-65 y.o.: 120 – 170 g/L (man)
115 – 155 g/L (woman)
adults more than 65 y.o.: 126 – 175 g/L (man)
117 – 160 g/L (woman)

### ASSAY PRINCIPLE:

In this method, ferricyanide and potassium cyanide convert hemoglobin to a more stable cyanmethemoglobin form that is measured photometrically. The intensity of color, measured at 540 nm, is directly proportional to hemoglobin concentration in the sample.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 540 nm.
2. Pipettes capable of accurately dispensing volumes of 0.01 mL and 1.0 mL.
5. Test tubes, 10 mL

### REAGENTS:

***1. Hemoglobin Detector***

This solution contains aceton cyanohydrin and oxidized reagent.

**NOTE:** Protect this solution from light.

***2. Hemoglobin Standard***

This standard solution contains such amount of hemoglobincyanid that equals exactly to sample of blood with hemoglobin concentration of 150 g/L.

**PROCEDURE**

| *Pipette the following solutions into the appropriately marked dry clean test tubes (B, S, T):* | | |
|---|---|---|
| **Reagent** | **Tube** | |
| | ***Blank (B)*** | ***Test (T)*** |
| ***1. Sample (blood)*** | - | 0.01 mL |
| ***2. Hemoglobin Detector*** | 2.5 mL | 2.5 mL |
| ***3. Hemoglobin Standard*** | - | - |

*Carefully mix each tube thoroughly.*
*Lat the test tubes stand for 15 minutes at room temperature.*
*Set wavelength of spectrophotometer at 540 nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 2 hours.

*Record the absorbance of the test tubes:*

$E_S$ _____ ***NOTE:*** *The $E_s$ value will be provided by your teacher!!!*

$E_T$ _____

**CALCULATION of hemoglobin concentration (C):**

**C, g/L** = $\frac{E_T}{Es}$ **× 150,** where 10 is concentration of glucose in standard solution

where 150 is the concentration of hemoglobin in the standard solution (g/L)

**Your calculation: _____**

**CONCLUSION:**

# *PRACTICAL WORK 11*

## DETERMINATION OF TOTAL AND DIRECT BILIRUBIN IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

BILIRUBIN, a degradation product of heme catabolism, is a non-polar molecule. There are two forms of bilirubin: water-soluble (conjugated or direct) and water-insoluble (unconjugated or indirect) bilirubin. Bilirubin is produced in the endoplasmic reticulum as unconjugated bilirubin, which binds to albumin in plasma and forms albumin-bilirubin complex. This complex is transported to the liver, where it is conjugated with glucuronic acid and forms conjugated bilirubin. The water-soluble (conjugated) bilirubin, is transported along with other bile constituents into the bile ducts, then to the intestines. In the intestines, the action of bacterial enzyme converts bilirubin to several related compounds, collectively referred to as urobilinogen. Bilirubin has potent antioxidant, anti-inflammatory and autoimmune properties.

**Diagnostic significance**:

A low concentration of bilirubin is found in normal plasma, almost all of which is indirect. The sum of the direct and indirect forms (or conjugated and unconjugated) is termed total bilirubin. Bilirubin concentration in human body depends on gender, drug intake, age, etc. Low serum bilirubin is directly correlated with pathological conditions including diabetes mellitus, metabolic syndrome, and cardiovascular diseases. However, high bilirubin indicates hemolysis, jaundice, Gilbert's syndrome, hepatitis, drug toxicity, and possible blockage of bile ducts.

*The determination of direct and total bilirubin is used in the differentiation of certain types of jaundice*, a condition characterized by an increase in the bilirubin level in the serum and the presence of a yellowish pigmentation in the skin. Jaundice may be classified as prehepatic, hepatic, or post-hepatic. In *prehepatic jaundice*, an excess bilirubin production (hemolysis) is responsible. *Hepatic jaundice* occurs when either the removal of bilirubin from the blood or conjugation of bilirubin by the liver is defective. This can have organic or genetic causes. *Post-hepatic jaundice* refers to anatomic obstruction of the extrahepatic biliary tree. The most common causes of jaundice are liver disease and blockage of the common bile duct. It is necessary to distinguish between the causes of jaundice early in the disease prior to the onset of complications, as the course of treatment is dependent on the cause of the jaundice. Hemolytic jaundice is caused by overproduction of bilirubin due to excessive hemolysis and the inability of the liver to adequately remove this pigment from the blood. This condition is usually associated with elevated values of serum *indirect* bilirubin. Cirrhosis of the liver and infectious or toxic hepatitis are caused by some type of intrahepatic obstruction, where production of bilirubin is not increased, but accumulates and is discharged back into the blood. In these conditions, the *indirect form of bilirubin predominates in the early phase, but as liver damage progresses the direct form also elevated.* Obstructive jaundice, caused by a post-hepatic blockage of the larger bile passages, particularly the common bile duct, results in a reflux of bilirubin into the blood. This condition, when uncomplicated, is associated with elevated serum bilirubin only of the *direct* type.

**Normal reference values**:

*Total bilirubin:*

Serum: children 0-1 day from birth: 24 – 149 μmol/L
1-2 day from birth: 58 – 197 μmol/L
3-5 day from birth: 26 – 205 μmol/L
more than 5 day from birth: 5 – 21 μmol/L
adults less than 60 y.o.: 5 – 21 μmol/L
adults 60-90 y.o.: 3 – 19 μmol/L

*Indirect bilirubin:* 6,3 – 15.4 μmol/L

*Direct bilirubin:* 2.2 – 5.13 μmol/L

**ASSAY PRINCIPLE**

Bilirubin is coupled with diazotized sulfanilic acid to form azobilirubin. The color of this derivative is pH dependent, occurring as pink in acid or neutral medium and blue under alkaline conditions. Direct (conjugated) bilirubin couples with diazotized sulfanilic acid (*p*-diazobenzenesulfonic acid), forming a pink-violet colored product. Indirect (unconjugated) bilirubin is diazotized only in the presence of an "accelerating" agent, caffeine-benzoate-acetate mixture. Thus, the azobilirubin produced in mixtures containing "accelerating" agent originates from both the Direct and Indirect fractions and reflects the Total bilirubin concentration. The indirect fraction is obtained by subtracting the direct value from the total value.

**APPARATUS:**

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 540 nm;
2. Pipettes capable of accurately dispensing volumes of 0.05, 0.1, and 1.0 mL;
3. Test tubes, 10 mL

**REAGENTS:**

***1. Caffeine reagent***:

This solution contains 50 g/L of caffeine, 0.5 mol/L of sodium benzoate and 1.5 mol/L of sodium acetate.

***2. Diazo reagent***

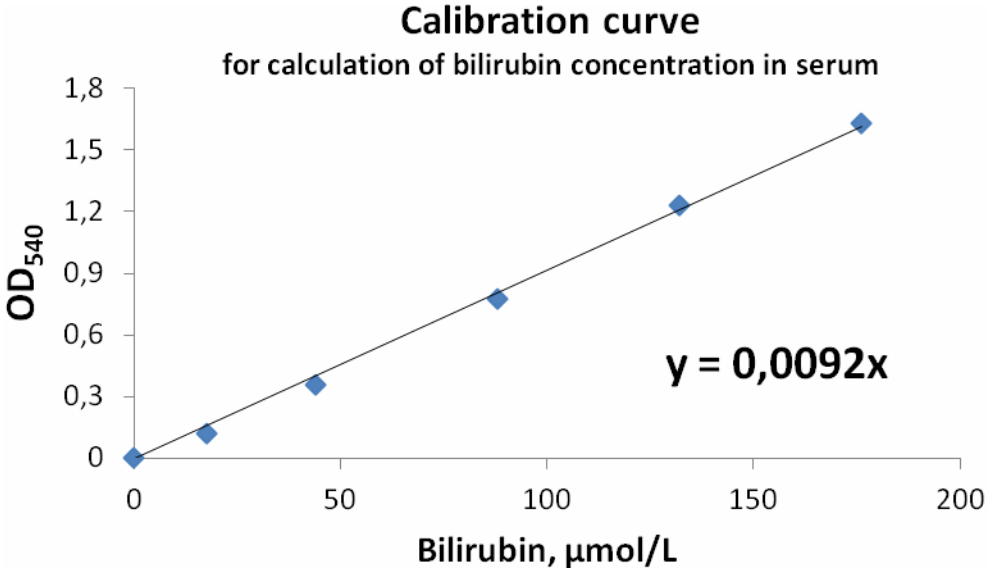
This solution has been made by mixing of two solutions: sulfanilic acid (with concentration of 25 mol/L) and sodium nitrite (with concentration of 0.35 mol/L) in proportion 100:3, respectively.

**PROCEDURE**

<table>
<tr><td colspan="5">Pipette the following solutions into the appropriately marked dry clean test tubes (B, T):</td></tr>
<tr><td rowspan="3">Reagent</td><td colspan="4">Tube</td></tr>
<tr><td colspan="2">Total bilirubin</td><td colspan="2">Direct bilirubin</td></tr>
<tr><td>Blank ($B_t$)</td><td>Test ($T_t$)</td><td>Blank ($B_d$)</td><td>Test ($T_d$)</td></tr>
<tr><td>1. Sample (serum)<br>2. Caffeine reagent<br>3. NaCl 0.9%<br>4. Diazo reagent</td><td>0.5 mL<br>1.75 mL<br>0.25 mL<br>-</td><td>0.5 mL<br>1.75 mL<br>-<br>0.25 mL</td><td>0.5 mL<br>-<br>2 mL<br>-</td><td>0.5 mL<br>-<br>1.75 mL<br>0.25 mL</td></tr>
<tr><td rowspan="3"></td><td colspan="4">Carefully mix each tube thoroughly.</td></tr>
<tr><td colspan="2">Lat the test tubes stand for<br>20 minutes at room temperature.</td><td colspan="2">Lat the test tubes stand for 5-10<br>minutes at room temperature.</td></tr>
<tr><td colspan="4">Set wavelength of spectrophotometer at 540 nm and zero the instrument with the appropriate "Blank".<br>Read the absorbance of all test tubes.<br><br>Record the absorbance of the test tubes:<br><br>$E(T_t)$ _____<br><br>$E(T_d)$ _____</td></tr>
</table>

**CALCULATIONS**

Use the prepared calibration curve to determine the concentration of different types of bilirubin in unknown samples.




1) Determine total and direct bilirubin levels from the calibration curve;
2) Calculate the indirect bilirubin level as the difference between the total and the direct.

***RECORD YOUR RESULTS:***

***Total bilirubin level***:_____

***Direct bilirubin level***:_____

***Indirect bilirubin level***:_____

**CONCLUSION:**

_____

_____

_____

_____

_____

_____

_____

# Lesson 14

The main topic
**"BIOCHEMISTRY OF BLOOD (part 2)"**

🕮 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Basic fractions of plasma proteins. Changes in their content in pathological conditions: hyper-, hypo-, dys- and paraproteinemia;
⇨ Acute phase proteins. Clinical and diagnostic significance of their determination;
⇨ Blood enzymes, their origin, clinical and diagnostic significance of their determination;
⇨ Non-protein nitrogen-containing compounds. Total and rest nitrogen levels. Clinical significance of their determination. Azotemia: types, causes, methods of determination;
⇨ Coagulation system, anticoagulation system and fibrinolysis in the blood.

✎ ***Complete the table of fractions of plasma proteins:***

| ***Fractions*** | | ***Consentration or Rel. amoumt (%)*** | ***Function*** | ***Example*** |
|---|---|---|---|---|
| ***albumins*** | | | | |
| ***globulins*** | ***$\alpha_1$-globulins*** | | | |
| | ***$\alpha_2$-globulins*** | | | |
| | ***β-globulins*** | | | |
| | ***γ-globulins*** | | | |
| ***fibrinogen*** | | | | |

✎ ***Define the term*** **"ACUTE PHASE PROTEINS":**

_____
_____
_____
_____
_____
_____

✎ ***Complete the table of diagnostic importance of nonfunctional plasma enzymes:***

| ***Name of the enzyme*** | ***Normal value*** | ***Diagnostic importance*** |
|---|---|---|
| Aspartate Amino transferase (AST) | | |
| Alanine Amino transferase (ALT) | | |
| Alkaline Phosphatase (ALP) | | |
| Acid Phosphatase (ACP) | | |
| γ-Glutamyl Transferase ( GT) | | |
| Creatine kinase (CK) | | |
| Lactate Dehydrogenase (LDH) | | |

✎ ***Define the term*** **"ISOENZYMES" and explain their role as diagnostic markers (use lactate dehydrogenase as an example in your explanation):**

_____
_____
_____
_____
_____
_____
_____

## Questions from KROK-1

1. 12 hours after an acute attack of retrosternal pain a patient presented a jump of aspartate aminotransferase activity in blood serum. What pathology is this deviation typical for?
   A. Viral hepatitis
   B. Diabetes insipidus
   C. Collagenosis
   D. Diabetes mellitus
   E. Myocardial infarction

2. A patient who had been working hard under condition of elevated temperature of the environment has now a changed quantity of blood plasma proteins. What phenomenon is the case?
   A. Absolute hyperproteinemia
   B. Relative hyperproteinemia
   C. Absolute hypoproteinemia
   D. Disproteinemia
   E. Paraproteinemia

3. 62 y.o. woman complains of frequent pains in the area of her chest and backbone, rib fractures. A doctor assumed myelomatosis (plasmocytoma).What of the following laboratory characteristics will be of the greatest diagnostic importance?
   A. Proteinuria
   B. Hypoproteinemia
   C. Hypoglobunemia
   D. Hyperalbuminemia
   E. Paraproteinemia

4. Diabetes mellitus causes ketosis as a result of activated oxidation of fatty acids. What disorders of acid-base equilibrium may be caused by excessive accumulation of ketone bodies in blood?
   A. Metabolic alkalosis
   B. Metabolic acidosis
   C. Respiratory alkalosis
   D. Respiratory acidosis
   E. Any changes won't happen

5. A 63-year-old woman developed symptoms of rheumatoid arthritis. Their increase of which blood values indicators could be most significant in proving the diagnosis?
   A. R-glycosidase
   B. Acid phosphatase
   C. Lipoproteins
   D. General cholesterol
   E. Additive glycosaminoglycans

6. Marked increase of activity of MB-forms of CPK (creatinephosphokinase) and LDH-1 was revealed by examination of the patient's blood. What is the most probable pathology?
   A. Myocardial infarction
   B. Hepatitis
   C. Pancreatitis
   D. Rheumatism
   E. Cholecystitis

7. There is high activity of LDH1,2, aspartate aminotransferase, creatine phosphokinase in the blood of patient. In what organs (tissues) the development of pathological process is the most probable?
   A. In the heart muscle {initial stage of myocardium infraction}
   B. In skeletal muscle {dystrophy, atrophy}
   C. In kidneys and adrenals
   D. In liver and kidneys
   E. In connective tissue

8. The high level of Lactate Dehydrogenase (LDH) isozymes concentration showed the increase of LDH-1 and LDH-2 in a patient's blood plasma. Point out the most probable diagnosis.
   A. Diabetes mellitus
   B. Skeletal muscle dystrophy
   C. Myocardial infarction
   D. Acute pancreatitis
   E. Viral hepatitis

9. Analysis of blood serum of a patient revealed the increase of alanine aminotransferase and aspartate aminotransferase levels. What cytological changes can cause such a situation?
   A. Disturbance of genetic apparatus of cells
   B. Cellular breakdown
   C. Disorder of enzyme systems of cells
   D. Disturbance of cellular interrelations
   E. Disturbed energy supply of cells

10. A worker has decreased buffer capacity of blood due to exhausting muscular work. What acidic substance that came to blood caused this phenomenon?
   A. 3-phosphoglycerate
   B. 1,3-bisphosphoglycerate
   C. Lactate
   D. α-ketoglutarate
   E. Pyruvate

11. Blood sampling for bulk analysis is recommended to be performed on an empty stomach and in the morning. What changes in blood composition can occur if to perform blood sampling after food intake?
   A. Reduced contents of erythrocytes
   B. Increased contents of erythrocytes
   C. Increased contents of leukocytes
   D. Increased plasma proteins
   E. Reduced contents of thrombocytes

12. Examination of a 43 y.o. anephric patient revealed anemia symptoms. What is the cause of these symptoms?
   A. Folic acid deficit
   B. Vitamin B12 deficit
   C. Reduced synthesis of erythropoietins
   D. Enhanced destruction of erythrocytes
   E. Iron deficit

13. A 55 y.o. women consulted a doctor about having continuous cyclic uterine hemorrhages for a year, weakness, dizziness. Examination revealed skin pallor. Hemogram: Hb – 70 g/L, erythrocytes-3.2 x 1012/L, color index – 0.6; leukocytes – 6.0 x 109/L, reticulocytes – 1%, erythrocyte hypochromia. What anemia is it?

A. Iron-deficiency anemia
B. B12-folate-deficiency anemia
C. Hemolytic anemia
D. Aplastic anemia
E. Chronic posthemorrhagic anemia

14. Blood plasma of healthy man contains several dozens of proteins. During an illness new proteins can originate named as the "proteins of acute phase». Select such protein from the listed below:

A. Albumin
B. Immunoglobulin G
C. Immunoglobulin E
D. C-reactive protein
E. Prothrombin

15. A patient complains about dyspnea provoked by the physical activity. Clinical examination revealed anaemia and presence of the para-protein in the zone of gamma-globulins. To confirm the myeloma diagnosis it is necessary to determine the following index in the patient's urine:

A. Ceruplasmin
B. Bilirubin
C. Antitrypsin
D. Bence Jones protein
E. Haemoglobin

16. Examination of 27-year-old patient revealed pathological changes in liver and brain. Blood plasma analysis revealed an abrupt decrease in the copper concentration, urine analysis revealed an increased copper, concentration. The patient was diagnosed with Wilson's degeneration. To confirm the diagnosis it is necessary to study the activity of the following enzyme in blood serum:

A. Leucine aminopeptidase
B. Xanthine oxidase
C. Alcohol dehydrogenase
D. Ceruloplasmin
E. Carbonic anhydrase

17. After a surgery a 36-year-old woman was given an intravenous injection of concentrated albumin solution. This has induced intensified water movement in the following direction:

A. From the intercellular fluid to the capillaries
B. No changes of water movement will be observed
C. From the intercellular to the cells
D. From the cells to the intercellular fluid
E. From the capillaries to the intercellular fluid

18. Electrophoretic study of a blood serum sample, taken from the patient with pneumonia, revealed an increase in one of the protein fractions. Specify this fraction:

A. γ-globulins
B. Albumins
C. α1-globulins
D. β-globulins
E. α2-globulins

19. Examination of a 56-year-old female patient with a history of type 1 diabetes revealed a disorder of protein metabolism that is manifested by aminoacidemia in the laboratory blood test values, and clinically by the delayed wound healing and decreased synthesis of antibodies. Which of the following mechanisms causes the development of aminoacidemia?

A. Increased proteolysis
B. Decrease in the concentration of amino acids in blood
C. Albuminosis
D. Increase in the oncotic pressure in the blood plasma
E. Increase in low-density lipoprotein level

20. A 49-year-old male patient with acute pancreatitis was likely to develop pancreatic necrosis, while active pancreatic proteases were absorbed into the blood stream and tissue proteins broke up. What protective factors of the body can inhibit these processes?

A. Immunoglobulin
B. Ceruloplasmin, transferrin
C. a2-macroglobulin, a1-antitrypsin
D. Cryoglobulin, interferon
E. Hemopexin, haptoglobin

21. A patient is diagnosed with hereditary coagulopathy that is characterized by factor VIII deficiency. Specify the phase of blood clotting during which coagulation will be disrupted in the given case:

A. Clot retraction
B. Thromboplastin formation
C. Fibrin formation
D. Thrombin formation

22. A 67-year-old male patient consumes eggs, pork fat, butter, milk and meat. Blood test results: cholesterol – 12.3 mmol/l, total lipids – 8.2 g/l, increased low-density lipoprotein fraction (LDL). What type of hyperlipoproteinemia is observed in the patient?

A. Hyporlipoproteinemia type I.
B. Hyperlipoproteinemia type IV
C. Cholesterol, hyperlipoproteinemia
D. Hyperlipoproteinemia type IIa
E. Hyperlipoproteinemia type IIb

23. Human red blood cells do not contain mitochondria. What is the main pathway for ATP production in these cells?

A. Creatine kinase reaction
B. Anaerobic glycolysis
C. Cyclase reaction
D. Aerobic glycolysis
E. Oxidative phosphorylation

24. A 28-year-old patient undergoing treatment in a pulmonological department has been diagnosed with pulmonary emphysema caused by splitting of alveolar septum by tripsin. The disease is caused by the congenital deficiency of the following protein:

A. Alpha-1-proteinase inhibitor
B. Haptoglobin
C. Cryoglobulin
D. Alpha-2-macroglobulin
E. Transferrin

25. Biochemical analysis of an infant`s erythrocytes revealed evident glutathione peroxidase deficiency and low concentration of reduced glutathione. What pathological condition can develop in this infant?

A. Hemolytic anemia
B. Megaloblastic anemia
C. Siclemia
D. Iron-deficiency anemia
E. Pernicous anemia

26. Lymphocytes and other cells of our body synthesize universal antiviral agents as a response to viral invasion. Name this protein factors

A. Interferon
B. Tumor necrosis factor
C. Cytokines 29
D. Interleukin-2
E. Interleukin-4

# *PRACTICAL WORK 12*

## DETERMINATION OF THE SERUM PROTEIN FRACTION

### 🕮 BACKGROUND

The serum protein components are separated into five major fractions: albumin, α1-globulins; α2-globulins; β-globulins and γ-globulins. Serum albumin accounts for 55% of blood proteins, and is a major contributor to maintaining the osmotic pressure of plasma to assist in the transport of lipids and steroid hormones. Globulins make up 38% of blood proteins and transport ions, hormones, and lipids assisting in immune function. Fibrinogen comprises 7% of blood proteins; conversion of fibrinogen to insoluble fibrin is essential for blood clotting. The remainder of the plasma proteins (1%) are regulatory proteins, such as enzymes, proenzymes, and hormones. All blood proteins are synthesized in liver except for the gamma globulins.

**Diagnostic significance**:

In disease states both the total plasma protein level and the ratio of the individual fractions may be dramatically altered from their normal values. Hypoproteinemia may be caused by such conditions as nephrotic syndrome, extensive bleeding, sprue (deficient protein absorption), severe burns, salt retention syndromes, and Kwashiorkor (acute protein starvation). Hyperproteinemia may be observed in cases of severe dehydration and disease states such as multiple myeloma. Changes in the proportions of the plasma proteins may occur in one or several of the protein fractions and often without alterations in the quantity of the total protein. The A/G ratio has commonly been used as an index of the distribution between the albumin and globulin fractions. This ratio can be significantly altered in such conditions as cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosis, and in some acute and chronic infections.

**Normal reference values (Rel. amount)**:

albumin: 56.6 – 66.8%
α1-globulins: 3.0 – 5.6%
α2-globulins: 6.9 – 10.5%
β-globulins: 7.3 – 12.5%
γ-globulins: 12.8 – 19.0%

### ASSAY PRINCIPLE

Serum proteins from various fractions can be segmented by phosphate buffer. The sedimentation of different functions was shown to be dependent on the concentration of phosphate buffer.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 640 nm.
2. Pipettes capable of accurately dispensing volumes of 0.5 and 1.0 mL.
3. Test tubes, 10 mL

### REAGENTS:

***1. Phosphate buffer 0 (PB 0) –*** *3.347 mol/L, pH 6.5*
***2. Phosphate buffer 1 (PB 1) –*** *3.084 mol/L, pH 6.5*
***3. Phosphate buffer 2 (PB 2) –****2.496 mol/L, pH 6.5*
***4. Phosphate buffer 3 (PB 3) –*** *2.359 mol/L, pH 6.5*
***5. Phosphate buffer 4 (PB 4) –*** *1.959 mol/L, pH 6.5*
***6. Phosphate buffer 5 (PB 5) –*** *1.622 mol/L, pH 6.5*

**PROCEDURE**

**Sample preparation:** *In the clean dry test tub mix:*

- 0.75 mL of distilled water,
- 3.75 mL of Phosphate buffer 0 (PB0)
- 0.5 mL of serum.

*Mix carefully and use this mixture in the further steps of experiment as SAMPLE.*
**NOTE:** Mix this tube every time before pippetin its content into another tube.

*Pipette the following solutions into the appropriately marked dry clean test tubes (B, from 1 to 5):*

| **Reagent** | **Tube** | | | | | |
|---|---|---|---|---|---|---|
| | ***Blank (B)*** | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** |
| ***1. Distilled water*** | 5 mL | - | - | - | - | - |
| ***2. Phosphate buffer (PB 1-5)*** | - | 5 mL PB1 | 5 mL PB2 | 5 mL PB3 | 5 mL PB4 | 5 mL PB5 |
| ***3. Sample*** | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL | 0.5 mL |

*Carefully mix each tube thoroughly.*
*Let the test tubes stand for 15 minutes at room temperature.*
**NOTE:** Carefully mix each tube thoroughly every time before measurement.
*Set wavelength of spectrophotometer at 640 nm and zero the instrument with the "Blank".*
*Read and record the absorbance of all test tubes.*

E1_____

E2_____

E3_____

E4_____

E5_____

**CALCULATIONS of the Rel. amount of each protein fractions:**

| Protein fractions | Formula | The results of calculation | Calculate the sum of fractions: | The sum of fractions is taken as 100% and calculate the Rel. amount of each protein fractions |
|---|---|---|---|---|
| ***albumin*** | E1-E2 | | | |
| ***α1-globulins*** | E2-E3 | | | |
| ***α2-globulins*** | E3-E4 | | | |
| ***ß-globulins*** | E4-E5 | | | |
| ***γ-globulins*** | E5 | | | |

**CONCLUSION:**

_____

_____

_____

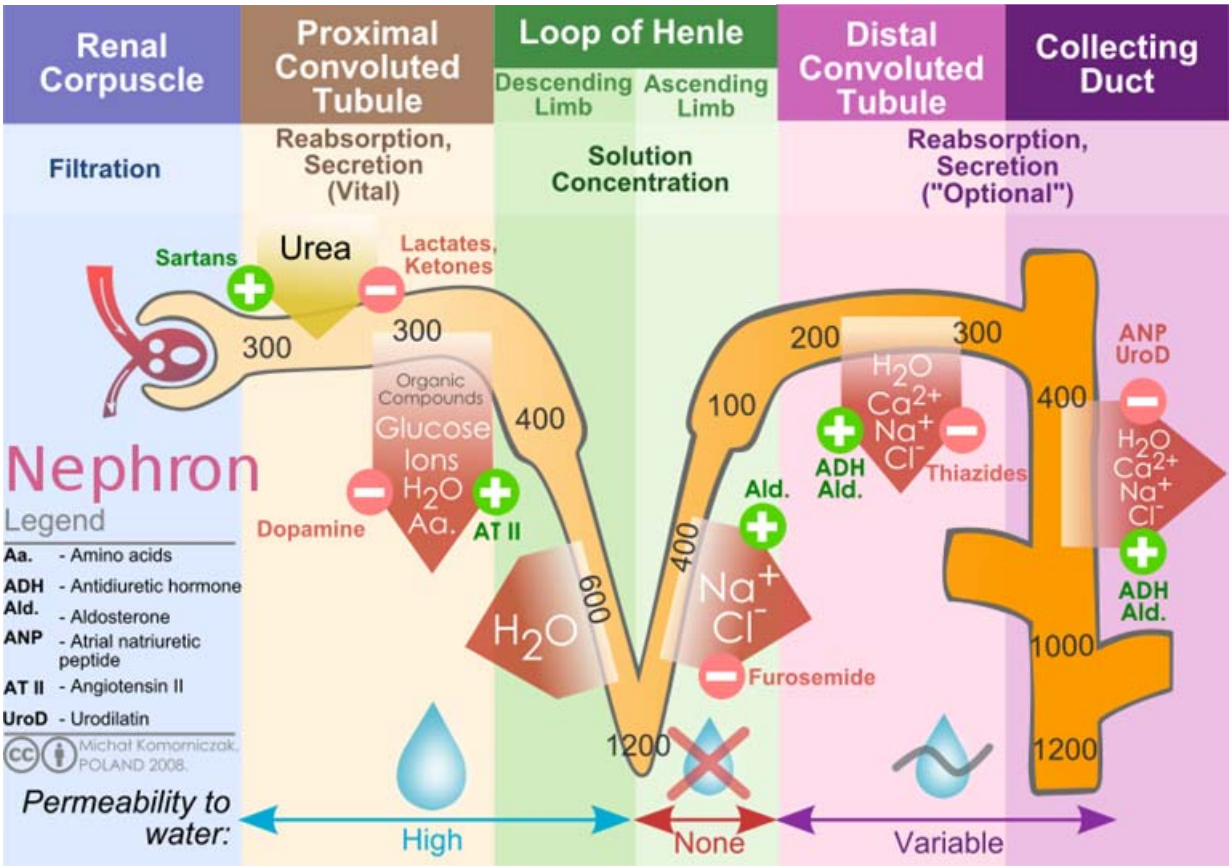
# Lesson 15

The main topic
**“BIOCHEMISTRY OF KIDNEY”**

📖 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Role of kidney tissue in the regulation of water and salts metabolism;
⇨ Role of kidney tissue in creation of acid-base balance;
⇨ General conceptions of urine formation by kidneys. Hormonal regulation of kidneys` filtration, adsorption and secretion (vasopressin, atrial diuretic peptide, aldosterone, renin-angiotensinogen system, calciotonin, calcitriols);
⇨ Physical and chemical properties of the urine at healthy adults;
⇨ Chemical composition of the urine. Characteristic of the main urine components at healthy adults. Pathological components of urine in patients.

✎ ***Using the figure below, describe the presses of urine formation by kidneys:***




✎ ***Define the following terms:***

1. **Filtration**: _____
_____

2. **Reabsorption**: _____
_____

3. **Secretion**: _____
_____

✎ ***Complete the table of urine analysis and pathological conditions:***

| *Parameter* | *Normal values* | *Pathological conditions* |
|---|---|---|
| Color | | |
| Clarity/Turbidity | | |
| pH | | |
| Glucose | | |
| Proteins | | |
| Ketones | | |
| Nitrates | | |
| Bilirubin | | |
| Urobilirubin | | |
| Blood | | |
| RBCs | | |
| WBCs | | |
| Epithelial cells | | |
| Bacteria / Yasts | | |

## Questions from KROK-1

1. A 34-year-old patient was diagnosed with chronic glomerulonephritis 3 years ago. Edema has developed within the last 6 months. What caused the edema?
   A. Liver dysfunction of protein formation
   B. Hyperosmolarity of plasma
   C. Proteinuria
   D. Hyperproduction of vasopressin
   E. Hyperaldosteronism

2. Examination of a 43 y.o. anephric patient revealed anemia symptoms. What is the cause of these symptoms?
   A. Folic acid deficit
   B. Vitamin B12 deficit
   C. Reduced synthesis of erythropoietin
   D. Enhanced destruction of erythrocytes
   E. Iron deficit

3. A biochemical urine analysis has been performed for a patient with progressive muscular dystrophy. In the given case muscle disease can be confirmed by the high content of the following substance in urine:
   A. Urea
   B. Porphyrin
   C. Hippuric acid
   D. Creatine
   E. Creatinine

4. Kidney insufficiency in patient is accompanied with:
   A. Excess levels of urea in the blood plasma
   B. Excess levels of potassium ions in the blood plasma
   C. Disturbed clearance
   D. Disturbed filtration and reabsorption processes
   E. All that is placed above

5. Point out the most important compensatory mechanism in metabolic acidosis:
   A. Hyperventilation
   B. Increased NH3 excretion by kidneys
   C. Increased filtration of phosphates
   D. Increased HCO3- production
   E. Urea production in the liver

6. Point out the main source of ammonia in kidney tissue:
   A. Urea
   B. Aspartate
   C. Glutamine
   D. Glutamate
   E. Uric acid

7. Choose normal amount of proteins excreted in urine/24 hours:
A. Less than 150 mg
B. 200 mg - 225 mg
C. 450 mg – 500 mg
D. More than 800 mg
E. 150 mg – 250 mg

8. Name organic compound which is terminal for humans and not reabsorbed in renal tubules:
   A. Globulins
   B. Glucose
   C. Albumin
   D. Creatinine
   E. Bilirubin

9. Choose the specific gravity region (g/ml) for urine of healthy person:
   A. 1.005-1.015
   B. 1.030-1.040
   C. 1.015-1.020
   D. 1.030-1.040
   E. Less then 1.010

10. Creatinine levels in the urine and blood are used to test kidney function. Creatinine is useful for this test because it is not significantly reabsorbed nor secreted by kidney, and metabolically it is:
   A. Produced at a constant rate
   B. Produced only in kidney
   C. A storage form of energy
   D. An acceptor of protons in renal tubules
   E. A precursor for phosphocreatine

11. Appearance of albumins in the urine of diseased person may be at:
   A. Acute nephritis
   B. Chronical nephritis
   C. Severe form of diabetes mellitus
   D. Pyelonephritis
   E. All that is placed above

12. Choose the main biochemical tests for diagnostics of kidney diseases:
   A. Urea content in the blood plasma and in the urine
   B. Creatinine content in the blood and urine
   C. Sodium ions content in the blood and urine
   D. N-acetyl-beta-D-glucose-aminidase activity (blood serum, urine)
   E. All that is placed above

13. What organic compounds may accumulate in final urine at severe form of diabetes mellitus?
   A. Albumins
   B. Glucose
   C. Ketone bodies
   D. Bilirubin conjugated
   E. All that is placed in positions A, B, C

14. Kidney insufficiency development will cause the infringements in those processes:
   A. Erythropoietin synthesis and secretion
   B. Calcitriol synthesis
   C. Mineralization of bone tissue
   D. Creatine synthesis
   E. All that is placed

15. The infringement in glomerular filtration mostly is associated with appearance in the urine of this class compounds. Name it:
   A. Lipids
   B. Proteins
   C. Amino acids
   D. Keto acids
   E. Carbohydrates

16. Renal clearance may be calculated using this compound concentration value in the blood serum and in the urine of patient. Name it:

A. Inulin
B. Creatine
C. Free ammonia
D. Ammonia salt
E. Indican

17. Utilization of excess protons in renal tubule lumen may be due to:

A. Aspartic acid
B. Creatinine
C. Uric acid
D. Ammonia
E. Water

18. This compound is impossible to find out in the urine of healthy person:

A. Globulin
B. Alanine
C. Pyruvate
D. Oxaloacetate
E. Carbonic acid

19. Acute tubular necrosis is associated with increase of this index in the blood serum of patient. Name it:

A. Creatine
B. Free amino acids
C. Pyruvate
D. Cholesterol total
E. Urea

20. This vitamin derivative is produced in renal tubules mainly to control calcium and phosphate ions levels in the blood. Name it:

A. FAD
B. $NAD^+$
C. Calcitonin
D. Calcitriol
E. Angiotensin II

*PRACTICAL WORK 13*

## DETERMINATION OF CREATININE IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

CREATININE is the result of the degradation of the creatine, component of muscles; it can be transformed into ATP, which is a source of high energy for the cells. The creatinine production depends on the modification of the muscular mass, and it varies little and the levels usually are very stable. It is excreted by the kidneys. With progressive renal insufficiency there is retention in blood of urea, creatinine and uric acid.

**Diagnostic significance**:

Measurements of creatinine are used in the diagnosis and treatment of renal disease. Serum creatinine measurements prove useful in evaluation of kidney glomerular function and in monitoring renal dialysis. However, the serum level is not sensitive to early renal damage and responds more slowly than blood urea nitrogen (BUN) to hemodialysis during treatment of renal failure. Both serum creatinine and BUN are used to differentiate prerenal and postrenal (obstructive) azotemia. An increase in serum BUN without concomitant increase of serum creatinine is key to identifying prerenal azotemia. With postrenal azotemia, both serum BUN and creatinine rise, but the rise is disproportionately greater for BUN. Serum creatinine varies with the subject's age, body weight, and sex. It is sometimes low in subjects with relatively small muscle mass, cachetic patients, amputees, and in older persons.

**Normal reference values**:

Serum: children less than 18 y.o.: 44 – 88 µmol/L
adults 18-60 y.o.: 80 – 115 µmol/L (man)
53 – 97 µmol/L (woman)
adults 60-90 y.o.: 71 – 115 µmol/L (man)
53 – 106 µmol/L (woman)
Urine: children less than 18 y.o.: 8 – 30 mg/kg/24h
adults 18-60 y.o.: 14 – 26 mg/kg/24h (man)
11 – 20 mg/kg/24h (woman)

### ASSAY PRINCIPLE:

At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 505 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 505 nm;
2. Low-speed centrifuge (3000 rpm required);
3. Pipettes capable of accurately dispensing volumes of 0.5 and 1.0 mL.
4. Test tubes, 10 mL

### REAGENTS:

***1. Picric Reagent***

This solution contains 0.04 mol/L of picric acid;

***2. Alkaline Reagent***

The concentration of NaOH in this solution is 4.6% (or 1.15N)

***3. Creatinine Standard***

This standard solution contains 50 mg/L of creatinine (or 442.5 µmol/L of creatinine).

***3. Trichloroacetic acid***

The concentration of Trichloroacetic acid (TCA) in this solution is 1.220 mol/L.

**PROCEDURE**

| *Pipette the following solutions into the appropriately marked dry clean test tubes (B, S, T):* | | | |
|---|---|---|---|
| **Reagent** | **Tube** | | |
| | ***Blank (B)*** | ***Standard (S)*** | ***Test (T)*** |
| ***1. Sample (serum)*** | - | - | 0.5 mL |
| ***2. Distilled water*** | 1.5 mL | 1.0 mL | 1.0 mL |
| ***3. Creatinine Standard*** | - | 0.5 mL | - |
| ***4. TCA solution*** | 0.5 mL | 0.5 mL | 0.5 mL |
| *Carefully mix each tube thoroughly.*<br>*Centrifuge test tubes at 3000 rpm for 5 min (but only if necessary).*<br>*Take the supernatant for the further of experiment.* | | | |
| ***5.Supernatant*** | 1.0 mL | 1.0 mL | 1.0 mL |
| ***6. Alkaline Reagent*** | 0.5 mL | 0.5 mL | 0.5 mL |
| ***7. Picric Reagent*** | 0.5 mL | 0.5 mL | 0.5 mL |
| *Carefully mix each tube thoroughly.*<br>*Incubate the test tubes for 20 minutes at room temperature.*<br>*Set wavelength of spectrophotometer at 505 nm and zero the instrument with the "Blank".*<br>*Read the absorbance of all test tubes.*<br>**NOTE:** The final color is stable for at least 20 minutes.<br><br>*Record the absorbance of the test tubes:*<br>$E_S$ _____<br>$E_T$ _____ | | | |

**CALCULATION of creatinine concentration (C):**

$$\textbf{C, µmol/L} = \frac{E_T}{Es} \times 442.5,$$

where 442.5 is the concentration of creatinine in the standard solution (µmol/L)

**Your calculation: _____**

**CONCLUSION:**

_____

_____

_____

_____

_____

_____

# Lesson 16

The main topic
**“BIOCHEMISTRY OF LIVER”**

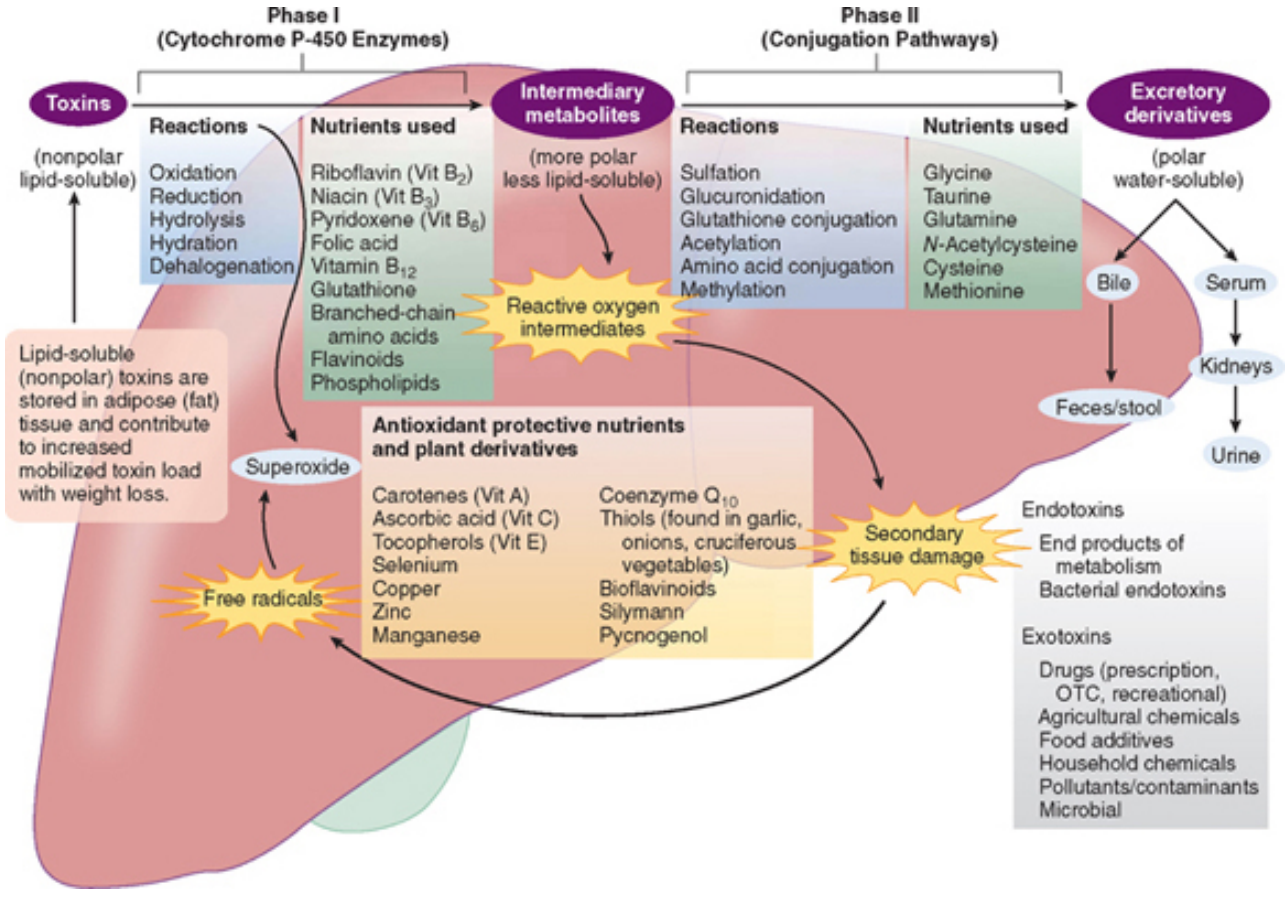
🕮 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Main biochemical functions of the liver;
⇨ The role of the liver in carbohydrate and lipid metabolism;
⇨ Bile-producing function of the liver. Chemical composition of bile;
⇨ The role of the liver in protein and pigment metabolism;
⇨ Detoxification in the liver. Types of reactions for biotransformation of xenobiotics and endogenous toxins. Systems of conjugation in the liver for detoxification of toxic substances.

✎ ***State the main functions of liver:***

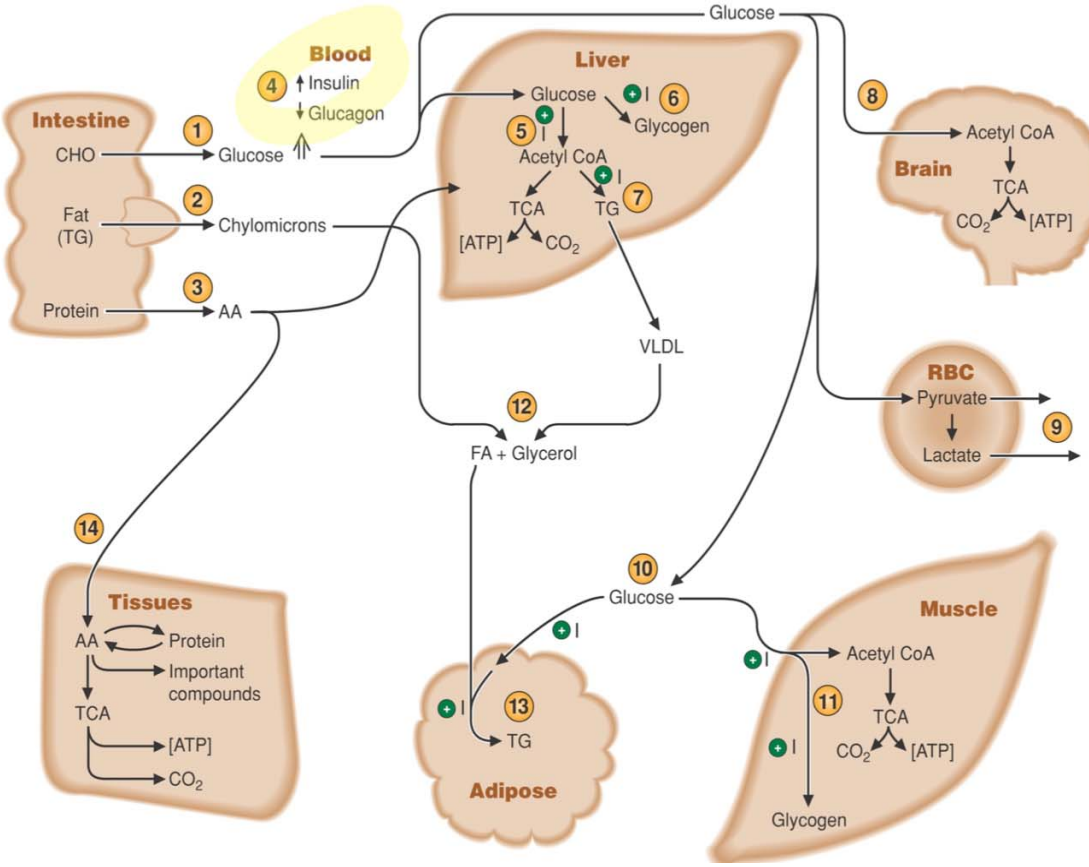
1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

✎ ***Describe the presses of detoxification in liver:***

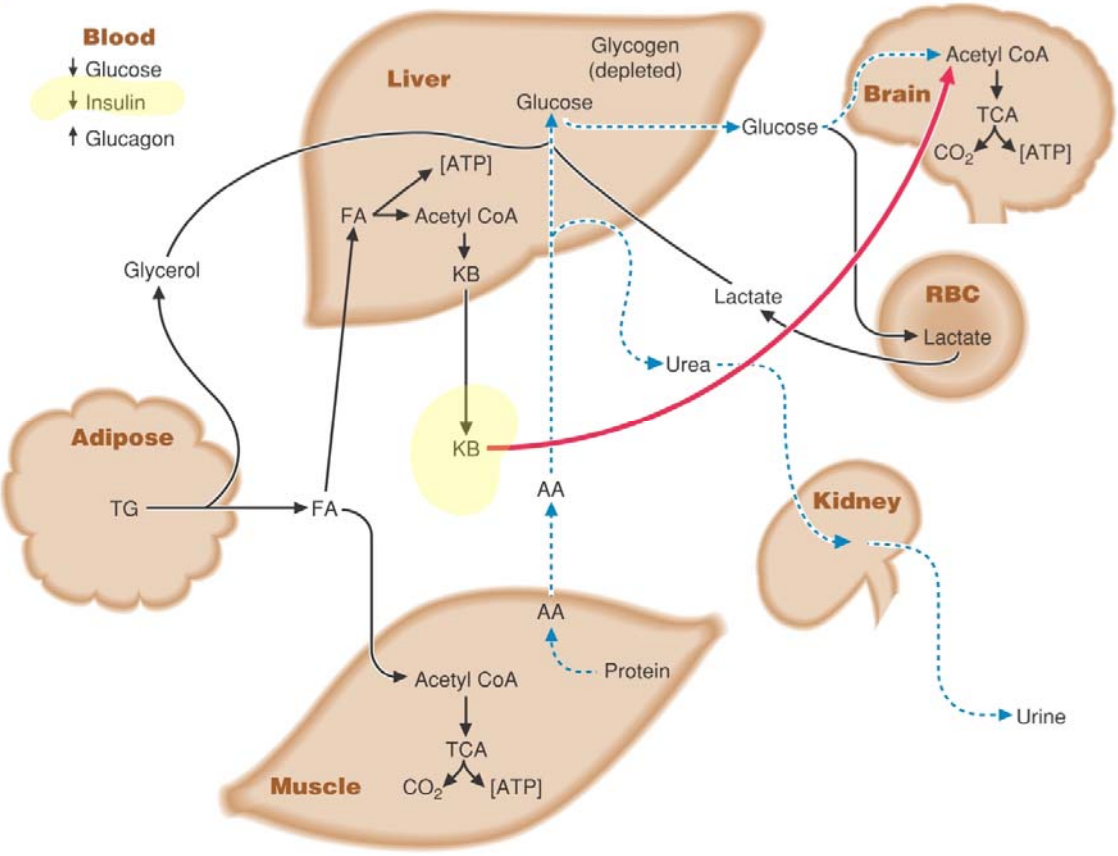


✎ ***Describe major metabolic pathways in liver during the fed (A) and the starved (B) states:***

***A. Fed state***




***B. Starved state***



# *PRACTICAL WORK 14*

## DETERMINATION OF ALANINE AMINOTRANSFERASE ACTIVITY IN SERUM BY COLORIMETRIC METHOD

### 🕮 BACKGROUND

ALANINE AMINOTRANSFERASE (ALT), also known as serum glutamic-pyruvic transaminase (SGPT), is a pyridoxal-phosphate-dependent enzyme that catalyzes the reversible transfer of an amino group from alanine to α-ketoglutarate. The products of this transamination reaction are pyruvate and glutamate. ALT is found primarily in liver and serum, but occurs in other tissues as well.

**Diagnostic significance**:

Elevated serum ALT is found in hepatitis cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction. Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver specific enzyme. Moreover, elevations of the ALT activity persist longer then elevations of the AST activity.

**Normal reference values**:

Serum: 0.1 – 0.68 μmol/(h·mL of serum) at 37ºC

### ASSAY PRINCIPLE:

The reaction involved in the assay system is as follows:

The amino group is enzymatically transferred by ALT present in the sample from L-alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.

$$\text{L-alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 546 nm;
2. Water bath or heating block capable of maintaining temperature at 37 ± 1 °C;
3. Pipettes capable of accurately dispensing volumes of 0.02, 0.2 and 1.0 mL;
4. Test tubes, 10 mL

### REAGENTS:

***1. Substrate-Buffer solution***

This solution contains 0.2 mol/L of L-alanine and 2 mmol/L of 2-oxoglutarate in 0.1 M phosphate buffer;

***2. Stop reagent***

This solution contains 1 mmol/L of 2,4-dinitrophenylhydrazine;

***3. Sodium hydroxide***

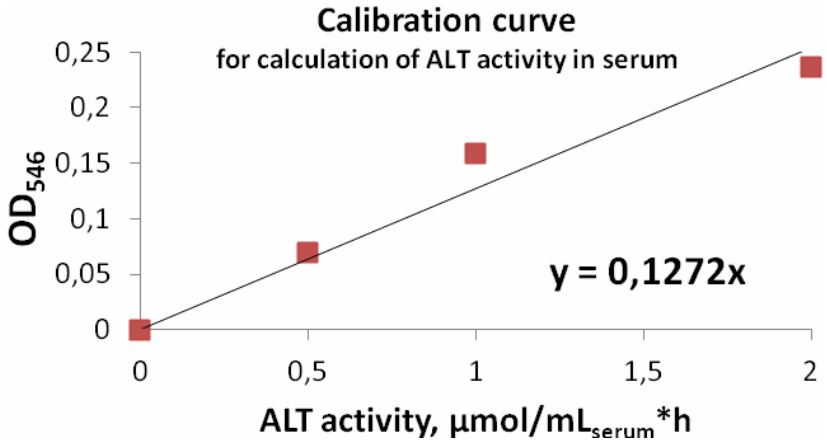
The concentration of NaOH in this solution is 4 mol/L

**PROCEDURE**

| *Pipette the following solutions into the appropriately marked dry clean test tubes (B, T):* | | |
|---|---|---|
| **Reagent** | **Tube** | |
| | ***Blank (B)*** | ***Test (T)*** |
| ***1. Substrate-Buffer solution*** | 0.2 mL | 0.2 mL |
| *Incubate the test tubes for 3 minutes at 37ºC* | | |
| ***2. Stop reagent***<br>***3. Sample (serum)*** | 0.2 mL<br>0.04 mL | -<br>0.04 mL |
| *Incubate the test tubes for 30 minutes at 37ºC* | | |
| ***4. Stop reagent*** | - | 0.2 mL |
| *Lat the test tubes stand for 20 minutes at room temperature.* | | |
| ***4. Sodium hydroxide*** | 2.0 mL | 2.0 mL |
| *Lat the test tubes stand for 5-10 minutes at room temperature.*<br>*Set wavelength of spectrophotometer at 546 nm and zero the instrument with the "Blank".*<br>*Read the absorbance of all test tubes.*<br>**NOTE:** The final color is stable for at least 60 minutes.<br><br>*Record the absorbance of the test tube:*<br>$E_T$ _____ | | |

**CALCULATIONS**

Use the prepared calibration curve to determine the activity of ALT in unknown samples.




***ALT activity,*** µmol/(h·mL of serum): _____

**CONCLUSION:**

_____

_____

_____

_____

# *PRACTICAL WORK 15*

## DETERMINATION OF THE ASPARTATE AMINOTRANSFERASE ACTIVITY IN SERUM BY COLORIMETRICAL METHOD

### 🕮 BACKGROUND

ASPARTATE AMINOTRANSFERASE (AST), formerly called serum glutamate-oxaloacetate transaminase (SGOT), is a pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the conversion of aspartate and α-keto-glutarate to oxaloacetate and glutamate. Depending on the sites of origin inside the cell there are two isoenzymes with different ph optimum: the mitochondrial m-AST, and the soluble cytosolic S-AST. The two izoenzymes can be separated by electrophoresis.

**Diagnostic significance**:

Similar to Alanine Aminotransferase (ALT), AST levels in blood are commonly used as a marker for liver function. However, AST has a broader tissue distribution than ALT and perturbations in AST levels can occur in response to diseases or injuries in multiple tissues including skeletal and heart. The activity of AST in the serum is significantly increased during heart, liver, kidney and muscle diseases (tissue injuries, functional disorders). The activity of the enzyme is increased 4-8 hours following a myocardial infarction, reaching its peak in 2-3 days and declining on the fifth and sixth days. Hepatobiliary diseases such as cirrhosis, metastatic carcinoma and viral hepatitis can show increased levels of AST. Other disorders which can lead to an elevated level of AST are muscular dystrophy, dermatomyositis, acute pancreatitis and infectious mononucleosis

**Normal reference values**:

Serum: 0.1 – 0.45 μmol/(h·mL of serum) at 37ºC

### ASSAY PRINCIPLE:

AST enables alpha-ketoglutaric acid and aspartic acid to displace amino and keto groups to form glutamic acid and oxaloacetic acid.

$$\textit{L-Aspartate} + \textit{2-oxoglutarate} \xrightarrow{\text{AST}} \textit{L-glutamate} + \textit{oxalacetate}$$

Oxaloacetic acid can decarboxylate itself to form Pyroracemic acid during the reaction. Pyroracemic acid reacted with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone showing reddish brown in alkaline solution.

### APPARATUS:

1. Spectrophotometer or colorimeter suitable for measuring absorbance at 546 nm;
2. Water bath or heating block capable of maintaining temperature at 37 ± 1 °C;
3. Pipettes capable of accurately dispensing volumes of 0.02, 0.2 and 1.0 mL;
4. Test tubes, 10 mL

### REAGENTS:

***1. Substrate-Buffer solution***

This solution contains 0.1 mol/L of L-aspartate and 2 mmol/L of 2-oxoglutarate in 0.1 M phosphate buffer;

***2. Stop reagent***

This solution contains 1 mmol/L of 2,4-dinitrophenylhydrazine;

***3. Sodium hydroxide***

The concentration of NaOH in this solution is 4 mol/L.

## PROCEDURE

| *Pipette the following solutions into the appropriately marked dry clean test tubes (B, T):* | | |
|---|---|---|
| **Reagent** | **Tube** | |
| | ***Blank (B)*** | ***Test (T)*** |
| ***1. Substrate-Buffer solution*** | 0.2 mL | 0.2 mL |
| *Incubate the test tubes for 3 minutes at 37ºC* | | |
| ***2. Stop reagent***<br>***3. Sample (serum)*** | 0.2 mL<br>0.04 mL | -<br>0.04 mL |
| *Incubate the test tubes for 30 minutes at 37ºC* | | |
| ***4. Stop reagent*** | - | 0.2 mL |
| *Lat the test tubes stand for 20 minutes at room temperature.* | | |
| ***4. Sodium hydroxide*** | 2.0 mL | 2.0 mL |

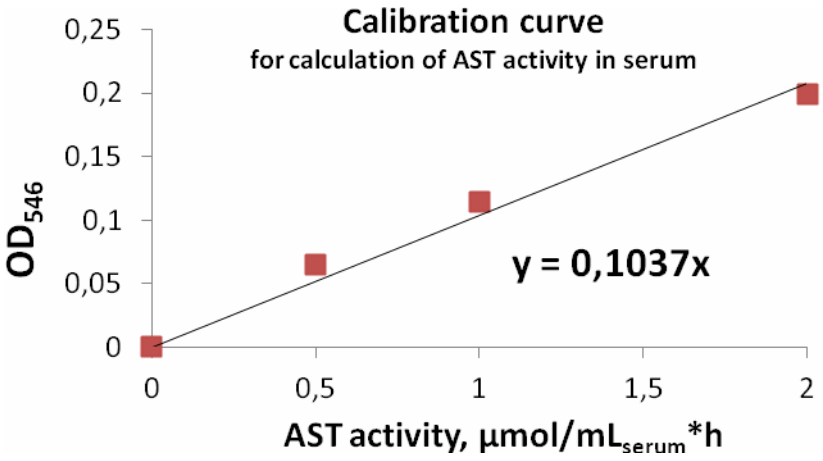
*Lat the test tubes stand for 5-10 minutes at room temperature.*
*Set wavelength of spectrophotometer at 546 nm and zero the instrument with the "Blank".*
*Read the absorbance of all test tubes.*
**NOTE:** The final color is stable for at least 60 minutes.

*Record the absorbance of the test tube:*

$E_T$ _____

## CALCULATIONS

Use the prepared calibration curve to determine the activity of AST in unknown samples.




***AST activity,*** µmol/(h·mL of serum): _____

**CONCLUSION:**

_____

_____

_____

_____

_____

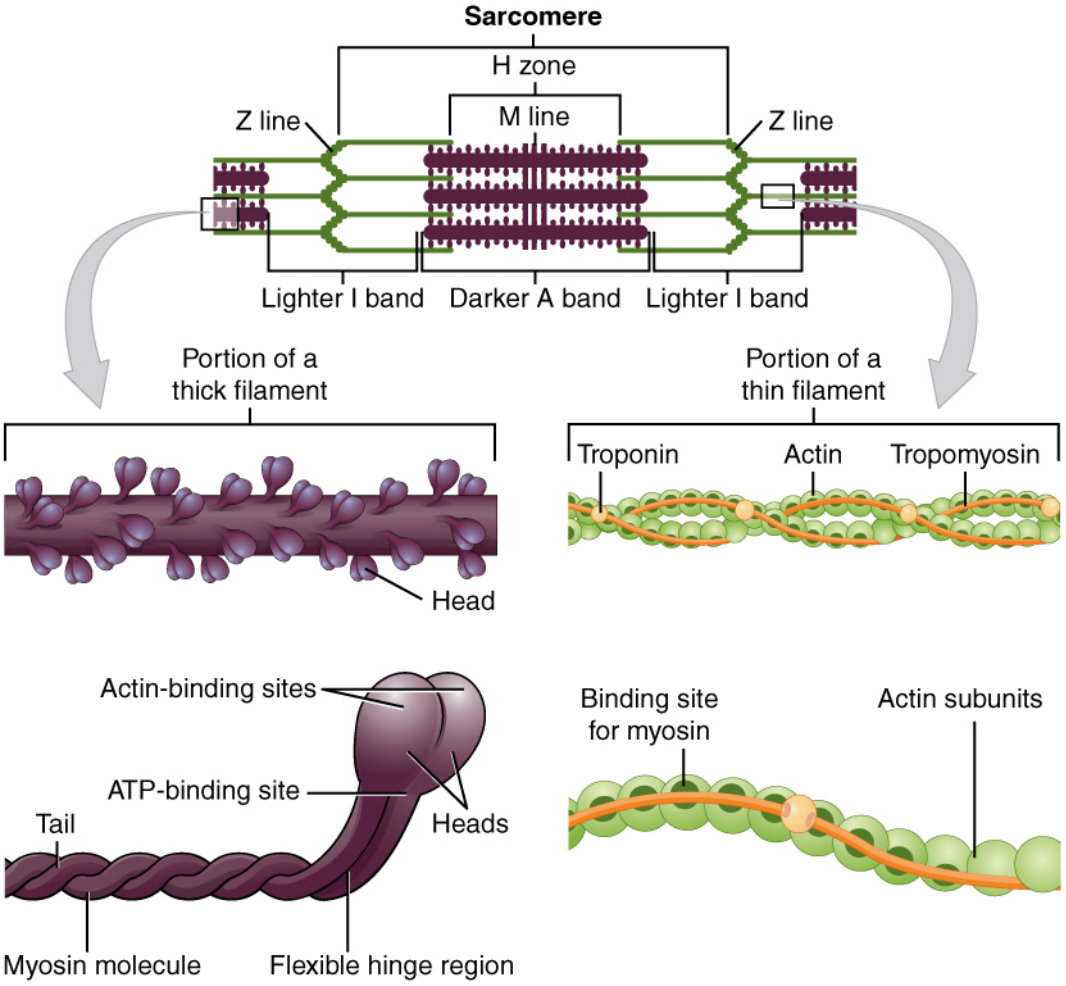
# Lesson 17

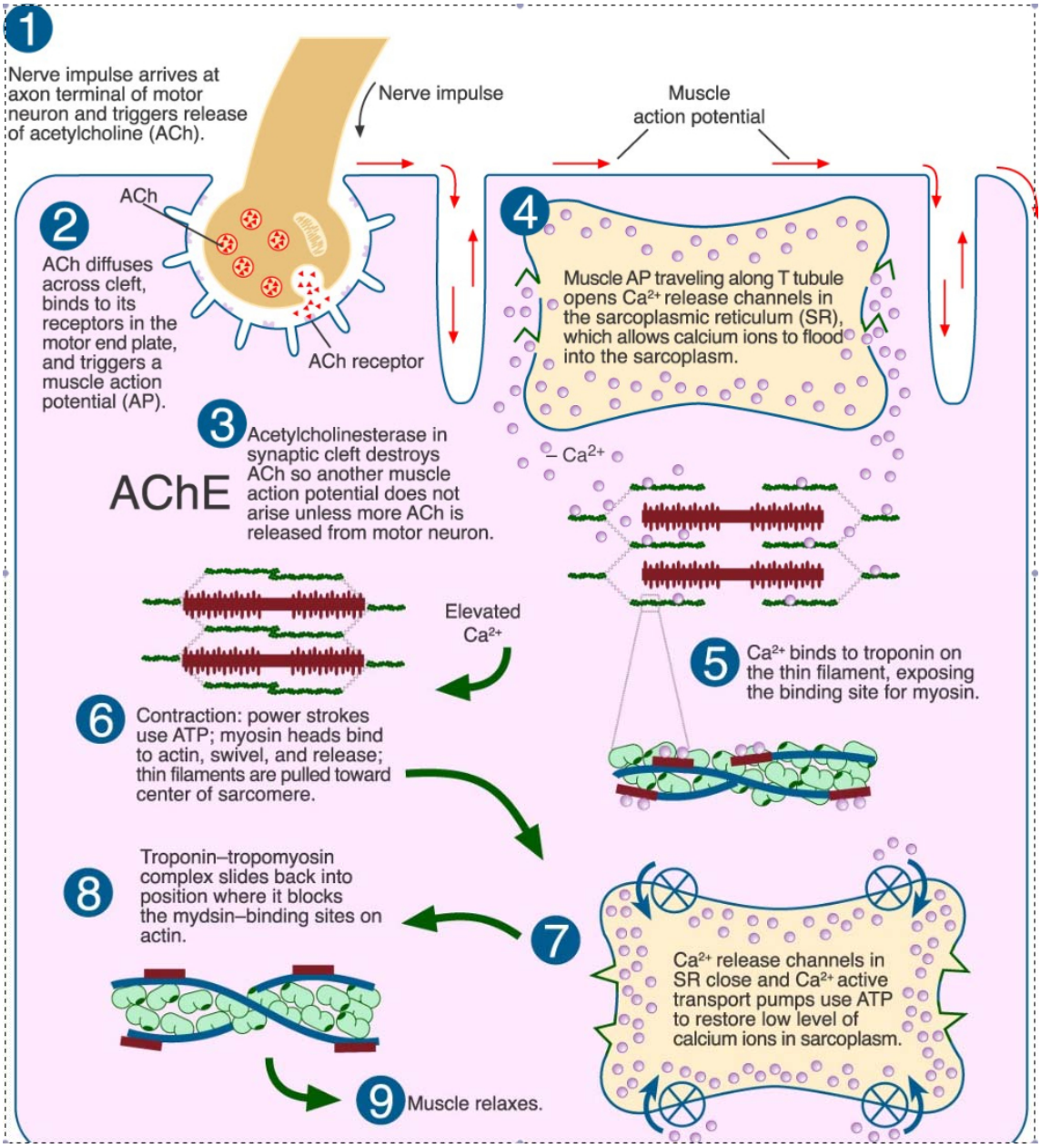
The main topic
**“BIOCHEMISTRY OF MUSCULAR AND CONNECTIVE TISSUES”**

🕮 ***Follow this plan at home to prepare for classroom discussion:***

⇨ Chemical composition of the muscle tissue;
⇨ Characteristics of muscle proteins and non-protein nitrogen-containing compounds;
⇨ Bioenergy of the muscle tissue: ATP sources and role of creatine phospate in muscular contraction;
⇨ Changes in muscular tissue in patients with muscular dystrophy, low physical activity, vitamin E deficiency;
⇨ Biochemistry of the connective tissue. Changes in the connective tissue with aging. Diseases of the connective tissue.
⇨ Structure, physical and chemical properties and biological significance of main proteins of connective tissue: fibronectin, elastin, collagen;
⇨ Basic classes of proteo- and glycosaminoglycans, their structure and functions.

✎ ***Describe the chemical composition of the muscle tissue:***





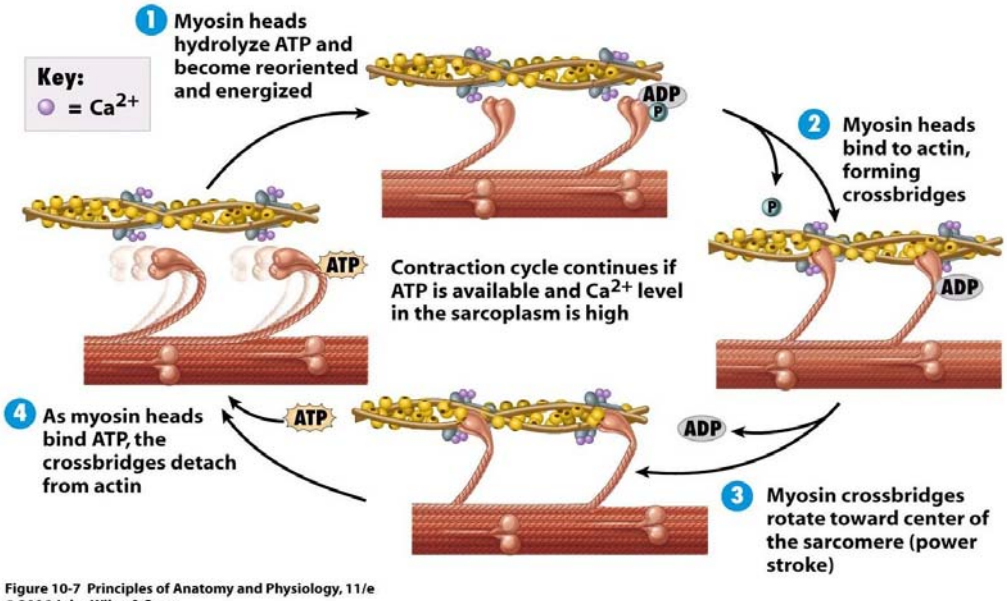




Figure 10-7 Principles of Anatomy and Physiology, 11/e

✎ ***Describe the major components of intracellular matrix:***

| ***Components*** | | ***Structure and function*** |
|---|---|---|
| **Protein fibers** | ***Collagen fibers*** | |
| | ***Elastic fibers*** | |
| | ***Reticular fibers*** | |
| **Ground Substances** | ***Healuronic acid*** | |
| | ***Proteoglycans*** | |
| | ***Adhesive molecules*** | |
| ***Fluid*** | | |

## RECOMMENDED LITERATURE

### Basic

### 1. Lecture notes

2. Harvey R. A. Lippincott's Illustrated Reviews: Biochemistry / R. A. Harvey, D. R. Ferrier -- 5th ed: Lippincott Williams & Wilkins, 2011. – 520 p.

3. Murray R. K. Harper's Illustrated Biochemistry / R. K. Murray, D. K. Granner, V. W. Rodwell. - 27th ed. - Boston [etc.] : McGraw Hill, 2006. - 692 p.

### Additional

1. Koolman J. Color Atlas of Biochemistry: textbook / J. Koolman, K.-H. Roehm. – 2nd ed. – Stuttgart-New York : Thieme, 2005. – 467 p.

2. Lieberman M. Medical Biochemistry: textbook / M. Lieberman; A. Marks, C. Smith. - 2nd ed. - New York : Lippincott Williams & Wilkins, 2007. – 540 p.

3. Marks D. B. Biochemistry: The Chemical Reactions of Living Cells / D. B. Marks, D. Metzler - [2nd ed., vol. 1,2] - USA : Elsevier Academic Press, 1994.- 1974 p.

4. Marshall J. W. Clinical Chemistry : textbook / J. W. Marshall, S. K. Bangert.- Fifth edition. – China : Mosby, 2004. – 422 p.

5. Newsholme E. A. Functional Biochemistry in Health and Disease / E. A. Newsholme, T. R. Leech. - UK : John Wiley & Sons Ltd, 2010.-543 p.

6. Smith C. Basic Medical Biochemistry: A Clinical Approach: textbook / C. Smith, A. Marks, M. Lieberman. - 2nd ed. - New York : Lippincott Williams & Wilkins, 2009. - 920 p.

**Навчальне видання**

# БІОЛОГІЧНА ТА БІООРГАНІЧНА ХІМІЯ

**Робочий зошит для іноземних студентів спеціалізації "Медицина"**

**Частина 2. Загальна та функціональна біохімія**

(Англійською мовою)


**Упорядники:**

**Галенова** Тетяна Іванівна,
**Савчук** Олексій Миколайович,
**Остапченко** Людмила Іванівна



**Формат 60x84$^{1/8}$. Ум. друк. арк. 13,5. Наклад 400. Зам. № 219-9327.**
**Гарнітура Times New Roman. Папір офсетний. Друк офсетний.**
**Підписано до друку**

**Видавець і виготовлювач**
**ВПЦ "Київський університет"**
**б-р Т. Шевченка, 14, м. Київ, 01601**
**☎ (044) 239 32 22; (044) 239 31 72; тел./факс (044) 239 31 28**
**e-mail: vpc_div.chief@univ.net.ua; redaktor@univ.net.ua**