МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

PART 3
METABOLISM OF PROTEINS AND NUCLEIC ACIDS

Self-Study Guide for Students
of General Medicine Faculty in Biochemistry

ЧАСТИНА 3
ОБМІН БІЛКІВ І НУКЛЕЇНОВИХ КИСЛОТ

Методичні вказівки
для підготовки до практичних занять з біологічної хімії
(для студентів медичних факультетів)

Затверджено вченою радою ХНМУ
Протокол № 1 від 26.01.2017 р.

Approved by the
Scientific Council of KhNMU.
Protocol 1 (January 26, 2017)

Харків
ХНМУ
2017

Metabolism of proteins and nucleic acids : self-study guide for students of general medicine faculty in biochemistry. Part 3: / Comp. : O. Nakonechna, S. Stetsenko, L. Popova, A. Tkachenko. – Kharkiv : KhNMU, 2017. – 56 p.

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Обмін білків та нуклеїнових кислот : метод. вказ. для підготовки до практ. занять з біологічної хімії (для студ. мед. ф-тів). Ч 3. / упоряд. О.А. Наконечна, С.О. Стеценко, Л.Д. Попова, А.С. Ткаченко. – Харків : ХНМУ, 2017. – 56 с.

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SOURCES

For preparing to practical classes in "Biological Chemistry"

Basic Sources

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CLASS 1 (4 hours)

TOPIC 1 (2 classes). Protein metabolism. Digestion and absorption of proteins in the gastrointestinal tract. Biochemical analysis of the gastric juice.

IMPORTANCE. Protein metabolism is fundamental in the various transformations of substances typical to all living organisms. Its study is needed to clarify the features of protein biosynthesis and catabolism, to understand molecular mechanisms of development and course of pathological processes. Proteins perform a number of functions in the body: plastic, catalytic, transport, contractile, receptor, regulatory, energy and others. Proteins and amino acids are directly involved in biosynthesis of biologically active substances: hormones, neurotransmitters, biogenic amines, etc. According to nutritional value, proteins can be divided into complete proteins, which contain all the essential amino acids, and incomplete ones lacking at least one essential amino acid. Proteins of food are not utilized by the body without prior cleavage in the gastrointestinal tract. The biological meaning of their digestion is the loss of species and tissue specificity, the transformation of proteins into immunologically indifferent amino acids, which are used in the future for the body's needs. Complete cleavage of dietary proteins by enzymes of the stomach and small intestine often does not occur. Undegraded peptides and amino acids, which are not absorbed in intestinal mucosa, are influenced by bacterial enzymes in the large intestine with the formation of toxic (phenol, cresol, indole, skatole, etc.) and nontoxic (alcohols, hydroxyacids, fatty acids, etc.) compounds. Conversion of amino acids, caused by bacteria of the large intestine, is called *protein putrefaction*. After absorption the toxic substances are transported to the liver where they are detoxified by conjugation with sulfate or glucuronic acid to form nontoxic "pairing" substances excreted in the urine.

AIM. Study the digestion of food proteins under the influence of proteolytic enzymes of the gastrointestinal tract. Characterize the specificity of action, mechanisms of activation and regulation of proteolytic enzymes, role of gastric hydrochloric acid; mechanisms of absorption of amino acids. Consider the conversion of amino acids in the large intestine. Study the mechanisms of detoxification of amino acid putrefaction products. Familiarize yourself with the method of quantitative determination of gastric acidity and its clinical and diagnostic significance.

THEORETICAL QUESTIONS

1. Role of proteins in the vital activity of organism.
2. Protein nutritional requirements. Complete and incomplete proteins. Nonessential, essential, conditionally essential and semi-essential amino acids.
3. Basic stages of the intestinal metabolism of proteins.
4. Proteolytic enzymes: the specificity of action, the biological sense of formation in the inactive form, the mechanisms of activation and regulation.

5. Chemical composition of gastric juice. Role of HCl. Forms of the acidity of gastric juice, the methods of determination.
6*. Clinical and diagnostic significance of gastric juice analysis.
7*. Risk factors for the formation of gastric ulcer and tumors. Diagnostic significance of the qualitative determination of lactic acid.
8. Mechanisms of amino acid absorption in intestine.
9. Chemical conversions of amino acids in large intestine.
10. Mechanisms of detoxification of protein putrefaction products in the large intestine.
11. Amines formed in the large intestine during the putrefaction from diaminomonocarboxylic acids, their detoxification.
12. Toxic substances formed in the large intestine during the putrefaction from tyrosine and phenylalanine, their detoxification.
13. Toxic substances formed in large intestine during the putrefaction from tryptophan, their detoxification.
15. UDP-glucuronic acid and PAPS: structure, role in detoxification of poisonous products.

Recommendations for self-study of theoretical questions

Question	Information
1. Clinical and diagnostical significance of gastric juice analysis	1.1. Analysis of the gastric juice has significant diagnostic significance in diseases of the stomach – gastritis, ulcer disease, cancer, etc. 2. Key parameters of the gastric juice: volume, acidity, HCl and pepsin, and the presence of lactate. Histamine hydrochloride, pentagastrin, and other substances are used to stimulate gastric secretion. 1.3. It is necessary to assess the level of HCl production to confirm the diagnosis of ulcer. The basal phase of secretion in the body of stomach: pH 1.6-2.0; stimulated secretion – pH 1.21–2.20. Hypoacidic gastritic: basal secretion phase – pH 2.1-5.9; stimulated secretion – pH 2.3–3.0. Anacidity: basal secretion phase – pH> 6; stimulated secretion – pH>5. Duodenal bulb ulcer - pH 2.8-3.4. The increased amount of gastric juice (normally ≈ 1.5 l) and its high fasting acidity are symptoms of hyperacidic gastritis and gastric ulcer. 1.4. There are basal secretion (BS) and stimulated secretion (SS). BS/SS ratio in healthy individuals is 1:3. This index is about 1 in atrophic gastritis. Prognosis for duodenal ulcer disease becomes negative if the maximum secretion is high → This may increase risk of complications (bleeding, perforation). 1.5. Color of the gastric juice: whitish (normal), yellow-green (the presence of bile), and brownish (blood).

Question	Information
2. Risk factors for the formation of gastric ulcers and tumors. Diagnostic significance of qualitative determination of lactate in the gastric juice	2.1. Determination of lactic acid in the gastric juice is used to assess the intensity of lactic fermentation in the stomach, which can serve as a result of metabolism of cancer cells. Early diagnosis of cancer is confirmed by determination of LDH and β-glucuronidase. 2.2 Risk factors: – Acid-peptic factor plays a pivotal role in the mechanisms of gastric ulcer development. Reverse H^+ diffusion across the stomach wall and impairment of the mucous barrier function → Imbalance of secretion of biologically active substances by the APUD system with elevated concentrations of histamine and gastrin → stimulation of HCl and pepsin secretion → aggressive effect of gastric juice on the mucous membrane; – An increase in pepsin production; – Proteolytic index (intensity of proteolysis in the stomach and acid production); – Role of proteases that have ulcerogenic properties; – Role of cyclic nucleotides; elevation of cAMP in the gastric mucosa, increased activity of adenylyl cyclase, reduced activity of phosphodiesterase; – Role of biologically active substances in the process of ulceration: histamine, acetylcholine, gastrin, calcium, prostaglandins, catecholamines, insulin (they affect metabolism of cyclic nucleotides and HCL secretion); – Increased vagal tone; – Autoimmune gastric secretagogue hypothesis; a special IgG-secretagogue that interacts with H2-receptor on the cell surface and stimulates HCl secretion is isolated; – Abnormal antroduodenal inhibition; – Abnormal mucous-bicarbonate barrier (hexoses, hexosamines, fucose, sialic acid, etc.); – Low lysozyme activity; – Imbalance in the prooxidant-antioxidant system; – Role of microorganisms (Helicobacter pylori).

TESTS FOR SELF-CONTROL

1. Which of the below mentioned proteinases belong to exopeptidases?

A. *Pepsin, trypsin.* ***B.*** *Elastase, collagenase.* ***C.*** *Carboxypeptidases, aminopeptidases.*
D. *Chymotrypsin, carboxypeptidases.* ***E.*** *Aminopeptidases, elastase.*

2. C-terminal amino acids are removed by:

A. *Dipeptidases.* ***B.*** *Aminopeptidases.* ***C.*** *Carboxypeptidases.* ***D.*** *Chymotrypsin.* ***E.*** *Pepsin.*

3. Analysis of gastric juice shows that he content of free hydrochloric acid is normal. Which of the below mentioned values corresponds to that?

A. *20–40 mmol\L.*
B. *10–20 mmol\L.*
C. *40–50 mmol\L.*
D. *30–50 mmol\L.*
E. *5–15 mmol\L.*

4. Putrefaction of proteins under the influence of intestinal microflora includes the following reactions:

A*. Conversion of proteins to peptides.*
B. *Conversion of conjugated proteins to simple ones.*
C. *Formation of amino acids from proteins.*
D. Transamination of amino acids.
E. *Deamination and decarboxylation of amino acids with the further formation of toxic products.*

5. N-terminal amino acids are removed by:

A. *Dipeptidases.*
B. *Aminopeptidases.*
C. *Carboxypeptidases.*
D. *Elastase.*
E. *Endopeptidases.*

6. Pancreatic juice contains enzymes:

A. *Trypsin, chymotrypsin, rennin.*
B. *Chymotrypsin, elastase, pepsin.*
C. *Carboxypeptidases, rennin, lipase.*
D. *Elastase, carboxypeptidases, aminopeptidases.*
E. *Chymotrypsin, trypsin, carboxypeptidases*

7. Which of the below mentioned enzymes is activated by trypsin?

A. *Pepsin.*
B. *Aminopeptidase.*
C. *Lipase.*
D. *Amylase.*
E. *Chymotrypsin.*

8. A patient had been hospitalized with diarrhea after consumption of protein-rich food. Physician believed the disturbance of protein digestion that resulted in their intensified putrefaction. Which of the below mentioned substances was the product of protein putrefaction in intestine?

A. *Bilirubin.*
B. *Lactate.*
C. *Porphobilinogen.*
D. *Cadaverin.*
E. *Tryptophan.*

9. A patient has bad appetite, belching. Total acidity of gastric juice is 10 units. This state is typical for:

A. *Anacidic gastritis.*
B. *. Hyperacidic gastritis.*
C. *Acute pancreatitis.*
D. *Hypoacidic gastritis.*
E. *Stomach ulcer.*

10. The daily diet of the adult man should be composed of lipids, proteins, carbohydrates, vitamins, mineral salts and water. Name the amount of protein, which ensures normal vital activity of the organism.

A. *100–120 g per day.*
B. *50–60 g per day.*
C. *10–20 g per day.*
D. *70–80 g per day.*
E. *40–50 g per day.*

11. In newborn the milk curdling (the conversion of soluble caseins into insoluble paracaseins) is performed with participation of enzymes and calcium ions. Which enzyme participates in this process?

A. *Secretin.* ***B.*** *Pepsin* ***C.*** *Gastrixin* ***D.*** *Rennin* ***E.*** *Lipase*

12. Toxic substances are formed in large intestine as a result of amino acid decarboxylation. Which substance is formed from ornithine?

A. *Skatole* ***B.*** *Indole* ***C.*** *Cadaverin* ***D.*** *Phenol* ***E.*** *Putrescine*

13. Negative nitrogen balance was found in a 45–year-old man after prolonged vegetable-based diet. Which feature of diet was the cause of that state?

A. *Excessive amount of water.* ***D.*** *Insufficient amount of fats.*
B. *Excessive amount of carbohydrates.* ***E.*** *Insufficient amount of fats and proteins.*
C. *Insufficient amount of proteins.*

14. A doctor decided to do laboratory blood tests for a patient to determine the electrophoretic spectrum of proteins before prescribing protein parenteral nutrition. The method is based on the following physical and chemical properties of proteins:

A. *Viscosity.* ***C.*** *Optical activity.* ***D.*** *Inability to be denatured.*
B. *Charge.* ***E.*** *Hydrophylicity and ability to be denaturated.*

15. Pancreatic diseases are associated with an increase in formation and secretion of trypsin. Select substances whose hydrolysis is affected in this case.

A. *Proteins.* ***B.*** *Lipids.* ***C.*** *Carbohydrates.* ***D.*** *Nucleotides.* ***E.*** *Phospholipids.*

16. Digestion of proteins in the stomach is the initial stage of their breakdown in the digestive tract. Which enzymes are involved in protein digestion in the stomach?

A. *Trypsin and cathepsins.* ***D.*** *Enteropeptidase and elastase.*
B. *Chymotrypsin and lysozyme.* ***E.*** *Carboxypeptidase and aminopeptidase.*
C. *Pepsin and gastrixin.*

17. In humans chymotrypsin is secreted by the pancreas and undergoes limited proteolysis in the intestinal cavity to form active chymotrypsin by:

A. *Enterokinase.* ***B.*** *Pepsin.* ***C.*** *Trypsin.* ***D.*** *Aminopeptidase.* ***E.*** *Carboxypeptidase.*

18. Daily diet of healthy adults should include fat, protein, carbohydrates, vitamins, minerals, and water. What is the lowest possible daily requirement for proteins (g/day), which provides normal vital function?

A. *50–60.* ***B.*** *10–20.* ***C.*** *40–50.* ***D.*** *70–80.* ***E.*** *100–120.*

19. A woman suffers from insufficient pacreatic function. Which nutrients are hydrolyzed improperly?

A. *Proteins, carbohydrates.* ***C.*** *Proteins.* ***D.*** *Proteins, lipids, carbohydrates.*
B. *Fats,carbohydrates.* ***E.*** *Proteins, lipids.*

20. Analysis of the gastric mucosa biopsies of a patient with chronic gastritis revealed a sharp decrease in the number of parietal cells. How did it affect parameters of gastric juice analysis?

A. *High production of enzymes.* ***D.*** *Low mucin formation.*
B. *High acidity.* ***E.*** *Low acidity.*
C. *More free hydrochloric acid.*

21. A patient with acute inflammation of the pancreas (pancreatitis) has disorders in the protein cavitary digestion. This may be due to insufficient synthesis and release of:

A. *Lipase.* ***B.*** *Pepsin.* ***C.*** *Dipeptidase.* ***D.*** *Trypsin.* ***E.*** *Amylase.*

22. A weak hydrochloric acid solution was injected in acute experiment to the duodenal cavity. Which gastrointestinal hormone is overproduced in this case?

A. *Neurotensin.* ***B.*** *Histamine.* ***C.*** *Secretin.* ***D.*** *Gastrin.* ***E.*** *Motilin.*

23. A patient complains of weight loss, stomach pain after meal. Analysis of gastric juice revealed that the total acidity is 20 U. Which nutrient is digested improperly?

A. *Starch.* ***B.*** *Phospholipids.* ***C.*** *Neutral fats.* ***D.*** *Oligosaccharides.* ***E.*** *Proteins.*

24. Blood proteins became available for intestinal microorganisms in a patient due to gastrointestinal bleeding. Select a substance whose concentration increases in the patient.

A. *Tryptophan.* ***B.*** *Cyanocobalamin.* ***C.*** *Thiamine.* ***D.*** *Indole.* ***E.*** *Creatine.*

25. To evaluate the functional state of the liver in patients, it is necessary to study animal indican excretion in the urine. It is formed during detoxification of amino acid putrefaction products. Select this amino acid.

A. *Serine.* ***B.*** *Tryptophan.* ***C.*** *Cysteine.* ***D.*** *Glycine.* ***E.*** *Valine.*

26. A male patient complains of severe abdominal pain after eating fatty food and drinking alcohol. Serum trypsin level reaches 850 mmol / (h • L) (the normal concentration is 60–240 mmol / (L • h)). Which pathology of the digestive system can be diagnosed?

A. *Dynamic bowel obstruction.* ***C.*** *Gastric ulcer.* ***D.*** *Acute pancreatitis.*
B. *Mechanical bowel obstruction.* ***E.*** *Hepatitis.*

27. A female patient who had had a surgery due to "acute abdomen" noticed brown urine. Urine indican levels exceeded 93 mmol / day. What can this parameter indicate?

A. *Activation of amino acid deamination.* ***D.*** *Intensified pretein putrefaction.*
B. *Low intensity of ornithine cycle.* ***E***. *Low glomerular filtration rate.*
C. *Gluconeogenesis inhibition.*

28. A patient was hospitalized to the neurological hospital with symptoms of encephalopathy. Correlation between the severety of encephalopathy and substances that enter the bloodstream from instestine was found. Which compounds formed in the intestine can cause endotoxemia?

A. *Indole* ***B.*** *Acetoacetate* ***C.*** *Biotin* ***D.*** *Ornithine* ***E.*** *Butyrate*

29. To prevent attacks of acute pancreatitis, physician prescribed trasilol (contrical), which is an inhibitor of:

A. *Chymotrypsin.* ***C.*** *Trypsin.* ***D.*** *Gastrixin.*
B. *Carboxypeptidase.* ***E.*** *Elastase.*

30. Lactate was found in the patient's gastric juice. What is the possible cause?

A. *Pepsin insufficiency.* ***C.*** *Renin deficiency.* ***D.*** *Excess HCI.*
B. *Lack of HCI.* ***E.*** *Gastrixin insufficiency.*

31. A patient has high indican levels in blood and urine reflecting activation of protein putrefaction in the intestine. Which amino acid is the source of indican?

A. *Tryptophan.* ***B.*** *Proline.* ***C.*** *Tyrosine.* ***D.*** *Phenylalanine.* ***E.*** *Histidine.*

32. To determine antitoxic function of liver, sodium benzoate was prescribed to patient. It is converted to hippuric acid in the liver. Which substance is used for this reaction?

A. *UDP-glucoronyl transferase.* ***C.*** *PAPS.* ***D.*** *Cysteine.*
B. *Methionine.* ***E.*** *Glycine.*

33. To lose weight, a female limited the amount of food products. Three months later she suffered of edema, increased diuresis. Which nutrient deficiency can cause such changes?

A. *Proteins.* ***B.*** *Fats.* ***C.*** *Carbohydrates.* ***D.*** *Vitamins.* ***E.*** *Minerals.*

34. Bowel dysmotility results in intense protein putrefaction to form toxic products, including phenol. Which amino acid can form phenol?

A. *Proline.* ***B.*** *Tryptophan.* ***C.*** *Threonine.* ***D.*** *Tyrosine.* ***E.*** *Histidine*

35. Hypochlorhydria was found during investigation of gastric secretory function. Which enzyme activity can be reduced?

A. *Pepsin.* ***B.*** *Hexokinase.* ***C.*** *Amylase.* ***D.*** *Dipeptidase.* ***E.*** *Lipase.*

36. Intake of gluten-containing food leads to celiac disease, which is characterized by degeneration of the intstinal villi with the loss of absorptive function, diarrhea, steatorrhea, bloating, weight loss, and other extraintestinal manifestations. Gluten is a protein of:

A. *Rice.* ***B.*** *Strawberry.* ***C.*** *Wheat.* ***D.*** *Egg.* ***E.*** *Corn.*

37. Motility of the gastrointestinal tract in newborns is by several orders of magnitude higher compared to adults. Which enzyme facilitates rapid sedimentation and digestion of milk caseinogens in the stomach of children?

A. *Renin.* ***B.*** *Trypsin.* ***C.*** *Chymotrypsin.* ***D.*** *Proelastase.* ***E.*** *Pepsin.*

38. A patient complains of intense hypersalivation, resulting in partial neutralization of hydrochloric acid in the stomach. Which substances are broken down improperly in this case?

A. *Lipids.* ***B.*** *Carbohydrates.* ***C.*** *Nucleic acids.* ***D.*** *Proteins.* ***E.*** *Cholesterol.*

39. A male who suffers from chronic intestinal obstruction has activated protein putrefaction in the colon. This is confirmed by:

A. *Hyperuricemia.* ***B.*** *Glucosuria.* ***C.*** *Creatinuria.* ***D.*** *Bilirubinuria.* ***E.*** *Indicanuria.*

40. A woman who has been limiting the amount of food for three months has weight loss, fatigue and decreased mental activity. Edema of the face and extremities is observed. Which nutrient is deficient?

A. *Proteins.* ***B.*** *B. Vitamins.* ***C.*** *Fats.* ***D.*** *Carbohydrates.* ***E.*** *Microelements.*

41. The action of bacterial enzymes in the colon can lead to the formation of toxic products - phenol and cresol – from tyrosine. Detoxification of phenol and cresol requires conjugation with:

A. *S-Adenosylmethionine.* ***C.*** *Glycine.* ***E.*** *All options are correct.*
B. *Glutathione.* ***D.*** *UDP- glucuronate.*

42. Pancreatic proenzymes - chymotrypsin, proelastase, procarboxypeptidase – have a common activator. Which of the following substances is it?

A. *Trypsinogen.* ***B.*** *Trypsin.* ***C.*** *Elastase.* ***D.*** *Chymotrypsin.* ***E.*** *HCl.*

43. Intake of the significant amount of protein caused an increase in pancreatic proteolytic enzymes. Which enzyme is elevated in this case?

A. *Renin.* ***B.*** *Pepsin.* ***C.*** *Enterokinase.* ***D.*** *Gastrixin.* ***E.*** *Trypsin.*

44. A patient with hypersecretion of gastric juice excluded vegetable soups and broths in accordance with a physician's reccomendation, since they stimulated secretion of:

A. *Secretin.* ***B.*** *Cholecystokinin.* ***C.*** *Gastrin.* ***D.*** *Neurotensin.* ***E.*** *Somatostatin.*

45. Which process is activated in gastric tumor cells causing apprearance of lactic acid in the gastric juice?

A. *Gluconeogenesis.* ***C.*** *Pentose phosphate pathway.* ***E.*** *Anaerobic glycolysis.*
B. *Beta-oxidation of fatty acids.* ***D.*** *Aerobic glucose oxidation.*

PRACTICAL WORK

Analysis of gastric juice

Task 1. Carry out a quantitative determination of gastric acidity.

Principle. The method is based on the determination of acidic substances in the gastric juice titrated with a solution of sodium hydroxide, using two different indicators: *para*-dimethylamino-azobenzene (color transition zone at pH = 2.3–4.2) and phenolphthalein (color transition zone at pH = 8.2–10.0). The color change of *para*-dimethylaminoazobenzene from red to orange indicates the free hydrochloric acid, and the color change of phenolphthalein from colorless to pink indicates total acidity of gastric juice.

Procedure. Pour 5 ml of filtrated gastric juice in a conical flask; add 1–2 drops of 0.5 % solution of *para*-dimethylaminoazobenzene and 2 drops of 0.5 % solution of phenolphthalein. Titrate with 0.1 N sodium hydroxide solution to the appearance of the orange-red color and note the amount of alkali (in ml), which is expended to the titration of free hydrochloric acid (I point titration).

Continue the titration to the appearance of the lemon-yellow color (II titration point) and note the amount of alkali, which was expended from the start of the titration to II point of the titration. Then, continue to titrate to the appearance of the pink color (III titration point) and note the amount of alkali that is expended to the titration from the beginning to point III. The unit of acidity of gastric juice is the volume of solution 0.1 mol/L sodium hydroxide (in ml), that is expended to the titration of 100 ml of gastric juice. For example, 1.5 ml of 0.1 mol/L sodium hydroxide is expended to the titration of 5 ml of gastric juice to I titration point, therefore the amount of free hydrochloric acid is: $(1.5 \times 100) / 5 = 30$ (units).

It is necessary to know the total concentration of hydrochloric acid to calculate the amount of bound hydrochloric acid. The latter is determined on the basis of the titration. It is known that the amount of alkali, needed to the binding of all hydrochloric acid, is equal to the arithmetic mean of the number of alkali consumed to points II and III of the titration. For example, for titration to II and III titration steps, 2 ml and 3 ml of alkali solution are consumed, respectively, the arithmetic mean is 2.5 ml. Therefore the total content of hydrochloric acid in 100 ml of gastric juice is: $(2.5 \times 100) / 5 = 50$ (units). Protein bound hydrochloric acid is determined by the difference between the amount of total and free hydrochloric acid: 50–30 = 20 (units). In addition, III titration is used to determine the total acidity. For example, if 3 ml of 0.1 mol/L sodium hydroxide is expended to the titration of 5 ml of gastric juice, the total acidity is: $(3.0 \times 100) / 5 = 60$.

Task 2. Determine the presence of lactic acid (Uffelman's reaction).

Principle. The method is based on the ability of lactic acid in the presence of ferric phenolate to form a ferrum lactate salt of yellow-green color.

Procedure. Add three drops of 1 % solution of ferric chloride to 10 ml of 1 % phenol solution to prepare ferrum phenolate. The content is mixed with a glass rod. Violet color develops. Add 5–6 drops of investigated gastric juice to 2–3 ml of the reagent to carry out the reaction. Changing the color to yellow-green indicates the presence of lactic acid.

Task 3. Determine the presence of blood (Adler's reaction with benzidine).

Principle. The reaction is based on the oxidation of benzidine by oxygen, which is formed during the decomposition of hydrogen peroxide under the action of blood catalase, with the formation of products of blue-green color.

Procedure. Put 5 drops of unfiltered gastric juice into the test tube. Add 5 drops of freshly prepared 1 % solution of benzidine in glacial acetic acid and 5 drops of 3 % solution of hydrogen peroxide. In the presence of blood in gastric juice a blue or green color (oxidized benzidine) appears by the time.

Practical significance. Gastric juice is almost colorless, strongly acidic multicomponent liquid, which contains water (99.4 %), in which the enzymes, hydrochloric acid, mucoids are dissolved. The main inorganic component is the hydrochloric acid in the free and bound to the protein states. It also includes chlorides, phosphates, sulfates, carbonates, sodium, potassium, calcium, etc. Proteins, mucin (mucus), lysozyme, enzymes pepsin and rennin, the products of metabolism constitute organic compounds. The gastric juice contains intrinsic Castle's factor, which promotes absorption of vitamin B_{12}. The digestion of proteins is strongly dependent on the acidity of gastric juice. The total acidity (all the acid substances of gastric juice), free and bound hydrochloric acid are distinguished. Normally, the total acidity is 40–60 units, the content of free hydrochloric acid is 20–40 units, and the content of bound hydrochloric acid is 5–20 units. The acidity of gastric juice in diseases of the stomach may be zero, low and high. The increase of the content of free HCl and total acidity (hyperchlorhydria) is observed in gastric ulcer or hyperacid gastritis. A decrease in free HCl and total acidity (hypochlorhydria) is observed in hypoacid gastritis and tumors of stomach. A complete absence of free HCl and a significant decrease in total acidity (achlorhydria) may be observed in malignant tumors of the stomach and chronic gastritis. Complete absence of hydrochloric acid and pepsin (achylia) may be observed in pernicious anemia, tumors of the stomach. Along with the normal components of the gastric juice other ingredients (lactic acid, blood, fatty acids, bile pigments) can appear in a number of diseases. In the clinic, they are often determined by specific reactions. In achlorhydria lactic, acetic, butyric acids are produced under the influence of microorganisms. Blood (blood pigments) may occur in the gastric ulcers. Bile pigments can come into the stomach from the duodenum due to reflux.

TOPIC 2 (2 hours). Tissue proteolysis. Amino acid pool of tissues. General reactions of amino acid catabolism. Decarboxylation of amino acids. Quantitative determination of histamine in the blood.

IMPORTANCE. Every day about 400 grams of proteins are renovated in organism of adults. A turnover rate of proteins in different tissues varies. Tissue hydrolysis of proteins by tissue proteases (or cathepsins) is necessary not only for renovation of them, but also for the destruction of defective molecules, mobilization of endogenous proteins for energy during starvation, etc. One-third of amino acid pool is formed by food proteins, and two-thirds of amino acid pool is formed by degradation of tissue proteins. Depending on the physiological needs of the body, the amino acid pool is used in biosynthesis of proteins and peptides, the formation of biologically active compounds, it is involved in energy metabolism. In the processes of catabolism amino acids undergo decarboxylation, deamination, transamination (the general pathways of metabolism). The study of these aspects will allow efficient use of knowledge about the metabolism of proteins and amino acids for the analysis of its numerous disorders.

AIM. Familiarize yourself with the tissue proteolysis, the classification and the mechanism of action of cathepsins. Study the ways of formation and using the pool of free amino acids in tissues. Study the main ways of amino acid metabolism, in detail study processes of decarboxylation; describe the role of biogenic amines (γ-amino-butyric acid, histamine, serotonin, dopamine, noradrenaline, adrenaline, etc.), enzymes of their formation and destruction. Familiarize yourself with the method of determining histamine in the blood and its clinical and diagnostic significance.

THEORETICAL QUESTIONS

1. Tissue proteolysis. Action, properties and the classification of cathepsins.

2*. Scheme of the basic ways of formation and using the amino-acid pool of tissues. Basic classes of the organic compounds, formed from the amino acids.

3. General pathways of the amino acid metabolism.

4. Decarboxylation of amino acids; the characteristic of decarboxylases, the role of vitamin B_6.

5. Biogenic amines: reactions of formation, role. Mechanisms of inactivation of biogenic amines by mono- and diamine oxidases.

6. Decarboxylation of phenylalanine and tyrosine, the role of the amines.

7. Decarboxylation of histidine, the role of the amine.

8. Decarboxylation of tryptophan and 5-hydroxytryptophan, the role of the amines.

9. Decarboxylation of glutamate and aspartate, the role of the amines.

10. Decarboxylation of cysteine and cysteic acid, the role of the amines.

Recommendations for self-study of theoretical questions

Question	Information
1. Major pathways for replenishment and use of amino acid pool in tissues	1.1. Ways of amino acid intake: – Dietary proteins (100–120 g/day); – Breakdown of ou own protein in tissues by cathepsins (approximately 400 g/day); – Synthesis from carbohydrates. 1.2. Use of amino acids: – Protein synthesis; – Breakdown to final products - CO_2, H_2O, NH_3; – Synthesis of non-protein nitrogen-containing compounds (peptides, biogenic amines, nucleotides, heme, choline, nicotinic acid, creatine, hormones, etc.). – Formation of α-keto acids; – Formation of glucose (from glucogenic amino acids); – Formation of ketone bodies (from ketogenic amino acids).

TESTS FOR SELF-CONTROL

1. Point out products of amino acid decarboxylation reactions:

A. *Aceton+CO_2.* ***C.*** *Glucose+CO_2.* ***E.*** *Biogenic amines+CO_2.*
B. *Glycerol+CO_2.* ***D.*** *Keto acids+CO_2.*

2. Decarboxylation of glutamate in CNS results in the formation of neurotransmitter called:

A. *Asparagine.* ***B.*** *B. Serotonin.* ***C.*** *Histamine.* ***D.*** *Glutathione.* ***E.*** *GABA.*

3. Point out the biological role of serotonin, which is the product of 5-hydroxytryptophan decarboxylation:

A. *Inhibitor of enzymes of proteosynthesis.*
B. *Activator of enzymes of gluconeogenesis.*
C. *Activator of enzymes of glycolysis.*
D. *Activator of enzymes of lipolysis.*
E. *Regulator of arterial pressure and body temperature.*

4. Point out the biological role of biogenic amine formed in decarboxylation of glutamate:

A. *Coenzyme of complex enzymes.* ***D.*** *Inhibitor of lipolysis.*
B. *Activator of protein synthesis.* ***E.*** *Inhibitor of gluconeogenesis.*
C. *Mediator of inhibition in CNS.*

5. Point out the biological role of histamine, which is the product of histidine decarboxylation:

A. *Activator of gastric juice secretion.*
B. *Inhibitor of gastric juice secretion.*
C. *Activator of bicarbonate secretion by pancreas.*
D. *Inhibitor of bicarbonate secretion by pancreas.*
E. *It has bactericidal effect.*

6. Point out biogenic amines, which are inhibitory neurotransmitters:

A. *Dopamine.* ***B.*** *Histamine.* ***C.*** *Serotonin.* ***D.*** *γ-Aminobutyric acid.* ***E.*** *Taurine.*

7. A child was admitted to the hospital in state of an allergic shock, developed after a sting of a wasp. The concentration of histamine in the blood was increased. Which reaction does this amine result from?

A. *Reduction.* ***C.*** *Dehydrogenation.* ***E.*** *Decarboxylation.*
B. *Hydroxylation.* ***D.*** *Deamination.*

8. Human organism has a peptide, which formation is performed with participation of γ-carboxylic group of glutamic acid. What is the name of this peptide?

A. *Vasopressin.* ***B.*** *Carnosine.* ***C.*** *Anserine.* ***D.*** *Oxytocine.* ***E.*** *Glutathione.*

9. Which food product should be excluded from diet during the treatment with antidepressants – inhibitors of monoaminoxidase?

A. *Beef.* ***B.*** *Cabbage.* ***C.*** *Potato.* ***D.*** *Solid cheese.* ***E.*** *Apples.*

10. β-Alanine is formed in decarboxylation of:

A. *Glutamate.* ***B.*** *Aspartate.* ***C.*** *Valine.* ***D.*** *Leucine.* ***E.*** *Histidine.*

11. Psychopharmacologic drugs with antidepressant effect inhibit the oxidative deamination of noradrenaline and serotonin in brain by means of inhibition of enzyme:

A. *L-Amino acid oxidase.* ***C.*** *Monoaminoxidase.* ***E.*** *D-Amino acid oxidase.*
B. *Cytochrome oxidase.* ***D.*** *Glutamate dehydrogenase.*

12. A patient with brain injury has epileptic convulsions, which are regularly repeated. Which biogenic amine is formed improperly?

A. *GABA.* ***B.*** *Histamine.* ***C.*** *Adrenaline.* ***D.*** *Serotonine.* ***E.*** *Dopamine.*

13. Biogenic amines (histamine, serotonin, dopamine, etc.) are active substances that affect various physiological functions in the body. Which process leads to the formation of biogenic amines in tissues?

A. *Amino acid decarboxylation.* ***D.*** *Amino acid oxidation.*
B. *Amino acid deamination.* ***E.*** *Reductive amination.*
C. *Amino acid transamination.*

14. A patient was hospitalized with "intestinal carcinoma". Tests showed an increased production of serotonin. It has been known that this substance is formed from tryptophan. What biochemical mechanisms underlie this process?

A. *Transamination.* ***C.*** *Formation of conjugates.* ***E.*** *Decarboxylation.*
B. *Deamination.* ***D.*** *Microsomal oxidation.*

15. Decarboxylation of histidine results in the formation of extremely active amine – mediators of inflammation and allergy, namely:

A. *γ-Aminobutyric acid.* ***C.*** *Dopamine.* ***E.*** *Tryptamine.*
B. *Serotonin.* ***D.*** *Histamine.*

16. It has been known that metabolism of catecholamine neurotransmitters (norepinephrine, epinephrine, dopamine) is significantly affected by monoamine oxidase. How does this enzyme inactivate catecholamine neurotransmitters?

A. *Removal of methyl group.* ***C.*** *Oxidative deamination.* ***E.*** *Hydrolysis.*
B. *Addition of amino group.* ***D.*** *Carboxylation.*

17. Antidepressants which are inhibitors of monoamine oxidase are used for treatment of depression of various origins. Which substance is a happiness neurotransmitter whose level increases in the brain under the influence of antidepressants?

A. *Dopamine.* ***B.*** *Serotonin.* ***C.*** *Glycine.* ***D.*** *Taurine.* ***E.*** *Noradrenaline.*

18. Which vitamin forms a part of glutamate decarboxylase and is involved in the formation of GABA, and whose failure manifests by convulsions?

A. *Ascorbic acid.* ***B.*** *Cobalamin.* ***C.*** *Folic acid.* ***D.*** *Pyridoxine.* ***E.*** *Tocopherol.*

19. Catabolism of histidine leads to the formation of a biogenic amine that has a powerful vasodilator effect. Select it.

A. *Noradrenaline.* ***B.*** *Dopamine.* ***C.*** *Serotonin.* ***D.*** *DOPA.* ***E.*** *Histamine.*

20. Pharmacological effects of antidepressants are associated with inhibition of an enzyme that catalyzes breakdown of biogenic amines - norepinephrine, serotonin – in mitochondria of cerebral neurons. Which enzyme is involved in this process?

A. *Monoaminooxidase.* ***B.*** *Lyase.* ***C.*** *Decarboxylase.* ***D.*** *Peptidase.* ***E.*** *Transaminase.*

21. An examination of a male revealed acute radiation sickness. A sharp reduction of serotonin in platelets was found. Which substance metabolism is a possible cause of low platelet serotonin?

A. *Tyrosine.* ***B.*** *Phenylalanine.* ***C.*** *5-Hydroxytryptophan.* ***D.*** *Serine.* ***E.*** *Histidine.*

22. A 9-month-old baby is exclusively fed with formula whose pyridoxine content is not balanced. The child ahs pellagra-like dermatitis, convulsions, anemia. Development of convulsions may be associated with abnormal formation of:

A. *Dopamine.* ***B.*** *Serotonin.* ***C.*** *DOPA.* ***D.*** *GABA.* ***E.*** *Histamine.*

23. Hypersensitivity tests include allergen injection under the skin. It promotes redness, swelling, and pain due to the action of histamine. Which process leads to its formation from the amino acid histidine?

A. *Deamination.* ***C.*** *Phosphorylation.* ***E.*** *Decarboxylation.*
B. *Methylation.* ***D.*** *Isomerization.*

24. Decarboxylation of glutamate forms a neurotransmitter gamma-aminobutyric acid (GABA). GABA breakdown forms the following metabolite of the citric acid cycle:

A. *Citrate.* ***B.*** *Malate.* ***C.*** *Oxaloacetate.* ***D.*** *Succinate.* ***E.*** *Fumarate.*

25. As a result of the action of boiling water on the hands, the affected skin became red, swollen, and painful. Which substance can cause such reaction?

A. *Histamine.* ***B.*** *Histidine.* ***C.*** *DOPA.* ***D.*** *Glutamine.* ***E.*** *Asparagine.*

26. A patient with riboflavin hypovitaminosis experiences a decrease in the activity of FAD-dependent enzyme monoamine oxidase. Which biochemical reaction is affected in this case?

A. *Isomerization.* ***C.*** *Deamination of biogenic amines.* ***E.*** *Hydration.*
B. *Transamination.* ***D.*** *Hidroxylation.*

27. Melatonin regulates the daily and seasonal changes in metabolism in the body. It is involved in the regulation of reproductive function. Which biogenic amine is a precursor of this hormone?

A. *Dopamine.* ***B.*** *Histamine.* ***C.*** *Tryptamine.* ***D.*** *GABA.* ***E.*** *Serotonin.*

28. The inhibitory action of GABA is explained by the increased permeability of post-synaptic membranes to chloride ions. This neurotransmitter is formed by decarboxylation of:

A. *Glutamine.* ***B.*** *Aspartate.* ***C.*** *Glutamate.* ***D.*** *Asparagine.* ***E.*** *Arginine.*

29. A patient has a dysfunction of the cerebral cortex that manifests as epileptic seizures. His physician prescribed a biogenic amine synthesized from glutamate and responsible for central inhibition. How is it called?

A. *Serotonin.* ***B.*** *GABA.* ***C.*** *Dopamine.* ***D.*** *Acetylcholine.* ***E.*** *Histamine.*

30. Most biogenic amines in tissues undergo oxidative deamination by the enzyme called:

A. *L-Amino acid dehydrogenases.* ***C.*** *Transaminases.* ***E.*** *Monoaminooxidases.*
B. *Isomerases.* ***D.*** *D-Amino acid oxidases.*

31. To determine the maximum secretion of gastric hydrochloric, histamine solution was given to a patient. This led to an increase in the secretion of one of the following pancreatic juice components:

A. *Lipases.* ***B.*** *Trypsinogen.* ***C.*** *Bicarbonates.* ***D.*** *Amylase.* ***E.*** *Mucin.*

PRACTICAL WORK

Quantitative determination of histamine in blood by method of N.V. Klimkina and S.I. Plitman

Task. Perform a quantitative determination of histamine in the blood.

Principle. The method is based on the reaction between histamine and diazotized *para*-nitroaniline with the yielding red-orange products.

Procedure. Whole blood is sampled and precipitated with 10 % trichloroacetic acid solution (in a blood-to-acid ratio 1:9) for histamine extraction; before use, the blood sample is kept in a refrigerator for a period of twenty-four hours. Filter the trichloroacetic acid extract from blood through a paper filter. Pour 2 ml of filtrate into a test tube (sample), and 0.2 ml standard histamine solution (200 μmol/L) and 1.8 ml of distilled water into another test tube (standard). Add 3 ml of distilled water and 1 ml of 4 % sodium nitrate solution to both test tubes. Mix the contents by vigorous shaking and place the test tubes in a boiling water bath. Wait for 2 min and then cool under a stream of tap water. Add 1 ml of diazotized *para*-nitroaniline (this reagent is prepared prior to use from a 0.1 % *para*-nitroaniline solution in 0.1 M hydrochloric acid; to this

effect, 1 ml of 4 % aqueous sodium nitrate solution is added to 10 ml of ice-cooled *para*-nitroaniline solution) to both test tubes. Mix thoroughly the contents and adjust their pH to 10.0 (check by reaction to universal indicator paper), by adding successively two portions, 1.5 and 0.5 ml, of sodium carbonate solution. Mix the contents with shaking, cool under running tap water and add 2 or 3 drops of sodium hydroxide solution to allow coloration to develop. Optical densities of the test and standard samples are measured on a photoelectrocolorimeter at the wavelength 520–540 nm (green filter) in a 5 mm thick cells against control (for its preparation, mix 10 ml of distilled water, 2 ml of sodium nitrate solution, 2 ml of diazotized *para*-nitroaniline soluion, and 4 ml of sodium carbonate solution, and then add 0.6 ml of sodium hydroxide solution). Histamine content (X) in μmol/L is calculated by the equation:

$X = (E_{test} \times 200)/E_{st}$, where E_{test} is the optical density of test sample; E_{st} is the optical density of standard sample; 200 is the concentration of standard solution of histamine, μmol/L.

Clinical and diagnostic significance. *Histamine is a biologically active substance, which is involved in the regulation of the vital functions of the body. It causes vasodilation at the site of inflammation, and thus speeds up the flow of white blood cells, resulting in the activation of protective forces of the body; it is directly related to the development of allergic and immune responses, the process of sensibilization and desensibilization; it acts as a mediator of pain. Histamine causes a spasm of smooth muscle, vasodilatation and a decrease in blood pressure. There is a swelling of the surrounding tissues due to haemostasia in the capillaries and an increase in the permeability of the walls. Under normal conditions histamine is predominantly in the inactive (bound) state. Its concentration in the blood of healthy perople is 0.02-0.04 μmol/L. Free histamine increases in some pathological processes (anaphylactic shock, burns, pollen fever and other allergic diseases), injection of chemicals, including drugs.*

The determination of level of some biogenic amines has diagnostic significance in clinical biochemical laboratory. Level of tyramine is increased in the blood plasma in severe nephrosclerosis; serotonin level is increased in the case of shock; histamine level is increased in allergic diseases.

1**. Prepare a report "Mechanisms of activation and inhibition of proteolytic enzymes of the gastrointestinal tract".

2**. Prepare a review "Regulation of secretion of gastrointestinal juices".

**** - questions for self-study**

CLASS 2 (4 hours)

TOPIC 3 (2 hours). Deamination and transamination of amino acids. Determination of transaminase activity in serum.

***IMPORTANCE*.** Deamination is a removal of amino group from amino acids with ammonia formation. Four types of amino acid deamination exist in living organisms: reductive, hydrolytic, intramolecular and oxidative. Transamination reaction is the intermolecular transfer of amino group from amino acids to α-keto acids without

the intermediate formation of ammonia. During the reaction new amino acids and α-keto acids are formed. Transamination reactions are reversible and universal for all living organisms; they are catalyzed by enzymes aminotransferases (transaminases). Transamination is very important for the synthesis of non-essential amino acids, transdeamination of most amino acids. The study of deamination and transamination reactions is necessary to efficient use of knowledge about the metabolism of proteins and amino acids for the analysis of its numerous disorders.

AIM. Learn deamination and transamination reactions of amino acids in the body, their significance and mechanism of transaminase action. Familiarize yourself with the method of determining the activity of aspartate and alanine aminotransferases in blood serum and its clinical and diagnostic significance.

THEORETICAL QUESTIONS

1. Major types of deamination of amino acids in the organism.
2. Direct and indirect deamination of L-amino acids. Mechanism of action and role of amino acid oxidases and glutamate dehydrogenase. Clinical significance of determination of glutamate dehydrogenase activity in blood.
3*. Deamination of serine, threonine, cysteine, and histidine.
4. Transamination, its role in metabolism of amino acids. Mechanism of aminotransferase action. Role of vitamin B_6. Clinical significance of determination of aminotransferases in blood.
5. Reaction of transamination between α-ketoglutarate and aspartate. Its significance. Clinical significance of determination of AsAT activity in blood.
6. Reaction of transamination between α-ketoglutarate and alanine. Its significance. Clinical significance of determination of AlAT activity in blood.
7. Reaction of α-ketoglutarate reductive amination, its role.

Recommendations for self-study of theoretical questions

Question	Information
1. Deamination of serine, threonine, cysteine, and histidine.	1.1. Nonoxidative deamination of serine, threonine, cysteine; location – liver: • deamination of serine; enzyme – serine dehydratase (PLP-dependent); end products – ammonia, pyruvate; • deamination of threonine; enzyme – threonine dehydratase (PLP-dependent); end products – ammonia, α-ketobutyrate; • deamination of cysteine; enzyme – cystathionine-γ-lyase (PLP-dependent); end products – ammonia, pyruvate, hydrogen sulfide. 1.2. Deamination of histidine – intramolecular; location – liver and skin; enzyme – histidinase; end products – ammonia, urocanic acid

TESTS FOR SELF-CONTROL

1. Which enzyme catalyzes the deamination of glutamate?

A. *Glutamate dehydrogenase.* ***C.*** *Glutamate decarboxylase.* ***E.*** *Cystathionine-γ-lyase.*
B. *γ-Glutamyl transferase.* ***D.*** *Glutaminase.*

2. Which pathology is the most probable in the increase of aspartate aminotransferase activity in blood serum?

A. *Chronic hepatitis.* ***C.*** *Diabetes insipidus.* ***E.*** *Myocardial infarction.*
B. *Renal insufficiency.* ***D.*** *Diabetes mellitus.*

3. Glutamate dehydrogenase belongs to the following class of enzymes:

A. *Transferases.* ***B.*** *Isomerases.* ***C.*** *Lyases.* ***D.*** *Oxidoreductases.* ***E.*** *Ligases.*

4. The activity of transminases in blood is determined for diagnostics of some diseases. Which vitamin is the coenzyme of these enzymes?

A. *Vitamin B_6.* ***B.*** *Vitamin B_2.* ***C.*** *Vitamin B_1.* ***D.*** *Vitamin B_3.* ***E.*** *Vitamin B_{12}.*

5. Which substance is the acceptor of amino groups in reactions of transamination?

A. *Argininosuccinate.* ***C.*** *Lactate.* ***E.*** *Ornithine.*

B. *α-Ketoglutarate.* ***D.*** *Citrulline.*

6. Pyridoxal phosphate was prescribed to a patient. Which process correction was observed:

A. *Synthesis of purine and pyrimidine nucleotides.*

B. *Oxidative decarboxylation of α-keto acids.*

C. *Deamination of amino acid.*

D. *Transamination and decarboxylation of amino acids.*

E. *Protein synthesis*

7. Transaminase activity sharply increases in blood plasma of patients with hepatitis, myocardial infarction. Point out the possible cause:

A. *The increase of enzyme activity by hormones.*

B. *Damage of cellular membrane and entering enzymes to the blood.*

C. *Deficiency of pyridoxine.*

D. *An increase in amino acid synthesis velocity in tissues.*

E. *An increase in amino acid degradation velocity in tissues.*

8. Which amino acid undergoes the most intensive oxidative deamination?

A. *Leucine.* ***B.*** *Valine.* ***C.*** *Glutamate.* ***D.*** *Serine.* ***E.*** *Aspartate.*

9. Which products are formed in transamination between α-ketoglutarate and alanine?

A. *Aspartate and lactate.* ***C.*** *Glutamate and pyruvate.*

B. *Glutamate and lactate.* ***D.*** *Glutamine and asparagine.*

10. In humans, amino acids are deaminated by transamination. As a result, amino group is transferred to:

A. *Alpha-ketoglutarate.* ***B.*** *Succinate.* ***C.*** *Citrate.* ***D.*** *Fumarate.* ***E.*** *Malate.*

11. High serum activity of alanine aminotransferase and aspartate aminotransferase was found in a patient. Which changes at the cellular level can lead to such situation?

A. *Damage to cellular genetic apparatus.* ***C.*** *Energy deficiency.* ***E.*** *Abnormal enzymatic systems.*

B. *Abnormal intercellular communication.* ***D.*** *Cell destruction.*

PRACTICAL WORK

Determination of aspartate aminotransferase activity (AsAT) in blood serum by King's method

Task. Perform a quantitative determination of AsAT activity in blood serum by the method of King.

Principle. The method is based on the development of color that occurs after the interaction of 2,4-dinitrophenylhydrazine with oxaloacetic or pyruvic acids.

Procedure. Pour 0.2 ml of serum into one test tube (experimental sample) and 0.2 ml of distilled water into another test tube (control sample). Add 0.5 ml of aspartic acid and 0.5 ml of α-ketoglutaric acid to both test tubes, put them to thermostat at 37° C for 60 minutes. After incubation, add 1 ml of 2,4 – dinitrophenylhydrazine to

both test tubes to stop the process of transamination. Put them again to thermostat for 15 minutes. Then add 10 ml of 0.4 N NaOH, mix and keep 1–2 minutes to the color occurence. The color intensity of test sample is determined on photoelectrocolorimeter (green filter) versus the control sample. The activity of AsAT is calculated according to the calibration curve.

Clinical and diagnostic significance. Wide distribution and high activity of transaminases in human organs and tissues, and relatively low values of basal activity in the blood are the basis for the determination of their level in the blood serum in patients with lesions of the heart and liver. Organic disorders in acute and chronic diseases are accompanied by destruction of cells and lead to the release of transaminases into the bloodstream. For example, in myocardial infarction AsAT activity of blood serum is 20–30 times higher (normal activity is 0.1–0.45 mmol/(h·L)) within 3–5 h after an attack. Liver damage (cirrhosis, hepatitis) is characterized by elevation of blood alanine aminotransferase (normal activity is 0.1–0.68 mmol/(h·L)) In the clinic the determination of the activity of transaminases is used not only for diagnosis but for prognosis, treatment efficacy monitoring.

TOPIC 4 (2 hours). Ammonia metabolism: sources, mechanisms of detoxification, transport forms. Ornithine cycle (Krebs – Henseleit cycle). Biological role of urea synthesis in the liver. Disorders of ornithine cycle. Formation of ammonium salts in the kidneys. Determination of urea and ammonia in biological fluids.

IMPORTANCE. Deamination reactions of amino acids, purine and pyrimidine nucleotides, biogenic amines and other processes produce ammonia, which is toxic to the organism. Central nervous system is particularly sensitive to ammonia. Content of ammonia in the blood should not exceed 60 μmol/L. Temporary detoxification of ammonia occurs in the places of its occurrence by reductive amination of α-ketoglutarate to form glutamate, amination or aspartate and glutamate with the formation of asparagine and glutamine, which, especially glutamine, have important role in the transport of ammonia to the liver and kidneys. The final stage of ammonia detoxification occurs in the liver via urea synthesis and in kidneys by means of the formation of ammonium salts. Urea and ammonium salts are excreted in the urine.

AIM. Familiarize yourself with the ways of ammonia formation in the body, its toxicity, mechanisms of transport and detoxification. Learn the reaction of urea biosynthesis in the liver, ammonium salts in the kidneys. Learn and be able to characterize the disorder of ammonia metabolism (hyperammonemia), its causes, manifestation and consequences. Study methods for determining urea and ammonia in biological fluids and their clinical and diagnostic significance.

THEORETICAL QUESTIONS

1. Pathways of the formation of ammonia in the body.
2. Mechanisms of temporary and final ammonia detoxification.
3. Transport of ammonia from tissues to liver and kidney.

4*. Role of alanine in transport of ammonia.

5*. Formation of ammonia salts in kidneys, the significance of process.

6. Ornithine cycle of urea formation in the liver: reactions, role. Genetic defects of ornithine cycle enzymes (enzymopathies).
7. Hyperammoniemia: its causes, manifestation, consequences.

Recommendations for self-study of theoretical questions

Question	Information
1. Role of alanine in ammonia transport	Intense physical activity – consumption of amino acids as an energy source → amino acids in muscle transfer amino groups to α-ketoglutarate to form glutamate → due to low activity of glutamate dehydrogenase in muscle glutamate gives amino group to pyruvate (always present in sufficient amount in muscle as a product of glycolysis) to form alanine → alanine diffuses from muscles into the bloodstream → it enters the liver, where it reacts by transamination with α-ketoglutarate to form pyruvate and glutamate: 1) glutamate undergoes oxidative deamination by glutamate dehydrogenase to form ammonia, which is detoxified in the urea cycle; 2) pyruvate is used as a substrate for gluconeogenesis; glucose formed in it enters the muscle
2. Formation of ammonium salts in kidney; significance of the process.	Glutamine is hydrolyzed by glutaminase to form ammonia in kidney. Biological role: mechanism of acid-base balance regulation; it preserves potassium and sodium cations to maintain osmotic pressure. Acidosis → induction of renal glutaminase → release of ammonia → neutralization of acidic products with ammonia → urinary excretion of ammonium salts. Alkalosis → a decrease in glutaminase activity. Daily urinary excretion of ammonium salts is about 0.5 g.

TESTS FOR SELF-CONTROL

1. The process of urea synthesis is located in:

A. *Kidney.* ***B.*** *Intestine.* ***C.*** *Liver.* ***D.*** *Muscles.* ***E.*** *Pancreas.*

2. Point out the transport form of ammonia in blood:

A. *Tryptophan.* ***B.*** *Isoleucine.* ***C.*** *Ammonium salts.* ***D.*** *Glutamine.* ***E.*** *Urea.*

3. Select the regulatory enzyme of ornithine cycle:

A. *Ornithine decarboxylase.*
B. *Ornithine carbamoyl transferase.*
C. *Carbamoyl phosphate synthetase.*
D. *Arginase.*
E. *Argininosuccinate lyase*

4. What is the name of the process of ammonia detoxification in kidney?

A. *Ammonia salts formation.* ***C.*** *Indirect deamination.* ***E.*** *Synthesis*
B. *Reductive amination.* ***D.*** *Urea synthesis.* *of biogenic amines.*

5. Which is the main process of ammonia detoxification in nervous tissue?

A. *Transamination.* ***C.*** *Formation of dicarboxylic acid amines.*
B. *Urea synthesis.* ***D.*** *Ammonia salts formation.* ***E.*** *Biogenic amines synthesis.*

6. Nitrogen of urea constitutes about 90 % of total nitrogen of urine. Which enzyme activity decrease results in inhibition of urea synthesis and ammonia accumulation in blood and tissues?

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

7. The main final product of protein metabolism excreting in the greatest amount with urine is:

A. *Glutamine.* ***B.*** *Ammonia salts.* ***C.*** *Uric acid.* ***D.*** *Asparagine.* ***E.*** *Urea.*

8. Point out the normal level of urea in blood:

A. *3.0–4.0 mmol/L.* ***C.*** *8.4–12.6 mmol/L.* ***E.*** *More than 9.0 mmol/L.*
B. *3.3–8.3 mmol/L.* ***D.*** *10.0–13.0 mmol/L.*

9. Hyperargininemia and argininuria are observed in the 25-year-old patient. The urea level is decreased in blood and urine. Which enzyme deficiency is observed?

A. *Glutamate dehydrogenase.* ***C.*** *Ornithine carbamoyl transferase.* ***E.*** *Tryptophan-*
B. *Arginase.* ***D.*** *Argininosuccinate synthetase.* *5-monooxigenase.*

10. Which amino acid is the intermediate of urea synthesis and is cleaved with formation of ornithine and urea?

A. *Leucine.* ***B.*** *Citrulline.* ***C.*** *Arginine.* ***D.*** *Valine.* ***E.*** *Glutamate.*

11. Ammonia is a toxic substance, especially for the nervous system. Which compound takes part in ammonia detoxification in the brain?

A. *Lysine.* ***B.*** *Proline.* ***C.*** *Glutamate.* ***D.*** *Histidine.* ***E.*** *Alanine.*

12. The newborn's urine has citrulline and high levels of ammonia. Which substance formation is probably affected?

A. *Urea.* ***B.*** *Uric acid.* ***C.*** *Ammonia.* ***D.*** *Creatinine.* ***E.*** *Creatine.*

13. The bulk of the nitrogen is excreted from the body as urea. Which enzyme reduced activity in the liver leads to inhibition of urea synthesis and ammonia accumulation in blood and tissues?

A. *Aspartate aminotransferase.* ***C.*** *Amylase.* ***E.*** *Carbamoylphosphate synthetase.*
B. *Urease.* ***D.*** *Pepsin.*

14. A child after a severe viral infection has repeated vomiting, loss of consciousness, seizures. Hyperammonemia was found. What can cause changes in such blood biochemical parameters?

A. *Activation of amino acid decarboxylation.* ***D.*** *Intensified protein putrefaction.*
B. *Inhibition of transaminases.* ***E.*** *Abnormal ammonia detoxification*
C. *Abnormal inactivation of biogenic amines.* *in ornithine cycle.*

15. Brain injury caused increased formation of ammonia. Which amino acid is involved in ammonia removal from the brain tissue?

A. *Glutamate.* ***B.*** *Tryptophan.* ***C.*** *Lysine.* ***D.*** *Tyrosine.* ***E.*** *Valine.*

16. A newborn sucks badly. Frequent vomiting and hypotension are observed. Citrulline concentration is significantly increased in blood and urine. Which metabolic process is affected?

A. *Krebs cycle.* ***C.*** *Glycolysis.* ***E.*** *Gluconeogenesis.*
B. *Ornithine cycle.* ***D.*** *Cori cycle.*

17. Carbamoylphosphate synthetase catalyzes the reaction of carbamoylphosphate synthesis from free ammonia. This enzyme provides carbamoylphosphate for synthesis of:

A. *Creatine.* ***B.*** *Lipids.* ***C.*** *Amino acids.* ***D.*** *Urea.* ***E.*** *Purines.*

18. Intense exercise causes formation of the significant amount of ammonia. Which amino acid plays a major role in transporting it to the liver and is used in reactions of gluconeogenesis?

A. *Arginine.* ***B.*** *Lysine.* ***C.*** *Ornithine.* ***D.*** *Aspartate.* ***E.*** *Alanine.*

19. A central role in amino acid metabolism in the nervous tissue is performed by glutamic acid. This is due to the fact that this amino acid participates in:

A. *Ammonia detoxification with the formation of glutamine.*

B. *Glucose synthesis.* ***D.*** *Synthesis of neurospecific proteins.*
C. *Lipid synthesis.* ***E.*** *Synthesis of ketone bodies.*

20. A patient shows signs of protein malnutrition after prolonged starvation. Choose a state that is typical for protein deficiency.

A. *Hyperglycemia.* ***C.*** *High blood oncotic pressure.* ***E.*** *Hematuria.*
B. *Positive nitrogen balance.* ***D.*** *Low urea synthesis in the liver.*

21. There are several pathways for ammonia detoxification in the body, but some organs have specific pathways. Which pathway prevails in brain cells?

A. *Asparagine formation.* ***C.*** *Urea formation.* ***E.*** *Creatine formation.*
B. *Glutamine formation.* ***D.*** *NH_{4+} formation.*

22. Secondary orotic aciduria developed in a patient with hereditary hyperammonemia due to disorders of ornithine cycle. Which metabolite of ornithine cycle is elevated and causes enhanced synthesis of orotic acid?

A. *Ornithine.* ***C.*** *Citrulline.* ***E.*** *Argininosuccinate.*
B. *Carbamoylphosphate.* ***D.*** *Urea.*

23. A child suffers from dysfunctions of the central nervous system. Clinical and biochemical investigations revealed hyperammonemia. Which enzyme is affected in this pathological condition?

A. *Glutathione transferase.* ***C.*** *Ornithinecarbamoyl transferase.* ***E.*** *Glucuronyl*
B. *Glycine transferase.* ***D.*** *Sulfate transferase.* *transferase.*

24. In high acidity (acidosis) acidic products are neutralized by ammonia and are excreted by kidney in the form of salts of:

A. *Magnesium.* ***B.*** *Potassium.* ***C.*** *Calcium.* ***D.*** *Sodium.* ***E.*** *Ammonia.*

25. Hyperammonemia manifests by cramps, vomiting, loss of consciousness. Blood biochemical analysis shows increased concentrations of ammonia. Biochemical mechanism of cramps formation in this case is associated with:

A. *Inhibiton of fatty acid β-oxidation.*
B. *Glycolysis inhibition.*
C. *Inhibition of pentose phosphate pathway.*
D. *Inhibition of GABA synthesis.*
E. *Uncoupling of oxidative phosphorylation and ETC.*

26. An unconscious patient was hospitalized. The objective examination revealed absent reflexes, occasional convulsions, irregular breathing. Hepatic coma was diagnosed after laboratory tests. Which metabolite accumulation is necessary for manifestations of disorders of the central nervous system?

A. *Urea.* ***B.*** *Ammonia.* ***C.*** *Glutamine.* ***D.*** *Bilirubin.* ***E.*** *Histamine.*

27. Brain cells are very sensitive to energy deficiency whose cause is high content of ammonia, which stimulates the outflow of alpha-ketoglutarate to:

A. *Pentose phosphate pathway.* ***C.*** *Glycolysis.* ***E.*** *Citric acid cycle.*
B. *Ornithine cycle.* ***D.*** *Glycogenolysis.*

28. A child has mental retardation, protein intolerance, severe hyperammonemia against the background of reduced urea in blood plasma due to congenital deficiency of the mitochondrial enzyme called:

A. *Succinate dehydrogenase.* ***C.*** *Malate dehydrogenase.* ***E.*** *Monaminooxidase.*
B. *Carbamoylphosphate synthetase.* ***D.*** *Citrate synthase.*

PRACTICAL WORK

Quantitative determination of urea and ammonia in biological fluids

Task 1. Determine the urea content in blood serum.

Principle. Urea and diacetyl monooxym in the presence of thiosemicarbazide and ferrum salts form a complex of pink-red color in strongly acidic medium. The color intensity is proportional to the amount of urea.

Procedure. Determination is carried out in test tubes that are filled in accordance with the following scheme:

	Test sample	Standart sample	Control
Blood serum (ml)	0.01		
Standard solution of urea (16.65 mmol/L) (ml)		0.01	
Distilled water (ml)			0.01
Working solution to color reaction (diacetyl monooxym, thiosemicarbazide, ferric chloride, sulphuric acid) (ml)	2.0	2.0	2.0

The test tubes are covered by aluminium foil lids and are heated strictly for 10 minutes in a boiling water bath, and they are cooled for 2–3 minutes under running tap water. Optical densities of the test and standard samples are measured for 15 minutes on a photoelectrocolorimeter at the wavelength 490–540 nm in 1 cm thick cells against control. The content of urea (X) in mmol/L in serum is calculated by the formula: $X = (E_{test} \times 16.65) / E_{st}$. Where E_{test} is the optical density of test sample; 16.65 is the concentration of urea in the standard sample (mmol/L); E_{st} is the extinction of the standard sample.

Task 2. Determine the content of urea in the urine.

Principle. The same.

Procedure. 0.1 ml of the urine collected during the day, filtered and diluted to 25 times and 0.1 ml of standard solution of urea are used for analysis. The samples are treated in the same way as blood serum. The content of urea (Y) in mmol/day in urine is calculated by the formula:

$$Y = (E_{test} \times V \times 25 \times 1.665) / (E_{st} \times 0.1 \times 1000),$$

where the E_{test} is the extinction of test sample; V is the volume of daily urine, 1.665 is the concentration of urea in 0.1 ml of a standard sample (μmol), 0.1 is the volume of urine taken for the study (ml); E_{st} is the extinction of the standard sample, 1000 is the scaling factor for micromol-to-millimol conversion, 25 is the dilution.

Task 3. Determine the content of urea in the blood serum by express method using "Ureatest".

Principle. The method is based on the ability of urease to break down urea to form ammonia, which stains the reactive chromatography paper strip in a blue color. The height of the painted area is proportional to the concentration of urea calculated from the calibration graph.

Procedure. Blood serum is diluted with water at a ratio of 1:1.Then 0.03 ml of diluted blood serum is applied at the end of the urease saturated paper strip at the distance of 3 mm to red paraffin line using a special pipette. Strip is immediately placed in a clean, dry test tube, hermetically stoppered and placed in a thermostat at 37° C.

After 20 minutes the strip is removed. The height of the zone, which is colored blue, is measured. The concentration of urea in the blood serum is calculated from the calibration scale of "Ureatest".

Task 4. Determine the content of ammonia in the urine.

Principle. The method is based on the interaction of ammonium salts with formaldehyde to form hexamethylene tetraamine and to release an acid, amount of which is equivalent to ammonia amount. An acid is titrated with a solution of the base.

Procedure. Pour 10 ml of urine into the flask, add 1–2 drops of phenolphthalein and neutralize with 0.1 M solution of sodium hydroxide. Add an equal volume of fresh formaldehyde solution. Due to the formation of acid a pink color disappears. Titrate the mixture with 0.1 M sodium hydroxide to the appearance of the pink color. Calculate the concentration of ammonia (Z) in grams per daily urine by the volume of base consumed in the titration according to the formula: $Z=(a\times0.0017 \times D)/10$, where *a* is the volume of the base, which is used for titration, ml; 0.0017 is the amount of ammonia corresponding to 1 ml of 0.1 M solution of sodium hydroxide (titer of ammonia) (g); D is the daily quantity of urine (ml); 10 is the amount of urine using for analysis (ml).

Clinical and diagnostic significance. *The normal content of urea in the blood varies between 3.3 and 8.3 mmol/L. About 75 % of urea is excreted in the urine. The concentration of urea in the blood depends on the intensity of its synthesis and excretion. Determination of urea is an important diagnostic test that characterizes not only the state of protein metabolism but also functional status of kidney and liver. Increased concentration of urea in the blood (uremia) is observed in kidney diseases (disorder of their excretory function), enhanced protein breakdown, excessive protein diet, in the case of dehydration (relative azotemia), in poisoning by phosphorus. The reduction of urea in the blood and excretion of it in the urine are observed in diseases of the liver (hepatodystrophy, cirrhosis and hepatitis), pregnancy, as well as genetic defects of enzymes of urea synthesis. Under such conditions, there is an increase in blood ammonia called ammoniemia. The symptoms of hyperammonemia (nausea, vomiting, convulsions, syncope and oedema of the brain in severe cases) are manifestations of its effects on the CNS. The increase of ammonia in the urine is also observed in a variety of processes accompanied by acidosis, fever, and diabetes mellitus. In diabetic acidosis the amount of ammonia in the urine exceeds the norm by more than 50 times. Excretion of ammonia in the urine decreases in some diseases that are accompanied by alkalosis (parathyroidotropic and children tetany, epylepsy, significant phosphaturia), as well as alkaline admission into the body.*

1.** Prepare a report "Clinical significance of aminotransferases".

2.** Create a scheme: 1) interrelation of urea synthesis and deamination, transamination of amino acids and energy metabolism; 2) interrelation of both Krebs cycles (citric acid cycle and ornithine cycle).

CLASS 3 (4 hours)

TOPIC 5 (4 hours). Specific metabolism of amino acids. Metabolism of nitrogen-free carbon skeletons of amino acids; interrelation with Krebs cycle. Glucogenic and ketogenic amino acids. Metabolism of phenylalanine, tyrosine and tryptophan. Qualitative reaction for phenylpyruvate in the urine.

***IMPORTANCE*.** Intermediate metabolism of amino acids involves different processes: anabolic (biosynthesis of proteins, peptides, amino acids, carbohydrates, lipids, ketone bodies), catabolic (breakdown to the final product), and specific metabolism accompanied by the formation of biologically active compounds. Tricarboxylic acid cycle is essential in nitrogen-free conversion of amino acid residues. The study of the specific reactions of phenylalanine, tyrosine, tryptophan conversion is necessary for understanding the pathogenesis of diseases (phenylketonuria, alkaptonuria, albinism, etc.) related to disorders of these conversions.

***AIM*.** Familiarize yourself with pathways of nitrogen-free amino acid residues metabolism, interrelation with the Krebs cycle; be able to prove gluco- and ketogenic properties of amino acids with specific reactions. Learn metabolism of phenylalanine, tyrosine and tryptophan, possible disturbances and ways of correction. Familiarize yourself with qualitative reaction for phenylpyruvic acid in urine and its clinical and biochemical significance.

THEORETICAL QUESTIONS

1. Scheme of metabolic pathways of nitrogen-free amino acid carbon skeletons. Their interrelation with Krebs cycle.

2. Glucogenic amino acids, their formulas. Prove that glutamate, aspartate and alanine are glucogenic amino acids.

3*. Physiologic meaning and regulation of gluconeogenesis processes from amino acids.

4*. Ketogenic amino acids, their formulas. Prove that leucine is ketogenic amino acid.

5*. Prove that phenylalanine and tyrosine are glucoketogenic amino acids.

6. Biologically active substances formed from phenylalanine and tyrosine, their role in organism.

7. Biologically active substances formed from tryptophan, their role in organism.

8. Disturbances of phenylalanine and tyrosine metabolism, ways of their correction.

9. Disorders of tryptophan metabolism, ways of their correction.

Recommendations for self-study of theoretical questions

Question	Information
1. Physiological significance and regulation of gluconeogenesis from amino acids	1.1. Amino acids that are used in the synthesis of glucose after entering Krebs cycle via acetyl-CoA, α-ketoglutarate, succinyl-CoA, fumarate are called glucogenic (e.g., alanine, glycine, aspartate, asparagine, valine, glutamate, glutamine, etc.). 1.2. Location – liver, renal cortical layer. 1.3. Physiological significance – ensurance of normal blood glucose concentration in reduced dietary intake and depletion of glycogen in the liver (postabsorptive state, prolonged starvation, exhausting

Question	Information
	physical exercise). 1.4. Regulation: – Allosteric: at the level of pyruvate carboxylase (acetyl-CoA is a positive modulator), fructose-1,6-bisphosphatase (ATP is a positive modulator, whereas ADP is a negative one); – Hormonal regulation of synthesis and activity of gluconeogenesis enzymes: – glucagon, epinephrine, glucocorticoids increase the rate of synthesis of phosphoenolpyruvate kinase, fructose-1,6-bisphosphatase, glucose-6-phosphatase; – insulin inhibits synthesis of phosphoenolpyruvate kinase, fructose-1,6-bisphosphatase, glucose-6-phosphatase
2. Ketogenic amino acids. Prove that leucine is a ketogenic amino acid	2.1. Ketogenic amino acids are amino acids that are converted to acetoacetate (lysine, leucine) or acetyl-CoA (leucine) and subsequently are used in ketogenesis. 2.2. Leucine → α-ketoisocaproate (transamination) → isovaleryl-CoA → β-hydroxy-β-metylglutaryl-CoA → acetoacetate (+ acetyl-CoA) → β-hydroxybutyrate (or decarboxylation with the formation of acetone)
3. Prove that phenyla-lanine and tyrosine are both gluco- and ketogenic amino acids	Some amino acids are used in the body for synthesis of both ketone bodies and glucose. Amino acids whose catabolism leads to the formation of Krebs cycle metabolites and acetoacetate (phenylalanine, tyrosine) or acetyl-CoA (isoleucine) are referred to as glucoketogenic. Phenylalanine → tyrosine → p-hydroxyphenylpyruvate → homogentisic acid → fumarylacetoacetate → fumarate (→ ... glucose) + acetoacetate (→ β-hydroxybutyrate + acetone)

TESTS FOR SELF-CONTROL

1. A child with mental and physical retardation suffers from frequent vomiting after meals. Phenylpyruvic acid is found in urine. Which kind of metabolism is affected?

A. *Carbohydrate.* ***B.*** *Phosphate-calcium.* ***C.*** *Amino acid.* ***D.*** *Water salt.* ***E.*** *Lipid.*

2. An ill child has elevated levels of phenylpyruvate (it is almost absent under normal conditions) in the urine. The content of phenylalanine is 350 mg/L (the normal level is about 15 mg/L). Which disease is characterized by such symptoms?

A. *Phenylketonuria.* ***B.*** *Albinism.* ***C.*** *Gout.* ***D.*** *Alkaptonuria.* ***E.*** *Tyrosinosis.*

3. The child's parents noticed that urine of their child darkened while exposing to the air. Body temperature is normal. Skin is pink. Liver is not enlarged. What is the most likely cause of this phenomenon?

A. *Phenylketonuria.* ***B.*** *Gout.* ***C.*** *Cushing disease.* ***D.*** *Alkaptonuria.* ***E.*** *Hemolysis.*

4. A child has increased levels of phenylpyruvic acid. What kind of treatment should be provided?

A. *Enzymopathy.* ***B.*** *Vitamins.* ***C.*** *Antibacterial therapy.* ***D.*** *Hormones.* ***E.*** *Diet.*

5. One of the features of tyrosine metabolism is its participation in the synthesis of hormones. Select a hormone formed from tyrosine in the cerebral layer of adrenal glands.

A. *Glucagon.* ***B.*** *Adrenaline.* ***C.*** *Thyroxine.* ***D.*** *Histamine.* ***E.*** *Serotonin.*

6. Tyrosine is used as a substrate for thyroxine synthesis. Choose a chemical element that is involved in this process.

A. *Calcium.* ***B.*** *Copper.* ***C.*** *Iron.* ***D.*** *Iodine.* ***E.*** *Zinc.*

7. To prevent pellagra, it is necessary to take an amino acid that is a precursor of vitamin PP. How is it called?

A. *Triptophan.* ***B.*** *Aspartate.* ***C.*** *Glutamate.* ***D.*** *Methionine.* ***E.*** *Glycine.*

8. Nutritional niacin deficiency (pellagra) can become less severe after intake of:

A. *Tyrosine.* ***B.*** *Threonine.* ***C.*** *Triptophan.* ***D.*** *Proline.* ***E.*** *Leucine.*

9. An infant has darkened sclera, mucous membranes, ears. Excreted urine darkens exposed to the air. Homogentisic acid is found in the blood and urine. What is the possible diagnosis?

A. *Albinism.* ***B.*** *Cystinuria.* ***C.*** *Porphyria.* ***D.*** *Alkaptonuria.* ***E.*** *Hemolytic anemia.*

10. A mentally and physically retarded boy was hospitalized. Blood biochemical analysis revealed increased amounts of phenylalanine. Which enzyme is blocked?

A. *Homogentisic acid oxidase.* ***B.*** *Glutamine transaminase.* ***C.*** *Aspartate aminotransferase.* ***D.*** *Glutamate decarboxylase.* ***E.*** *Phenylalanine 4-monooxygenase.*

11. Mother noticed too dark color of her child's urine. The child does not have any complaints. Bile pigments in the urine are absent. Alkaptonuria was diagnosed. Wich enzyme deficiency is observed?

A. *Phenilalanine hydroxylase.* ***B.*** *Tyrosinase.* ***C.*** *Hydroxyphenylpyruvate oxidase.* ***D.*** *Homogentisic acid oxidase.* ***E.*** *Phenylpyruvate decarboxylase.*

12. One of the forms of congenital anomalies is accompanied by inhibition of the conversion of phenylalanine to tyrosine. Biochemical basis of the disease is accumulation of some organic acids, including:

A. *Citrate.* ***B.*** *Pyruvate.* ***C.*** *Lactate.* ***D.*** *Phenylpyruvate.* ***E.*** *Glutamine.*

13. A boy was hospitalized with frequent vomiting, especially after meals. The child does not gain weight. He is physically retarded. Hair is dark, somewhere grey. What treatment should be provided?

A. *Enzymotherapy.* ***B.*** *Low phenylalanine diet.* ***C.*** *Amino acid mixtures.* ***D.*** *High-carbohydrate/lipid and low-protein diet.* ***E.*** *Protein-free diet.*

14. A young man has signs of skin depigmentation caused by disorders of melanin synthesis. Which amino acid metabolism is affected?

A. *Tryptophan.* ***B.*** *Histidine.* ***C.*** *Tyrosine.* ***D.*** *Proline.* ***E.*** *Glycine.*

15. Under the influence of ultraviolet rays human skin darkens that can be considered to be defensive reaction. Which protective substance – an amino acid derivative – is synthesized in cells under the influence of ultraviolet light?

A. *Melanin.* ***B.*** *Arginine.* ***C.*** *Methionine.* ***D.*** *Phenylalanine.* ***E.*** *Thyroxine.*

16. The repeated action of UV leads to darkening of the skin as a result of melanin synthesis that protects cells from damage. What is the major mechanism of this protection?

A. *Tyrosinase inhibition.*

B. *Homogentisic acid oxidase activation.*

C. *Homogentisic acid oxidase inhibition.*

D. *Tyrosinase activation.*

E. *Phenilalanine hydroxylase inhibition.*

17. A newborn has dark spots on diapers, indicating the formation of homogentisic acid. Which substance metabolism is affected?

A. *Tyrosine.* ***B.*** *Cholesterol.* ***C.*** *Galactose.* ***D.*** *Tryptophan.* ***E.*** *Methionine.*

18. Muscle proteins break down to free amino acids during starvation. These compounds will primarily serve as substrates for:

A. *Fatty acid synthesis.* ***C.*** *Gluconeogenesis in muscles.* ***E.*** *Glycogenolysis.*
B. *Gluconeogenesis in the liver.* ***D.*** *Decarboxylation.*

19. A girl has mental and physical retardation, light skin and hair, a decrease in blood catecholamines. Addition of a few drops of 5 % trichloroacetic iron solution to her urine leads to appearance of olive-green color. Which pathology of amino acid metabolism can be diagnosed?

A. *Albinism.* ***B.*** *Tyrosinosis.* ***C.*** *Phenylketonuria.* ***D.*** *Xanthinuria.* ***E.*** *Alkaptonuria.*

20. A patient was diagnosed with alkaptonuria. Select an enzyme whose defect can cause this disease.

A. *Pyruvate dehydrogenase.* ***C.*** *Phenylalanine hydroxylase.* ***E.*** *Homogentisic*
B. *Glutamate dehydrogenase.* ***D.*** *DOPA decarboxylase.* *acid oxidase.*

21. Albinos do not tolerate ultraviolet light. Tanning can cause burns. Which amino acid metabolism abnormality underlies this phenomenon?

A. *Methionine.* ***B.*** *Tryptophan.* ***C.*** *Glutamate.* ***D.*** *Phenylalanine.* ***E.*** *Histidine.*

22. High content of homogentisic acid is found in child's urine. Urine turns black while exposing to the air. Which disease is characterized by such changes?

A. *Phenylketonuria.* ***B.*** *Albinism.* ***C.*** *Aminoaciduria.* ***D.*** *Alkaptonuria.* ***E.*** *Cystinuria.*

23. A newborn has excessive amount of phenylpyruvate and phenylacetate on the 6th day of life. Which amino acid metabolism is impaired?

A. *Tryptophan.* ***B.*** *Phenylalanine.* ***C.*** *Methionine.* ***D.*** *Histidine.* ***E.*** *Arginine.*

24. A patient with alkaptonuria has signs of arthritis and ochronosis. Joint pain in this case is caused by the deposition of:

A. *Homogentisic acid.* ***B.*** *Urates.* ***C.*** *Phosphates.* ***D.*** *Oxalates.* ***E.*** *Carbonates.*

25. All proteinogenic amino acids in the body are divided into essential, conditionally essential and nonessential depending on their ability to be synthesized in the organism. Select an essential amino acid.

A. *Serine.* ***B.*** *Phenylalanine.* ***C.*** *Proline.* ***D.*** *Glutamine.* ***E.*** *Tyrosine.*

26. Glucose synthesis from non-carbohydrate components is an important biochemical process. Gluconeogenesis from amino acids actively occurs when in high-protein diet. Which amino acid is glucogenic?

A. *Isoleucine.* ***B.*** *Leucine.* ***C.*** *Valine.* ***D.*** *Alanine.* ***E.*** *Lysine.*

27. A child has vomiting, diarrhea, and mental retardation. Tyrosinosis was diagnosed. Which enzyme hereditary defect is the cause of the disease?

A. *Tyrosinase.* ***C.*** *Tyrosine aminotransferase.* ***E.*** *Fumaryl aceto-*
B. *Phenylalanine hydroxylase.* ***D.*** *Homogentisic acid oxidase.* *acetate hydrolase.*

28. Hypothyroidism in newborns leads to cretinism, which manifests by multiple congenital defects and severe irreversible mental retardation. This condition is related to abnormal thyroxine and triiodothyronine synthesis from:

A. *Tryptophan.* ***B.*** *Serine.* ***C.*** *Tyrosine.* ***D.*** *Glycine.* ***E.*** *Threonine.*

29. A patient who suffers from hereditary Hartnup disease has pellagra-like skin lesions,

mental retardation as a result of nicotinic acid deficiency. The cause of this disease is abnormal:

A. *Absorption and reabsorption of methionine in the kidney.*

B. *Decarboxylation of tryptophan.*

C. *Transamination of phenylalanine.*

D. *Absorption and reabsorption of cysteine.*

E. *Absorption and reabsorption of tryptophan in the kidney.*

30. High levels of serotonin and hydroxyanthranilic acid are found in a patient with bladder cancer. Which amino acid excess intake is observed?

A. *Tryptophane.* ***B.*** *Alanine.* ***C.*** *Histidine.* ***D.*** *Methionine.* ***E.*** *Tyrosine.*

31. Melatonin metabolism defect is found. This may be due to the lack of amino acid, which is a precursor of melatonin. How is it called?

A. *Alanine.* ***B.*** *Histidine.* ***C.*** *Tryptophan.* ***D.*** *DOPA.* ***E.*** *Glutamate.*

32. Administration of corticosteroid analogues causes breakdown of muscle proteins to free amino acids. These amono acids are used for:

A. *Glycolysis in muscles.* ***C.*** *Gluconeogenesis in the liver.* ***E.*** *Fatty acid synthesis.*
B. *Decarboxylation.* ***D.*** *Glycogenolysis.*

33. A child has motor and mental disorders, lighter hair, skin and iris. Fehling test is positive. What is the possible diagnosis?

A. *Albinism.* ***C.*** *Phenylketonuria.* ***E.*** *Alkaptonuria.*
B. *Down syndrome.* ***D.*** *Galactosemia.*

34. In bowel carcinoma about 60% of tryptophan is oxidized to serotonin. This increases daily requirements for one of the following vitamins:

A. *Folic acid.* ***B.*** *Pantothenic acid.* ***C.*** *Pyridoxine.* ***D.*** *Riboflavin.* ***E.*** *Niacin.*

35. Citric acid cycle plays an important role in the glucoplastic effect of amino acids. This is due to obligatory conversion of their nitrogen-free skeletons to:

A. *Citrate.* ***B.*** *Succinate.* ***C.*** *Malate.* ***D.*** *Oxaloacetate.* ***E.*** *Fumarate.*

36. Which biologically active substance is formed from tryptophan?

A. *Corticosterone.* ***B.*** *Thyroxine.* ***C.*** *Serotonin.* ***D.*** *Histamine.* ***E.*** *Adrenaline.*

37. Which amino acid is formed by phenylalanine oxidation?

A. *Serine.* ***B.*** *Tryptophane.* ***C.*** *Alanine.* ***D.*** *Tyrosine.* ***E.*** *Glutamine.*

PRACTICAL WORK

Qualitative reaction to phenylpyruvate in urine (Fehling's test)

Principle. Phenylpyruvate forms a complex compound of blue-green color with trivalent ferrum-ions.

Procedure. Add 8–10 drops of 10 % ferric chloride solution to 2 ml of freshly filtered urine. In the presence of phenylpyruvate in the urine a blue-green color appears within 30–60 seconds that gradually disappears after 5–30 minutes, depending on the concentration of phenylpyruvate in the urine. This test can be performed on filter paper. A strip of filter paper is wetted with urine, air-dried and a drop of 10 % ferric chloride solution is added to it. Positive test gives a blue-green color.

Clinical and diagnostic significance. *Diagnostic criterion for phenylketonuria (phenylpyruvate oligophrenia) is an increase in the content of phenylalanine in the blood and the presence of phenylpyruvate in the urine.*

CLASS 4 (4 hours)

TOPIC 6 (4 hours). Metabolism of glycine, serine, arginine, methionine, cysteine, dicarboxylic amino acids and branched-chain amino acids. Creatine as a product of arginine, glycine and methionine metabolism. Determination of creatine and creatinine in biological fluids.

IMPORTANCE. There are specific ways of their transformation in addition to common pathways of amino acid metabolism in tissues. Although some of them are quantitatively minor, but the reaction products have an important and sometimes leading role in the life of the organism. The study of the specific reactions of amino acids conversion is necessary for a better understanding of the pathogenesis of diseases related to their disorders.

AIM. Familiarize yourself with the specific pathways of glycine and serine, arginine, methionine and cysteine, amino acids, dicarboxylic amino acids and branched-chain amino acids transformation. Know the role of the tripeptide glutathione, nitric oxide and other biologically active substances that are formed in the metabolic pathways of above mentioned amino acids. Learn metabolism of creatine in the body: the reaction, enzymes, and disorders. Learn the methods of quantitative determination of creatinine in biological fluids and their clinical and biochemical significances.

THEORETICAL QUESTIONS

1. Scheme of glycine and serine metabolic pathways.
2. Glutathione as a product of glycine, glutamate and cysteine metabolism; its role.
3*. Scheme of pathways of arginine metabolism. Nitric oxide as a metabolite of arginine, its role.
4. Scheme of pathways of sulfur-containing amino acids metabolism.
5*. Scheme of pathways of dicarboxylic amino acids metabolism.
6. Scheme of pathways of valine, leucine, isoleucine metabolism. Maple syrup urine disease.
7. Creatine synthesis, its role in the body.
8. Creatine metabolism, disorders. Creatine kinase: isoforms, clinical and diagnostic significance of its determination in blood serum and urine.
9. Inherited disorders of amino acid metabolism, biochemical methods of their diagnostic, possibilities of their treatment.

Recommendations for self-study of theoretical questions

Question	Information
1. Arginine metabolism pathways; nitric oxide as a product of arginine metabolism, its role in the body	1.1. Arginine is a partially essential amino acid (i.e. essential in childhood). 1.2. Arginine metabolism is related to: – Reactions of ornithine cycle (considered as a pathway of arginine synthesis in the body); – Reaction of creatine synthesis; – Arginine is a precursor of ornithine, which is used for synthesis of polyamines – spermidine and spermine (components of chromatin; they participate in DNA replication, transcription and translation); – Arginine is a source of nitric oxide (NO). 1.3. Synthesis and biological role of NO: – It is synthesized from arginine by NO-synthase using O_2, NADPH, tetrahydrobiopterin, FMN/FAD, glutathione, Ca^{2+}, calmodulin; L-citrulline is also formed in the reaction;

Question	Information
	– Most cells of the human body are able to synthesize and secrete NO, but three cellular populations have been studied at most: vascular endothelium, cells of the nervous tissue, macrophages → therefore, there are three isoforms of NO-synthases (NOS): endothelial (eNOS), neuronal (nNOS), inducible (iNOS); eNOS and nNOS are constantly expressed by the cells (constitutive enzymes), whereas macrophagic NOS is inducible (i.e. it is synthesized as a response to certain factors). 1.4. Roles of NO: – Signaling molecule → it increases activity of guanylyl cyclase → stimulation of cGMP production →a decrease in heart rate, regulation of vascular tone; – Relaxation of smooth muscles, vasodilation; – Dilation of coronary vessels; – Neurotransmitter; – Lysis of phagocytized bacteria, participation in immune processes; – Participation in the regulation of apoptosis; – Participation in the regulation of secretion of neurotransmitters and hormones; – Antitumor effect; – Prevention of platelet aggregation; – Inhibits the production of vasoconstrictor. 1.5. Excessive NO production → hypotension, inflammatory diseases, carcinogenesis, hemorrhagic stroke. 1.6. Insufficient NO production → hypertension, atherosclerosis, erectile dysfunction, low immunity.
2. Metabolic pathways of dicarboxylic amino acids	2.1. Glutamate metabolism: – Glucogenic amino acid → via α-ketoglutarate it is converted to oxaloacetate → ... → glucose; – Formation of GABA (inhibitory neurotransmitter) via decarboxylation under the influence of glutamate decarboxylase; – Formation of glutamine, i.e. an ammonia transport form; – It is a precursor of tripeptide glutathione, proline, arginine, and ornithine. 2.2. Aspartate metabolism: – It is a glucogenic amino acid that is converted to oxaloacetate → ... → glucose; – Formation of asparagine, which is an ammonia transport form; – Aminotransferase reaction: L-aspartate + α-ketoglutarate ↔ oxaloacetate + L-glutamate (aspartate aminotransferase); – Participation in biosynthesis of purine ring; – Synthesis of pyrimidines; – Participation in ornithine cycle; – Participation in malate-aspartate shuttle.

TESTS FOR SELF-CONTROL

1. Diagnosis of a young man is muscle dystrophy. The increased level of which substance in blood serum is the most possible in this pathology?

A. *Myoglobin.* ***B.*** *Myosin.* ***C.*** *Creatine.* ***D.*** *Lactate.* ***E.*** *Alanine.*

2. The considerable increase of creatine kinase MB-form activity was found in patient's blood. Point out a possible pathology:

A. *Hepatitis.* ***C.*** *Rheumatism.* ***E.*** *Cholecystitis.*
B. *Myocardial infarction.* ***D.*** *Pancreatitis.*

3. Hormones and mediators are formed in metabolism of some amino acids. Which amino acid metabolism leads to the formation of gaseous mediator NO?

A. *Methionine.* ***B.*** *Leucine.* ***C.*** *Glycine.* ***D.*** *Arginine.* ***E.*** *Serine.*

4. A young patient complains of general weakness, easy fatigability. High concentration of valine, isoleucine, leucine in blood and urine has been detected on examination. The urine has a spesific odor. What can be the cause of this condition?

A. *Histidinemia.* ***C.*** *Alkaptonuria.* ***E.*** *Maple syrup urine disease.*
B. *Addison's disease.* ***D.*** *Phenylketonuria.*

5. It has been known more than 20 inherited disturbances of amino acids metabolism. The general symptom of these diseases is:

A. *Hyperammonemia.* ***C.*** *Creatinuria.* ***E.*** *Abnormal development*
B. *Albinism.* ***D.*** *Stone in the urinary bladder.* *and functions of brain.*

6. Point out a peptide, which formation is performed with participation of γ-carboxylic group of glutamic acid:

A. *Vasopressin.* ***B.*** *Carnosine.* ***C.*** *Anserine.* ***D.*** *Oxytocine.* ***E.*** *Glutathione.*

7. The increase of urea and creatinine levels and the decrease of these parameters in the urine were revealed in patient. Point out possible causes of this state:

A. *Renal disease.* ***C.*** *Muscle disease.* ***E.*** *Disturbance of acid-base balance.*
B. *Liver disease.* ***D.*** *Disturbances of binding, transport and excretion of ammonia with urine.*

8. Obesity, necrotic changes in liver, adrenal gland insufficiency, low levels of phospholipids, choline and adrenaline in blood are revealed in 32-year-old patient. The most possible cause of this state development is the deficiency of:

A. *Alanine.* ***B.*** *Methionine.* ***C.*** *Valine.* ***D.*** *Arginine.* ***E.*** *Glycine.*

9. Methyl groups are used in organism for the synthesis of the important substances such as creatine, choline, and adrenaline. The source of these groups is the essential amino acid:

A. *Valine.* ***B.*** *Glycine.* ***C.*** *Methionine.* ***D.*** *Cysteine.* ***E.*** *Arginine.*

10. A patient has ischemic disease of heart, arterial and venous thromboses, neuronal and psychiatric disorders, renal insufficiency. The level of homocysteine in blood is 3.5 times more than the normal one (normal value is less than 10 mmol/L). The patient probably has:

A. *Hartnup's disease.* ***C.*** *Hyperhomocysteinemia.* ***E.*** *Phenylketonuria.*
B. *Albinism.* ***D.*** *Alkaptonuria.*

11. A baby refuses to suck the breast. Breathing is arrhythmic. Urine has an odor of beer or maple syrup. Which enzyme congenital defect is observed in this case?

A. *Glucose-6-phosphate dehydrogenase.*
B. *Glycerol kinase.*
C. *Aspartate aminotransferase.*
D. *UDP-glucoronyl transferase.*

E. *Dehydrogenase of branched-chain alpha-ketoacids.*

12. A patient has been suffering from progressive muscular dystrophy (Duchenne disease) for a long time. Blood diagnostic test includes measurement of:

A. *Creatine phosphokinase.* ***C.*** *Pyruvate dehydrogenase.* ***E.*** *Adenylate kinase.*
B. *Lactate dehydrogenase.* ***D.*** *Glutamate dehydrogenase.*

13. A ten-year boy has high content of all aliphatic amino acids in the urine. However, the highest excretion of cystine and cysteine is observed. In addition, renal ultrasound showed the presence of stones. Which pathology should be diagnosed?

A. *Cystitis.* ***B.*** *Hartnup disease.* ***C.*** *Cystinuria.* ***D.*** *Alkaptonuria.* ***E.*** *Phenylketonuria.*

14. A two-year child with renal insufficiency has hyperoxaluria, oxalate urolithiasis, which caused deposition of calcium oxalate in kidney. Which amino acid metabolism is impaired?

A. *Histidine.* ***B.*** *Arginine.* ***C.*** *Methionine.* ***D.*** *Glycine.* ***E.*** *Lysine.*

15. A central role in amino acid metabolism in the nervous tissue is played by glutamic acid. This is due to the fact that this amino acid:

A. *Is used for glucose synthesis.* ***D.*** *Binds ammonia to form glutamine.*
B. *Is used for lipid synthesis.* ***E.*** *Is used for synthesis of ketone bodies.*
C. *Is used for synthesis of neurospecific proteins.*

16. Cheese was recommended to an aged patient in order to prevent the development of fatty liver disease. Which essential amino acid present in cheese is necessary for synthesis of phospholipids?

A. *Lysine.* ***B.*** *Methionine.* ***C.*** *Valine.* ***D.*** *Arginine.* ***E.*** *Proline.*

17. A precursor of nitric oxide, which is an important regulator of smooth muscle tone, neurotransmission and immune processes, is:

A. *Ornithine.* ***B.*** *Citrulline.* ***C.*** *Arginine.* ***D.*** *Carbamoylphosphate.* ***E.*** *Lysine.*

18. Which amino acid is an intermediate in urea biosynthesis in the liver and is broken down to form ornithine and urea?

A. *Arginine.* ***B.*** *Citrulline.* ***C.*** *Valine.* ***D.*** *Leucine.* ***E.*** *Tryptophan.*

19. An examination of a woman suffering from diabetes mellitus I type revealed a protein metabolism disorder, confirmed by hyperaminoacidemia in laboratory blood tests and by slow wound healing and low synthesis of antibodies clinically. Which mechanism is responsible for hyperaminoacidemia?

A. *High blood plasma oncotic pressure.* ***D.*** *High LDL.*
B. *Low blood levels of amino acids.* ***E.*** *Activated proteolysis.*
C. *Hyperproteinemia.*

20. *Heptral*, which is used in treatment of liver diseases, contains S-adenosylmethionine. This active amino acid participates in synthesis of :

A. *Phospholipids.* ***B.*** *Cholesterol.* ***C.*** *Heme.* ***D.*** *Triacylglycerols.* ***E.*** *Bile acids.*

PRACTICAL WORK

Determination of creatinine content in biological fluids

Task 1. Determine the creatinine content in blood serum.

Principle. The interaction of creatinine with picric acid in alkaline medium leads to formation of compound of orange-red color. The intensity of color is determined by photometer.

Procedure. *Test sample* contains 0.5 ml of blood serum, 1 ml of distilled water and 0.5 ml of trichloroacetic acid; *standard sample* contains 0.5 ml of standard solution (creatinine concentration - 177 μmol/L), 1 ml of distilled water and 0.5 ml of trichloroacetic acid. Mix a content of each test tube. Keep the test tubes for 5 minutes. Centrifuge them for 5 min under 2500-3000 rpm. Pour the supernatants into other test tubes. Fill two new centrifuge test tubes in the following manner: 1 ml of corresponding supernatant, 0.5 ml of picric acid (0.04 mol/L) and 0.5 ml of NaOH (0.75 mol/L). Mix and make colorimetry in 1-cm thick cells against at 505 nm against control sample strictly after 20 min. *Control sample:* Pour 1.5 ml of distilled water and 0.5 ml of trichloroacetic acid, mix. Carry 1 ml of this solution to other test tube; add 0.5 ml of picric acid and 0.5 ml of sodium hydroxide.

Creatinine concentration (X) in μmol/L in blood serum is calculated by the formula: $X = (C_{st} \times E_{test})/E_{st}$. Where C_{st} is the concentration of creatinine in the standard sample (177 μmol/L); E_{test} is the optical density of test sample; E_{st} is the optical density of the standard sample. *Normal content of creatinine in blood serum (on an empty stomach) is 61–115 μmol/L for males and 53–97 μmol/L for females.*

Attention! At work should strictly adhere to the rules of working with toxic substances in chemical laboratories, as picric acid is the poison, and trichloroacetic acid and NaOH are caustic substances!

Task 2. Determine the content of creatinine in the urine.

Principle. The same.

Procedure. Determination is carried out in a centrifuge tube filled in accordance with the scheme:

	Test sample	Control sample
Urine diluted 1:100 (ml)	0.5	-
Distilled water (ml)	0.25	0.75
Trichloroacetic acid (ml)	0.25	0.25
Picric acid (ml)	0.5	0.5
NaOH (ml)	0.5	0.5

Mix the contents of test tubes and measure the absorbance of experimental (test) sample and calibrator (standard) sample against the control sample in 1 cm cuvette at 505 nm exactly after 20 minutes. The calibration solution is prepared as in the case of determination of serum creatinine. The amount of creatinine (U) in mmol/day in the urine using the formula:

$$U=(C_{st} \times E_{test} \times D \times 50)/(E_{st} \times 1000),$$

where C_{st} is the concentration of creatinine in the standard sample (177 micromol/L); E_{test} is the optical density test sample; E_{st} is the optical density of the standard sample; D is the daily urine output (1.5 L); 1000 is the conversion of micromol in mmol; 50 is the dilution of urine. Normally, the content of creatinine in urine is: for males – 8.8–17.7 mmol/day (1.0–2.0 g), for females – 7.1–15.9 mmol/day (0.8–1.8 g).

Clinical and diagnostic significance. *The concentration of creatinine in the blood serum of healthy people is relatively constant as a result of strict relationship between the formation and excretion. Creatinine is a non-threshold substance, it is*

only filtered by glomerulus but not reabsorbed. Tubular secretion is possible only in case of its high concentration in blood. Determination of creatinine is performed to investigate the function of kidneys. Creatinine concentration in plasma is inversely proportional to the filtration. Critical upper concentration is 115 μmol/L. The increase of creatinine concentration in blood indicates the degree of chronic renal failure. Also, elevated creatinine levels (hypercreatininemia) may occur in bowel obstruction, hyperfunction of adrenal glands, gout.

Increased excretion of creatinine in the urine is observed while eating a lot of meat, crush syndrome of muscle, heavy physical exertion, fever, pneumonia. Decreased excretion of creatinine in the urine is observed in chronic nephritis with uremia, muscle atrophy, leukemia, etc.

1.** Prepare a presentation "Nitric oxide (II): formation and biological functions".

2.** Prepare a review "Protein and amino acid metabolism disorders."

CLASS 5 (4 hours)

TOPIC 7 (4 hours). Functions and metabolism of nucleotides. Its disorders. Analysis of the final products of nitrogen metabolism. Determination of total nitrogen in the urine. Determination of uric acid in biological fluids. Nitrogen balance, its types.

IMPORTANCE. Nucleotides and their derivatives serve a variety of functions in the body, taking part in the synthesis of nucleic acids and nucleotide coenzymes; in reactions of energy storage and use; in the formation of active forms of carbohydrates, nitrogenous bases, sulfates, methionine; in the signal transduction in cells, etc. Synthesis of nucleotides involves different metabolites of all types of metabolism of substances and specific enzymes. Enzymes synthesizing ribo- and deoxyribonucleotides are the target for the antiviral and anticancer drugs. The final product of transformation of purine nucleotides in humans is uric acid. Final products of pyrimidine nucleotide catabolism are CO_2, ammonia, urea, β-alanine, β-aminobutyric acid. The state of nucleotide metabolism is indicated by the activity of enzymes involved in various stages of breakdown and transformation, as well as the content of uric acid (the end product of purine metabolism).

AIM. Consider the structure and role of nucleotides in the body. Familiarize yourself with the process of digestion and absorption of nucleoproteins; synthesis and degradation of purine and pyrimidine nucleotides. Learn the basic mechanisms of regulation of nucleotides metabolism and its disorders: gout, inherited orotic aciduria, Lesch-Nyhan syndrome. Familiarize yourself with the method of quantitative determination of uric acid in biological fluids and its clinical and biochemical significance. Sum up the catabolism of simple and complex proteins (nucleoproteins), consider all the end products of nitrogen metabolism, whose nitrogen constitutes the total nitrogen of urine. The total nitrogen of urine is used for the study of nitrogen balance.

THEORETICAL QUESTIONS

1*. Nucleotides, their structure and role in the body.

2*. Digestion and absorption of nucleoproteins.

3. Formation of 5'-phosphoribosyl-1'-pyrophosphate in cells; its participation in synthesis of purine and pyrimidine nucleotides. Role of phosphoribosyl pyrophosphate synthetase.

4. Synthesis of pyrimidine nucleotides. Regulation. Disorders.

5. Synthesis of purine nucleotides. Amino acids participating in the synthesis of purine skeleton. Regulation of synthesis and possible disorders.

6*. Synthesis of deoxyribonucleotides.

7. Role of carbamoyl phosphate in metabolism of proteins and nucleotides.

8. Role of hypoxanthine-guanine phosphoribosyl transferase in synthesis of purine nucleotides; its deficiency.

9. Breakdown of pyrimidine nucleotides.

10. Degradation of purine nucleotides.

11. Disorders of metabolism of purine nucleotides. Hyperuricemia, its causes, biochemical mechanisns of correction.

12. Disorders of pyrimidine nucleotide metabolism. Orotic aciduria.

13*. Correlation between metabolism of proteins and nucleic acids.

14*. Final products of catabolism of simple and conjugated (nucleoproteins) proteins. Nitrogen balance, its types.

Recommendations for self-study of theoretical questions

Question	Information
1. Nucleotides, their structure and role in the body	1.1. Nucleotides are three-component compounds made up of purine (adenine, guanine) or pyrimidine (uracil, thymine, cytosine) nitrogenous base, pentose sugar (ribose or deoxyribose), and phosphoric acid. 1.2. Biochemical functions: – Structural (monomers of nucleic acids – DNA and RNA); – Energy (ATP, ADP); – Coenzyme (NAD, FMN, FAD); – Metabolic (UTP, UDP, CTP participate in the reactions of glycogen and phospholipid synthesis); – Regulatory (allosteric modulators of regulatory enzymes of glycolysis and biosynthesis of purine nucleotides)
2. Digestion and absorption of nucleoproteins	2.1. Digestion in the stomach: – Breakdown and denaturation of the protein component under the influence of gastric HCl; – Digestion of protein components by gastric peptidases; 2.2. Digestion in the intestine: – Further digestion of protein components by pancreatic and intestinal proteinases; – Hydrolysis of polynucleotides by pancreatic RNases with the formation of purine and pyrimidine mononucleotides, di- and trinucteotides, as well as oligonucleotides that are resistant to RNases; – Hydrolysis of polynucleotides by pancreatic Dnases to dinucleotides, oligonucleotides, and mononucleotides; – Hydrolysis of oligonucleotides by pancreatic phosphodiesterases to mononucleotides; – Hydrolysis of mononucleotides by nucleotidases and nonspecific phosphatases to nucleosides; – Hydrolysis of nucleosides by intestinal nucleoside phosphorylases

Question	Information
	to form ribose- or deoxyribose-1-phosphate, purine and pyrimidine bases. 2.3. Absorption: – Products of protein component digestion are a mixture of amino acids → intestinal absorption; – Basic products of nucleoprotein digestion, which are absorbed in the intestine, are nucleosides and phosphate.
3. Synthesis of deoxyribonucleotides	3.1. Location: cells that enter S-phase of the cell cycle and are prepared for DNA synthesis and cell division. 3.2. Ribonucleotide reductase complex consists of ribonucleotide reductase (RNR), thioredoxin, thioredoxin reductase : – RNR is an olihgomeric protein (2β1- and 2β2 subunits) nonheme iron serves as a cofactor; – Thioredoxin acts as a hydrogen donor for ribose reduction; – Thioredoxin reductase catalyzes reduction of oxidized thioredoxin using NADPH. Ribonucleotide reductase complex catalyzes the formation of dADP, dGDP, dUDP, and dCDP. 3.3. Biosynthesis of thymidine nucleotides: – dTMP is formed from dUMP by thymidylate synthase (its cofactor is tetrahydrofolate); – dUMP formation pathways: dUDP dephosphorylation; hydrolytic deamination of dCMP by deaminase (the major pathway in humans); – dTMP synthesis inhibitors as anticancer agents: a) antimetabolites (5-fluorouracil) that interact with thymidylate synthase (competitive inhibition); b) pterine derivatives (aminopterine, methotrexate) are competitive inhibitors of dihydrofolate reductase. 3.4. Salvage pathways of deoxyribonucleotide synthesis: 1) Thymine + deoxyribose-1-phosphate → thymidine + H_3PO_4 (catalyzed by thymidine phosphorylase); thymidine + ATP → ADP + dTMP (catalyzed by thymidine kinase); 2) Deoxycitidine + ATP → ADP + dCMP (catalyzed by deoxycitidine kinase). 3.5. Regulation of deoxyribonucleotide synthesis: ribonucleotide reductases, thymidylate synthases, thymidine kinases are regulated by induction and repression at the genetic level
4. Interaction between protein metabolism and nucleic acid metabolism	4.1. Nucleoside triphosphates are substrates for DNA and RNA synthesis, which are indispensable for protein synthesis and cell proliferation. 4.2. Macroergic compounds (ATP, GTP, UTP, etc.) are necessary for anabolic processes, including protein synthesis. 4.3. Derivatives of nucleotides are donors of substrates for protein synthesis. 4.4. Some amino acids and their derivatives (aspartate, glutamate, glycine, glutamine) are necessary for nucleotide formation.
5. Final products of metabolism of simple and complex proteins (nucleoproteins). Nitrogen balance, its types	5.1. End products are urea, amino acids, uric acid, creatine, creatinine, ammonia, indican, bilirubin, polypeptides (rest blood nitrogen is 14.3–28.5 mmol/L; total nitrogen in the urine is 15–16 g/day). 5.2. Nitrogen balance is a difference between the amount of nitrogen that enters the body with food and the amount of nitrogen excreted from the organism. 5.3. Types of nitrogen balance:

Question	Information
	– Nitrogen equilibrium is observed when the amount of nitrogen intake is equal nitrogen excretion (typical for healthy adults); – Positive nitrogen balance occurs when the amount of nitrogen intake exceeds the amount of excreted nitrogen (typical for children, pregnant women); – Negative nitrogen balance is observed when the amount of nitrogen intake is less than the amount of exreted nitrogen (typical for old people, for patients with cancer, in case of protein deficiency)

TESTS FOR SELF-CONTROL

1. Allopurinol was prescribed to a patient with gout. It inhibits uric acid synthesis by inactivation of:

A. *Xanthine oxidase.* ***C.*** *Hyaluronidase.* ***E.*** *Transaminase.*
B. *Deaminase.* ***D.*** *Lactate dehydrogenase.*

2. Pterine derivatives - aminopterine and methotrexate - are competitive inhibitors of dihydrofolate reductase. Therefore, they inhibit regeneration tetrahydrofolate to dihydrofolate. These drugs lead to inhibition of intermolecular one-carbon unit transport. Which polymer biosynthesis is suppressed?

A. *Protein.* ***C.*** *DNA.* ***E.*** *Glycosaminoglycans.*
B. *Homopolysaccharides.* ***D.*** *Gangliosides.*

3. Allopurinol that is a structural analogue of hypoxanthine is used for treatment of gout. Its intake leads to increased hypoxanthine excretion in the urine. Which process is blocked?

A. *Salvage pathway of purine nucleotide synthesis.*
B. *De novo pathway of purine nucleotide synthesis.*
C. *Urea synthesis.*
D. *Pyrimidine nucleotide breakdown.*
E. *Uric acid synthesis.*

4. Prolonged intake of methotrexate by cancer patients leads to loss of sensitivity to it by target tumor cells.Which enzyme gene amplification is observed?

A. *Thioredoxin reductase.* ***C.*** *Thyminase.* ***E.*** *All options mentioned*
B. *Dihydrofolate reductase.* ***D.*** *Deaminase.* *above are correct.*

5. A 19-month-old child with physical retardation and self-mutilative behaviour has uric acid levels of 1.96 mmol/L. Which metabolic disorder is observed?

A. *Lesch-Nyhan syndrome.* ***C.*** *Von Gierke's disease.* ***E.*** *Gout.*
B. *AIDS.* ***D.*** *Cushing disease.*

6. A male patient complains of periodic intense bouts of pain in joints of the big toe and their swelling. Urine is characterized by sharply acidic reaction and pink color. Which substances are present in his urine?

A. *Ammonium salts.* ***C.*** *Calcium phosphate.* ***E.*** *Urates.*
B. *Magnesium sulfate.* ***D.*** *Chlorides.*

7. Biosynthesis of purine ring requires ribose-5-phosphate. It gradually accepts nitrogen and carbon atoms with further cyclization of rings. The source of ribose-5-phosphate is:

A. *Glycolysis.* ***C.*** *Gluconeogenesis.* ***E.*** *Lipolysis.*
B. *Pentose phosphate pathway.* ***D.*** *Glycogenolysis.*

8. Orotic aciduria is an inherited disease characterized by severe metabolic anemia and orotic acid crystal deposition in organs and tissue. Which drug should be prescribed?

A. *Adenosine.* ***B.*** *Guanosine.* ***C.*** *Asparagine.* ***D.*** *Uridine.* ***E.*** *Glutamine.*

9. A man went to a doctor complaining of pain in his joints. The examination revealed increased uric acid concentration in the blood and urine. Uric acid is formed during catabolism of:

A. *Amino acids.* ***C.*** *Purine nucleotides.* ***E.*** *Chromoproteins.*
B. *Proteins.* ***D.*** *Pyrimidine nucleotides.*

10. A male was diagnosed with gout. Biochemical blood analysis revealed hyperuricemia. Which of the following enzymes is involved in the formation of uric acid?

A. *Pyruvate dehydrogenase.* ***C.*** *Epimerase.* ***E.*** *Xanthine oxidase.*
B. *Succinate dehydrogenase.* ***D.*** *Transaminase.*

11. Gout develops in impaired nucleotide metabolism as a result of accumulation of:

A. *Uric acid.* ***B.*** *Urea.* ***C.*** *Beta-alanine.* ***D.*** *Homogentisic acid.* ***E.*** *Phenylpyruvate.*

12. A patient with gout went to a physician. Allopurinol, which is an inhibitor ofxanthine oxidase, was prescribed. This enzyme takes part in breakdown of:

A. *Pyrimidine nucleotides.* ***C.*** *Glycoproteins.* ***E.*** *Lipoproteins.*
B. *Purine nucleotides.* ***D.*** *Phosphoproteins.*

13. Normal replication requires thymidylate nucleotides, whose synthesis needs participation of thymidylate synthase. Its co-enzyme is:

A. *Carboxybiotin.* ***C.*** *Pyridoxal phosphate.* ***E.*** *Nicotinamideadenin-*
B. *Thiamine pyrophosphate.* ***D.*** *Methyltetrahydrofolate.* *dinucleotide.*

14. A child has growth and mental retardation. The significant amount of orotic acid is exreted in urine. This hereditary disease develops as a result of impaired:

A. *Purine nucleotide synthesis.*
B. *Pyrimidine nucleotide breakdown.*
C. *Pyrimidine nucleotide synthesi.*
D. *Purine nucleotide breakdown.*
E. *Conversion of ribonucleotides to deoxyribonucleotides.*

15. Methotrexate, which is a structural analogue of folic acid, is prescribed to treat malignant tumors. It is a competitive inhibitor of dihydrofolate reductase and therefore inhibits the synthesis of:

A. *Nucleotides of DNA.* ***C.*** *Monosaccharides.* ***E.*** *Glycogen.*
B. *Fatty acids.* ***D.*** *Clycerophosphatides.*

16. Formation of purine deoxyribonucleosidetriphosphates occurs from the corresponding ribonucleotides by:

A. *Decarboxylation.* ***C.*** *Reductionof ribose into deoxyribose.*
B. *Transamination.* ***D.*** *Hydroxylation.* ***E.*** *Phosphorylation.*

17. Synthesis of purine nucleotides requires some amino acids, vitamin derivatives, phosphate esters of ribose. Which vitamin coenzyme form is a transfer form of one-carbon atom fragments for synthesis of purine nucleotides?

A. *Pyridoxine.* ***B.*** *Riboflavin.* ***C.*** *Pantothenic acid.* ***D.*** *Nicotinic acid.* ***E.*** *Folic acid.*

18. Children with Lesch-Nyhan syndrome have severe form of hyperuricemia, which is accompanied by tophi, urate stones in the urinary tract and severe neuro-psychiatric disorders. Which enzyme activity is reduced in patients with this disease?

A. *Hydrofolate reductase.* ***C.*** *Hypoxanthine guanine phosphoribosyl transferase.* ***E.*** *Carbamoylphosphate synthetase.*
B. *Xanthine oxidase.* ***D.*** *Thymidilate synthase.*

19. A patient went to a doctor complaining of severe pain, swelling, redness over the joints, fever up to 38° C. High content of urates was found in the blood. The possible cause is abnormal metabolism of:

A. *Purines.* ***B.*** *Collagen.* ***C.*** *Cholesterol.* ***D.*** *Pyrimidines.* ***E.*** *Carbohydrates.*

20. Purine nucleotides include:

A. *Adenine, guanine.* ***C.*** *Cytosine.* ***E.*** *All options are correct.*
B. *Uracil.* ***D.*** *Pseudouridine.*

21. Pyrimidine nucleotides include:

A. *Adenine.* ***C.*** *Uracil, thymine, cytosine.* ***E.*** *All options are correct.*
B. *Guanine.* ***D.*** *Pseudouridine.*

22. Which substance is initial in biosynthesis of adenylate and guanylate?

A. *Inosinic acid.* ***C.*** *Ribose-5-phosphate.* ***E.*** *5-Phosphoribosyl-1-diphosphate.*
B. *Hypoxanthine.* ***D.*** *Carbamoylphosphate.*

23. Which compound is a source of amino groups for biosynthesis of adenylate from inosinate?

A. *Aspartate.* ***B.*** *Glutamine.* ***C.*** *Glycine.* ***D.*** *Asparagine.* ***E.*** *Carbamoylphosphate.*

24. A patient experiences pain in small joints. They are enlarged. Serum urate levels are increased. Which metabolism is affected?

A. *Amino acids.* ***B.*** *Disaccharides.* ***C.*** *Purines.* ***D.*** *Pyrimidines.* ***E.*** *Glycerol.*

25. A boy with hereditary Lesch-Nyhan syndrome has symptoms of gout and neuropsychological changes. Which metabolic pathway is impaired?

A. *Purine nucleotide synthesis from free nitrogenous bases.*
B. *Purine nucleotide synthesis from amino acids.*
C. *Pyrimidine base synthesis from amino acids.*
D. *Breakdown of purines.*
E. *Breakdown of pyrimidines.*

26. High concentration of uric acid in the blood serum was found in a boy with symptoms of cerebral palalysis. Lesch-Nyhan syndrome was diagnosed. Which enzyme genetic defect is the cause of this disease?

A. *UDP-glucoronyltransferase.* ***C.*** *Hyaluronidase.* ***E.*** *Hypoxanthine guanine phosphoribosyl transferase.*
B. *UDP-glycosyltransferase.* ***D.*** *Lactate dehydrogenase.*

27. A three-year child suffers from mental retardation and severe megaloblastic anemia that is resistant to treatment with vitamins B_9 and B_{12}. Orotic acid is found in the urine. Megaloblastic anemia is caused by impaired synthesis of:

A. *AMP, GMP.* ***B.*** *GDP.* ***C.*** *ATP.* ***D.*** *GTP.* ***E.*** *UMP.*

28. A female patient has enlarged joints. High levels of urates are found in her blood. What is the name of the disease?

A. *Rickets.* ***B.*** *Scurvy.* ***C.*** *Pellagra.* ***D.*** *Caries.* ***E.*** *Gout.*

29. Which biochemical test should be done to confirm a diagnosis of gout?

A. *Determination of amino acids in blood.*
B. *Determination of urea in blood and urine.*
C. *Determination of creatin in blood.*
D. *Determination of urikase activity in blood.*
E. *Determination of uric acid blood and urine levels.*

30. Nitrogen metabolism was evaluated in a patient after prolonged starvation. What is the most probable result?

A. *Higher nitrogen excretion.* ***C.*** *Nitrogen equilibrium.* ***E.*** *Ketonemia.*
B. *Lower nitrogen excretion.* ***D.*** *Unaffected nitrogen balance.*

31. A child has positive nitrogen balance. What is its cause?

A. *Growth.* ***C.*** *Intense exercise.* ***E.*** *Starvation.*
B. *Emotional stress.* ***D.*** *Protein-poor diet.*

32. High blood concentrations of urates, xanthine, and hypoxanthine are found in a boy. Which genetic defect is observed?

A. *Arginase.* ***C.*** *Xanthine oxidase.* ***E.*** *Glycerol kinase.*
B. *Urease.* ***D.*** *Ornithine carbamoyltransferase.*

33. A patient went to a doctor complaining of joint pain whose intensity depends on the weather. High concentrations of uric acid were found in blood. Which substance increased breakdown is the most likely cause of such changes?

A. *CMP.* ***B.*** *UTP.* ***C.*** *UMP.* ***D.*** *TMP.* ***E.*** *AMP.*

34. A boy was diagnosed with Lesch-Nyhan disease. Blood concentration of uric acid is elevated. What is the cause of hyperuricemia?

A. *Purine nucleotide breakdown.* ***D.*** *Pyrimidine nucleotide breakdown.*
B. *Purine nucleotide breakdown.* ***E.*** *Deoxyribonucleotide synthesis.*
C. *Pyrimidine nucleotide synthesis.*

35. A man had underwent a prostate cancer surgery. Three months later he had underwent radiation and chemotherapy. The complex of drugs included 5-fluorodeoxyuridine, which is an inhibitor of thymidylate synthase. Which substance synthesis is blocked?

A. *DNA.* ***B.*** *mRNA.* ***C.*** *rRNA.* ***D.*** *tRNA.* ***E.*** *Protein.*

36. A man suffers from gout and complains of pain in the region of kidneys. Ultrasound examination revealed the presence of kidney stones. Which substance concentration is increased and causes stone formation in this case?

A. *Cholesterol.* ***B.*** *Bilirubin.* ***C.*** *Urea.* ***D.*** *Uric acid.* ***E.*** *Cystine.*

37. A man suffers from gout and complains of pain in the region of kidneys. Ultrasound examination revealed the presence of kidney stones. Which process leads to their formation?

A. *Protein catabolism.* ***C.*** *Heme breakdown.* ***E.*** *Purine nucleotide*
B. *Ornithine cycle.* ***D.*** *Cysteine reduction.* *breakdown.*

38. Allopurinol was prescribed to a patient with urolithiasis. It is a competitive inhibitor of xanthine oxidase. The reason for its prescription was the chemical analysis of kidney stones, which consisted primarily of:

A. *Dihydrate of calcium oxalate.* ***C.*** *Sodium urate.* ***E.*** *Calcium sulfate.*
B. *Calcium phosphate.* ***D.*** *Monohydrate of calcium oxalate.*

39. In hereditary orotic aciduria excretion of orotic acid is many times higher than under normal conditions. Which substance synthesis is impaired in this pathology?

A. *Urea.* ***C.*** *Purine nucleotides.* ***E.*** *Uric acid.*

B. *Biogenic amines.* ***D.*** *Pyrimidine nucleotides.*

40. Methotrexate was prescribed to a patient with cancer. Its target cells lost sensitivity to it. Which enzyme gene expression is impaired?

A. *Folate oxidase.* ***C.*** *Deaminase.* ***E.*** *Folate decarboxylase.*

B. *Dihydrofolate reductase.* ***D.*** *Thyminase.*

PRACTICAL WORK

Determination of uric acid content in blood serum by the method of Muller and Seifert. Determination of total nitrogen in the urine by the Kjeldahl method

Task 1. Determine the content of uric acid in blood serum.

Principle. The method is based on the ability of uric acid to reduce phosphotungstic reagent (Folin's reagent) yielding colored compounds, the color intensity of which is proportional to the concentration of the acid.

Procedure. Transfer 1.5 ml of blood serum, 1.5 ml of distilled water, and 1.5 ml of 20 % solution of trichloroacetic acid to a centrifuge tube. Mix the contents, wait for 5 min, and then centrifuge the mixture at 3,000 rpm for 10 min. Take two clean graduated test tubes (experimental and standard), and add to them the reagents according to the scheme.

	Experimental	Standard
Supernatant (equivalent to 0.5 ml of blood serum)	1.5 ml	–
Standard uric acid solution (0.2 mg/ml)	–	0.5 ml
20 % Solution of trichloroacetic acid	–	0.5 ml
Distilled water	–	0.5 ml
Saturated sodium carbonate solution	0.7 ml	0.7 ml
Folin's reagent (handle with care!)	1 drop	1 drop

Wait for 10 min and measure the absorbance for two samples against distilled water on a photocolorimeter at 510–560 nm (green light filter) using 0.5 cm thick cells. Uric acid (A) in mmol/L in serum is calculated by the formula:

$$X = (E_{exp} \times 0.01 \times 200) / (E_{st.} \times 168),$$

where E_{exp} is the extinction of the test sample; $E_{st.}$ is the extinction of the standard sample; 0.01 is the mass of uric acid contained in standard solution, used for reaction, mg; 200 is the scaling factor for conversion to per liter of blood serum; 168 is the molecular mass of uric acid.

Clinical and diagnostic significance. Uric acid is the end product of purine nucleotide metabolism. The normal concentration of uric acid in the blood serum is 0.10–0.40 mmol/L, and the uric acid excretion with urine is 2.36–5.90 mmol/day. Elevated levels of uric acid in the blood (hyperuricemia) occur in many pathological conditions associated with enhanced degradation of nucleoproteins (leukemia, diabetes mellitus, allergies), in defects of enzymes, such as phoshoribosyl pyrophosphate synthetase, hypoxanthine-guanine phosphoribosyl transferase (gout, Lesch-Nyhan syndrome), adenine phosphoribosyl transferase (urolithiasis) and von Gierke's disease (deficiency of glucose-6-phosphatase in the liver). The increase in uric acid concentration is the main

diagnostic criterion of gout. It is obvious that when medium becomes acidic, uric acid is deposited in tissues, cartilages, joint capsules. Hypouricemia and increased excretion of hypoxanthine and xanthine may be due to lack of xanthine oxidase in result of disturbances in gene structure of this enzyme, or the result of liver damage. Hypouricuria is observed in nephritis, kidney insufficiency. Hyperuricuria may occur due to increased intake and enhanced breakdown of nucleoproteins. Children excrete relatively more uric acid than adults.

Task 2. Determine the total nitrogen of urine by the Kjeldahl method.

Principle. Urine is mineralized in concentrated sulphuric acid. In this case the nitrogen of organic and inorganic compounds in the form of ammonia is bound with sulphuric acid to form ammonium sulfate, which reacts with Nessler's reagent, yielding a compound of yellow-orange color. The intensity of the solution color is proportional to the concentration of nitrogen.

Procedure. 1. *Mineralization.* Pour 0.5 ml of urine into the test tube; add 0.05 ml of saturated sulphuric acid to it. Mineralization is carried out on a sand bath: a test tube should touch only the top layer of sand. Firstly, water evaporates; the urine acquires a brown color. Remove the test tube from the bath, cool it. Add 2 drops of perhydrol and again put up for mineralization to decolorate the liqui. You should check the color of the liquid in the test tube after its cooling, as the fluid, which is colorless when it is hot, often becomes dark after cooling. After cooling, add 10 ml of boiled distilled water to the test tube, neutralize acid with 12.5 M sodium hydroxide until slightly alkaline reaction appears, which is determined by changing the color of lacmus paper from red to blue. Excessive amount of base leads to the clouding of the solution. Deficiency of base causes precipitation of mercury salts from the Nessler's reagent, and the experiment is considered spoiled.

2. *Color reaction (nesslerization).* Add 0.5 ml of Nessler's reagent to the test tube. The solution in the test tube becomes yellow of varying intensity depending on the nitrogen content.

The standard sample is treated in parallel with the test sample: 0.2 ml of standard solution of ammonium sulfate, 0.05 ml of saturated sulphuric acid, 0.3 ml of 12.5 M sodium hydroxide solution, 0.5 ml of Nessler's reagent and 9.8 ml of distilled water. Experimental and standard samples are measured against the control at 440–450 nm using 0.5 cm thick cells. Control is prepared in the same way as the standard sample, but water is added instead of a standard solution of ammonium sulfate. The control sample should have a light yellowish tinge. More intense color of the control indicates the presence of nitrogen (ammonia) in distilled water. The calculation is carried out as follows: $C_{test} = C_{st} \times (E_{test} / E_{st})$, where C_{test} is the concentration of total nitrogen in urine (mmol/L); C_{st} is the concentration of total nitrogen in the standard; E_{test} is test sample extinction; E_{st} is standard sample extinction.

Clinical and diagnostic significance. *Person excretes 10–17 grams of nitrogen per day. Nitrogen of urea accounts for 80–90 % of total nitrogen. Total nitrogen of urine is the indicator of the nitrogen balance, and functional status of the liver, kidneys and other organs.*

1**. Prepare a presentation "Pyrimidine derivatives as drugs".

2**. Create a scheme of nucleic acid structural organization.

3**. Explain a mechanism of formation of tRNA hairpins.

CLASS 6 (4 hours)

TOPIC 8 (2 hours). Biosynthesis of nucleic acids and proteins (template biosyntheses). Transmission of genetic information. Principles of molecular genetics

IMPORTANCE. One of the major achievements of modern biochemistry is an elucidation of the mechanisms of nucleic acid and protein biosynthesis. Amino acids are incorporated in the polypeptide chain in nonchaotic way, forming well-defined sequence, which provides a unique structure and function. The mechanism of protein biosynthesis must have the exact coordination system that automatically programs the inclusion of each amino acid residue in a specific location of the polypeptide chain. The coordinating system determines the primary structure, but secondary and tertiary structures of the protein molecule are determined by the primary structure, its physicoal and chemical properties, as well as chemical structure. Nucleic acids (DNA, RNA) perform the conservation, realization and transmission of genetic information. DNA acts as a carrier of genetic information, which is transmitted through a more labile than DNA structures called RNA. Proteins are the only substances in which genetic information is realized. The process of transfer of genetic information determines the development and vitality of a living organism. Specialization of cell functions (of liver, brain, muscle, etc.) depends on of protein composition, primarily enzymes that control metabolic processes at the cellular level.

AIM. Learn in detail the template biosynthesis in order to use the knowledge for understanding the mechanisms regulating the activity of genes in prokaryotes and eukaryotes, actions of template biosynthesis inhibitors (drugs and bacterial toxins), tmolecular mechanisms of genetic variability, molecular pathology of protein biosynthesis, principles of treatment and prevention of molecular diseases, use of recombinant DNA and gene cloning in medicine.

THEORETICAL QUESTIONS

1. DNA replication, its biological significance; semiconservative replication mechanism. Discovery of DNA double helix by Watson and Crick.
2. General scheme of biosynthesis of DNA strands. Enzymes of DNA replication in prokaryotes and eukaryotes, scheme of DNA replication.
3. Molecular mechanisms of DNA replication: significance of DNA antiparallel structure, Okazaki fragments. Stages of synthesis of daughter strands of DNA molecules.
4. Total RNA transcription scheme: coding and non-coding DNA strands. RNA polymerases of prokaryotes and eukaryotes.
5. Stages and enzymes of RNA synthesis. Signals of transcription: promoter, initiator and terminator sequences of genome.
6. Processing (posttranscriptional modification of RNA). Antibiotics, which are inhibitors of transcription.
7. Genetic (biological) code; triplet structure of the code and its properties.
8. Ribosomal protein-synthesizing system; its components. The structure of eukaryotic ribosomes.
9. Transfer RNAs and activation of amino acids. Aminoacyl-tRNA synthetases.
10. Stages and mechanisms of translation: initiation, elongation, termination. Codons of initiation and termination, the role of ribosomal protein factors in translation.

11. Posttranslational modification of peptide chains. Regulation of translation. Molecular mechanisms controlling translation on the example of the biosynthesis of globin.

12. Effects of physiologically active substances on the process of translation. Antibiotics, which are inhibitors of translation in prokaryotes and eukaryotes, their application in medicine.

13*. Regulation of gene expression in prokaryotes. Scheme of structural and control genes according to F. Jacob and J. Monod: promoter, regulatory genes.

14*. Features of the molecular organization of DNA and expression of eukaryotic genome (exons, introns, repeated sequences).

15*. Genetic recombinations in prokaryotes (transformation, transduction, conjugation).

16*. Biological significance and mechanisms of DNA reparation. Repair of UV-induced gene mutations, xeroderma pigmentosum.

17*. Genetic engineering or recombinant DNA technology: general concepts, biomedical importance.

18*. Technology of genes transplantation and making hybrid DNA molecules. Cloning of genes to obtain biotechnology drugs (hormones, enzymes, antibiotics, interferons, etc.).

19*. Mutations: genome, chromosome and gene mutations. Role of mutations in the development of enzymopathies and inherited diseases in human.

Recommendations for self-study of theoretical questions

Question	Information
1. Regulation of prokaryotic gene expression. Jacob-Monod hypothesis: structural and control gene	Regulation of gene expression is one of the mechanisms of adaptation to the environmental changes. 1.1. In prokaryotes, specific interaction of protein regulators with different DNA sequences (along with transcription initiation sites). 1.2. Results of interaction: 1) activation, 2) inhibition. 1.3. Jacob-Monod theory of expression regulation in prokaryotes: – Operon theory is a model that explains molecular mechanisms of protein synthesis induction and repression oat the level of transcription; • operon structure (complex of genetic elements that is responsible for synthesis of enzymatic proteins): structural genes (information on the protein primary structure); control sites (promoter is a DNA sequence that initially interacts with RNA polymerase); operator is a DNA sequence that can specifically bind to repressor proteins; • regulatory gene – its expression leads to production of repressor ptoteins that block information reading from the operon structural genes
2. Features of DNA molecular organization and eukaryotic gene expression (exons, introns, repetitive sequences)	2.1. The bulk of the nuclear DNA is not translated into amino acid sequences of proteins; a) introns are "silent" fragments of the genome, DNA segments that are not transcribed; b) exons are genome fragments that are transcribed to mRNA, which carries information necessary for synthesis of specific proteins. 2.2. Only 2 % of DNA contains information for coding proteins. 2.3. Repeated DNA sequences: – Frequency – 20–30 % of repeated DNA in the human genome; – Classes of repetitive nucleotide sequences: a) highly repetitive (their

	length varies from 5 to 500 base pairs); b) moderately repetitive can be either short or long
3. Genetic recombinations in prokaryotes (transformation, transduction, conjugation)	3.1. Genetic recombinations are gene restructuring, exchange of DNA fragments between genes or combining genes from different biosources; their role includes formation of biochemical individuality; formation of species during evolution. 3.2. Transformation is a process of incorporation of DNA from dead donor cells into the genome of recipient microorganisms. 3.3. Transduction is a transfer of bacteriophagic DNA fragments of infected cells into the genome of another recipient organism. 3.4. Conjugation is a process of reproduction by transferring DNA fragments from donor cells to the recipient
4. Biological significance and mechanisms of DNA repair. Repair of UV-induced gene mutations, xeroderma pigmentosum	4.1. All living organisms are subject to constant action of physical and chemical mutagenic factors (UV radiation, ionizing radiation, xenobiotics). Mechanism of action: formation of free radicals, changes in the covalent structure of nitrogenous bases, DNA instability, point mutations (depurination, depyrimidination). 4.2. DNA repair is a special molecular mechanism of fighting with permanent DNA damage and restoring changes. 4.3. Repair of UV-induced damage (mainly observed in human skin): – DNA restriction occurs from the left side by UV-specific endonuclease; – Formation of polydeoxyribonucleotide "patch" in the DNA segment by DNA polymerase; – Removal of the damaged DNA fragment; – Ligation of 3'-end of the "patch" with the 5'-end of the cut DNA chain. 4.4. Xeroderma pigmentosum is a hereditary disease associated with disorders of UV-induced DNA repair: – Autosomal recessive disease; – Patients' skin is sensitive to sunlight; skin cancer may develop; – Defects of UV-specific endonuclease
5. Genetic engineering or recombinant DNA technology: general conception, biomedical significance	5.1. Genetic engineering (recombinant DNA technology) is a modern biomedical field, which is based on the isolation of individual cellular DNA, DNA fragments of different biological species (molecular chimeras). 5.2. Biomedical significance: – Creation of new genotypes of organisms (gene transplantation from one organism to genotype of the other); – Synthesis of drugs (interferons, insulin); – Treatment of inherited diseases (gene therapy)
6. Gene therapy technology and hybrid DNA. Gene cloning for synthesis of biotechnological drugs (hormones, enzymes, antibiotics, interferons, etc.)	6.1. Technology of transplantation: – Creation of a required gene (DNA molecule) by chemical gene synthesis, isolation of a required gene, construction of complementary DNA using mRNA as a template; – Construction of recombinant DNA using restrictases (recombinant DNA consists of nDNK and a specific DNA molecule that serves as a vector, which is able to penetrate into the recipient cell); – Incorporation of recombinant DNA into the recipient cell and amplication of the desired gene. 6.2. Significance: synthesis of human interferon, insulin, growth hormone, somatostatin, plasminogen activator, protein products for diagnosis of AIDS

7. Mutations: genome, chomosomal, gene. Role in the formation of enzymopathies and inherited human diseases	7.1. Mutations are changes in genetic properties as a result of quantitative and qualitative changes in the genotype of the organism, changes in sequence of purine or pyrimidine bases in the gene: – Basis of genetic variability; – Spontaneous and induced mutations. 7.2. Classification of mutations by their nature: – **Genomic**: changes in the number of the complete set of chromosomes or specific chromosomes in the diploid set (development of chromosomal human diseases); – **Chromosomal** mutations are associated with structural changes in chromosomes; their types: • transposition is a transfer DNA fragment to another fragment of the same chromosome; • translocation is a transfer of the chromosome fragment to the homologous chromosome; • inversion is a change in gene sequences to the reverse sequence in the chromosome fragment; • deletion is a loss of DNA fragment; • duplication is a repeat of certain chromosome fragments; – **Gene (point)** mutations are changes in the structure of the genome caused by disorders of nucleotide sequence; their types: • replacement of nucleotides by transition (substitution of a purine to another purine base, or pyrimidine base to another pyrimidine) or transversion (replacement of purine to pyrimidine and vice versa); • deletion in the DNA chain; • insertion. 7.3. Mutagens: – Analogues of nitrogenous bases (5-bromouracil, 2-aminopurine); – Chemicals (deaminating agents – nitrosamines, alkylating compounds); – UV- and ionizing radiation. 7.4. Role of mutations in etiology of enzymopathies and hereditary diseases: – Blockage of enzyme synthesis, which is encoded by the corresponding gene; – Formation of protein with the altered primary structure. Examples: α- and β-thalassemias, associated with disorders of hemoglobin α- or β-chain synthesis (replacement or deletion of one or more nucleotides)

TESTS FOR SELF-CONTROL

1. A patient with xeroderma pigmentosum has sensitive to light skin, because excision repair is impaired. Which process is affected in this case?

A. *Removal of DNA defects.* ***C.*** *mRNA maturation.* ***E.*** *Removal of introns and ligation of exons.*
B. *mRNA synthesis.* ***D.*** *Synthesis of primary protein structure.*

2. The use of certain medications, is associated with a hundredfold increase in the number of copies of specific genes and synthesis of the corresponding enzymes, respectively. Which gene amplification enzyme caused the loss of sensitivity of target cells to the antitumor action of methotrexate?

A. *Thymidilate synthase.* ***C.*** *Thyoredoxin reducatse.* ***E.*** *Orotate phosphoribosyl transferase.*
B. *Ribonucleotide reductase.* ***D.*** *Dihydrofolate reductase.*

3. Antitumor antibiotics doxorubicin and daunomicyn are able to be integrated into the DNA between GC pairs, inhibiting:

A. *Replication and translation.* ***C.*** *DNA repair and replication.* ***E.*** *Replication and transcription.*
B. *DNA repair and transcription.* ***D.*** *Reverse transcription.*

4. Cytological investigation revealed a large number of different tRNA molecules, which deliver amino acids to ribosomes. The number of various types of tRNAs in a cell is equal to the number of:

A. *Nucleotides.* ***C.*** *Triplets that encode amino acids.* ***E.*** *Various types of iRNA.*
B. *Amino acids.* ***D.*** *Proteins synthesized in the cell.*

5. Antibiotics (streptomycin, erythromycin, chloramphenicol) are used for the treatment of infectious bacterial diseases. Which of the microbial processes mentioned below is inhibited by them?

A. *Translation.* ***B.*** *Replication.* ***C.*** *Processing.* ***D.*** *Transcription.* ***E.*** *Splicing.*

6. It has been proven that UV-exposed skin cells of patients with xeroderma pigmentosum repair DNA structure slower than cells of healthy people due to the defect of a DNA repair enzyme. Which enzyme is responsible for this process?

A. *RNA ligase.* ***C.*** *Primase.* ***E.*** *DNA-gyrase.*
B. *DNA polymerase III.* ***D.*** *Endonuclease.*

7. It has been proven that immature mRNA (pro-mRNA) contains a higher number of triplets than the amount of amino acids in synthesized proteins. This is due to the fact that translation is normally preceded by:

A. *Processing.* ***B.*** *Replication.* ***C.*** *DNA repair.* ***D.*** *Mutation.* ***E.*** *Initiation.*

8. All types of RNA are synthesized in the form of RNA precursors, which undergo maturation (processing). One of the stages of processing is called splicing. Splicing is:

A. *DNA fragmentation.*
B. *Addition of 7-methylguanosine to 5'-terminal residue.*
C. *Addition of 100-200 adenylate residues to 3'-terminus.*
D. *Chemical modification of nitrogenous bases.*
E. *Removal of non-coding introns and ligation of coding exons.*

9. Translation occurs in the rough endoplasmic reticulum. During it mRNA moves along ribosomes. Amino acids are linked by peptide bonds in a chain – polypeptide. The sequence of amino acids in the polypeptide should match the sequence of:

A. *mRNA codons.* ***C.*** *Nucleotides of tRNA.* ***E.*** *Nucleotides of rRNA.*
B. *tRNA antocodons.* ***D.*** *rRNA anticodons.*

10. iRNA synthesis requires DNA as a template and occurs in accordance with the principle of complementarity. If there are the following DNA triplets ATG-CGT, the corresponding RNA codons are:

A. *ATG-CGT.* ***B.*** *UAG-CGU.* ***C.*** *TAG-UGU.* ***D.*** *UAC-GCA.* ***E.*** *AUG-CGU.*

11. Translation occurs in a cell. When a ribosome reaches the codons UAA, UAG, or UGA, synthesis of polypeptide chain ends. These codons cannot be recognized by any tRNA and therefore they are signals of:

A. *Initiation.* ***C.*** *Termination.* ***E*** *Posttranslational modofication .*
B. *Elongation.* ***D.*** *Transcription initiation.*

12. During reproduction of some RNA-containing viruses that cause tumors in animals, genetic information can be transferred in the opposite direction from RNA to DNA by a special enzyme called:

A. *Ligase.* ***B.*** *Revertase.* ***C.*** *Topoisomerase.* ***D.*** *DNA polymerase.* ***E.*** *Primase.*

13. The antibiotic chloramphenicol is attached to ribosomal 50S subunit and inhibits translocation that leads to impaired:

A. *Translation elongation.* ***C.*** *Translation initiation.* ***E.*** *Transcription elongation.*
B. *Translation termination.* ***D.*** *Transcription initiation.*

14. During the experimental study of toxic effects of some drugs, significantly lower activity of aminoacyl-tRNA synthetases was found. Which process is inhibited?

A. *Processing.* ***B.*** *Replication.* ***C.*** *Transcription.* ***D.*** *Splicing.* ***E.*** *Translation.*

15. Erythromycin was prescribed to a patient with bacterial pneumonia. It has been known that erythromycin has antibacterial properties due to the ability to interact with a free 50S ribosome subunit. Which subtances cannot be produced due to this antibiotic in bacterial cells?

A. *RNA.* ***B.*** *Proteins.* ***C.*** *Polysaccharides.* ***D.*** *Fats.* ***E.*** *DNA.*

16. During presynthentetic period of the mitotic cycle, synthesis of DNA-dependent DNA polymerase was impaired. Which consequences can be observed?

A. *Abnormal cytokinesis.* ***C.*** *Abnormal DNA replication.* ***E.*** **–** .
B. *Shorter mitosis.* ***D.*** *Abnormal formation of spindle apparatus.*

17. Chloramphenicol was prescribed to a patient. It blocks protein synthesis in microorganisms by inhibiting:

A. *Gene amplification.* ***C.*** *Transcription.* ***E.*** *Translation elongation.*
B. *Formation of polyribosomes.* ***D.*** *Processing.*

18. Infectionists widely use antibiotics that inhibit synthesis of nucleic acids. Which stage of biosynthesis is inhibited by rifampicin?

A. *Initiation of transcription in prokaryotes.*
B. *Replication in prokaryotes.*
C. *Termiantion of transcription in prokaryotes and eukaryotes.*
D. *Splicing in prokaryotes and eukaryotes.*
E. *Transcription in prokaryotes and eukaryotes.*

19. Treatment of viral RNA with nitric acid led to changes in UCA codon to UGA codon. What type of mutation occurred?

A. *Nucleotide deletion.* ***B.*** *Missense.* ***C.*** *Transition.* ***D.*** *Inversion.* ***E.*** *Insertion.*

20. Protein biosynthesis is energy-dependent. Which macroergic substrate is directly used in this process at the stage of elongation?

A. *ATP.* ***B.*** *GTP.* ***C.*** *ADP.* ***D.*** *UTP.* ***E.*** *CTP.*

21. Nucleic acids are used by the body to store and transmit genetic information. Select a type of RNA that contains information about location of amino acids in the protein.

A. *70sRNA.* ***B.*** *tRNA.* ***C.*** *30sRNA.* ***D.*** *mRNA.* ***E.*** *40sRNA.*

22. The process of mRNA maturation of requires some transformations. Choose an amino acid that is the first one in protein synthesis in prokaryotes.

A. *Cysteine.* ***B.*** *Glycine.* ***C.*** *Serine.* ***D.*** *Alanine.* ***E.*** *Formylmethionine.*

23. Under the influence of physical factors defects may develop in DNA molecules. Ultraviolet rays cause formation of dimers, which are two adjacent pyrimidine bases. Select them.

A. *Guanine and cytosine.* ***C.*** *Thymine and cytosine.* ***E.*** *Guanine and thymine.*
B. *Adenine and thymine.* ***D.*** *Adenine and guanine.*

24. A mutation occurred at the level of DNA in a worker in the Chernobyl zone. However, the primary structure of DNA molecule was restored in the damaged area by a special enzyme. Which process took place?

A. *Replication.* ***C.*** *Transcription.* ***E.*** *Translation.*
B. *DNA repair.* ***D.*** *Reverse transcription.*

25. Nitric acid is formed in the body from nitrates, nitrites, and nitrosamines. It causes oxidative deamination of nitrogenous bases of nucleotides. This can lead to a point mutation – replacement of cytosine by:

A. *Inosine.* ***B.*** *Guanine.* ***C.*** *Thymine.* ***D.*** *Adenine.* ***E.*** *Uracil.*

26. The antibiotic rifampicin is used to treat tuberculosis. It affects certain biochemical processes. Select them.

A. *Inhibition of DNA ligase.*
B. *Inhibition of DNA polymerase at the level of initiation.*
C. *Inhibition of RNA polymerase at the level of initiation.*
D. *Inhibition of aminoacyl-tRNA synthestase.*
E. *Inhibition of protein factors during protein synthesis.*

27. Quinolones that are DNA gyrase inhibitors are used for the treatment of urogenital infections. Which process is affected by quinolones?

A. *DNA replication .* ***C.*** *Gene amplification.* ***E.*** *Reverse transcription.*
B. *DNA repair.* ***D.*** *Gene recombination.*

28. Patients with xeroderma pigmentosum have extremely sensitive to sunlight skin. This may cause skin cancer due to UV endonuclease genetic deficiency. Which process is impaired?

A. *Reverse transcription.* ***C.*** *Transcription.* ***E.*** *Translation.*
B. *DNA replication.* ***D.*** *DNA repair.*

29. Genetic information is stored in DNA, but it is not directly involved in protein synthesis in cells. Which process ensures the implementation of hereditary information in the polypeptide chain?

A. *rRNA formation.* ***C.*** *iRNA formation.* ***E.*** *Replication.*
B. *Translation.* ***D.*** *mRNA formation.*

30. To form the transport form of amino acids during protein synthesis, ribosomes should have:
A. *Revertase.* ***B.*** *GTP.* ***C.*** *mRNA.* ***D.*** *Ribosome.* ***E.*** *Aminoacyl-tRNA synthestase.*

31. Amanitin (death cap poison) blocks RNA polymerase B (II). This inhibits:

A. *Reverse transcription.* ***C.*** *mRNA synthesis.* ***E.*** *mRNA maturation.*
B. *tRNA synthesis.* ***D.*** *Synthesis of primers.*

32. A child suffering from diphtheria has fibrinous deposits on tonsils. Which process is inhibited by diphtheria toxin?

A. *Fatty acid synthesis.* ***C.*** *Protein synthesis.* ***E.*** *Synthesis*
B. *Gluconeogenesis.* ***D.*** *Beta-oxidation of fatty acids.* *of biogenic amines.*

33. Watson and Crick found that DNA double helix was stabilized by bonds between complementary nitrogenous bases. What bonds are they?

A. *Phosphodiester.* ***B.*** *Hydrogen.* ***C.*** *Peptide.* ***D.*** *N-Glycosidic.* ***E.*** *Ester.*

34. All living organisms have the same triplets that encode the same amino acids. This allows transfering E. coli insulin gene to humans. How is this property of genetic code called?

A. *Universality.* ***B.*** *Non-ambiguity.* ***C.*** *Degeneracy.* ***D.*** *Triplet.* ***E.*** *Commaless.*

35. A patient has folate deficiency. This may lead to abnormal synthesis of:

A. *Citrate and ketone bodies.* ***D.*** *Purine nucleotides and cholesterol.*

B. *Thymidilate and fatty acids.* ***E.*** *Heme and creatine.*

C. *Purine nucleotides and thymidilate.*

36. During cell division a signal for DNA replication comes from the cytosol and a certain section of the DNA helix unwinds and splits into two chains. Which enzyme is necessary for this?

A. *DNA polymerase.* ***B.*** *RNA polymerase.* ***C.*** *Ligase.* ***D.*** *Restrictase.* ***E.*** *Helicase.*

37. According to the model of the DNA double helix proposed by Watson and Crick, it has been found that one of the chains is not kept during replication, whereas the second one is synthesized complementary to the first strand. How is this type of replication called?

A. *Disperse.* ***B.*** *Analogous.* ***C.*** *Identical.* ***D.*** *Semi-conservative.* ***E.*** *Conservative.*

38. Molecular mechanisms that fix damaged DNA have developed during evolution. This process is called:

A. *DNA repair.* ***B.*** *Transcription.* ***C.*** *Translation.* ***D.*** *Replication.* ***E.*** *Processing .*

39. Human genome contains about 30,000 genes, and the number of antibodies reaches millions. Which process is used to form new genes responsible for synthesis of so many antibodies?

A. *DNA replication.* ***C.*** *Gene recombination.* ***E.*** *Okazaki fragments.*

B. *Gene amplification.* ***D.*** *DNA repair.*

40. A patient has reduced content of magnesium ions required to attach ribosomes to the rough endoplasmic reticulum. It has been known that this leads to disorders of protein synthesis. Which stage of protein synthesis is affected?

A. *Activation of amino acids.* ***C.*** *Termination.* ***E.*** *Transcription.*

B. *Translation.* ***D.*** *Replication.*

41. Degeneracy of genetic code is the ability of several triplets to encode a single amino acid. Which amino acid is encoded by a single triplet?

A. *Serine.* ***B.*** *Alanine.* ***C.*** *Leucine.* ***D.*** *Methionine.* ***E.*** *Lysine.*

42. It has been established that some compounds, such as fungal toxins and some antibiotics, can inhibit the activity of RNA polymerase. Which process is impaired due to inactivation of this enzyme?

A. *Transciption.* ***B.*** *Translation.* ***C.*** *Processing.* ***D.*** *DNA repair.* ***E.*** *Replication.*

43. HIV virus attacks T lymphocytes. Viral enzyme reverse transcriptase (RNA-dependent DNA polymerase) catalyzes synthesis of:

A. *DNA using viral rRNA as a template.* ***D.*** *Viral RNA using DNA as a template.*

B. *Viral DNA using DNA as a template.* ***E.*** *DNA using viral RNA as a template.*

C. *mRNA using viral protein as a template.*

44. A chemical plant worker underwent the action of nitrous acid and nitrites as a result of violation of safety rules. They cause deamination of cytosine in DNA. Which enzyme triggers repair processes?

A. *Cytidinetriphosphate synthetase.* ***D.*** *DNA-dependent RNA polymerase.*

B. *Thymidilate synthase.* ***E.*** *Uridine DNA glycosidase.*

C. *Orotidylmonophosphate decarboxylase.*

45. Pro-mRNA is initially synthesized in nuclei of eukaryotic cells. It is complementary to exons and introns of the structural gene. However, only RNA complementary to exons

can reach ribosomes. This indicates that one of the following processes take place in the nucleus:

A. *Processing.* ***C.*** *Transcription.* ***E.*** *DNA repair.*
B. *Reverse transcription.* ***D.*** *Replication.*

46. mRNA containing exons and introns enters rough endoplasmic reticulum in humans. Which process will not occur?

A. *Replication.* ***B.*** *Prolongation.* ***C.*** *Translation.* ***D.*** *Processing.* ***E.*** *Transcription.*

47. Transcription occurs in human cells. The enzyme RNA polymerase, moving along the DNA molecule, reaches a specific sequence of nucleotides. Transcription stops. This DNA sequence is called:

A. *Operator.* ***B.*** *Terminator.* ***C.*** *Repressor.* ***D.*** *Regulator.* ***E.*** *Promotor.*

48. Experimental studies showed that steroids affected proteosynthesis. Which stage of the process is affected?

A. *Synthesis of specific rRNA.* ***C.*** *Synthesis of specific tRNA.* ***E.*** *Synthesis*
B. *Synthesis of ATP.* ***D.*** *Synthesis of GTP.* *of specific mRNA.*

49. Cells of AIDS patients, infected with the HIV virus, have revertase activity. Which nucleic acid is synthesized with this enzyme?

A. *rRNA.* ***B.*** *mRNA.* ***C.*** *preRNA.* ***D.*** *DNA.* ***E.*** *tRNA.*

50. Streptomycin is used for the treatment of infectious diseases. Which compound synthesis is inhibited by the action of this antibiotic?

A. *Proteins.* ***B.*** *DNA.* ***C.*** *mRNA.* ***D.*** *tRNA.* ***E.*** *rRNA.*

FINAL MODULE CONTROL № 3 – METABOLISM OF PROTEINS AND NUCLEIC ACIDS (2 hours)

CONTROL QUESTIONS

1. Role of proteins in the vital activity of organism.
2. Protein norms in the nutrition. Complete and incomplete proteins. Non-essential, essential, conditionally essential and semiessential amino acids.
3. Nitrogen balance, its types.
4. The biological meaning of protein digestion in the gastrointestinal tract. Enzymes of protein digestion. The mechanism of proteolytic enzymes activation.
5. The chemical composition of gastric juice. Forms of gastric juice acidity, the methods of determination. Role of HCl.
6. Mechanisms of amino acids absorption in the intestine.
7. Biochemical mechanisms of protein digestion regulation. Hormones of gastrointestinal tract.
8. Disorders of protein digestion in the gastrointestinal tract. Parenteral nutrition.
9. Chemical conversions of amino acids in large intestine. Mechanisms of detoxification of protein putrefaction products in the large intestine.
10. Amines formed in the large intestine during the putrefaction from diaminomonocarboxylic acids, their detoxification.
11. Toxic substances formed in the large intestine during the putrefaction from tyrosine and phenylalanine, their detoxification.
12. The toxic substances formed in large intestine during the putrefaction from tryptophan, their detoxification.

13. Tissue proteolysis. Action, properties and classification of cathepsins.

14. Scheme of the basic pathways of formation and using the amino-acid pool of tissues. Basic classes of the organic compounds formed from the amino acids.

15. General pathways of the amino acid metabolism.

16. Decarboxylation of amino acids: enzymes, physiological significance.

17. Biogenic amines: reactions of formation, role. Mechanisms of inactivation of biogenic amines.

18. Major types of deamination of amino acids in organism.

19. Direct and indirect deamination of L-amino acids. Mechanism of action and role of amino acid oxidases and glutamate dehydrogenase.

20. Transamination of amino acids. Mechanism of aminotransferase action. Role of aminotransferases in metabolism of amino acids. Clinical value of determination of aminotransferases in blood.

21. Reaction of reductive amination of α-ketoglutarate, its role.

22. The pathways of ammonia formation in the body, its toxic action.

23. Transport of ammonia from tissues to liver and kidneys. Reactions of formation of glutamine and asparagine, their role. Role of alanine in transport of ammonia.

24. Mechanisms of temporary and final detoxification of ammonia.

25. Formation of ammonia salts in kidneys, the significance of process.

26. Ornithine cycle of urea formation in the liver: reactions, role. Genetic defects of ornithine cycle enzymes (enzymopathies).

27. Hyperammoniemia: its causes, manifestation, consequences.

28. Scheme of metabolic pathways of nitrogen-free amino acid residues. Their interrelation with Krebs cycle.

29. Glucogenic and ketogenic amino acids.

30. Physiologic meaning and regulation of gluconeogenesis processes from amino acids.

31. Metabolism of phenylalanine and tyrosine, disturbances, ways of correction.

32. Metabolism of tryptophan, disturbances, ways of correction.

33. Metabolism of glycine and serine. Glutathione as a product of amino acid metabolism; its structure and role.

34. Arginine metabolism. Nitric oxide as a metabolite of arginine, its role.

35. Metabolism of sulfur- containing amino acids.

36. Metabolism of dicarboxylic amino acids.

37. Metabolism of valine, leucine, isoleucine. Maple syrup urine disease.

38. Creatine synthesis, its role, disorders. Creatine kinase: isoforms, clinical and diagnostic significance of its determination in blood serum and urine.

39. Congenital defects of amino acids metabolism, biochemical methods of their diagnostics, possibilities of their correction.

40. Nucleotides, their structure and role in the body.

41. Digestion and absorption of nucleoproteins.

42. Synthesis of pyrimidine nucleotides. Regulation. Disorders.

43. Synthesis of purine nucleotides. Regulation. Disorders.

44. Synthesis of deoxyribonucleotides.

45. Role of carbamoyl phosphate in metabolism of proteins and nucleotides.

46. Breakdown of pyrimidine nucleotides.

47. Degradation of purine nucleotides. Hyperuricemia, its causes, biochemical mechanisms of correction.

48. Correlation between metabolism of proteins and nucleic acids.

49. DNA replication, the biological significance; semiconservative replication mechanism. Discovery of DNA double helix by Watson and Crick.

50. General scheme of biosynthesis of DNA strands. Enzymes of DNA replication in prokaryotes and eukaryotes, scheme of DNA replication.

51. Molecular mechanisms of DNA replication. Stages of synthesis of daughter strands of DNA molecules.

52. Total RNA transcription scheme. RNA-polymerases of prokaryotes and eukaryotes.

53. Stages and enzymes of RNA synthesis. Signals of transcription: promoter, initiator and terminator sequences of genome.

54. Processing (posttranscriptional modification of RNA). Antibiotics,which are inhibitors of transcription.

55. Genetic (biological) code; triplet structure of the code and its properties.

56. Ribosomal protein-synthesizing system: its components. The structure of eukaryotic ribosomes.

57. Transfer RNAs and activation of amino acids. Aminoacyl-tRNA synthetases.

58. Stages and mechanisms of translation: initiation, elongation, termination. Codon of initiation and termination, the role of protein factors of ribosomes in translation.

59. Posttranslational modification of peptide chains. Regulation of translation. Molecular mechanisms controlling translation on the example of the globin biosynthesis .

60. Effects of physiologically active substances on the process of translation. Antibiotics, which are inhibitors of translation in prokaryotes and eukaryotes, their application in medicine.

61. Regulation of gene expression in prokaryotes. Scheme of structural and control genes according to F. Jacob and J. Monod: promoter, regulatory genes.

62. Features of the molecular organization of DNA and expression of eukaryotic genome (exons, introns, repeated sequences).

63. Genetic recombinations in prokaryotes (transformation, transduction, conjugation).

64. The biological significance and mechanisms of DNA reparation. Reparation of UV-induced gene mutations, xeroderma pigmentosum.

65. Genetic engineering or recombinant DNA technology: general concepts, biomedical importance.

66. Technology of genes transplantation and making hybrid DNA molecules. Cloning of genes to obtain biotechnology drugs (hormones, enzymes, antibiotics, interferon).

67. Mutations: genome, chromosome and gene mutations. Role of mutations in the development of enzymopathies and inherited diseases in humans.

Навчальне видання

ЧАСТИНА 3
ОБМІН БІЛКІВ І НУКЛЕЇНОВИХ КИСЛОТ

Методичні вказівки
для підготовки до практичних занять з біологічної хімії
(для студентів медичних факультетів)

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Формат 60×84/16. Ризографія. Ум. друк. арк. 3,5.
Зам. № 17-33344.

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Свідоцтво про внесення суб'єкта видавничої справи до Державного реєстру видавництв, виготівників і розповсюджувачів видавничої продукції серії ДК № 3242 від 18.07.2008 р.