MINISTRY OF HEALTH OF THE REPUBLIC OF MOLDOVA
STATE UNIVERSITY OF MEDICINE AND PHARMACY
NICOLAE TESTEMITANU

V. Vovc, S. Lozovanu, A. Ganenco, V. Chihai,
O. Arnaut, T. Besleaga

EXPERIMENTAL PHYSIOLOGY

Guide for Practical Classes in Physiology

Chisinau
2013

MINISTRY OF HEALTH OF THE REPUBLIC OF MOLDOVA
STATE UNIVERSITY OF MEDICINE AND PHARMACY
NICOLAE TESTEMITANU

V. Vovc, S. Lozovanu, A. Ganenco, V. Chihai,
O. Arnaut, T. Besleaga

EXPERIMENTAL PHYSIOLOGY

Guide for Practical Classes in Physiology

Chisinau
Editorial Polygraphic Center *Medicina*
2013

CZU 612.1/.8(076.5)
E 97

Approved for edition by the Central Methodic Council of the SUMPh *Nicolae Testemitanu* (protocol Nr. 2 from 01.11.2012).

Also cooperated:
A. Saulea, B. Dragan, N. Demiscan, V. Ojog, S. Grosu

Translated by **B. Toma** and **L. Toma**
Verified by **N. Samsurina**

Referents: *O.Tagadiuc* – M.D., Ph.D, Professor
V.Lisnic – M.D., Professor

DESCRIEREA CIP A CAMEREI NAȚIONALE A CĂRȚII

Experimental physiology: Guide for Practical Classes in Physiology / V. Vovc, S. Lozovanu, A. Ganenco [et al.]; State Univ. of Medicine and Pharmacy *Nicolae Testemitanu*. – Ch.: CEP *Medicina*, 2013. – 102 p.
300 ex.
ISBN 978-9975-113-78-6
612.1/.8(076.5)
E 97

ISBN 978-9975-113-78-6

Foreword

In the system of higher medical education physiology is one of the basicsubjects. Physiology as a training course designed to teach future doctors to understand functioning mechanisms of living organisms and their component as cells, tissues and organs.

In 1628 the founder of human and animal physiology as a science William Harvey published a treatise "On the motion of the heart and blood". This treatise generalized concepts and ideas about the functions of the cardiovascular system, formulated on the basis of physiological experiments on animals. Thus, physiology became an experimental science and that its main feature is relevant to the present.

The university course in human physiology consists of theoretical lectures and practical (lab) classes. Carrying out laboratory works, students receive experimental support for theoretical material of the lecture course and the skills of the experimental work with biological objects. Some of the laboratory works introduce students to the functional methods of studies that are applied in the clinic.

Most of the lab works is intended for independent performance by students. A number of works is performed in a demonstration experiment by a teacher or lab assistant.

This guide has been compiled in accordance with the syllabus for human physiology. It has been elaborated on a basis of "Collection of practical work in physiology Experimental Physiology" published by the staff of the Department of human physiology and biophysics of the SUMPh Nicolae Testemitanu in 2008. The sequence of chapters follows the logic of the teaching course during an academic year. Procedures for experiments and research which are specific to the various functional systems of the organism are set out in the chapters. The text is accompanied by illustrations for better learning.

A systematic independent work of students over the course during the academic year is indispensable for a successful mastery of the basic course of physiology. At the beginning of each theme there is a checklist designed to facilitate and systematize the process of students' preparation for classes.

We hope that this guide will be an effective aid for students in preparation for practical classes in human physiology.

The authors will be grateful for the advice and comments designed to improve the content of the guide.

Professor Victor Vovc

MEMBRANE PHYSIOLOGY, NEURON, NERVE AND MUSCLE

Theme 1. **Purpose of physiology. the structure and the functions of the cell membrane.**

Check questions:

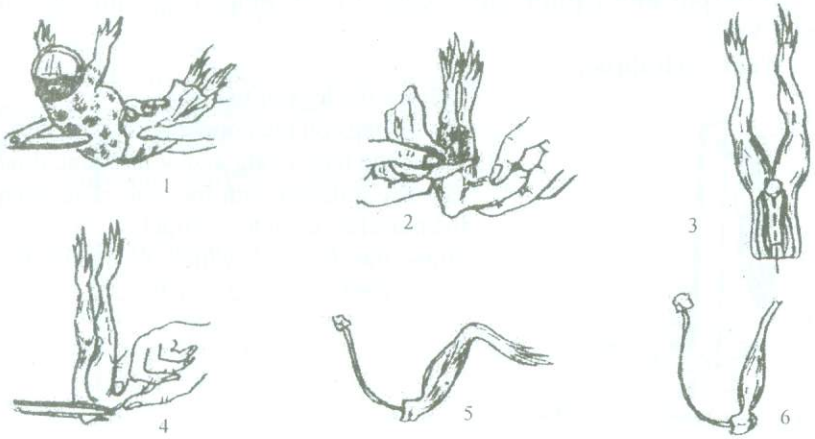
1.1 Introduction to human physiology. The purpose and methods of human physiology.

1.2 Cells as living units of the body. Homeostasis. Regulation of the body functions.

1.3 The structure and the functions of the cell membrane. Transport of substances through the cell membrane. Passive transport (diffusion, facilitated diffusion, osmosis). Types of ionic channels. Active transport (primary active transport and secondary active transport).

Laboratory Work Nr.1 The preparation of the neuromuscular sample:

1. The preparation of a frog with the destruction of the spinal cord.
2. An incision is made on the posterior side, 1.5 cm above the sacrum, after that another incision is made along the sacrum and the anterior side of the torso is removed along with the organs.
3. Remove the skin from the posterior limbs with a mull paper.
4. Divide the sample in two equal parts along the spinal cord and symphysis.
5. Place the inferior limb on the board, find the exiting place of the fibers which form the sciatic nerve. Prepare this nerve up to the coxofemural joint. For that turn the limb with the dorsal part up, with the glass stick, split the hip muscle, find the sciatic nerve and carefully release it from its origin in the spine down to the knee joint. Remove the muscles and bones above the knee.
6. After an incision of the Achilles tendon, the gastrocnemian muscle and also the leg below the knee joint are removed. What remains are the gastrocnemian muscle, the knee joint and sciatic nerve. For the preparation it is also necessary to remove the sciatic nerve.



Steps of neuromuscular sample preparation

Theme 2. **Electrophysiology of the cell membranes. The nervous fibers.**

Check questions:

2.1 Basic physics of membrane potential. Membrane potentials caused by diffusion of the ions. Nernst's equation. Measurement of membrane potential. Resting membrane potential of nerve. Origin of the normal resting membrane potential.

2.2 Nerve action potential. Stages. Voltage-gated sodium and potassium channels. Record of the action potential. Refractory period.

2.3 Excitation – the process of eliciting action potential. Threshold. Strength – duration curve. Rheobase and chronaxy. Acute local potential and its characteristics. Electrotonic potentials.

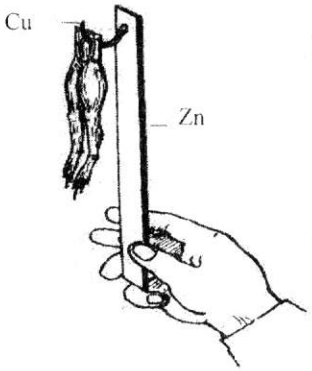
2.4 Propagation of action potential. Special characteristics of signal transmission in nerve trunks (myelinated and unmyelinated nerve fibers). Laws of signal transmission in nerve fibers. Functional classification of nerve fibers.

Laboratory Work Nr.1 Galvani's first experiment.

Work goal: to perform Galvani's experiment in order to study the history of discovery the "animal electricity".

Materials and equipment: a copper hook welded to a zinc plate, a frog, a vivisection kit.

Work technique:



1. Prepare the legs of the frog.
2. Fix the legs on the copper hook.
3. When the legs swing and at the same time they are touched with the zinc plate, then the muscles begin to contract.
4. Draw the *Fig. I.1* which illustrates the experiment and make conclusions.

***Figure I.1* Galvani's first experiment**

Laboratory Work Nr.2 Galvani's second experiment (contraction without metal).

Work goal: to demonstrate that there is a potential difference between the injured and the intact parts of the muscle which has an excitant action.

Materials and equipment: a frog, a vivisection kit, a dissecting board.

Work technique:

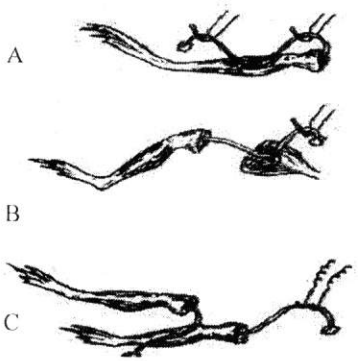


***Figure I.2* Galvani's second experiment (A, B) and Matteuci's experiment (C).**

1. Prepare the legs of the frog.
2. Make the incision on the gastrocnemian muscle near the knee.
3. Put the sciatic nerve on the muscle in such a way that will assure the simultaneous contact of the nerve with the both- injured and intact parts of the muscle. When touching, the contraction of the muscle is observed.
4. Draw the picture (A, B) which illustrates the experiment and make conclusions.

Laboratory Work Nr. 3 Matteuci's experiment – muscle excitation with action currents.

Work goal: to demonstrate the apparition of action potentials after muscle excitation which can spread out and excite the neuromuscular preparation.

Materials and equipment: a frog, a vivisection kit, a board, a stimulator.

Work technique:

1. Put the legs on the board so that the nerve of the first one is placed on the muscle of the second leg, and the nerve of the second leg must be connected to the electrodes of the excitation device.
2. Apply frequent excitations and observe the muscle contraction of the both legs.
3. Draw the picture above (C) which illustrates the experiment and make conclusions.

Laboratory Work Nr. 4 The determination of the threshold intensity of the stimulus at direct and indirect excitation of the muscle with unique stimuli.

Work goal: to determine the excitation threshold of one muscle and one nerve in order to demonstrate the different excitability of the nervous and muscular tissue.

Materials and equipment: a frog, a vivisection kit, a board, a stimulator.

Work technique:

1. Prepare the leg of the frog.
2. Connect the exciting electrodes to the stimulator (to the clips of direct current) and fix the indicator of the stimulus intensity in "0" position.
3. Place the electrodes on the sciatic nerve. Determine the threshold intensity of the stimulus. By rotating the button "Intensity", determine its position when a minimal contraction of the muscle appears. In this way, we find out the threshold intensity of the stimulus.
4. Place the electrodes directly on the muscle and determine the excitation threshold of the muscle.
5. Note the obtained data and make conclusions.

Laboratory Work Nr. 5 Determination of rheobase and cronaxy of the flexor muscles of the hand.

Work goal: to determine the rheobase and cronaxy of the hand flexor muscles.

Materials and equipment: cronaximeter, 2 electrodes, gauze pads, ether, Ringer solution, volunteer

Work technique:

1. Fix the electrode on the anterior surface of the forearm. Before this, the skin must be cleaned with ether, and a gauze pad wetted with Ringer solution must be applied on the place where the electrodes will be fixed.
2. The active electrode wetted with Ringer solution is applied on one of the motor points of the forearm or the palm.
3. Pressing the button in the position "unique stimulus", an electric stimulus is applied, the intensity being regulated according to the scale. Gradually, increasing the intensity of the stimulus, find the intensity which causes the sensation of excitation (sensorial rheobase); increasing a little bit more the intensity of the stimulus the muscle contraction is observed (motor rheobase).
4. Apply stimuli with double intensity on the skin and rotating the button which controls the duration of the stimuli determine the cronaxy: according to the sensations - sensorial chronaxy, and according to the muscle contraction - motor cronaxy.
5. Write in the report: the principles of determination of rheobase and cronaxy, the definitions, the obtained results. Explain which proprieties of the excitable tissues are characterized by rheobase and cronaxy.

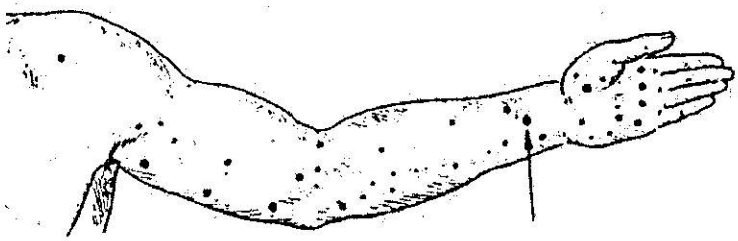


Figure I.3 **Motor points of the arm's muscles. The arrow indicates the *flexor digitorum communis* muscle point.**

Laboratory Work Nr. 6 The laws of propagation of excitation through the nerve fibers.

Work goal: to observe during the experiment the basic laws of propagation of excitation through the nerve fibers.

Materials and equipment: a frog, a vivisection kit, a board, a stimulator, 2 electrodes, a stand, Ringer solution, ammonia or chloroform solution, cotton-wool wads.

Work technique:

1. Prepare the legs of the frog and fix them on the stand.
2. Using the tweezers apply one ligature under each spinal root. Holding one nerve root by the ligature, place the electrodes under it and excite it with a threshold intensity excitation. Observe which groups of muscles contract. The experiment is repeated placing the electrodes under other roots (the law of isolate propagation through the nerve fibers).
3. Place the legs of the frog on the cork plates of the board. Prepare the sciatic nerve on the dorsal surface of the thigh. Cut the femur and the muscles with the scissors keeping the sciatic nerve intact. Apply frequent excitations using an over-threshold stimulus on the sciatic nerve and observe the contractions of the thigh and leg muscles higher and lower the section (the law of bilateral propagation of excitation through the nerve fiber).

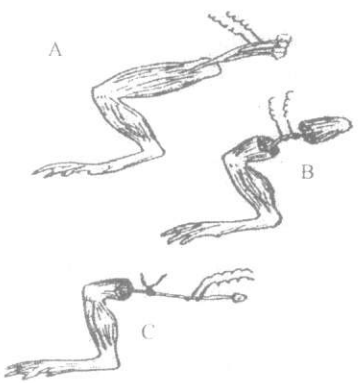


Figure I.4 **Experimental demonstration of the propagation laws of excitation through nerves and nerve fibers.**
A - Isolated propagation of excitation through nerves; B - Bilateral propagation of excitation; C - The necessity of physiological integrity of the nerves.

4. Apply cotton-wool wads wetted with chloroform, or a ligature on the sciatic nerve. Excite the nerve applying a current of

threshold intensity higher and lower the altered place and fix the case when the gastrocnemian muscle contracts (the law of physiological integrity of the muscle fibers).

5. Draw the schemes of the experiment and formulate the conclusions.

Theme 3. **The physiology of the nervous cells and synapses.**

Check questions:

3.1 Neuron as the basic functional unit of the nervous system. The central nervous systems synapses. The types of synapses. Physiologic anatomy of the synapse. The mechanism of neurotransmitter release. Special characteristics of synaptic transmission (one way conduction, synaptic delay, fatigue).

3.2 Electrical phenomena during neuronal excitation. Chemical substances that function as excitatory neurotransmitters. The ionic mechanism of EPSP (excitatory postsynaptic potential). Generation of action potential in the initial segment of the axon.

3.3 Electrical phenomena during neuronal inhibition. Chemical substances that function as inhibitory neurotransmitters. The ionic mechanism of IPSP (inhibitory postsynaptic potential). Postsynaptic inhibition. Presynaptic inhibition.

3.4 Transmission and processing of signals in neuronal pools. Relaying of signals through neuronal pools. One way conduction, convergence, divergence, after discharge, rhythmical output, facilitation, occlusion, fatigue.

Laboratory Work Nr.1 The reflex receptive field.

Work goal: experimental demonstration of the fact that each reflex has its field of reception.

Materials and equipment: a frog, dissection kit, a stand with hook, sulfuric acid solution (1%), a glass of water, filter paper.

Work technique:

I. Examination of the receptive field corresponding to the various reflexes on the intact frog *(Fig.I.5).*

a. Blink reflex.

When touching the frog's cornea with tweezers, notice the reflex reaction of the blink. Excitation of other regions of the body show that this reflex does not occur.

b. Noise reflex.

Compress the lateral sides of the frog's body. Notice the appearance of the noise reflex. In other parts of the body this reflex does not appear.

c. Gripping reflex.

Press the frog's paw or the sternum region. Observe the gripping reflex that cannot be revealed by stimulating other reflex regions.

II. Study of spinal frog reflexes that appear at the excitation of different fields of reception.

1. Prepare the spinal frog through decapitation and fix it on the stand.
2. A piece of filter paper (1x1 cm) soaked in sulfuric acid solution is applied on different regions of the frog's skin.
3. Excite different regions of skin: the paw region, the lateral surface of the thigh, sacral and pectoral regions and observe various motor reflexes.
4. In the report note the obtained results, draw the scheme of the reception fields, make conclusions.

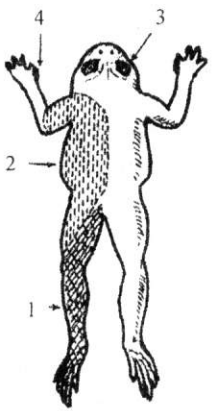


Figure I.5 **Receptive field of the reflexes:**
1-flexion reflex; 2-scraching reflex; 3-blink reflex; 4- gripping reflex.

Figure I.6 **Determination of the reflex time using the method of Tűrck.**

Laboratory Work Nr. 2 The determination of the reflex time using the method of Tűrck.

Work goal: the determination of the reflex reaction time depending on the intensity of the excitant.

Materials and equipment: a frog, stand with hook, solution of sulfuric acid (0.25, 0.5, 1.0 %), glass of water, filter paper, stopwatch.

Work technique:

1. Prepare the frog and fix it on the stand.
2. After 1-2 minutes, placed the frog paw in solution of sulfuric acid (0.25%). Measure the time from the moment of the application until the excitant response appears *(Fig. I.6)*.
3. Repeat the experiment three times.
4. Repeat this experiment with 0.5% and 1.0% solution of sulfuric acid. The results are written in the table below.
5. Compare the results and write the conclusions.

Reflex time depending on the concentration of sulfuric acid.

Sample nr.	Reflex time		
1	0.25%	0.5%	1.0%
2			
3			
Average			

Laboratory Work Nr.3 The analysis of the reflex arch.

Work goal: determination of the importance of all links of the reflex arch in achieving it.

Materials and equipment: a frog, a stand with hook, a vivisection kit, a glass of water, solution of sulfuric acid (1.0%), thread, chloroform solution, filter paper.

Work technique:

1. Prepare the spinal frog and fix it on the stand.
2. Observe the flexion reflex after the excitation of the posterior paw with sulfuric acid.
3. Make a circular incision of the skin below the knee and then remove it like a sock. This way, the first link of the reflex arch, the receptors, is removed.
4. Apply a filter paper soaked with sulfuric acid and then observe the lack of reflex.

5. Remove the skin and the muscles from the other paw. The sciatic nerve is ligatured in order to stop the conductivity with a thread soaked in chloroform.
6. Destroy the spinal cord with a probe and apply stimuli over any reception field. No reflexes occur.
7. In the report describe the experiment, explain the observed phenomena, and write conclusions. Draw the scheme of the reflex arch and mark its main links (*Fig. I.7*).

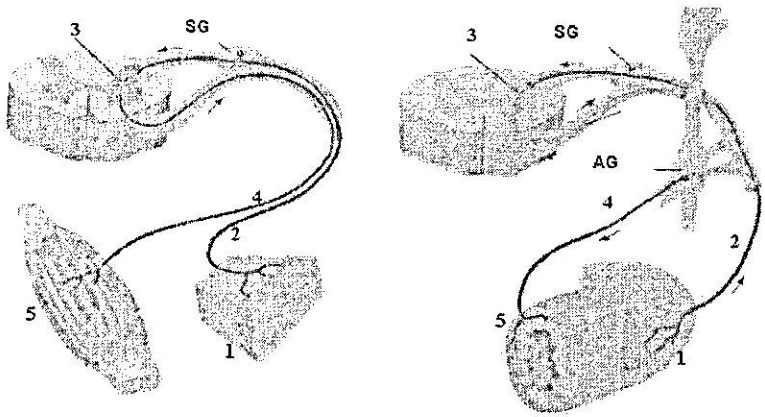


Figure I.7 **The links of the somatic and autonomic reflex arch:** 1-receptors; 2-afferent pathway; 3-nervous center; 4-efferent pathway; 5-effector organ.

Laboratory Work Nr. 4 The inhibition of the spinal reflexes (I. Secenov's experiment).

Work goal: study of the descendent inhibitory influence of diencephalon on spinal reflexes.

Materials and equipment: a frog, a stand with hook, a vivisection kit, a glass of water, solution of sulfuric acid (0.25%), NaCl crystals, stopwatch.

Work technique:

1. Prepare the thalamic frog by: remove the skin and open the skull between the eyes from the anterior parts of the orbits to the posterior ones on the area 1x2 cm^2, the brain is visible. Cut the brain on the upper edge of the optic thalami and remove the hemispheres.

2. Fix the frog on the stand by the lower jaw.

3. Determine the flexion reflex time by exciting the paw with the sulfuric acid solution (0.25%). After repeating this step three times, the average time is calculated.

4. In 2-3 minutes, a crystal of NaCl is placed on the optic thalami. In 1-2 minutes, the time of the same reflex is determined by repeating the same experience until the reflex is completely gone.

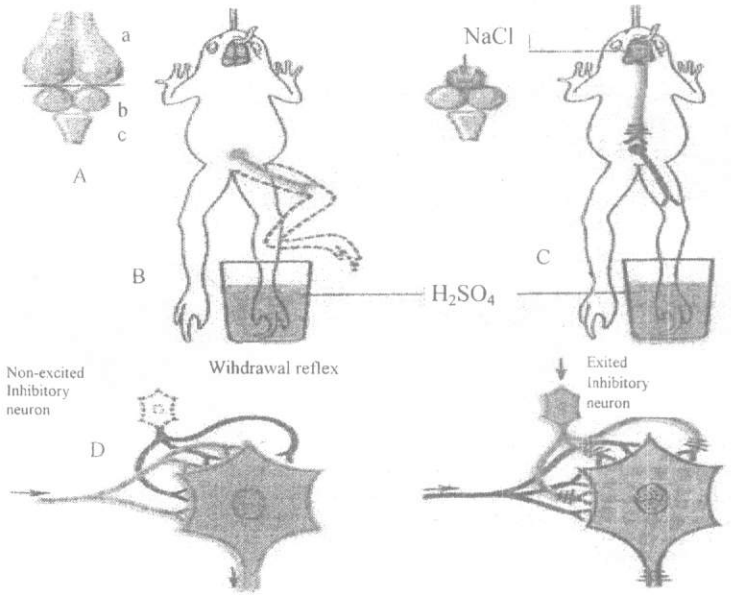


Figure I.8 **The scheme of Secenov's experiment and the probable mechanism of central inhibition.**

A - Secenov's level of section of the frog's encephalon: *a-the hemispheres; b-mesencephalon; c-spinal bulb. B* - Determination of the reflex time without application of the NaCl crystal. C - Determination of the reflex time after the application of the NaCl crystal. D - The phenomenon of neuronal inhibition.

Laboratory Work Nr.5 Mutual inhibition of the spinal reflexes.

Work goal: experimental demonstration of the mutual inhibition.

Materials and equipment: a frog, a stand with hook, a vivisection kit, a glass of water, solution of sulfuric acid (0.25%), tweezers, filter paper, stopwatch.

Work technique:

1. Prepare the spinal frog and fix it on the stand.
2. In 1-2 minutes determine the flexion reflex time by the method of Tűrck exciting with sulfuric acid (0.25%).
3. In 2 minutes the same step is made, meanwhile the other paw is compressed with tweezers. The reflex is inhibited.
4. Write the results and conclusions in the report.

Laboratory Work Nr. 6 Examination of the action of curare toxin on muscle contraction.

Work goal: to observe during the experiment the basic laws of propagation of the excitation through the nerve fibers.

Materials and equipment: a frog, a vivisection kit, a board, an excitation device, 2 electrodes, Ringer solution, curare solution.

Work technique:

1. Prepare the legs of the frog.
2. Place the legs on the board and apply the electric threshold stimulus first on the nerve, and then on the muscle. In both cases, muscle contraction is observed.
3. Put the muscle in the Petri dish with Ringer solution which contains a myorelaxant drug.
4. In 1-2 minutes apply again a stimulus on the muscle and observe its contraction. When applying the stimulus on the nerve, no muscle contraction is observed. After 1-2 minutes, repeat the experiment.
5. Draw the scheme of experiment, explain what happened and formulate conclusions.

Theme 4. **The physiology of skeletal and smooth muscles**

Check questions:

4.1 The neuromuscular junction. Physiological anatomy of neuromuscular junction. The mechanism of synaptic transmission. The release of acetylcholine, the end-plate potential, the role of acetylcholinesterase. Drugs that enhance or block transmission in the neuromuscular junction.

4.2 Physiologic anatomy of the skeletal muscles. Myofibrils, actin and myosin filaments, sarcomere. Molecular characteristics of contractile

filaments. Transverse tubule – sarcoplasmic reticulum system. Excitation-contraction coupling.

4.3 Molecular mechanism of muscular contraction. Sliding filament mechanism. The "walk-along" theory of contraction. Effect of muscle length on the force of contraction. Muscle relaxation.

4.4 Isometric versus isotonic contraction. Fast and slow muscle fibers. Motor unit. Multiple fiber summation and frequency summation. Muscle hypertrophy and muscle atrophy. Fatigue.

4.5 Contraction of smooth muscles. Types of smooth muscles. Contractile mechanism in smooth muscles. Nervous and hormonal control of smooth muscle contraction.

Laboratory Work Nr. 1 Unique and tetanic contractions of the skeletal muscles.

Work goal: to record unique and tetanic contractions of the skeletal muscle and to study the conditions when different types of muscle contractions summation appear.

Materials and equipment: a frog, a vivisection kit, Engelmann levergraph with pen, a kymograph, paper, glue, an electrical stimulator, Ringer solution, ink.

Work technique:

1. Prepare the gastrocnemian muscle of the frog. Fix the muscle on the myograph and regulate recording on the kymograph.
2. Connect the wire with the electrical stimulator using the clips of the myograph.
3. Apply one stimulus and record a unique contraction of the muscle. In order to obtain a developed curve, remove the screw which fixes the tambour of the kymograph and in the moment of excitation, rotate it fast with the hands.
4. Fix the tambour, turn on the kymograph and record the curves of complete and incomplete titanic contractions.
5. Attach the kymograms to the report; describe the phases of unique muscle contraction and the conditions under which complete and incomplete tetanic contractions appear.

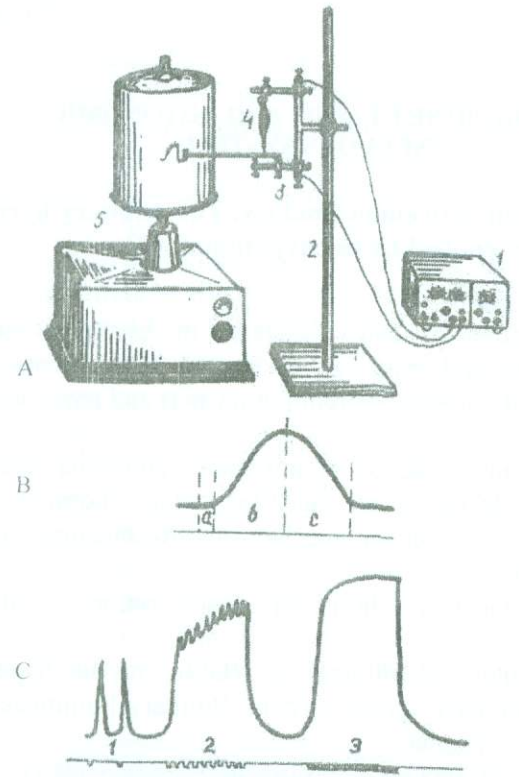


Figure I.9 **Myography**

A - Device for recording muscular contractions: 1 – electric stimulator; 2 – stand; 3 – myograph; 4 – gastrocnemian muscle; 5 – kymograph. B - The curve of unique contraction: a-latent period; b-contraction phase; c-relaxation phase. C - The curves of tetanic contractions: 1 – unique contraction; 2 – incomplete titanic contraction; 3 – complete titanic contraction.

ENDOCRINE GLANDS AND AUTONOMIC NERVOUS SYSTEM

Theme 1: **Introduction to endocrinology. The pituitary hormones and their control by the hypothalamus.**

Check questions:

1.1 Coordination of body functions by chemical messengers. Chemical structure and synthesis of hormones. Hormone secretion: feedback control of hormone secretion, transport and removing from the blood.

1.2 Mechanisms of action of hormones. Hormone receptors and their activation. Intracellular signalling after hormone receptor activation. Second messenger mechanisms to mediate intracellular hormonal function.

1.3 Measurement of hormone concentration in the blood. Radioimmunoassay.

1.4 The pituitary gland and its relation to the hypothalamus, hypothalamic – pituitary portal system. Pituitary hormones and their control by the hypothalamus.

1.5 Physiologic function of Growth hormone (GH). Metabolic effects of GH. Somatomedins. Regulation of GH secretion. Abnormalities of GH secretion.

1.6 The posterior pituitary gland and its relation to the hypothalamus. Chemical structures of ADH and Oxytocin. Physiologic function of ADH and Oxytocin

Laboratory Work Nr.1 The influence of pituitrine and adrenaline on the skin melanocytes of the frog.

Work goal: to determine the role of hormones in the mechanisms of biological adaptation at the cellular level of the organism.

Materials and equipment: a frog, a vivisection kit, pituitrine and adrenaline solutions (1:1000), Ringer solution, 3 glasses, microscope with small objective, glass blade.

Work technique:

1. Immobilize the frog, cut 3 square-shaped pieces of skin (2x2 cm^2) from the lateral side of the body or from the thigh and put them in 3 glasses. Pour 5 ml of Ringer solution on each of the pieces. In one glass add 3 drops of pituitrine, in the second glass add 3 drops of adrenaline and the third glass remains for control.
2. Examine the pieces of skin under the microscope every 10-15 minutes *(Fig.II.1)*.
3. The action of the melanocyte-stimulating hormone (the pituitrine is the extract of the posterior lobe of the pituitary gland which contains this hormone) begins after 30 minutes. The granules of pigment move from the center of the cell to the ramifications of melanocytes. The maximal dispersion of the pigment is observed in 2 hours (the skin becomes hyperpigmentated).
4. Adrenaline action is observed in 15-20 minutes: the pigment granules concentrate in the centre of the cell, causing skin whitening/decolouring.
5. In the piece of skin from the Ringer solution, melanocytes are seen under the microscope like black stars.
6. Draw the intact melanocytes, those influenced by adrenaline and pituitrine. Explain the biological importance of these reactions.

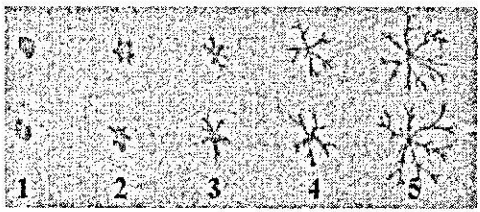


Figure II.1 **Repartition of the melanine pigment in melanocytes.**
1, 2 – adrenaline influence; 3 – control; 4, 5 – pituitrine influence.

NOTE: Frog's melanocytes have no innervation and their functional condition is regulated by hormones only. The melanocyte-stimulating hormone has an important role in the regulation of pigment distribution.

Theme 2: **The thyroid, parathyroid and pancreatic hormones**

Check questions:

2.1 Synthesis, secretion and transport of the thyroid metabolic hormones (T3 and T4).

2.2 Physiologic functions of the thyroid hormones. The effect of T3 and T4 on growth, and on specific body mechanisms. Regulation of thyroid hormones secretion.

2.3 Diseases of the thyroid. Hyperthyroidism. Hypothyroidism.

2.4 Insulin and its metabolic effects. Glucagon and its functions. Effects on glucose metabolism. Regulation of glucagon and insulin secretion. Diabetes mellitus.

2.5 Parathyroid hormone. Pathology of the parathyroid glands, vitamin D, and bone diseases. Primary hyperparathyroidism, secondary hyperparathyroidism. Rickets – vitamin D deficiency. Osteoporosis.

2.6 Calcitonin. Overview of calcium and phosphate regulation in the extracellular fluid and plasma. Bone and its relation to extracellular calcium and phosphate.

2.7 Physiology of the teeth. Functions of the different parts of the teeth. Dentition. Mineral exchange in the teeth. Dental abnormalities.

Laboratory Work Nr.1 The influence of insulin on the organism.

Work goal: metabolic action of insulin on the organism.

Materials and equipment: 2 white hungry mice (24 hours), syringe (1ml), insulin, glucose solution (10%).

Work technique:

1. Inject 0.2-0.5 units of insulin diluted in 0.1 ml of distilled water under the skin of the both mice.
2. Simultaneously one of the mice is being injected intraperitoneally at the same moment 1ml of glucose solution (10%). The mouse that was injected only insulin without glucose has the symptoms of hypoglycemic shock (unusual position, frequent respiration, convulsions). The convulsions appear faster if after the injection of insulin the mouse is placed in a warm place. The mouse that was injected both insulin and glucose, has no hypoglycemic shock.
3. Write the obtained results and the mechanism of action of insulin.

Theme 3. **The adrenocortical hormones. Male and female reproductive and hormonal functions. pregnancy and lactation.**

Check questions:

3.1 Synthesis and secretion of adrenocortical hormones

3.2 Functions of mineralocorticoids – aldosterone. Functions of glucocorticoids. Adrenal androgens.

3.3 Abnormalities of adrenocortical secretion. Hypoadrenalism – Addison's Disease. Hyperadrenalism – Cushing's syndrome. Primary aldosteronism – Conn's syndrome. Adrenogenital syndrome.

3.4 Testosterone and other male sex hormones. Abnormalities of male sexual functions. The pineal gland – its function in controlling seasonal fertility in some animals.

3.5 Function of female sexual organs. Female hormonal system. The monthly ovarian cycle; effects of gonadotropic hormones. Function of the ovarian hormons – estradiol and progesterone.

3.6 Regulation of the female monthly rhythm – interplay between the ovarian and hypothalamic – pituitary hormones. Maturation and fertilization of the ovum. Early nutrition of the embryo. Function of the placenta. Hormonal factors in pregnancy.

Laboratory Work Nr.3 Galli-Mainini test.

The diagnosis of pregnancy before the apparition of clinic signs is performed according to a few biological, immunological methods etc. The biological tests are based on the presence of a big quantity of chorionic gonadotropin hormone (CGH) in the female blood serum and urine, which has the property of modification the genital tract in laboratory animals. The most frequently used are: Galli-Mainini and Ascheim-Zondek tests.

Galli-Mainini test is based on the fact that the apparition of spermatozoids in the genital tract of a frog male is caused by the injection of gonadotropin hormone, because the spermatozoids are found in the genital tract just in the period of mating.

Work goal: the precocious establishment of pregnancy diagnosis.

Materials and equipment: a frog male, a syringe with needle, pregnant woman urine (or the chorionic gonadotropin hormone), glass blade, pipette, microscope with a small objective, Ringer solution.

Work technique:

1. The frog male is injected 4 ml of pregnant woman urine in the dorsal lymph sacs. To avoid the outflow, the injection is made with the needle through the thigh muscles. It is used the morning urine of the examined person.
2. In 45 minutes extract the urine from the cloaca of the frog using the pipette.
3. Put a drop of the extracted urine on the glass blade and examine it under the microscope.
4. In case of pregnancy in the extracted urine there are many mobile spermatozoids.
5. If in 45 minutes there are no spermatozoids, the extraction is repeated in 4-7 hours after the injection (the period of time when the reaction has the maximal intensity).
6. The absence of spermatozoids in the urine indicates no pregnancy.
7. Draw the picture and make conclusions.

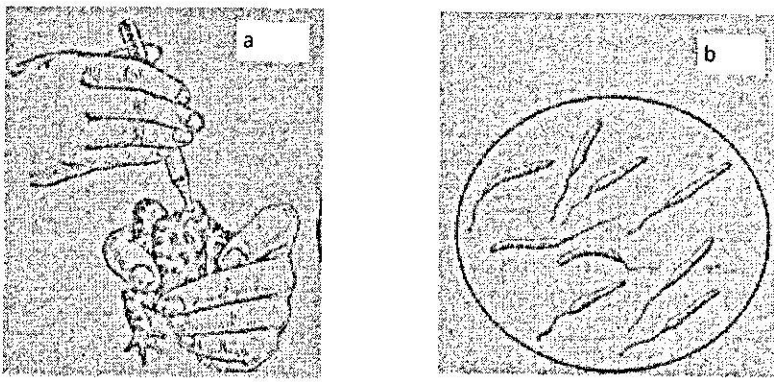


Figure II.2 **Galli-Mainini test.** a. Extracting the content; b. Frog's spermatozoids.

Theme 4. **The autonomic nervous system and the adrenal medulla.**

Check questions:

1. General organization of the Autonomic Nervous System. Sympathetic preganglionic and postganglionic neurons. Parasympathetic preganglionic and postganglionic neurons.

2. Basic characteristics of sympathetic and parasympathetic functions. Cholinergic and adrenergic fibers – secretion of acetylcoline or norepinephrine. Receptors on the effector organs.

3. Mechanisms of sympathetic and parasympathetic mediators. The effects of sympathetic and parasympathetic stimulation on the human body.

4. Excitatory and inhibitory actions of sympathetic and parasympathetic stimulation on the organs. Sympathetic and parasympathetic stimulation on specific organs.

5. The function of the adrenal medulla. The relation of stimulus rate to degree of sympathetic and parasympathetic effect. „Alarm" or „stress" response of the sympathetic nervous system.

6. Control of autonomic functions by the superior brain stem and hypothalamic centers. Autonomic reflexes.

Laboratory Work Nr. 1. The influence of adrenaline on the pupil of an enucleated frog eye.

Work goal: to observe *in vitro* the kinetic influence of adrenaline on the organ.

Materials and equipment: a frog, a vivisection kit, Ringer solution, adrenaline(1:1000), 2 glasses.

Work technique:

1. Immobilize the frog and enucleate the eye balls (extract them from the orbits).
2. Pour 5 ml of Ringer solution in two glasses and put an eye ball in each of them.
3. Add 0.5 ml of adrenaline in one of the glasses. In 15 min examine the pupil and compare it with the other pupil.
4. In the report explain the mechanism of adrenaline action on the pupil.

NOTE: Adrenaline causes contraction of the dilator muscle of the pupil. Its action can be observed not only in the integral organism, but also in isolated organs.

Laboratory Work Nr.2 Functional tests for the determination of the autonomic nervous system tonus condition.

Work goal: to study different vegetative reflexes in order to appreciate the vegetative system tonus.

Materials and equipment: stopwatch, sphygmomanometer, stethoscope, an examined person.

Work technique:

Orthostatic test

In 5 minutes in the clinostatic position determine the frequency of the cardiac contractions and measure the arterial blood pressure. Then, the examined person stands up slowly on his feet in the orthostatic position.

During the 1st and 3rd minutes take the pulse, and during the 3rd and 5th minutes measure the arterial blood pressure. The results are determined according to the pulse and arterial blood pressure in the three-grade system.

The obtained results are compared with the ones from the chart.

Parameters	Test tolerability		
	good	sufficient	insufficient
Frequency of the cardiac contractions(*FCC*)	The acceleration does not exceed 11 contractions	Acceleration with 12-18 contractions	Acceleration with 19 contractions and even more
Systolic pressure(*SP*)	Increases	Does not change	Decreases within the limits of 5-10 mmHg
Diastolic pressure (*DP*)	Decreases	Does not change or increases slightly	Increases
Pulse pressure(*PP*)	Increases	Does not change	Decreases
Vegetative reactions	No	Sweat	Sweat and buzzing in the ears

Aschner-Dagnini reflex

1. Determine the frequency of the cardiac contractions.
2. Press the eyeballs slowly with the middle and index fingers during 10 seconds.
3. Take the pulse immediately after pressing. Usually a decrease of the frequency of the cardiac contractions ranged from 6 to 10 beats is observed.
4. Write the conclusions and draw the scheme.

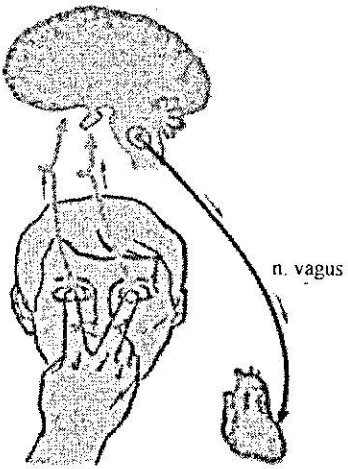


Figure II.3 **Dagnini-Aschner reflex arch**

Laboratory Work Nr.2 Calculation of the Kredo's autonomic index.

Work goal: to study the method of determining the autonomic index in order to determine the prevalence of the tonus of one of the parts of the vegetative nervous system.

Materials and equipment: sphygmomanometer, stethoscope, stopwatch.

Work technique:

Kredo autonomic index is calculated according to the formula:

$$IV\ (Kredo) = \left(1 - \frac{\text{Diastolic pressure}}{\text{Heart rate}}\right) \times 100$$

If the value of the index is negative ‚-' then the tonus of the parasympathetic system is prevalent - **vagotonic**, and if the value is positive ‚+' then the tonus of the sympathetic system is prevalent - **sympathotonic**. If the result is neither negative, nor positive then the autonomic nervous system is equilibrated - **normotonic**.

THE CARDIOVASCULAR SYSTEM

Theme 1. **The heart**

Check questions:

1.1 Physiology of the cardiac muscle. Physiological characteristic features of the cardiac muscle (excitability, conductibility, contractility (principle „all or nothing"), rhythmicity, refracterity, tone). Action potentials in the cardiac muscle. Chemical energy required for a cardiac contraction: oxygen utilization by the heart.

1.2 The cardiac cycle, its phases and their duration. Functions of the valves. Functions of the atria as primer pumps. The function of the ventricles as pumps. Pressure in the atria and ventricles during each phase of the cardiac cycle. Aortic pressure curve. Stroke volume and cardiac output. Graphical analysis of the ventricular pumping.

1.3 Specialized excitatory and conductive system of the heart. Sinus (sinoatrial) node. The sinus node as a pacemaker of the heart. The origin of cardiac automaticity. Gradient of cardiac automaticity (Stannius experiment). Control of excitation and conduction in the heart.

Laboratory Work Nr. 1 Observation and graphical representation of the mechanical activity of the heart. Cardiogram.

Work goal: to record frog's heart contractions in acute experiments.

Materials and equipment: a frog, a dissection kit, kymograph, Engelmann lever graph, a pin (pliers-shaped), thread, entomological pins, Ringer's solution, a board, accessories (cotton swabs, gauze pads).

Work Technique:

1. Prepare the spinal frog by decapitation and fix it on the board in the dorsal decubitus.
2. The heart is opened via "V"-shaped incision with the origin at the bottom of the chest; it is isolated from the pericardial sac and the heart frenulum is sectioned.
3. The pin with thread fastened to the Engelmann lever graph is fixed to the apex of heart.

4. The recording pen of the Engelmann lever graph is placed near the recording device of the kymograph. Suspend the balance lever in the attachment region of the pin, device that facilitates the activity of heart and increases the amplitude of recording (*Fig. III.1*).
5. Pace the recording pen nearer to the kymograph tambour, which is connected at that moment.
6. The movement of kymograph tambour allows the recording of cardiogram – the phases of cardiac cycle.
7. The cardiogram with the above mentioned phases of the cardiac cycle is attached to the report.

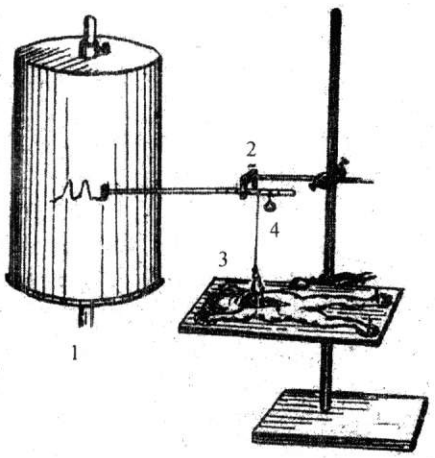


Figure III.1 **Installation for cardiogram recording.**

Laboratory Work Nr. 2 The forms of the heart's excitability. The extrasystole.

Work goal: the experimental demonstration of the modification of cardiomyocytes excitability during the cardiac cycle.

Materials and equipment: a frog, a scalpel, a kymograph, Engelmann levergraph, a pin (pliers-shaped), a balance, thread, entomologic needles, a board, an electric stimulator.

Work technique:

1. Register the cardiogram (see Laboratory Work Nr.1).
2. Apply to the back of the frog an electrode wrapped in gauze soaked with Ringer solution and connected to the stimulator.

3. Register 2-3 cardiac cycles, then connect the excitant electric circuit and with the apply induction shocks on the ventricle with the second electrode.
4. Note that contractions may appear or not according to the moment of stimulus application:
   - The stimulus applied during the ventricular systole does not produce any reaction to the excitation;
   - The stimulus applied during the ventricular diastole produces an additional contraction (extrasystolar) followed by a longer period of time (a compensatory pause) till the next contraction;
   - The amplitude of the extrasystole depends on the moment of the excitant application: at the beginning or at the end of the diastole.

NOTE: The compensatory postextrasystolic pause is explained by the fact that the physiologic stimulus (generated by the cardiac pacemaker) keeps the heart in an additional contraction (the absolute refractory period of the extrasystole) and thus remains unreacted. A pause appear in the activity of the heart till a new contraction determined by a physiologic stimulus.

5. The cardiogram in which the extrasystole and the compensatory pause is marked is attachedto the report. The role of the phase of refraction in the function of the heart and the origin of the compensatory pause in ventricular extrasystole are explained

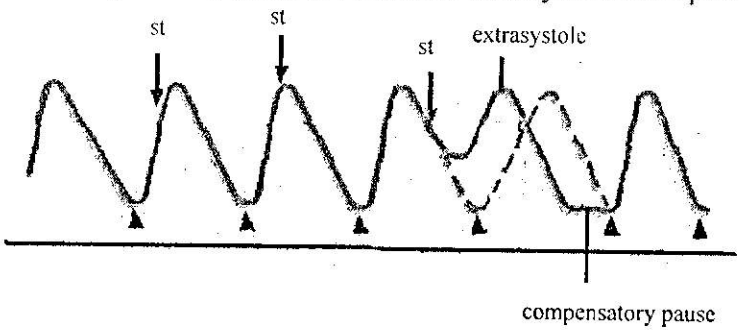


Figure III.2. **The scheme of the registration of an extrasystole with a compensatory pause.**

Laboratory Work Nr.3 Study of the degree of automaticity in different regions of the frog's heart – Stannius experiment.

Work goal: during the experiment to isolate the structures of the excito-conductor system in order to determine the descendent gradient of the cardiac automaticity.

Materials and equipment: a frog, a dissection kit, kymograph, Engelmann lever graph, a pin (pliers-shaped), stand, stand holder, thread (non-stretch nylon or equivalent), entomological pins, a board.

Work Technique:

Prepare the frog to record the cardiogram (see Laboratory Work Nr. 1).

1. Record the normal cardiogram and count the heart contractions per one minute *(Fig III. 3 A)*.
2. **The First Stannius Ligature.** The thread is passed under the venous sinus at the boundary between it and the auricle. It is ligated obtaining in this way the total isolation of the venous sinus from the rest of the heart. Observe the activity of the heart on kymograph (the heart does not contract) and count the contractions of the venous sinus *(Fig. III. 3 B)*.
3. **The Second Stannius Ligature** is applied between the atria and the ventricle on the heart which contains the first ligature. The kymograph continues recording of the cardiogram. Count the contractions of the functional segment of the heart per one minute. Compare the outcome with the frequency of the venous sinus contraction *(Fig. III. 3 C)*.
4. Apply the **Third Stannius Ligature** on the lower third part of the ventricle. Notice that the isolated apex of the heart contracts only in the mechanical and electrical excitations *(Fig. III. 3.D)*.
5. The report contains the scheme regarding the application of ligatures and is attached the recorded kymograms and notes on the number of contractions of the heart before and after the application of each ligature.

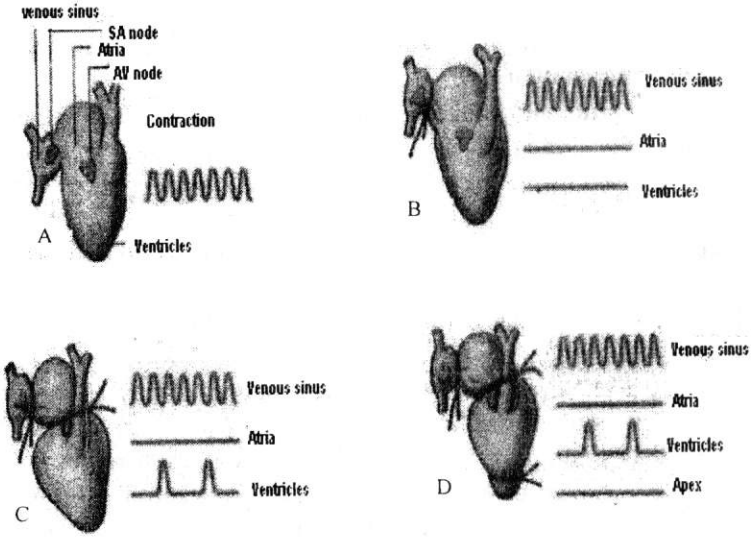


Figure III. 3. **Stannius ligatures:**
A– cardiogram recording; *B*– 1st ligature; *C* - 2nd ligature; D - 3rd ligature.

Theme 2. Overview of circulation. Microcirculation.

Check questions:

2.1 Physical characteristics of the circulation. The basic theory of the circulatory function. Blood flow. Blood pressure. Resistance to blood flow. Interrelationships among pressure, flow, and resistance.

2.2 Vascular distensibility. Vascular compliance (or vascular capacitance). Volume-pressure curves of the arterial and venous circulations. Arterial pressure pulsations. Venous pressures – right atrial pressure (central venous pressure) and peripheral venous pressures. Blood reservoir function of the veins.

2.3 Microcirculation and the lymphatic system: capillary fluid exchange, interstitial fluid and lymph flow. The structure of the microcirculation and capillary system. The flow of blood in the capillaries – vasomotion. Exchange of water, nutrients, and other substances between the blood and interstitial fluid. Hydrostatic and colloid osmotic pressures in fluid filtration across capillary walls.

2.4 Local and humoral control of the blood flow by the tissues. Local control of the blood flow in response to tissue needs. Acute and long-term control of the local blood flow. Humoral control of the circulation. Vasoconstrictor and vasodilator agents. Vascular control by ions and other chemical factors.

Laboratory Work Nr.1 The particularities of microcirculation in organs and tissues.

Work goal: the establishment of the functional particularities of the microcirculation in the tongue, mezenterium, lungs and in the swimming membrane.

Materials and equipment: a microscope, special board, cotton-wool, entomological needles, Ringer's solution, ether, a frog.

The technique of the task:

1. Observation the circulation in the lung vessels.

Narcotize the frog with ether. Make a lateral incision of the rib cage, and get out the lungs. Fix the frog on the board with the abdomen down, extend and fix the lungs over the orifice of the board. Look through the microscope the network of capillaries situated around the alveoli. Notice the speed of the blood flow and the change of the configuration of erythrocytes while they pass through the capillaries.

2. Observation the circulation through the mezenterium vessels.

The same frog is fixed in the dorsal position. Open the frog's abdomen by the incision in the lateral side. Get out the intestinal loop with the mesenterium. Irrigate the mezenterium with Ringer's solution and extend it over the orifice of the board. Pay attention to the anatomic distribution of the arteries, veins, and capillaries. The speed of blood in different portions of the vascular system does not differ. The speed of the erythrocytes will be higher in the arteries in comparison with that from veins. In the mesenterium the number of capillaries is much smaller than in the lungs and the swimming membranes, but the movement of the erythrocytes is much slower.

3. Observation the blood circulation in the tongue' vessels.

Fix the frog's tongue above the board's orifice. Wet the tongue with Ringer's solution and study it at the microscope. Notice blood vessels of different dimensions.

4. By analogy research the vessels of the swimming membrane. *(Fig. III.4)*

5. In the report write the particularities of the blood circulation in different vessels and the conclusions.

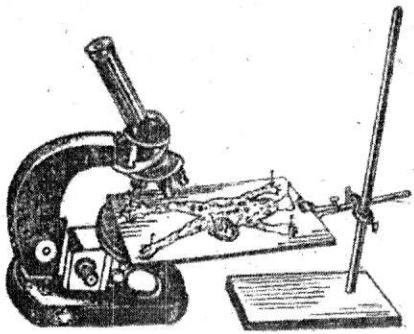


Figure III.4. **The scheme of installation to study microcirculation in different tissues.**

NOTE: The researching of the blood circulation in vessels is done at low magnification of the microscope. The researches on microcirculation may be completed by studying the action of vasoconstrictive and vasodilator substances. For this purpose we use the following solutions: epinephrine (1:1000), acetylcholine (1:10000), histamine (1:1000), Ringer's solution and a pipette. At low magnification of the microscope notice the blood flow. Then dribble histamine (or other substance with similar properties) on the swimming membrane (or on other studied region). We make obvious the change of the vessels' lumen and the deviations in the blood speed. After using of the substance, the portion under experiment is cleaned with Ringer's solution, after that another solution is used and so on.

Laboratory Work Nr. 2 Claude Bernard's experiment.

Work goal: to study the effects of sympathetic nerves on the vascular tonus.

Materials and equipment: a white rabbit, a vivisection kit, source of light, eletrothermometer, 20% solution of urethane, 2% solution of novocaine.

Work technique:

1. Narcotize the rabbit, and place it on a special table. Bend the head to the back and fix it with a stand. Cut the fur in the cervical region.
2. Make a longitudinal section of the skin in the middle cervical line. Remove the fascia and muscles that are laterally of the trachea, and find the vasculonervous fascicle of the neck: the carotid artery, the vagus nerve, the sympathetic nerve and the depressor nerve. Separate the sympathetic nerve (it is thin and grey), ligature, and cut it lower down the ligature.

3. Using the electrothermometer compare the colour and temperature of the both ears in 30 – 60 minutes.
4. Remove the ligature from the sympathetic nerve, apply the electrodes on it and excite it with the frequency of 10 – 20 impulses/second and the voltage of 20 – 30 V. Notice how the lumen of the ear vessels of the same part with the excited nerve has changed.
5. In your report describe the work technique and write the results of the experiment. Note the results of thermometer and explain why the blood vessels have changed their lumen after the ligaturation and stimulation of the sympathetic nerve.

Topic 3. **Regulation of the circulation, cardiac output and arterial pressure.**

Check questions:

3.1 Cardiac output, venous return, and their regulation. Normal values for the cardiac output at rest and during activity. Control of the cardiac output: Frank-Starling (heterometric) mechanism of the heart and homeometric mechanism. Hypertrophy of the heart. Pathologically high and pathologically low cardiac outputs.

3.2 Arterial pressure. Systolic and diastolic pressures, normal values. The mean arterial pressure. Clinical methods of measurement and record of the arterial pressure. Respiratory and vasomotor waves of the arterial pressure. Auscultatory method to measure the arterial pressure.

3.3 Nervous regulation of the circulation. The role of the nervous system in the rapid control of arterial pressure. Reflex mechanism of the maintenance of the normal arterial pressure. The ischemic response of the central nervous system. Special features of nervous control of arterial pressure.

3.4 The dominant role of the kidney in a long-term regulation of arterial pressure and in hypertension: the integrated system for pressure control. Renal-body fluid system for arterial pressure control. The renin-angiotensin system: its role in pressure control and in hypertension.

Laboratory Work Nr.1 Blood pressure measurement using the method of Riva-Rocci (the palpatory method).

Work goal: to fell the first heart beat of the radial artery at the moment of slow decompression of the cuff, placed around the arm in order to measure only the systolic pressure.

Materials and equipment: Riva-Rocci sphygmomanometer, a volunteer

Work technique:

1. The sphygmomanometer is composed of the following elements: cuff, inflation bulb and valve and measuring unit (manometer). The hose joins the cuff and manometer. With the help of the inflation bulb, the air is inflated in the cuff, which pressure is recorded by the manometer.
2. Place the cuff around the upper arm. The cuff is inflated until the pressure becomes higher than the maximum blood pressure. At the same time, palpate the pulse on radial artery. The heart beat can no longer be felt on this vessel (*Fig. III. 5*).
3. Decompress the cuff smoothly and slowly by opening the valve.
4. Record the readings of the manometer at the moment when the pulse can be felt. This can be noticed when the air pressure in the cuff becomes a little bit lower than the maximum blood pressure in vessel and pulsatile wave propagates through it.
5. In this very moment, manometer's readings will correspond to the maximal blood pressure (or systolic). The official report will include a description that depicts the above used method and the result of systolic blood pressure measurements.

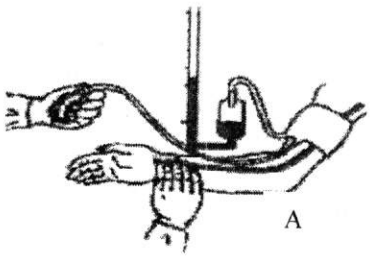


Figure III. 5 **Blood pressure measurement using the method of Riva-Rocci (the palpatory method).**

Laboratory Work Nr. 2 Measurement of the arterial pressure using the method of Korotkoff (the auscultatory method).

Work goal: listening to tapping sounds caused by blood passing through the partly occlused vessel when the cuff pressure gradually is reduced with the stethoscope placed over the brachial artery.

Materials and equipment: sphygmomanometer, stethoscope, a volunteer.

Work technique:

1. Place the cuff on the arm. Its inferior edge must be 2 – 3 cm above the cubital region. The cuff must not be placed over the clothes and at least of 12 cm wide.
2. Palpating find the brachial artery (in the cubital region) and place the stethoscope over it but not under the cuff.
3. Using the rubber pear, elevate the pressure in the cuff with 30 – 40 mmHg above the pressure at which the pulse of the radial artery disappears *(Fig. III.6)*.
4. Reduce gradually the pressure in the cuff by opening the valve. The pressure when you begin to hear tapping sounds is the ***systolic pressure***; when Korotkoff's sounds cease to be audible, the manometer pressure is about equal to the ***diastolic pressure.*** Repeat the experiment with both arms in the clinostatic and orthostatic positions.

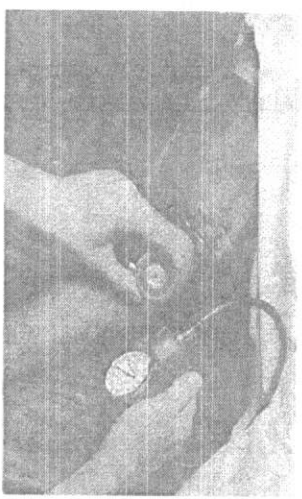


Figure III.6 **Korotkoff's method (the auscultatory method) for measurement systolic and diastolic pressures.**

NOTE: The origin of Korotkoff's sounds *(Fig. III.6)*. The pressure in the cuff is first elevated well above the arterial systolic pressure, so that the brachial artery remains collapsed and no blood jets into the lower artery (the pulse of the radial artery disappears). The cuff pressure reduces gradually. Just as soon as the pressure in the cuff falls below the systolic pressure, the blood begins to slip through the artery (turbulent circulation) and causes the first Korotkoff's sound during the peak of the systolic pressure. Korotkoff's sounds can be heard as long as the blood passes through the squeezed artery. When the pressure in the cuff falls to equal the diastolic pressure, the sounds cease to be audible. When the artery lumen changes to the normal one, the blood flowing through the artery becomes laminary and Korotkoff's sounds disappear. This is a clinical method for measurement of systolic and diastolic pressures.

Laboratory Work Nr.3 Holtz experiment.

Work goal: observation of the reflex influence from receptors and the sensitive fibres of the vagus nerve situated in the abdomen on the heart activity.

Materials and equipment: a frog, vivisection equipment, glass sticks, a board.

Work technique:

1. Decapitate the frog and fix it on the board in the dorsal decubitus.
2. Open the heart and count the cardiac contractions.
3. Make 2-3 strikes with a glass stick on the abdomen, then count the frequency of the heart contractions in a minute. Notice a temporary cardiac stop. Repeat the experiment some times.
4. In the report describe the modifications found in the cardiac activity and explain their mechanisms.

Laboratory Work Nr.4 The influence of electrical stimulation of the vagosympathetic trunk upon heart activity.

Work goal: to study the antagonistic action of the vegetative sympathetic and parasympathetic fibers upon the heart activity and determine "escape" phenomenon of the cord from the influence of vague.

Materials and equipment: a frog, a kymograph, electrical stimulator, electrodes, a dissection kit, a board, Ringer's solution, a tube.

Work Technique:

1. Immobilize the frog and fix it on the board in the dorsal decubitus.
2. Connect the electrical exciting circuit to stimulate the vagosympathetic trunk.
3. Open the thoracic-abdominal cavity; open the heart, dissect the clavicles making an incision on the lower edge of the jaw.
4. Raise the posterior wall of the esophagus, introduce the tube through the mouth to the stomach.
5. Divergent sublingual and glossopharyngeal nerve fibers can be easily observed in the region between the low jaw and clavicle. In the posterior part, on the dorsal wall of the esophagus, there is a nervous fascicle composed of the joint core of the vagal nerve and sympathetic nerve.

6. Ligature the vagosympathetic core. Take a tube. *(Fig. III. 7)*
7. Fix the apex of the heart with a pin (pliers-shaped) and connect it to the lever graph.
8. Raise the ligatured nervous fascicle and place it under stimulating electrodes. Connect the installation and record the cardiogram.
9. Excite the vagosympathetic core during 20 sec. continuing the record.
10. Notice the change in the cardiogram after electrical stimulation (the modification of amplitude and frequency of cardiac contractions).
11. The next step is the "escape" phenomenon of the heart from the action of vagal nerve. To establish this phenomenon, keep on electrical stimulation of the nerve after heart stop (restore of the heart rhythm).
12. The results are recorded in the report, the cardiogram is attached to it and all the mechanisms of the observed phenomena are explained in details.

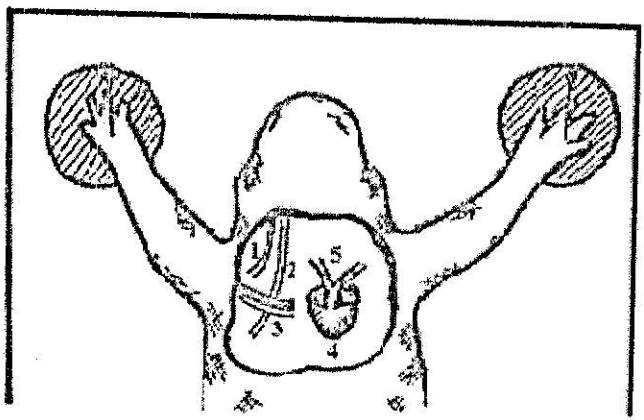


Figure III.7 **Frog's vasculo-nervous bundle.**
1 - Glossopharyngeal nerve. 2 - Sublingval nerve. 3 - Vasculo-nervous bundle.
4 - The heart. 5 - The bifurcation of the aorta.

Topic 4. **Clinical physiological methods to study the heart activity.**

Check questions:

4.1 The heart valves. Heart sounds, causes, characteristics, chest surface areas for auscultation. Phonocardiogram. Heart murmurs, abnormal circulatory dynamics in valvular heart disease and in congenital heart defects.

4.2 Characteristics of the normal electrocardiogram. Methods for recording electrocardiogram. Flow of electric current around the heart during the cardiac cycle. Electrocardiographic leads.

4.3 Electrocardiographic interpretation. Principles of vectorial analysis of electrocardiograms. Vectorial analysis of the normal electrocardiogram. Electrical axis of the normal electrocardiogram. The mean electrical axis of the ventricular QRS and its significance. Electrocardiographic interpretation of the cardiac muscle and coronary blood flow abnormalities.

4.4 Cardiac arrhythmias and their electrocardiographic interpretation. Abnormal rhythms and blocks. Premature contractions. Paroxysmal tachycardia.Ventricular fibrillation. Atrial fibrillation. Atrial flutter. Cardiac arrest.

Laboratory Work Nr. 1 Auscultation of the human heart.

Work goal: auscultation of the sonorous phenomena (the heart sounds) that appear during the cardiac activity and the optimal points of auscultation of these.

Materials and equipment: stethoscope, the examinee.

Work technique:

First it is necessary to distinguish the first cardiac sound from the second one (the first sound begins after a longer interval). We palpate the pulse on the radial artery, listen to the sounds of the heart simultaneously and determine to which phases of cardiac activity they correspond. Taking into account the anatomic projection of the valves on the torax (*Fig. III.8*) we establish the coincidence of this projection with the maximal point of the sounds. We may listen to the sounds after a physical effort (25 squattings).

Usually the sound produced by **bicuspid valve** is better heard in the apex region; that of the **tricuspid valve** – in the region of the xiphoid process; that of the **aortal valve** – in the II right intercostal

space on the parasternal line; that of the **pulmonary artery valve** – in the II left intercostal space on the parasternal line.

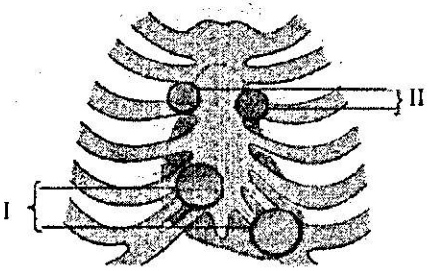


Figure III.8 **The thoracic projection of the points of heart auscultation.**
I – auscultation points of the first heart sound; II – auscultation points of the second heart sound.

Laboratory Work Nr. 2 Electrocardiography.

Work goals:

I) Record electrocardiogram;

II) Analysis of the main parameters of the electrocardiogram;

III) Determination of the direction of the heart electrical axis.

Materials and equipment: electrocardiograph with electrodes, gauze, physiological salt solution (saline solution), ether, a volunteer.

Work technique:

I. Methods of record of Electrocardiograms

I.I. **Standard leads.** These are bipolar limb leads, developed by W.Einthoven, which explore heart activity in the frontal plane. There are three points to place the electrodes *(Fig. III.9)*:

* Right arm (**R** = right); * Left arm (**L** = left); * Left foot (**F** = foot).

If we note electrical potentials of the points described above with VR, VL and VF respectively, then, a standard lead measures potential differences between the remained pair of limbs (other two limbs) as it is shown in *Fig. III.9 B.*

In the graphic representation, the electrical axis of each lead is represented by the corresponding side of an equilateral triangle (the triangle apices correspond to the points where electrodes are fixed), called Einthoven's triangle; the heart being an electromotor source, is placed in the centre of this triangle. *(Fig. III.9 A.)*

As we obtained a closed electrical circuit, we can apply the second Kirchhoff's theorem in order to prove the fundamental law of a standard lead:

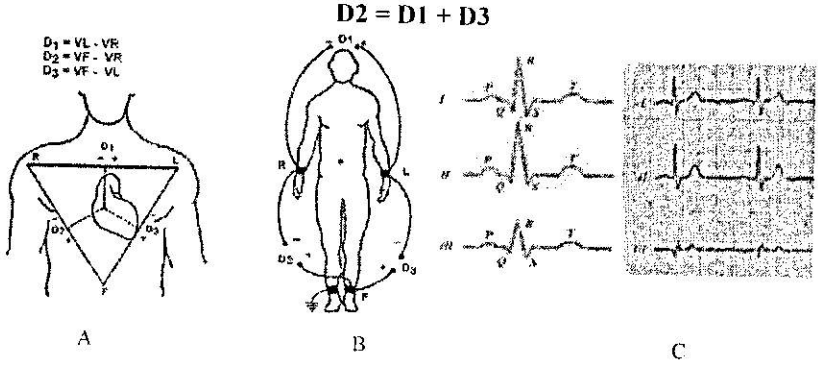


Figure III.9 A - Einthoven's triangle and standard leads represented by its sides, with positive and negative zones; B - Standard leads and conventional arrangement of electrodes to obtain them; C - Normal electrocardiograms recorded from three standard electrocardiographic leads.

I.II. **Unipolar limb leads:** Electrodes are placed as if we have standard bipolar leads. These leads are called unipolar because one of the electrodes, considered to be indifferent, permanently records a null electrical potential; in this case, the electrocardiograph measures the potential collected by the other electrode (explorer electrode).
According to Goldberger's method, the indifferent electrode can be obtained by connecting the electrodes of two limbs, different from the explorer electrode. Obtained leads are noted aVR, aVL and aVF (*Fig. III. 10*). "a" means "augmented" because, the potentials obtained by this technique are sufficiently bigger than in other techniques.
Using Kirchhoff's theorems it is possible to deduct the fundamental law of unipolar limb leads:

$$\mathbf{VR + VL + VF = 0}$$

The axis of an unipolar limb lead is perpendicular to the corresponding axis of a standard lead, at the same time passing through the point where the explorer electrode is placed. Thus, we added an additional triaxial system to Einthoven's triangle, that permits to analyze the electrical vector in the frontal plane, in a hexaaxial system *(Fig III. 10).*

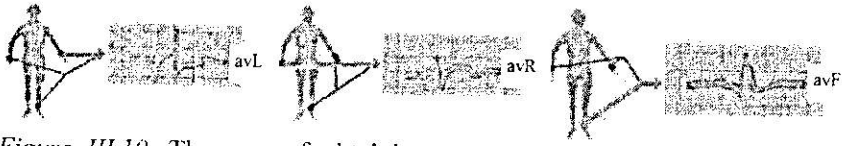
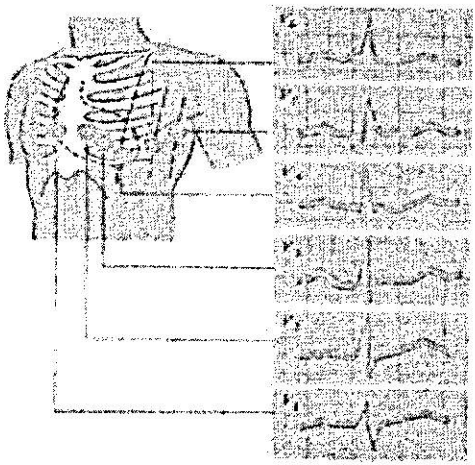


Figure III.10. The way of obtaining augmented unipolar limb leads in accordance with Goldberger's method.

I.III. **Chest leads (precordial leads)** are unipolar leads which explore heart activity in the horizontal plane.

In accordance with Wilson's method, the indifferent electrode can be obtained by connecting all three electrodes: R, L and F. This point is called *central terminal jack.* The explorer electrode is placed in different conventional points on the anterior thoracic wall marked from V1 to V6 *(Fig. III. 11).* The axis of the chest lead starts from the heart electric centre and ends on the anterior chest face, under the explorer electrode.



- **V1-** the fourth intercostal space, on the right parasternal line.
- **V2-** the fourth intercostal space, on the left parasternal line.
- **V3-** the in the middle between V2 and V4.
- **V4-** the fifth intercostal space, on the mammary line.
- **V5-** the fifth intercostal space, on the anterior axillary line.
- **V6-** the fifth intercostal space on the medium axillary line.

***Figure III.11.*Precordial leads.**

Record of electrocardiogram

The electrocardiograph is an apparatus for recording heart activity and consists of the following:

- **the system for taking over the signal** that consists of electrodes, cables for connecting to the volunteer, entrance block with necessary resistances needed for making different unipolar leads. The electrodes are metallic plates, covered with gauze.

The gauze must be moistened with physiological salt solution. Each cable has its standardized colour and must be connected to the corresponding limb:

- **red** is for the right arm;
- **yellow** is the for left arm;
- **green** – the left foot;
- **black**- the right foot.

- **the amplification system;**
- **the system for displaying result.** Some of electrocardiographs post the result directly on a moving sheet of scale paper, others display it on a cathodic oscilloscope. Different electrocardiographs can record in a single or more channels.

Recording technique:

- the volunteer is recumbent in the dorsal decubitus. He must be relaxed. The air temperature has to be about 18-22 C, that is the comfort temperature. In this case, the volunteer will not have abnormal muscular contractions that influence the electrocardiogram;
- fix steadily the electrodes in the mentioned points;
- record the voltage calibrating curve. Usually **1mV = 10mm**;
- record electrocardiograms from each lead. Usually, the paper moving speed is 25 mm per second, so 1mm = 0.04 seconds.

II. Principles of analysis of electrocardiograms (*Fig. III.12.*; *Tab. III. 1*)**:**

The analysis of the electrocardiogram is made in accordance with standard lead II:

a. Determination of the amplitude of P, Q, R, S, T waves in accordance with electrocardiograph calibration (1 mV = 10 mm).
b. Determination of the length of waves and P – Q, Q – T, T– P, R – R intervals (25 mm/s).

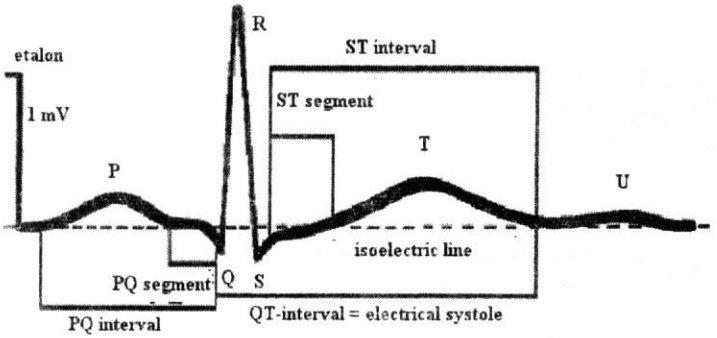


Figure III.12. **Electrocardiogram reading. The main elements of ECG.**

Table III. 1

Write obtained data in the table and compare them with norma:

Waves	Volunteer (mV)	Norma	Segments, intervals	Volunteer	Norm (seconds)
P		0,05-0,3	P-Q		0,12-0,2
Q		0,2-0,3	Q-T		0,32-0,50
R		0,3-1,6	T-P		0,25-0,52
S		0,26-0,48	R-R		0,7-1,3
T		0,25-0,6	FCC=60/(R-R) FCC-frequency of cardiac cycle		45-82

II. **Determination of the heart position** – the equilateral triangle method.

The resultant of all vectors that appears during the ventricular depolarization constitutes the **electrical axis of the heart**. This resultant is a vector that shows the preponderant direction of the potential during depolarization. The vector that constitutes the electrical axis can be projected on the sides of Einthoven's triangle, the projection corresponds to the amplitude of the R wave from a standard lead.

The result can be interpreted according to the next relations *(Fig. III. 13)*:

1. $R_2 > R_1 > R_3$ – oblique position.
2. $R_1 > R_2 > R_3$ – horizontal position.
3. $R_2 > R_3 > R_1$ – vertical position.

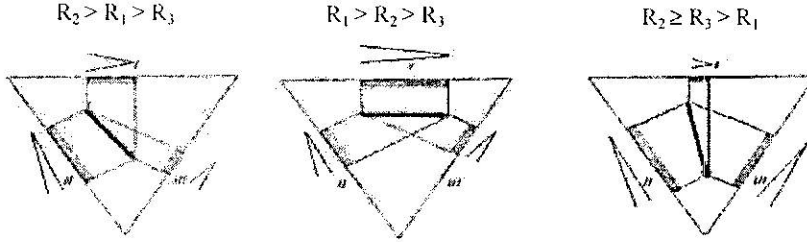


Figure III.13. **Projection of the summary vector on the sides of Einthoven's triangle.**

The electrocardiogram attach to your report, write the analysis results in the table and compare them with the norm. Draw the scheme of summary vector projections *(Fig. III. 13)* and determine the heart position of the person under examination.

CHAPTER IV

DIGESTION, METABOLISM AND RESPIRATION

Topic 1. The secretory and motor functions of the gastrointestinal tract

Check questions:

1.1General principles of gastrointestinal motility. Physiologic anatomy of the gastrointestinal wall. Neural control of gastrointestinal function. Enteric nervous system, types of neurotransmitters secreted by enteric neurons. Autonomic control of the gastrointestinal tract.

1.2 Gastrointestinal blood flow - "splanchnic circulation". Effect of intestinal activity and metabolic factors on the gastrointestinal blood flow. Anatomy of the gastrointestinal blood supply. Nervous control of the gastrointestinal blood flow.

1.3 Functional types of movements in the gastrointestinal tract. Propulsive movements – peristalsis, mixing movements. Ingestion of food. Mastication (Chewing).Swallowing (Deglutition).

1.4 Motor functions of the stomach. Storage function of the stomach. Mixing and propulsion of food in the stomach – the basic electrical rhythm of the stomach wall. Regulation of stomach emptying.

1.5 Movements of the small intestine. Mixing contractions (segmentation contractions). Propulsive movements. Function of the ileocecal valve. Movements of the colon. Defecation.

1.6 General principles of alimentary tract secretion. Anatomical types of glands. Basic mechanisms of stimulation of the alimentary tract glands. Basic mechanism of secretion by glandular cells. Lubricating and protective properties of mucus, and importance of mucus in the gastrointestinal tract.

1.7 Secretion of saliva, salivary glands. Characteristics of saliva. Nervous regulation of salivary secretion. Esophageal secretion. Characteristics of the gastric secretions. Stimulation of gastric acid secretion. Pyloric glands – secretion of mucus and gastrin. Regulation of pepsinogen secretion. Phases of gastric secretion Chemical composition of gastrin and other gastrointestinal hormones.

1.8 Pancreatic secretion. Pancreatic digestive enzymes. Secretion of bicarbonate ions. Regulation of pancreatic secretion. Phases of pancreatic secretion. Secretion of bile by the liver. Functions of the biliary tree. Function of bile salts in fat digestion and absorption. Liver secretion of cholesterol and gallstone formation.

1.9 Secretions of the small intestine. Secretion of mucus by Brunner's glands in the duodenum. Secretion of intestinal digestive juices by the crypts of Lieberkühn. Regulation of small intestine secretion – local stimuli. Secretions of the large intestine.

Laboratory Work Nr.1 The movements of the cilia of the esophageal mucosa epithelium in the frog.

Work goal: to study the rhythmic movements of the cilia of esophageal mucosa to the frog - one of the automatism features of digestive tract activity.

Materials and equipment: a frog, a vivisection kit, entomological pins, an operation table, a stopwatch, a ruler, some poppy seeds, Ringer's solution, acetylcholine solution (1:10000), adrenaline solution (1:1000).

Work technique:

1. Immobilize the frog, fix it on the operation table with the abdomen up. Open the thoracic cavity, cut the lower jaw and the esophagus along its whole. Fix the esophagus on the operation table with the entomological pins and wash the esophageal mucosa with Ringer's solution.
2. Put some poppy seeds in the proximal part of the esophagus, then measure the esophageal length and the time of the seeds movement through the esophagus until they enter the stomach. Calculate the speed.
3. Pour adrenaline solution on the surface of the esophagus and repeat the same experiment with acetylcholine solution (wash the esophagus with Ringer solution after using each solution).
4. Describe the experiment in the report and note the results.

Laboratory Work Nr.2 The masticatiography.

Work goal: determination the dependence between the rhythm and the amplitude of jaw movements during chewing and the properties and content of ingested food.

Materials and equipment: a pneumatic rubber sleeve, a T-shaped tube, Marey capsule, the kymograph, a clamp, bread, some crumbs.

Work technique:

1. Apply the rubber sleeve on the low jaw and fix it. Open the clamp and pump air in the cuff through the rubber tube. Connect it with Marey capsule, afterwards check the registration (on kymograph).
2. Register the mastication first with bread, then with crumbs.
3. Attach the masticatiogrames to the report.

On the masticatiogramme note: 1) the resting phase; 2) the food intake; 3) the beginning of chewing; 4) the basic phase of the chewing; 5) the formation of the food bolus and the beginning of swallowing *(Fig.IV.1; Tab. IV.1).*

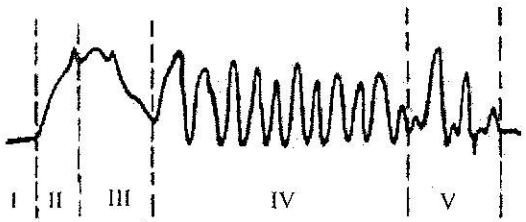


Figure IV.1. **The masticatiogram.**
I. In the resting phase, the lower jaw is immobile, the muscle tone is minimal, between the upper and lower teeth – 2-3 mm. II. The food intake in the oral cavity (the first ascension). III. The early mastication for test (it corresponds to the process of adaptation to the food mastication). IV. The basic phase of chewing (successive masticatory waves). V. The formation of the bolus (the beginning of swallowing).

Table IV.1

The masticator cycle phases	Soft bread	Crumbs
I	no apparent differences	no apparent differences
II	it depends on the speed of food ingestion	it depends on the speed of food ingestion
III	low amplitude, slow rhythm of masticatory movements	high amplitude , frequent rhythm of masticatory movements
IV	the masticatory waves go up and down frequently and uniformly	the first masticatory wave takes a scale shape and a longer duration
V	the food bolus is formed in a single phases	the food bolus is formed in several phases

Laboratory Work Nr.3 The enzymatic action of gastric juice.

Work goal: to establish the optimal conditions of the enzymatic action of gastric juice.

Materials and equipment: gastric juice, fibrin, graduated tubes, a lamp with alcohol, ice, a water bath or a thermostat, Fehling I (10% NaOH) and Fehling II (0.3% $CuSO_4$) solution, HCl solution (0.5%).

Work technique:

1. Put in 6 numbered tubes:
   №1 – 1 ml of gastric juice + 0.2 g fibrin
   №2 - 1 ml of gastric juice (boiled at the lamp with alcohol, then cooled) + 0.2 g fibrin
   №3 - 1 ml of gastric juice + 0.2 g fibrin + 10 drops of Feling I reagent (10% solution of NaOH)
   №4 - 1 ml of HCl (0.5%) + 0.2 g fibrin
   №5 - 1 ml of distilled water + 0.2 g fibrin
   №6 - 1 ml of gastric juice, cooled on ice + 0.2 g fibrin.
2. Put the first 5 tubes into the water bath at 37-38^{0}C, for 15 minutes, then cool them under running water.

3. Put tube Nr.6 into a bolus with ice for 15 minutes.

4. The **burette** reaction: add – 2 ml of 10% solution of NaOH (Fehling I) and 1-2 drops of $CuSO_4$ sol. (0.3%) (Fehling II) in each tube.

NOTE: the color of proteins is blue-violet; that of peptones is red-violet.

5. Describe the results and analyze them, write them down in the report and make the conclusions.

Laboratory Work Nr.4 The influence of acetylcholine and adrenaline on the motility of frog's intestine.

Work goal: to study the influence of acetylcholine and adrenaline on the movements of the frog's colon.

Materials and equipment: a frog, a vivisection set, entomological pins, Ringer's solution, acetylcholine solution (1:10000), adrenaline solution (1:10000), kymograph, pin (pliers- shaped), Enghelman's lever graph, pipettes.

Work technique:

1. Immobilize the frog by the brain and spinal cord destruction.
2. Fix it on the board in the dorsal position.
3. Divide and remove the skin from the abdominal muscles up to the symphysis.
4. Prepare the large intestine by removing the mesenterium.
5. Apply a ligature at the junction between the small and large intestines. Irrigate the intestines with Ringer's solution permanently. Divide the intestine above the ligature.
6. Fix the rectal end by the Enghelman's lever with a pin.
7. Record the colon movements and observe the automatism of the intestines on the sheet of paper attached to the kymograph.
8. Pour some drops of acetylcholine on the isolated rectum and observe the motility of the intestines.
9. Wash the intestines with Ringer's solution several times, until the contractions of intestine will normalize.
10. Repeat the experiment with adrenaline solution, then compare the contraction curves of both experiments.

Laboratory Work Nr.5 The influence of bile on lipids.

Work goal: to determine the role of bile in emulsification and hydrolysis process of fats into the digestive tract.

Materials and equipment: a stand, some tubes, funnels, a pipette, fresh bile, vegetal oil, filter paper, water.

Work technique:

1. Place the filter paper into the funnels, soak one of them with water and the other one – with bile, then put the funnels into the tubes from the stand.
2. Pour 10 ml of vegetal oil in each tube. In 45 min, determine the quantity of the filtrated fats through each filter paper.
3. Describe the experiment and draw it scheme in the report, note the results and explain the action of bile on lipids.

Topic 2. **Digestion and absorption in the gastrointestinal tract. Metabolism.**

Check questions:

2.1 Anatomical basis of Absorption. Absorption in the small intestine. Absorption of water. Absorption of ions. Absorption of nutrients: absorption of carbohydrates, absorption of fats, absorption of proteins. Absorption in the large intestine: formation of feces.

2.2 Disorders of swallowing and of the esophagus. Disorders of the stomach: peptic ulcer. Abnormal digestion of food in the small intestine – pancreatic failure. Malabsorption by the small intestinal mucosa – sprue. Disorders of the large intestine: Constipation. Diarrhea. General disorders of the gastrointestinal tract: vomiting, nausea, gastrointestinal obstruction.

2.3 Physiologic anatomy of the liver. Metabolic functions of the liver. Measurement of bilirubin in the bile as a clinical diagnostic tool.

2.4 Control of protein metabolism, lipid metabolism, carbohydrate metabolism. Metabolism of phospholipids and cholesterol. Atherosclerosis, prevention of atherosclerosis.

2.5 Energy used for processing food – thermogenic effect of food. Energy available in foods. Methods for determination of metabolic utilization of proteins, carbohydrates, and fats. Regulation of food intake and energy storage. Factors that regulate quantity of food intake. Inanition. Anorexia and cachexia. Starvation. Obesity. Treatment of Obesity.

2.6 Control of energy release in the cell. Metabolic rate. Measurement of the whole body metabolic rate. Energy metabolism – factors that influence Energy Output. Overall energy requirements for daily activities. Basal Metabolic Rate (BMR) - the minimum energy expenditure for the body to exist. The methods for recording BMR (calorimetry, complete gaseous analyze).

2.7 Normal body temperatures. Heat production. Heat loss. Regulation of body temperature – role of the hypothalamus. Temperature - decreasing, mechanisms when the body is too hot. Temperature - increasing mechanisms when the body is too cold. Abnormalities of body temperature regulation.

Laboratory Work Nr.1 The absorption of potassium iodate.

Work goal: to study the absorption function of the intestines by using potassium iodate.

Materials and equipment: potassium iodate (0.25 g dissolved in 250 ml of water), starch milk of starch (10% amidine solution), a chronometer, some tubes for saliva collection.

Work technique:

1. Drink 250 ml of solution in which 0.25 g potassium iodate were dissolved.
2. Determine the time of appearance of iodine in the saliva: for that you need to collect the saliva for 12 minutes, every 2 minutes; then collective saliva for one hour every 5 minutes.
3. Pour 1-2 drops of amidine solution (10%) into the collected saliva. If the colour becomes blue, it means iodine is present. Usually iodine appears in the saliva in 6-12 minutes.

Laboratory Work Nr.2 Determination of basal metabolism by the method of direct calorimetry.

Work goal: familiarization with the principle of basal metabolism determination by the method of direct calorimetry.

Materials and equipment: calorimeter, water (cold), a rabbit, receiver for water, balance, thermometer.

Work technique:

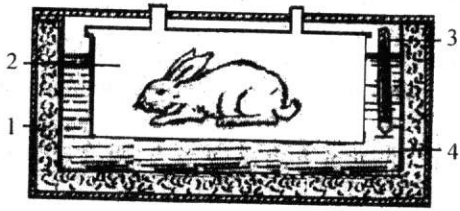
1. Study and draw the scheme of the calorimeter (*Fig. IV.2*).
2. Pour 3 liters of water in the extern chamber of the calorimeter and measure its initial temperature (t_1).
3. Weigh and place the rabbit in the interior chamber of the calorimeter and close it.

4. In 1h extract the rabbit and measure the temperature of the water (t_2).
5. Determine the basal metabolism using the formula:

$$Q = \frac{mc(t2 - t1)}{MT}$$

Where: Q - basal metabolism(kcal); m - weight of the water (kg); c - thermal capacity of water; t_2 - final temperature of the water; t_1 - initial temperature of the water; M-weight of the rabbit; T-time (1 hour).

6. Determine the basal metabolism of the rabbit for an hour then for 24 hours.



1. External chamber;
2. Internal chamber;
3. Thermometer;
4. Water.

***Figure IV.2.*The scheme of calorimeter.**

Laboratory Work Nr. 3 Calculation of the standard basal metabolism *(Tab. IV.2; Tab. IV.3.a.; Tab. IV.3.b; Tab. IV.4)*

Work goal: to study the method of calculation the standard basal metabolism depending on sex, weight, height, age and body surface.

Materials and equipment: tables for calculation of the basal metabolism, balance, anthropometer, a volunteer.

Work technique:

1. Measure the anthropometric data of the volunteer (weight, heights).
2. Determine basal metabolism with the help of the tables taking into account the anthropometric data.
3. The calculation of the basal metabolism can be made using the surface of the body (*Tab. IV.5*). In this case, initially the energetic use is determined for $1m^2$ of the body surface depending on the age. Using the table this value is calculated.
4. The standard value can be compared with the value determined by the apparatus.
5. Describe the method in the report and all obtained and conclusions should be formulated and written down.

Table IV.2

Data for the determination of the basal metabolism according to weight

Women				Men			
Weight	Kcal	Weight	Kcal	Weight	Kcal	Weight	Kcal
45	1085	68	1305	46	699	72	1057
46	1095	70	1325	48	727	74	1084
47	1105	72	1344	50	754	76	1112
48	1114	74	1363	52	782	78	1139
50	1133	76	1382	54	809	80	1167
52	1152	78	1401	56	837	82	1194
54	1172	80	1420	58	864	84	1222
56	1191	82	1439	60	892	86	1249
58	1210	84	1458	62	919	88	1277
60	1229	86	1478	64	947	90	1304
62	1248			66	974		
64	1267			68	1002		
66	1286			70	1029		

Table IV.3.a.

Data for the determination of the basal nictimeral metabolism in men according to age and height.

Height	Age										
	17-18	19	20-21	23-24	25-26	27-28	29-32	33-40	41-50	51-62	63
144	593	568									
148	633	608									
152	673	648	619	605	592	578	565	538	484	416	335
156	713	678	639	625	612	598	585	558	504	436	355
160	743	708	659	645	632	618	605	578	524	456	375
164	773	738	679	665	652	638	625	598	544	476	395
168	803	668	699	685	672	658	645	618	564	496	415
172	823	788	719	705	692	678	665	638	584	516	435
176	843	808	739	725	712	698	685	658	604	536	455
180	863	828	759	745	732	718	705	678	624	556	475
184	883	848	779	865	752	738	725	698	644	576	495

Table IV.3.b

Data for the determination of the basal nictimeral metabolism in women according to age and heights.

Heights	Age										
	17-18	19-20	21-22	23-24	25-26	27-28	29-32	33-40	41-50	51-62	63
144	171	162									
148	187	178									
152	201	192	183	174	164	155	146	127	89	43	-13
156	215	206	190	181	162	162	153	134	97	50	-6
160	229	220	198	188	179	199	160	142	104	57	1
168	255	256	213	203	194	184	175	156	119	72	17
172	267	258	220	211	201	192	183	164	126	80	24
176	279	270	227	218	209	99	190	171	134	87	31
180	291	282	235	225	216	207	197	179	141	94	38

Table IV.4

Basal nictimeral metabolism values in children according to weight

Weight	b	g	Weight	b	g	Weight	b	g
	kcal			kcal			kcal	
3	150	136	14	700	678	30	1140	1063
4	210	205	15	725	718	32	1190	1101
5	270	274	16	750	747	34	1230	1137
6	330	336	17	780	775	36	1270	1173
7	390	395	18	810	802	38	1305	1207
8	445	448	19	840	827	40	1340	1241
9	495	496	20	870	852	42	1370	1274
10	545	541	22	910	898	44	1400	1305
11	590	582	24	980	942			
12	625	620	26	1070	984			
13	665	665	28	1100	1025			

Table IV.5

Energy consumption (kcal) per 1 m^2 of body surface according to age, during 24h

Age	Men	Women
16-18	1032	960
18-20	984	942
20-30	948	888
30-40	948	876

Laboratory Work Nr.4 The determination of energy use by the method of incomplete gas analysis.

Work goal: to study the principle of the determination of energy use of the organism using the method of incomplete gas analysis.

Materials and equipment: metatest or spirometabolograph, alcohol, cotton-wool, ink.

Work technique:

Spirometabolograph is a close system made of a spirograph, an absorbent of CO_2 and water steam, a valve block. The chamber of the spirograph (volume of 6.0 l) is connected to the recording pen of the spirograme. The movement of the chamber of spirograph depending on the depth of the respiration records the spirograme. The expired air passes through the absorbents of carbon dioxide and water steam and returns to the chamber of the spirograph where is mixed with the oxygen from the system. The volume of the circulating oxygen from the system decreases with the volume used by the volunteer. This modification of the volume is recorded in the form of a descendent curve of the respiratory movements. The research is made by the lecturer in form of demonstration. Students are given the data of the value of consumed oxygen for a unit of time for calculating the basal metabolism and then compare the obtained value with the standard level of basal metabolism.

Laboratory Work Nr.5 Measurement of the body temperature.

Work goal: to study the temperature in different parts of the human body surface (temperature chart).

Materials and equipment: electronic thermometer.

Work technique:

1. Determine with the electronic thermometer the temperature of different parts of the body: the tip of a finger, the palm, the jugular fossa, the forehead and in the armpit.
2. Write down the obtained results and make conclusions.

Theme 3. **Pulmonary ventilation. pulmonary circulation. Gas diffusion in the lungs and tissues.**

Check questions:

1.1 Primary and secondary functions of the respiratory system. Stages of respiration. Biomechanics of quiet and forced breathing. Pleural, alveolar and transpulmonary pressures, and their changes within the respiratory cycle.

1.2 Compliance of the thorax and lungs. Surface tension and the role of surfactant. Pleural fluid, pleural pressure – its origin and importance. Work of breathing. Atelectasis, its causes. Pneumothorax.

1.3 Principles of spirometry and helium dilution methods, principles and importance. Pulmonary volumes and capacities – normal values and factors that affect them. Definition of anatomic and physiologic "dead spaces", normal values and factors that can affect them. Dead space volume determination. Minute respiratory volume and alveolar ventilation.

1.4 Functions of the respiratory passageways. Resistance to airflow in the bronchial tree. Nervous and humoral control of bronchial tonus. Evaluations of maximum expiratory flow, forced expiratory vital capacity and forced expiratory volume, their normal values and practical importance.

1.5 Physics of gas diffusion and gas partial pressures. Composition of atmospheric and alveolar air. Partial pressures of O_2 and CO_2 in alveoli, arterial blood, venous blood and tissues and their importance for the exchange of gases in tissues and lungs.

1.6 Respiratory unit, respiratory membrane and factors that affect the rate of gas diffusion in lungs. Diffusing capacity for O_2, CO_2 and CO of the respiratory membrane. Ventilation – perfusion ratio, its relation with the physiologic dead space and the influence of gravity.

1.7 Blood pressures in the pulmonary system. Blood volume in the lungs. Blood flow through the lungs and its distribution, influence of

gravity, physical exercise. Nervous and humoral regulation of pulmonary circulation. Capillary exchange of fluid in the lungs, and pulmonary interstitial fluid dynamics.

Laboratory Work Nr.1 The study of the inspiration and expiration mechanisms. Donders' model.

Work goal: to establish the importance of the negative pressure in the pleural cavity during inspiration and expiration.

Materials and equipment: a dissection kit, a rat, a tightly stoppered carboy with two tubes, a rubber bag.

Work technique:

The work is demonstrated by the lecturer. Firstly we give anesthesia to the rat and extract the lungs with the airways; fix the trachea with the lungs on the edge of the glass tube and introduce it in the carboy with a small amount of water (*Fig. IV.3.*). We close tightly the carboy with the stopper through which the glass tube connected with the trachea is brought outside. The second tube which passes through the carboy stopper and is connected with the rubber tube to the rubber pump, which is used to change the air pressure inside the carboy.

1. The rubber pump produces negative pressure inside the carboy. Observe the expansion of the lungs. By varying the negative pressure in the lungs, their breathing movements can be observed.

2. After the pressure in the carboy becomes equal to the atmospheric one observe the lungs collapse, like in case of an "open pneumothorax".

3. Draw the scheme of the Donders' model in the written work; explain the mechanism and the importance of the pressure changing in the pleural cavity for the participation of the lungs in the respiratory process.

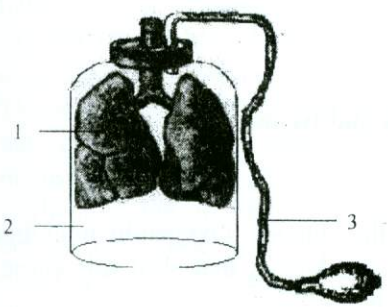


Figure IV.3. **The scheme of modified Donders' model.**
1 - Rat's lungs; 2 - Glass carboy; 3 - Tube and rubber pump.

Laboratory Work Nr. 2 Determination of minute respiratory volume and alveolar ventilation at rest and physical exertion.

Work goal: acquaintance with methods of calculation of the minute respiratory volume (MRV) and alveolar ventilation (AV) in order to assess the respiration efficiency.

Materials and equipment: transducer for registration of velocity of respiratory flow (SS 11LA), bacteriologic filter (AFT 1), mouth piece (AFT2), calibration syringe (AFT 6), computer, Biopac Student Lab 3.7.1 software, acquisition system MP35/30.

Work technique: Start the acquisition system Biopac and make the calibration in volume of air flow transducer. Respiration is registered at rest and after physical exertion (20 sit-ups).

1. MRV (the volume of air that passes through the lungs and airways per minute) can be determined analyzing the respiratory volume curve. MRV = CV x f, where CV - respiration volume, f – respiration frequency. Introduce the results in the table. The alveolar ventilation (AV), that is the quantity of air that passes through the pulmonary alveoli per minute, is calculated using the formula: AV = (CV – 150) x f, where 150 is the volume of dead space. Introduce the results in the table below.
2. In the report describe briefly the technique of work and the principles of calculation of different volumes (*Tab. IV.6*).

Table IV.6.

Modifications of respiratory indices at rest and physical exertion

Conditions of experiment	Research results		Calculated data	
	CV	f	MVR	AV
At rest				
After physical exertion				

Theme 4. **Transport of gases in blood and tissues. Regulation of breathing**

Check questions:

2.1 Transport of oxygen in the blood. Oxygen-hemoglobin dissociation curve, its characteristics and factors that shift this curve. Oxygen content in arterial and venous blood.

2.2 Transport of carbon dioxide in the blood – forms and importance of carbonic anhydrase. Carbon dioxide content in the arterial and venous blood. Carbon dioxide dissociation curve.

2.3 Respiratory center in the medulla oblongata and pons. The role of the spinal cord in regulation of respiration. Control of respiration by the upper nervous centers (hypothalamus, limbic system, cortex).

2.4 Nervous and chemical control of respiration. Central and peripheral respiratory chemoreceptors, mechanism of their stimulation. Control of respiration in hypoxemia, hypercapnia and hypocapnia and variations of blood pH. Congenital central hypoventilation syndrome or Ondine's curse.

2.5 Characteristics and role of the airways and lungs receptors (stretch-, irritant- and J receptors). Hering-Breuer inflation and deflation reflexes. Respiratory protective reflexes.

2.6 Acute and chronic effects of hypobaric hypoxia and mechanisms of acclimatization to high altitude. Effects of hyperbaric conditions and importance of decompression.

2.7 Respiratory control during physical exercise. Causes of sleep apneas.

Laboratory Work Nr. 1 Oxyhemometry.

Work goal: acquaintance with the method of determination of blood saturation with oxygen.

Materials and equipment: oxyhemograph, ethanol, a volunteer, cotton-wool.

Work technique: Study the principle of work of the oxyhemograph. The device consists of an electrical bulb that heats the pavilion of the ear and makes blood vessels dilate, on the other part a light source is situated, so the light that passes through the tissue of the ear and reaches the photo-element. The intensity of the light fascicule depends on the absorption properties of the tissue of the ear pavilion.
The absorption coefficient of light of reduced hemoglobin especially in the red spectrum is higher than of the oxyhemoglobin. Thus, the intensity of light is modified in function of the quantity of oxyhemoglobin in blood. So, the indications of the quantity of the device show the approximate value of the content of oxyhemoglobin in blood or the grade of arterial blood saturation with oxygen. The saturation degree of blood with oxygen is called the ratio between the content of

O_2 in arterial blood and its oxygenic capacity, expressed in volume-per cent.

1. Clean the ear pavilion with alcohol.
2. Fix the device on the superior part of the pavilion.
3. Turn on the device and wait for 10-15 minutes.
4. The volunteer must make 2 inspirations and expirations, turning the button "Installation initial saturation" the indicator will stay on 96% of oxihemoglobin that corresponds to the internal value.
5. Record oxyhemoglobin for 2-3 minutes, at a normal respiration.
6. The volunteer makes a maximal expiration, and then we record the oxyhemogram.
7. To receive the results immediately the volume of air retained in the time of inspiration using the spirometer. The calculation of residual volume is based on the fact that during inspiration the retention of the blood oxygenation goes down till the same value is directly proportional to the volume of air in the lungs.

Laboratory Work Nr. 2 Functional experiment with the retention of breathing.

Work goal: to study the methods to determine the development of the external breathing levels

Materials and equipment: stopwatch, a volunteer

Work technique:

1. The volunteer makes a deep inspiration and holds the respiration for the maximum possible time that is fixed with the stopwatch.
2. Repeat the experiment, but the respiration holding is made not during the inspiration, but during the expiration, the time is fixed with the stopwatch.
3. In the report should be written the respiration retention time during the inspiration and expiration.

Laboratory work Nr. 3 Time of breath retention after hyperventilation and exercise.

Work goal: to study the influence of the initially content of the CO_2 in the blood on the retention of breath.

Materials and equipment: a chronometer, a volunteer.

Work technique:

1. The examinee makes quiet inspirations during 3 minutes.

2. Then, retains respiration on inspiration and other person calculates the time of breath retention (repeat the experiment three times and find the average value).
3. The volunteer hyperventilates the lungs with 10 deep inspirations and expirations.
4. Then retains respiration once again and we calculate the time of retention
5. Further on, the volunteer makes exercise – 20 sit-ups or running in place for 30 seconds (sportsmen run for 1-2 minutes).
6. Immediately after the exercise, the volunteer retains respiration and we calculate this time.

Compare the obtained data and introduce them in the table IV.7.

Table IV.7.

The time of breath retention in different conditions

Nr.	Body state before breath retention	Time of breath retention			
		1	2	3	Average value
1.	At rest				
2.	Hyperventilation				
3.	After exercise				

Laboratory work Nr. 4 Herring-Breuer inflation reflex. Observation of afferent impulses transmitted through the vagal nerves during respiration using the cathode oscillograph.

Work goal: observation of the conduction of afferent impulses through the vagal nerves and comparing them with respiratory phases.

Materials and equipment: a vivisection kit, a rabbit, a cathode oscillograph, electrodes, chloralhydrate solution for anesthesia, novocaine, amplifier.

Work technique.

1. Preparation of the animal: fix the rabbit on the surgical table. It must be fixed on the back, the head being placed in a special device. Shear the fur on the middle line of the neck and make an incision of the skin and soft tissues under the skin. Using a glass stick, separate the tissues and reach the carotid artery. In this region the sympathetic, vagus and depressor nerves are located.

Find the vagus nerve (it is thicker and white) and ligature it. Using special needles and a glass stick dissect the vagus nerve trunk in many fibers. Ligature and cut each fiber; prepare 4 - 5 such fibers. The animal prepared this way serves for the observation of afferent impulses.

2. The device for screening afferent impulses consists of: 2 silver electrodes, an amplifier of biocurrents and an oscillograph. Apply the peripheral extremity of the nervous fiber on the electrodes, switch on the amplifier and observe nervous impulses on the display of the oscillograph. If the fiber conducts nervous impulses from stretch receptors of the lung then the impulses will have a periodic character: they will appear during inspiration and disappear during expiration *(Fig. IV. 4).*
3. Describe briefly in your report the preparation technique of the animal and characterize the afferent impulses you have seen on the display. Explain the role of Herring - Breuer reflex in self-regulation of respiration.

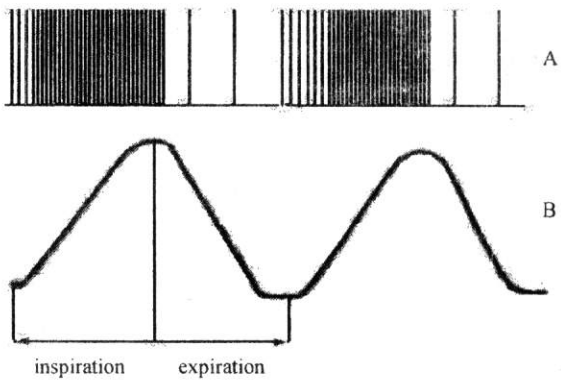


Figure IV.4. **Herring-Breuer reflex:** A - vagal nerve neurogram; B - respiratory volume.

THE BODY FLUIDS AND KIDNEYS. BLOOD CELLS, IMMUNITY AND BLOOD CLOTTING

Topic 1. **Urine formation by the kidneys.**

Check questions:

1.1 Multiple functions of the kidneys in homeostasis. Physiologic anatomy of the kidneys. Organs related to excretion. Micturition.

1.2 Urine formation. Glomerular filtration. Renal blood flow. Physiologic control of glomerular filtration and renal blood flow.

1.3 Reabsorption and secretion by the renal tubules. Mechanisms.

1.4 Reabsorption and secretion along different parts of the nephron.

1.5 Regulation of tubular reabsorption. Clearance methods to quantify kidney function

1.6 Diuretics and their mechanisms of action

1.7 Acute renal failure. Chronic renal failure. Specific tubular disorders. Treatment of renal failure by dialysis with an artificial kidney

Laboratory Work Nr.1 The determination of urine density.

Work goal: the determination of urine density (also named as specific gravity).

Materials and equipment: glass cylinder, urodensimeter, urine.

Work technique:

The urine is poured into a cylinder, sufficiently large for the urodensimeter to float in it without producing any foam. The urodensimeter is plunged into the cylinder (*Fig. V.1*) with the urine. The results are taken from the division which corresponds to the liquid level. In cases when the measurements are made at a temperature different from 15°C, it is necessary to make some corrections. When the temperature is lower than 15°C, we subtract the corresponding number from Bouchardat table from the obtained value. If the temperature is higher, we add the corresponding number from Bouchardat table *(table V.1)* to the obtained number. If there is glucose in the urine, then for the corrections the data from the third column of the table are used.

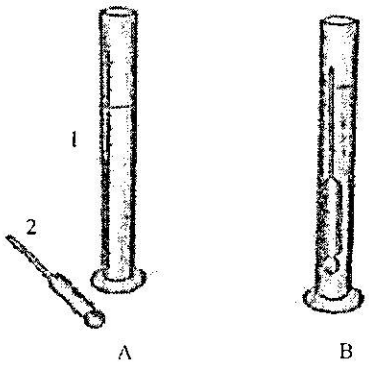


Figure V.1 **Determination of urine density using the urodensimeter.**
A - 1 – densimeter; 2 – glass cylinder;
B - determination of the specific density.

The normal value in physiological conditions of urine density is 1015-1030 g/L.

This value depends on:

- The alimentary diet;
- The physical activity;
- The quantity of ingested liquids;
- The volume of urine eliminated in a certain period of time.

Modifications of urine density

1. Hyposthenuria – the urine has the concentration (<1015) lower than the density of the plasma.
   - ✓ Hyperhydration;
   - ✓ Diabetes insipidus;
   - ✓ In some phases of acute renal insufficiency and chronic renal insufficiency.
2. Isosthenuria – the urine density is equal to the plasma density (1008-1012).
3. Hypersthenuria – the urine's density is higher than 1035;
   - ✓ Dehydration;
   - ✓ Diabetes mellitus;
   - ✓ After the administration of contrast substances.

In the report describe the work, note and reason the results.

Table V.1

Bouchardat table to make corections according to the relation between the temperature and the presence of glucose in urine

Temperature, C^0	Urine without sugar	Urine with sugar
1	-0,9	-1,3
2	-0,9	-1,3
3	-0,9	-1,3
4	-0,9	-1,3
5	-0,9	-1,3
6	-0,8	-1,2
7	-0,8	-1,1
8	-0,7	-1,0
9	-0,6	-0,9
10	-0,5	-0,8
11	-0,4	-0,7
12	-0,3	-0,6
13	-0,2	-0,4
14	-0,1	-0,2
16	+0,1	+0,2
17	+0,2	+0,4
18	+0,3	+0,6
19	+0,5	+0,8
20	+0,9	+1,0
21	+0,9	+1,2
22	+1,1	+1,4
23	+1,3	+1,6
24	+1,5	+1,9
25	+1,7	+2,2
26	+2,0	+2,5
27	+2,3	+2,8
28	+2,5	+3,1
29	+2,7	+3,4
30	+3,0	+3,7

Theme 2. **Body fluid compartments. Mechanisms for control of blood volume and extracellular fluid volume**

Check questions:

2.1 Functions of water. Fluid intake and output are balanced during steady-state conditions. Body fluid compartments. Constituents. Blood, functions, volum, composition and plasma constants, plasma proteins.

2.2 Measurement of fluid volumes in different body fluid compartments. Regulation of fluid exchange and osmotic equilibrium between intracellular and extracellular fluid.

2.3 Volume and osmolality of extracellular and intracellular fluids in abnormal states. Edema."Potential spaces" of the body.

2.4 Regulation of extracellular fluid osmolarity and sodium concentration

2.5 Regulation of potassium excretion and potassium concentration in extracellular fluid

2.6 Control of renal calcium excretion and extracellular calcium ion concentration. Regulation of renal phosphate excretion

2.7 Integration of renal mechanisms for control of extracellular fluid. The importance of pressure natriuresis and pressure diuresis in maintenance of the body sodium and fluid balance. Nervous and hormonal factors for the renal – body fluid feedback control.

Laboratory Work Nr. 1 Determination of the osmotic resistance of red blood cells.

Work goal: to assess the osmotic resistance of the red blood cells in hypotonic solutions.

Materials and equipment: 8 tubes, a stand, 5-10 ml cylinder, sodium chloride solutions of the following concentrations: 85.4; 76.9; 72.6; 68.3; 64.1; 59.8; 55.5; 51.3; mM/l (0.50%, 0.45%, 0.426%, 0.40%, 0.375%, 0.35%, 0.325%, 0.30%); sterile lancet, alcohol, cotton-wool.

Work technique:

1. Pour 3 ml of hypotonic solution of sodium chloride with different concentration in each tube (from 0.50% to 0.30%). Number the tubes.
2. Add 20 mm^3 of blood with Sahli capillary in each tube. Shake slightly the content, avoiding the formation of air bubbles.

3. In 30-40 minutes examine the content of each tube without shaking them. Analyze the results.
4. Osmotic resistance of the red blood cells is determined by the degree of hemolysis of the red cells from different tubes with hypotonic solutions.
5. The results are introduced into the table.
6. Designate the tubes in which:
   a. Hemolysis lacks;
   b. Partial hemolysis is noticed;
   c. Complete hemolysis is present (the content is completely transparent).

In conclusions (complete *Tab. V.2*) indicate the upper and lower limits of erythrocyte resistance and make an abstract upon the comparison of the achieved results and physiological norms.

Table V.2

Nr.	Concentration of NaCl solution	Observation results		Conclusions		
		Colour of superior transparent layer	Aspect of the other part of solution	Precipitate	Degree of Hemolysis	Level of resistance
1.	0.50%					
2.	0.45%					
3.	0.425%					
4.	0.40%					
5.	0.375%					
6.	0.35%					
7.	0.325%					
8.	0.30%					

Theme 3. **Regulation of acid-base balance (abb). Red blood cells. Blood types, transfusion**

Check questions:

3.1 Regulation of acid-base balance. Acids and bases–their definitions and meanings. Acidosis and alkalosis. Defenses against changes in hydrogen ion concentration: buffers, lungs, and kidneys

3.2 Buffering of hydrogen ions in the body fluids. Respiratory regulation of acid-base balance.

3.3 Renal control of acid-base balance.

3.4 The primary disturbance of ABB. Clinical causes of acid-base disorders. Treatment of acidosis or alkalosis. Clinical measurements and analysis of acid-base disorders

3.5 Red blood cells (Erythrocytes). Anemias. Polycythemia.

3.6 O-A-B Blood types. Rh Blood types. Erythroblastosis fetalis. Transplantation of tissues and organs.

Laboratory Work Nr.1 The technique of blood collection.

Work goal: to study the method of collecting capillary blood.

Materials and equipment: disposable sterile lancet, cotton-wool, alcohol, ether, Sahli's capillary, a tube and rubber bulb, a glass plate

Work technique:

1. Disinfect with alcohol and degrease with ether the tip of the ring or middle finger (in newborns and kids do the same with the toe or the heel).
2. After the evaporation of alcohol and ether, pierce the skin quickly and deeply in the lateral part of the finger with the disposable sterile lancet.
3. The first drop of blood is cleaned with a cotton swab, after that collect the blood for the analyses.
4. Collecting blood should be done rapidly and correctly – draw off the blood in Sahli's capillary by decompressing the rubber bulb gradually *(Fig. V.2)*.

NOTE: Do not squeeze the region of the piercing when you get the drop, because it can be diluted with the lymph.

5. After collecting the blood, clean out the drop of the blood and put a cotton swab soaked with alcohol on the finger.
6. In the report describe briefly the rules that should be kept in collecting blood from the finger.

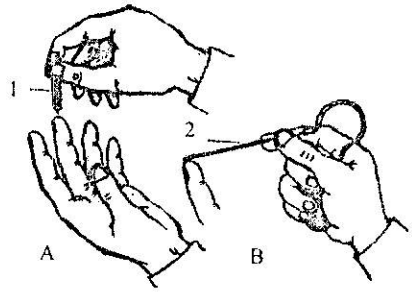


Figure V.2. **Taking blood from the finger (A, B);** 1 - Sterile lancet; 2 - Sahli capillary.

Laboratory Work Nr.2 Determination of the hemoglobin blood level using the method of Sahli.

Work goal: to study the colorimetric method of determination of the hemoglobin quantity after Sahli method.

Materials and equipment: Sahli hemometer (a graduated tube, Sahli comparator, Sahli capillary), a sterile lancet, a pipette, alcohol, cotton-wool, 0,1N HCl solution, distilled water.

NOTE:

The graduated tube has a graduated scale (gr/%) used for measurement of the level of hemoglobin.

Sahli comparator – the standard tubes have the colour of 1% hematin hydrochloride solution identical to hemoglobin blood level of 16 gr/100 ml.

Sahli capillary with the mark permits collecting of 0,02 ml (20 mm^3) of blood.

Work technique:

1. Introduce with the pipette in the graduated tube of Sahli hemometer 0,2 ml (till the mark) of 0,1N HCl solution.
2. Collect 20 mm^3 of blood with Sahli capillary.
3. Add the blood from the tube to the solution of HCl and clean the capillary by drawing off the mixture of the HCl and blood 2-3 times.
4. Put the graduated tube into the hemometer for 5 minutes until the complete hemolysis and the formation of hematin of a reddish-brown colour occurs.
5. Dilute slowly the content of the tube with distilled water until the colour of the mixture becomes the same as the colour of standard solution. Mix periodically the solution with a glass stick.

6. The hemoglobin level corresponds to the level of solution on the scale of the tube. In the report write shortly the process of the task, introduce the received results and write the conclusions.

Laboratory Work Nr. 3 Spectral analysis of blood.

Work goal: to determine the hemoglobin compounds by spectroscopic method.

Materials and equipment: a spectroscope, a stand with tubes, distilled water, sodium hydrosulphite, 10% potassium ferricyanide solution, a sterile blood lancet, ethanol, cotton-wool.

Work technique:

1. Introduce 3 ml of distilled water in 4 tubes.
2. Install the spectroscope.
3. Collect some blood (see the technique of collecting blood in Lab. Work nr. 1)
4. Dilute 0,2 ml of blood with distilled water to obtain 10 ml of solution (diluted as 1: 50) for ***oxyhemoglobin***; 1 ml of blood and 10 of distilled water (1:10) and 2-3 drops of 10% potassium ferricyanide solution for ***methemoglobin***; if you bubble up with a flow of gas the content of the tube with oxyhemoglobin, you will obtain ***carboxyhemoglobin***; if you add some crystals of sodium hydrosulphite in the tube with oxygen-hemoglobin you will obtain reduced hemoglobin.
5. Each tube is fixed on the stand one by one and analyzed by the spectroscope. Observe the absorption stripes which are characteristic of each compound of hemoglobin.
6. Study the number of absorption stripes and their location in the spectrum for each hemoglobin compound.
7. Draw the absorption spectra for each hemoglobin compound *(Fig. V.3)* in your report and make conclusions.

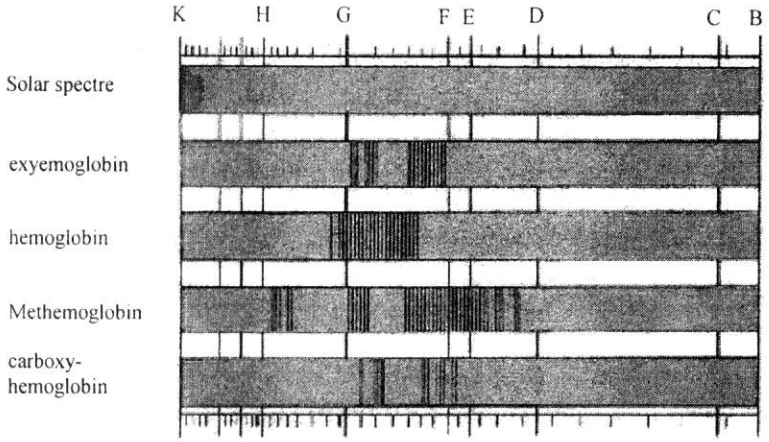


Figure V.3. **Absorption spectra of hemoglobin compounds.**

Laboratory Work Nr.4 Determination of erythrocyte sedimentation rate (ESR).

Work goal: to study the phenomenon of erythrocytes sedimentation in the collected blood mixed with the anticoagulant; used as a non-specific test in the diagnosis of some pathologic cases.

Materials and equipment: Pancencov capillary with the appropriate stand, two glass slides, 5% solution of sodium citrate, a sterile lancet, tweezers, alcohol, cotton-wool.

Work technique:

1. Study Pancencov capillary, its marking.
2. Pour a little of sodium citrate on the glass slide and wash the capillary with it.
3. Draw off with Pancencov capillary up to the sign "P" 0,5 ml of the solution of sodium citrate and pour it on the glass slide.
4. Collect the blood (see the technique of collecting in Laboratory Work Nr.1).
5. Add to the solution of sodium citrate 100 mm^3 of blood (without air bubbles) drawn off with Pancencov capillary up to the sign "K" two times and mix the mixture.
6. Draw off in the capillary the received mixture up to the sign "K" so that the column of blood should be continuous. The blood dilution is 4:1.

7. Close the top of the capillary with the index finger to stop the flow of the blood, put the capillary on the stand and fix it strictly vertically *(Fig V.4)*.
8. Read the results in one hour, and express it in mm of the column of plasma freed from erythrocytes per one hour (mm/h).
9. Write briefly the process of the task in the report, draw the capillary with its marks, write the value of ESR and compare it with the physiologic norms.

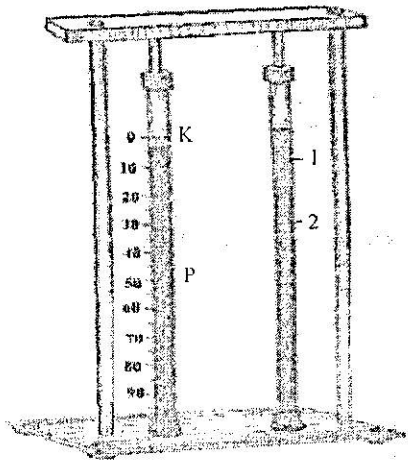


Figure V.4. **Pancencov device.**
1. 1. The column of plasma;
2. 2. The column of blood.

Laboratory Work Nr. 5 Determination of blood types using blood test serums: Anti-A, Anti-B and Anti-AB.

Work goal. Anti-agglutinogen blood test serums: Anti-A, Anti-B and Anti-AB are used for the determination of blood types in A-B-0 system by direct agglutination reactions.

Materials and equipment: a ceramic plate with cups; blood test serums Anti-A, Anti-B and Anti-AB; glass sticks, a sterile lancet, alcohol, cotton-wool.

Work technique:

1. Drip a drop of each blood test serum: Anti-A, Anti-B and Anti-AB (about 0,1 ml) in different cups of the plate.
2. Collect some blood and add a drop of blood (0,1-0,3 ml) to each drop of serum. Blood drops must be applied with different glass sticks, separately for each solution.

3. First, mix the blood and blood test serum with glass sticks but later continue to mix by a slow and turning movement of the plate.
4. Look after the result and continue to do periodically those slow movements of the plate during 3-5 minutes. As a rule, erythrocyte agglutination occurs during first 3-5 seconds, but you must look after the plate during 3 minutes because agglutination may occur with erythrocytes that contain variations of A and B agglutinogens (A1 A3; B2 B4).
5. The result of each reaction can be positive or negative. Positive result is represented by agglutination of erythrocytes, which represent small erythrocyte units. In case of negative result the solution remains homogeneously coloured *(Fig. V.5)*
6. The result of erythrocyte agglutination is presented in the table below:

The result of reaction with anti-agglutinogen			Determined blood type
A	B	AB	
0	0	0	0(I)
+	0	+	A(II)
0	+	+	B(III)
+	+	+	AB(IV)

"+" – agglutination is present "0" – no agglutination

7. Draw in your report the picture of *Fig. V.5* and explain the results you have got.

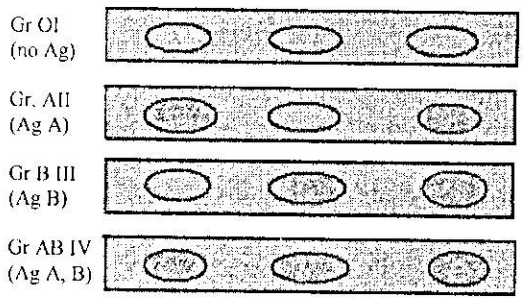


Figure V.5. **Determination of blood types with blood test serums: Anti-A, Anti-B and Anti-AB.**

Laboratory Work Nr. 6 Determination of Rh blood type with blood test serum Anti-D.

Work goal: blood test serum Anti-D (IgM) is applied to reveal agglutinogene D from the erythrocytes membrane using the reaction of direct agglutination.

Materials and equipment: a ceramic plate with cups, hemotest serum Anti-D, glass sticks, a sterile lancet, alcohol, cotton-wool.

Work technique:

1. Put a drop of blood test serum (0,1 ml) on the plate.
2. Collect the blood and add a drop of blood (0,01-0,03 ml) to the blood test serum then mix the mixture with the glass stick and turn continuously the plate.
3. The result is in 20-30 seconds. We find the formation of some erythrocyte aggregates. The agglutination of the erythrocytes begins in 10-15 seconds and is maximal in 30-60 seconds.
4. The result is read in 3 minutes.
5. The presence of agglutination of the erythrocytes confirms that the blood is Rh+ (positive), the absence of agglutination is Rh- (negative).
6. Draw the figure in the report and explain the results.

Theme 4. Resistance of the body to infection, immunity. Hemostasis and blood coagulation

Check questions:

4.1 Leukocytes. Neutrophils and macrophages in inflammation. Monocyte-macrophage cell system (reticuloendothelial system). Eosinophils. Basophils. Leukopenia. Leukemias.

4.2 Immunity. Types. Innate imunity. Acquired imunity. Allergy and hypersensitivity.

4.3 Events in hemostasis. Mechanism of Blood Coagulation

4.4 Conditions that cause excessive bleeding. Thromboembolic conditions. Anticoagulants for clinical use. Blood coagulation tests.

Laboratory Work Nr. 1 Count of erythrocytes (red blood cells) under the microscope.

Work goal: to count under microscope the number of erythrocytes contained in a definite volume of liquid diluted in a definite proportion.

The dilution makes possible the count of erythrocytes which are very numerous and it also prevents blood coagulation.

Materials and equipment: a microscope with ocular 15, Goreaev counting chamber, polished glass slide, a sterile blood lancet, Sahli capillary with tube and rubber bulb, tube with diluter, alcohol, cotton-wool.

Work technique (according to the method of dilution in the tube of N.M. Nicolaev):

1. Collect some blood (see the technique of collecting blood in the Laboratory Work Nr.1).
2. Using Sahli capillary, collect 20 mm^3 of blood, in order to obtain a column of blood which is not interrupted by air bubbles.
3. Pour the blood into a tube containing 4 ml of 2% solution of NaCl (in hypertonic solution, erythrocytes become wrinkled and can be easily counted). Using Sahli capillary connected to the bulb mix the content of the tube. The obtained dilution of 1: 202 can be considered as 1: 200.
4. Fix the glass slide on Goreaev chamber until you observe Newton's rings (these are the rings of light diffraction).
5. Fill up the chamber with diluted blood. Move a drop of mixture (diluted blood) on Goreaev chamber network using the top of Sahli capillary (do not use the rubber bulb). Work carefully in order to avoid the appearance of air bubbles.
6. Examine Goreaev chamber network under the microscope *(Fig. V.6)* and count the erythrocytes (using microscope ocular 15).

The technique of calculating erythrocytes number.

Count the erythrocytes in 5 big squares which are arranged on the diagonal of Goreaev chamber network. Each big square is divided into 16 smaller squares, so that altogether, there are 80 small squares to be counted. Erythrocytes from these small squares must be counted according to Egorov's rule *(Fig.V.6)*.

Calculate the number of erythrocytes in 1 mm^3 of blood using the formula:

$$E = S_e \times 200 \times 4000/80$$

NOTE: 1/4000 mm^3 represents the volume of a small square (square side = 1/20 mm; height = 1/0.1 mm).

7. Draw the counting chamber, explain the rule of counting erythrocytes (Egorov's rule) - *Figure V.6*, write obtained data and make conclusions in your report.

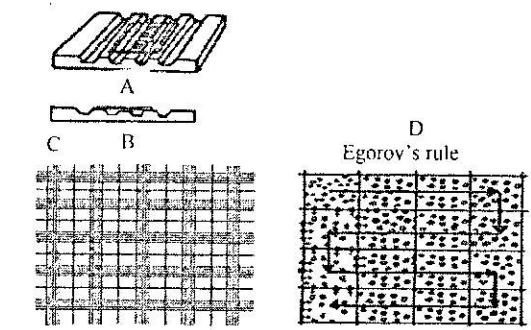


Figure V. 6. **Goreaev chamber of counting:** A - upper view; B - lateral view; C - Goreaev network; D - Egorov rule.

Laboratory Work Nr.2 White blood cell count using microscope.

Work goal: to count white blood cells contained in a definite volume of liquid diluted in a definite proportion

NOTE. *Diluting Solution:* 0.4 ml acetic acid + 2-3 drops of 1% methylene blue.

Acetic acid subjects the red cells to hemolysis, while methylene blue colours the white cells to count them easier.

Materials and equipment: Microscope with ocular x15, Goreaev counting camera, polished glass slide, a sterile lancet, Sahli capillary with rubber tube and bulb, a tube with diluting solution, alcohol, iodine, cotton-wool.

Work Technique:

1. Collect the blood (see collecting blood technique in Laboratory Work Nr.1).
2. Collect 20 mm^3 of blood using Sahli capillary and pour it into the tube with diluting solution.
3. Using Sahli capillary connected to the bulb mix the content of the tube. The obtained dilution is 1:20.
4. Continue in accordance with steps 4-6 of the previous Work.

Count Technique of the White Cells: we count the white cells in 25 big unlined squares in different regions of the grid. Calculate the number of white cells according to the formula:

$$L = \frac{St \times 20 \times 4000}{400}$$

Present a brief description of the performed steps within this work in the report, introduce obtained data and make conclusions.

Laboratory Work Nr.3 Calculation of colour index of blood (chromatic index).

Work goal: to calculate the colour index of the blood.

Materials and equipment: data regarding the hemoglobin blood level and the number of red blood cells (see the previous laboratory works).

Work Technique:

To calculate the chromatic index of the blood is necessary to divide the hemoglobin blood level (expressed in per cents), to the standard hemoglobin blood level, considered as 100%, and to divide again to the number of red blood cells, divided to the given number of red cells ($N=5000000/mm^3$).

Ideal conditions would be as follows: hemoglobin level 140 g/l = 100%, number of red cells – 5 mln / 1 mm^3 of blood, colour index = 1.

$$CI = \frac{Hbcalc\ (\%)}{Hb\ (N=100\%)} : \frac{Number\ of\ counted\ red\ cells}{Number\ of\ red\ cells\ (N=500000/mm3)}$$

Colour Index (chromatic) --- CI = 1 normochromatic;
CI< 1 hypochromatic;
CI> 1 hyperchromatic.
Task: To calculate Colour Index and compare it with the standard

Laboratory Work nr. 4 Fixing the coagulation time of the blood.

Work goal: to fix the coagulation time of the blood, proceeding from Agadjanean's method.

Materials and equipment: paraffined glass slide, a sterile lancet, alcohol, cotton-wool.

Work technique:

1. Pierce the finger and collect the first drop of blood on the paraffined glass slide.
2. Every 30 seconds insert the lancet in the drop and look after fibrin filament formation.
3. Fix the time from the moment when the drop of blood was applied on the glass slide until the first fibrin filaments appear, this time corresponds to the time of blood coagulation.

4. According to this method the normal time of coagulation about 3-5 minutes.
5. Note the result of the experiment in your report and compare with the normal value.

CHAPTER VI

THE NERVOUS SYSTEM: GENERAL PRINCIPLES AND SENSORY PHYSIOLOGY. THE SPECIAL SENSES

Theme 1. **Somatic sensations: general organization, the tactile and position senses. Pain, headache, and thermal sensations**

Check questions:

1.1 Sensory receptors. Types of sensory receptors and the sensory stimuli they detect. differential sensitivity of receptors.

1.2 Transduction of sensory stimuli into nerve impulses. Local electrical currents at nerve endings - receptor potentials. Adaptation of receptors.

1.3 Nerve fibers that transmit different types of signals, and their physiologic classification.

1.4 Classification of somatic senses. Detection and transmission of tactile sensations. Detection of vibration.

1.5 Sensory pathways for transmitting somatic signals into the central nervous system. Dorsal column–medial lemniscal system. Anterolateral system.

1.6 Transmission in the dorsal column-medial lemniscal system. Anatomy of the dorsal column-medial lemniscal system. Somatosensory cortex. Somatosensory association areas. Overall characteristics of signal transmission and analysis in the dorsal column–medial lemniscal system. Interpretation of sensory stimulus. Judgment of stimulus intensity. Position senses.

1.7 Transmission of less critical sensory signals in the anterolateral pathway. Anatomy of the anterolateral pathway.

1.8 Some special aspects of somatosensory function. Function of the thalamus in somatic sensation. Cortical control of sensory sensitivity - "corticofugal" signals. Segmental fields of sensation-the dermatomes.

1.9 Types of pain and their qualities – fast pain and slow pain. Pain receptors and their stimulation. Dual pathways for transmission of pain signals into the central nervous system. Dual pain pathways in the cord and brain stem – the neospinothalamic tract and the paleospinothalamic tract.

1.10 Pain suppression ("analgesia") system in the brain and spinal cord. Brain's opiate system – endorphins and enkephalins. Inhibition of pain transmission by simultaneous tactile sensory signals. Treatment of pain by electrical stimulation.

1.11 Referred pain. Visceral pain. Causes of true visceral pain. "Parietal pain" Caused by visceral disease. Localization of visceral pain – "visceral" and the "parietal" pain transmission pathways.

1.12 Some clinical abnormalities of pain and other somatic sensations. Hyperalgesia. Herpes Zoster (Shingles). Tic douloureux. Brown-Séquard syndrome. Headache. Headache of Intracranial Origin.

1.13 Thermal sensations. Thermal receptors and their excitation. Transmission of thermal signals in the nervous system.

Laboratory Work Nr.1 Determination of the space threshold of tactile perception.

Work goal: determination of the space threshold of tactile perception in different zones of the skin.

Materials and equipment: a special compass or an ordinary compass with blunt ends, ethanol, cotton-wool.

Work technique:

1. Apply simultaneously both compass arms maximally approached (1mm) on the investigated sector of the skin. A single touch sensation will be perceived.
2. Repeat the procedure and increase the distance between the arms of the compass until two tactile sensations are perceived (for each of the compass arms).
3. Determine the minimal distance at which two distinct tactile sensations were perceived.
4. Determine the threshold space on other skin sectors in the same way.
5. Introduce the results in the table and compare them with the average values of the space threshold.

Table VI.1

Space threshold of tactile sensibility (mm)

Skin sector	Space threshold of tactile sensibility
Intern al surface of the finger tip	2-3
Dorsal surface of the third phalange	6-7
Palm	11
Dorsal surface of the hand	20
Neck	54
Tip of the tongue	1
Nose	3
Middle of the back, arm and thigh	67
Thigh	35

Make conclusions about the variations of the space threshold and the causes that produce such different results in different zones.

Laboratory Work Nr.2 Aristotel's experiment.

Work goal: to study the role of the visual and kinetic analyzers in the control of the localization of tactile sensations.

Materials and equipment: a ball made of paraffin or metal.

Work technique:

1. Take the ball between the index and the middle finger and roll it on the table. A single ball is perceived. Cross the fingers, put the ball between the ulnar surface of the middle finger and the radial surface of the index and roll it again. The experience can be repeated by touching the nose with crossed fingers. In this case two balls are perceived.
2. Describe the experiment and the character of tactile sensations in the report. In the conclusions will be mentioned that the determination of the character of sensations depending on the activity of the analyzers.

Topic 2. **The eye. Optics of vision. Receptor and neural function of the retina. Central neurophysiology of vision.**

Check questions:

2.1 Physical principles of optics. Refraction of light. Application of refractive principles to lenses. Focal length of a lens. Formation of an

image by a convex lens. Measurement of the refractive power of a lens-"dioptre".

2.2 Optics of the eye. The eye as a camera. Mechanism of "accommodation". Pupillary diameter. Errors of refraction. Visual acuity. Determination of distance of an object from the eye-"depth perception". Ophthalmoscope.

2.3 Fluid system of the eye–intraocular fluid. Formation of aqueous humor by the ciliary body. Outflow of aqueous humor from the eye. Intraocular pressure.

2.4 Anatomy and function of the structural elements of the retina.

2.5 Photochemistry of vision. Rhodopsin-retinal visual cycle, and excitation of the rods. Automatic regulation of retinal sensitivity- light and dark adaptation.

2.6 Color vision. Tricolor mechanism of color detection. Color blindness.

2.7 Neural function of the retina. Neural circuitry of the retina. Ganglion cells and optic nerve fibers. Excitation of the ganglion cells.

2.8 Visual pathways. Function of the dorsal lateral geniculate nucleus of the thalamus.

2.9 Organization and function of the visual cortex. Layered structure of the primary visual cortex. Two major pathways for analysis of visual information - (1) the fast "position" and "motion" pathway; (2) The accurate color pathway.

2.10 Neuronal patterns of stimulation during analysis of the visual image. Detection of color. Effect of removing the primary visual cortex. Fields of vision. Perimetry.

2.11 Eye movements and their control. Fixation movements of the eyes. "Fusion" of the visual images from the two eyes. Autonomic control of accommodation and pupillary aperture. Control of accommodation (focusing the eyes). Control of pupillary diameter.

Laboratory Work Nr.1 Pupillary reflexes.

Work technique:

- The direct photomotor reflex:
  - ✓ Note the pupils' diameter of the volunteer.
  - ✓ Cover the eyes for about 30-60 seconds.

- ✓ Open the eyes and note the degree of pupil diameter modification. In light the pupil diameter narrows (myosis) and in the dark the pupil diameter increases (mydriasis).

- The consensual photomotor reflex is determined for each eye separately:
  - ✓ The volunteer covers an eye. The modification of the diameter of the pupil of the other eye (mydriasis) is observed.
  - ✓ Open the eye and observe the modification of the pupil diameter (miosis) of both eyes.
- The pupillary reflex with the secondary role, distance accommodation reflex:
  - ✓ The volunteer looks at a distant object. Observe the diameter of the pupils.
  - ✓ Then he looks quickly at an object situated close to the eye (15cm), on the median line.
  - ✓ Observe the convergence of the ocular axes and the myosis of equal intensity at both eyes.
  - ✓ If the look is no longer fixated on the close object, the symmetric dilatation of the pupils occurs.

In the report note the ways of constriction and dilatation of the pupillary reflex.

Laboratory Work Nr.2 Determination of visual acuity.

Work goal: to study the method of determination of the static visual acuity at a distance.

Materials and equipment: a standard table for determination of visual acuity, indicator.

Work technique:

1. Students work in pairs and determine the visual acuity for each other and for each eye and for both eyes.
2. The volunteer stands at a distance of 5 m from the table.
3. The unexamined eye is covered.
4. The other student indicates a row on the table which the volunteer must read (without any confirmation if he reads right or wrong).
5. Note the last row that was recognized half plus one of the total characters.

6. The procedure of determination of visual acuity is repeated for the other eye and then for the both eyes simultaneously.
7. Visual acuity is calculated by the formula of Snellen:

$$Vis = \frac{d}{D}$$

Where: **d**-distance from which the optotype is read; **D**-distance from which letters of that row can be read by an emmetropic eye.

***Example**: If the volunteer sees at the distance of 5 m only the first row, which he should normal see at 50 m, that means that his visual acuity is 5/50.*

Another method of determination of the visual acuity for values under 1/10 is realized by counting of the fingers (the width of the fingers is approximately equal to the width of the letters on the optotype). The volunteer is requested to count the fingers of the examiner. If the person cannot perceive hand movements, the light perception is tested and the person is requested to specify in which part of the visual field a light fascicule is projected on the examined eye (up, down, left, right).

The conclusion of the report must specify if the visual acuity corresponds or not corresponds to the emmetropic eye, which is equal to 1.

Laboratory Work Nr.3 Determination of the visual field.

Work goal: examination of the visual field for chromatic and achromatic target-tests by the method of kinetic parametric examination.

Materials and equipment: perimeter of Foster, standard target-tests.

Work technique:

1. The volunteer fixes the chin on the apparatus stand and the examined eye looks straight the white spot in the middle of the semicircle. The other eye is covered.
2. The examiner moves gradually chromatic and achromatic target-tests from the periphery of the circle to center. In the moment that the subject sees the target, the examiner notes the angle on a standard form.
3. Then the semicircle is rotated 15 degrees and the operation is repeated. Finally, after the semicircle makes a full rotation, a graph with the total points seen by the volunteer will be obtained (*Fig VI.1*). This represents the visual field. The visual field of the other eye also is determined.

4. Determine the visual field of the volunteer.

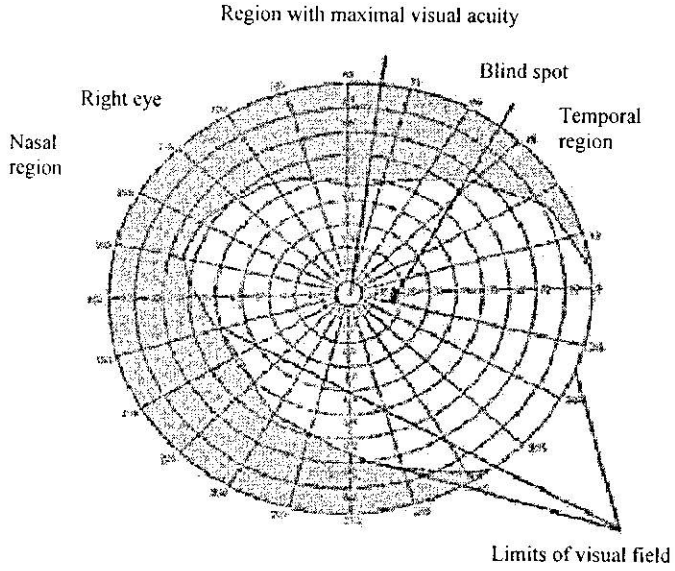


Figure VI.1 **Standard form for visual field examining**

Topic 3. **The sense of hearing. Chemical senses – sensation of taste and smell.**

Check questions:

3.1 Tympanic membrane and the ossicular system. Conduction of sound from the tympanic membrane to the cochlea. Transmission of sound through bone.

3.2 Cochlea. Functional anatomy of the cochlea. Transmission of sound waves in the cochlea – "travelling wave". Function of the organ of Corti. Determination of sound frequency – the "place" principle. Determination of loudness.

3.3 Central auditory mechanisms. Auditory nervous pathways. Function of the cerebral cortex in hearing. Determination of the direction from which sound comes. Centrifugal signals from the central nervous system to lower auditory centers. Hearing abnormalities. Types of deafness.

3.4 The sense of smell. Olfactory membrane. Stimulation of olfactory cells. Transmission of smell signals to the central nervous system.

3.5 The sense of taste and functional aspects of the taste organs. Taste bud and its function, transmission of taste signals to the central nervous system. The mechanism of taste reception. Taste abnormalities. Functional relations between smell and taste senses.

Additional questions for students of Dentistry Faculty

3.6 Sensitive function of maxillofacial system, types of sensation. Classification of receptors within the mouth. Contact sensory systems of the oral cavity (tactile, thermic, taste, nociception and proprioceptive senses)

3.7 Tactile and thermic sensations. Tactile receptors (static, phasic) and thermic receptors of mouth mucosa. Conducting segments and central regions of tactile and thermic sensory systems.

3.8 Pain sensations in the maxillofacial region. Nociceptive system of the trigeminal nerve. Upward tracts of the trigeminal complex. Toothache. Receptors, conducting pathways and the main link of toothache. Reflected pain in dental practice.

3.9 The antinociceptive system in modulating painful stimuli. Levels of the antinociceptive system activity. Endogenous mechanisms (short-term, long-term, urgent, tonic) of modulating pain. Physiological basis of the analgesia in dental practice. Anesthesia.

Laboratory Work Nr. 1 Instrumental acumetry (Weber's probe)

Work goal: evaluation of acoustic acuity in volunteers.

Materials and equipment: a tuning fork that vibrates at 512 Hz frequency.

Work technique:

1. The tuning fork is applied on the median line of the head with the vertical arms and in frontal plan.
2. The volunteer in asked to determine the sound localization. This can be "lateralized" to an ear or "indifferent" (it is heard all over or in middle of the head). The response can be explained as: W "indifferent" – means a normal hearing or hearing symmetrical disturbances, but W "lateralized" – means conduction deafness. W is lateralized in the affected ear in case

of unilateral conduction or in the most affected ear in bilateral conduction; in case of sensory hypoacusis the sound is heard only by a healthy ear; in case of mixed hypoacusis there are some particularities, but a rule can be applied: Weber is lateralized for a certain frequency in the side with bigger difference Rinne's test value and the value of threshold.

3. Explain the results and make conclusions.

Laboratory Work Nr.2 Determination of the taste sensation.

Work goal: determination of simple (primary or fundamental) taste sensations.

Materials and equipment: quinine solution (0.1%), sugar (20%), salt (10%) and citric acid (0.2%); water, cotton-wool, glass sticks.

Work technique:

1. Pour each of the solutions in a separate tubes (the examined person must not be informed about the content of the tubes). Check the temperature of the solutions (25°C).
2. The volunteer after each determination rins the mouth with distilled water (38°C), and will make a pause between tasting of about 1 minute.
3. Soak a cotton wool swab in the tested solution. First paint tip of the tongue, then the lateral parts, then the base and the middle portion (**ATTENTION!** The solution must not be swallowed!).
4. The volunteer will identify, describe, name the tested substance or describe its characteristics.
5. The same procedure is repeated for each solution separately. A new cotton wool swab and a glass stick are used for each solution.
6. It is found that:
   - There is a zonal sensibility of the tongue:
   - ✓ The sweet taste is perceived at the apex of the tongue;
   - ✓ The sour taste is perceived at the lateral parts of the tongue;
   - ✓ The salty taste is perceived at the apex and the lateral parts of the tongue;
   - ✓ The bitter taste is perceived at the base of the tongue;
   - The middle portion of the dorsal surface of the tongue has no taste sensibility.

7. In conclusion note the presence of 4 fundamental taste sensations.

Laboratory Work Nr.3 Determination of taste thresholds.

Work goal: to study the method of determination of taste thresholds.

Materials and equipment: solutions of quinine, sugar, salt and citric acid in different concentrations (0.0001; 0.001; 0.0025; 0.005; 0.05; 0.1; 0.5; 1.0%); glass stick, distilled water, cotton-wool.

Work technique:

1. The volunteer before and after each determination will wash the mouth with distilled water (38°C) and will make a pause of about 1 minute between the tastings.
2. The volunteer has a small quantity of solution (5-10ml) that is spat out in 30 seconds. The test is started with the smallest concentrations.
3. The volunteer has to identify the tasted substance or to describe it qualities.
4. Determine the taste threshold sensibility for each substance. The sensibility threshold is expressed by the minimal concentration of substance that can be perceived and it varies depending on the substance.
5. The obtained data must be introduced in the table, marking the taste threshold for each substance with "+".

Concentration of solutions (%)	Sensation			
	Quinine	Sugar	Salt (NaCl)	Citric acid
0.001				
0.05				
0.1				
0.5				
1.0				

CHAPTER VII

THE NERVOUS SYSTEM: MOTOR AND INTEGRATIVE NEUROPHYSIOLOGY

Topic 1. **Motor functions of the spinal cord; the cord reflexes. cortical and brain stem control of motor function.**

Check questions:

1.1 Organization of the spinal cord for motor functions.

1.2 Muscle sensory seceptors-muscle. Spindles and Golgi tendon organs. Their roles in muscle control. Muscle stretch reflex. Clinical applications of the stretch reflex. Golgi tendon reflex. Tendon organs and motor control from higher levels of the brain.

1.3 Flexor reflex and the withdrawal reflexes. Crossed extensor reflex. Reciprocal inhibition and reciprocal innervation. Reflexes of posture and locomotion. Autonomic reflexes in the spinal cord. Spinal cord transection and spinal shock.

1.4 Primary motor cortex. Premotor area. Supplementary motor area. Some specialized areas of motor control found in the human motor cortex. Transmission of Signals from the Motor Cortex to the Muscles. Incoming Fiber pathways to the motor cortex. Red nucleus as an alternative pathway for transmitting cortical signals to the spinal cord. "Extrapyramidal" system.

1.5 Role of the brain stem in controlling motor function. Support of the body against gravity – roles of the reticular and vestibular nuclei.

1.6 Vestibular sensations and maintenance of equilibrium. Vestibular apparatus. Function of the utricle and saccule in the maintenance of static equilibrium. Detection of head rotation by the semicircular ducts. Vestibular mechanisms for stabilizing the eyes. Other factors concerned with equilibrium.

Laboratory work Nr. 1 (A, B, C, D). Human reflexes of clinical importance.

Work goal: to examine human reflexes which are used in clinics; to learn methods to study stretch reflexes.

Materials and equipment: a reflex hammer, a volunteer.

Work technique:

Knee jerk (Fig. VII.1 a)

The volunteer sits on a chair so that the muscles of both legs are relaxed. Palpate the patellar tendon and then observe the lower leg to jerk forward on striking the patellar tendon with a reflex hammer. Compare reflexes of both legs. If the knee jerk is weakly manifested, then the volunteer grasp his hands and makes a lateral extension. This increases essentially the knee jerk (Iendrassik phenomenon) because of the lack of inhibitory influences from the cortex on spinal cord motor centers.

Achilles reflex (Fig. VII.1 b)

This reflex appears on striking the Achillan tendon and it manifests the extension of the foot that results from an instantaneous stretching of the sural triceps muscle. Compare the reflexes of both legs.

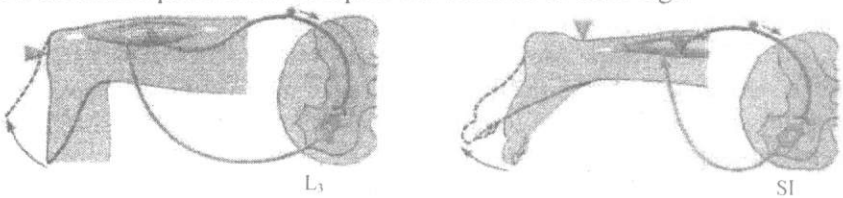


***Figure VII.1*a - Knee jerk; *1b* - Achilles reflex.**

Stretch reflex of the brachial biceps muscle (Fig. VII.2)

Place the volunteer's arm (it must be a little flexed) on the left examiner's forearm. The examiner palpates the brachial biceps muscle's tendon. The forearm jerk can be elicit by striking this tendon with the reflex hammer.

Stretch reflex of the brachial triceps muscle. (Fig. VII.2)

The volunteer's arm is fixed in the elbow region by the examiner and laterally extended (the arm and forearm form a straight angle). Strike the muscle's tendon and observe the forearm extension.

Draw and describe the arch reflexes from figures *VII.1* and *VII.2* in your report and show the segments of the spinal cord where the centers of these reflexes are localized.

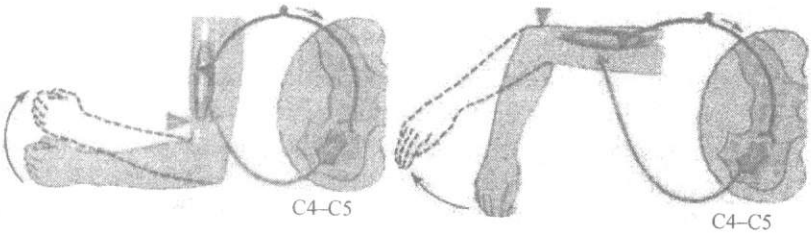


Figure VII.2 **Stretch reflex of the brachial biceps and triceps muscles.**

Laboratory Work Nr.2 (A, B, C) Somatic reflexes of the brainstem.

Work goal: examination of somatic reflexes in order to appreciate the functional state of the brainstem.

Materials and equipment: a reflex hammer.

Work technique:

A. Supraorbitary reflex

With the reflex hammer strike slightly on the supraorbitary arch of the examined person in the exit point of the trigeminal nerve terminal branches. The closure of eyelids is noticed.

B. Corneal reflex

Touch easily the eye above the iris with a corneal cotton-wool pad. Notice the closure of the eyes.

C. Mandibulary reflex

Strike with the reflex hammer on the volunteer's mandible (the mouth is half-open) in the exit point of the sensitive branches of the mandibular nerve (of the trigeminal nerve). Observe contractions of the masseter muscle.

In the report analyze the reflex arches, note reflex centres localization of these.

Laboratory Work Nr.3 Reflex regulation of muscular tonus (Brongest Experiment)

Work goal: to prove experimentally the role of the afferent impulses of the proprioreceptors of the muscles in the maintenance of the muscular tonus.

Materials and equipment: a frog, a dissection kit, stand with metal hook fixed on the board.

Work Technique:

1. Prepare the (bulbar) frog.
2. Section the skin and the lateral muscles of the pelvis (approximately 1 cm). With the help of incision, find lumbar nervous plexus. Introduce a ligature under the plexus and suspend the frog by its jaw on the hook of the stand.
3. Notice the position of the hind limbs: the angles formed by the hip and shank, shank and feet of the both limbs are equal.
4. Ligature tightly or section the lumbar plexus.
5. Some minutes later, compare the length of the both extremities, stating the increase of the angles between the segments of the limb on the operated part. The extension of the limb is conditioned by the disappearance of the tonic reflex *(Fig. VII.3)*.
6. This experiment may be performed in a simpler way. To accomplish this, it is necessary to cut the skin of the spinal frog, to separate the semimembranosus muscle from the semitendinosus muscle, and to stretch the under the sciatic open nerve thread ligaturing tightly or sectioning it.
7. Attach the drawings (pictures) and description regarding the position of the feet of the frog before and after the unilateral denervation. To the report explain the cause of the tonic contraction of the muscles of the intact foot.

Figure VII.3. **Brongest Experiment:**
A - limb with sectioned nerve; *B* - limb with intact nerve

Laboratory work Nr. 4 The influence of labyrinths on muscular tonus

Work goal: to determine the role of the vestibular apparatus receptors in regulation of muscular tonus.

Materials and equipment: a frog, a vivisection kit, a board, gauze, a big vessel of water.

Work technique:

1. Observe the usual position and behavior of the frog (jumping and swimming movements).
2. Wrap up the frog in gauze and open its mouth. Separate the jaws from each other and fix with your hand the low jaw on the thorax. Using cut with scissors the mucous membrane near the sphenoid bone and open this region. Introduce the needle in the junction between the sphenoid and temporal bones (a whitish protrusion) and destroy the labyrinth by rotative movements of the needle.
3. Set free the frog, place it on the table and notice its position and jumps (when jumping the frog falls down backwards or on the affected side). Place the frog in water and observe its swimming movements: the frog is circling in the direction that corresponds to the injured labyrinth.
4. Describe observed phenomena in your report. Explain the role of the labyrinths in redistribution and maintenance of the muscular tonus. Make conclusions.

Topic 2. **Contributions of the cerebellum and basal ganglia to overall motor control. Cerebral blood flow, cerebrospinal fluid, and brain metabolism.**

Check questions:

2.1 Cerebellum and its motor functions. Anatomical functional areas of the cerebellum. Neuronal circuit of the cerebellum. Function of the cerebellum in overall motor control. Clinical abnormalities of the cerebellum.

2.2 Basal ganglia and their motor functions. Putamen circuit and caudate nucleus circuit. Functions of specific neurotransmitter substances in the basal ganglia system. Abnormalities of the basal ganglia.

2.3 Cerebral blood flow. Normal rate of cerebral blood flow. Regulation of cerebral blood flow. Cerebral microcirculation.

2.4 Cerebrospinal fluid system. Formation, flow and absorption of cerebrospinal fluid. Cerebrospinal fluid pressure. Blood–cerebrospinal fluid and blood-brain barriers. Brain edema. Brain metabolism.

Laboratory Work Nr.1 The role of different parts of the central nervous system in formation of the muscular tonus and phasic movements.

Work goal: to determine the role of different parts of CNS in regulation of the muscular tonus and phasic movements.

Materials and equipment: live frog, a vivisection kit, a board, gauze.

Work technique:

1. Trepanate the skull in the anterior part of the orbits on the surface of a 1x2 cm^2 surface and open the brain. Find the cerebral hemispheres, in the posterior part of which the optical thalami (2 small formations), mesencephalon (the bigeminal bodies), cerebellum and the spinal bulb are situated.
2. Remove different parts of the encephalon (the cerebral hemispheres, diencephalon, mesencephalon and spinal bulb) by gradual cutting and removal.
3. After each extirpation of different parts of the brain, notice the frog's posture in sitting position and check the postural recurrence reflex.
4. Note the results in the table below:

Phenomena observed	Intact frog	Thalamic frog	Bulbar frog	Spinal frog
Formation of the muscular tonus	Normal	Plastic	Contractual	
The posture presence of the animal in seitting position	+	+	-	-
The postural recurrence reflex	+	+	+	-

Conclusions: The normal posture characteristic of the animal in sitting position is absent in bulbar and spinal frog. Therefore, the normal motor activity and muscular tonus are regulated by means of all parts of the CNS.

Theme 3. **Cerebral cortex, intellectual functions of the brain. Sleep. the limbic system and the hypothalamus.**

Check questions:

3.1 Physiologic anatomy of the cerebral cortex. Functions of specific cortical areas. Association areas. Comprehensive interpretative function of the posterior superior temporal lobe – "Wernicke's area". Functions of the parieto-occipitotemporal cortex in the nondominant hemisphere. Higher intellectual functions of the prefrontal association areas.

3.2 Function of the brain in communication – language input and language output. Function of the corpus callosum and anterior commissure to transfer thoughts, memories, training, and other information between the two cerebral hemispheres.

3.3 Thoughts, consciousness, and memory. Memory – roles of synaptic facilitation and synaptic inhibition. Short-term memory. intermediate long-term memory. Long-term memory. Consolidation of memory.

3.4 Activating-driving systems of the brain. Control of cerebral activity by continuous Excitatory signals from the brain stem. Neurohormonal control of brain activity.

3.5 Functional anatomy of the limbic system. Key position of the hypothalamus. Vegetative and endocrine control functions of the hypothalamus. Behavioral functions of the hypothalamus and associated limbic structures. "Reward" and "punishment" function of the limbic system.

3.6 Specific functions of other parts of the Limbic System: hippocampus, amygdale and limbic cortex.

3.7 Sleep. Slow-Wave Sleep. REM Sleep. Basic Theories of Sleep. Physiologic Effects of Sleep.

3.8 Brain Waves. Origin of Brain Waves. Effect of Varying Levels of Cerebral Activity on the Frequency of the EEG. Changes in the EEG at Different Stages of Wakefulness and Sleep.

3.9 Epilepsy. Grand mal epilepsy. Petit mal epilepsy. Focal epilepsy.

3.10 Psychotic behavior and dementia – role of specific neurotransmitter systems. Depression and manic-depressive psychoses – decreased activity of the norepinephrine and serotonin

neurotransmitter systems. Schizophrenia – possible exaggerated function of part of the dopamine system. Alzheimer's disease.

Laboratory Work Nr.1 Development of the conditioned defensive reflex in rats.

Generals:

The conditioned reflexes are formed during the life summing individual experience and accumulated knowledge, determining the specific behavior in case of repeated situations. They improve continuously the adaptation function of the organism to variable conditions of the external or internal environment and represent the neurophysiological substrate of the learning process.

Some conditions and ratios between the unconditioned stimuli and the indifferent (conditioned) ones are necessary for the formation of conditioned reflexes, especially:

- The conditioned stimuli should precede the unconditioned ones.
- The conditioned and unconditioned stimuli act together for a period of time.
- The indifferent stimuli must be weaker (like a biologic meaning) than the unconditioned ones.
- The intensity of the conditioned stimuli must be adequate for not subjecting adverse reactions at some high intensities (of conditioned reflex stop) and sufficient to be felt.
- The repeated associations of the conditioned stimuli with the unconditioned ones to strengthen the elaborated conditioned reflex, avoiding the phenomena of stopping the reflex.
- The state developed of wakefullness (the conditioned reflexes are not developed during sleep).
- At the cortex level there should not be any other active centers of some other nature, because that additional center can be dominant and bother the conditioned reflex formation.
- The absence of severe cortex injuries.

The association of some unconditioned excitants with indifferent stimuli determines the formation of new interneuronal connections, followed by the formation of the reflex reaction when using of the conditioned stimuli. The conditioned reflex arches, unlike the elementary ones, are variable, are formed and disappear in dependence of the request.

At the base of the new created functional connection there is neuro-chemical and plastical modifications of the interneuron synapses (the growth and multiplication of axons and dendrites, the enlargement of terminal parts, the narrowing of the synapse space).

The problem of conditioned reflex development is complex, a the cortico-subcortical reverberant circuits, in which participate the reticular mesencephalic part, the limbic structures and the thalamic centers, near the cortex, like the main place of integration and elaboration of the conditioned reflex reaction, play a very important role.

Work goal: to study the process of development of the conditioned reflex of adaptation in rats.

Materials and equipment: an isolated chamber for the elaboration of the conditioned defensive reflex, bell, stopwatch.

Work technique:

1. Study the installation scheme for the development of the conditioned reflex of adaptation in rats.
2. Place the rat in the section of the chamber with the metallic floor, on which painful electric stimuli will be applied.
3. Respecting the necessary conditions for the formation of the conditioned reflex described above:

- Connect the bell for 3 seconds (conditioned stimulus) after which apply the electric stimuli (unconditioned stimulus).
- Repeat the association of the conditioned stimuli with the unconditioned ones several times.
- Note the number of associations of stimuli as a result of which the conditioned defensive reflex appeared in rats.

4. In the report the method of the conditioned defensive reflex is described, the isolated chamber, reflex arch and the elaborated reflex arch are drawn.

Laboratory Work Nr.2 Development of the conditioned reflex of defense in human being.

Work goal: to perform the experiment on the conditioned reflex of blinking in human being.

Materials and equipment: the installation necessary to develop the corneal conditioned reflex of blinking in human being.

Work Technique:

1. Set the installation presented in *Figure VII.4.*
2. The glass cannula (3), united through an elastic tube with a rubber ball (pear-shaped) (1) is installed so that the air jet would stimulate mechanically the cornea.
3. The electric bell (2) is connected to the grid. Be cautious! The intensity of the sound should not be high not to provoke the auditory blink reflex, which is the unconditioned reflex of defense of the eyeballs.
4. The cornea is stimulated by a jet of air with the view to observe the unconditioned reflex of blinking.
5. To develop the conditioned reflex of blinking, it is necessary to apply a sound stimulus (indifferent and conditioned stimulus) and 1-2 seconds later, it is associated with the jet of air (unconditioned stimulus). Such a kind of association is repeated 5-6 times at intervals of 1 minute.
6. Only the sound stimulus is applied and thus it is stated the conditioned reflex of blinking is developed. When the conditioned reflex becomes stable, the noise of the bell may be replaced with a verbal stimulation pronouncing the word "SOUND". As a consequence, the same reflex is developed.
7. The conditioned reflex of blinking is developed in at different persons and the number of stimuli associations is determined for each subject.
8. The report will include attachments of the description regarding the technique on conditioned reflex development, record of the obtained results (how many stimuli associations were necessary to cause the formation of the conditioned reflex in different persons) and explanatory notes containing arguments concerning the question why the replacement of the noise of the bell with the word "SOUND" provokes the appearance of the conditioned reflex of blinking.

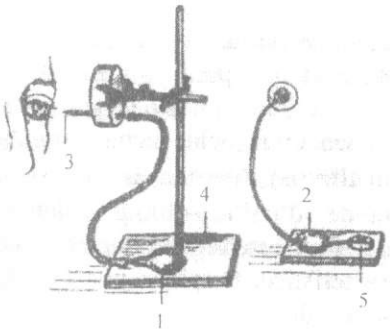


Figure VII.4. **Installation for the formation of corneal conditioned reflex of blinking (see explanations in the text).**

Laboratory Work Nr.3 Determination of the dominant motivation form in the animals under the conditions of free choice of food and water.

Work goal: to establish the role of the motivated excitement in behavior formation.

Materials and equipment: rats, special room or cage for keeping rats, food and water.

Work Technique:

Within a period of 24 hours, food is not given to one rat and water to another one. We mark the rats and place them into the room for the experiments, where they have the possibility to choose food and water. In accordance with what the rats choose, we come to the conclusion with regard to the nature of the dominant motivation.

The report will contain the scheme of the situation regarding the possibility of the animals to choose food and water.

As a result, conclusions concerning the type of dominant motivation are drawn.

Laboratory work Nr. 4 Aminazin effect on the central nervous system.

Work goal: to determine the behavioral changes in the rat after administration of aminazin.

Materials and equipment: experimental chamber with 2 sections (one has an iron floor connected to a source of electric power, so that it can be stimulated, in the other the floor is electrically isolated, so that it is harmless; 2 rats; 2.5 % aminazin solution; syringe.

Work technique:

1. Inject intramuscularly 0.3 ml of 2.5 % solution of aminazin to a rat. The other rat must not be injected.
2. Place the both of rats into the section with iron floor. The sections are communicated by through an orifice so that rats can easily move from one section to the other. When the iron floor is stimulated with electric power, the rat without aminazin injection leaves rapidly this section because of the defence reaction that appears. The same reaction is absent or weakly manifested in the rat with aminazin.
3. In your report draw the experimental chamber *(Fig. VII. 5.)*, explain the observed phenomena and the role of aminazin in maintaining the wakeful state of the brain cortex, make conclusions.

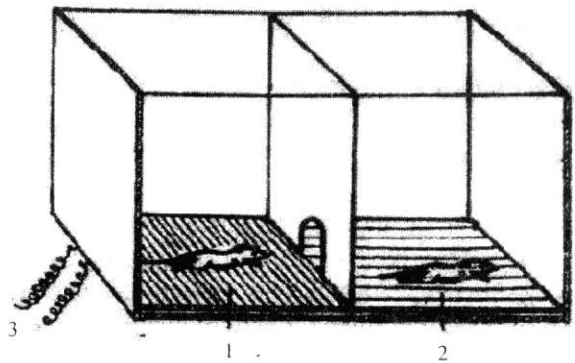


Figure VII. 5. **Chamber to study defence and orientation reflexes:** 1 - iron floor that can be stimulated; 2- harmless section of the chamber; 3- source of electric power.

CONTENTS