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Buyanova S. V. Konevalova N. Yu.

WORKING NOTE-BOOK FOR THE PRACTICAL CLASSES ON BIOCHEMISTRY

**FOR THE SECOND-YEAR STUDENTS
IN THE SPECIALITY «GENERAL MEDICINE»**

GROUP № __________

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Authors: S.V.Buyanova
N.Yu.Konevalova

Editor: professor N.Yu.Konevalova

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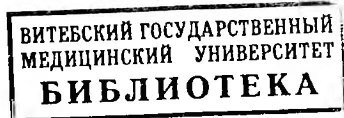

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VALIDITY OF NUMERICAL VALUES IN REPORTING LABORATORY RESULTS

The value reported from a clinical laboratory after determination of the concentration or amount of a substance in a specimen represents the best value obtainable with the method, reagents, instruments, and technical personnel involved in obtaining and processing the material.

Accuracy is the degree of agreement of the determination with the "true" value (eg, the known concentration in a control sample). **Precision** denotes the reproducibility of the analysis and is expressed in terms of variation among several determinations on the same sample. **Reliability** is a measure of the congruence of accuracy and precision.

Precision is not absolute but is subject to variation inherent in the complexity of the method, the stability of reagents, the accuracy of the primary standard, the sophistication of the equipment, and the skill of the technical personnel. Each laboratory should maintain data on precision (reproducibility) that can be expressed statistically in terms of the standard deviation from the mean value obtained by repeated analyses of the same sample. For example, the precision in determination of cholesterol in serum in a good laboratory may be the mean value ± 5 mg/dL. The 95% confidence limits are ± 2 SD, or ± 10 mg/dL. Thus, any value reported is "accurate" within a range of 20 mg/dL. Thus, the reported value 200 mg/dL means that the true value lies between 190 and 210 mg/dL. For the determination of serum potassium with a variance of 1 SD of ± 0.1 mmol/L, values ± 0.2 mmol could be obtained on the same specimen. A report of 5.5 could represent at best the range 5.3-5.7 mmol/L. That is, the 2 results - 5.3 and 5.7 mmol/L - might be obtained on analysis of the same sample and still be within the limits of precision of the test.

Physicians should obtain from the laboratory the values for the variation of a given determination as a basis for deciding whether one reported value represents a change from another on the same patient.

Interpretation of laboratory tests

Normal values are those that fall within 2 standard deviations from the mean value for the normal population. This normal range encompasses 95% of the population. Many factors may affect values and influence the normal range; by the same token, various factors may produce values that are normal under the prevailing conditions but outside the 95% limits determined under other circumstances. These factors include **age; race; sex; environment; posture; diurnal and other cyclic variations; fasting or postprandial state, foods eaten; drugs; and level of exercise.**

Normal or reference values vary with the method employed, the laboratory, and conditions of collection and preservation of specimens. The normal values established by individual laboratories should be clearly expressed to ensure proper interpretation.

Interpretation of laboratory results must always be related to the condition of the patient. A low value may be the result of deficit or of dilution of the substance measured, eg, low serum sodium. Deviation from normal may be associated with a specific disease or with some drug consumed by the subject - eg, elevated serum uric acid levels may occur in patients with gout or may be due to treatment with chlorothiazides or with antineoplastic agents. The reader should consult an appropriate text for lists of drugs interfering with chemical tests.

Values may be influenced by the method of collection of the specimen. Inaccurate collection of a 24-hour urine specimen, variations in concentration of the randomly collected urine specimen, hemolysis in a blood sample, addition of an inappropriate anticoagulant, and contaminated glassware or other apparatus are examples of causes of erroneous results.

Note: Whenever an unusual or abnormal result is obtained, all possible sources of error must be considered before responding with therapy based on the laboratory report. Laboratory medicine is a specialty, and experts in the field should be consulted whenever results are unusual or in doubt.

THE INSTRUCTION ON SAFE WORK OF STUDENTS IN EDUCATIONAL LABORATORIES IN THE CHAIR OF THE GENERAL AND CLINICAL BIOCHEMISTRY

1. The student should put on white coat, pick up long hair, if necessary, before the beginning of lesson.

2. Each student should be trained established laboratory work safety methods before conduct of laboratory work.

3. All manipulations with reactants during conduct of laboratory work are necessary to carry out on a workplace (a laboratory table or hood).

4. Work with the cracked glass laboratory ware is strictly prohibited.

5. It should be treated a tip of a pipette by little piece of cotton wool moistened with alcohol before mouth pipetting.

6. Precautions for a work with strong reactants:

6.1. Chemicals (concentrated acids, alkalies, etc.) stored in hoods should not take out on a laboratory table.

6.2. Mouth pipetting of strong reactants is strictly prohibited. For this purpose it is necessary to apply a syringe with a rubber tube.

6.3. The test tube should be stood in a support during strong reactants pipetting.

6.4. Prevent spilling of strong reactants splashes on the skin and in to the eyes.

First aid in case of spilling of acid and alkali splashes on the skin:

1) Wash the affected part with plenty of water; 2) apply cotton wool soaked in 2% sodium carbonate solution if acid spilling, in 2% acetic acid, if alkali spilling over the affected part; 3) consult a physician.

First aid in case spilling of acid and alkali splashes into the eye:

1) Wash the eyes immediately with plenty of tap water. Squirt water into the corner of the eye near the nose; 2) consult ophthalmologist immediately.

The indicated solutions and medicine chest are in laboratory room (room № 415).

7. Precautions for a work with a spirit-lamp:

7.1. It is necessary to observe rules of fire-prevention safety.

7.2. Spirit-lamp taking out a on a laboratory table is strictly prohibited.

7.3. Before spirit-lamp ignition it is necessary to check up, whether will shift a match from its case (for prevention of spirit steams ignition).

7.4. It is necessary to use the test-tube holder and carefully to move a test tube over a flame for its even heating.

Thus the test tube should be directed in a safe place (or wall), but not to itself or side by side with working student.

7.5. It is impossible to leave a burning spirit-lamp without supervision.

7.6. It is necessary to put on a cap on a wick of spirit-lamp at end of work.

8. Any questions regarding the safety precautions and labour safety should be addressed to teacher or laboratory assistant.

WITH SAFETY PRECAUTIONS REGULATIONS IS ACQUAINTED AND INSTRUCTED

Student____

The signature of the student _______________

Teacher ____

The signature of the teacher _______________

Date ___________________

THE MAIN BIOCHEMICAL VALUES IN BLOOD SERUM AND URINE

Values	Diagnostic significance
In serum	
K serum 3.5-5.5 mmol/l	Potassium concentration in plasma determines neuromuscular and muscular irritability. Elevated or decreased concentrations impair the capability of muscle tissue to contract. **1. Elevated in** renal insufficiency (especially in the presence of increased rate of protein or tissue break down); adrenal insufficiency (especially hypoaldosteronism, Addison's disease); hyporeninemic hypoaldosteronism; dehydration; use of spironolactone; too rapid administration of potassium salts, especially intravenously; and use of triamterene or phenformin. **2. Decreased in** inadequate intake (starvation); inadequate absorption or unusual enteric losses – Vomiting, diarrhea, malabsorption syndrome, or use of sodium polystyrene sulfonate resin; unusual renal loss – secondary to hyperadrenocorticism (especially hyperaldosteronism, Conn's syndrome) and to adrenocorticosteroid therapy, metabolic alkalosis, use of diuretics such as chlorothiazide and its derivatives and the mercurials; renal tubular defects such as the de Toni-Fanconi syndrome and renal tubular acidosis; treatment with antibiotics that are excreted as anions (carbenicillin, ticarcillin); use of phenothiazines, amphotericin B, and drugs with high sodium content; and use of degraded tetracycline; abnormal redistribution between extracellular and intracellular fluids – Familial periodic paralysis or testosterone administration.
Na serum 130-150 mmol/l	Most of the exchangeable sodium is in the extracellular fluid. With its associated anions sodium provides the bulk of osmotically active solute in the plasma, thus affecting the distribution of body water significantly. A shift of sodium into cells or a loss of sodium from the body results in a decrease of extracellular fluid volume, affecting circulation, renal function, and nervous system function. **1. Elevated in** dehydration (water deficit), central nervous system trauma or disease, and hyperadrenocorticism with hyperaldosteronism or

	corticosterone of corticosteroid excess. **2. Decreased in** adrenal insufficiency; in renal in sufficiency, especially with inadequate sodium intake; in renal tubular acidosis; as a physiologic response to trauma or burns (sodium shift into cells); in unusual losses via the gastrointestinal tract, as with acute or chronic diarrhea or with intestinal obstruction or fistula; and in unusual sweating with inadequate sodium replacement.
Ca serum **2-3 mmol/l**	Endocrine, renal, gastrointestinal, and nutritional factors normally provide for precise regulation of calcium concentration in plasma and other body fluids. Since some calcium is bound to plasma protein, especially albumin, determination of the plasma albumin concentration is necessary before the clinical significance of abnormal serum calcium levels can be interpreted accurately. **1. Elevated** in hyperparathyroidism, secretion of parathyroidlike hormone by malignant tumors, vitamin D excess, milk-alkali syndrome, osteolytic disease such as multiple myeloma, invasion of bone by metastatic cancer, Paget's disease of bone, Boeck's sarcoid, immobilization, and familial hypocalciuria. Occasionally elevated with hyperthyroidism and with ingestion of thiazide drugs. **2. Decreased** in hypoparathryoidism, vitamin D deficiency (rickets, osteomalacia), renal insufficiency, hypoproteinemia, malabsorption syndrome (sprue, ileitis, celiac disease, pancreatic insufficiency), severe pancreatitis with pancreatic necrosis, and pseudohypoparathyroidism.
Alanin Aminotransferase (ALT) **<40 U/L,** **0.1-0.68 mmol/ (h*l)**	ALT is a cytoplasmic enzyme found in high concentrations in liver, heart muscle. Elevations of concentrations of this enzyme in the blood indicate liver disease. **Elevated** after acute infectious hepatitis of viral or toxic origin (ALT usually elevated more than AST); cirrhosis of the liver (AST usually elevated more than ALT); metastatic or primary liver neoplasm and jaundice.
Aspartate Aminotransferase (AST) **<40 U/L,**	AST is found in heart muscle, liver, some also in skeletal muscle, kidneys and the pancreas. AST is found in both cytoplasm and mitochondria. It may be noted that AST is more specific for the diagnosis of heart

0.1-0.45 mmol/ (h*l)	diseases. **1. Elevated** after myocardial infarction This enzyme shows an elevation 8-12 hours after infarction. Peak levels are reached 24-48 hours after the MI; cirrhosis of the liver (AST usually elevated more than ALT). AST is elevated also in muscular dystrophy, dermatomyositis, and paroxysmal myoglobinuria, pancreatitis. **2. Decreased** with pyridoxine (vitamin B_6) deficiency (often as a result of repeated hemodialysis), renal insufficiency, pregnancy, diabetic ketoacidosis, and beriberi.
Amylase (α-amylase) **16-30 g/l*h**	Amylase helps to digest starch and glycogen in the mouth, stomach, and intestine. Normally, small amounts of amylase, originating in the pancreas and salivary glands, are present in the blood. Inflammatory disease of these glands or obstruction of their ducts results in regurgitation of large amounts of enzyme into the blood and increased excretion via the kidney. **1. Elevated in** acute and chronic pancreatitis, pseudocyst of the pancreas, obstruction of pancreatic ducts (carcinoma, stone, stricture, duct sphincter spasm after morphine), and acute parotitis (mumps). Occasionally elevated in renal insufficiency, in diabetic acidosis, and in inflammation of the pancreas from a perforating peptic ulcer. **2. Decreased** in acute and chronic hepatitis, in pancreatic insufficiency, and occasionally, in toxemia of pregnancy.
Albumin **35-52 g/l**	Albumin is the major plasma protein and is synthesized by the liver. It accounts for about 50 % of the total hepatic protein production. Albumin has a biological half-life in plasma of about 20 days. Albumin makes the biggest contribution to the plasma oncotic pressure. If the albumin concentration falls very low oedema is the result. **1. Elevated in** dehydration, shock, hemoconcentration, and administration of large quantities of concentrated albumin "solution" intravenously. **2. Decreased in** acute or chronic glomerulonephritis, nephrosis, acute or chronic hepatic insufficiency, neoplastic disease, leukemia, nephrotic syndrome, alcoholic cirrhosis, inflammatory bowel disease, metastatic cancer, Hodgkin's disease.

	There are three main reasons for the occurrence of a low plasma albumin concentration: Decreased .synthesis. This may be due to malnutrition or malabsorption. Decreased synthesis is also a feature of advanced chronic liver disease. Abnormal distribution or dilution. Hypoalbuminaemia can be induced hy overhydration or if there is increased capillary permeability as occurs in septicaemia. Abnormal excretion or degradation. The causes include the nephrotic syndrome, protein-losing enteropathies, burns, haemorrhage and calabolic states.
Total protein **65-85 g/l**	The concentration of protein in plasma is influenced by the nutritional state, hepatic function, renal function, occurrence of disease such as multiple myeloma, and metabolic errors. **1. Increased levels of total protein** observed in cases of dehydration. An increase in total protein concentration in a serum specimen is usually due to an increase in the globulin fraction and may indicate the presence of a paraprotein. **2. Decreased levels of total protein** are usually due to hypoalbuminemia. Measurement of a number of specific proteins gives useful information in the diagnosis and management of disease. Characteristic changes in the concentration of certain plasma proteins are seen following surgery or trauma, or during infection or tumour growth. The proteins involved are called acute phase reactants. These acute phase proteins may be used to monitor progress of the condition or its treatment.
Fraction of plasma proteins:	
Albumins **- 53-66 %**	(see albumin above)
Globulins: **20-30 g/L**	Globulins constitute several proteins that are separated into 4 distinct bands (α1, α2, β and γ) on electrophoresis. **1. Elevated in** hepatic disease, infectious hepatitis, cirrhosis of the liver, biliary cirrhosis, and hemochromatosis; disseminated lupus erythematosus; plasma cell myeloma; lymphoproliferative disease; sarcoidosis; and acute or chronic infectious diseases, particular-

	ly lymphogranuloma venereum, typhus, leishmaniasis, schistosomiasis, and malaria. 2. **Decreased in** malnutrition, congenital agammaglobulinemia, acquired hypogammaglobulinemia, and lymphatic leukemia.
α_1 - 2-5.5 %	***a1-Antitrypsin*** is a protease inhibitor with a normal concentration of about 200mg/dl. It inhibits trypsin, elastase, and certain other proteases by forming complexes with them. It is synthesized in the liver. A deficiency of this protein has a role in certain cases (approximately 5%) of emphysema and in one type of cirrhosis.
α_2 - 6-12 %	***Haptoglobin (Hp)*** plasma concentration is increased in several inflammatory conditions. The levels of Hp in human plasma vary and are of some diagnostic use. Low levels of Hp are found in patients with hemolytic anemias. Hp binds with the free hemoglobin that spills into the plasma due to hemolysis. The haptoglobin-hemoglobin complex cannot pass through glomeruli of kidney. Hp, therefore, prevents the loss of free Hb into urine. ***Ceruloplasmin***, an oxidase enzyme with a blue color, binds to about 95% of serum copper. In Wilson's disease, serum copper and Ceruloplasmin are low and urinary copper is high. Elevated in pregnancy, hyperthyroidism, infection, aplastic anemia, acute leukemia. Hodgkin's disease, cirrhosis of the liver, and with use of oral contraceptives. Decreased in Wilson's disease (accompanied by increased urinary excretion of copper), malabsorption, nephrosis, and copper deficiency that may accompany total parenteral nutrition. ***Macroglobulin*** inhibits protease activity and serves as an anticoagulant. Its concentration in plasma is elevated in nephrotic syndrome.
β - 8-15 %	Iron is transported as a complex of the metal-binding globulin transferrin (siderophilin). Normally, this transport protein carries an amount of iron that represents about 30-40% of its capacity to combine with iron. Interpretation of Saturation of Transferrin: 1. **Elevated** in iron excess (iron poisoning, hemolytic disease, thalassemia, hemocnromatosis, pyridoxine de-

	ficiency, nephrosis, and, occasionally, hepatitis). **2. Decreased** in iron deficiency, chronic infection, cancer, and late pregnancy.
γ - 11-22 %	There are five classes of immunoglobulins - namely IgG, IgA, IgM, IgD and IgE - containing the heavy and light chains. **1. Elevated** in liver diseases, persistent infections, systemic lupus erythematosus, myeloma, and lymphoma. **2. Decreased** in chronic lymphatic leukemia, acquired immunodeficiency syndrome.
Total bilirubin 3.5-20.5 μmol/l	Destruction of hemoglobin yields bilirubin, which is conjugated in the liver to the diglucuronide and excreted in the bile. Bilirubin accumulates in the plasma when liver insufficiency exists, biliary obstruction is present, or the rate of hemolysis increases. Rarely, abnormalities of enzyme systems involved in bilirubin metabolism in the liver (eg, absence of glucuronyl transferase) result in abnormal bilirubin concentrations. Total bilirubin can be significantly elevated in normal and jaundiced subjects by fasting 24-48 hours (in some instances even 12 hours) or by prolonged caloric restriction.
Bilirubin conjugated (direct bilirubin) 2.2-5.1 μmol/l	Direct forms of serum bilirubin are elevated in acute or chronic hepatitis; biliary tract obstruction (cholangiolar, hepatic, or common ducts); toxic reactions to many drugs, chemicals, and toxins; and Dubin- Johnson and Rotor's syndromes. Direct can be significantly elevated in normal and jaundiced subjects or by prolonged caloric restriction.
Bilirubin unconjugated (indirect bilirubin) 1.7-17.1 μmol/l	Indirect serum bilirubin is elevated in hemolytic diseases or reactions and absence or deficiency of glucuronyl transferase, as in Gilbert's disease and Crigler-Najjar syndrome, in acute or chronic hepatitis; biliary tract obstruction (cholangiolar, hepatic, or common ducts); toxic reactions to many drugs, chemicals, and toxins; and Dubin- Johnson and Rotor's syndromes.
Glucose 3.65-6.11 mmol/l	The glucose concentration in extracellular fluid is normally closely regulated, with the result that a source of energy is available to tissues, and no glucose is excreted in the urine. Hyperglycemia and hypoglycemia are nonspecific signs of abnormal glucose me-

	tabolism. **1. Elevated in** diabetes mellitus, hyperthyroidism, adrenocortical hyperactivity (cortical excess - Cushing's syndrome), hyperpituitarism, feochromocytoma, pancreas diseases and hepatic disease (occasionally), thiazide diuretics. **2. Decreased in** hyperinsulinism, adrenal insufficiency, hypopituitarism, hepatic insufficiency (occasionally), functional hypoglycemia, and by hypoglycemic agents, malnutrition, sepsis, endocrine tumors.
Creatinine **female - 44-97 μmol/l** **male - 44-115 μmol/l**	Creatinine is the anhydride of creatine as a product from metabolism in the muscle tissues. Endogenous creatinine is excreted by filtration through the glomerulus and by tubular secretion. Creatinine clearance is an acceptable measure of glomerular filtration rate. Creatinine **is elevated** in acute or chronic renal insufficiency, urinary tract obstruction, and impairment of renal function induced by some drugs. Materials other than creatinine may react to give falsely high results with the alkaline picrate method (Jaffe reaction): acetoacetate, acetone, β-hydroxybutyrate, α-ketoglutarate, pyruvate, glucose, bilirubin, hemoglobin, urea, and uric acid. Values below 0.7 mg/dL are of no known significance.
Creatine **Phosphokinase (CPK)** **female - 25-175 U/l** **male - 25-200 U/l**	CPK splits creatine phosphate in the presence of ADP to yield creatine and ATP. Skeletal and heart muscle and brain are rich in the enzyme. **1. Elevated** in the presence of muscle damage such as with myocardial infarction, trauma to muscle, muscular dystrophies, polymyositis, severe muscular exertion (jogging), hypothyroidism, and cerebral infarction (necrosis). Following myocardial infarction, serum CPK concentration increases rapidly (within 3-5 hours), and it remains elevated for a shorter time after the episode (2 or 3 days) than does AST or LDH. **2. Not elevated** in pulmonary infarction or parenchymal liver disease.
Creatine Phosphokinase Isoenzymes	CPK consists of 3 proteins separable by electrophoresis. Skeletal muscle is characterized by isoenzyme MM, myocardium by isoenzyme MB, and brain by isoenzyme BB. CPK-MM is elevated in injury to skeletal muscle, myocardial muscle, and brain; in muscle disease (eg, dystrophies, hypothyroidism, dermato-

	myositis, polymyositis); in rhabdomyolysis; and after severe exercise. CPK-MB is elevated soon (within 2-4 hours) after myocardial infarction and for up to 72 hours afterward (high levels are prolonged with extension of infarct or new infarction); also elevated in extensive rhabdomyolysis or muscle injury, severe muscle disease, Reye's syndrome, or Rocky Mountain spotted fever. CPK-BB is occasionally elevated in severe shock, in some carcinomas (especially oat cell carcinoma or carcinoma of the ovary, breast, or prostate), or in biliary atresia.
γ-Glutamyl Transpeptidase female – 7-32 U/l male – 11-50 U/l	The microsomal enzyme is present in liver, kidney, and pancreas and transfers C-terminal glutamic acid from a peptide to other peptides or L-amino acids. It is induced by alcohol. **Elevated in** acute infectious or toxic hepatitis, chronic and subacute hepatitis, cirrhosis of the liver, intrahepatic or extrahepatic obstruction, primary or metastatic liver neoplasms, and liver damage due to alcoholism. It is elevated occasionally in congestive heart failure and rarely in postmyocardial infarction, pancreatitis, and pancreatic carcinoma.
Lactate dehydrogenase (LGH) 225-450 U/l	LDH catalyzes the interconversion of lactate and pyruvate in the presence of NADH or $NADH_2$. It is distributed generally in body cells and fluids and used for the diagnosis of myocardial infarction, infective hepatitis, leukemia and muscular dystrophy. LDH has five isoenzymes. **Elevated in** all conditions accompanied by tissue necrosis, particularly those involving acute injury of the heart, red cells, kidney, skeletal muscle, liver, lung, and skin. Marked elevations accompany hemolytic anemias, the anemias of vitamin B_{12} and folate deficiency, and polycythemia rubra vera. The course of rise in concentration over 3-4 days followed by a slow decline during the following 5-7 days may be helpful in confirming the presence of a myocardial infarction (increased levels of enzyme takes place after 8-12 hours after myocardial infarction); however, pulmonary infarction, neoplastic disease, and megaloblastic anemia must be excluded. Although elevated during the acute phase of infectious hepatitis, enzyme activity is seldom increased in chronic liver disease. In myocardial infarction, the α isoenzymes are elevated - particularly LDH 1 to yield a ratio of LDH 1: LDH 2

	of greater than 1. Similar α isoenzyme elevations occur in renal cortex infarction and with hemolytic anemias. LDH 5 and 4 are relatively increased in the presence of acute hepatitis, acute muscle injury, dermatomyositis, and muscular dystrophies.
Urea **2.5-8.32 mmol/l**	Urea, an end product of protein metabolism, is excreted by the kidney. The urea concentration in the glomerular filtrate is the same as in the plasma. Tubular reabsorption of urea varies inversely with rate of urine flow. Thus, urea is a less useful measure of glomerular filtration than is creatinine, which is not reabsorbed. Blood urea nitrogen varies directly with protein intake and inversely with the rate of excretion of urea. **1. Elevated in** 1)Extra-renal conditions are due to increased nitrogen metabolism associated with diminished renal blood flow or impaired renal function - dehydration (from any cause) and upper gastrointestinal bleeding (combination of increased protein absorption from digestion of blood plus decreased renal blood flow), diabetic coma, thyrotoxicosis; 2) Prerenal conditions are due to decreased renal blood flow - shock, adrenal insufficiency, diabetes mellitus, dehydration, cardiac failure, etc.; 3) Renal conditions are due to renal insufficiency - nephritis, acute and chronic; acute renal failure (tubular necrosis); 4) Post-renal conditions are due to enlargement of prostate, urinary tract obstruction, due to tumors, stones, etc. **2. Decreased in** hepatic failure, nephrosis not complicated by renal insufficiency, cachexia, pregnancy, malnutrition and starvation.
Uric acid **female –** **0.12-0.34 mmol/l** **male –** **0.2-0.415 mmol/l**	Uric acid, an end product of purine metabolism, is excreted by the kidney. Gout, a genetically transmitted metabolic error, is characterized by an increased plasma or serum uric acid concentration, an increase in total body uric acid, and deposition of uric acid in tissues. An increase in uric acid concentration in plasma and serum may accompany increased purine catabolism (blood dyscrasias, therapy with antileukemic drugs), use of thiazide diuretics, or decreased renal excretion. **1. Elevated in** gout, preeclampsia-eclampsia, leuke-

ВИТЕБСКИЙ ГОСУДАРСТВЕННЫЙ
МЕДИЦИНСКИЙ УНИВЕРСИТЕТ

	mia, polycythemia, therapy with antileukemic drugs and a variety of other agents, renal insufficiency, glycogen storage disease (type I), Lesch-Nyhan syndrome (X-linked hypoxanthine-guanine phosphoribosyltransferase deficit), and Down's syndrome. The incidence of hyperuricemia is greater in Filipinos than in whites. **2. Decreased in** acute hepatitis (occasionally), treatment with allopurinol, and treatment with probenecid.
Triacylglycerol **0.5-1.8 mmol/l**	Dietary fat is hydrolyzed in the small intestine, absorbed and resynthesized by the mucosal cells, and secreted into lacteals in the form of chylomicrons. Triglycerides in the chylomicrons are cleared from the blood by tissue lipoprotein lipase (mainly adipose tissue), and the split products are absorbed and stored. Free fatty acids derived mainly from adipose tissue are precursors of the endogenous triglycerides produced by the liver. Transport of endogenous triglycerides is in association with β-lipoproteins, the very low density lipoproteins. In order to ensure measurement of endogenous triglycerides, blood must be drawn in the post-absorptive state. **1. Elevated** (hyperlipoproteinemia) 1) Primary-Type I hyperlipoproteinemia (exogenous hyperlipidemia), type II hyperbetalipoproteinemia, type III broad beta hyperlipoproteinemia, type IV hyperlipoproteinemia (endogenous hyperlipidemia), and type V hyperlipoproteinemia (mixed hyperlipidemia); 2) Secondary-Hypothyroidism, diabetes mellitus, nephrotic syndrome, chronic alcoholism with fatty liver, ingestion of contraceptive steroids, biliary obstruction, and stress. **2. Decreased** (hypolipoproteinemia) 1) Primary-Tangier disease (α-lipoprotein deficiency), abetalipoproteinemia, and a few rare, poorly defined syndromes; 2) Secondary-Malnutrition, malabsorption, and, occasionally, with parenchymal liver disease.
Alkaline phosphatase **<117 U/l**	Alkaline phosphatase is present in high concentration in growing bone, in bile, and in the placenta and is mainly derived from bone and liver (the cells lining the bile canaliculi). In serum, it consists of a mixture of isoenzymes. The isoenzymes may be separated by

	electrophoresis; liver alkaline phosphatase migrates faster than bone and placental alkaline phosphatase, which migrate together. Alkaline phosphatase is useful for the diagnosis of rickets, hyperparathyroidism, carcinoma of bone, and obstructive jaundice. **1. Elevated in** 1) Children (normal growth of bone); 2) Osteoblastic bone disease - Hyperparathyroidism, rickets and osteomalacia, neoplastic bone disease (osteosarcoma, metastatic neoplasms), ossification as in myositis ossificans, Paget's disease (osteitis deformans), and Boeck's sarcoid; 3) Hepatic duct or cholangiolar obstruction due to stone, stricture, and neoplasm; 4) Hepatic disease resulting from drugs such as chlorpromazine and methyltestosterone; 5) Pregnancy. **2. Decreased in** hypothyroidism and in growth retardation in children.
Total cholesterol **3.65-5.2 mmol/l**	Cholesterol concentrations are determined by metabolic functions, which are influenced by heredity, nutrition, endocrine function, and integrity of vital organs such as the liver and kidney. Cholesterol metabolism is intimately associated with lipid metabolism. **1. Elevated** in familial hypercholesterolemia (xanthomatosis), hypothyroidism, poorly controlled diabetes mellitus, nephrotic syndrome, chronic hepatitis, biliary cirrhosis, obstructive jaundice, hypoproteinemia (idiopathic, with nephrosis or chronic hepatitis), and lipidemia (idiopathic, familial). **2. Decreased** in acute hepatitis and Gaucher's disease. Occasionally decreased in hyperthyroidism, acute infections, anemia, malnutrition, apolipoprotein deficiency, carcinoma, and acute pancreatitis.
Hemoglobin **female - 120-150 g/l** **male - 130-170 g/l**	Hemoglobin is the pigment part of the erythrocyte, and the oxygen-carrying part of the blood. A low hemoglobin level indicates anemias: posthemorrhagic anemia, iron-deficient anemia, hemodilution.
In urine	
Protein, g/l —	Normally only trace amounts of proteins (0,03 g/24 h) are present in the urine that are not readily detected. When chemically detectable quantities of proteins are present in urine, the condition is called as **proteinuria**. Albumin is the major protein present in proteinuria. Causes of proteinuria: a) Physiological proteinuria (0.5% of protein present) - severe excercise, standing

	for a long time (due to temporary impairment of renal circulation), pregnancy. b) Pathological proteinuria: 1) Prerenal conditions - dehydration, heart diseases causing an impaired renal circulation, multiple myeloma, etc; 2) Renal conditions - when the lesion is in the kidney as in glomerularnephritis; 3) Post renal conditions - lesions of pelvis, bladder, prostate and urethra, inflammation of the lower urinary tract, etc.
Glucose, mmol/daily —	Small amount of glucose (2-20 mg or 0,3-1,1 mmol) may be present in fasting urine which cannot be detectable by chemical methods. When glucose is present, the condition is called **glucosuria**. Glycosuria occurs in hyperglycaemia with values above renal threshold for glucose (7,99-9,99 mmol/l or 160-180 mg/dl). Glycosuria is the good first line screening test-line for diabetes mellitus. **Renal glycosuria** which is caused by a disorder of the renal tubular function. It is not accompanied by the classical symptoms of diabetes. **Alimentary glycosuria:** In certain individuals, blood glucose level raises rapidly after meals resulting in its spill over into urine. It is observed in some normal people after anesthesia, or emotional stress, and in patients of hepatic diseases, hormonal disorders, medications, pregnancy, hyperthyroidism and peptic ulcer.
Bilirubin —	It is not present normally in significant amounts since it is mainly excreted through bile, unusual amounts of bilirubin is detected in urine in cases of obstructive jaundice.
Urobilinogen 0.08-4.23 µmol/daily	Unusual amounts of uribilinogen seen in cases of hemolytic jaundice.
Amylase (α-amylase) 28-160 g/l*h (U/L)	In cases of suspected acute pancreatic disease, measurement of urine amylase is the most important laboratory test. After the onset of acute pancreatitis, amylase levels begin to rise within 2 hours, peak within 12 to 48 hours, and return to normal within 3 to 4 days. Determination of urine levels should follow normal serum amylase results to rule out pancreatitis.
Vanillylmandelic acid 0.7-3.8 mg/daily, 2.5-38 µmol/daily	Vanillylmandelic acid is the major urinary excretory product of both epinephrine and norepinephrine catabolism. Moderately elevated vanillylmandelic acid can be caused by a variety of factors such as essential hypertension, intense anxiety, intense physical exer-

	cise, and numerous drug interactions (including some over-the-counter medications and herbal products), pheochromocytoma, neuroblastoma, ganglioma.

Date___________________

LESSON 1

THEME: INTRODUCTION TO BIOCHEMISTRY. STRUCTURES OF AMINO ACIDS AND PEPTIDES. STRUCTURE, PHYSICO-CHEMICAL PROPERTIES AND FUNCTIONS OF PROTEINS

The purpose of the lesson: studying of role and problems of biological chemistry; familiarizing with features of work in biochemical laboratory, with safety rules, application of the international system of units (SI) in biochemical laboratory practice; studying of protein structure and properties: levels and kinds of the macromolecular organization of proteins molecules and dependence on their biological properties; familiarizing with qualitative reactions on proteins and amino acids, with reactions of protein sedimentation.

THEORETICAL QUESTIONS:

1. Object and aims of biological chemistry. Most important attributes of living matter. Manifestations of dialectic laws in organization and functions of living matter.
2. Major divisions and trends in biochemistry. Place of biochemistry among other biological subjects. Biochemistry and medicine.
3. Most important stages in the history of biochemistry. Leading native and foreign biochemists, their contribution to the development of biochemistry. Biochemistry and development of molecular biology, physical-and-chemical biology and biotechnology.
4. Proteins as the most important components of the body. Classification and functions of the proteins.
5. Structures of amino acids. Classification of amino acids. Role of amino acids in organism.
6. Physico-chemical properties of proteins. Isoelectric point (pI). Molecular mass, shape and charge of molecules. The factors determining the solubility of proteins; sedimentation reversible and irreversible. Denaturation and renaturation of proteins.
7. Protein structure:
   7.1. Primary structure (properties of peptide bonds), secondary structure, tertiary structure, quaternary structure.
   7.2. Native structure of proteins. Protein structure and functional significance. Chaperones, chaperonins.
   7.3. The relationship between protein structure and function. Domain structure and polymorphism of proteins. Functioning of oligomeric

proteins (cooperative interaction between protomers). Hemoglobin and myoglobin - structure and function. Sickle-cell anemia.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 6, 7, p. 113.
2. Task from «Clinical Biochemistry» №№ 2, 15, 18, 22, 26, 34, p. 103, 105-109.

LABORATORY WORK:

1) Colour reactions of proteins and amino acids.

Colour reactions are applied to find out the presence of the protein in biological fluids, solutions and to establish the amino-acid structure of various natural proteins. These reactions are used both for qualitative, and quantitative determination of protein and amino acids containing in it.

Reagents: 1. Sodium hydroxide solution, 100 g/L. 2. Copper sulphate solution, 10 g/L. 3. Ninhydrin solution, 1 g/L. 4. Nitric acid concentrated. 5. Millon's reagent. 6. Phenol solution, 1 g/L. 7. Solution of egg white for colour reactions.

1. Biuret test on peptide bond.

Proteins in solution answer the biuret reaction, i.e. they develop a pink or a purple colour with a solution of dilute copper sulphate and excess of NaOH. The colour is due to the presence of at least two peptide bonds. Insoluble proteins like keratin and dipeptides do not answer this reaction.

Practical procedure:

Place 5 drops of protein solution in the test-tube. Add 5 drops of NaOH solution and add 2 drops of copper (II)-sulphate ($CuSO_4$) solution alongside the test-tube. Red-violet colour is produced.

Write down the results and make a conclusion: ________________

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2. Ninhydrin reaction.

A delicate colour reaction for amino acids is the ninhydrin reaction. Most amino acids give a blue or a purple colour on warming with a solution of ninhydrin in acetone. Ninhydrin oxidizes the amino acids releasing ammonia. The reduced ninhydrin reacts with the liberated ammonia and another molecule of ninhydrin to form the coloured complex. The keto acids formed from α amino acids are immediately and quantitatively decomposed to their

corresponding aldehydes liberating CO_2 which is not the case with β and other amino acids. (Ninhydrin is triketohydrindene hydrate).

Practical procedure:

Place 5 drops of protein solution in the test-tube. Add 5 drops of ninhydrin solution and boil 1-2 minutes. Pink-violet colour or blue-violet colour is produced.

Write down the results and make a conclusion: ________________

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3. Xanthoproteic reaction.

When a native protein is treated with concentrated HNO_3, a white precipitate (meta protein) is formed and this turns yellow on heating. The yellow colour is due to the nitration of the benzene rings of aromatic amino acids (tryptophan, tyrosine and phenylalanine) of the protein (xantho-yellow).

Practical procedure:

Place 5 drops of protein solution in the test-tube. Add 3 drops of concentrated nitric acid. Warm the test-tube carefully, shaking it all the time. Solution and precipitate take in yellow colour. Cool the test-tube. Carefully add 1-3 drops of NaOH solution. Brightly-orange colour is produced.

Write down the results and make a conclusion: ________________

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4. Millon`s test.

This test is given by the amino acid tyrosine, or any other compound containing hydroxyphenyl ring. When a solution of tyrosine is heated with Millon`s reagent (a solution of mercurous and mercuric nitrates containing nitric acid) or 10% mercuric sulphate in 10% H_2SO_4 and 1% sodium nitrite, a red colour is produced.

Practical procedure:

Place 5 drops of protein solution in the test-tube. Add 3 drops of Millon`s reagent. When white protein precipitate is produced, test-tube need boil. The protein precipitate is painted in brick-red colour. To 10 drops of phenol solution add 5 drops and carefully to heat up. Pink colour is produced.

Write down the results and make a conclusion: ________________

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2) Reactions of protein sedimentation.

The solubility of proteins is strongly dependent on the salt concentration (ionic strength) of the medium. Proteins are usually poorly soluble in pure water. Their solubility increases as the ionic strength increases, because more and more of the well-hydrated inorganic ions (blue circles) are bound to the protein's surface, preventing aggregation of the molecules (salting in). At very high ionic strengths, the salt withdraws the hydrate water from the proteins and thus leads to aggregation and precipitation of the molecules (salting out). For this reason, adding salts such as ammonium sulfate $(NH_4)_2SO_4$ makes it possible to separate proteins from a mixture according to their degree of solubility (fractionation).

Reagents: 1. Sodium chloride solution. 2. Ammonium sulfate solution. 3. Salicylsulphonic acid 200 g/L. 4. Trichloroacetic acid 100 g/L. 5. Ethanol. 6. Solution of egg white for sedimentation.

1. Fractional sedimentation of proteins from a sample of blood plasma.

Principle of salting-out: This a reverse sedimentation of proteins, by adding divalent salts, such as sodium chloride and ammonium sulfate $(NH_4)_2 SO_4$ to a sample of blood plasma. This method is used to separate and purify proteins and medicines containing globulins for treatment.

Practical procedure:

Step 1. Add 15 drops of proteins sample into 15 drops of saturated ammonium sulfate (test-tube 1). 50% saturation is reached - globulins are as a precipitate.

Step 2. Filter the sample in 5 min.

Step 3. Wash the filter paper with distilled water (4-5ml) and put it in a separate clean test-tube 2.

Step 4. Add ammonium sulfate powder to the filtrate while it reaches full saturation. The precipitant is formed by albumens.

Step 5. Filter the sample in 10 min.

Step 6. Wash the filter paper with distilled water (4-5ml) and put it in a separate clean test-tube 3.

Preserve the solution without proteins for further uses test-tube 4.

Step 7. Then take test-tubes 2, 3, 4 with its contents and carry out the biuret test. The positive reaction must be in the test-tubes 2, 3 and the negative reaction must be in test tube 4.

Write down the results and make a conclusion: ________________

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2. Precipitation of proteins by organic acids.

Practical procedure:

Add 5 drops of proteins solution into each of 2 test-tubes. Add 2 drops of solution of trichloroacetic acid into the first and add 2 drops of solution of salicylsulphonic acid into the second of the test-tubes. These reactions are used in practical for detecting and separating proteins from solutions. The white precipitate indicates the presence of protein.

Write down the results and make a conclusion: ______________

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3. Precipitation of proteins by alcohol.

Dehydrating agents such as alcohols precipitate the proteins.

Practical procedure:

Add 15-20 drops of alcohol to 5 drops of protein solution into a test-tube. A white opalescent appears. You will notice the precipitation of proteins. If distilled water is added into the test tube, opalescent disappears.

Write down the results and make a conclusion: ______________

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MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 4-9.

Literature for essay:

1. D.B. Marks. Biochemistry, 1994, p. 23-32.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 14-50.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 29-81.

Lesson is passed______________________

(The signature of the teacher)

Date___________________

LESSON 2

THEME: STRUCTURE, PHYSICO-CHEMICAL PROPERTIES AND FUNCTIONS OF PROTEINS. METHODS OF BIOCHEMICAL RESEARCH

The purpose of the lesson: studying of physico-chemical and biological properties of proteins; studying methods of purification of individual proteins.

Theoretical questions:

1. Ability of proteins to specific interactions.
   1.1. Self-organization of polymolecular protein structures.
   1.2. The protein-ligand interaction.
   1.3. Quantitative determination of proteins.
2. Methods of protein purification.
   2.1. Ammonium sulfate precipitation.
   2.2. Dialysis.
   2.3. Chromatography of proteins (gel filtration chromatography, ion exchange chromatography, affinity chromatography).
   2.4. Electrophoresis (native PAGE, SDS-PAGE, isoelectric focusing).

QUESTIONS AND EXERCISES:

1. Task from «Clinical Biochemistry» №№ 103, p. 103.

CONTROL TEST «STRUCTURE AND FUNCTIONS OF PROTEINS»

Main theoretical questions:

1. Structures of 20 amino acids.
2. Isoelectric point (pI).
3. Primary structure of proteins.
4. Secondary structure of proteins.
5. Tertiary structure of proteins.
6. Quaternary structure of proteins.
7. Ammonium sulfate precipitation.
8. Dialysis.
9. Chromatography of proteins (gel filtration chromatography, ion exchange chromatography, affinity chromatography).
10. Electrophoresis (Native PAGE, SDS-PAGE, isoelectric focusing).

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 10-17.

Literature for essay:

1. D.B. Marks. Biochemistry., 1994, p. 27-32.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 14-50.
3. B. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 29-81.

Lesson is passed____________________

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Date__________________

LESSON 3

THEME: METHODS OF PROTEIN METABOLISM STUDYING

The purpose of the lesson: studying of clinical significance of protein metabolism indexes determination in blood; quantitative methods mastering of the blood total protein and albumin determination; learning of analysis and clinical interpretation of protein metabolism indexes during pathology.

THEORETICAL QUESTIONS:

1. Sources and ways of protein expenditure in organism: intake, synthesis, consumption, excretion. Participation of various organs in protein metabolism: role of gastrointestinal tract, liver, kidneys. Blood proteins defined in laboratory practice: the total proteins, albumins, globulins, enzymes.
2. Methods of indicators determination of a protein metabolism in various biological liquids. Qualitative reactions and the physical and chemical properties of proteins underlying quantitative determination of protein metabolism indicators.
3. Clinical significance of total protein determination in blood serum. The normal content of the total protein in blood serum in adults and children. Absolute and relative hypo-and hyperproteinemia: the reasons, effects.
4. Clinical significance of albumin determination in blood serum. The normal content of the albumin in blood serum in adults and children. Absolute and relative hypo-and hyperalbuminemia: the reasons, effects.
5. Clinical significance of plasma proteins electrophoretical researches. Blood proteins defined electrophoretically, proteinogram, the normal content of blood proteins various fractions.
6. Clinical significance of qualitative detection of protein in urine by Geller`s test.
7. Interpretation of laboratory researches.

LABORATORY WORK:

1. Quantitative determination of total protein by the biuret method in the serum.

Principle: Peptide bonds (-CO-NH) of proteins react with cupric ions in alkaline medium to form a purple colour complex. The intensity of the color is proportional to the total protein concentration.

Diagnostic significance:

A. Physiologic Basis: Concentration of protein determines colloidal osmotic pressure of plasma. The concentration of protein in plasma is influenced by the nutritional state, hepatic function, renal function, occurrence of disease such as multiple myeloma, and metabolic errors. Variations in the fractions of plasma proteins may signify specific disease.

B. Interpretation:

Increased levels of total protein (hyperproteinemia) observed in cases of dehydration. An increase in total protein concentration in a serum specimen is usually due to an increase in the globulin fraction and may indicate the presence of a paraprotein.

Decreased levels of total protein (hypoproteinemia) are usually due to hypoalbuminemia. Measurement of a number of specific proteins gives useful information in the diagnosis and management of disease. Characteristic changes in the concentration of certain plasma proteins are seen following surgery or trauma, or during infection or tumour growth. The proteins involved are called acute phase reactants. These acute phase proteins may be used to monitor progress of the condition or its treatment.

Total protein, Serum: Normal – SI: 65-85 g/L, for baby - 56-85 g/L.

Reagents: 1. Biuret reagent (10% NaOH + 1% $CuSO_4$ 10:1); 2. Protein standard solution; 3. Blood serum.

Practical procedure:

Take 3 test tubes and label as test (T), standard (S) and blank (B) as shown in the table below:

Pipette	Test-tube 1 Test	Test-tube 2 Standard	Test-tube 3 Blank
Blood serum	0.02 ml	-	-
Protein standard solution	-	0.02 ml	-
Biuret reagent	1.0 ml	1.0 ml	1.0 ml

Add 0.02 ml of researched serum and 1 ml of biuret reagent in a test-tube 1, 0.02 ml of a protein standard solution and 1.0 ml of biuret reagent in a test-tube 2 and 1.0 ml of biuret reagent in a test-tube 3. Mix thoroughly the contents of the test-tubes and incubate at a room temperature for 30 minutes to let the colors develop. The coloured solutions (test-tubes 1 or 2) are placed in cuvettes (the layer thickness=1cm) and analyzed on a photoelectric colorimeter against blank (test-tube 3) at 546 nm wavelength. Determine the optical densities of standard and test.

Calculation

Determine the concentration of blood protein using the formula:

$$Ct = (Et * Cs) / Es,$$

Where Ct - concentration of the protein in test-tube 1 (Test), Et - optical density of test in test-tube 1 (Test), Cs - concentration of the protein standard solution in test-tube 2 (Standard), Es - optical density of standard in test-tube 2 (Standard).

Write down the results and make a conclusion: ________________

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2. Quantitative determination of albumin by the bromocresol green dye in the serum.

Principle: Bromocresol dye reacts with albumin to form an intense blue-green coloured complex in succinate buffer (pH 4,2). Intensity of the color is proportional to albumin concentration in serum.

Diagnostic significance:

A. Physiologic Basis: Albumin makes the biggest contribution to the plasma oncotic pressure. If the albumin concentration falls very low, oedema is the result. Albumin functions as a transport protein for long-chain fatty acids, bilirubin, drugs (sulfonamides, penicillin G, dicoumarol, aspirin), and some steroid hormones and vitamins. In addition, serum albumin binds Ca^{2+} and Mg^{2+} ions.

B. Interpretation:

1. Elevated in dehydration, shock, hemoconcentration, and administration of large quantities of concentrated albumin "solution" intravenously.

2. Decreased in malnutrition, malabsorption syndrome, acute or chronic glomerulonephritis, nephrosis, acute or chronic hepatic insufficiency, neoplastic disease, leukemia, nephrotic syndrome, alcoholic cirrhosis, inflammatory bowel disease, metastatic cancer, Hodgkin's disease.

There are main reasons for the occurrence of a low plasma albumin concentration:

1. Decreased synthesis. This may be due to malnutrition or malabsorption. Decreased synthesis is also a feature of advanced chronic liver disease.
2. Abnormal distribution or dilution. Hypoalbuminaemia can be induced by overhydration or if there is increased capillary permeability as occurs in septicaemia.
3. Abnormal excretion or degradation. The causes include the nephrotic syndrome, protein losing enteropathies, burns, haemorrhage and catabolic states.

Albumin, Serum: Normal – SI: 35-52 g/L.

Reagents: 1. Bromocresol green reagent; 2. Albumin standard solution; 3. Blood serum.

Practical procedure:

Take 3 test tubes and label as test (T), standard (S) and blank (B) as shown in the table below:

Pipette	Test-tube 1 Test	Test-tube 2 Standard	Test-tube 3 Blank
Blood serum	0.01ml	-	-
Albumin standard solution	-	0.01ml	-
Bromocresol green reagent	1.0ml	1.0ml	1.0ml

Add 0.01ml of researched serum and 1 ml of bromocresol green reagent in a test-tube 1, 0.01ml of albumin standard solution and 1.0 ml of bromocresol green reagent in a test-tube 2 and 1.0 ml of bromocresol green in a test-tube 3. Mix thoroughly the contents of the test-tubes and incubate at 37^0C for 10 minutes to let the colors develop. The coloured solutions (test-tubes 1 or 2) are placed in cuvettes (the layer thickness=lcm) and analyzed on a photoelectric colorimeter against blank (test-tube 3) at 630-690 nm wavelength. Determine the optical densities of standard and test.

Calculation

Determine the concentration of blood albumin using the formula:

$$Ct = (Et * Cs) / Es,$$

Where Ct - concentration of the albumin in test-tube 1 (Test), Et - optical density of test in test-tube 1 (Test), Cs - concentration of the albumin standard solution in test-tube 2 (Standard), Es - optical density of standard in test-tube 2 (Standard).

Write down the results and make a conclusion: ________________

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3. Qualitative determination of protein in urine (Heller test).

Diagnostic significance: Proteinuria - the presence of abnormal quantities of protein in the urine. Proteinuria may be a sign of renal (kidney) damage. Since serum proteins are readily reabsorbed from urine, the presence of excess protein indicates either an insufficiency of absorption or impaired filtration. Diabetics may suffer from damaged nephrons and develop proteinuria. Conditions causing proteinuria are of three types: 1) Prerenal conditions - dehydration, heart diseases etc.; 2) Renal conditions - all forms

of renal diseases; 3) Post renal conditions - lesions of pelvis, bladder, prostate & urethra etc. With severe proteinuria, general hypoproteinemia can develop which results in diminished oncotic pressure. Symptoms of diminished oncotic pressure may include ascites, edema, and hydrothorax.

Reagents: 1. Acetic acid solution 10 g/L; 2. Concentrated nitric acid with sodium chloride; 3. Urine, containing protein and normal urine.

Heller test. It is necessary to work cautiously at conducting of Heller test with the concentrated nitric acid. Place 1 ml of concentrated nitric acid in the test-tube by dropper. Then cautiously add equal volume of the filtered urine from a pipette to test tube wall so that liquids did not mix up. In case of protein presence on border of liquids there is a muddy, whitish ring of a protein precipitate.

Write down the results and make a conclusion: ________________

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MAIN LITERATURE:

1. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., p. 33-37.

Lesson is passed____________________

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LESSON 4

THEME: PROPERTIES OF ENZYMES, COENZYMES AND CATALYTIC EFFICIENCY OF ENZYMES. ENZYME KINETICS. FACTORS AFFECTING REACTION VELOCITY

The purpose of the lesson: studying of enzymes as biological catalysts, their features, a structure and properties.

THEORETICAL QUESTIONS:

1. History of discovering and studying of enzymes.
2. Specific features of biocatalysts. Substrate and reaction specificity of enzymes. Specific features of enzyme separation.
3. The classification and nomenclature of enzymes. Units of enzyme activity measurement.
4. Mechanism of enzyme action, "lock and key" and "induced fit models" for substrate binding, catalytic efficiency of enzymes.
5. Structural and functional organization of enzymes.
   5.1. Simple and complex enzymes.
   5.2. Active and allosteric sites, their structures, properties, significance.
   5.3. Apoenzyme, cofactor, coenzyme, prosthetic group, holoenzyme.
6. Factors affecting reaction velocity (substrate concentration, pH, temperature, enzyme concentration). V max and Km of enzymes.
7. Mechanism and stages of enzyme catalysis.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 9, 10 p. 113.
2. Task from «Clinical Biochemistry» №№ 3, p. 103.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 18-26.

Literature for essay:

1. D.B. Marks. Biochemistry., 1994, p. 33-35.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 51-61.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 83-101.

Lesson is passed______________________
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Date____________________

LESSON 5

THEME: ENZYMES. REGULATION OF ENZYMES. ENZYME INHIBITION. ENZYMES IN MEDICINE

The purpose of the lesson: considering of regulation mechanisms of enzymes activity, change of enzymes activity during diseases, application of enzymes in medicine.

THEORETICAL QUESTIONS:

1. Activators and inhibitors of enzymes.
2. Inhibition of enzyme activity, reversible and irreversible inhibition, competitive, noncompetitive inhibition
3. Antimetabolites. Drugs as inhibitors of enzyme activity. Use of inhibitors as medicines.
4. Regulation of enzyme activity: allosteric mechanisms, cooperative effects, chemical modification, feedback regulation, notion of proenzymes and multienzyme complexes.
5. Medical enzymology
   5.1. Enzymodiagnostics,
   5.2. Enzymotherapy,
   5.3. Hereditary enzymopathies.
   5.4. The using of tissue-specific enzymes and isoenzymes in laboratory diagnostic (lactate dehydrogenase, creatine kinase).

QUESTIONS AND EXERCISES:

Task from «Clinical Biochemistry» №№ 8, 12, 38, p. 104-105, 110.

LABORATORY WORK:

Amylase (diastase) activity analysis in the serum and urine.

Diagnostic significance: Normally, very small amylase activity is present in urine. But its concentration increases highly in acute pancreatitis, pancreatic duct obstruction, and bacterial parotitis. It is elevated in the same situations in which the activity of serum amylase is elevated.

Amylase, Serum: Normal – 16-30 g/L h.

A. Physiologic Basis: Normally, small amounts of amylase (diastase), molecular weight about 50,000, originating in the pancreas and salivary glands, are present in the blood. Inflammatory disease of these glands or ob-

struction of their ducts results in regurgitation of large amounts of enzyme into the blood and increased excretion via the kidney.

B. Interpretation:

1. Elevated in acute pancreatitis, pseudocyst of the pancreas, obstruction of pancreatic ducts (carcinoma, stone, stricture, duct sphincter spasm after morphine), and mumps. Occasionally elevated in renal insufficiency, in diabetic acidosis, and in inflammation of the pancreas from a perforating peptic ulcer. Rarely, combination of amylase with an immunoglobulin produces elevated serum amylase activity (macroamylasemia) because the large molecular complex (molecular weight at least 160,000) is not filtered by the glomerulus.

2. Decreased in acute and chronic hepatitis, in pancreatic insufficiency, and occasionally, in toxemia of pregnancy.

Principle of method: The Caraway method is based on the ability of starch solution to change its colour into blue on interaction with iodine solution. The intensity of colouring is proportional to the starch concentration in solution. Amylase activity is determined by decrease the intensity of colouring.

Reagents: 1. Starch solution, 20 g/L; 2. Phosphate-buffer solution, 0.1 mol/L; 3. Sodium chloride solution, 30 g/L; 4. HCl solution 1N; 5. Iodine solution 0,01N; 6. Blood serum; 7. Urine.

Procedure:

Take 2 test-tubes as shown in the table below:

Reagents	Test, ml	Blank, ml
Starch solution	0,5	0,5
Phosphate-buffer solution	0,3	0,3
NaCl	0,1	0,1
HCl	-	0,1
Serum (or Urine)	0,1	0,1
incubate for 30 min at 37^0C		
HCl	0,1	-
Place into 50 ml flask	0,2	0,2
H_2O	40	40
HCl	0,5	0,5
I_2	0,1	0,1
H_2O	till 50 ml	till 50 ml

The solutions are placed in cuvettes (the layer thickness = 1cm) and analyzed on a photoelectric colorimeter against distilled water at wavelength 597±10 nm.

Calculation: The activity of amylase in urine is defined as the number of starch mg digested by 1 of sample at 37^0C in 1 second.

Amylase activity can be calculated from the following equation:

$$\textbf{Amylase activity, mg/(sec)} = \frac{Ec - Es}{Ec} \cdot 10 \cdot 20 = X,$$

Where Ec - optical density of test-tube with control solution, Es - optical density of test-tube with sample, 10 - starch mass (mg), 20 - coefficient of sample dilution.

Write down the results and make a conclusion: ________________

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MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 27-37.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 35-38.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 75-83.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 102-112.

Lesson is passed____________________

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Date__________________

LESSON 6

COLLOQUIM: PROTEINS. ENZYMES

The purpose of the lesson: knowledge imprinting in memory about the structure and functions of the proteins and enzymes.

THEORETICAL QUESTIONS:

1. Proteins as the most important components of the body: their functions, classification. Shape and size of protein molecules, molecular weight, physical and chemical properties.
2. Primary structure of proteins, its role. Hereditary and acquired proteinpathies. Polymorphism of proteins.
3. Conformation of protein molecule (secondary and tertiary levels). Types of intramolecular bonds in protein. Native structure and denaturation of proteins. Protein structure and functional significance. Chaperones, chaperonins.
4. Quaternary structure of proteins. Cooperative changes of protomers conformation (hemoglobin as compared with myoglobin). Ability of proteins to specific interactions. Self-organization of polymolecular protein structures.
5. Overall scheme and methods of purification of individual proteins and characteristics of homogeneity of purified proteins. Quantitative determination of proteins.
6. Specific features of biocatalysts. Specificity of enzyme effect on the substrate and type of catalytic reaction. Specific features of enzyme separation. Classification and nomenclature of enzymes.
7. Structural and functional organization of enzymes. Active and allosteric centers. Isoenzymes (lactate dehydrogenase, creatine kinase). Mechanism and stages of enzyme catalysis. Units of enzyme activity measurement.
8. Effect of substrate and enzyme concentration, temperature and pH on enzymatic reaction rate.
9. Regulation of enzyme action: allosteric mechanisms, cooperative effects, chemical modification, action based on feedback regulation, notion of proenzymes and multienzyme complexes. Examples of metabolic pathways regulated by these mechanisms. Physiological significance of enzyme action regulation.
10. Activators and inhibitors of enzymes. Use of inhibitors as medicines. Antimetabolites. Enzymodiagnostics, enzymotherapy, hereditary enzymopathies.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 6,7,9,10 p. 113
2. Task from «Clinical Biochemistry» №№ 1-3, 8, 12, 15, 18, 22, 26, 34, 38, p. 103-110.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., p. 4-37.

Lesson is passed______________________
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LESSON 7

THEME: INTRODUCTION TO METABOLISM. INTRODUCTION IN DIGESTION AND ABSORPTION. STRUCTURE AND FUNCTIONS OF MEMBRANES

The purpose of the lesson: metabolism studying and its regulation; familiarizing with processes of food digestion, digestion regulation, absorption of the digestion products, biochemical bases of the balanced food; studying structure and functions of the membranes, types of transport through membranes.

THEORETICAL QUESTIONS:

1. Characteristics of metabolism.
   1.1. Metabolic classification of organism according to sources of energy, reducing power, and starting materials for biosynthesis.
   1.2. Metabolism and its functions.
   1.3. Regulation of metabolism in the cell.
   1.4. Catabolism and anabolism, their integration.
2. Introduction to the biochemistry of digestion.
   2.1. Properties of food.
   2.2. Digestive enzymes.
   2.3. Regulation of digestion with hormones of the gastrointestinal tract.
3. General characteristics and functions of membranes.
   3.1. Structure of cell membranes components (lipids, proteins and carbohydrates).
   3.2. Models of membrane structure.
4. Transmembrane transport.
   4.1. Passive transport (simple diffusion, facilitated diffusion).
   4.2. Active transport (primary active transport, secondary active transport).

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 1-5, 11-22, 100, 101, p. 114-115, 127.
2. Task from «Clinical Biochemistry» №№ 10, p. 104.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 38-58.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 1-4, 185-189.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 131-142, 406-424, 459-466.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 131-155.

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LESSON 8

THEME: BIOENERGETICS OF THE CELL. ATP FORMATION. MITOCHONDRIAL ELECTRON TRANSPORT CHAIN

The purpose of the lesson: studying of transfer of protons and electrons by respiratory chain enzymes.

THEORETICAL QUESTIONS:

1. Bioenergetics general laws of thermodynamics. Endergonic and exergonic reactions in metabolism.
2. ATP and other high energy compounds. ADP/ATP cycle. Types of ATP phosphorylation (oxidative, substrate and photophosphorylation). Generation of ATP from metabolic fuels.
3. Biological oxidation and pathways of oxygen uptake.
4. Tissue respiration. Structure of mitochondria and structural organization of electron transport chain. Types of oxygenated substrates.
5. Complete respiratory chain. Mechanism of proton and electron transport. NAD (NADP)-linked-dehydrogenases. NADH-dehydrogenase (FMN-linked- dehydrogenase). Structure and functions of ubiquinone. Structure and functions of cytochromes. Cytochrome oxidase.
6. Incomplete respiratory chain. FAD-linked-dehydrogenases: succinate dehydrogenase and acyl-CoA-dehydrogenase.
7. Structural organization of respiratory chain. Respiratory chain complexes.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 33-38, 41, 47-56, 58-62, p. 117-121.
2. Task from «Clinical Biochemistry» №№ 5, 9, 14, p. 103-105.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 59-83.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 99-110, 116-119.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 92-102.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 372-383.

Lesson is passed____________________

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Date___________________

LESSON 9

THEME: OXIDATIVE PHOSPHORYLATION. OXIDATIVE SYSTEMS NOT ASSOCIATED WITH PRODUCTION OF ENERGY

The purpose of the lesson: studying of the oxidative phosphorylation and the oxidative systems not connected with production of energy.

THEORETICAL QUESTIONS:

1. Respiratory chain is a key component of the mitochondrial oxidative phosphorylation system. Mitochondrial structure and localization of components of oxidative phosphorylation system in it. The structural organization of the respiratory chain. The mitochondrial electron transport chain as a part of respiratory system of all organisms.
2. Oxidative phosphorylation: essence of the process, the generalized scheme, substrates, P/O ratio.
3. Hypotheses of the mechanism of oxidative phosphorylation (chemical hypothesis, mechano-chemical hypothesis). The chemiosmotic theory of Mitchell.
4. Coupling of oxidation and phosphorylation in the respiratory chain. Transmembrane electrochemical potential as the intermediate form of energy at oxidative phosphorylation. H-ATP-synthetase: biological role, localization, structure, mechanism of ATP synthesis.
5. Inhibitors of oxidative phosphorylation, and uncouplers of oxidative phosphorylation and their proposed loci of action.
6. Regulation of functioning of oxidative phosphorylation system. The respiratory control. Infringements of energetic metabolism. Hypoenergetic conditions as result of hypoxia, hypovitaminosis and other reasons.
7. Oxidative systems not associated with energy production. Microsomal oxidation. Free radical oxidation.
8. Active form of oxygen. Formation of reactive oxygen species during biological oxidation in mitochondria. Physiological and toxic effects of reactive oxygen species. Its role in cell pathology.

QUESTIONS AND EXERCISES:

MSQ from «Clinical Biochemistry» №№ 39, 40, 42-46, 57, 63-66, p. 118-122.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 74-77, 85-81.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, 119-122.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 103-112.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 372-383.

Lesson is passed____________________

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Date__________________

LESSON 10

COLLOQUIM: INTRODUCTION TO METABOLISM. MEMBRANES. BIOLOGICAL OXIDATION AND PATHWAYS OF OXYGEN UPTAKE

The purpose: knowledge imprinting in memory about metabolism, its functions and regulation; digestion biochemistry; biological oxidation and tissue respiration.

MAIN THEORETICAL QUESTIONS:

1. General characteristics and functions of membranes, membrane components.
2. Models of membrane structure, transmembrane transport.
3. Metabolism and its functions, regulation of metabolism in the cell. Introduction to the biochemistry of digestion, properties of food, digestive enzymes. Regulation of digestion with hormones of the gastrointestinal tract.
4. Catabolism and anabolism, their integration. Endergonic and exergonic reactions in metabolism. ATP and other high energy compounds. ADP/ATP cycle. Types of ATP phosphorylation and pathways of APT uptake.
5. Biological oxidation and pathways of oxygen uptake. Structure of mitochondria and structural organization of electron transport chain. Types of oxygenated substrates. NAD-linked dehydrogenases. FAD-linked dehydrogenases: succinate dehydrogenase and acyl-CoA-dehydrogenase.
6. NAD-linked dehydrogenases, composition, mechanism of proton and electron transport.
7. Internal mitochondrial membrane and respiratory chain: NADH-dehydrogenase, ubiquinone, cytochromes, their structure and mechanism of proton and electron transfer to oxygen.
8. Oxidative phosphorylation, P/O ratio. Hypotheses of the oxidative phosphorylation mechanism.
9. Coupling of oxidation and phosphorylation in the respiratory chain. H-ATP-synthetase. Respiratory control. Uncouplers of oxidative phosphorylation. Hypoenergetic conditions.
10. Oxidative systems not associated with energy accumulation. Microsomal oxidation. Free radical oxidation. Its role in cell pathology.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 1-5, 11-22, 33-66, 100, 101, p. 112-122, 127.

2. Task from «Clinical Biochemistry» №№ 5, 9, 10, 14, p. 103-105.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 38-85.

Lesson is passed ____________________
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LESSON 11

THEME: CARBOHYDRATE METABOLISM. DIGESTION OF DIETARY CARBOHYDRATES. GLUCOSE METABOLISM UNDER AEROBIC CONDITION. GLYCOLYSIS

The purpose of the lesson: knowledge imprinting in memory about chemistry of carbohydrates, their biological role, considering of glucose catabolism under aerobic and anaerobic conditions, studying of carbohydrate metabolism by oral glucose tolerance test.

THEORETICAL QUESTIONS:

1. Major carbohydrates of human tissues, their biological role.
2. Digestion and absorption of carbohydrates in digestive tract, characteristics of enzymes.
3. Scheme of sources and pathways of glucose uptake in the body. Key role of glucose-6-phosphate.
4. Glucose metabolism under aerobic condition: sequence of reactions, physiological significance. Energetic and regulation of glycolysis.
5. Shuttle mechanisms of reductive equivalent transfer: glycerol phosphate shuttle and malate-aspartate shuttle.
6. Anaerobic glycolysis: sequence of reactions, physiological significance. Central oxidation-reduction reaction of glycolysis. Energetic of glycolysis.
7. Alcoholic fermentation. Ethanol metabolism in the body.
8. Oxidative decarboxylation of pyruvate: composition of pyruvate dehydrogenase complex and sequence of reactions.
9. Citric acid cycle: sequence of reactions and characteristics of enzymes, regulation. Biological role of CAC.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 67-81,83, 85, 94, 97, p.122-127.
2. Task from «Clinical Biochemistry» №№ 29, p. 108.

LABORATORY WORK:

Oral glucose tolerance test (OGTT).

WHO recommendation on the retention of the OGTT as a diagnostic test: it should be used in individuals who have fasting venous plasma glucose of 6.1–6.9 mmol/l, to determine glucose tolerance status. The test reveals how quickly glucose is metabolized from the bloodstream for use by cells as an energy source. The test can be used to diagnose diabetes, or prediabetes (a condition characterized by higher-than-normal blood sugar levels that can lead to type 2 diabetes).

With an oral glucose tolerance test, the person fasts overnight. Then first, the fasting plasma glucose is tested. After this test, the person receives 75 grams of glucose. Usually, the glucose is in a 300 ml of sweet-tasting liquid that the person drinks within 5 minutes. Blood samples are taken at specific intervals to measure the blood glucose. Plasma glucose levels are measured every 30 minutes for 3 hours. Some physicians simply get a baseline blood sample followed by a sample two hours after drinking the glucose solution.

In a person without diabetes, the glucose levels in the blood rise following drinking the glucose drink. Normally, blood glucose levels peak within an hour. Then they fall quickly back to normal (because insulin is produced in response to the glucose, and the insulin has a normal effect of lowing blood glucose.). Normal response: A person is said to have a normal response when the 2-hour glucose level is less than 6.1-7.8 mmol/l (110 - 140 mg/dl), and all values between 0 and 2 hours are less than 11.1 mmol/l (200 mg/dl).

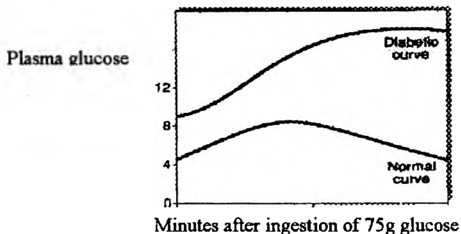
In a diabetic, glucose levels rise higher than normal after drinking the glucose drink and come down to normal levels much slower (insulin is either not produced, or it is produced but the cells of the body do not respond to it).

Diabetes: A person has diabetes when two diagnostic tests done on different days show that the blood glucose level is high. A person has diabetes when oral glucose tolerance tests show that the blood glucose fasting level at or above 7.0 mmol/l (126 mg/dl). Levels between this and 11.1 mmol/l (200 mg/dl) indicate "impaired glucose tolerance. Glucose levels above 11.1 mmol/l (200 mg/dl) at 2 hours confirms a diagnosis of diabetes.

1999 WHO Diabetes criteria - Interpretation of Oral Glucose Tolerance Test								
Glucose levels	NORMAL		impaired fasting glycaemia (IFG)		impaired glucose tolerance (IGT)		Diabetes Mellitus (DM)	
Venous Plasma	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs
(mmol/l)	<6.1	<7.8	> 6.1 & <7.0	<7.8	<7.0	>7.8	>7.0	>11.1
(mg/dl)	<110	<140	>110 & <126	<140	<126	>140	>126	>200

Diagnosis	Capillary Blood	Venous Plasma	Blood plasma
Diabetes Mellitus	> 11	> 10	> 11
Impaired glucose tolerance	7,8 - 11	7-10	7,8-11
Normal	< 7,8	< 7	< 7,8

Normal and diabetic responses to an oral glucose load.



MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 86-100, 122-126.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 131-141, 149-157.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 113-120, 143-156.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 293-314, 367-371.

Lesson is passed___________________

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Date___________________

LESSON 12

THEME: CARBOHYDRATE METABOLISM. GLUCONEOGENESIS. PENTOSE PHOSPHATE PATHWAY. GLYCOGEN METABOLISM. METABOLISM OF FRUCTOSE AND GALACTOSE

The purpose of the lesson: knowledge imprinting in memory about glucose catabolism and glucose synthesis; considering of regulation mechanisms of carbohydrate metabolism under normal and pathological states; mastering of colorimetric dinitrophenylhydrazine method of lactate dehydrogenase activity.

THEORETICAL QUESTIONS:

1. Glucose biosynthesis (gluconeogenesis), physiological function. Glucose-lactate cycle (Cori cycle).
2. Pentose phosphate pathway of glucose metabolism: sequence of reactions, biological role.
3. Biosynthesis and glycogen degradation: sequence of reactions.
4. Physiological significance of biosynthesis and glycogen degradation. Regulation of glycogen phosphorylase and synthase activity. Glycogenosises and aglycogenosises.
5. Metabolism of fructose and galactose.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 82, 84, 86-93, 95, 96, p. 124-126.
2. Task from «Clinical Biochemistry» №№ 21, p. 107.

LABORATORY WORK:

Lactate dehydrogenase activity analysis in blood serum by colorimetric dinitrophenylhydrazine method according to Sevel and Tovarek.

Principle: The LDH activity is measured by colorimetric method, because LDH can catalysis lactate to form pyruvate which is able to combine 2,4-dinitrophenylhydrazine to produce pyruvate - clinitrophenylhydrazone that is shown brownish red in the basic solution.

Diagnostic significance:

A. Physiologic Basis: LDH catalyzes the interconversion of lactate and pyruvate in the presence of NADH or $NADH_2$. It is distributed generally in body

cells and fluids. LDH consists of 5 separable proteins, each made of tetramers of 2 types, or subunits, H and M. The 5 isoenzymes can be distinguished by kinetics, electrophoresis, chromatography, and immunologic characteristics. By electrophoretic separation, the mobility of the isoenzymes corresponds to serum proteins α_1, α_2, β, γ_1; and γ_2. These are usually numbered 1 (fastest moving), 2, 3, 4, and 5 (slowest moving). Isoenzyme 1 is present in high concentrations in heart muscle (tetramer H H H H) and in erythrocytes and kidney cortex; isoenzyme 5 in skeletal muscle (tetramer M M M M) and liver.

B. Interpretation:

Elevated in all conditions accompanied by tissue necrosis, particularly those involving acute injury of the heart, red cells, kidney, skeletal muscle, liver, lung, and skin. Marked elevations accompany hemolytic anemias, the anemias of vitamin B_{12} and folate deficiency, and polycythemia rubra vera. The course of rise in concentration over 3-4 days followed by a slow decline during the following 5-7 days may be helpful in confirming the presence of a myocardial infarction (increased levels of enzyme takes place after 8-12 hours after myocardial infarction); however, pulmonary infarction, neoplastic disease, and megaloblastic anemia must be excluded. Although elevated during the acute phase of infectious hepatitis, enzyme activity is seldom increased in chronic liver disease. In myocardial infarction, the α isoenzymes are elevated - particularly LDH 1 to yield a ratio of LDH 1: LDH 2 of greater than 1. Similar α isoenzyme elevations occur in renal cortex infarction and with hemolytic anemias. LDH 5 and 4 are relatively increased in the presence of acute hepatitis, acute muscle injury, dermatomyositis, and muscular dystrophies.

Lactate dehydrogenase, Serum – Normal: 225-450 U/L.

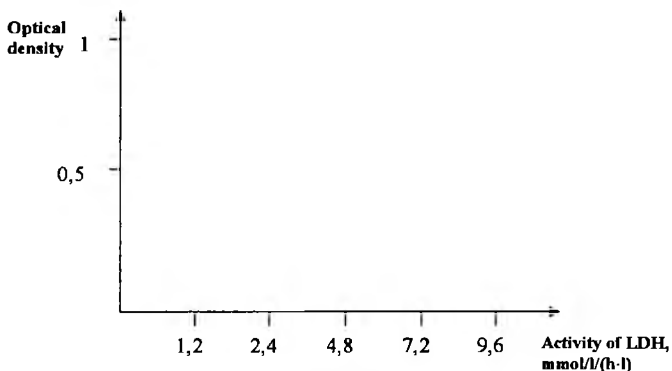
Practical procedure:

0,1ml of blood diluted 1:2 is added into 0.3 ml NAD solution and then incubated at 37°C for 5 min. After both 0.8 ml of sodium pyrophosphate ($Na_4P_2O_7 \bullet 10H_2O$) and 0.2 ml of sodium pyruvate are added, mixed and incubated at 37 °C for 15 min. After 0.5 ml of 2,4-dinitrophenylhydrazine is added into above reaction system, mixed and incubated at room temperature for 20 min. Then 5 ml of sodium hydroxide is added, mixed and incubated at room temperature for 10 min. Absorbance (A) value is measured at 500-560 nm with the spectrophotometer. Measurement control samples are performed like test samples but blood diluted 1:2 is added after incubation.

Calculate enzyme activity according to calibrating graph. For construction of calibrating graph, it is necessary prepare number of dilutions from sodium pyruvate standard according to table:

Sodium pyruvate standard	Sodium pyrophosphate	Distilled water	Pyruvate in standard sample		Activity of pyruvate in 1L of serum for 1 hour of incubation
(ml)	(ml)	(ml)	μg	μmol	mmol
0,1	0,8	0,5	0,88	0,01	1,2
0,2	0,8	0,4	1,76	0,02	2,4
0,4	0,8	0,2	3,52	0,04	4,8
0,6	0,8	-	5,28	0,06	7,2
0,8	0,6	-	7,04	0,08	9,6

After 0.5 ml of 2,4-dinitrophenylhydrazine is added into test tubes, mixed and incubated at room temperature for 20 min. Then 5 ml of sodium hydroxide is added, mixed and incubated at room temperature for 10 min. Absorbance (A) value is measured at 500-560 nm with the spectrophotometer. Measurement control samples are performed like standard samples but distilled water is instead of standard solution.
Write down values of enzyme activity on axes of abscises at construction of calibrating graph.



Conclusion: ____

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MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 101-121.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, 142-149, 157-174.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 157-183.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 315-334.

Lesson is passed____________________

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Date________________

LESSON 13

THEME: METHODS OF CARBOHYDRATE METABOLISM STUDYING

The purpose of the lesson: studying of clinical value of carbohydrate metabolism indexes determination in blood and urine; mastering of quantitative and qualitative methods of glucose determination in blood and urine; learning of analysis and clinical interpretation of carbohydrate metabolism indexes during pathology.

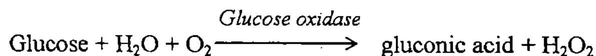
THEORETICAL QUESTIONS:

1. Sources and pathways of blood glucose uptake. Role of gastrointestinal tract, pancreas, liver, kidneys in carbohydrate metabolism.
2. The normal content of glucose in blood. The clinical importance of glucose determination in blood. Insulin and extrainsulin hypoglycemias. Hypoglycemia, the reasons, symptoms.
3. The normal content of glucose in urine. The clinical importance of glucose determination in urine. A renal threshold of glucose. The reasons and types of glucosuria.

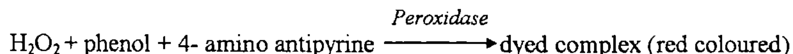
LABORATORY WORK:

1. Quantitative determination of glucose by enzymatic method in the serum.

Principle: Glucose gets oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. The enzyme peroxidase converts hydrogen peroxide to water and oxygen. The oxygen in turn reacts with 4- amino antipyrine in the presence of phenol to form a red coloured complex. Concentration of coloured product is measured on photoelectric colorimeter. The intensity of the color is proportional to the glucose concentration in solution.



$$\text{Glucose} + H_2O + O_2 \xrightarrow{\textit{Glucose oxidase}} \text{gluconic acid} + H_2O_2$$



$$H_2O_2 + \text{phenol} + \text{4- amino antipyrine} \xrightarrow{\textit{Peroxidase}} \text{dyed complex (red coloured)}$$

Reagents: 1. Glucose reagent; 2. Glucose standard solution; 3. Blood serum.

Practical procedure:

Take 3 test tubes and label as test (T), standard (S) and blank (B) as shown in the table below:

Pipette	Test-tube 1 Test	Test-tube 2 Standard	Test-tube 3 Blank
Blood serum	0.01 ml	–	–
Glucose standard solution	–	0.01 ml	–
Glucose reagent	1.0 ml	1.0 ml	1.0 ml

Add 0.01ml of researched serum and 1 ml of glucose reagent in a test-tube 1, 0.01ml of glucose standard solution and 1.0 ml of glucose reagent in a test-tube 2 and 1.0 ml of glucose reagent in a test-tube 3. Mix thoroughly the contents of the test-tubes and incubate at 37^0C for 15 minutes to let the colors develop. The coloured solutions (test-tubes 1 or 2) are placed in cuvettes (the layer thickness=lcm) and analyzed on a photoelectric colorimeter against blank (test-tube 3) at 500 nm wavelength. Determine the optical densities of standard and test.

Calculation

Determine the concentration of blood glucose using the formula:

$$Ct = (Et * Cs) / Es,$$

Where Ct - concentration of the glucose in test-tube 1 (Test), Et - optical density of test in test-tube 1 (Test), Cs - concentration of the glucose standard solution in test-tube 2 (Standard), Es - optical density of standard in test-tube 2 (Standard).

Write down the results and make a conclusion: ____________________

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Glucose, Serum: Normal – 3.65-6.11 mmol/L or 65-110 mg/dL.

Diagnostic significance:

A. Physiologic Basis: The glucose concentration in extracellular fluid is normally closely regulated, with the result that a source of energy is available to tissues, and no glucose is excreted in the urine. Hyperglycemia and hypoglycemia are nonspecific signs of abnormal glucose metabolism.

B. Interpretation:

1. Elevated in diabetes mellitus, hyperthyroidism, adrenocortical hyperactivity (cortical excess - Cushing's syndrome), hyperpituitarism, feochromocytoma, pancreas diseases and hepatic disease (occasionally), thiazide diuretics.

2. Decreased in hyperinsulinism, adrenal insufficiency, hypopituitarism, hepatic insufficiency (occasionally), functional hypoglycemia, and by hypoglycemic agents, malnutrition, sepsis, endocrine tumors.

2. Qualitative determination of sugar in urine (reaction of Nilander).

Principle: The Nilander`s reagent containing bismuth nitrate for qualitative detection of sugar in urine is used. Bismuth oxide hydrate in the alkaline medium is produced, which is reduced by glucose to the bismuth tincturing a liquid in black-brown colour.

Reaction according to Nilander is used specifically for sugar detection in urine since bismuth nitrate is not reduced by uric acid (a normal component of urine).

Reagents: 1. Urine 1; 2. Urine 2; 3. The Nilander`s reagent.

Practical procedure:

Prepare 2 test tubes as shown in the table below:

Pipette	Test- tube 1	Test-tube2
Urine 1	20 drops	–
Urine 2	–	20 drops
The Nilander`s reagent	20 drops	20 drops

Add 20 drops of Urine 1 and 20 drops of the Nilander`s reagent in the test-tube 1. Then add 20 drops of Urine 2 and 20 drops of the Nilander`s reagent in the test-tube 2. Boil two test-tubes 1-2 minutes.

Write down the results and make a conclusion: ____________________

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Diagnostic significance: Glucose is normally not present in urine. Small amount of glucose (2-20 mg or 0,3-1,1 mmol) may be present in fasting urine which cannot be detectable by chemical methods. Condition of presence of chemically detectable quantities of glucose in urine is called as glycosuria. Glycosuria occurs in hyperglycaemia with values above renal threshold for glucose (7,99-9,99 mmol/l or 160-180 mg/dl). Glycosuria is the good first line screening test-line for diabetes mellitus.

Renal glycosuria: Renal glycosuria is a benign condition due to a reduced renal threshold for glucose. It is unrelated to diabetes and, therefore,

should not be mistaken as diabetes. Further, it is not accompanied by the classical symptoms of diabetes.

Alimentary glycosuria: In certain individuals, blood glucose level raises rapidly after meals resulting in its spill over into urine. This condition is referred to as alimentary glycosuria. It is observed in some normal people, and in patients of hepatic diseases, hormonal disorders, medications, pregnancy, hyperthyroidism and peptic ulcer.

3. Express diagnostics of sugar determination in urine.

Principle: For the express diagnostics of sugar determination in urine test-strips «Urotest», «Urotest II» or «Glucotest» are used. Strips are plastic strips to which chemically specific reagent pads are affixed. The reagent pads react with the sample urine to provide a standardized visible color reaction within 30 seconds. Urine is added to the pads for reaction by dipping the plastic strip into the urine and then slowly withdrawing it. Results are obtained by noting the presence of a visible color change on the test pad of the strip. Urine testing for sugar is not an accurate way to measure how much sugar is in your blood.

Procedure:

Place 5 ml of urine in the pure dry test tubes from all researching samples. Dip a strip into a urine sample for 30 seconds.

Write down the results and make a conclusion: ____________________

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MAIN LITERATURE:

1. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 122-126.

Lesson is passed____________________

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LESSON 14

COLLOQUIM: CARBOHYDRATE METABOLISM

The purpose of the lesson: knowledge imprinting in memory about the carbohydrates metabolism, their connection with energy.

MAIN THEORETICAL QUESTIONS:

1. Common and specific pathways of catabolism. Oxidative decarboxylation of pyruvate: composition of pyruvate dehydrogenase complex and sequence of reactions.
2. Citric acid cycle: sequence of reactions and characteristics of enzymes, regulation. Biological role of CAC.
3. Major carbohydrates of human tissues, their biological role. Digestion of carbohydrates, characteristics of enzymes. Scheme of sources and pathways of glucose uptake in the body. Key role of glucose-6-phosphate.
4. Sources and pathways of blood glucose uptake. Regulation of blood glucose level by insulin, glucagon, epinephrine and glucocorticoids.
5. Glucose metabolism under aerobic condition: sequence of reactions, physiological significance. Shuttle mechanisms of reductive equivalent transfer.
6. Anaerobic glycolysis: sequence of reactions, physiological significance. Central oxidation-reduction reaction of glycolysis. Alcoholic fermentation. Ethanol metabolism in the body.
7. Glucose biosynthesis (gluconeogenesis), physiological function. Glucose-lactate cycle (Cori cycle).
8. Pentose phosphate pathway of glucose metabolism: sequence of reactions, biological role.
9. Biosynthesis and glycogen degradation: sequence of reactions, physiological significance. Regulation of glycogen phosphorylase and synthase activity. Glycogenoses and aglycogenoses.
10. Metabolism of fructose and galactose. Genetic diseases of metabolism of fructose and galactose.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 67-97, p. 122-126.
2. Task from «Clinical Biochemistry» №№ 21, 29, p. 107-108.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 86-126.

Lesson is passed____________________
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LESSON 15

THEME: LIPID METABOLISM. DIGESTION AND ABSORPTION OF LIPIDS. LIPOPROTEINS. OXIDATION OF FATTY ACIDS. METABOLISM OF EICOSANOIDS

The purpose of the lesson: studying of lipid biological functions, their transport, fatty acids oxidation. Mastering of total phospholipid determination method in blood.

THEORETICAL QUESTIONS:

1. Major human tissue lipids: fatty acids, triacylglycerols, phospholipids and cholesterol. Functions of lipids. Classification. Physiological norms of daily lipids intake. The essential components of dietary lipids for the human organism.
2. Eicosanoids and their role in regulation of metabolism and physiological functions.
3. Digestion of dietary fats as hydrolysis of fats under lipases action, necessary conditions. Digestion of phosphoacylglycerols and cholesterol esters. Absorption of lipid products. Role of bile acids. Resynthesis of lipids.
4. Transport blood lipoproteins: characteristics, synthesis, physiological role. Classification of lipoproteins by density, electrophoretic mobility, their functions. Structure of blood lipoprotein.
5. The place of lipoprotein formation, features of lipid structure of various lipoproteins; apolipoproteins, their functions.
6. Lipid transport in the body. Exogenous and endogenous transport of lipids. Lipoprotein lipase.
7. Dislipoproteinaemia. Hyperchylomicronaemia. Hypercholesterolaemia.
8. Chemical mechanism of glycerol oxidation and its energy yield.
9. Activation and oxidation of fatty acids: sequence of reactions, physiological significance: formation of fatty acylcarnitine and it transport into mitochondria, steps of β- oxidation, oxidation of odd chain fatty acids, oxidation of unsaturated fatty acids, the energy yield from β-oxidation of saturated and unsaturated fatty acids, regulation of β-oxidation.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 102 -137, p.127-132.
2. Task from «Clinical Biochemistry» №№ 11, 13, p. 104-105.

LABORATORY WORK:

Determination of the total phospholipids in blood serum according to the content of phosphorus.

Principle of method: Lipoproteins containing phospholipids are precipitated by trichloroacetic acid according to Davis. It is established the maintenance of lipid phosphorus by thermal hydrolysis and destruction of organic substances of the protein precipitate in the presence of perchloric acid. It is possible to know the level of the total phospholipids according to phosphorus concentration (phosphorus content is 4% of phospholipid molecular weight).

Diagnostic significance:

Increased levels of phospholipids in the blood are associated with hyperlipoproteinemias IIa и IIb types, glycogenosis I type, diabetes, nephrosis, chronic nephritis, essential hyperglycemia, obstructive jaundice, post-haemorrhagic anemia, renal coma and other pathological conditions.

Decreased levels of phospholipids in the blood are associated with atherosclerosis, anaemia, acute feverish condition, alimentary dystrophy, cachexia, acute hepatitis, portal cirrhosis and fatty degeneration of liver, multiple sclerosis, and hyperthyroidism.

Normal content of phosphorus in lipids is 1,97-4,68 mmol/l in healthy adult.

Reagents: 1. Trichloroacetic acid solution, 100 g/L; 2. Perchloric acid (conc) solution, 570 g/L; 3. Ammonium molybdate solution, 40 g/L; 4. Ascorbic acid solution, 10 g/L; 5. Standard phosphate solution (KH_2PO_4) containing 0.01 mg of phosphorus in 1 ml; 6. Blood serum.

Practical Procedure:

0.2 ml of blood is added into 3 ml of water in the test-tube. Then 3 ml of trichloroacetic acid is added (first 1,5 ml – on drops, shaking test-tube). Wait 1-2 min. Then centrifuge during 6 minutes at 2000 revolutions per minute before precipitate is produced. Pour out supernatant and turn up test-tube on filter paper. Add 1 ml of perchloric acid ($HClO_4$) to precipitate. Warm up test-tube in sandy bath (T=+180^{0}C) till mix is decoloured. At once after burning and cooling, add 5 ml of water, 1 ml of ammonium molybdate solution (shake) and 1 ml of ascorbic acid in the test-tube. Add water till 10 ml is made in the test-tube.
In same time prepare 4 test-tubes as shown in the table below:

Pipette	Control test-tube 1	Standard test-tube 2	Standard test-tube 3	Standard test-tube 4
Perchloric acid (conc.)	0,8 ml	0,8 ml	0,8 ml	0,8 ml
Standard phosphate	-	2 ml	2 ml	2 ml
Water 1	5,2 ml	3,2 ml	3,2 ml	3,2 ml

Ammonium molybdate	1 ml	1 ml	1 ml	1 ml
Ascorbic acid	1 ml	1 ml	1 ml	1 ml
Water 2	2 ml	2 ml	2 ml	2 ml

Mix all test-tubes. In 20 minutes samples are placed in cuvettes (the layer thickness=10 mm) and analyzed on a photoelectric colorimeter supplied with a red light filter. Calculate value of phosphorus using the formula:

$$\frac{\mathbf{Et \cdot 0.02 \cdot 1000 \cdot 0.03223}}{\mathbf{Est \cdot 0{,}2}} = \mathbf{X\ mol/l},$$

where 0,02 ml - quantity of phosphorus (mg) containing in 2 ml of the standard solution; 0,2 - volume of serum (ml); 1000 - 1000 ml of serum; 0,03223 - quantity of the phosphorus in mmol/L.

Write down the results and make a conclusion: ____________________

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MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 127-136.

Literature for essay:

1. D.B. Marks, et al. "Biochemistry", 1994, p. 185-187, 189-190, 199-206, 212-214.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 184-204, 212-223.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 335-345, 363-366.

Lesson is passed____________________

(The signature of the teacher)

Date___________________

LESSON 16

THEME: LIPID METABOLISM. ACETYL-CoA METABOLISM

The purpose of the lesson: studying of the basic ways of Acetyl-CoA using: ketogenesis, cholesterol synthesis, fatty acids synthesis; familiarizing with qualitative reaction on ketone bodies.

THEORETICAL QUESTIONS:

1. Sources of Acetyl-CoA and pathways where it is used.
2. Metabolism of ketone bodies. Ketogenesis. Oxidation of ketone bodies. Regulation of ketone bodies utilization. Ketonemia, ketonuria, ketosis.
3. Metabolism and functions of cholesterol. Biosynthesis of cholesterol: sequence of reactions, regulation.
4. Biosynthesis of fatty acids: synthesis and role of malonyl-CoA, characteristics of palmitate synthase complex, regulation.
5. Production of long-chain fatty acids and unsaturated fatty acids.
6. Synthesis of triacylglycerols and glycerophospholipids.
7. Major pathological processes associated with disorders of acetyl-CoA metabolism (fasting, obesity, atherosclerosis, cholelithiasis, diabetes).

QUESTIONS AND EXERCISES:

Task from «Clinical Biochemistry» №№ 24, p. 107.

LABORATORY WORK:

Qualitative determination of ketone bodies in urine.

Principle: The method is based on the tendency of acetone and acetoacetic acid to form, when allowed to react with sodium nitroprusside in an alkaline medium, complexes coloured orange-red which turn to cherry-red products in an acidified medium.

Diagnostic significance:

In normal individuals, there is a constant production of ketone bodies by liver and their utilization by extrahepatic tissues. The concentration of ketone bodies in blood is maintained around 1 mg/dl. Their excretion in urine is very low and undetectable by routine tests. When the rate of synthesis of ketone bodies exceeds the rate of utilization, their concentration in blood increases, this is known as ketonemia. This is followed by ketonuria - excretion of ketone bodies in urine. The overall picture of ketonemia and ketonuria is commonly referred to

as ketosis. Smell of acetone in breath is a common feature in ketosis. Ketosis is most commonly associated with starvation and severe uncontrolled diabetes mellitus.

Starvation: Starvation is accompanied by increased degradation of fatty acids (from the fuel reserve triacylglycerol) to meet the energy needs of the body. This causes an overproduction of acetyl-CoA which cannot be fully handled by citric acid cycle. Furthermore, TCA cycle is impaired due to deficiency of oxaloacetate, since most of it is diverted for glucose synthesis to meet the essential requirements (often unsuccessful) for tissues like brain. The result is an accumulation of acetyl-CoA and its diversion for overproduction of ketone bodies.

Diabetes mellitus: Diabetes mellitus is associated with insulin deficiency. This results in impaired carbohydrate metabolism and increased lipolysis, both of them ultimately leading to the accumulation of acetyl-CoA and its conversion to ketone bodies. In severe diabetes, the ketone body concentration in blood plasma may reach 100 mg/dl and the urinary excretion may be as high as 500 mg/day.

Reagents: 1. Urine; 2. Sodium nitroprusside solution, 100 g/L; 3. Acetate solution; 4. NaOH solution, 100 g/L.

Practical procedure:

Add 1 drop of 10% sodium nitroprusside solution and 2 drops of 10% NaOH solution to 5 drops of urine. The orange-red colour is produced. Add 3 drops of acetate solution. The cherry colour is produced.

Write down the results and make a conclusion: ____________________

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MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 137-146.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 190-197, 206-212, 214-217.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 193-204, 224-233.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 346-362.

Lesson is passed____________________

(The signature of the teacher)

Date________________

LESSON 17

THEME: ADIPOSE TISSUE. BIOCHEMISTRY OF ATHEROSCLEROSIS

The purpose of the lesson: knowledge imprinting in memory about structure and biological role of lipids, adipose tissue; studying of cholesterol metabolism and functions, considering of cholesterol transport and biochemical aspects of obesity and atherosclerosis development.

THEORETICAL QUESTIONS:

1. Deposition and mobilization of fats in adipose tissue. Regulation. Transport and uptake of fatty acids.
2. Obesity. Pathogenesis, classification and adiposity treatment.
3. Metabolism and functions of cholesterol. Direct transport of cholesterol. Role of LDL. Reverse transport of cholesterol. Role of HDL and LCAT.
4. Biochemistry of atherosclerosis.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 147-153.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 197-199, 217-219.

Lesson is passed________________
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Date__________________

LESSON 18

THEME: METHODS OF PROTEIN AND LIPID METABOLISM STUDYING

The purpose of the lesson: studying of the clinical importance of protein and lipid metabolisms values determination; mastering of the quantitative methods of determination of urea, total cholesterol, triacylglycerols and HDL cholesterol determination in blood; mastering of the methods of phenyl pyruvate qualitative determination and diagnostics of phenylketonuria; mastering of the calculation procedure of lipoprotein electrophoregram indexes; learning of analysis and clinical interpretation of protein and lipid metabolism values at the pathology.

THEORETICAL QUESTIONS:

1. Urea of serum: formation sources, excretion. Role of liver, kidneys in ammonia detoxification. Normal concentration of urea. Clinical significance of urea content change. The reasons, consequences.
2. Sources and ways of cholesterol and triacylglycerols using in organism. Liver role, endocrine systems in lipid metabolism.
3. The normal total cholesterol content in blood. The clinical importance of total cholesterol determination: hyper-and hypocholesterolemia, the reasons, symptoms.
4. The normal triacylglycerols content in blood. The clinical importance of triacylglycerols determination: hyper-and hypotriacylglycerolemia, the reasons, symptoms, consequences.
5. Calculation of the cholesterol-LDL content, atherogenity index. Normal values. Clinical interpretation of results.
6. The normal content of HDL cholesterol in blood. The clinical importance of HDL cholesterol determination: hyper-and hypo-α-cholesterolemia, the reasons, consequences.
7. Lipidogram. Calculation of the LDL cholesterol content, atherogenity index. Normal values. Clinical interpretation of results.
8. Electrophoresis of lipoproteins. Classification of the lipoprotein classes according to electrophoretical mobility. The changes of blood lipoprotein electrophoregram during various types of hyperlipoproteinemias.

LABORATORY WORK:

Quantitative determination of urea by enzymatic method in the serum.

Principle: Urea in the blood is converted to ammonia and CO_2 by the action of urease enzyme. Ammonia reacts with chromogen in the presence of sodium hypochlorite to form coloured complex. Concentration of coloured product is measured on photoelectric colorimeter. Concentration of blood urea is directly proportional to the colour intensity.

Diagnostic significance:

A. Physiologic Basis: Urea, an end product of protein metabolism, is excreted by the kidney. The urea concentration in the glomerular filtrate is the same as in the plasma. Tubular reabsorption of urea varies inversely with rate of urine flow. Thus, urea is a less useful measure of glomerular filtration than is creatinine, which is not reabsorbed. Blood urea nitrogen varies directly with protein intake and inversely with the rate of excretion of urea.

B. Interpretation:

1. Elevated in

- Extra-renal conditions are due to increased nitrogen metabolism associated with diminished renal blood flow or impaired renal function - dehydration (from any cause) and upper gastrointestinal bleeding (combination of increased protein absorption from digestion of blood plus decreased renal blood flow), diabetic coma, thyrotoxicosis.
- Pre-renal conditions are due to decreased renal blood flow - shock, adrenal insufficiency, diabetes mellitus, dehydration, cardiac failure, etc.
- Renal conditions are due to renal insufficiency - nephritis, acute and chronic; acute renal failure (tubular necrosis).
- Post-renal conditions are due to enlargement of prostate, urinary tract obstruction, due to tumors, stones, etc.

2. Decreased in hepatic failure, nephrosis not complicated by renal insufficiency, cachexia, pregnancy, malnutrition and starvation.

Urea, Serum: Normal – 2,5-8,3 mmol/l (8-25 mg/dL),
Urea, Urine: 333,0-587,7 mmol/L (21-53 mg/dL).

Reagents: 1. Reagent 1 (enzymatic reagent), Reagent 2 (chromogen), Reagent 3 (sodium hypochlorite); 2. Urea standard solution; 3. Blood serum.

Practical procedure:

Take 3 test tubes and label as test (T), standard (S) and blank (B) as shown in the table below:

Pipette	Test-tube 1 Test	Test-tube 2 Standard	Test-tube 3 Blank
Blood serum	0.01ml	-	-
Urea standard solution	-	0.01ml	-
Reagent 1	0.25ml	0.25ml	0.25ml
incubation at 37^0C for 10 min			
Reagent 2 (chromogen)	0.5ml	0.5ml	0.5ml
Reagent 3 (sodium hypochlorite)	0.5ml	·0.5ml	0.5ml
incubation at 37^0C for 5 min			

Add 0.01ml of researched serum and 0.25ml of Reagent 1 in a test-tube 1, 0.01ml of urea standard solution and 0.25ml of Reagent 1 in a test-tube 2 and 0.25ml of Reagent 1 in a test-tube 3. Mix thoroughly the contents of the test-tubes and incubate at 37^0C for 10 minutes

After incubation add 0.5 ml of Reagent 2 and Reagent 3 in all test-tubes (test- tube 1, test-tube 2, and test-tube 3) sequentially, incubate at 37^0C for 5 minutes. The coloured solutions (test-tubes 1 or 2) are placed in cuvettes (the layer thickness=lcm) and analyzed on a photoelectric colorimeter against blank (test-tube 3) at 590 nm wavelength. Determine the optical densities of standard and test.

Calculation

Determine the concentration of blood urea using the formula:

$$Ct = (Et * Cs) / Es,$$

Where Ct - concentration of the urea in test-tube 1 (Test), Et - optical density of test in test-tube 1 (Test), Cs - concentration of the urea standard solution in test-tube 2 (Standard), Es - optical density of standard in test-tube 2 (Standard).

Write down the results and make a conclusion: ____________________

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2. Quantitative reaction on phenylpyruvate (Feling`s sample).

Principle: Phenylpyruvate reacts with iron ions to form the complex of blue-green color.

Diagnostic significance: Phenylketonuria is inherited as an autosomal recessive trait (both parents must pass on the defective gene for the child to be affected). The genetically-determined abnormality in phenylketonuria is a missing enzyme called phenylalanine hydroxylase. In PKU, phenylalanine cannot be used in a normal fashion because of the missing enzyme. Subsequently, high

levels of phenylalanine, and 2 closely-related phenylalanine derivatives, build up in the body. These compounds are toxic to the central nervous system and cause brain damage. Damage to the brain causes marked mental retardation by the end of the first year of life if the offending proteins are not scrupulously avoided. Older children may develop movement disorders (athetosis), rocking, and hyperactivity. PKU is a treatable disease that can be easily detected by a simple blood test. Most states require a screening test for all newborns, generally done with a heelstick shortly after birth. Blood is routinely drawn from newborn infants for testing. Blood is obtained by "heel stick" and collected on a special blotter paper.

Because phenylalanine is involved indirectly in the production of melanin, children with phenylketonuria often have lighter complexions than their unaffected siblings. There is a characteristic "mousy" odor that results from the accumulation of phenylacetic acid. This odor may be detected on the breath, skin, and urine if the condition has not been treated immediately from birth or if foods containing phenylalanine are consumed.

Reagents: 1. Urine 1 and 2; 2. Iron chloride solution; 3. Filter papers.

Practical procedure:

Put several urine drops on 2 filter papers; add 8-10 drops of iron chloride solution.

Write down the results and make a conclusion: ____________________

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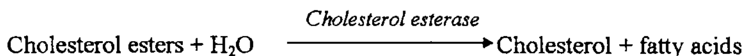
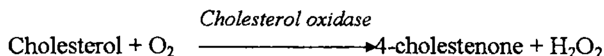
3. Quantitative determination of total cholesterol by enzymatic method in the serum.

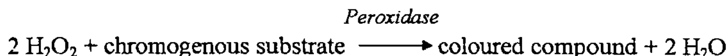
Principle: Cholesterol is product of cholesterol esters hydrolysis by cholesterol esterase. It is oxidized by air oxygen by cholesterol oxidase. Hydrogen peroxide is product of this reaction resulting in chromogenous substrate oxidation catalyzed by peroxidase with pink coloured compounds appearance. The intensity of the colour is proportional to the cholesterol concentration in plasma.



$$\text{Cholesterol esters} + H_2O \xrightarrow{\textit{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$



$$\text{Cholesterol} + O_2 \xrightarrow{\textit{Cholesterol oxidase}} \text{4-cholestenone} + H_2O_2$$



$$2\,H_2O_2 + \text{chromogenous substrate} \xrightarrow{\textit{Peroxidase}} \text{coloured compound} + 2\,H_2O$$

Diagnostic significance: Cholesterol is transported in the blood as a component of VLDL and LDL, 60-70% - in a form of cholesterol esters and 30-40% - as free cholesterol. Total blood serum cholesterol contains cholesterol and its esters. Cholesterol level increases during the life.

A. Physiologic Basis: Cholesterol concentrations are determined by metabolic functions, which are influenced by heredity, nutrition, endocrine function, and integrity of vital organs such as the liver and kidney. Cholesterol metabolism is intimately associated with lipid metabolism.

B. Interpretation:

1. **Elevated** in familial hypercholesterolemia (xanthomatosis), hypothyroidism, poorly controlled diabetes mellitus, nephrotic syndrome, chronic hepatitis, biliary cirrhosis, obstructive jaundice, hypoproteinemia (idiopathic, with nephrosis or chronic hepatitis), and lipidemia (idiopathic, familial).
2. **Decreased** in acute hepatitis and Gaucher's disease. Occasionally decreased in hyperthyroidism, acute infections, anemia, malnutrition, apolipoprotein deficiency, carcinoma, and acute pancreatitis.

Hypercholesterolemia

Norm – at the 3.65- 5.2 mmol/L or 140-200 mg/dL

Low risk – at the 5.2-6.5 mmol/L or 200-250 mg/dL

Moderate risk – at the 6.5-7.8 mmol/L or 250-300 mg/dL

High risk – at the greater than 7.8 mmol/L or 300 mg/dL.

Cholesterol, Serum: Normal – 3.65- 5,2 mmol/l or 140-200 mg/dL.

Reagents: 1. Working reagent 1; 2. Cholesterol standard solution - 5,17 mmol/L (200mg/100ml); 3. Blood serum.

Practical procedure:

Take 3 test tubes and label as test (T), standard (S) and blank (B) as shown in the table below:

Pipette	Test-tube 1 Test	Test-tube 2 Standard	Test-tube 3 Blank
Blood serum	0.01 ml	-	-
Cholesterol standard solution	-	0.01 ml	-
Reagent 1	1.0 ml	1.0 ml	1.0 ml

Add 0.01 ml of researched serum and 1 ml of Reagent 1 in a test-tube 1, 0.01 ml of cholesterol standard solution and 1.0 ml of Reagent 1 in a test-tube 2 and 1.0 ml of Reagent 1 in a test-tube 3.

Mix thoroughly the contents of the test-tubes and incubate at 37^{0}C for 10 minutes to let the colors develop. The coloured solutions (test-tubes 1 or 2) are placed in cuvettes (the layer thickness=lcm) and analyzed on a photoelectric

colorimeter against blank (test-tube 3) at 500 nm wavelength. Determine the optical densities of standard and test.

Calculation

Determine the concentration of blood cholesterol using the formula:

$$Ct = (Et * Cs) / Es,$$

Where Ct - concentration of the cholesterol in test-tube 1 (Test), Et - optical density of test in test-tube 1 (Test), Cs - concentration of the cholesterol standard solution in test-tube 2 (Standard), Es - optical density of standard in test-tube 2 (Standard).

Write down the results and make a conclusion: ____________________

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4. Quantitative determination of triacylglycerol by enzymatic method in the serum.

Principle: Triacylglycerols are hydrolyzed by the action of lipoprotein lipase to glycerol and fatty acids. Glycerol is oxidized by glycerolkinase, after that by glycerolphosphateoxidase. Hydrogen peroxide is product of this reaction resulting in 4-chlorphenol and 4-aminephenazon oxidation catalyzed by peroxidase with red coloured compounds appearance. The intensity of colouring is proportional to the triacylglycerol concentration in plasma.

Diagnostic significance:

A. Physiologic Basis: Dietary fat is hydrolyzed in the small intestine, absorbed and resynthesized by the mucosal cells, and secreted into lacteals in the form of chylomicrons. Triglycerides in the chylomicrons are cleared from the blood by tissue lipoprotein lipase (mainly adipose tissue), and the split products are absorbed and stored. Free fatty acids derived mainly from adipose tissue are precursors of the endogenous triglycerides produced by the liver. Transport of endogenous triglycerides is in association with β-lipoproteins, the very low density lipoproteins. In order to ensure measurement of endogenous triglycerides, blood must be drawn in the postabsorptive state.

B. Interpretation: Concentration of triglycerides, cholesterol, and lipoprotein fractions (VLDL, LDL, and HDL) is interpreted collectively. Disturbances in normal relationships of these lipid moieties may be primary or secondary in origin.

1. Elevated (hyperlipoproteinemia)-

a. Primary-Type I hyperlipoproteinemia (exogenous hyperlipidemia), type II hyperbetalipoproteinemia, type III broad beta hyperlipoproteinemia, type IV hyperlipoproteinemia (endogenous hyperlipidemia), and type V hyperlipoproteinemia (mixed hyperlipidemia).

b. Secondary-Hypothyroidism, diabetes mellitus, nephrotic syndrome, chronic alcoholism with fatty liver, ingestion of contraceptive steroids, biliary obstruction, and stress.

2. Decreased (hypolipoproteinemia)-

a. Primary-Tangier disease (α-lipoprotein deficiency), abetalipoproteinemia, and a few rare, poorly defined syndromes.

b. Secondary-Malnutrition, malabsorption, and, occasionally, with parenchymal liver disease.

Hypertriacylglycerolemia

Norm – at the 0.5 - 1.8 mmol/L

Low risk – at the 1.81-2.25 mmol/L

Moderate risk – at the 2.26-4.50 mmol/L

High risk – at the greater than 4.50 mmol/L.

Triacylglycerol, Serum: Normal – 0.5 -1.8 mmol/L.

Reagents: 1. Reagent 1; 2. Glycerol standard solution; 3. Blood serum.

Practical procedure:

Take 3 test tubes and label as test (T), standard (S) and blank (B) as shown in the table below:

Pipette	Test-tube 1 Test	Test-tube 2 Standard	Test-tube 3 Blank
Blood serum	0.01 ml	-	-
Glycerol standard solution	-	0.01 ml	-
Reagent 1	1.0 ml	1.0 ml	1.0 ml

Add 0.01 ml of researched serum and 1 ml of Reagent 1 in a test-tube 1, 0.01 ml of glycerol standard solution and 1.0 ml of Reagent 1 in a test-tube 2 and 1.0 ml of Reagent 1 in a test-tube 3.

Mix thoroughly the contents of the test-tubes and incubate at a room temperature for 10 minutes to let the colors develop. The coloured solutions (test-tubes 1 or 2) are placed in cuvettes (the layer thickness=lcm) and analyzed on a photoelectric colorimeter against blank (test-tube 3) at 500 nm wavelength. Determine the optical densities of standard and test.

Calculation

Determine the concentration of blood triacylglycerol using the formula:

$$Ct = (Et * Cs) / Es,$$

Where Ct - concentration of the triacylglycerol in test-tube 1 (Test), Et - optical density of test in test-tube 1 (Test), Cs - concentration of the glycerol standard solution in test-tube 2 (Standard), Es - optical density of standard in test-tube 2 (Standard).

Write down the results and make a conclusion: ____________________

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5. Calculation of lipid atherogenity (atherogenicity) index according to determination of total cholesterol, HDL and triacylglycerol concentrations.

The **index of atherogenity (IA)** is the ratio between cholesterol of atherogenic lipoprotein classes to cholesterol of antiatherogenic lipoprotein classes:

$$\mathbf{IA = (Ch_{(total)} - Ch\text{-}HDL) / Ch\text{-}HDL \text{ (absolute units)}}$$

The **index of atherogenity** is used for estimation of a atherosclerosis risk degree.

Norm – up to 3

low risk – at the 3-4

average risk – at the 4-5

High risk – at the greater than 5.

It is necessary to calculate Ch-LDL:

$$\mathbf{Ch\text{-}LDL = Ch_{(total)} - Ch\text{-}HDL - Ch\text{-}VLDL;}$$
$$\mathbf{Ch\text{-}VLDL = TG * 0{,}458}$$

According to classification of the European society on struggle against an atherosclerosis LDL level is divided on:

Normal level LDL - at the 1,91-2,60 mmol/l;

easy increasing of LDL level - at the 2,61-3,40 mmol/l;

moderate increasing of LDL level - at the 3,41-5,05 mmol/l;

the expressed increase in level LDL > 5,05 mmol/l.

Procedure:

According to a clinical task (the teacher gives) calculate an atherogenity index and Ch-LDL concentration.

Write down the results and make a conclusion: ____________________

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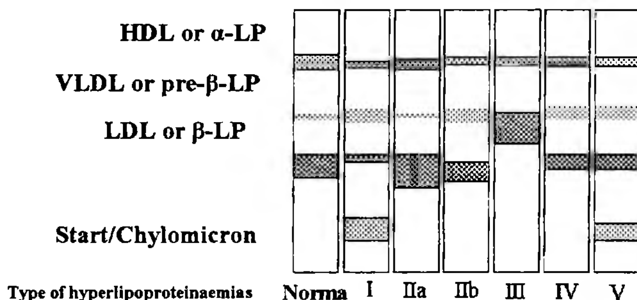
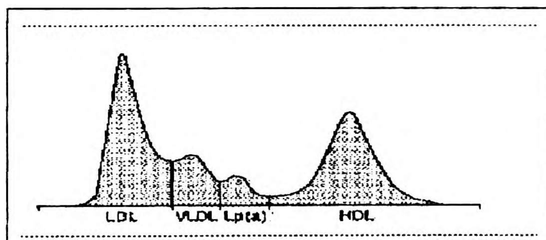
6. Determination of lipoprotein electrophoretic separation.

Agarose gel electrophoresis with staining for lipids. During this type of electrophoresis, the quantity of each lipoprotein class is measured based on its movement in an electrical field. Lipoprotein electrophoresis is used for determination of hyperlipoproteinaemias.

Schematically the arrangement of lipoprotein fractions is shown in the picture.



Lipoprotein electrophoregram of the blood serum is shown:



The fastest are α-lipoproteins 22,3-53,3%, then LP (a) 0% (if they present), then pre-β-lipoproteins 4,4-23,1%, then β-lipoproteins 38,6-69,4%; chylomicrons remain on start 0% (they are detected only after meal).

Hyperlipidemias are classified according to the Fredrickson classification which is based on the pattern of lipoproteins on electrophoresis. The six types of hyperlipoproteinaemia are defined in the Fredrickson Classification.

Fredrickson phenotype	Synonyms	Defect	LP abnormality	Associated clinical disorders	Ch	TG
Type I (rare)	Primary hyperlipoproteinaemia, or Familial hyperchylomicronemia	Decreased lipoprotein lipase or altered ApoC2	Chylomicrons	Diabetes, lupus, dysglobulinemia	→	↓↓
Type IIa	Polygenic hypercholesterolaemia or Familial hypercholesterolemia	LDL receptor deficiency	LDL	Nephrosis, hypothyroidism, myxedema, dysgammaglobulinemia, obstructive liver disease acute intermittent porphyria	↑↑	→
Type IIb	Combined hyperlipidemia	Decreased LDL receptor and Increased ApoB	LDL and VLDL	Myxedema, dysgammaglobulinemia, obstructive liver disease acute intermittent porphyria	↑↑	↑
Type III rare)	Familial dysbetalipoprotein-emia	Defect in Apo E2 synthesis	IDL	myxedema, dysgammaglobulinemia	↑	↑
Type IV	Familial hyperlipemia	Increased VLDL production and Decreased elimination	VLDL	diabetes, corticosteroid therapy, acute alcohol intoxication, acute pancreatitis, gout, gram-negative sepsis, glycogen storage disease I, oral contraceptive use, nephrotic syndrome, chronic renal failure, obesity.	→↑	↑↑
Type V rare)	Endogenous hypertriglyceridemia	Increased VLDL production and Decreased LPL	Chylomicrons and VLDL	Diabetes, nephrotic syndrome, acute alcohol intoxication, myeloma disease		↑

Hyperlipoproteinemia type I is a form of hyperlipoproteinemia associated with deficiencies of lipoprotein lipase.

Hyperlipoproteinemia type II, by far the most common form, is further classified into type IIa and type IIb, depending mainly on whether there is elevation in the triglyceride level in addition to LDL cholesterol.

Type IIa: This may be sporadic (due to dietary factors), polygenic, or truly familial as a result of a mutation either in the LDL receptor gene on chromosome 19 (0.2% of the population) or the ApoB gene (0.2%). The familial form is characterized by tendon xanthoma, xanthelasma and premature cardiovascular disease. The incidence of this disease is about 1 in 500 for heterozygotes, and 1 in 1,000,000 for homozygotes.

Type IIb: The high VLDL levels are due to overproduction of substrates, including triglycerides, acetyl CoA, and an increase in B-100 synthesis. They may also be caused by the decreased clearance of LDL. Prevalence in the population is 10%.

Hyperlipoproteinemia type III: This form is due to high chylomicrons and IDL. Also known as broad beta disease or dysbetalipoproteinemia, the most

common cause for this form is the presence of ApoE E2/E2 genotype. It is due to cholesterol-rich VLDL (β-VLDL). Prevalence is 0.02% of the population.

Hyperlipoproteinemia type IV (type 4 = familial): This form is due to high triglycerides. It is also known as hypertriglyceridemia (or pure hypertriglyceridemia). According to the NCEP-ATPIII determination of high triglycerides (>200 mg/dl), prevalence is about 16% of adult population.

Hyperlipoproteinamia type V (type 5 = endogenous): This type is very similar to type I, but with high VLDL in addition to chylomicrons. It is also associated with glucose intolerance and hyperuremia.

Procedure:

On electrophoregram (a clinical task the teacher gives) define the basic classes of lipoproteins and what type of hyperlipoproteinaemias in each sample.

Write down the results and make a conclusion: ____________________

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MAIN LITERATURE:

1. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 147-153.

Lesson is passed____________________

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Date____________________

LESSON 19

THEME: DIGESTION OF PROTEINS. METABOLISM AND FUNCTIONS OF AMINO ACIDS. TRANSAMINATION. DEAMINATION

The purpose of the lesson: knowledge imprinting in memory about chemical content of gastric juice, digestion of proteins with proteolytic enzymes of digestive juices, mechanisms of their activation; studying of amino acids reactions by amino group.

THEORETICAL QUESTIONS:

1. Digestion and absorption of amino acids.
2. Dynamic state of proteins in the body. Nitrogen balance. Biological significance of nutritional proteins and protein norms in nutrition.
3. Regulation of digestion by hormones of the gastrointestinal tract.
4. Types of gastric juice acidity.
5. Transamination of amino acids: sequence of reactions, significance. Characteristics of transaminases.
6. Indirect deamination of amino acids.
7. Oxidative deamination of amino acids: chemical mechanism, characteristics of enzymes.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 154-161.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 231-237.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 248-261.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 399-406.

Lesson is passed____________________

(The signature of the teacher)

Date___________________

LESSON 20

THEME: METABOLISM AND FUNCTIONS OF AMINO ACIDS. DECARBOXYLATION OF AMINO ACIDS. DETOXICATION OF AMMONIA

The purpose of the lesson: studying of amino acids reactions by carboxylic group, ammonia detoxication in organism; mastering of the quantitative methods of determination of urea.

THEORETICAL QUESTIONS:

1. Decarboxylation of amino acids.
   1.1. Characteristics of the enzymes, their activity. Chemism of the process. Role of amines.
   1.2. Decarboxylation of tyrosine, tryptophan, 5-hydrohytryptophan, histidine. Chemism. Pole of formed amines.
   1.3. Decarboxylation of glutamic acid and aspartic acid, cysteine, phenylalanine, ornithine, lysine. Chemism. Pole of amines formed.
   1.4. Detoxification of biogenic amines. Characteristics of MAO and DAO. Chemism.
2. Detoxication of ammonia in organism.
   2.1. Sources of ammonia in organism.
   2.2. Local detoxication of ammonia, mechanisms. Role of glutamine. Reductive amination of α-ketoglutarate, its significance. Glucose-alanine cycle.
   2.3. General detoxication of ammonia, localization. Urea synthesis. Chemism of the process.
   2.4. Hyperammonemias.
   2.5. Ammonium salts synthesis in the kidney, significance of the process.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 138-139, p. 133.
2. Task from «Clinical Biochemistry» №№ 27, 30, 35, p. 108-110.

LABORATORY WORK:

Quantitative determination of urea by enzymatic method in the serum.

Principle: Urea in the blood is converted to ammonia and CO_2 by the action of urease enzyme. Ammonia reacts with chromogen in the presence of sodium hypochlorite to form coloured complex. Concentration of coloured

product is measured on photoelectric colorimeter. Concentration of blood urea is directly proportional to the colour intensity.

Diagnostic significance:

A. Physiologic Basis: Urea, an end product of protein metabolism, is excreted by the kidney. The urea concentration in the glomerular filtrate is the same as in the plasma. Tubular reabsorption of urea varies inversely with rate of urine flow. Thus, urea is a less useful measure of glomerular filtration than is creatinine, which is not reabsorbed. Blood urea nitrogen varies directly with protein intake and inversely with the rate of excretion of urea.

B. Interpretation:

1. Elevated in

- Extra-renal conditions are due to increased nitrogen metabolism associated with diminished renal blood flow or impaired renal function - dehydration (from any cause) and upper gastrointestinal bleeding (combination of increased protein absorption from digestion of blood plus decreased renal blood flow), diabetic coma, thyrotoxicosis.
- Pre-renal conditions are due to decreased renal blood flow - shock, adrenal insufficiency, diabetes mellitus, dehydration, cardiac failure, etc.
- Renal conditions are due to renal insufficiency - nephritis, acute and chronic; acute renal failure (tubular necrosis).
- Post-renal conditions are due to enlargement of prostate, urinary tract obstruction, due to tumors, stones, etc.

2. Decreased in hepatic failure, nephrosis not complicated by renal insufficiency, cachexia, pregnancy, malnutrition and starvation.

Urea, Serum: Normal – 2,5-8,3 mmol/l (8-25 mg/dL),

Urea, Urine: Normal – 333,0-587,7 mmol/L (21-53 mg/dL).

Reagents: 1. Reagent 1 (enzymatic reagent), Reagent 2 (chromogen), Reagent 3 (sodium hypochlorite); 2. Urea standard solution; 3. Blood serum.

Practical procedure:

Take 3 test tubes and label as test (T), standard (S) and blank (B) as shown in the table below:

Pipette	Test-tube 1 Test	Test-tube 2 Standard	Test-tube 3 Blank
Blood serum	0.01ml	-	-
Urea standard solution	-	0.01ml	-
Reagent 1	0.25ml	0.25ml	0.25ml
incubation at 37^0C for 10 min			
Reagent 2 (chromogen)	0.5ml	0.5ml	0.5ml

Reagent 3 (sodium hypochlorite)	0.5ml	0.5ml	0.5ml
incubation at 37^0C for 5 min			

Add 0.01ml of researched serum and 0.25ml of Reagent 1 in a test-tube 1, 0.01ml of urea standard solution and 0.25ml of Reagent 1 in a test-tube 2 and 0.25ml of Reagent 1 in a test-tube 3. Mix thoroughly the contents of the test-tubes and incubate at 37^0C for 10 minutes

After incubation add 0.5 ml of Reagent 2 and Reagent 3 in all test-tubes (test- tube 1, test-tube 2, and test-tube 3) sequentially, incubate at 37^0C for 5 minutes. The coloured solutions (test-tubes 1 or 2) are placed in cuvettes (the layer thickness=lcm) and analyzed on a photoelectric colorimeter against blank (test-tube 3) at 590 nm wavelength. Determine the optical densities of standard and test.

Calculation

Determine the concentration of blood urea using the formula:

$$Ct = (Et * Cs) / Es,$$

Where Ct - concentration of the urea in test-tube 1 (Test), Et - optical density of test in test-tube 1 (Test), Cs - concentration of the urea standard solution in test-tube 2 (Standard), Es - optical density of standard in test-tube 2 (Standard).

Write down the results and make a conclusion: ____________________

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MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 162-169.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 237-249.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 339-247.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 395-398, 407-412.

Lesson is passed____________________

(The signature of the teacher)

Date___________________

LESSON 21

THEME: METABOLISM AND FUNCTIONS OF INDIVIDUAL AMINO ACIDS

The purpose of the lesson: knowledge imprinting in memory about conversions of amino acids in the tissues; studying of the some amino acids metabolism; mastering of the methods of phenyl pyruvate qualitative determination and diagnostics of phenylketonuria by thin layer chromatography of blood amino acids.

THEORETICAL QUESTIONS:

1. Phenylalanine and tyrosine metabolism.
   1.1. Use of tyrosine for synthesis of catecholamines, thyroid hormones, melanin.
   1.2. Tyrosine metabolism into fumarate and acetoacetate.
   1.3. Hereditary defects in phenylalanine and tyrosine metabolism: phenylketonuria, alkaptonuria, albinism, tyrosinosis, Parkinson's disease.
2. Methylation and transmethylation reactions.
   2.1. S-adenosylmethionine – universal donor of methyl radicals for substrates, itsformation.
   2.2. Methylation reactions: synthesis of creatinine, epinephrine, phosphatidylcholine.
   2.3. Role of tetrahydrofolic acid in methylation.

QUESTIONS AND EXERCISES:

MSQ from «Clinical Biochemistry» №№ 140, 145, p. 133-134.

LABORATORY WORK:

1. Quantitative reaction on phenylpyruvate (Feling`s sample).

Principle: Phenylpyruvate reacts with iron ions to form the complex of blue-green color.

Diagnostic significance: Phenylketonuria is inherited as an autosomal recessive trait (both parents must pass on the defective gene for the child to be affected). The genetically-determined abnormality in phenylketonuria is a missing enzyme called phenylalanine hydroxylase. In PKU, phenylalanine cannot be used in a normal fashion because of the missing enzyme. Subsequently, high levels of phenylalanine, and 2 closely-related phenylalanine derivatives, build

up in the body. These compounds are toxic to the central nervous system and cause brain damage. Damage to the brain causes marked mental retardation by the end of the first year of life if the offending proteins are not scrupulously avoided. Older children may develop movement disorders (athetosis), rocking, and hyperactivity. PKU is a treatable disease that can be easily detected by a simple blood test. Most states require a screening test for all newborns, generally done with a heelstick shortly after birth. Blood is routinely drawn from newborn infants for testing. Blood is obtained by "heel stick" and collected on a special blotter paper.

Because phenylalanine is involved indirectly in the production of melanin, children with phenylketonuria often have lighter complexions than their unaffected siblings. There is a characteristic "mousy" odor that results from the accumulation of phenylacetic acid. This odor may be detected on the breath, skin, and urine if the condition has not been treated immediately from birth or if foods containing phenylalanine are consumed.

Reagents: 1. Urine 1 and 2; 2. Iron chloride solution; 3. Filter papers.

Practical procedure:

Put several urine drops on 2 filter papers; add 8-10 drops of iron chloride solution.

Write down the results and make a conclusion:____________________

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2. Diagnostics of phenylketonuria by thin layer chromatography of blood amino acids.

Principle: At phenylketonuria the content of phenylalanine in blood exceeds normal in 30 and more times (norm of 85-115 μmol/l). It is possible to detect such quantity of phenylalanine by thin layer chromatography of amino acids on plates "Fixion 50x8".

All amino acids should be absorbed in a start position by their exchange with Na^+ when sample is applied on a plate. Hence, all amino acids in initial test should be in form of cations, that is reached by finishing of a put solution till pH less than 2,2. Deduction of amino acids by pitch depends on degree of dissociation of the amine- and carboxylic groups of amino acid (i.e. pH), number of these groups in a molecule, ability to hydrophobic interactions with pitch and some other conditions. Aromatic and basic amino acids (Arg, His, Lys, Phe, Leu, Tyr) are strongly attached to cationite. It is applied passing through a pitch layer nitrate buffer pH 5.25 with concentration Na^+ 0,35M for their division. In these conditions all other amino acids (acid and neutral) get high mobility and pass with solvent front. For revealing of separate amino acids a plate is sprayed

lightly with ninhydrin. There are violet spots of the amino acids which are settling down on length of the plate in specified above sequence on degree of amino acids mobility increase. The semiquantitative estimation of the amino acid maintenance in the mix is reached by comparison of spot size from the analyzed sample with the spot of corresponding standard amino acid.
Test preparation consists in separation of blood serum from proteins by sulfosalicylic acid sedimentation: add 1 ml of 3% sulfosalicylic acid to 1 ml of sick and healthy child serum. Shake mix by spare capillary. Separate protein precipitate by centrifugation at 3000 revolutions per minute for 10 minutes.

Reagents: 1. Sulfosalicylic acid solution, 30 g/L; 2. Phenylalanine standard solution, 4 µmol/ml; 3. Citrate buffer, pH 5,28; 4. Ninhydrin solution; 5. Blood serum.

Practical procedure:

Place samples of deproteinized serum of the sick and healthy child in starting points (7) on the plate "Fixion 50x8" by 15-20 contacts on to each point (2) with a capillary tube. Place phenylalanine working solution in the others 5 points by 1, 3, 5, 7, and 9 contacts that corresponds 0,5, 1,5, 2,5, 3,5 and 4,5 µL (0,5 µL of solution contains – $2{,}10^{-3}$ (!) µmol of phenylalanine).
Then spend chromatographic separation and colouring of spots. Amino acids present in the mixture should appear as purple/blue spots. Compare phenylalanine spot of the serum with phenylalanine spot of the standard during semiquantitative estimation. Thus it is necessary to remember, that concentration of amino acids in blood (C_b) and standard solution of amino acid (C_{st}) are inversely proportional to the put volumes (or quantity of contacts) at the equal intensity of colouring and size of compared spots. Besides, blood has been twice diluted during sedimentation of proteins. Therefore:

$$Cb = \frac{Cst \cdot Vb}{Vst} \cdot 2 \cdot 1000$$

Usually result is expressed by two limiting values, corresponding to the phenylalanine content in two spots of standard scale between which it is possible to arrange corresponding phenylalanine blood spot by intensity of colouring.

Write down the results and make a conclusion: ____________________

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MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 170-174.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 249-264.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 262-270.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 399-406.

Lesson is passed___________________

(The signature of the teacher)

Date___________________

LESSON 22

COLLOQUIM: PROTEIN AND LIPID METABOLISM

The purpose of the lesson: knowledge imprinting in memory about the proteins and lipids metabolism, their connection with energy.

MAIN THEORETICAL QUESTIONS:

1. Digestion of proteins. Absorption of amino acids. Dynamic state of proteins in the body. Nitrogen balance. Biological value of nutritional proteins and protein norms in nutrition. Regulation of digestion by hormones of the gastrointestinal tract. Types of acidity of gastric juice.
2. Transamination of amino acids: sequence of reactions, significance. Characteristics of transaminases. Indirect deamination of amino acids.
3. Oxidative deamination of amino acids: chemical mechanism, characteristics of enzymes. Reductive amination of α-ketoglutarate, its significance.
4. Decarboxylation of amino acids. Production of biogenic amines, their role in regulation of metabolism and functions, detoxication.
5. General detoxication of ammonia: synthesis of urea and ammonium salts, significance of the processes.
6. Transmethylation. Methionine and S-adenosylmethionine (participation in the synthesis of creatinine, epinephrine, phosphatidylcholine and methylation of xenobiotics). Role of tetrahydrofolic acid in methylation.
7. Phenylalanine and tyrosine metabolism. Use of tyrosine for synthesis of catecholamines, thyroid hormones, melanins. Tyrosine metabolism into fumarate and acetoacetate. Hereditary defects in phenylalanine and tyrosine metabolism.
8. Major human tissue lipids. Functions of lipids. Deposition and mobilization of fats in adipose tissue. Regulation. Transport and uptake of fatty acids. Chemical mechanism of glycerol oxidation.
9. Eicosanoids and their role in regulation of metabolism and physiological functions.
10. Nutritional fats: digestion, absorption of the products of digestion. Role of bile acids. Lipid transport in the body.
11. Transport blood lipoproteins: characteristics, synthesis, physiological role. Lipoprotein lipase. Types of hyperlipoproteinamias. Biochemistry of atherosclerosis.
12. Activation and oxidation of fatty acids: sequence of reactions, physiological significance. Biosynthesis and use of ketone bodies.

13. Biosynthesis of fatty acids: synthesis and role of malonyl-CoA, characteristics of palmitate synthase complex, regulation. Production of long-chain fatty acids and unsaturated fatty acids.
14. Biosynthesis of triacylglycerols, glycerophospholipids.
15. Metabolism and functions of cholesterol, direct and reverse transport. Biosynthesis of cholesterol: sequence of reactions, regulation.
16. Major pathological processes associated with disorders of acetyl-CoA metabolism (fasting, obesity, atherosclerosis, cholelithiasis, diabetes).

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 102-137, 138-140, 145, p. 127-132, 133-134.

2. Task from «Clinical Biochemistry» №№ 11, 13, 24, 27, 30, 35, p. 104-107, 108-110.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 127-174.

Lesson is passed__________________
(The signature of the teacher)

Date____________________

LESSON 23

THEME: STRUCTURE OF NUCLEIC ACIDS. METABOLISM OF NUCLEOTIDES

The purpose of the lesson: studying of structure and nitrogenous bases biosynthesis, their degradation.

THEORETICAL QUESTIONS:

1. Nucleoproteids, structure, role in the cell. Histones, characteristics. Nucleic acids are prosthetic groups of nucleoproteids, their structural monomers.
2. Structural components of the mononucleoproteids.
3. Nucleosides, nucleotides.
4. DNA. Primary and secondary structures of DNA. Localization in the cell.
5. Tertiary structure of DNA. Structural organization of DNA in chromatin and chromosomes.
6. Physico-chemical properties of DNA.
7. RNA. Types of RNA, structure, localization in the cell, functions. Primary and secondary structures of RNA.
8. Purine and pyrimidine nucleotides biosynthesis.
9. Purine and pyrimidine nucleotides degradation.
10. Nucleotide metabolism disorders (gout, xanthinuria, orotaciduria).

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 23-28, 31, p. 116-117.
2. Task from «Clinical Biochemistry» №№ 16, p. 106.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 175-180, 185, 191-194.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 47-55.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 285-311.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 173-177, 193-194, 228-240.

Lesson is passed____________________
(The signature of the teacher)

Date___________________

LESSON 24

THEME: REPLICATION. TRANSCRIPTION. TRANSLATION

The purpose of the lesson: studying of the processes of nucleic acids biosynthesis, protein biosynthesis; knowledge imprinting in memory about nucleotide metabolism, structure and functions of nucleic acids, their role in protein biosynthesis.

THEORETICAL QUESTIONS:

1. DNA biosynthesis (replication): mechanism and biological significance.
2. Replication and phases of the cellular cycle.
3. DNA damages and replication errors repair.
4. RNA biosynthesis (transcription).
5. Genetic code.
6. Biosynthesis of proteins (translation). Major components of protein-synthesizing system.
7. Amino acid activation. Amino acyl-t-RNA synthesis. Substrate nature of amino acyl-t-RNA synthases. Adaptive function of t-RNA, role of m-PNA.
8. Initiation stage of translation. Structure of ribosomes and polyribosomes. Ribosome functioning.
9. Elongation stage of translation.
10. Termination stage of translation.
11. Regulation of gene activity. Induction and repression of protein synthesis in the human body. Role of hormones in the gene activity regulation.
12. Medicinal preparations as matrix synthesis inhibitors in prokaryotes and eukaryotes.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 29, 30, 32, p. 166-117.
2. Task from «Clinical Biochemistry» №№ 17, 19, p. 106.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 180-184, 186-191, 195-202.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 55-85.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 312-368.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 195-227, 241-256.

Lesson is passed_______________

(The signature of the teacher)

Date___________________

LESSON 25

THEME: REGULATION OF METABOLISM. HORMONES. PITUITARY AND HYPOTHALAMIC HORMONES. HORMONES OF THE GONADS

The purpose of the lesson: studying of the chemical nature of hormones and mechanisms of metabolism hormonal regulation; studying of the hypothalamic and pituitary hormones, hormones of the gonads.

THEORETICAL QUESTIONS:

1. Hierarchy of regulatory systems.
2. Definition of hormones. Role of hormones in the system of regulation of metabolism and organ functions.
3. Classification of hormones according to their chemical structure and mechanism of action. Major mechanisms of metabolic regulation.
4. Central regulation of endocrine system: hormones of hypothalamus and tropic hormones of the pituitary.
5. Male hormones: structure, effect on metabolism. Anabolics of steroid structure.
6. Female hormones: structure, role in the sex cycle, effect on metabolism.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 148, p. 135.
2. Task from «Clinical Biochemistry» №№ 31, p. 109.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 203-237.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 275-294.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 444-458.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 156-166.

Lesson is passed___________________

(The signature of the teacher)

Date__________________

LESSON 26

THEME: HORMONES OF THE ADRENAL CORTEX. HORMONES OF THE ADRENAL MEDULLA. HORMONES OF THE PANCREAS

The purpose of the lesson: studying of the hormones of the adrenal cortex, adrenal medulla and pancreas.

THEORETICAL QUESTIONS:

1. Insulin: structure, synthesis, biological action.
2. Characteristics of metabolic disorders in diabetes mellitus. Biochemical mechanisms of development of complications in diabetes mellitus.
3. Epinephrine and norepinephrine: structure, synthesis, effect on metabolism.
4. Glucocorticosteroids. Their structure, effect on metabolism. Manifestation of hyper- and hypofunction.
5. Mineralocorticosteroids. Their structure, effect on metabolism. Manifestation of hyper- and hypofunction. Renin-agiotensin system. Biochemical mechanisms of renal hypertension development.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 149, p. 135.
2. Task from «Clinical Biochemistry» №№ 6, 20, 23, 28, 33, p. 104, 107-109.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 238-268.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 275-294.

Lesson is passed__________________

(The signature of the teacher)

Date____________________

LESSON 27

THEME: THYROID HORMONES. HORMONES THAT REGULATE CALCIUM METABOLISM

The purpose of the lesson: studying of the thyroid hormones; studying of the calcium and phosphates metabolism; studying of the hormones that regulate calcium metabolism; knowledge imprinting in memory about theme "Regulation of metabolism" (control test).

THEORETICAL QUESTIONS:

1. Characteristics of mineral metabolism: calcium, phosphates, zink, magnesium. Functions, metabolism regulation, disorders. Special features of mineral metabolism in connection with Chernobyl accident.
2. Metabolism of calcium and phosphates: role of parathyroid hormone, calcitonin, vitamin D_3.
3. Iodothyronines: structure, synthesis, metabolism, their effect on metabolism. Hypo- and hyperthyroidism. Features of thyroid gland functioning in connection with Chernobyl accident.

QUESTIONS AND EXERCISES:

MSQ from «Clinical Biochemistry» №№ 146, 147, 150, p. 134-135.

CONTROL TEST: «HORMONS»

Main theoretical questions:

1. Hierarchy of regulatory systems. Role of hormones in the system of regulation of metabolism and organ functions. Classification of hormones according to their chemical structure and mechanism of action. Major mechanisms of metabolic regulation.
2. Central regulation of endocrine system: hormones of hypothalamus and tropic hormones of the pituitary.
3. Insulin: structure, synthesis, biological action. Characteristics of metabolic disorders in diabetes mellitus. Biochemical mechanisms of development of complications in diabetes mellitus.
4. Regulation of water-salt metabolism. Structure, mechanism of action of vasopressin and aldosterone. Renin-agiotensin system. Biochemical mechanisms of renal hypertension development.

5. Epinephrine and norepinephrine: structure, synthesis, effect on metabolism.
6. Iodothyronines: structure, synthesis, metabolism, their effect on metabolism. Hypo- and hyperthyroidism. Features of thyroid gland functioning in connection with Chernobyl accident.
7. Hormones of adrenal cortex: gluco- and mineralocorticosteroids. Their structure, effect on metabolism. Manifestation of hyper- and hypofunction.
8. Metabolism of calcium and phosphates: role of parathyroid hormone, calcitonin, vitamin D_3.
9. Male hormones: structure, effect on metabolism. Anabolics of steroid structure. Female hormones: structure, role in the sex cycle, effect on metabolism.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 269-284.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 275-294.

Lesson is passed_________________

(The signature of the teacher)

Date__________________

LESSON 28

THEME: INTRODUCTION IN VITAMINOLOGY. LIPID-SOLUBLE VITAMINS

The purpose of the lesson: knowledge imprinting in memory about structure and biological role of fat-soluble vitamins.

THEORETICAL QUESTIONS:

1. Vitamins, general characteristics, classification, functions. Pathologic states due to vitamin metabolism disorders. Specificity of fat-soluble vitamins.
2. Vitamin A: structure, biological role, sources, recommended dietary allowance. Hypo-, a- and hypervitaminosis.
3. Vitamin D. Structure, biological role, natural sources, recommended dietary allowance. Hypo-, a- and hypervitaminosis.
4. Vitamin K: structure, biological role, natural sources, recommended dietary allowance, manifestations of deficiency. Water-soluble drugs.
5. Vitamin E: its structure, biological role, natural sources, recommended dietary allowance. Manifestations of deficiency.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 285, 300-308.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 109-110.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 467-481.

Lesson is passed__________________

(The signature of the teacher)

Date____________________

LESSON 29

THEME: WATER-SOLUBLE VITAMINS. VITAMINS C, P, B_1, B_2, B_6, PP

The purpose of the lesson: knowledge imprinting in memory about structure and biological role of water-soluble vitamins.

THEORETICAL QUESTIONS:

1. Vitamin C: structure, biological role, natural sources, recommended dietary allowance. Hypo- and avitaminosesis.
2. Vitamin P: structure, biological role, natural sources, recommended dietary allowance. Hypo- and avitaminosesis.
3. Vitamin B_1: structure, biological role, natural sources, recommended dietary allowance. Avitaminosis.
4. Vitamin B_2: its structure (co-enzyme forms), biological role, natural sources, recommended dietary allowance.
5. Vitamin B_6: its structure (co-enzyme forms), biological role, natural sources, recommended dietary allowance.
6. Vitamin PP: its structure (co-enzyme forms), biological role, natural sources, recommended dietary allowance.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 8, 158-166, p. 113, 136-137.
2. Task from «Clinical Biochemistry» №№ 25, p. 108.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 286-290, 291-293, 298-299.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 103-109.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 467-481.

Lesson is passed____________________
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Date____________________

LESSON 30

THEME: WATER-SOLUBLE VITAMINS. VITAMINS B_{12}, FOLIC ACID, PANTOTHENIC ACID, BIOTIN

The purpose of the lesson knowledge imprinting in memory about structure and biological role of water-soluble vitamins. (control test); familiarizing with vitamin C quantitative determination in blood.

THEORETICAL QUESTIONS:

1. Biotin. Its structure, biological role (examples of carboxylation reaction), sources.
2. Pantothenic acid. Its structure, biological role (examples of carboxylation reaction), sources.
3. Folic acid. Structure, folate co-enzymes, recommended dietary allowance. Sulfanilamides as antimetabolites.
4. Vitamin B_{12}. Structure, biological role, natural sources, recommended dietary allowance. Pernicious anemia.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 143, 144, 151-157, p. 134-136.
2. Task from «Clinical Biochemistry» №№ 7, 32, p. 104, 109.

LABORATORY WORK:

Quantitative determination of vitamin C in blood.

Clinical importance: In febrile conditions, infections and stress, vitamin C is lost heavily from the body. Hence, adequate quantities of this vitamin should be taken in such conditions. The usefulness of this vitamin in many pathological conditions like atherosclerosis, deep vein thrombosis, diabetes mellitus, cancer, skin diseases, frost-bite and in common cold and maintenance of youthfulness invites one to call it the 'versatile' vitamin. The determination of the plasma levels of ascorbic acid is an indicative of the ascorbic acid status of an individual.

Reagents: 1. 2,6-dichlorophenol indophenol solution, 0,0001 N; 2. Sodium chloride solution, 9 g/L; 3. Sulfosalicylic acid solution, 200 g/L; 4. Blood.

Practical procedure:

Place 1 ml of blood and 3 ml of physiological sodium chloride solution in the rotary test tube. Separate red blood cells from plasma during 15 minutes by centrifugation (3000 revolutions per minute). Separate plasma mix with sodium chloride solution from precipitate. Place it in another rotary test tube and add 2 ml of sulfosalicylic acid solution. Separate protein by centrifugation (15 minutes). Pour out supernatant in a glass and titrate with 2,6-dichlorophenol indophenol, which is reduced by vitamin, till pink colouring is produced.
Calculate using the formula: $0{,}05 \cdot 1000 = X$ μmol/l,
Where 0,005 - quantity of the ascorbic acid in μmol, corresponding 1 ml 0,001N of 2,6 dichlorophenol indophenol. Make conclusion.

CONTROL TEST: «VITAMINS»

Main theoretical questions:

1. Vitamins, general characteristics, classification, functions. Pathologic states due to vitamin metabolism disorders. Specificity of fat-soluble vitamins.
2. Vitamin A: structure, biological role, sources, recommended dietary allowance. Hypo-, a- and hypervitaminosis.
3. Vitamin D. Structure, biological role, natural sources, recommended dietary allowance. Hypo-, a- and hypervitaminosis.
4. Vitamin K: structure, biological role, natural sources, recommended dietary allowance, manifestations of deficiency. Water-soluble drugs.
5. Vitamin E: its structure, biological role, natural sources, recommended dietary allowance. Manifestations of deficiency.
6. Vitamins C and P, their structure, biological role, natural sources, recommended dietary allowance. Hypo- and avitaminosesis.
7. Vitamin B_1: structure, biological role, natural sources, recommended dietary allowance. Avitaminosis.
8. Vitamin B_2 and PP: their structure (co-enzyme forms), biological role, natural sources, recommended dietary allowance.
9. Biotin. Pantothenic acid. Its structure, biological role (examples of carboxylation reaction), sources.
10. Folic acid. Structure, folate co-enzymes, recommended dietary allowance. Sulfanilamides as antimetabolites.
11. Vitamin B_{12}. Structure, biological role, natural sources, recommended dietary allowance. Pernicious anemia.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 290-291, 294-298.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 103-109, 249-253.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 467-481.

Lesson is passed________________

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LESSON 31

THEME: BIOCHEMISTRY OF BLOOD. BLOOD PROTEINS. HEMOGLOBIN. METABOLISM OF IRON AND COPPER

The purpose of the lesson: knowledge imprinting in memory about chemical composition and biological role of blood, hemoglobin role, about mechanisms of oxygen and carbon dioxide transport; studying of the hemoglobin synthesis, kinds of its heterogeneity. Studying of the iron and copper metabolism; familiarizing with benzidine test on blood and quantitative method of hemoglobin determination according to Drabkin.

THEORETICAL QUESTIONS:

1. General characteristics and functions of blood.
2. Blood plasma proteins and enzymes.
   2.1. Albumins, their functions. Hyper- and hypoalbuminemias.
   2.2. Globulins, their functions. Hyper- and hypoglobulinemias.
3. Erythrocyte proteins.
   3.1. Hemoglobin and its derivatives. Methemoglobinemia.
   3.2. Types of hemoglobin. Hemoglobinopathy.
   3.3. Synthesis of heme and hemoglobin, its regulation.
   3.4. Erythrocytes metabolism: role of glycolysis and pentose phosphate pathway.
4. Iron metabolism: transport, deposition, daily requirements.
5. Copper metabolism: transport, deposition, daily requirements.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 141, p. 133.
2. Task from «Clinical Biochemistry» №№ 36, 40, p. 110-111.

LABORATORY WORK:

1. Benzidine test.

Principle: Blood can be detected by preparing derivatives of hemoglobin like hemochromogen and hemin crystals. Hemoglobin decomposes hydrogen peroxide to liberate nascent oxygen ($O^{\cdot}$) which oxidizes benzidine to deeply greenish-blue coloured complex. This is due to the catalytic effect of hemoglobin-haptoglobin complex which has peroxidase activity.

Diagnostic significance:

Reaction is very sensitive and detects the presence of blood. This reaction is taken as a principle detection of the latent blood in faeces, and also for control of presterilization clearing of toolkit and ware in clinical practice.

Reagents: 1. Defibrinated blood diluted by water; 2. Benzidine solution, 50 g/L; 3. Hydrogen peroxide solution, 30g/L.

Practical procedure:

Place 5 drops of blood and 5 drops of benzidine in the test-tube 1. Then add 5 drops of hydrogen peroxide. In the test-tube 2 place 5 drops of distilled water, 5 drops of benzidine and 5 drops of hydrogen peroxide. Compare received results.

Write down the results and make a conclusion:____________________________

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2. Quantitative determination of hemoglobin by Drabkin`s method.

Principle: Hemoglobin reacts with potassium hexacyanoferrate(III) to form methemoglobin in appropriate medium. Methemoglobin in blood is converted to cyanmethemoglobin. The absorbance at 530-550 nm is proportional to the hemoglobin concentration in blood.

Diagnostic significance: Quantitative determination of hemoglobin is used to recognize anemia. Anemia is typically defined as hemoglobin <120 g/l. Increased levels of hemoglobin are associated with clotting, erythremia.

Hemoglobin: Normal: m-130-170 g/L (2,09-2,79 mmol/L),
f-120-150 g/L (1,86-2,48 mmol/L).

Reagents: 1. Drabkin's reagent (potassium hexacyanoferrate (III), Na_2CO_3, CO_2, KCN);
2. Blood.

Note: for studying purposes Drabkin`s reagent is prepared without KCN!

Practical procedure:

Mix 0,02ml of blood and 5ml of Drabkin`s reagent in the test-tube. The coloured solution in 10 minutes placed in cuvettes (the layer thickness=l0mm) and analyzed on a photoelectric colorimeter supplied with a green light filter (X=630-690nm).

Multiply received extinction on 361 which is established by practical consideration.

The received result corresponds to concentration of hemoglobin in g/l. The multiplication of received result on 0.0153 is concentration of hemoglobin in mmol/l.

Write down the results and make a conclusion: ____________________
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MAIN LITERATURE:

1. Lecture,
2. Clinical biochemistry. Materials for the state examination in biochemistry. Konevalova N.Yu., Buyanova S.V., 2005 p. 11-18.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 30-31, 260-262.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 566-582, 593-608.

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Date___________________

LESSON 32

THEME: BIOCHEMISTRY OF KIDNEYS

The purpose of the lesson: Studying of the kidneys functional biochemistry.

THEORETICAL QUESTIONS:

1. Functional biochemistry of kidneys, specific features of metabolism, functions of kidneys.
2. Urine formation, notion of renal threshold substances.
3. Renal insufficiency, its manifestations. Renal stones.
4. Urine components, proteinuria.

MAIN LITERATURE:

1. Lecture.

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LESSON 33

COLLOQUIM: INTEGRATION OF METABOLISM. REGULATION OF METABOLISM

The purpose of the lesson: knowledge imprinting in memory about metabolism integration of proteins, lipids and carbohydrates; regulation of metabolism.

MAIN THEORETICAL QUESTIONS:

1. Integration of amino acid, fat, carbohydrate metabolism. Manifestations of this integration. Major sites of integration (glucose-6-phosphate, pyruvate, acetyl-CoA, CAC substrates).
2. Sources and pathways of blood glucose uptake. Regulation of blood glucose level by insulin, glucagon, epinephrine and glucocorticoids.
3. Hierarchy of regulatory systems. Role of hormones in the system of regulation of metabolism and organ functions. Classification of hormones according to their chemical structure and mechanism of action. Major mechanisms of metabolic regulation.
4. Central regulation of endocrine system: hormones of hypothalamus and tropic hormones of the pituitary.
5. Insulin: structure, synthesis, biological action. Characteristics of metabolic disorders in diabetes mellitus. Biochemical mechanisms of development of complications in diabetes mellitus.
6. Regulation of water-salt metabolism. Structure, mechanism of action of vasopressin and aldosterone. Renin-agiotensin system. Biochemical mechanisms of renal hypertension development.
7. Epinephrine and norepinephrine: structure, synthesis, effect on metabolism.
8. Iodothyronines: structure, synthesis, metabolism, their effect on metabolism. Hypo- and hyperthyroidism. Features of thyroid gland functioning in connection with Chernobyl accident.
9. Hormones of adrenal cortex: gluco- and mineralocorticosteroids. Their structure, effect on metabolism. Manifestation of hyper- and hypofunction.
10. Metabolism of calcium and phosphates: role of parathyroid hormone, calcitonin, vitamin D_3.
11. Male hormones: structure, effect on metabolism. Anabolics of steroid structure. Female hormones: structure, role in the sex cycle, effect on metabolism.

12. Major mechanisms of metabolic regulation: changes in enzyme activity, amount of enzymes in the cell, membrane permeability. Regulation of carbohydrate, fat and amino acid metabolism by glucagon, cortisol and insulin.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 146-150, p. 134-135.
2. Task from «Clinical Biochemistry» №№ 6, 20, 23, 28, 31, 33, p. 104, 107-109.

MAIN LITERATURE:

1. Lecture,
2. Biochemistry. Lecture course. Konevalova N.Yu., Buyanova S.V., 2005, p. 203-284.
3. Clinical biochemistry. Materials for the state examination in biochemistry. Konevalova N.Yu., Buyanova S.V., 2005 p. 4-10.

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Date____________________

LESSON 34

THEME: BIOCHEMISTRY OF LIVER. CARCINOGENESIS

The purpose of the lesson: studying of the liver role in various kinds of metabolism and detoxication of substances; understanding of «carcinogenesis» concept, role of oncogenes and growth factors.

THEORETICAL QUESTIONS:

1. Liver, its functions. Specific features of metabolism in the liver. Role of the liver in metabolism of carbohydrates, lipids, proteins, amino acids, vitamins.
2. Catabolism of heme. Unconjugated and conjugated bilirubin. Disorders of bilirubin metabolism.
3. Types of jaundice (hemolytic, parenchimal, obstructive).
4. Biochemical mechanisms of pathogenesis of hepatic insufficiency, serous-biochemical hepatic syndromes.
5. Major mechanisms of detoxication of substances in the liver: microsomal oxidation, reactions of conjugation. Examples of detoxication of foreign substances (phenol), protein putrifaction products (cresol, indole).
6. Significance of drug metabolism, factors influencing drug metabolism.
7. Role of physical, chemical and biological factors in carcinogenesis. Laboratory diagnosis of cancer. Key role of DNA in carcinogenesis. Risk factors responsible for human carcinogenesis.
8. Role of oncogenes in tumour pathogenesis. Types of oncogenes. Mechanisms of protooncogene activation.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 142, p. 133.
2. Task from «Clinical Biochemistry» №№ 4, 37, 39, p. 103, 110-111.

MAIN LITERATURE:

1. Lecture,
2. Clinical biochemistry. Materials for the state examination in biochemistry. Konevalova N.Yu., Buyanova S.V., 2005 p. 19-45.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, p. 9, 84-85, 260-261.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 271-284, 609-615.

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LESSON 35

THEME: THE INTERMEDIATE CONTROL OF PRACTICAL SKILLS

The purpose of the lesson: knowledge imprinting in memory received during studying of protein, carbohydrate and lipid metabolism research methods: the clinical importance of indexes determination, principle of methods, clinical interpretation of the received data in norm and at the pathology.

THE LIST OF THE PRACTICAL SKILLS:

1. Quantitative determination of total protein by the biuret method in the serum.
2. Quantitative determination of albumin by the bromocresol green in the serum.
3. Quantitative determination of glucose by enzymatic method in the serum.
4. Qualitative determination of sugar in urine (reaction of Nilander).
5. Quantitative determination of urea by enzymatic method in the serum.
6. Quantitative determination of total cholesterol by enzymatic method in the serum.
7. Quantitative determination of triacylglycerols by enzymatic method in the serum.
8. Qualitative determination of ketone bodies in urine.
9. Benzidine test.
10. Qualitative determination of protein in urine (Geller`s test).
11. Quantitative reaction on phenylpyruvate (Feling`s sample).
12. Calculation of lipid atherogenity (atherogenicity) index according to determination of total cholesterol, HDL and triacylglycerol concentrations.
13. Glucose, ketone bodies, proteins determination in urine with method of "dry" chemistry by Boehringer Mannheim test strips.

QUESTIONS AND EXERCISES:

Practical skills from «Clinical Biochemistry» p. 66-102.

MAIN LITERATURE:

1. Clinical biochemistry. Materials for the state examination in biochemistry. Konevalova N.Yu., Buyanova S.V., 2005 p. 19-45.

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Date____________________

LESSON 36

THEME: MUSCLE. NERVE. CONNECTIVE TISSUE

The purpose of the lesson: studying of the chemical composition and metabolism features in nervous, muscular and connective tissues.

THEORETICAL QUESTIONS:

1. Biochemistry of muscle.
   1.1. Sarcoplasmic and myofibrillar proteins of muscles.
   1.2. Molecular structure of myofibrils.
   1.3. Biochemical mechanisms of muscular contraction and relaxation.
   1.4. Muscular energetics: role of creatine phosphate, carnosine and anserine.
   1.5. Biochemistry of connective tissue.
2. General characteristics of connective tissue.
   2.1. Glycosamineglycanes and proteoglycanes, fibronectin, their structure and biological role.
   2.2. Fibrillar protein: collagen, its characteristics and biosynthesis. Role of vitamin C in collagen synthesis. Changes of connective tissue during aging, collagenoses, wound healing.
   2.3. Fibrillar protein: elastin, its characteristics and biosynthesis.
3. Biochemistry of nervous tissue.
   3.1. General characteristics and chemical composition of nervous tissue.
   3.2. Specific features of nervous tissue metabolism.
   3.3. Molecular mechanisms of adrenergic and cholinergic transfer, specific features, disorders.

QUESTIONS AND EXERCISES:

1. MSQ from «Clinical Biochemistry» №№ 98-99, p. 127.
2. Task from «Clinical Biochemistry»

MAIN LITERATURE:

1. Lecture,
2. Clinical biochemistry. Materials for the state examination in biochemistry. Konevalova N.Yu., Buyanova S.V., 2005 p. 46-65.

Literature for essay:

1. D.B. Marks «Biochemistry», 1994, 31-32, 37-38, 136-139.
2. Robert K. Murray et al. Harper's Illustrated Biochemistry, 2009, p. 527-565.
3. D. Hames and N. M. Hooper. Biochemistry, 2005, p. 167-172.

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LESSON 37

THEME: METHODS OF EXPRESS-DIAGNOSTICS

The purpose of the lesson: familiarizing with principles, methods and possibilities of express diagnostics; studying of the "dry chemistry" method importance for express diagnostics; studying of the clinical value of aminotransferase, creatinkinase, and amylase activity determination; learning of the test strips using for urine research.

THEORETICAL QUESTIONS:

1. Concept express-diagnostics. Express diagnostics methods, principles, significance and a role in clinical practice.
2. Principles of work and the device of test strips of the dry chemistry "Reflotron" analyzer.
3. Clinical significance of determination of aminotransferase, creatine kinase, amylase activities and cholesterol level in blood serum by analyzer "Reflotron".

LABORATORY WORK:

1) The quantitative determination of aminotransferases, creatine kinase, cholesterol and amylase levels in blood serum with Reflotron.

The reflotron Plus Chemistry Analyzer is an in vitro diagnostic device designed for the quantitative determination of clinical chemistry parameters using reflotron Test Tabs. It works on the principle of reflectance photometry and ensures rapid and reliable results while being easy to use. Reflectance measurement based on the color change in the test strips. The results are shown on a clear LC display. Reflotron Tests are reagent strips for specific testing of important clinical chemistry parameters directly from whole blood, plasma or serum. The parameters measured cover the most relevant indications in primary care: anemia, diabetes, gout, lipid disorders, liver diseases, muscle diseases, pancreatitis, renal diseases and bone disorders.

Procedure of with Reflotron:

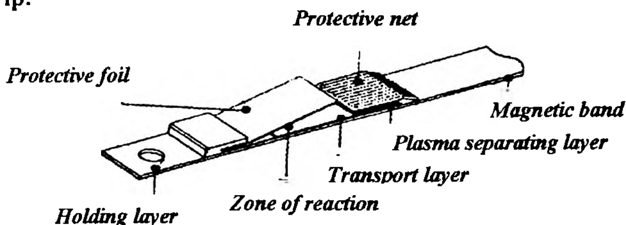
1- Switch on the device and allow getting warm 1-2 minutes.
2- Remove a protective foil.
3- Put 32 µL of serum on a protective net.

4- Place a test strip in a special measuring cell of the device to click, close a cover of a measuring cell.

5- Read results on a display in 120 seconds.

6- Open the cover, remove a test strip.

Test-strip:



1. Test for the quantitative determination of aminotransferases (Aspartate transaminase and Alanine transaminase) by Reflotron.

Diagnostic significance:

A. Physiologic Basis: Aspartate aminotransferase (AST), alanine aminotransferase (ALT) are intracellular enzymes involved in amino acid or carbohydrate metabolism. They catalyze the α-amino group of amino acid transfer to α-keto acid to form the corresponding amino acid and α-keto acid. These enzymes are present in high concentrations in muscle, liver, and brain. Elevations of concentrations of these enzymes in the blood indicate necrosis or disease, especially of these tissues.

B. Interpretation:

1. Elevated after myocardial infarction (especially AST); acute infectious hepatitis (ALT usually elevated more than AST); cirrhosis of the liver (AST usually elevated more than ALT); and metastatic or primary liver neoplasm. Elevated in transudates associated with neoplastic involvement of serous cavities. AST is elevated in muscular dystrophy, dermatomyositis, burns and paroxysmal myoglobinuria. ALT is elevated in billary obstruction. AST>ALT in alcoholic hepatitis

ALT>AST in viral hepatitis

2. Decreased with pyridoxine (vitamin B_6) deficiency (often as a result of repeated hemodialysis), renal insufficiency, and pregnancy.

Aminotransferases, Serum: Normal – AST 8-40 U/L; ALT 5-32 U/L

Write down the results and make a conclusion: ___________________

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2. Test for the quantitative determination of potassium with Reflotron.

Diagnostic significance:

A. Physiologic Basis: Potassium concentration in plasma determines neuromuscular and muscular irritability. Elevated or decreased concentrations impair the capability of muscle tissue to contract.

B. Interpretation:

1. Elevated in renal insufficiency (especially in the presence of increased rate of protein or tissue break down); adrenal insufficiency (especially hypoaldosteronism, Addison's disease); hyporeninemic hypoaldosteronism; dehydration; use of spironolactone; too rapid administration of potassium salts, especially intravenously; and use of triamterene or phenformin.

2. Decreased in-

a. Inadequate intake (starvation).
b. Inadequate absorption or unusual enteric losses – Vomiting, diarrhea, malabsorption syndrome, or use of sodium polystyrene sulfonate resin.
c. Unusual renal loss – Secondary to hyperadrenocorticism (especially hyperaldosteronism, Conn's syndrome) and to adrenocorticosteroid therapy, metabolic alkalosis, use of diuretics such as chlorothiazide and its derivatives and the mercurials; renal tubular defects such as the de Toni-Fanconi syndrome and renal tubular acidosis; treatment with antibiotics that are excreted as anions (carbenicillin, ticarcillin); use of phenothiazines, amphotericin B, and drugs with high sodium content; and use of degraded tetracycline.
d. Abnormal redistribution between extracellular and intracellular fluids – Familial periodic paralysis or testosterone administration.

Potassium, Serum or Plasma: Normal: – Serum: 3.6-5.0 mmol/l; Plasma: 3.8-4.5 mmol/l

Write down the results and make a conclusion: ___________________

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3. Test for the quantitative determination of creatine phosphokinase with Reflotron.

Diagnostic significance:

A. Physiologic Basis: creatine phosphokinase (CPK) splits creatine phosphate in the presence of ADP to yield creatine and ATP. Skeletal and heart muscle and brain are rich in the enzyme. CPK consists of 3 proteins separable by electrophoresis. Skeletal muscle is characterized by isoenzyme MM, myocardium by isoenzyme MB, and brain by isoenzyme BB.

B. Interpretation:

1. Elevated in the presence of muscle damage such as with myocardial infarction, trauma to muscle, muscular dystrophies, polymyositis, severe muscular exertion (jogging), hypothyroidism, and cerebral infarction (necrosis). Following myocardial infarction, serum CPK concentration increases rapidly (within 3-5 hours), and it remains elevated for a shorter time after the episode (2 or 3 days) than does AST or LDH.

CPK-MM is elevated in injury to skeletal muscle, myocardial muscle, and brain; in muscle disease (eg. dystrophies, hypothyroidism, dermatomyositis, polymyositis); in rhabdomyolysis; and after severe exercise. CPK-MB is elevated soon (within 2-4 hours) after myocardial infarction and for up to 72 hours afterward (high levels are prolonged with extension of infarct or new infarction); also elevated in extensive rhabdomyolysis or muscle injury, severe muscle disease, Reye's syndrome, or Rocky Mountain spotted fever. CPK-BB is occasionally elevated in severe shock, in some carcinomas (especially oat cell carcinoma or carcinoma of the ovary, breast, or prostate), or in biliary atresia.

2. Not elevated in pulmonary infarction or parenchymal liver disease.

Creatine Phosphokinase, Serum: Normal – female- 25-175 U/L, male -25-200U/L.

Write down the results and make a conclusion:____________________

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4. Test for the quantitative cholesterol determination with Reflotron.

Diagnostic significance:

A. Physiologic Basis: Cholesterol concentrations are determined by metabolic functions, which are influenced by heredity, nutrition, endocrine function, and integrity of vital organs such as the liver and kidney. Cholesterol metabolism is intimately associated with lipid metabolism.

B. Interpretation:

1. Elevated in familial hypercholesterolemia (xanthomatosis), hypothyroidism, poorly controlled diabetes mellitus, nephrotic syndrome, chronic hepatitis, biliary cirrhosis, obstructive jaundice, hypoproteinemia (idiopathic, with nephrosis or chronic hepatitis), and lipidemia (idiopathic, familial).

2. Decreased in acute hepatitis and Gaucher's disease. Occasionally decreased in hyperthyroidism, acute infections, anemia, malnutrition, apolipoprotein deficiency, carcinoma, and acute pancreatitis.

Cholesterol, Serum: Normal – 3.65- 5,2 mmol/l or 140-200 mg/dL.

Write down the results and make a conclusion:____________________

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5. Test for the quantitative determination of pancreatic α-amylase with Reflotron.

Diagnostic significance:

A. Physiologic Basis: Normally, small amounts of amylase (diastase), molecular weight about 50,000, originating in the pancreas and salivary glands, are present in the blood. Inflammatory disease of these glands or obstruction of their ducts results in regurgitation of large amounts of enzyme into the blood and increased excretion via the kidney.

B. Interpretation:

1. Elevated in acute pancreatitis, pseudocyst of the pancreas, obstruction of pancreatic ducts (carcinoma, stone, stricture, duct sphincter spasm after morphine), and mumps. Occasionally elevated in renal insufficiency, in diabetic acidosis, and in inflammation of the pancreas from a perforating peptic ulcer. Rarely, combination of amylase with an immunoglobulin produces elevated serum amylase activity (macroamylasemia) because the large molecular complex (molecular weight at least 160,000) is not filtered by the glomerulus.

2. Decreased in acute and chronic hepatitis, in pancreatic insufficiency, and occasionally, in toxemia of pregnancy.

Amylase, Serum or Urine: Normal – serum: 16-30 g/L·h; urine: 28-160 g/L·h.

Write down the results and make a conclusion: ____________________

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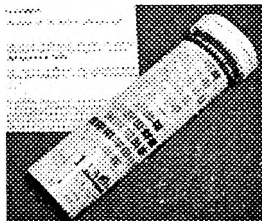
2) Glucose, ketone bodies, proteins determination in urine with method of "dry" chemistry by BOEHRINGER MANNHEIM test strips.



A plastic test strip is used, which contains pads that have incorporated within them the reagents for chemical reactions for the detection of a number of urine constituents. Urine is added to the pads for reaction by dipping the plastic strip into the urine and then slowly withdrawing it. The subsequent colorimetric reactions are timed to an endpoint; the extent of colors formation is directly related to the level of the urine constituent. The colors can be read manually by comparison with color charts.

Strips test simultaneously are used for the presence of glucose, ketones, blood, protein, nitrite, pH, urobilinogen, bilirubin, leucocytes in urine.

Clinical significance:

Proteins. Reagent-strip test for protein is a semi-quantitative screening procedure for proteinuria. Most of the urine protein is albumin, which has crossed the glomerular membrane. Smaller-molecular-weight proteins such as globulins may also be present in urine. Once filtered at the glomerulus, proteins are almost completely reabsorbed in the proximal tubule. Proteinuria, therefore, can be the result of either increased filtration at the glomerulus or decreased tubular reabsorption. Glomerular proteinuria is associated with the presence of larger molecular weight proteins and larger protein losses, usually > 2 g/day. The nephrotic syndrome is associated with very large losses of protein, usually >2-3 g/day. Tubular proteinuria is associated with smaller amounts (1-3 g/day) of lower molecular weight protein molecules. Small losses of protein in urine can be seen with vigorous exercise and pregnancy. Normal - a healthy person will excrete up to approximately 100 mg/day, a very small fraction of the plasma protein that is filtered at the glomerulus.

Sugars: Glucose is the predominant sugar in urine. It is not detectable by reagent strips in the urine of healthy individuals. Temporary elevation of glucose excretion measurable by test strips can occur after treatment with some drugs, cases of shock and during pregnancy. Repeated positive testing is almost always diagnostic for diabetes or pancreatitis. Normal - health individuals normally will have no detectable sugars in their urine.

Ketones: Ketones are spilled into urine when the body cannot utilize glucose (as in diabetes) and metabolize fatty acids. This catabolism is incomplete, resulting in the formation of large amounts of acetoacetic acid, acetone and beta-hydroxybutyric acid (ketone bodies). The presence of elevated levels of ketones in human urine is a primary indicator that the body is in ketosis. Ketones are not stored by the body and are eliminated as detectable waste products in urine. Ketone test strips are used routinely to help measure the progress of di-

eters especially those maintaining a low carbohydrate diet. Additionally, ketones are monitored in individuals with Type 1 diabetes. In diabetics, high ketone levels indicate ketoacidosis, a complication of inadequate insulin levels that can lead to diabetic coma. In overall health considerations, high ketone levels are generally an indication of abnormal nutritional conditions, including starvation, fasting, anorexia, vomiting, diarrhea, aspirin overdose, high protein and fat or low carbohydrate diets. Normal - health individuals normally will have no detectable ketones in their urine.

Blood: A positive test indicates the presence of red blood cells in the urine (hematuria). Hematuria can be renal and extrarenal. The renal is caused by pathology of kidneys; extrarenal- by pathology of excretory tract (stones, infection, Foley cath). Normal - health individuals normally will have no detectable blood in their urine.

Practical procedure:

Place 10-15 ml of urine in the test tube. Dip a strip in urine and wait five minutes. If the test color appears somewhat mottled at higher concentrations, match the darkest color to the color on the reagent pad. The results can be read by visual comparison with a color chart printed on the side of each vial.

Write down the results and make a conclusion: ____________________

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Lesson is passed__________________

(The signature of the teacher)

PROGRAM QUESTIONS

Introduction

1. Objects of biochemical studies. Major divisions and trends in biochemistry. Place of biochemistry among other biological subjects. Biochemistry and medicine.
2. Most important stages in the history of biochemistry. Leading native and foreign biochemists, their contribution to the development of biochemistry. Biochemistry and development of molecular biology, physical-and-chemical biology and biotechnology.
3. Object and aims of biological chemistry. Most important attributes of living matter. Manifestations of dialectic laws in organization and functions of living matter.

Proteins

4. Proteins as the most important components of the body: their functions, classification. Shape and size of protein molecules, molecular weight, physical and chemical properties.
5. Primary structure of proteins, its role. Hereditary and acquired proteinpathies. Polymorphism of proteins.
6. Conformation of protein molecule (secondary and tertiary levels). Types of intramolecular bonds in protein. Native structure and denaturation of proteins. Protein structure and functional significance. Chaperones, chaperonins.
7. Quaternary structure of proteins. Cooperative changes of protomers conformation (hemoglobin as compared with myoglobin). Ability of proteins to specific interactions. Self-organization of polymolecular protein structures.
8. Overall scheme and methods of purification of individual proteins and characteristics of homogeneity of purified proteins. Quantitative determination of proteins.

Enzymes

9. History of discovering and studying enzymes. Specific features of biocatalysts. Specificity of enzyme effect on the substrate and type of catalytic reaction. Specific features of enzyme separation. Classification and nomenclature of enzymes.
10. Structural and functional organization of enzymes. Active and allosteric centres. Isozymes (lactate dehydrogenase, creatine kinase). Mechanism and stages of enzyme catalysis. Units of enzyme activity measurement.
11. Effect of substrate and enzyme concentration, temperature and pH on enzymatic reaction rate.

12. Regulation of enzyme action: allosteric mechanisms, cooperative effects, chemical modification, action based on feedback regulation, notion of proenzymes and multienzyme complexes. Examples of metabolic pathways regulated by these mechanisms. Physiological significance of enzyme action regulation.
13. Activators and inhibitors of enzymes. Use of inhibitors as medicines. Antimetabolites. Enzymodiagnostics, enzymotherapy, hereditary enzymopathies.

Membranes

14. General characteristics and functions of membranes, membrane components.
15. Models of membrane structure, transmembrane transport.

Energy

16. Metabolism and its functions, regulation of metabolism in the cell. Introduction to the biochemistry of digestion, properties of food, digestive enzymes. Regulation of digestion with hormones of the gastrointestinal tract.
17. Catabolism and anabolism, their integration. Endergonic and exergonic reactions in metabolism. ATP and other high energy compounds. ADP/ATP cycle. Types of ATP phosphorylation and pathways of APT uptake.
18. Biological oxidation and pathways of oxygen uptake. Structure of mitochondria and structural organization of electron transport chain. Types of oxygenated substrates. NAD-linked dehydrogenases. FAD-linked dehydrogenases: succinate dehydrogenase and acyl-CoA-dehydrogenase.
19. NAD-linked dehydrogenases, composition, mechanism of proton and electron transport.
20. Internal mitochondrial membrane and respiratory chain: NADH-dehydrogenase, ubiquinone, cytochromes, their structure and mechanism of proton and electron transfer to oxygen.
21. Oxidative phosphorylation, P/O ratio. Hypotheses of the mechanism of oxidative phosphorylation.
22. Coupling of oxidation and phosphorylation in the respiratory chain. H^+-ATP-synthase. Respiratory control. Uncouplers of oxidative phosphorylation. Hypoenergetic conditions.
23. Oxidative systems not associated with energy accumulation. Microsomal oxidation. Free radical oxidation. Its role in cell pathology.
24. Common and specific pathways of catabolism. Oxidative decarboxylation of pyruvate: composition of pyruvate dehydrogenase complex and sequence of reactions.

25. Citric acid cycle: sequence of reactions and characteristics of enzymes, regulation. Biological role of CAC.

Carbohydrates

26. Major carbohydrates of human tissues, their biological role. Digestion of carbohydrates, characteristics of enzymes. Scheme of sources and pathways of glucose uptake in the body. Key role of glucose-6-phosphate.
27. Glucose metabolism under aerobic condition: sequence of reactions, physiological significance. Shuttle mechanisms of reductive equivalent transfer.
28. Anaerobic glycolysis: sequence of reactions, physiological significance. Central oxidation-reduction reaction of glycolysis. Alcoholic fermentation. Ethanol metabolism in the body.
29. Glucose biosynthesis (gluconeogenesis), physiological function. Glucose-lactate cycle (Cori cycle) and glucose-alanine cycles.
30. Pentose phosphate pathway of glucose metabolism: sequence of reactions, biological role.
31. Biosynthesis and glycogen degradation: sequence of reactions, physiological significance. Regulation of glycogen phosphorylase and synthase activity. Glycogenosises and aglycogenosises.

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32. Major human tissue lipids. Functions of lipids. Deposition and mobilization of fats in adipose tissue. Regulation. Transport and uptake of fatty acids. Chemical mechanism of glycerol oxidation.
33. Eicosanoids and their role in regulation of metabolism and physiological functions.
34. Nutritional fats: digestion, absorption of the products of digestion. Role of bile acids. Lipid transport in the body.
35. Transport blood lipoproteins: characteristics, synthesis, physiological role. Lipoprotein lipase. Types of hyperlipoproteinamias. Biochemistry of atherosclerosis.
36. Activation and oxidation of fatty acids: sequence of reactions, physiological significance. Biosynthesis and use of ketone bodies.
37. Biosynthesis of fatty acids: synthesis and role of malonyl-CoA, characteristics of palmitate synthase complex, regulation. Production of long-chain fatty acids and unsaturated fatty acids.
38. Biosynthesis of triacylglycerols, glycerophospholipids.
39. Metabolism and functions of cholesterol, direct and reverse transport. Biosynthesis of cholesterol: sequence of reactions, regulation.
40. Major pathological processes associated with disorders of acetyl-CoA metabolism (fasting, obesity, atherosclerosis, cholelithiasis, diabetes).

41. Integration of amino acid, fat, carbohydrate metabolism. Manifestations of this integration. Major sites of integration (glucose-6-phosphate, pyruvate, acetyl-CoA, CAC substrates).

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42. Digestion of proteins. Absorption of amino acids. Dynamic state of proteins in the body. Nitrogen balance. Biological value of nutritional proteins and protein norms in nutrition. Regulation of digestion by hormones of the gastrointestinal tract. Types of acidity of gastric juice.
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44. Oxidative deamination of amino acids: chemical mechanism, characteristics of enzymes. Reductive amination of α-keto-glutarate, its significance.
45. General detoxication of ammonia: synthesis of urea and ammonium salts, significance of the processes.
46. Phenylalanine and tyrosine metabolism. Use of tyrosine for synthesis of catecholamines, thyroid hormones, melanins. Tyrosine metabolism into fumarate and acetoacetate. Hereditary defects in phenylalanine and tyrosine metabolism.
47. Decarboxylation of amino acids. Production of biogenic amines, their role in regulation of metabolism and functions, detoxication.
48. Transmethylation. Methionine and S-adenosylmethionine (participation in the synthesis of creatinine, epinephrine, phosphatidylcholine and methylation of xenobiotics). Role of tetrahydrofolic acid in methylation.

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49. Notion of biosynthesis and degradation of purine and pyrimidine nucleotides. Nucleotide metabolism disorders (gout, xanthinuria, protic aciturea).
50. Nucleoproteids. DNA, its structural organization, molecular size, ways of packing in chromatin and chromosomes. DNA replication: mechanism and biological significance. Replication and phases of the cellular cycle. DNA damages and replication errors repair.
51. Primary and secondary structures of RNA. Types of RNA, structure, localization in the cell, functions. RNA biosynthesis (transcription). Composition of ribosomes and polyribosomes. Amino acyl-t-RNA synthesis. Substrate nature of amino acyl-t-RNA synthases.
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53. Regulation of gene activity. Induction and repression of protein synthesis in the human body. Role of hormones in the gene activity regulation. Medi-

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55. Hierarchy of regulatory systems. Role of hormones in the system of regulation of metabolism and organ functions. Classification of hormones according to their chemical structure and mechanism of action. Major mechanisms of metabolic regulation.
56. Central regulation of endocrine system: hormones of hypothalamus and tropic hormones of the pituitary.
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58. Regulation of water-salt metabolism. Structure, mechanism of action of vasopressin and aldosterone. Renin-agiotensin system. Biochemical mechanisms of renal hypertension development.
59. Epinephrine and norepinephrine: structure, synthesis, effect on metabolism.
60. Iodothyronines: structure, synthesis, metabolism, their effect on metabolism. Hypo- and hyperthyroidism. Features of thyroid gland functioning in connection with Chernobyl accident.
61. Hormones of adrenal cortex: gluco- and mineralocorticosteroids. Their structure, effect on metabolism. Manifestation of hyper- and hypofunction.
62. Metabolism of calcium and phosphates: role of parathyroid hormone, calcitonin, vitamin D_3.
63. Male hormones: structure, effect on metabolism. Anabolics of steroid structure. Female hormones: structure, role in the sex cycle, effect on metabolism.
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65. Vitamins, general characteristics, classification, functions. Pathologic states due to vitamin metabolism disorders. Specificity of fat-soluble vitamins.
66. Vitamin A: structure, biological role, sources, recommended dietary allowance. Hypo-, a- and hypervitaminosises.
67. Vitamin D. Structure, biological role, natural sources, recommended dietary allowance. Hypo-, a- and hypervitaminosises.

68. Vitamin K: structure, biological role, natural sources, recommended dietary allowance, manifestations of deficiency. Water-soluble drugs.
69. Vitamin E: its structure, biological role, natural sources, recommended dietary allowance. Manifestations of deficiency.
70. Vitamins C and P, their structure, biological role, natural sources, recommended dietary allowance. Hypo- and avitaminosises.
71. Vitamin B_1: structure, biological role, natural sources, recommended dietary allowance. Avitaminosis.
72. Vitamin B_2 and PP: their structure (co-enzyme forms), biological role, natural sources, recommended dietary allowance.
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74. Folic acid. Structure, folate co-enzymes, recommended dietary allowance. Sulfanilamides as antimetabolites.
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79. Synthesis of heme and hemoglobin, its regulation. Iron metabolism: transport, deposition, daily requirements. Erythrocytes metabolism: role of glycolysis and pentose phosphate pathway.
80. Functional biochemistry of kidneys, specific features of metabolism, functions of kidneys. Urine formation, notion of renal threshold substances.
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84. Catabolism of heme. Unconjugated and conjugated bilirubin. Disorders of bilirubin metabolism. Types of jaundice. Biochemical mechanisms of pathogenesis of hepatic insufficiency, serous-biochemical hepatic syndromes.
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Редактор Н.Ю. Коневалова
Технический редактор И.А. Борисов
Компьютерная верстка В.Г. Букатая
Корректор С.В Буянова, В.Г. Букатая

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