MINISTRY OF HEALTH OF UKRAINE
ZAPORIZHZHIA STATE MEDICAL UNIVERSITY

Biological Chemistry Department

Biochemistry of Blood and Kidneys

Manual for «Biological chemistry» discipline for teachers

Zaporizhzhia
2017

UDC 577.12(075.8)
B60

Ratified on meeting of the Central methodical committee
of Zaporozhye State Medical University
(protocol N _3__ from _02_03_2017_)
and it is recommended for the use in educational process for foreign students.

Reviewers:

Prihodko O. B., Dr. Hab, assoc. Professor, Head of Department of Medical Biology, Parasitology and Genetics. Zaporizhzhia State Medical University.

Gancheva O. V., Dr. Hab., Professor, Head of Department of Patological physiology, Zaporizhzhia State Medical University.

Compilers:
Aleksandrova K. V., Dr. Hab., Professor;
Krisanova N. V., Ph. D., Assoc. Professor;
Levich S. V., Ph. D., Sen. Teacher.

Biochemistry of Blood and Kidneys : manual for «Biological chemistry» discipline for teachers / comp. : K. V. Aleksandrova, N. V. Krisanova, S. V. Levich. – Zaporizhzhia : [ZSMU], 2017.- 85 p.

Manual was made in accordance with the program of biological chemistry for training of students of higher educational institutions of III-IV levels of accreditation for specialty 7.12010001 «General Medicine».

This manual is recommended to use for teachers that works with students of International Department (the second year of study).

CONTENT

RELEVANCE

Blood is a body fluid in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells.

Kidney – the couple organ, which is responsible for excreting of final products of metabolism and for homeostasis. They regulate water and mineral metabolism, acid-base balance, excreting of nitrogenous slags, osmotic pressure. Also they regulate arterial pressure and erhythropoesis.

Urine – fluid with different organic and inorganic compounds, which must be excreted (excess of water, final products of nitrogen metabolism, xenobiotics, products of protein's decay, hormones, vitamins and their derivates). Most of them present in urine in a bigger amount than in blood plasma. So, urine formation – is not passive process (filtration and diffusion only).

Changes in the chemical composition of blood or urine are important diagnostic markers of many deseases.

1. LEARNING OBJECTIVES OF LESSON № 16

To learn theoretical material about biochemistry of blood tissue, proteins of blood plasma, non-protein components of blood plasma at healthy and diseased people.

Necessary to know:

1. Blood biochemical functions.
2. Chemical composition for blood plasma and blood serum
3. Physical and chemical properties of the blood.
4. Blood plasma proteins.
5. Acute phase proteins in the blood plasma in some diseases.
6. The enzymes of blood.
7. Coagulation system, anticoagulation system and fibrinolysis in the blood.
8. Non-protein nitrogen-containing compounds of the blood plasma of healthy and diseased people.
9. The main carbohydrates, organic acids of the blood plasma.
10. Blood electrolytic composition.
11. Buffer systems of the blood.
12. A transport of hydrophobic organic compounds and ions across the blood stream.

Necessary to be able to do:

1. Determination of aspartate aminotransferase activity in the blood serum
2. Determination of sodium in blood serum
3. Determination of chloride in biological fluids (serum or urine)

2. EDUCATIONAL OBJECTIVES

Knowledge about biochemistry of blood tissue, proteins of blood plasma, non-protein components of blood plasma at healthy and diseased people are necessary for proper understanding of clinical subjects and formation of clinical thinking.

3. CONTENT OF EDUCATIONAL MATERIAL

1. A characteristic of blood biochemical functions (examples).
2. A comparative characteristic of chemical composition for blood plasma and blood serum
3. Physical and chemical properties of the blood.
4. Blood plasma proteins: their characteristics for structure and function.
5. Acute phase proteins in the blood plasma in some diseases.
6. The enzymes of blood: classification, diagnostic role.
7. General notions about coagulation system, anticoagulation system and fibrinolysis in the blood.
8. The major non-protein nitrogen-containing compounds of the blood plasma of healthy and diseased people (urea, uric acid, bilirubins, amino acids, creatine, creatinine, indican, hippuric acid, some hormones, etc.)
9. The main carbohydrates, organic acids of the blood plasma.
10. Blood electrolytic composition. Mineral components of the blood.
11. Buffer systems of the blood. Acid-base balance of the blood and its disorders (respiratory and metabolic acidosis, alkalosis).
12. A transport of hydrophobic organic compounds and ions across the blood stream (bilirubin, all the lipids, some hormones, Fe^{2+}/ Fe^{3+}, Ca^{2+}, Zn^{2+}, Cu^{2+}).

4. PLAN AND ORGANIZATIONAL STRUCTURE OF LESSON

Stage	Time (min)	Educational materials		Place
		Content of the plan point	Equipment	
1.Organizational moment	5 min	Check of present students		Classroom
2. Discussion of biochemistry of blood tissue, proteins of blood plasma, non-protein components of blood plasma at healthy and diseased people; organization of laboratory work carrying out	25 min	Clarification of the most important terms: blood plasma, albumins, globulins, fibrinogen, aminotransferases, anemia, buffer system, erythrocytes, thrombocytes, acid-base disbalance.	Textbook, Methodical recommendations for practical lesson, Tables of blood components	Classroom
To divide group on two subgroups. Subgroup №1: The determination of aspartate aminotransferase activity in the blood serum Subgroup №2: Determination of sodium in blood serum Subgroup №3: Determination of chloride in biological fluids Each subgroup makes its laboratory task, but records results of all tasks.				
Brake	10 minutes			
4. Beginning of laboratory work procedure	25 min	Subgroups make their laboratory tasks and explain obtained results	Equipment and reagent for laboratory work	Classroom
5. Record of results into protocols	5 min		Textbook, protocol	Classroom
6. Control of knowledge of lesson material (written independent work)	25 min		Tasks	Classroom
7. Final conversation about results of all types of students works during the lesson, motivation for the next practical class	5 min	Check and sign of protocols, analysis of students marks, informing about next lesson topic and tasks for independent work		Classroom

5. LABORATORY WORK ALGORITHM

1. The determination of aspartate aminotransferase activity in the blood serum

Principle of method: Glutamate and oxaloacetate are formed under the action of AsAT from α-ketoglutarate and aspartate. Oxaloacetate decarboxylation gives pyruvate. The pyruvate can act with 2.4-dinitrophenylhydrazine to produce dinitrophenylhydrazone of a brown color. The intensity of coloring is proportional to the quantity of pyruvic acid released during the reaction.

Equipment and reagents: glass tubes, pipettes, micropipettes, substrate-buffer solution, stop reagent, blood serum, 0.4 N aqueous solution of sodium hydroxide (NaOH), cuvettes.

***Course of the work*:** Prepare reactive solutions according to scheme:

Add, in ml	**Test sample**	**Control sample**
Substrate-buffer solution	0.5	0.5
Incubation in a dry-air thermostat at 37^0C for 3 min		
Stop reagent	-	0.5
Blood serum	0.1	0.1
Incubation in a dry-air thermostat at 37^0C for 30 min		
Stop reagent	0.5	-
Let them stay at room temperature for 20 min		
0.4 N NaOH	5	5
Let them stay at room temperature for 10 min		

Measure the optical density of experimental test against control one in cuvettes (10mm) at light-green filter (490-540 nm). Make the calculation of enzyme activity in the blood serum using the graph curve.

Result: Aspartate aminotransferase activity should be 0.1-0.45 μmole/ml (5-40 units/ml) · 60 min.

Clinical significance: Organic infringements at sharp and chronic lesions are accompanied with cell destruction and as result there is an output of aminotransferases from the center of lesion into the blood. Change activity of AsAT increases as early as in 3-6 hours after the attack of acute paroxys pains (20-30 times as much) and is high during 3-7 days. De Ritis's factor (AsAT/AlAT = 1.33±0.42 at healthy people) grows considerably at severe infarction of myocardium.

2. Determination of sodium in blood serum

Principle of method: Sodium, contained in the sample, binds precipitant reagent. Precipitant reagent ions remaining in solution form a colored complex with thioglycolate (mercaptoacetate***)***. The concentration of sodium is proportional to the difference between the control (without precipitation) and experimental samples.

Equipment and reagents: glass tubes, pipettes, micropipettes, reagent №1, reagent № 2, distilled water, blood serum, cuvettes, centrifuge.

Course of the work: Collect reaction mixtures according to scheme*:*

Solution	**Experimental sample, ml**	**Control sample, ml**
Reagent №1	1.0	1.0
Blood serum	0.02	-
Distilled water	-	0.02

Samples are thoroughly mixed and incubated for 5 minutes at a temperature of 18-25^{0}C, and then again stirred (not less than 30 seconds) and incubated for 30 minutes in the dark. Then the samples are centrifuged at 1000 rpm for 10 minutes. Transparent supernatant is used for further analysis.

Mix 0.02 mL of experimental and control samples supernatant with 2 ml of reagent № 2 and the optical density of the experimental and control samples are measured against water at a wavelength of 365 or 405 nm in 5 minutes after

mixing. Color is stable for 25 minutes after incubation if the samples are protected from direct light.

Note: the intensity of color of the samples is inversely proportional to the concentration of sodium in the sample.

The calculation of sodium concentration of in the experimental sample is performed according to the formula:

$$C = \frac{E_{contr} - E_{exp}}{E_{contr} - E_{st}} \times 150$$, where

C - concentration of sodium in the experimental sample, mmol/L;

E exp -extinction of experimental sample;

E contr - extinction of a control sample;

E st - extinction of the standard sample (the result is given by a laboratory assistant);

150 - sodium concentration in the standard sample, mmol/L.

Result: Reference values of sodium concentration in blood plasma are 135 - 155 mmol/dL.

Clinical significance:

Hyponatremia is reduced sodium concentration in plasma below 135 mmol/L. Contribute to the development of hyponatremia:

- diuretics intake, osmotic diuresis (diseases for which osmotically active compounds in blood such as glucose, urea are accumulated), kidney disease (acute and chronic pyelonephritis, urinary obturation, polycystic kidney disease);

- loss of sodium associated with diseases of the gastrointestinal tract (vomiting, fistula of the small intestine, etc.);

- the use of aminoglycoside antibiotics (gentamicin);

- adrenal insufficiency (Addison's disease);

Hypernatremia is increased concentration of sodium in the blood serum above 150 mmol/L. Always associated with hyperosmolarity. Hypernatremia can cause:

- dehydration when water depletion: prolonged sweating without a pertinent water compensation, loss of water by the gastrointestinal tract (diarrhea, vomiting), skin (burns);

- diabetes insipidus (decreased sensitivity of renal receptors to ADH);

- kidney disease proceeding with oliguria;

- hyperaldosteronism (Cushing's syndrome).

Sodium belongs to a threshold substance and increase its concentration in the blood leads to an increase in its excretion.To assess the balance of sodium in the body need to determine simultaneously its content in blood and urine.

3. Determination of chloride in biological fluids (serum or urine)

Principle of method: In strongly acidic medium chloride ion releases thiocyanate ion from mercury thiocyanate (II). Then thiocyanate ion reacts with ions of iron (III) to form a coloured product.The color intensity of the thiocyanate iron is proportional to the concentration of chloride ions in the sample.

Equipment and reagents: glass tubes, pipettes, micropipettes, working reagent, blank reagent, blood serum (urine), cuvettes.

Course of the work: Preparation of urine sample: urine is 2-fold diluted with distilled water and a drop of nitric acid is added to acidic pH.

The analysis is conducted in accordance with the scheme set out in the table below:

Solution	**Experimental sample, ml**	**Control sample, ml**
Working reagent	5.0	-
Blood serum or deluted urine	0.05	0.05
Blank reagent	-	5.0

Combine all the ingredients of the reaction mixture and kept it for 10 minutes at room temperature. Measure the optical density of experimental sample

against the control one. Photometry is carried out at a wavelength of 450 (440-480) nm in cuvette of 10 or 5 mm.

The calculation of chloride concentration is carried out according to the formula:

$$C = \frac{E_{exp}}{E_{st}} \times 100,$$ where

C - concentration of chlorides in the experimental sample, mmol/L;

E exp - extinction of experimental sample;

E st - extinction of the standard sample (the result is given by a laboratory assistant);

100 - chloride concentration in the standard solution, mmol/L.

To calculate the concentration of chloride in the daily urine: the above value is multiplied by 2 (dilution factor) and the volume of daily urine, expressed in liters (get mmol / day).

Result: Reference values of chloride concentration: in blood serum is 98-107 mmol/L; in urine is 250 mmol/day.

Clinical significance:

Hypochloremia may cause such diseases and conditions as:

- increased allocation of chlorine in sweat in a hot climate, with fever states, with diarrhea;
- diabetic acidosis, which is usually accompanied by a transition of chlorine from the blood into the tissues;
- renal diabetes, due to the large loss of chloride in the urine.
- diseases of the adrenal glands in violation of the mineralocorticoid formation.

Hyperchloremia is divided into absolute developing under violation of renal excretory function, and the relative associated with dehydration of body and clotting of blood. When nephrosis, nephritis, and especially nephrosclerosis occurs then salts are delayed in the body and hyperchloremia is developed. Insufficient intake of water in the body, diarrhea, vomiting, loss of water and salts with burns can lead to dehydration and development of relative hyperchloremia.

Hyperchloremia may occur during decompensation of the cardiovascular system, the development of edema, comes with food large quantity of sodium chloride. In addition, hyperchloremia possible with alkalosis, with resorption of edema, exudate and transudate.

6. TESTS

1. Name the enzyme which is the indicator of myocardium damage if its activity will be increased in the blood plasma in 10 times or more:

A. Alkaline phosphatase

B. Malate dehydrogenase

C. Glutamate dehydrogenase

D. Guanine transaminase

E. Aspartate transaminase

2. Point out the most probable location of the plasma proteins synthesis:

A. Kidneys

B. Muscle tissue

C. Nervous tissue

D. Liver

E. Lungs

3. Point out the blood plasma protein, participating in the blood oncotic pressure maintaining:

A. Globulin

B. Lipoprotein

C. Ceruloplasmin

D. Hemoglobin

E. Albumin

4. Point out the protein, which is not observed in the blood serum of healthy people:

A. Cryoglobulin

B. Albumin

C. Transferin

D. Haptoglobin

E. Alpha2-macroglobulin

5. Name the excretory enzyme of the blood plasma:

A. Alkaline phosphatase

B. Malate dehydrogenase

C. Glutamate dehydrogenase

D. Alanine transaminase

E. Aspartate transaminase

6. Blood is the tissue needed for the transport of all absorbed products in the gut after digestion processes. Name the function of the blood described above:

A. Body temperature regulatory function

B. Transport of hormones

C. Nutrition function

D. The maintenance of acid-base balance in the organism

E. Protection against microbial agents

7. Name the blood plasma protein used as inhibitor of some proteolytic enzymes:

A. Albumin

B. Immunoglobulin G

C. C-reactive protein

D. Alpha1-antitrypsin

E. Ceruloplasmin

8. Name the method used now as modern technique for the separation and determination of the content of some proteins in the blood plasma at the same time:

A. Dialysis

B. Immunoelectrophoresis

C. Spectrophotometry method

D. X-ray radiation method

E. Densitometry method

9. Name the factor of blood coagulation system needed for fibrin formation from fibrinogen:

A. Plasmin

B. Heparin

C. Thrombin

D. Prothrombin

E. Lysine

10. Point out the protease of blood that helps to solvate the fibrin clot:

A. Plasminogen

B. Lysolipase

C. Plasmin

D. Antifibrinogen

E. Tromboplastin

11. Point out the permissible range of the pH fluctuation in the blood:

A. 8.0-8.61

B. 7.37-7.44

C. 7.81-7.94

D. 6.2-6.84

E. 6.85-7.0

12. Point out the non-protein nitrogenous component of the blood plasma that is in a level about 50% of total non-protein nitrogen:

A. Uric acid

B. Creatine

C. Creatinine

D. Amino acids

E. Urea

13. Point out the blood microelement:

A. Sodium

B. Copper

C. Calcium

D. Potassium

E. Magnesium

14. Point out the most powerful buffer system of the blood:

A. The bicarbonate buffer system

B. The phosphate buffer system

C. The protein buffer system

D. Hemoglobin buffer system

E. The acetate buffer system

15. Name the index of blood plasma which helps to recognize the change in biliary system function at cholestasis state:

A. Fibrinogen

B. Conjugated bilirubin

C. Uric acid

D. Urea

E. Creatine

16. Point out the major transport form of triacylglycerols from the intestine to the liver and other tissues:

A. Chylomicrons

B. LDL

C. VLDL

D. IDL

E. HDL

17. Creatine level is much higher then normal, creatinine level is lower then normal in the blood plasma of patient. Choose the probable diagnosis for this patient:

A. Myocardium infarction

B. Cholestasis

C. Viral hepatitis

D. Phenylketonuria

E. Muscular dystrophy

18. Metabolic acidosis is observed in patient` organism due to the accumulation of:

A. Sodium ions

B. Glucose

C. Pyruvate

D. Fructose

E. Glycerol

19. Renal insufficiency was proposed to look at patient due to the change of the ratio [Urea]/Residual nitrogen (80%). Name the index of the blood plasma whose content will prove this diagnosis:

A. High levels of sodium ion

B. Low levels of copper ion

C. High levels of glucose

D. High levels of creatinine

E. High levels of creatine

20. Name the indexes of blood plasma whose content may be higher at insulin-dependent diabetes mellitus:

A. Glucose

B. Cholesterol

C. Pyruvate

D. Ketone bodies

E. All the indexes named above

7. TESTS FOR PREPARATION TO «KROK-1» EXAMINATION

1. 12 hours after an acute attack of retrosternal pain a patient presented a jump of aspartate aminotransferase activity in blood serum. What pathology is this deviation typical for?

A. Viral hepatitis
B. Diabetes insipidus
C. Collagenosis
D. Diabetes mellitus
E. Myocardial infarction

2. A patient who had been working hard under condition of elevated temperature of the environment has now a changed quantity of blood plasma proteins. What phenomenon is the case?

A. Absolute hyperproteinemia
B. Relative hyperproteinemia
C. Absolute hypoproteinemia
D. Disproteinemia
E. Paraproteinemia

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

A. Proteinuria
B. Hypoproteinemia
C. Hypoglobunemia
D. Hyperalbuminemia
E. Paraproteinemia

4. Diabetes mellitus causes ketosis as a result of activated oxidation of fatty acids. What disorders of acid-base equilibrium may be caused by excessive accumulation of ketone bodies in blood?

A. Metabolic alkalosis
B. Metabolic acidosis
C. Respiratory alkalosis
D. Respiratory acidosis
E. Any changes won't happen

5. A 63-year-old woman developed symptoms of rheumatoid arthritis. Their increase of which blood values indicators could be most significant in proving the diagnosis?

A. R-glycosidase
B. Acid phosphatase
C. Lipoproteins
D. General cholesterol
E. Additive glycosaminoglycans

6. Marked increase of activity of MB-forms of CPK (creatinephosphokinase) and LDH-1 was revealed by examination of the patient's blood. What is the most probable pathology?

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

7. There is high activity of $LDH_{1,2}$, aspartate aminotransferase, creatine phosphokinase in the blood of patient. In what organs (tissues) the development of pathological process is the most probable?

A. In the heart muscle {initial stage of myocardium infraction}
B. In skeletal muscle {dystrophy, atrophy}
C. In kidneys and adrenals
D. In liver and kidneys
E. In connective tissue

8. The high level of Lactate Dehydrogenase (LDH) isozymes concentration showed the increase of LDH-1 and LDH-2 in a patient's blood plasma. Point out the most probable diagnosis.

A. Diabetes mellitus
B. Skeletal muscle dystrophy
C. Myocardial infarction
D. Acute pancreatitis
E. Viral hepatitis

9. Analysis of blood serum of a patient revealed the increase of alanine aminotransferase and aspartate aminotransferase levels. What cytological changes can cause such a situation?

A. Disturbance of genetic apparatus of cells
B. Cellular breakdown
C. Disorder of enzyme systems of cells
D. Disturbance of cellular interrelations
E. Disturbed energy supply of cells

10. A worker has decreased buffer capacity of blood due to exhausting muscular work. What acidic substance that came to blood caused this phenomenon?

A. 3-phosphoglycerate
B. 1,3-bisphosphoglycerate
C. Lactate

D. α-ketoglutarate

E. Pyruvate

11. Blood sampling for bulk analysis is recommended to be performed on an empty stomach and in the morning. What changes in blood composition can occur if to perform blood sampling after food intake?

A. Reduced contents of erythrocytes

B. Increased contents of erythrocytes

C. Increased contents of leukocytes

D. Increased plasma proteins

E. Reduced contents of thrombocytes

12. Examination of a 43 y.o. anephric patient revealed anemia symptoms. What is the cause of these symptoms?

A. Folic acid deficit

B. Vitamin B_{12} deficit

C. Reduced synthesis of erythropoietins

D. Enhanced destruction of erythrocytes

E. Iron deficit

13. A 55 y.o. women consulted a doctor about having continuous cyclic uterine hemorrhages for a year, weakness, dizziness. Examination revealed skin pallor. Hemogram: Hb – 70 g/L, erythrocytes-3.2 x 10^{12}/L, color index – 0.6; leukocytes – 6.0 x 10^{9}/L, reticulocytes – 1%, erythrocyte hypochromia. What anemia is it?

A. Iron-deficiency anemia

B. B_{12}-folate-deficiency anemia

C. Hemolytic anemia

D. Aplastic anemia

E. Chronic posthemorrhagic anemia

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

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

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

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

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

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

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

A. From the intercellular fluid to the capillaries

B. No changes of water movement will be observed

C. From the intercellular to the cells

D. From the cells to the intercellular fluid

E. From the capillaries to the intercellular fluid

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

A. γ-globulins

B. Albumins

C. α_1-globulins

D. β-globulins

E. α_2-globulins

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

A. Increased proteolysis

B. Decrease in the concentration of amino acids in blood

C. Albuminosis

D. Increase in the oncotic pressure in the blood plasma

E. Increase in low-density lipoprotein level

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

A. Immunoglobulin

B. Ceruloplasmin, transferrin

C. a_2-macroglobulin, a_1-antitrypsin

D. Cryoglobulin, interferon

E. Hemopexin, haptoglobin

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

A. Clot retraction

B. Thromboplastin formation

C. Fibrin formation

D. Thrombin formation

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

A. Hyporlipoproteinemia type I.

B. Hyperlipoproteinemia type IV

C. Cholesterol, hyperlipoproteinemia

D. Hyperlipoproteinemia type IIa

E. Hyperlipoproteinemia type IIb

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

A. Creatine kinase reaction

B. Anaerobic glycolysis

C. Cyclase reaction

D. Aerobic glycolysis

E. Oxidative phosphorylation

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

A. Alpha-1-proteinase inhibitor

B. Haptoglobin

C. Cryoglobulin

D. Alpha-2-macroglobulin

E. Transferrin

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

A. Hemolytic anemia

B. Megaloblastic anemia

C. Siclemia

D. Iron-deficiency anemia

E. Pernicous anemia

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

A. Interferon

B. Tumor necrosis factor

C. Cytokines

D. Interleukin-2

E. Interleukin-4

8. THEORETIC MATERIAL FOR LESSON 16

Blood is a body fluid in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells.

FUNCTIONS

Blood has three main functions: transport, protection and regulation.

Transport

Blood transports the following substances:

- Gases, namely oxygen (O_2) and carbon dioxide (CO_2), between the lungs and rest of the body
- Nutrients from the digestive tract and storage sites to the rest of the body
- Waste products to be detoxified or removed by the liver and kidneys
- Hormones from the glands in which they are produced to their target cells
- Heat to the skin so as to help regulate body temperature

Protection

Blood has several roles in inflammation:

- Leukocytes, or white blood cells, destroy invading microorganisms and cancer cells
- Antibodies and other proteins destroy pathogenic substances
- Platelet factors initiate blood clotting and help minimise blood loss

Regulation

Blood helps regulate:

- pH by interacting with acids and bases
- Water balance by transferring water to and from tissues

COMPOSITION OF BLOOD

Blood is classified as a connective tissue and consists of two main components:

1. Plasma, which is a clear extracellular fluid
2. Formed elements, which are made up of the blood cells and platelets

The formed elements are so named because they are enclosed in a plasma membrane and have a definite structure and shape. All formed elements are cells except for the platelets, which are tiny fragments of bone marrow cells.

Formed elements are:

- Erythrocytes, also known as red blood cells (RBCs)
- Leukocytes, also known as white blood cells (WBCs)
- Platelets

Human RBCs do not contain mitochondria, so the main pathway for ATP production in these cells is anaerobic glycolysis.

Platelets play important role in blood clotting. Deficiency of VIII factor lead to hereditary coagulopathy caused by blockage of thromboplastin formation

Leukocytes are further classified into two subcategories called granulocytes which consist of neutrophils, eosinophils and basophils; and agranulocytes which consist of lymphocytes and monocytes. Lymphocytes synthesize interferon – universal antiviral agents as a response to viral invasion.

The formed elements can be separated from plasma by centrifuge, where a blood sample is spun for a few minutes in a tube to separate its components according to their densities. RBCs are denser than plasma, and so become packed into the bottom of the tube to make up 45% of total volume. This volume is known as the haematocrit. WBCs and platelets form a narrow cream-coloured coat known as the buffy coat immediately above the RBCs. Finally, the plasma makes up the top of the tube, which is a pale yellow colour and contains just under 55% of the total volume.

ANEMIA

Anemia, also spelled **anaemia**, is usually defined as a decrease in the amount of red blood cells (RBCs) or hemoglobin in the blood. It can also be defined as a lowered ability of the blood to carry oxygen. When anemia comes on slowly, the symptoms are often vague and may include: feeling tired, weakness, shortness of breath or a poor ability to exercise. Anemia that comes on quickly often has greater symptoms, which may include: confusion, feeling like one is going to pass out, loss of consciousness, or increased thirst. Anemia must be significant before a person becomes noticeably pale. Additional symptoms may occur depending on the underlying cause.

There are three main types of anemia: that due to blood loss, that due to decreased red blood cell production, and that due to increased red blood cell breakdown. Causes of blood loss include trauma and gastrointestinal bleeding, among others. Causes of decreased production include iron deficiency, a lack of vitamin B_{12}, thalassemia, and a number of neoplasms of the bone marrow. Causes of increased breakdown include a number of genetic conditions such as sickle cell anemia, infections like malaria, and certain autoimmune diseases. It can also be classified based on the size of red blood cells and amount of hemoglobin in each cell. If the cells are small, it is microcytic anemia. If they are large, it is macrocytic anemia while if they are normal sized, it is normocytic anemia. Diagnosis in men is based on a hemoglobin of less than 130 to 140 g/L (13 to 14 g/dL), while in women, it must be less than 120 to 130 g/L (12 to 13 g/dL). Further testing is then required to determine the cause.

Signs and symptoms

Anemia goes undetected in many people and symptoms can be minor. The symptoms can be related to an underlying cause or the anemia itself. Most commonly, people with anemia report feelings of weakness, or fatigue, general malaise, and sometimes poor concentration. They may also report dyspnea (shortness of breath) on exertion. In very severe anemia, the body may compensate

for the lack of oxygen-carrying capability of the blood by increasing cardiac output. The patient may have symptoms related to this, such as palpitations, angina (if pre-existing heart disease is present). There may be signs of specific causes of anemia, e.g., koilonychia (in iron deficiency), jaundice (when anemia results from abnormal break down of red blood cells — in hemolytic anemia), bone deformities (found in thalassemia major) or leg ulcers (seen in sickle-cell disease). In severe anemia, there may be signs of a hyperdynamic circulation: tachycardia (a fast heart rate), bounding pulse, flow murmurs, and cardiac ventricular hypertrophy (enlargement). There may be signs of heart failure. Pica, the consumption of non-food items such as ice, but also paper, wax, or grass, and even hair or dirt, may be a symptom of iron deficiency, although it occurs often in those who have normal levels of hemoglobin.

Causes

Figure shows normal red blood cells flowing freely in a blood vessel. The inset image shows a cross-section of a normal red blood cell with normal hemoglobin.

The causes of anemia may be classified as impaired red blood cell (RBC) production, increased RBC destruction (hemolytic anemias), blood loss and fluid overload (hypervolemia). Several of these may interplay to cause anemia eventually. Indeed, the most common cause of anemia is blood loss, but this usually does not cause any lasting symptoms unless a relatively impaired RBC production develops, in turn most commonly by iron deficiency.

Impaired production

- Disturbance of proliferation and differentiation of stem cells
  - Pure red cell aplasia
  - Aplastic anemia affects all kinds of blood cells. Fanconi anemia is a hereditary disorder or defect featuring aplastic anemia and various other abnormalities.
  - Anemia of renal failure by insufficient erythropoietin production
  - Anemia of endocrine disorders
- Disturbance of proliferation and maturation of erythroblasts

- Pernicious anemia is a form of megaloblastic anemia due to vitamin B_{12} deficiency dependent on impaired absorption of vitamin B_{12}. Lack of dietary B_{12} causes non-pernicious megaloblastic anemia
- Anemia of folic acid deficiency, as with vitamin B_{12}, causes megaloblastic anemia
- Anemia of prematurity, by diminished erythropoietin response to declining hematocrit levels, combined with blood loss from laboratory testing, generally occurs in premature infants at two to six weeks of age.
- Iron deficiency anemia, resulting in deficient heme synthesis
- Thalassemias, causing deficient globin synthesis
- Congenital dyserythropoietic anemias, causing ineffective erythropoiesis
- Anemia of renal failure (also causing stem cell dysfunction)

- Other mechanisms of impaired RBC production

- Myelophthisic anemia or myelophthisis is a severe type of anemia resulting from the replacement of bone marrow by other materials, such as malignant tumors or granulomas.
- Myelodysplastic syndrome
- anemia of chronic inflammation

Increased destruction

Anemias of increased red blood cell destruction are generally classified as hemolytic anemias. These are generally featuring jaundice and elevated lactate dehydrogenase levels. Glutathione peroxidase deficiency and low concentration of reduced glutathione also lead to the RBCs restruction.

Blood loss

- Anemia of prematurity from frequent blood sampling for laboratory testing, combined with insufficient RBC production
- Trauma or surgery, causing acute blood loss

Fluid overload

Fluid overload (hypervolemia) causes decreased hemoglobin concentration and apparent anemia:

- General causes of hypervolemia include excessive sodium or fluid intake, sodium or water retention and fluid shift into the intravascular space.
- Anemia of pregnancy is induced by blood volume expansion experienced in pregnancy.

BLOOD PLASMA

Blood plasma is a mixture of proteins, enzymes, nutrients, wastes, hormones and gases. The specific composition and function of its components are as follows:

Proteins

These are the most abundant substance in plasma by weight and play a part in a variety of roles including clotting, defence and transport. Collectively, they serve several functions:

- They are an important reserve supply of amino acids for cell nutrition. Cells called macrophages in the liver, gut, spleen, lungs and lymphatic tissue can break down plasma proteins so as to release their amino acids. These amino acids are used by other cells to synthesise new products.
- Plasma proteins also serve as carriers for other molecules. Many types of small molecules bind to specific plasma proteins and are transported from the organs that absorb these proteins to other tissues for utilisation. The proteins also help to keep the blood slightly basic at a stable pH. They do this by functioning as weak bases themselves to bind excess H+ ions. By doing so, they remove excess H+ from the blood which keeps it slightly basic.
- The plasma proteins interact in specific ways to cause the blood to coagulate, which is part of the body's response to injury to the blood vessels (also known as vascular injury), and helps protect against the loss of blood and invasion by foreign microorganisms and viruses.
- Plasma proteins govern the distribution of water between the blood and tissue fluid by producing what is known as a colloid osmotic pressure.

There are three major categories of plasma proteins, and each individual type of proteins has its own specific properties and functions in addition to their overall collective role:

Albumin: This is the most abundant class of plasma proteins (2.8 to 4.5 gm/100ml) with highest electrophoretic mobility. It is soluble in water ad is precipitated by fully saturated ammonium sulphate. Albumin is synthesized in liver and consists of a single polypeptide chain of 610 amino acids having a molecular weight of 69,000. It is rich in some essential amino acids such as lysine, leucine, valine, phenylalanine, threonine, arginine and histidine. The acidic amino acids like aspartic acid and glutamic acid are also concentrated in albumin. The presence of these residues makes the molecule highly charged with positive and negative charge. Besides having a nutritive role, albumin acts as a transport carrier for various biomolecules such s fatty acids, trace elements and drugs. Another important role of albumin is in the maintenance of osmotic pressure and fluid distribution between blood and tissues.

Globulins, which can be subdivided into three classes from smallest to largest in molecular weight into alpha, beta and gamma globulins. The globulins include high density lipoproteins (HDL), an alpha-1 globulin, and low density lipoproteins (LDL), a beta-1 globulin. HDL functions in lipid transport carrying fats to cells for use in energy metabolism, membrane reconstruction and hormone function. HDLs also appear to prevent cholesterol from invading and settling in the walls of arteries. LDL carries cholesterol and fats to tissues for use in manufacturing steroid hormones and building cell membranes, but it also favours the deposition of cholesterol in arterial walls and thus appears to play a role in disease of the blood vessels and heart. HDL and LDL therefore play important parts in the regulation of cholesterol and hence have a large impact on cardiovascular disease.

By electrophoresis plasma globulins are separated into α1, α2,β and ¥-globulins are synthesized in liver, whereas ¥-globulins are formed in the cells of reticulo-endothelial system. The average normal serum globulin (total)

concentration is 2.5 gm / 100 ml (Howe method) or 3.53 gm/100 ml by electrophoresis.

α1-Globulin: This fraction includes several complex proteins containing carbohydrates and lipids. These are, orosomucoid, α1-glycoprotein and α-lipoproteins. The normal serum level of α1-globulin is 0.42 gm/100 ml.

Orosomucoid is rich in carbohydrates. It is water-soluble, heat stable and has a molecular weight of 44,000. It serves to transport hexosamine complexes to tissues.

Lipoproteins are soluble complexes which contain non-covalently bound lipid. These proteins act mainly as transport carrier to different types of lipids in the body. Increasing of low-density lipoprotein fraction (LDL) could cause hyperlipoproteinemia type IIa.

α_1-antitrypsin (α_1-proteinase inhibitor) – glycoprotein with a molecular weight 55 kDa. Its concentration in blood plasma is 2-3 г/л. The main biological property of this inhibitor is its capacity to form complexes with proteinases oppressing proteolitic activity of such enzymes as trypsin, chemotrypsin, plasmin, trombin. The content of α_1-antitrypsin is markedly increased in inflammatory processes. The inhibitory activity of α_1-antitrypsin is very important in pancreas necrosis and acute pancreatitis because in these conditions the proteinase level in blood and tissues is sharply increased. The congenital deficiency of α_1-antitrypsin results in the lung emphysema.

α2-Globulins: This fraction also contains complex proteins such as α2-glycoproteins, plasminogen, prothrombin, haptoglobulin, ceruloplasmin (transports Cu) and α2-macroglobulin. The normal serum value of this fraction is 0.67 gm/100ml.

Plasminogen and prothrombin are in the inactive precursors of plasmin and thrombin respectively. Both these proteins play an important role in blood clotting.

Haptoglobulins are also glycoproteins having a molecular weight of 85,000. These are synthesized in liver and can bind with any free hemoglobin that may

arise in plasma due to lysis of erythrocytes and thus prevent excretion of Hb and iron associated with it.

Ceruloplasmin **-** glycoprotein of the α_2-globulin fraction. It can bind the copper ions in blood plasma. Up to 3 % of all copper contents in an organism and more than 90 % copper contents in plasma is included in ceruloplasmin. Ceruloplasmin has properties of ferroxidase oxidizing the iron ions. The decrease of ceruloplasmin in organism (Wilson disease) results in exit of copper ions from vessels and its accumulation in the connective tissue that shows by pathological changes in a liver, main brain, cornea.

α_2-Macroglobulin - protein of α_2-globulin fraction, universal serum proteinase inhibitor. Its contents (2,5 g/l) in blood plasma is highest comparing to another proteinase inhibitors.

The biological role of α_2-macroglobulin consists in regulation of the tissue proteolysis systems which are very important in such physiological and pathological processes as blood clotting, fibrinolysis, processes of immunodefence, functionality of a complement system, inflammation, regulation of vascular tone (kinine and renin-angiothensine system).

β-Globulins: This fraction of plasma proteins contain these different β-lipoproteins which are very rich in lipid content. It also contains transferrin (siderophilin) which transports non-heme iron in plasma. The normal serum value of β-globulins is 0.91 gm/100ml.

Transferrin is an iron transport protein. In plasma it can be saturated even up to 33% with iron. It has a low content of carbohydrate.

γ-Globulins:

Immunoglobulins (Ig A, Ig G, Ig E, Ig M) - proteins of γ-globulin fraction of blood plasma executing the functions of antibodies which are the main effectors of humoral immunity. They appear in the blood serum and certain cells of a vertebrate in response to the introduction of a protein or some other macromolecule foreign to that species.

Immunoglobulin molecules have bindind sites that are specific for and complementary to the structural features of the antigen that induced their formation. Antibodies are highly specific for the foreign proteins that evoke their formation.

Molecules of immunoglobulins are glycoproteins. The protein part of immunoglobulins contain four polipeptide chains: two heavy H-chains and two light L-chains.

Fibrinogen: It is a fibrous protein with a molecular weight of 340,000. It has 6 polypeptide chains which are held together by disulphide linkages. Fibrinogen plays an important role in clothing of blood where it is converted to fibrin by thrombin.

In addition to the above mentioned proteins, the plasma contains a number of enzymes such as ***acid phosphatase*** and ***alkaline phosphatase*** which have great diagnostic value.

Functions of Plasma Proteins:

1. ***Protein Nutrition:*** Plasma proteins act as a source of protein for the tissues, whenever the need arises.

2. ***Osmotic Pressure and water balance:*** Plasma proteins exert an osmotic pressure of about 25 mm of Hg and therefore play an important role in maintaining a proper water balance between the tissues and blood. Plasma albumin is mainly responsible for this function due to its low molecular weight and quantitative dominance over other proteins. During the condition of protein loss from the body as occurs in kidney diseases, excessive amount of water moves to the tissues producing edema.

3. ***Buffering action:*** Plasma proteins help in maintaining the pH of the body by acting asampholytes. At normal blood pH they act as acids and accept captions.

4. ***Transport of Lipids:*** One of the most important functions of plasma proteins us to transport lipids and lipid soluble substances in the body. Fatty acids and bilirubin are transported mainly by albumin, whereas cholesterol and

phospholipids are carried by the lipoproteins present in β-globulins also transport fat soluble vitamins (A, D, K and E)

5. ***Transport of other substances:*** In addition to lipids, plasma proteins also transport several metals and other substances α2-Globulins transport copper (Ceruloplasmin), bound hemoglobin (haptoglobin) and thyroxine (glycoprotein) and non-heme iron is transported by transferrin present in β-globulin fraction. Calcium, Magnesium, some drugs and dyes and several cations and anions are transported by plasma albumin.

6. ***Blood Coagulation:*** Prothrombin present in α2-globulin fraction and fibrinogen, participate in the blood clotting process as follows.

Causes and consequences of protein content changes in blood plasma.

Hypoproteinemia - decrease of the total contents of proteins in blood plasma. This state occurs in old people as well as in pathological states accompanying with the oppressing of protein synthesis (liver diseases) and activation of decomposition of tissue proteins (starvation, hard infectious diseases, state after hard trauma and operations, cancer). Hypoproteinemia (hypoalbuminemia) also occurs in kidney diseases, when the increased excretion of proteins via the urine takes place.

Hyperproteinemia - increase of the total contents of proteins in blood plasma. There are two types of hyperproteinemia - absolute and relative.

Absolute hyperproteinemia – accumulation of the proteins in blood. It occurs in infection and inflammatory diseases (hyperproduction of immunoglobulins), rheumatic diseases (hyperproduction of C-reactive protein), some malignant tumors (myeloma) and others.

Relative hyperproteinemia – the increase of the protein concentration but not the absolute amount of proteins. It occurs when organism loses water (diarrhea, vomiting, fever, intensive physical activity etc.).

Paraproteinemia, also known as **monoclonal gammopathy**, is the presence of excessive amounts of paraprotein or single monoclonal gammaglobulin in the blood. It is usually due to an underlying immunoproliferative disorder or

hematologic neoplasms, especially multiple myeloma (presence of Bence Jones protein).

Enzymes

Blood plasma contains many enzymes, which are classified into functional and non-functional plasma enzymes.

Differences between functional and non-functional plasma enzymes represents in table 1

Table 1

	Functional plasma enzymes	**Non-functional plasma enzymes**
Concentration in plasma	Present in plasma in higher concentrations in comparison to tissues	Normally, present in plasma in very low concentrations in comparison to tissues.
Function	Have known functions	No known functions
The substrates	Their substrates are always present in the blood	Their substrates are absent from the blood
Site of synthesis	Liver	Different organs e.g. liver, heart, brain and skeletal muscles
Effect of diseases	Decrease in liver diseases	Different enzymes increase in different organ diseases
Examples	Clotting factors e.g. prothrombin, Lipoprotein lipase and pseudo-choline esterase	ALT, AST, CK, LDH, alkaline phosphatase, acid phosphatase and amylase,

Sources of non-functional plasma enzymes :

1. Increase in the rate of enzyme synthesis) e.g. bilirubin increases the rate of synthesis of alkaline phosphatase in obstructive liver diseases.
2. Obstruction of normal pathway e.g. obstruction of bile ducts increases alkaline phosphatase.
3. Increased permeability of cell membrane as in tissue hypoxia.
4. Cell damage with the release of its content of enzymes into the blood e.g. myocardial infarction and viral hepatitis.

Medical importance of non-functional plasma enzymes :

Measurement of non-functional plasma enzymes is important for:

1. Diagnosis of diseases as diseases of different organs cause elevation of different plasma enzymes.

2. Prognosis of the disease; we can follow up the effect of treatment by measuring plasma enzymes before and after treatment.

Examples of medically important non-functional plasma enzymes :

1. Amylase and lipase enzymes increase in diseases of the pancreas as acute pancreatitis.
2. Creatine kinase (CK) enzyme increases in heart, brain and skeletal muscle diseases.
3. Lactate dehydrogenase (LDH) enzyme increases in heart, liver and blood diseases.
4. Alanine transaminase (ALT) enzyme, it is also called serum glutamic pyruvic transaminase (SGPT). It increases in liver and heart diseases.
5. Aspartate transaminase (AST) enzyme, it is also called serum glutamic oxalacetic transaminase (SGOT). It increases in liver and heart diseases.
6. Acid phosphatase enzyme increases in cancer prostate.
7. Alkaline phosphatase enzyme increases in obstructive liver diseases, bone diseases and hyperparathyroidism.

For example, high activity of $LDH_{1,2}$, aspartate aminotransferase, creatine phosphokinase (MB isoform) in the blood are caused by myocardial infarction.

Amino acids

These are formed from the break down of tissue proteins or from the digestion of digested proteins. Significant proteolisys of proteins could lead to the development of aminoacidemia (increasing of aminoacid content in blood).

Nitrogenous waste

Being toxic end products of the break down of substances in the body, these are usually cleared from the bloodstream and are excreted by the kidneys at a rate that balances their production.

Nutrients

Those absorbed by the digestive tract are transported in the blood plasma. These include glucose, amino acids, fats, cholesterol, phospholipids, vitamins and minerals.

Gases

Some oxygen and carbon dioxide are transported by plasma. Plasma also contains a substantial amount of dissolved nitrogen.

Electrolytes

The most abundant of these are sodium ions, which account for more of the blood's osmolarity than any other solute.

ACID-BASE BALANCE

The body's acid–base balance is normally tightly regulated by buffering agents, the respiratory system, and the renal system, keeping the arterial blood pH between 7.36 and 7.42. Several buffering agents that reversibly bind hydrogen ions and impede any change in pH exist.

Acid-base imbalance

Acid–base imbalance is an abnormality of the human body's normal balance of acids and bases that causes the plasma pH to deviate out of the normal range (7.35 to 7.45). In the fetus, the normal range differs based on which umbilical vessel is sampled (umbilical vein pH is normally 7.25 to 7.45; umbilical artery pH is normally 7.18 to 7.38). It can exist in varying levels of severity, some life-threatening.

An excess of acid is called acidosis or acidaemia and an excess in bases is called alkalosis or alkalemia. The process that causes the imbalance is classified based on the etiology of the disturbance (respiratory or metabolic) and the direction of change in pH (acidosis or alkalosis).

Metabolic acidosis is a condition that occurs when the body produces excessive quantities of acid or when the kidneys are not removing enough acid from the body. If unchecked, metabolic acidosis leads to acidemia, i.e., blood pH is

low (less than 7.35) due to *increased* production of hydrogen ions by the body or the inability of the body to form bicarbonate (HCO_3^-) in the kidney. Its causes are diverse, and its consequences can be serious, including coma and death. Together with respiratory acidosis, it is one of the two general causes of acidemia.

Metabolic acidosis occurs when the body produces too much acid (for example, during intensive musle work too much lactate is produced), or when the kidneys are not removing enough acid from the body. There are several types of metabolic acidosis. The main causes are best grouped by their influence on the *anion gap*.

It bears noting that the anion gap can be spuriously normal in sampling errors of the sodium level, e.g. in extreme hypertriglyceridemia. The anion gap can be increased due to relatively low levels of cations other than sodium and potassium (e.g. calcium or magnesium).

Respiratory acidosis is a medical emergency in which decreased ventilation (hypoventilation) increases the concentration of carbon dioxide in the blood and decreases the blood's pH (a condition generally called acidosis).

Carbon dioxide is produced continuously as the body's cells respire, and this CO_2 will accumulate rapidly if the lungs do not adequately expel it through alveolar ventilation. Alveolar hypoventilation thus leads to an increased $PaCO_2$ (a condition called hypercapnia). The increase in $PaCO_2$ in turn decreases the $HCO_3^-/PaCO_2$ ratio and decreases pH.

Acute respiratory acidosis occurs when an abrupt failure of ventilation occurs. This failure in ventilation may be caused by depression of the **central respiratory center** by cerebral disease or drugs, inability to ventilate adequately due to **neuromuscular disease** (e.g., **myasthenia gravis**, **amyotrophic lateral sclerosis**, **Guillain-Barré syndrome**, **muscular dystrophy**), or airway obstruction related to asthma or chronic obstructive pulmonary disease (COPD) exacerbation.

Chronic respiratory acidosis may be secondary to many disorders, including COPD. Hypoventilation in COPD involves multiple mechanisms, including decreased responsiveness to hypoxia and hypercapnia, increased ventilation-

perfusion mismatch leading to increased dead space ventilation, and decreased diaphragm function secondary to fatigue and hyperinflation.

Metabolic alkalosis is a metabolic condition in which the pH of tissue is elevated beyond the normal range (7.35-7.45). This is the result of decreased hydrogen ion concentration, leading to increased bicarbonate, or alternatively a direct result of increased bicarbonate concentrations.

The causes of metabolic alkalosis can be divided into two categories, depending upon urine chloride levels.

Chloride-responsive **(Urine chloride < 20 mEq/L)**

- *Loss of hydrogen ions* - Most often occurs via two mechanisms, either vomiting or via the kidney. Vomiting results in the loss of hydrochloric acid (hydrogen and chloride ions) with the stomach contents. In the hospital setting this can commonly occur from nasogastric suction tubes. Severe vomiting also causes loss of potassium (hypokalaemia) and sodium (hyponatremia). The kidneys compensate for these losses by retaining sodium in the collecting ducts at the expense of hydrogen ions (sparing sodium/potassium pumps to prevent further loss of potassium), leading to metabolic alkalosis.

- *Congenital chloride diarrhea* - rare for being a diarrhea that causes alkalosis instead of acidosis.
- *Contraction alkalosis* - This results from a loss of water in the extracellular space, such as from dehydration.
- *Diuretic therapy* - loop diuretics and thiazides can both initially cause increase in chloride, but once stores are depleted, urine excretion will be below < 25 mEq/L.
- *Posthypercapnia* - Hypoventilation (decreased respiratory rate) causes hypercapnia (increased levels of CO2), which results in respiratory acidosis.

Chloride-resistant **(Urine chloride > 20 mEq/L)**

- *Retention of bicarbonate* - retention of bicarbonate would lead to alkalosis
- *Shift of hydrogen ions into intracellular space* - Seen in hypokalemia. Due to a low extracellular potassium concentration, potassium shifts out of the cells. In

order to maintain electrical neutrality, hydrogen shifts into the cells, raising blood pH.

- *Alkalotic agents* - Alkalotic agents, such as bicarbonate (administrated in cases of peptic ulcer or hyperacidity) or antacids, administered in excess can lead to an alkalosis.
- *Hyperaldosteronism* - Renal loss of hydrogen ions occurs when excess aldosterone (Conn's syndrome) increases the activity of a sodium-hydrogen exchange protein in the kidney.

Respiratory alkalosis is a medical condition in which increased respiration elevates the blood pH beyond the normal range (7.35-7.45) with a concurrent reduction in arterial levels of carbon dioxide. This condition is one of the four basic categories of disruption of acid-base homeostasis

Respiratory alkalosis may be produced as a result of the following causes: stress, pulmonary disorder, thermal insult, fever, hyperventilation, liver disease.

The presence of only one of the above derangements is called a *simple* acid–base disorder. In a *mixed* disorder more than one is occurring at the same time. Mixed disorders may feature an acidosis and alkosis at the same time that partially counteract each other, or there can be two different conditions affecting the pH in the same direction.

The body's acid–base balance is tightly regulated. Several buffering agents exist which reversibly bind hydrogen ions and impede any change in pH. Extracellular buffers include bicarbonate and ammonia, while proteins and phosphate act as intracellular buffers. The bicarbonate buffering system is especially key, as carbon dioxide (CO_2) can be shifted through carbonic acid (H_2CO_3) to hydrogen ions and bicarbonate (HCO_3^-).

Acid–base imbalances that overcome the buffer system can be compensated in the short term by changing the rate of ventilation. This alters the concentration of carbon dioxide in the blood, shifting the above reaction according to Le Chatelier's principle, which in turn alters the pH. For instance, if the blood pH drops too low (*acidemia*), the body will compensate by increasing breathing,

expelling CO_2, and shifting the reaction above to the right such that fewer hydrogen ions are free – thus the pH will rise back to normal. For *alkalemia*, the opposite occurs.

ACUTE-PHASE PROTEINS

Acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the *acute-phase reaction* (also called acute-phase response).

In response to injury, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL1, IL6 and IL8, and TNFα. The liver responds by producing a large number of **acute-phase reactants**. At the same time, the production of a number of other proteins is reduced; these are, therefore, referred to as "negative" acute-phase reactants. Increased acute phase proteins from the liver may also contribute to the promotion of sepsis

Positive acute-phase proteins serve (part of the innate immune system) different physiological functions for the immune system. Some act to destroy or inhibit growth of microbes, e.g., C-reactive protein, mannose-binding protein,[2] complement factors, ferritin, ceruloplasmin, serum amyloid A and haptoglobin. Others give negative feedback on the inflammatory response, e.g. serpins. Alpha 2-macroglobulin and coagulation factors affect coagulation, mainly stimulating it. This pro-coagulant effect may limit infection by trapping pathogens in local blood clots. Also, some products of the coagulation system can contribute to the innate immune system by their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells.

Measurement of acute-phase proteins, especially C-reactive protein, is a useful marker of inflammation in both medical and veterinary clinical pathology. It correlates with the erythrocyte sedimentation rate (ESR), however not always

directly. This is due to the ESR being largely dependent on elevation of fibrinogen, an acute phase reactant with a half-life of approximately one week. This protein will therefore remain higher for longer despite removal of the inflammatory stimuli. In contrast, C-reactive protein (with a half-life of 6-8 hours) rises rapidly and can quickly return to within the normal range if treatment is employed. For example, in active systemic lupus erythematosus, one may find a raised ESR but normal C-reactive protein. They may also indicate liver failure. During rheumatoid arthritis in the blood appear additive glycosaminoglycans as acute-phase proteins

9. LEARNING OBJECTIVES OF LESSON № 17

To learn theoretical material about biochemistry of kidney, role of kidneys in the regulation of mineral metabolism, components of urine at healthy and diseased people.

Necessary to know:

1. Main biochemical functions of kidney tissue based on metabolic pathways
2. Role of kidney tissue in the regulation of water and salts metabolism.
3. Role of kidney tissue in creation of acid-base balance.
4. Physicochemical properties and pathological components of urine of healthy and diseased persons.

Necessary to be able to do:

1. Determination of urine total acidity, pH, specific gravity.
2. Qualitative reactions for protein in the urine.
3. Qualitative test for blood pigments in the urine.
4. Qualitative reaction for ketone bodies in the urine.
5. Qualitative reaction for glucose in the urine.

10. EDUCATIONAL OBJECTIVES

Knowledge about biochemistry of kidney, role of kidneys in the regulation of mineral metabolism, components of urine at healthy and diseased people are necessary for proper understanding of clinical subjects and formation of clinical thinking.

11. CONTENT OF EDUCATIONAL MATERIAL

1. General conceptions of urine formation by kidneys. Hormonal regulation of kidneys` filtration, adsorption and secretion (vasopressin, atrial diuretic peptide, aldosterone, renin-angiotensinogen system, calciotonin, calcitriols).
2. Physical and chemical properties of the urine at healthy adults.
3. Chemical composition of the urine. Characteristic of the main urine components at healthy adults. Pathological components of urine in patients.

12. PLAN AND ORGANIZATIONAL STRUCTURE OF THE LESSON

Stages	Time/ minutes	Materials for lesson		Place for Duration Of lesson
		Learning tools\content	Equipment	
1.Organizing time	5			Auditory 507,509, 511, 514
2. A conversation	25	Consideration of biochemical functions of kidney tissue in human organism based on metabolic pathways in it	1.Textbook on Biochemistry 2. A Manual for submodule 4	
3.Initial control	10	Testing	Blocks of tests Variants 1-3	
Brake	10 minutes			
4.Laboratory works	25	The group of students is divided in three subgroups with aim to start laboratory works from the beginning up to the end : each subgroup must make investigation with urine of healthy donor (pH, total acidity, specific gravity) and pathological components investigation in urine tasks NN1-3 to discover proteins, ketone bodies and glucose (each subgroup will take one number task for investigation)		
		BIOCHEMISTRY LABORATORY MANUAL Module 2	Reagents for laboratory works; Test tubes Thermostat	
5. Final results discussion with the use of theoretical part of the lesson	25	Each subgroup must report teacher investigation results with explanation for them, results for tasks NN1-3 may be explained with proposed probable diagnosis for patient.		

13. LABORATORY WORK ALGORITHM

Equipment and reagents: Test tubes, pipettes, an urinometer, indicator strips for determination of pH, 10% NaOH solution, 1% copper sulfate solution, 1% sodium nitroprusside solution (extempera prepared), 0.5% benzidine solution, 20% sulfosalicylic acid solution, concentrated nitric acid, 0.1N NaOH solution, distilled water in a flask, indicator phenolphthalein.

1.1. Determination of urine specific gravity

Course of the work*:* The specific gravity of urine samples is measured with the aid of a urinometer. Place the bulb in a cylinder. Add sufficient urine to the cylinder to make the bulb float. Read the specific gravity of the sample from the stem of the urinometer where the meniscus of the urine intersects the calibration lines. Be sure the urinometer is freely floating and does not touch the walls of the cylinder.

Clinical significance: The specific **gravity** of urine of healthy adults varies in the region 1.012-1.020 g/ml.

Abnormal specific gravity values may indicate:

- ***reduced specific gravity***: diabetes insipidus, certain renal diseases, excess fluid intake;
- ***increased specific gravity***: dehydration, adrenal insufficiency, nephrosis, congestive cardiac-failure, liver disease.

Further action of investigator required (if low or high specific gravity values are found in urine) also to check urine pH value , protein presence.

1.2. Determination of urine total acidity, pH.

Principle of method: Urine total acidity is estimated in volume of 0.1N of NaOH solution used for titration 100 ml of urine in the presence of phenolphthalein.

***Course of the work**:* Pour 5 ml of urine and 5-10 ml of distilled water into a flask; add 2-3 drops of phenolphthalein, add by drops 0.1N NaOH solution until the pink coloring will appear.

pH of urine may be determined by special indicator strips whose color is compared with scale colors on flask -keeper of these strips.

Clinical significance: Total acidity of normal urine varies within 25-30ml of 0.1N NaOH solution for 100 ml of urine titration. Normal pH of urine varies within 5-6.5.

The concentration of alkaline components in the urine is increased at vomiting, at high acidity of gastric juice, alkaline therapy, chronic infections of urine excretion ways, during using of big amounts of vegetables, fruits, milk products in diet.

Acidity is increased at diabetes mellitus, a tuberculosis and kidneys insufficiency.

Estimation of the total acidity takes place during differential diagnostic of alkalosis and acidosis of different etiology.

2. Qualitative reaction for the protein in the urine

2.1. Heller's test (with concentrated nitric acid)

***Course of the work**:* Pour 1-2 ml of concentrated nitric acid into a test-tube and stratify cautiously 1-2 ml of urine. A white ring of denaturated protein will appear between two layers of liquid. If there's a little quantity of protein in urine, the ring will form in 2-4 minutes.

2.2. Test with sulphosalicylic acid

***Course of the work**:* Pour 2 ml of urine into a test-tube and add 5-6 drops of 20% sulfosalicylic acid solution. You can see the appearance of the precipitate at the presence of protein. This test belongs to the most sensitive reactions.

Clinical significance: The protein appears in the urine during nephritis, some cardiac diseases, during some forms of idiopathic hypertension and during pregnancy pathology.

3. Qualitative test for blood pigments in the urine (Benzidine test)

Principle of method: Reaction is based on oxidation of benzidine with active oxygen, which is released from hydrogen peroxide under the action of peroxidase.

Course of the work*:* Pour 2 ml of fresh unstrained urine into a test-tube. Add an equal quantity of benzidine solution and some drops of hydroxyperoxide. Blue or green coloring products will appear at the presence of blood.

Clinical significance:

The renal hematuria is the main symptom of acute nephritis. The extrarenal hematuria is observed in inflammation or traumatism of the urinary tract.

4. Qualitative reaction for ketone bodies (Lugol's reaction)

Principle of method: Acetone and acetoacetate in alkaline environment form with sodium nitroprusside an orange-red coloring complex. After oxidation by glacial acetic acid a cherry-colored compound is formed.

Course of the work*:* Pour on Petri dish 1 drop of urine, 1 drop of 10% NaOH solution and 1 drop of sodium nitroprusside solution (extempera). An orange-red coloring will appear. Add 3 drops of glacial acetic acid - a cherry-red coloring will appear. The reaction can be carried out in a test-tube as well.

Clinical significance. An increase of ketone bodies content in urine takes place during diabetes mellitus and during a long-term starvation. Acetone is absent usually in the urine of patients.

5. Qualitative reaction for glucose in the urine (Trommer's test)

Course of the work*:* Add 1 ml of 10% NaOH solution and 0.5 ml of 1% copper sulfate solution into 1ml of urine in a test tube. Heat cautiously the mixture

of the test tube up to simmer and boil for 1min precisely. A red coloring will appear at glucose presence.

Clinical significance: Urine contains traces of glucose at healthy people.

Glucosuria is observed in such pathologic state as diabetes mellitus (if glucose concentration in the plasma is more 9 mmol/L). This state is observed during the emotional stress, hyperthyroidism, Cushing's syndrome, during some kidney pathology.

14. TESTS

1. Point out the substance that appears in the urine in a case of alkaptonuria at patient:

A. Fructose

B. Protein

C. Homogentisic acid

D. Glucose

E. Tryptophan

2. A patient suffers from pain attacks in the right hypochondriac region after the fats intake, skin and sclera are yellow, urine has a color of dark beer, and feces are acholic. What substance in the urine is promoter of its dark color?

A. Ketone bodies

B. Glucose

C. Stercobilin

D. Urobilinogen

E. Bilirubin glucuronides

3. Point out the pathological component of urine:

A. Hemoglobin

B. Urea

C. Uric acid

D. Creatinine

E. Amino acids

4. The urine, taken from a child with mental retardation becomes green after adding of 5% $FeCl_3$ solution. Point out, what amino acid metabolism is confirmed by the above-mentioned diagnostic test:

A. Arginine

B. Phenylalanine

C. Cysteine

D. Glutamine

E. Tryptophan

5. What color is for urine when intestinal rotting processes are extensive:

A. Brown

B. Straw-yellow

C. Red

D. Green or blue

E. Beer color

6. Choose amino acids whose levels in the urine are higher then normal at Maple syrup urine disease in patient:

A. Valine, leucine, isoleucine

B. Histidine, proline, glutamine

C. Tyrosine, phenylalanine, tryptophan

D. Alanine, glycine, proline

E. Methionine, cysteine, homocysteine

7. What normal urine component concentration is decreased in a case of liver cirrhosis in patient?:

A. Ketone bodies

B. Protein

C. Urea

D. Lipids

E. Carbohydrates

8. A nephritic filter dimension increases at nephron damage. Point out the pathological urine component that appears at this state:

A. Amino acids

B. Urea

C. Uric acid

D. Hippuric acid

E. Protein

9. The urine of healthy people contains the minimal quantity of protein that can't be determined by the qualitative tests. Point out the disease that causes an out-of-kidney proteinuria:

A. Phenylketonuria

B. Avitaminosis

C. Cystitis

D. Alkaptonuria

E. Diabetes mellitus

10. Point out the qualitative reaction for protein in the urine:

A. Heller's test

B. Benzidine test

C. Lugol's test

D. Trommer's reaction

E. Rozine's reaction

11. Patient's skeletal muscular tissue was smashed. Point out the urine index that will increase in this case:

A. Mineral salts

B. Glucose

C. Total lipids

D. Uric acid

E. Creatinine

12. The diuresis in healthy adults is about:

A. 400-700 ml/day

B. 1000-2000 ml/day

C. 2000-3000 ml/day

D. 700-900 ml/day

E. 3000-4000 ml/day

13. A patient suffers from the constant thirst. Hyperglycemia, polyuria and abnormally high concentration of 17-ketosteriods in urine are revealed. Point out the probable diagnosis:

A. Addison's disease

B. Myxedema

C. Glycogen storage disease (I type)

D. Steroidal diabetes

E. Insulin dependent diabetes mellitus

14. Point out the normal component of urine:

A. Coniugated bilirubin

B. Glucose

C. Ketone bodies

D. Uric acid

E. Protein

15. A patient has an abnormally high volume of dilute urine (over than 15 lit/day), urine has a straw-yellow color and its specific gravity is very low. Point out the disease of this patient:

A. Diabetes mellitus

B. Diabetes insipidus

C. Viral hepatitis

D. Addison’s disease

E. Acromegaly

16. Choose the urine component whose concentration increases at consuming of meat meals:

A. Glucose

B. Protein

C. Uric acid

D. Ketone bodies

E. Fructose

17. A child (of 2 years) has delayed mental and physical development, suffers from frequent vomiting after meals. A phenylpyruvic acid is determined in the urine. What kind of metabolism is damaged?

A. Lipid metabolism

B. Protein and amino acid metabolism

C. Carbohydrate metabolism

D. Water-salt metabolism

E. Phosphate-calcium metabolism

18. Point out the qualitative reaction for blood pigments in the urine:

A. Heller's test

B. Benzidine test

C. Lugol's test

D. Trommer's reaction

E. Rozine's reaction

19. The violation of the hormone secretion is followed by polyuria. Choose this hormone:

A. Adrenalin

B. Insulin

C. Sex hormone

D. Vasopressin

E. Oxytocin

20. It is suspected that a child has a progressing muscular dystrophy. What urine component is increased and will confirm the diagnosis:

A. Urea

B. Hippuric acid

C. Homogentisic acid

D. Creatine

E. Creatinine

21. Appearance of albumins in the urine of diseased person may be at:

A. Acute nephritis

B. Chronical nephritis

C. Severe form of diabetes mellitus

D. Pyelonephritis

E. All that is placed above

22. Choose the main biochemical tests for diagnostics of kidney diseases:

A. Urea content in the blood plasma and in the urine

B. Creatinine content in the blood and urine

C. Sodium ions content in the blood and urine

D. N-acetyl-beta-D-glucose-aminidase activity (blood serum, urine)

E. All that is placed above

23. What organic compounds accumulate in final urine at severe form of diabetes mellitus?

A. Albumins

B. Glucose

C. Ketone bodies

D. Bilirubin conjugated

E. All that is placed in positions A, B, C

24. Kidney insufficiency development will cause the infringements in those processes:

A. Erythropoietin synthesis and secretion

B. Calcitriol synthesis

C. Mineralization of bone tissue

D. Creatine synthesis

E. All that is placed

25. The infringement in glomerular filtration mostly is associated with appearance in the urine of this class compounds. Name it:

A. Lipids

B. Proteins

C. Amino acids

D. Keto acids

E. Carbohydrates

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

A. Inulin

B. Creatine

C. Free ammonia

D. Ammonia salt

E. Indican

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

A. Aspartic acid

B. Creatinine

C. Uric acid

D. Ammonia

E. Water

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

A. Globulin

B. Alanine

C. Pyruvate

D. Oxaloacetate

E. Carbonic acid

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

A. Creatine

B. Free amino acids

C. Pyruvate

D. Cholesterol total

E. Urea

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

A. FAD

B. NAD+

C. Calcitonin

D. Calcitriol

E. Angiotensin II

15. TESTS FOR PREPARATION TO «KROK-1» EXAMINATION

1. A 34-year-old patient was diagnosed with chronic glomerulonephritis 3 years ago. Edema has developed within the last 6 months. What caused the edema?

A. Liver dysfunction of protein formation
B. Hyperosmolarity of plasma
C. Proteinuria*
D. Hyperproduction of vasopressin
E. Hyperaldosteronism

2. Examination of a 43 y.o. anephric patient revealed anemia symptoms. What is the cause of these symptoms?

A. Folic acid deficit
B. Vitamin B_{12} deficit
C. Reduced synthesis of erythropoietin*
D. Enhanced destruction of erythrocytes
E. Iron deficit

3. A biochemical urine analysis has been performed for a patient with progressive muscular dystrophy. In the given case muscle disease can be confirmed by the high content of the following substance in urine:

A. Urea
B. Porphyrin
C. Hippuric acid
D. Creatine*
E. Creatinine

4. Kidney insufficiency in patient is accompanied with:

A. Excess levels of urea in the blood plasma

B. Excess levels of potassium ions in the blood plasma

C. Disturbed clearance

D. Disturbed filtration and reabsorption processes

E. All that is placed above*

5. Point out the most important compensatory mechanism in metabolic acidosis:

A. Hyperventilation

B. Increased NH_3 excretion by kidneys*

C. Increased filtration of phosphates

D. Increased HCO_3^- production

E. Urea production in the liver

6. Point out the main source of ammonia in kidney tissue:

A. Urea

B. Aspartate

C. Glutamine*

D. Glutamate

E. Uric acid

7. Choose normal amount of proteins excreted in urine/24 hours:

A. Less than 150 mg*

B. 200 mg - 225 mg

C. 450 mg – 500 mg

D. More than 800 mg

E. 150 mg – 250 mg

8. Name organic compound which is terminal for humans and not reabsorbed in renal tubules:

A. Globulins

B. Glucose

C. Albumin

D. Creatinine*

E. Bilirubin

9. Choose the specific gravity region (g/ml) for urine of healthy person:

A. 1.005-1.015

B. 1.030-1.040

C. 1.015-1.020

D. 1.030-1.040

E. Less then 1.010

10. Creatinine levels in the urine and blood are used to test kidney function. Creatinine is useful for this test because it is not significantly reabsorbed nor secreted by kidney, and metabolically it is:

A. Produced at a constant rate*

B. Produced only in kidney

C. A storage form of energy

D. An acceptor of protons in renal tubules

E. A precursor for phosphocreatine

11. Appearance of albumins in the urine of diseased person may be at:

A. Acute nephritis

B. Chronical nephritis

C. Severe form of diabetes mellitus

D. Pyelonephritis

E. All that is placed above*

12. Choose the main biochemical tests for diagnostics of kidney diseases:

A. Urea content in the blood plasma and in the urine

B. Creatinine content in the blood and urine
C. Sodium ions content in the blood and urine
D. N-acetyl-beta-D-glucose-aminidase activity (blood serum, urine)
E. All that is placed above*

13. What organic compounds may accumulate in final urine at severe form of diabetes mellitus?

A. Albumins
B. Glucose
C. Ketone bodies
D. Bilirubin conjugated
E. All that is placed in positions A, B, C*

14. Kidney insufficiency development will cause the infringements in those processes:

A. Erythropoietin synthesis and secretion
B. Calcitriol synthesis
C. Mineralization of bone tissue
D. Creatine synthesis
E. All that is placed *

15. The infringement in glomerular filtration mostly is associated with appearance in the urine of this class compounds. Name it:

A. Lipids
B. Proteins*
C. Amino acids
D. Keto acids
E. Carbohydrates

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

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

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

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

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

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

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

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

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

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

16. THEORETIC MATERIAL FOR LESSON 17

STRUCTURAL ORGANIZATION OF KIDNEY TISSUE AND ITS FUNCTIONS

Renal tissue is divided in two types:

1) outer or cortical coloured brown-red;
2) inner or medullary coloured lilac-red.

Nephron is the functional unit of renal parenchyma, the two kidneys of human number about 2 million nephrons. Cortical nephrons are about 85% of the total number; about 15% of total number is juxtamedullary nephrons whose glomeruli are located at the boundary between the cortex and medulla of the kidney.

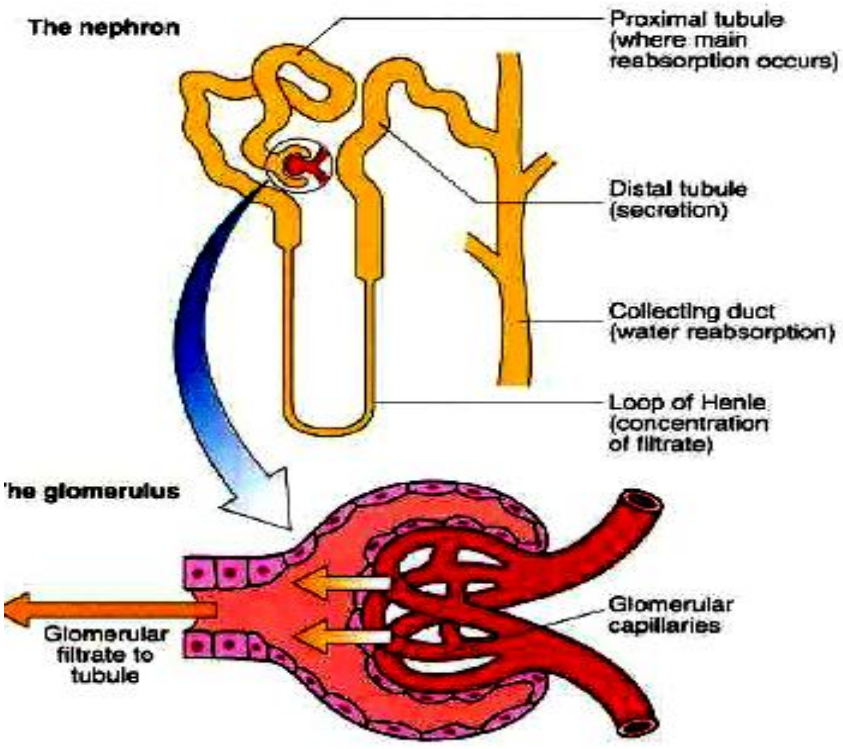


Figure 1. A structural unit of kidney tissue - a nephron.

The kidney is involved in:

1) the regulation of water and salt balance;

2) the maintenance of acid-base balance and of osmotic pressure of fluid media of the organism;

3) the removal of terminal products of metabolic processes;

4) the blood pressure control;

5) the stimulation of erythropoiesis, etc;

6) in hormonal control of a lot metabolic pathways..

Filtration at the glomerulus, tubular reabsorption and tubular secretion are observed in the nephron

Primary urine is formed due to filtration process. The composition of primary urine is very similar to blood plasm, but there is no any protein in the primary urine. The pores in the glomerular basal membrane, which are made up by collagen type IV, have an effective mean diameter of 2.9 nm. This allows all plasma components with a molecular mass about 15 kDa to pass through the membrane, that is because proteins are completely unable to enter to primary urine.

Reabsorption. The most of all low-molecular weight plasma components are transported back into the blood by reabsorption, to prevent losses of valuable metabolites and electrolytes. In the proximal tubule, organic metabolites (glucose, amino acids, lactate, ketone bodies) are recovered by secondary active transport. There are several group-specific transport systems for resorbing amino acids, with which hereditary disorders can be associated (cystinuria, glycinuria, cystinosis, Hartnup`s disease). Bicarbonate, sodium ion, phosphate and sulfate are also resorbed by ATP dependent active mechanisms in the proximal tubule. The later sections of nephron may only serve for additional water recovery and for regulated resorption of Na^+ and Cl^-. These processes are controlled by hormones.

Secretion helps also to form final urine. Some excreted substances are released into the urine by active transport in the renal tubules: protons, potassium ions, urea, creatinine, some drugs (penicillin).

These two processes help to keep useful substances and to maintain acid-base balance for organism. The molecular mechanism for resorption and secretion of materials by renal tubular cells are quite well understood.

The renal tubule cells are capable of secreting protons (H^+) from the blood into the urine against a concentration gradient. Despite the fact that the H^+ concentration in the urine is up to a thousand times higher than in the blood.

To achieve this, carbon dioxide (CO_2) is taken up from the blood and together with water (H_2O) and with a help of carbonate dehydratase is converted into hydrogen carbonate (bicarbonate, HCO_3^-) and one proton H^+. Formally, this yields carbonic acid, but it is not released during the reaction. The hydrogen carbonate returns to the plasma where it contributes to the blood base reserve. The proton is exported into the urine by secondary active transport in anti-port for Na^+. The driving force for proton excretion is Na^+ gradient established by the Na^+, K^+-ATPase. This integral membrane protein on the basal side (towards the blood) of tubule cells keeps the Na^+ concentration in the tubule cell low, thereby maintaining Na^+ inflow. In addition to this secondary active H+ transport mechanism, there is a V-type H+-transporting ATPase in the distal tubule and collecting duct.

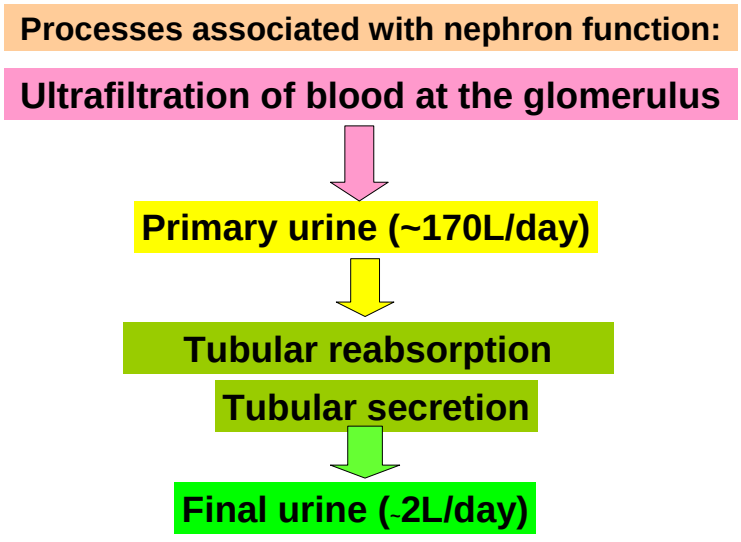


Figure 2. Main processes promoted by the function of nephron.

THE FUNCTION OF KIDNEY IN MAINTENANCE OF ACID-BASE BALANCE IN HUMAN ORGANISM

An important function of the secreted H^+ ions is to promote HCO_3^- reabsorption. Hydrogen carbonate, the most important buffering base in the blood, passes into primary urine quantitatively, like all ions. In the primary urine HCO_3^- reacts with proton ion to form water and carbon dioxide, which returns by free diffusion to the tubule cells and from there into the blood. In this way kidneys also influence the CO_2/HCO_3^- buffering balance in the plasma. Approximately 60 mmol of protons are excreted with urine every day. Buffering systems in the urine catch a large proportion of H^+ ions, so that the urine becomes weakly acidic (down to about pH=4.8).

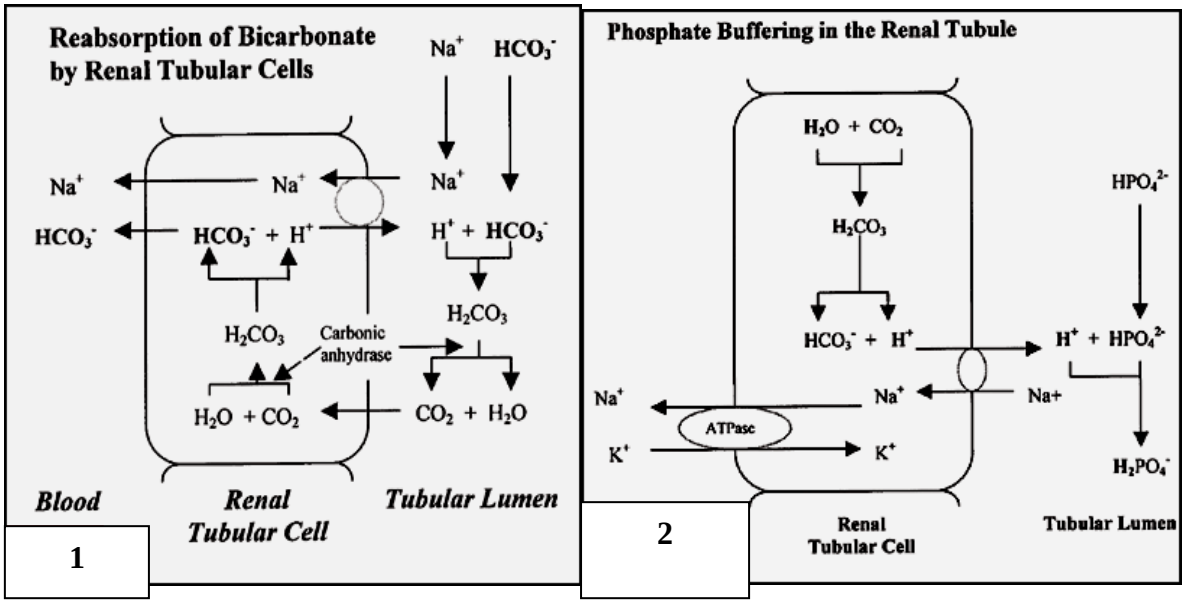
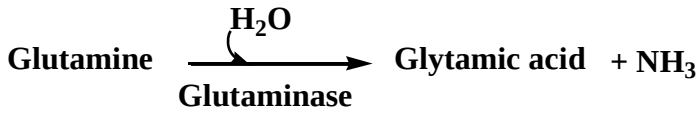
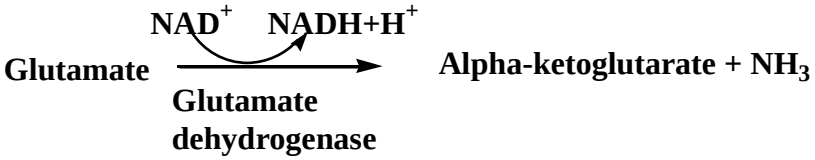


Figure 3. Sodium, bicarbonate ions reabsorption (1, 2) and phosphates buffering (2) is in close relation to protons secretion.

An important buffer in the urine is the hydrogen phosphate/dihydrogen phosphate system. The conversion of disubstituted phosphates to mono substituted phosphates is in need to keep sodium ions and calcium ions for human organism and to remove the accumulated protons from the blood at acidosis state.

In addition, ammonia also makes a vital contribution to buffering the secreted protons. Since plasma concentration of ammonia are low, the kidneys release ammonia from glutamine due to glutaminase action and during oxidative deamination of glutamate:





Ammonia can diffuse freely into the urine through the tubule membrane while the ammonium ions that are formed as charged particles and can no longer return to the cell. Acidic urine therefore promotes ammonia excretion as ammonia salts, it is normally 30-50 mmol per day.

At metabolic acidosis glutaminase activity usually is induced and ammonium ions excretion is increased. At metabolic alkalosis renal excretion of ammonia is reduced. But the production of the urea in the liver is stimulated.

The excess levels of hydrogen ions are considered at humans during starvation and metabolic acidosis caused by the accumulation of some substances such as lactate, pyruvate, some ketone bodies, amino acids and others. This condition causes the stimulation of gluconeogenesis in kidney, and this way is considered as the way for maintenance of acid-base balance, too. The synthesized glucose is very important source of ATP (due to oxidative phosphorylation after glucose aerobic oxidation way) that is used for the active transport mechanism.

METABOLIC PATHWAYS AND ENERGY FORMATION IN KIDNEY

The main energy sources are glucose, fatty acids, ketone bodies, some amino acids. To a lesser extent lactate, glycerol, and citric acid are used. The endothelial cells in the proximal tubule are capable of gluconeogenesis from amino acids

mainly. Amino groups of amino acids are used as ammonia for buffering of urine. Enzymes for protein degradation and the amino acid metabolism occur in the kidney at high levels of activity (amino acid oxidases, amino oxidases, glutaminase). The most important metabolic pathways for kidney tissue are:

- *Aerobic oxidation of monosacharides*
- *Gluconeogenesis*
- *Hexose Monophosphate Shunt*
- *Fatty acids oxidation*
- *Ketone bodies utilization*
- *Replication;Transcription;Translation*
- *Transport systems function in cellular membrane*
- *Antioxidant enzyme systems function*

KIDNEYS AND HORMONES

Kidneys have also endocrine function (fig.4). They produce erythropoietin and calcitriol and play a decisive part in producing the hormone angioteinsin II by releasing the enzyme *rennin*.

The activity of calcidiol-1-monooxygenase (hydroxylase) is enhanced by the hormone PTH. Calcitriol stimulates the resorption of both calcium and phosphates ions in renal tubules. The proportion of Ca^{2+} resorbed is over 99%, while for phosphate the figure is 80-90%. PTH stimulates resorption of Ca^{2+} but inhibits the resorption of phosphate.

The erythropoietin is a peptide hormone that is formed predominantly by the kidneys, but also by the liver. Together with colony-stimulating factors it regulates the differentiation of stem cells in the bone marrow. Erythropoietin release is stimulated by hypoxia. The hormone ensures that erythrocyte precursor cells in the bone marrow are converted to erythrocytes, so that their numbers in the blood increase. Renal damage leads to reduced erythropoietin release which in turn results in anemia. Forms of anemia with renal causes can now be successfully treated using erythropoietin produced by genetic engineering techniques. The hormone is also administered to dialysis patients.

The angiotensin II (A-II) is not secreted by any hormonal gland, it is produced in the blood from precursor angiotensin I secreted by kidney tissue. Angiotensin I is produced from angiotensinogen (a plasma glycoprotein in the alpha-2-fraction synthesized in the liver) due to enzyme rennin in kidney tissue. Angiotensin I is cleaved by peptidyl dipeptidase A (a membrane enzyme located on the vascular endothelium in the lungs and other tissues) to form octapeptide A-II. The plasma level of A II is mainly determined by the rate at which rennin is released by kidneys. The production of rennin by juxtaglomerular cells is when sodium ion levels decline in blood plasma or there is a fall in blood pressure.

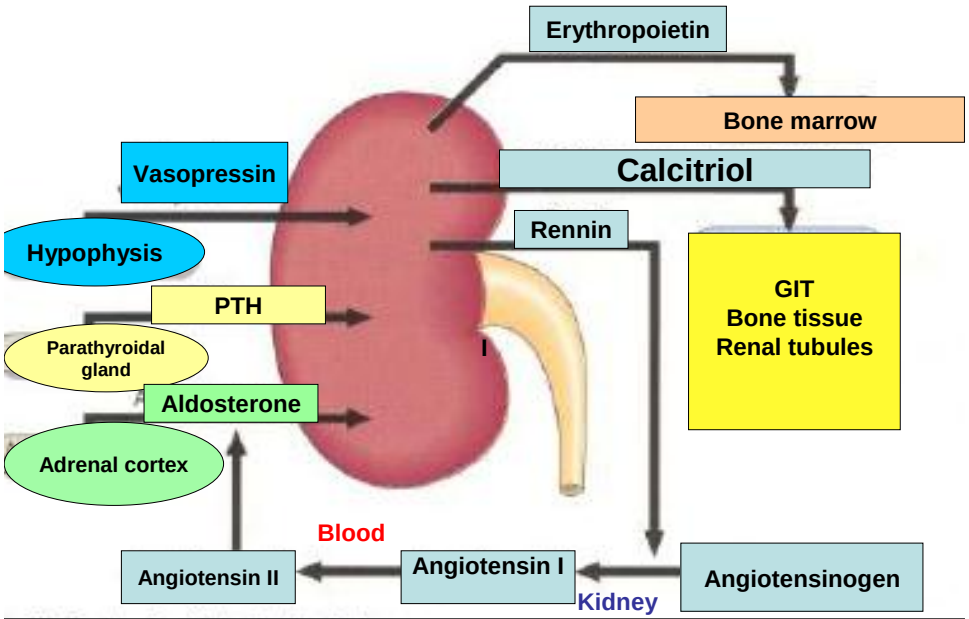


Figure 4.The role of kidney in hormonal regulation

A-II has effects on the kidneys, brain stem, pituitary gland, adrenal cortex, blood vessel walls and heart via membrane-located receptors.

In the brain stem and at nerve endings in sympathetic nervous system the effects of A-II lead to increased tonicity (neurotransmitter effect). In pituitary gland A-II stimulates vasopressin and ACTH secretion. In adrenal cortex stimulation of aldosterone synthesis and secretion.

All of the effects of angiotensin II lead directly or indirectly to increased blood pressure as well as increased sodium ions and water retention. This

important hormonal system for blood pressure regulation may be pharmacologically influenced by inhibitors at various points:

- using angiotensinogen analogs that inhibit rennin;
- using angiotensin I analogs that competitively inhibit peptidyl dipeptidase A;
- using hormone antagonists that block the binding of A-II to its receptors.

The regulation of sodium, potassium ions, chloride ions content and water volume in the blood depends on the secretion of some hormones: Aldosterone, Atrial natriuretic peptide, Vasopressin. Kidney is the main target for them. Controlled resorption of sodium ions from primary urine is one of the most important functions of the kidney. Sodium ion resorption is highly effective with more than 97% being resorbed. Several mechanisms are involved: some portion of Na^+ ions is taken up passively in the proximal tubule through the junctions between cells. There is secondary active transport together with glucose and amino acids, too. These two pathways are responsible for 60-70% of total Na^+ resorption. In ascending part of Henle`s loop there is another transporter which takes up one Na+ ion and one K^+ ion together with two Cl- ions. This symport is also dependent on the activity of Na^+/K^+-ATPase, which pumps the Na^+ resorbed from primary urine into the plasma in exchange for K+. This transport system is controlled by aldosterone an atrial natriuretic peptide.

Water resorption in the proximal tubule is a passive process in which water follows the osmotically active particles, particularly the Na^+. Final regulation of water excretion takes place in cells of distal tubules and the collecting ducts where the peptide hormone vasopressin operates.

CLEARANCE OF KIDNEYS

Renal clearance is used as quantitative measure of renal function. It is defined as plasma volume cleared of a given substance per unit of time. Inulin (fructose polysaccharide with M=6 kDa) or creatinine is used for the determination of this index. These organic compounds are not reabsorbed in kidneys, and ratio of their content in the urine to the content in the blood plasma estimates the rate of

glomerular filtration in patient. The index is named as the clearance © of kidney. You can see the formula of its determination:

$$C = \frac{U_{Cr} * V}{P_{Cr}} \text{ ml/min}$$

U_{Cr} - concentration of Creatinine in the urine;
P_{Cr} - concentration of Creatinine in the blood plasm;
V -minute diuresis.

Normal clearance index in adults equals 120 ml/min. It is usually determined at patients which may be potential donors of kidney; or in a case of to check the initial dose of some toxic drug to treat patients.

INDEXES OF THE BLOOD PLASMA AND URINE TO ESTIMATE KIDNEY FUNCTION

Creatinine and urea contents in the blood plasma are very important indexes for glomeruli function estimation. There is the increase of both indexes in the blood plasma at renal insufficiency in patients.

Urea, as you remember, is a relatively nontoxic substance made by the liver as a means of disposing of ammonia from protein metabolism.

Urea has MW 60, of which 28 comes from the two nitrogen atoms. Normal blood urea nitrogen (BUN) is 8-25 mg/dL (2.9-8.9 mmol/L).

Blood urea levels are quite sensitive indicators of renal disease, becoming elevated when renal function drops to around 25-50% of normal (remember the kidney has great functional reserve).

Increased BUN is, by definition, azotemia. It is due either to increased protein catabolism or impaired kidney function. Increased protein catabolism results from:

- a really big protein meal
- severe stress or damage of tissue (myocardial infarction, high fever, etc.)
- upper gastrointestinal bleeding (blood being digested and absorbed)

Impaired kidney function may be associated with "prerenal", "renal", or "postrenal" azotemia. *Prerenal azotemia* results from under-perfusion of the kidney: dehydration, hemorrhage, shock, congestive heart failure; glomerulonephritis is likely also to be "prerenal" if mild, since it comprises renal blood flow more than tubular function

Renal azotemia has several familiar causes: acute tubular necrosis, chronic interstitial nephritis, some glomerulonephritis, etc.

Postrenal azotemia results from obstruction of urinary flow: prostate trouble, renal stones, surgical mishaps, tumors .

In acute renal failure, BUN increases around 20 mg/dL each day.

ENZYMES OF BLOOD PLASMA AND URINE TO PROVE SOME PATHOLOGIC STATES OF KIDNEY

The most active enzymes of kidney are involved in aerobic type of metabolic processes to produce energy in a form of ATP, and the enzymes used for all type of transport across the membranes of glomeruli, renal tubules cells.

Glycine amidinotransferase -_(first enzyme from creatine synthesis). It is used in diagnostic of kidney parenchyma damage (its activity is increased in the blood serum).

N-acetyl-beta-D-glucosaminidase ("glucosaminidase", NAG) is a lysosomal enzyme (MW 140,000) found in serum and urine. Urinary NAG is a proposed marker for *tubular disease, especially subtle industrial poisoning, acute pyelonephritis, early acute tubular necrosis, and early transplant rejection.*

Lactate dehydrogenase isozymes: In acute renal insufficiency the activity of LDH_1 and LDH_2 is observed to increase. LDH_1 and LDH_2 isozymes are from renal cortex.LDH_4 and LDH_5 isozymes found in kidney medulla.

Alanine aminopeptidase isozyme 3 (AAP_3) in the blood plasma and urine is observed as the specific sign of the affected kidney tissue.

Adenosine Deaminase Binding Protein is an enzyme from the brush borders of the proximal tubule. Like NAG, *its presence in urine indicates tubular disease.*

Urinary alkaline phosphatase in urine comes from the proximal tubular brush border, detects tubular necrosis, too.

THE CHEMICAL COMPOSITION OF URINE OF HEALTHY ADULTS

1. Ions: $Na^{+,}$ K^{+}. Ca^{2+}, Mg^{2+}, SO_4^{2-}, HCO_3^{-}, HPO_4^{2-}, $H_2PO_4^{-}$, PO_4^{3-} and water.
2. The main nitrogen-containing compounds are ammonia salts and urea, other ones: uric acid, creatinine, amino acids, hippuric acid, stercobilin, indican.
3. Nitrogen free organic compounds such as acids: lactic, pyruvic, citric, oxalic, acetoacetic, and others.
4. Hormone derivatives such as 17-ketosteroids and others.
5. Terminal products of xenobiotics transformation.
6. Some vitamins and their derivatives.

THE PHYSICOCHEMICAL PROPERTIES OF THE URINE OF HEALTHY AND DISEASED HUMANS

1. Diuresis (urine output)

It is the average volume of urine (ml) excreted by individual person under ordinary dietary condition in 24 hours or per day. Normal diuresis: for men – 1500 ml/day; for women – 1200 ml/day.

Polyuria state is indicated in patients when urine output is much higher than normal (more then 3 L/day). It is considered at patients with chronic nephritis, diabetes insipidus, chronic pyelonephritis.

Oligouria state is the diminished excretion of daily urine, and it is observed at patients with febrile state, toxicosis, diarrhea, vomiting, and acute nephritis.

Anuria (nearly complete suppression of urinary excretion) is observed at patients: 1) under nervous shock; 2) at acute diffuse nephritis caused by poisoning with lead, mercury or arsenic compounds.

2. The pH of urine

All the acids, ammonia salts and urea make special pH of urine; the average value of it is about 5,3-6,5. The pH of urine depends on the diet of patient. Strong vegetarians` urine in pH is higher then 6,5. After animal food intake the pH of urine changes to value lower then 5, 3. The pH of urine may be decreased at diabetes mellitus associated with ketoacidosis, at diseases accompanied with extensive excretion of amino acids (aminoaciduria state). The alkaline urine is observed in cystitis and pyelitis, at intake of some drugs, also as sequent to strong vomiting. The pH of the urine is inversely proportional to the acidity of the urine .

3. ***The specific gravity of urine***

The normal value must be in region 1,012 – 1,020 g/ml. The specific gravity of urine may be very low (about 1,001-1,004 g/ml) in patients with diabetes insipidus. At oligouria state it may be lower then normal, too. Polyuria state at patients with diabetes mellitus may be accompanied with the increase of this index (higher then 1,020 g/ml) at the expense of glucose present in the urine.

4. ***The color of the urine***

The urine of healthy humans is transparent, straw yellow or amber liquid. The presence of pigments such as stercobilin, urochrome, uroerythrin gives those colour for urine. Abnormal pigments observed in the urine at pathologic states can change it to colour:

1) dark (urobilin formed from excess urobilinogen that is not transformed in the liver at liver parenchyma damage);

2) green or blue (intensive putrefaction of proteins in the intestine causes the accumulation of indoxyl sulpharic acid in the urine); uroglaucin at scarlatina state;

3) dark brown like beer (there is the conjugated bilirubin presence in the urine);

4) black (due to presence of homogentisic acid oxidation product at Alkaptonuria state);

5) red shade at presence of blood pigments, they include red blood cells (hematuria state) and hemoglobin (hemoglobinuria state).These pigments are observed at the damage of urinary tracts by kidney stones or at acute cystitis.

The urine may be not transparent when sediments are present in it. It may be at pathologies associated with the damage of urinary tracts by kidney stones, with the accumulation in the urine some salts (calcium oxalates), with the appearance of epithelial cells in the urine and with excretes from vagina of women.

5. Special smell of the urine

The urine slight smell is associated with the presence of ammonia salts and urea in it usually. But it may be changed at:

1) Maple syrup urine disease (like maple syrup odor);
2) Phenylketonuria (like mouse odor);
3) Intensive putrefaction of proteins in the intestine (the smell of rotten meat);
4) Glucosuria state at diabetes mellitus (special fruity odor);
5) The appearance of excretes from vagina of diseased women at pathologies such as syphilis and gonorrhoea can also change the smell of the urine (like the smell of rotten meat).

THE PATHOLOGICAL COMPONENTS OF THE URINE

Proteins of the urine

Proteins are found in minimal amounts (less 150 mg/day) in the urine of healthy people and they can't be detected by color reactions used for proteins. If the color reaction for proteins is positive in the urine, this component is considered as pathological and proves proteinuria state. This state is determined at patients with acute glomerular nephritis; extra renal reasons: inflammation of urinary tracts, affected prostate gland, at the burns and fever, at the trauma of urinary tract (hemoglobinuria). Proteinuria state is accompanied with the change of physicochemical properties of the urine: 1) hemoglobinuria is associated with pink-red color of the urine; 2) the density of urine becomes higher then normal at

proteinuria state at the expense of proteins; 3) the urine has the big foam after shaking.

Ketone bodies

In minimal amounts they are at healthy people but not detected by color reactions in the urine. If the color reaction for ketone bodies in the urine is positive they are considered as pathologic components. The high levels of them are accompanied with long time starvation or with diabetes mellitus (severe form). The pH of urine becomes lower then normal in this case.

Glucose

It is practically absent in the urine of healthy people, but is observed at patients with diabetes mellitus (all types) when the levels of glucose in the blood are higher then 9,5 mmole/L. Glucosuria state is observed in patients in this case. Polyuria state in this case accompanied with the increase of urinary density (higher then 1,020 g/ml) at the expense of glucose present in the urine.

Bile pigments

Urobilin formed from excess urobilinogen and conjugated bilirubin are pathological components and must be absent in the urine of healthy persons. Their appearance in the urine first of all is the signal for the problems with liver function, and is accompanied with the jaundice development in patient. The color of the urine is changed at their presence (see above).

Creatine

It is practically absent in the urine of healthy adults, and is considered as pathologic component. Creatine is determined in the urine at developed muscular dystrophy in patients and at old people with the deficiency of motor function for skeletal muscles in person.

RECOMMENDED LITERATURE

Basic

1. Berezov T. T. Biochemistry : translated from the Russian / T. T. Berezov, B. E. Korovkin. - M. : Mir, 1992. - 514 p.
2. Murray R. K. Harper's Illustrated Biochemistry / R. K. Murray, D. K. Granner, V. W. Rodwell. - 27th ed. - Boston [etc.] : McGraw Hill, 2006. - 692 p.
3. Satyanarayana U. Biochemistry : with clinical concepts & case studies / U. Satyanarayana, U. Chakra Pani. - 4th ed. - India : Elsevier, 2015. - 812 p.

Additional

1. Koolman J. Color Atlas of Biochemistry: textbook / J. Koolman, K.-H. Roehm. – 2nd ed. – Stuttgart-New York : Thieme, 2005. – 467 p.
2. Lieberman M. Medical Biochemistry: textbook / M. Lieberman; A. Marks, C. Smith. - 2nd ed. - New York : Lippincott Williams & Wilkins, 2007. – 540 p.
3. Marks D. B. Biochemistry: The Chemical Reactions of Living Cells / D. B. Marks, D. Metzler - [2nd ed., vol. 1,2] - USA : Elsevier Academic Press, 1994.- 1974 p.
4. Marshall J. W. Clinical Chemistry : textbook / J. W. Marshall, S. K. Bangert.- Fifth edition. – China : Mosby, 2004. – 422 p.
5. Newsholme E. A. Functional Biochemistry in Health and Disease / E. A. Newsholme, T. R. Leech. - UK : John Wiley & Sons Ltd, 2010.-543 p.
6. Smith C. Basic Medical Biochemistry: A Clinical Approach: textbook / C. Smith, A. Marks, M. Lieberman. - 2nd ed. - New York : Lippincott Williams & Wilkins, 2009. - 920 p.

Розглянуто і затверджено на засіданні циклової методичної комісії хімічних дисциплін Запорізького державного медичного університету (протокол №_____ від_____ 2017 року)