MINISTRY OF HEALTH CARE REPUBLIC OF BELARUS
GOMEL STATE MEDICAL UNIVERSITY

Normal Physiology Department

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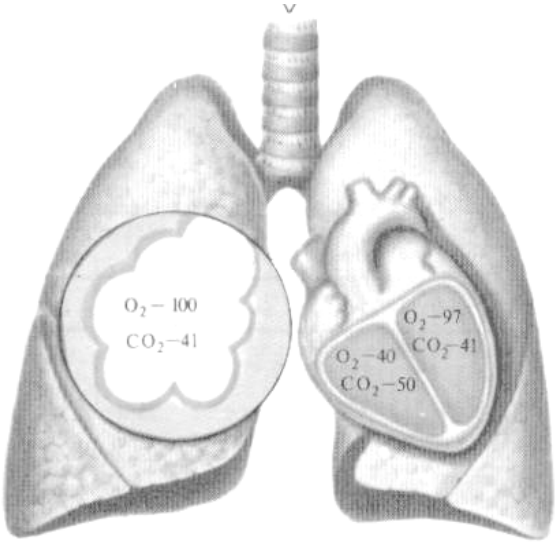
GUIDANCE IN NORMAL PHYSIOLOGY

laboratory course in normal physiology
for overseas students in English medium

Part I



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Рецензент: зав. кафедрой патологической физиологии УО «Гомельский государственный медицинский университет», кандидат медицинских наук, доцент **Т.С. Угольник**.

Киеня А.И.

К 39 Guidance in normal physiology (laboratory course in normal physiology for overseas students in English medium)=Практикум по нормальной физиологии (методическое пособие для лабораторных работ по нормальной физиологии для иностранных студентов, обучающихся на английском языке) / А.И. Киеня, Э.М. Заика, В.А. Мельник; под ред. Э.С. Питкевича; пер. на англ. яз. Р.А. Карпова, В.А. Мельника. Ч. I. — Гомель: УО «Гомельский государственный медицинский университет». 2006. — 42 с.

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Представлены лабораторные работы по физиологии крови, сердечно-сосудистой системы, дыхания и пищеварения, необходимые для проведения практических занятий по данным разделам нормальной физиологии со студентами факультета по подготовке специалистов для зарубежных стран, которые занимаются на английском языке. Изучение методик лабораторных работ, включенных в пособие позволит закрепить полученные теоретические знания и освоить некоторые важные клинико-диагностические методы исследования.

При составлении и переводе данного пособия использовались материалы, опубликованного ранее руководства к практическим занятиям по нормальной физиологии под редакцией профессора А.И. Киени.

Рассмотрено и утверждено на заседании Центрального учебно-научно-методического Совета УО «Гомельский государственный медицинский университет» протокол № 5 от 19 мая 2006 г.

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INTRODUCTION

The practical guidance has been developed for practical classes in normal physiology with students of the Faculty of General Medicine of Overseas Students who study medicine in English. The content of the guidance meets the Normal Physiology Program for students of higher medical schools No. 08-14/5941, approved by Ministry of Health Care of the Republic of Belarus on September 3, 1997.

Teaching normal physiology to overseas students put forward certain difficulties due to the absence of special references in English adapted for the academic program in normal physiology. Hence, one of the main concern for the staff of the Department was developing of a manual in normal physiology written in English.

The practical guidance contains laboratory tasks in physiology of blood, cardio-vascular system, breathing and digestion necessary for practical classes in these areas of the normal physiology. Study of techniques of laboratory tasks included into the guidance allows to improve obtained theoretical knowledge and to learn the issues of clinico-diagnostic methods of research. Each section has in the end main constants expressed in the International system of physical units (SI).

Authors will be grateful to all who will make any comments upon this guidance; these will be taken into consideration contributed into developing of a new edition of the guidance.

ВВЕДЕНИЕ

Методическое пособие предназначается для проведения лабораторных работ по нормальной физиологии со студентами факультета по подготовке специалистов для зарубежных стран, обучающихся на английском языке. Материал пособия соответствует Программе по нормальной физиологии для студентов высших медицинских учебных заведений № 08-14/5941, утвержденной Министерством здравоохранения Республики Беларусь от 3 сентября 1997 г.

При организации учебного процесса по нормальной физиологии со студентами факультета по подготовке специалистов для зарубежных стран, обучающихся на английском языке одной из трудностей является отсутствие учебно-методической литературы, адаптированной к учебной программе по нормальной физиологии. Поэтому одной из важнейших задач, стоящих перед преподавателями кафедры, явилось составление и перевод на английский язык данного пособия.

В методическом пособии представлены лабораторные работы по физиологии крови, сердечно-сосудистой системы, дыхания и пищеварения необходимые для проведения практических занятий по данным разделам нормальной физиологии. Изучение методик лабораторных работ включенных в пособие позволит закрепить полученные теоретические знания и освоить некоторые важные клинико-диагностические методы исследования. В конце каждого раздела приведены основные константы, выраженные в Международной системе физических единиц (СИ).

Авторы будут весьма благодарны всем, кто сочтет возможным высказать свои критические замечания в адрес предлагаемого пособия, которые будут восприняты как выражение желания оказать помощь в его улучшении при последующим переиздании.

1. PHYSIOLOGY OF BLOOD

Lab. work 1.1. Method of blood taking from finger

When taking blood one should remember of its possible infection with AIDS, hepatitis B and associated high infection risk to which laboratory staff are subjected during clinical investigation. In this respect when making an analysis it is necessary to follow regulations of AIDS prevention in medical staff involved in blood sampling and its examination.

Purpose of work: to learn the technique of blood taking from a finger.

Necessary material: sterile scarificator, alcohol, cotton wool, iodine, diethyl ether. Object of research — a person.

Course of work

1. The patient should sit opposite to the staff-person taking blood, his hand (better left) on the table.
2. Blood is taken from the 4^{th} finger since its synovial vagina is isolated preventing expansion of inflammatory process in case it gets into wrist.
3. Skin of the finger is disinfected and degreased with alcohol-ether.
4. Open package with disposable scarificators at the side opposite to the piercing tip.
5. Pierce the skin in the center of the finger-pad (one time only!), piercing tip immersed in full into the finger-pad.
6. The first drop of blood is swept clean with dry cotton wool, the finger is carefully wiped (skin should be dry).
7. The following drop of blood should have convex meniscus and not spread on the finger, from these and subsequent drops blood is taken for analysis.
8. After taking of blood the pierced place is treated with alcohol or iodine.

Lab. work 1.2. Fresh blood sample under the microscope

Blood consists of liquid part — plasma, and cells weighed in it (uniform elements): erythrocytes (red blood cells), leucocytes (white blood cells) and thrombocytes (platelets).

Purpose of work: to study features of fresh blood preparation.

Necessary materials: drop of fresh blood, microscope, slides and cover slips, sterile scarificator, cotton wool, alcohol, diethyl ether, iodine.

Course of work

To look erythrocytes under the microscope, place the drop of blood on one of the sides of the slide, then cover it with cover slip, select field for observation with not thick layer of blood. Field of vision is occupied with erythrocytes located most often as «monetary columns». Make a drawing, write down a conclusion of the regular location of erythrocytes in the fresh blood sample.

Lab. work 1.3. Detection of the hematocrit number

Hematocrit number is a percent-marked relation of the uniform elements volume to the volume of the whole blood.

There are several techniques of hematocrit number detection. All of them are based on centrifugation of blood prevented from coagulation and placed into the capillary, and measuring of blood columns and the sedimented erythrocytes.

Purpose of work: to learn technique of definition of the hematocrit number and to determine its index in the examined blood.

Necessary materials: hematocrit capillary, centrifuge, cotton wool, alcohol, iodine. Object of research — a person.

Course of work

Pierce a finger by the known technique. Fill in hematocrit capillary at not less than 2/3 of its volumes. Seal up the ends of the tubule with plasticine and place into the centrifuge (not less than two capillars in opposite cells). Rotate the cover to close the centrifuge tightly. Once centrifugation is over, measure the blood column in the tubule with a ruler. Measure the erythrocytes column in mm, calculate percentage relation. Take the length of blood column as 100%, length of erythrocytes column as X. The obtained value of X% corresponds to the parameter of hematocrit of blood. To convert the %-marked hematocrit into the international units system, multiply the obtained value by 0,01.

In conclusion compare the obtained parameter of hematocrit with the norm.

Lab. work 1.4. Definition of erythrocytes amount

Purpose of work: to learn the technique and to define amount of erythrocytes in the examined blood.

Necessary materials: microscope, erythrocytometer (Gorjaev's count chamber), melangeur for erythrocytes, 3% solution of sodium chloride, cover slips, adjustable pipette, tubes, sterile scarificator, cotton wool, alcohol, diethyl ether, iodine. Object of research — a person.

Course of work

Prior to work, it is necessary to examine the count chamber under the microscope, to detect small squares and the big squares of a frame (one big square consists of 16 small) (Fig. 1). Gorjaev's count chamber should be preliminary prepared by rubbing of the cover slip to lateral extended edges of the chamber till color (Newtonian) rings similar to gasoline stains in a pool appear. Place the tip of the melangeur (with red bead at widening) for erythrocytes into the drop of the fresh blood taken from the finger, and collect blood up to mark 0,5 watching for air bubbles not to get inside the capillary. Instantly, till the blood is not coagulated, immerse the tip of the melangeur into 3% solution of sodium chloride and collect it to the mark of 101, i.e. dissolve blood in 200 times. Then, take the filled melangeur, close its both ends with the 1st and the 3rd fingers and shake it for 1 minute. Once

the blood has been mixed carefully, remove preliminary 1–2 drops of it and place a small droplet onto the frame of the Gorjaev's count chamber.

If necessary, the same dilution can be made in other way. For example, 0,02 ml of blood is mixed with 4 ml of the 3% NaCl in a tube. The content of the tube in this case is carefully mixed.

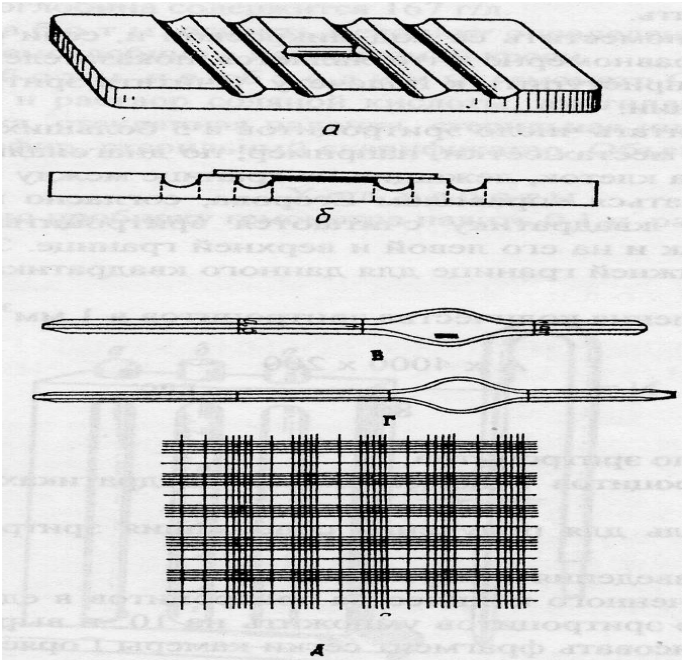

Fig. 1. Devices for determination of uniform elements of blood
Left — Gorjaev's count chamber (A — top view, B — lateral view).
Right — melangeur for erythrocytes (c) and leucocytes (d) count,
frame of Gorjaev's count chamber (f).

If the drop of dissolved blood will be too big, liquid can get onto the lateral sides of the chamber and the height of the layer above the frame will be more than 0,1 mm. In this case the chamber should be rinsed with distilled water, wiped dry and filled in again. Dissolved (in melangeur) blood should be mixed once again.

Having filled in the chamber, place it under the microscope and, if uniform elements are regularly distributed (the evidence of the appropriate mixing of blood), make calculation. Calculation of erythrocytes is more convenient at large magnification.

It is necessary to calculate the number of erythrocytes in 5 big squares of different places of the frame, for example, on diagonal. To prevent double count of cells laying on the border of the two small squares, apply Egorov’s rule: a square includes erythrocytes laying both inside it and on its left and top boundary. Erythrocytes on the right and the bottom boundary are not counted for the given square.

Equation for calculation of erythrocytes amount in 1 mm^3 of blood:

$$N = \frac{A \times 4000 \times 200}{80},$$

where: N — number of erythrocytes;

A — number of erythrocytes in 80 small squares (or in 5 big);

4000 — multiplier for calculation of the erythrocytes content in 1 mm^3 of blood;

200 — dilution degree of erythrocytes.

To convert the received amount of erythrocytes into the international units system, multiply the calculated amount of erythrocytes by 10^6 and express as N x 10^{12}/l (tera per liter). Make a drawing of Gorjaev's count chamber with small and big squares.

In conclusion compare the obtained amount of erythrocytes in the examined blood with the norm.

Lab. work 1.5. Definition of amount of hemoglobin in blood by Sali method

Definition of hemoglobin content in blood is made by colorimetric methods, one of which (Sali hematin method) is based on the formation of steady solution of brown color at interaction of hemoglobin with the hydrochloric acid. The principle of the colorimetric method is that if the examined solution is diluted to the color similar to that of the standard solution, concentration of soluted substances in both solutions will be identical, and amounts of substance will correlate as their volumes. Standard solution contains 167 g/l of hemoglobin.

Purpose of work: to learn technique of definition of hemoglobin and to detect the amount of hemoglobin in the examined blood.

Necessary materials: Sali hemometer, capillary with mark 0,02 ml, 0,1 normality (n) solution of the hydrochloric acid, distilled water, fresh blood, pipette, glass rod, sterile injection needle, cotton wool, alcohol, iodine, diethyl ether, sterile scarificator. Object of research — a person.

Course of work

Pour in 0,1 n solution of hydrochloric acid into the middle tube of the hemometer to the low ring mark. Take blood from a finger into capillary as usual (Fig. 2) without air bubbles up to the mark.

Surplus of blood can be removed from the capillary by applying cotton wool or filter paper to the tip of the capillary. Blow out the blood onto the bottom of a tube from the capillary so that the top layer of the acid remained uncolored. Not taking out capillary, wash it with the solution of hydrochloric acid from the top layer of the hemometer tube. Then, mix the content of the tube with the glass rod and leave it in the hemometer for 5–10 minutes. This time is necessary for complete transformation of hemoglobin into hydrochloride hematin.

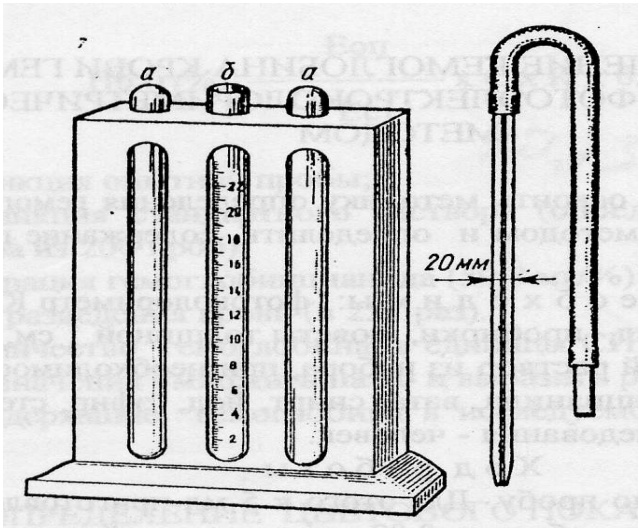

Fig. 2. Sali hemoglobinometer and capillary. (Explanatory in the text)

Then add distilled water, one drop at a time, to the contents of the tube, continue mixing solution with the glass rod until the color of the obtained solution becomes identical with that of the standard solution of lateral ampules of hemometer. Digit marked at the lower boundary of the meniscus of the obtained solution indicates hemoglobin amount in the examined blood in grams-percent (g %).

To convert the received amount of hemoglobin into the international units system, multiply measured amount of hemoglobin in g% by 10.

Make a drawing of hemometer and the capillary.

In conclusion compare the obtained amount of hemoglobin in the examined blood with the norm.

Lab. work 1.6. Definition of hemoglobin by hemoglobin-cyanide (photoelectrocolorimetric) method

Purpose of work: to learn the definition technique of hemoglobin of blood by hemoglobincyanide method and to determine the amount of hemoglobin in the examined blood.

Necessary materials: photocolorimeter, 0,02 ml and 5,0 ml pipettes, tubes, cuvette of 1 cm depth, prepared transforming solution, standard of solution of hemoglobincyanide (if necessary), cotton wool, alcohol, iodine, diethyl ether, sterile scarificator. Object of research — a person.

Course of work

Prepare test solution. For this purpose, add 0,02 ml of finger blood to 5 ml of the prepared transforming solution. Dilution of blood in this case will make 251. Mix the obtained solution carefully, let it stand for 5–10 minutes, then place it to the photocolorimeter cuvette.

Prepare cuvette with transforming solution. For this, measure 5 ml of the transforming solution and place it to the similar cuvette (as described above).

Measure as described in the instruction manual of the device with the help of the laboratory assistant. Measure the amount of hemoglobin not less than 3 times, then calculate arithmetic mean.

Concentration of hemoglobin in the examined blood to make with formula:

$$Hb(g/\%) = \frac{Etest}{Est} \times C \times K \times 0{,}001,$$

where: E_{test} — extinction of experienced test;

E_{st} — extinction of standard solution (determined unitary for one set of 200 samples);

C — concentration hemoglobincyanide (59,8 mg /%);

K — degree of blood dilution (in 251 times).

Convert the obtained amount of hemoglobin into the international units system: multiply the measured amount of hemoglobin by 10 and express the result in g/l.

In conclusion compare the obtained amount of hemoglobin in the examined blood with the norm.

Lab. work 1.7. Definition of color index of blood

The color index (CI) is the saturation of erythrocytes with hemoglobin.

Purpose of work: to learn the technique and to calculate CI of blood.

Necessary materials: data of erythrocytes and hemoglobin amount in blood.

Course of work

The examined person is determined the amount of erythrocytes and hemoglobin in blood. Calculation of the color index is made with the formula:

$$CI = \frac{Hb(g/l) \times 3}{A},$$

where: A — number of erythrocytes in 1 microliter (first three digits).

In conclusion compare the obtained size of CI with the norm.

Lab. work 1.8. Blood grouping

Blood grouping is the definition of the contents of agglutinogens (A and B) on the membrane of erythrocytes and agglutinin (α and β) in blood plasma.

Purpose of work: to learn technique of blood grouping and to define group blood of the examined person.

Necessary materials: slide, glass rods, standard serums of I, II, III groups, cotton wool, alcohol, iodine, diethyl ether, sterile scarificator. Object of research — a person.

Course of work

Place the slide on a white sheet of paper and put (without mixing) drops of standard serum of I, II and III groups one by one. Place small drops of finger blood (approximately 1/3 of the drop of serum) on the slide close to the drops of standard serum. Carefully mix each pair of serum and blood drops with dry clean glass rod (different rod for each pair!) until the fusion is of regular color.

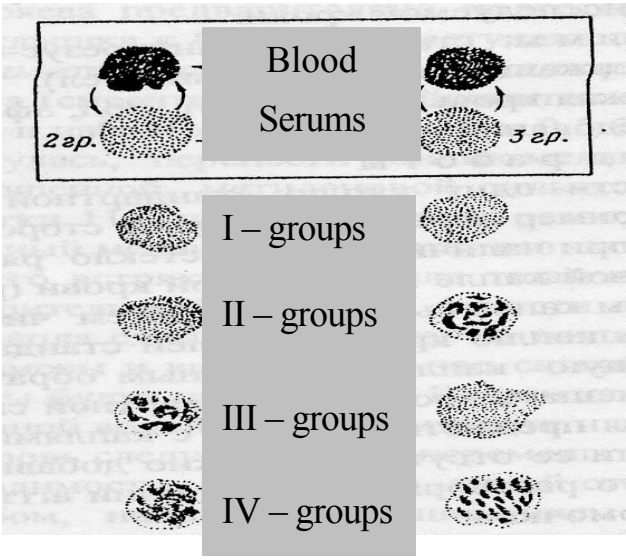

Fig. 3. Scheme of blood groups definition

The agglutination reaction will happen in 1–5 minutes (Fig. 3). If agglutination presents, the drop becomes transparent and erythrocytes stick together as nubbins.

1. Absence of agglutination in each of the three mixed drops of serum refers the examined blood to 0 (I) group.

2. If agglutination occurred in drops of serums of I and III groups, the examined blood belongs to A (II) group.

3. If agglutination occurred in drops of serums of I and II groups, the examined blood belongs to B (III) group.

4. At presence of agglutination in drops of serums of I, II and III groups, the examined blood belongs to AB (IY) group.

In conclusion specify blood group.

Lab. work 1.9. Definition of rhesus-factor (Rh) of blood

Membrane of erythrocytes of 85% of people, apart from agglutinogens A and B, has a special antigen called rhesus-factor (Rh). When transfusing rhesus-positive (Rh^+) blood to a rhesus-negative (Rh^-) recipient, antibodies to the Rh-factor of donor are developed the latter. At the repeated transfusion of the rhesus-positive blood these antibodies induce agglutination of erythrocytes of donor resulting in pathologic state.

Purpose of work: to learn the technique of definition of Rh-factor and to define Rh-factor of the examined blood.

Necessary materials: standard antirhesus serum and control serum (antirhesus of antibodies free), slide, glass rod, blood samples, cotton wool, alcohol, iodine, diethyl ether, sterile scarificator. Object of research — a person.

Course of work

Place one drop of standard antirhesus serum on one side of slide (for example, on the right), and the drop of the control serum (if it is available) — on the another side. Place a drop of the examined blood next to each drop of the serum

(size of blood drop to be twice less than that of serum). Then mix drop of blood with drop of standard antirhesus serum with clean dry glass rod thus making one general drop. In similar way mix blood with standard serum with clean dry glass rod. Watch the reaction, rocking slightly slide with drops. For better revealing of the agglutination or its absence it is possible to add drop of physiological solution into both test. At presence of agglutination erythrocytes stick together as nubbins.

If the examined blood is rhesuses-positive, agglutination of erythrocytes will be observed in the test with standard antirhesus serum (it should not be in the control). If the blood is Rh-negative, agglutination is absent in both tests.

In conclusion note Rh-factor of the examined blood.

Lab. work 1.10. Definition of leucocytes amount

Leucocytes are cell elements directly participating in immune-protective reactions. Unlike erythrocytes, they have nuclei and can leave blood channel and move independently.

Purpose of work: to learn technique and to define amount of leucocytes in the examined blood.

Necessary materials: microscope, Gorjaev's count chamber, melangeur for leucocytes, 5% solution of acetic acid with methylene-blue, cover slips, regulated pipette, tubes, cotton wool, alcohol, iodine, diethyl ether, sterile scarificator. Object of research — a person.

Course of work

Prepare the Gorjaev's chamber as described earlier (Lab. work 1.4.).

Place the tip of the melangeur (with white bead at widening) for leukocytes into the drop of the fresh blood taken from the finger, and collect blood up to mark 0,5 watching without air bubbles and instantly, till the blood is not coagulated, immerse the tip of the melangeur into solution of acetic acid stained with methylene-blue. Collect the acetic acid solution to the mark 11, i.e. dissolve blood in 20 times. Then, take the filled melangeur, close its both ends with the 1st and the 3rd fingers and shake it for 1 minute. Once the blood has been mixed carefully, remove preliminary 1–2 drops of it and place a small droplet onto the frame of the Gorjaev's count chamber. If the drop is too large, liquid can get onto the lateral sides of the chamber and the liquid layer above the frame will be more than 0,1 mm and further calculations will be wrong. In this case rinse the chamber carefully under the distilled water, dry it and fill in again. Soluted blood in melangeur should be mixed once again.

If necessary, the same dilution can be made in other way. For example, 0,1 ml of blood is mixed with 2,0 ml of the 5% acetic acid in a tube. The content of the tube in this case is carefully mixed.

Having filled in the chamber, place it under the microscope and start calculation. Leucocytes are much easier to count with small magnification. Perform counting in 25 big squares that compounds 400 small squares. Use the Egorov's rule when counting.

Formula for calculation of the amount of leucocytes in 1 mm^3 of blood:

$$N = \frac{B \times 4000 \times 20}{400},$$

where: N — number of leucocytes;

B — number of leucocytes in 25 big (400 small) squares;

4000 — multiplier to get the contents of leucocytes in 1 microliter of blood;

400 — number of small squares in 25 big squares;

20 — degree of dilution of blood.

To convert the received amount of leucocytes into the international units system, multiply the calculated amount of leucocytes by 10^6 and express in $N \times 10^9$/l (giga per liter).

In conclusion compare the obtained amount of leucocytes in the examined blood with the norm.

Lab. work 1.11. Definition of blood coagulation time by Althousen

There is a number of methods for definition of blood coagulation time the use of which leads to different data. Normal parameters of blood coagulation time when using this method are 5–6 minutes at room temperature.

Purpose of work: to learn technique and to define blood coagulation time by Althousen.

Necessary materials: stop-watch, sterile scarificator, alcohol, cotton wool, iodine, diethyl ether, slide. Object of research — a person.

Course of work

Warm up slide in your hand to the body temperature and put on it 2–3 drops of finger blood by known technique. Pass the scarificator over the blood each half minute till first thread of fibrin reaches for the needle. Hold the glass either on your palm or on gauze. Repeat the procedure with a cold slide.

In conclusion compare the received results with the norm.

Lab. work 1.12. Definition of erythrocyte sedimentation rate (ESR) by T.P. Panchenkov

Blood protected from coagulation with sodium citrate divides at sedimentation into the upper light layer (plasma) and the lower red layer (erythrocytes).

Purpose of work: to learn technique of definition of ESR and to define its value in the examined blood.

Necessary materials: Panchenkov's device, 5% solution of sodium citrate, sterile scarificator, clock glass or other vessel for blending, cotton wool, alcohol, iodine. Object of research — a person.

Course of work

With the Panchenkov's device capillary (Fig. 4) take 5% solution of sodium citrate up to mark P and place it on the clock glass.

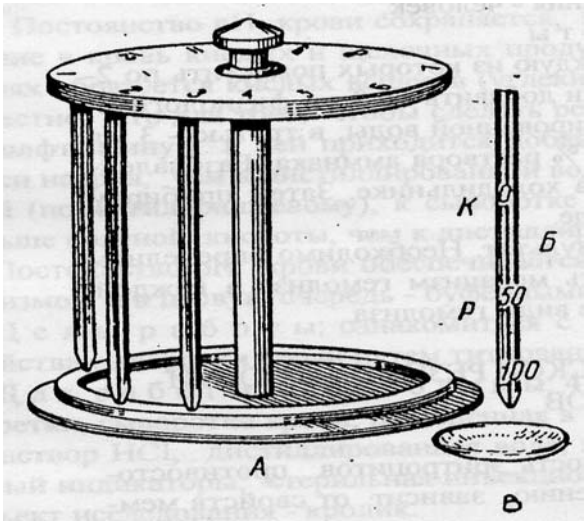

Fig. 4. Panchenkov's device for ESR definition
A — general view, B — capillary, C — clock glass.

Pierce the finger, immerse the tip into the drop of blood and take blood up to mark K. Blow out the contents on the clock glass into the solution of sodium citrate. Instantly take blood for the second time up to mark K and blow this portion out on the clock glass again. Rapidly mix blood on the clock glass, take the mixture into the capillary up to mark 0 (K) and close the top end of the capillary with finger so that its contents did not leak. Place firmly the lower end of the capillary into the lower rubber strip of the Panchenkov's device and then insert the upper end into above rubber strip.

At laboratory work in order to learn the definition of ESR it is possible to use donor's blood which already contains anticoagulant. In this case it is possible to take blood into the capillary up to mark 0 (K) at once and close the top end of the capillary with finger so that its contents did not leak. Place firmly the lower end of the capillary into the lower rubber strip of the Panchenkov's device and then insert the upper end into above rubber strip.

Note the time and in one hour sharply measure height of the column of plasma. Write down the ESR value in mm/hour.

Compare the received parameters of ESR with norm.

Lab. work 1.13. Examination of various kinds of hemolysis

Destruction of erythrocytes with release of hemoglobin into the blood plasma is called hemolysis. Hemolyzed blood is transparent and has specific red color («varnish blood»).

Purpose of work: to learn technique and study various kinds of hemolysis.

Necessary materials: stand with five tubes, pipettes, saline solution, distilled water, 5% solution of ammonia, 0,1% solution of HCI, citrated blood, cotton wool, alcohol, iodine, diethyl ether, sterile scarificator. Object of research — a person.

Course of work

Put 5 tubes into stand, each containing 2–3 drops of blood. In the first and the fifth tubes add 3 ml of saline solution, in the second — 3 ml of the distilled water, in third — 3 ml of 0,1% of HCl solution, in the fourth — 3 ml of 5% solution of ammonia. Freeze soluted blood in the fifth tube in a fridge. Then take the tube out and warm contents up in hot water.

Examine all tubes, compare results. It is necessary to define presence or absence of hemolysis. Describe mechanism of hemolysis in each tube.

In conclusion note observed kinds of hemolysis.

Lab. work 1.14. Examination of osmotic resistance of erythrocytes

The osmotic resistance of erythrocytes is an ability of erythrocytes to resist the decreased osmotic pressure; it depends on the properties of erythrocytes membrane.

Purpose of work: to learn technique and to define limits of osmotic resistance of erythrocytes.

Necessary materials: stand with 8 tubes, solutions of NaCl of descending concentration from 0,85 up to 0,3%, donor's blood, pipettes, cotton wool, alcohol, iodine, diethyl ether, sterile scarificator. Object of research — a person.

Course of work

Consecutively number the tubes and place them into stand. With the pipette pour in 3 ml of solution of NaCl into the tubes as described:

1 — tube — 0,85%
2 — tube — 0,70%
3 — tube — 0,60%
4 — tube — 0,50%
5 — tube — 0,45%
6 — tube — 0,40%
7 — tube — 0,35%
8 — tube — 0,30%

Then add 3–4 drops of blood with pipette into each tube. Check for results in 5 minutes — presence or absence of hemolysis.

Write down in which of the tubes and at what concentration of NaCl solution first signs of hemolysis are marked (blood becomes clear), at what concentration blood is fully hemolyzed.

Define the upper and the lower limits of the erythrocytes resistance and in conclusion compare the data to the norm.

CONSTANTS OF BLOOD SYSTEM

Amount of blood: in adults	4,5–6,l or 6–8% of body weight
in newborns	15% of body weight
Hematocrit (m)	0,44–0,46
(f)	0,41–0,43
Blood: deposited	45–50%
circulating	50–55%
Volume of blood plasma	approx. 3 l

Structure of blood plasma:	
Water	90–92%
Solid residual	8–10%
General protein	65–80 g/l
Albumins	45 g/l
Globulins	20–35 g/l
Fibrinogen	3 g/l
Residual nitrogen	14,3–28,5 millimole/l
Glucose (whole blood)	3,30–5,55 millimole/l
(plasma)	3,88–6,10 millimole/l
Triglycerides	0,40–1,81 millimole/l
Inorganic substances	0,9 %
Osmotic pressure	7,6–8,1 atm
Oncotic pressure	0,03–0,04 atm
Viscosity of blood in adults	5
in newborns	10,0–14,8
Relative density: in adults	1,050–1,060
in newborns	1,060–1,080
pH of arterial bloods	7,40
venous	7,35
pH borders compatible with life	7,0–7,8
Amount of erythrocytes: (m)	$4,5–5,0 \times 10^{12}$ /l (tera per litre)
(f)	$3,8—4,5 \times 10^{12}$ /l (tera per litre)
Erythrocytes: diameter thickness volume	7,2—7,7 microns 2,2 microns 76—96 micrometer^3 (femtoliter)
General surface of all erythrocytes	3800 m^2
Structure of erythrocytes:	
water	60%
dry residual	of 40% (of it 90% Hb)
lifetime of erythrocyte	120–130 days
Amount of hemoglobin (m)	130–160 g/l
(f)	115–145 g/l
Types of Hb:	HbP appears in 7–12 weeks of antenatal period
	HbF — in 9 week of antenatal period
	HbA — before birth and in adults
Contents of Hb in erythrocyte	27–31 picogram
Concentration Hb in erythrocyte	30–38%

Color index: adults	0,8–1,0
newborns	0,9–1,3
Osmotic resistance of erythrocytes: Min	0,46–0,48% solution of NaCI
Max	0,32–0,34% solution of NaCI
Erythrocyte sedimentation rate (m)	1–10 mm / hr
(f)	2–15 mm / hr
Neonatal	1–2 mm / hr
Leucocytes: amount in adults	4–9 $\times 10^9$ /l (giga per litre)
in newborns	15–20 $\times 10^9$ /l (giga per litre)
The leukocytic formula (%): Neutrophils:	50–70%
Myelocytes	0
Metamyelocytes	0–1
stab neutrophil	1–5
segmentonuclear	45–70
Eosinocytes	1–5
Basophils	0–1
Lymphocytes	20–40
Monocytes	2–10
Index of regeneration (shift to the left)	0,05–0,1
Amount of thrombocytes	180–320 $\times 10^9$/l (giga per litre)
Lifetime of thrombocyte	5–11 days
Blood coagulation time (by Lee-White)	5–7 min
Lymph:	
pH	7,35–9,0
Relative density	1,012–1,023
Proteins	20 g/l

2. PHYSIOLOGY OF HEART

Lab. work 2.1. Electrocardiography

Electrocardiography — method of registration of electrical phenomena which arise in the heart during the cardiac cycle. The electric potential generated by the cardiac muscle may be register from the body surface. Electrocardiogram (ECG) usually has three upward-directed positive waves P, R, T, and two downward-directed negative waves Q, S. It has five intervals:

P-Q, QRS, S-T, Q-T, R-R. P wave — atrial complex of ECG; it is the algebraic sum of the potentials arising in the right and left atriums at their excitation. QRST — ventricular potentials, they reflect processes of the excitation of ventricles (Fig. 5). State of heart is judged upon the voltage of waves (it is measured from the isoline to top) and duration of intervals.

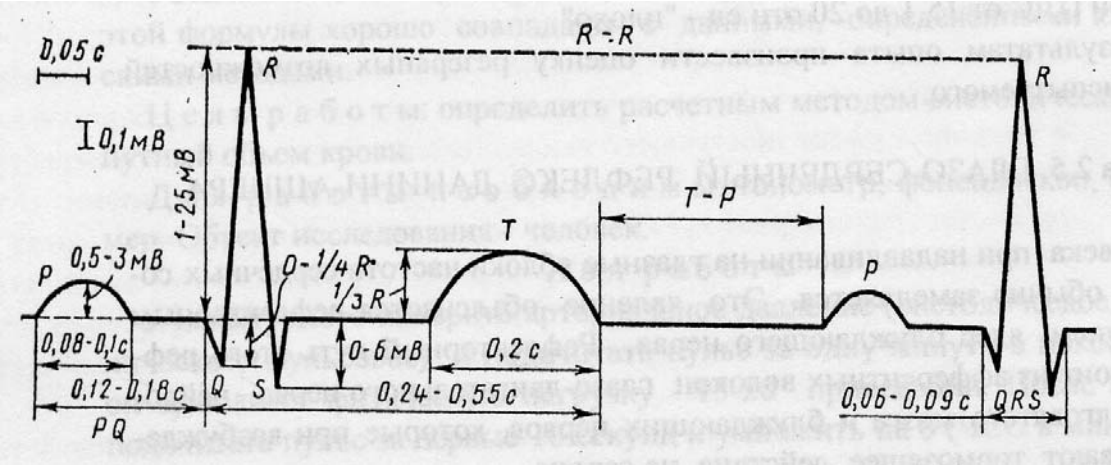

Fig. 5. Scheme of electrocardiogram (explained in the text).

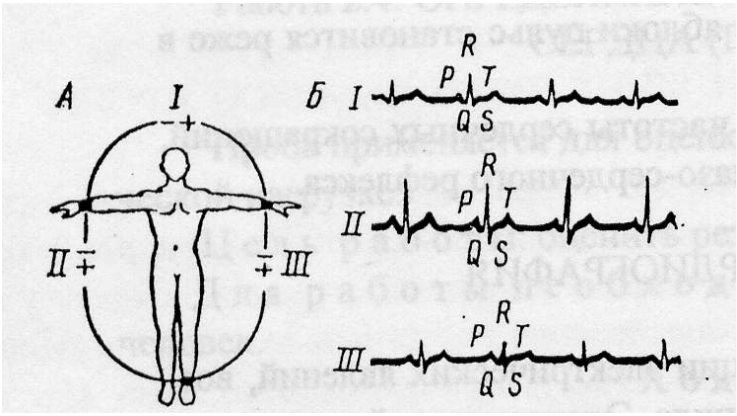

Fig. 6. Scheme of electrocardiography leads and curves features received.
A — standard leads, B — recording of ECG in 3 standard leads.

At electrocardiography the method of bipolar and unipolar leads is used. Perform electrocardiography, three standard bipolar leads are applied with which potential difference between extremities is recorded:

I — right hand — left hand; II — right hand — left leg; III — left hand — left leg. (Fig. 6).

Purpose of work: to learn technique, to record and interpret the ECG.

Necessary material: electrocardiograph, electrodes, electrode paste or 1,0% solution of NaCl, gauze or filter paper, glue, alcohol-ether. Object of research — a person.

Course of work

A. Application of electrodes. The patient lays on a couch. Treat electrodes and spots of body where electrodes will be applied with alcohol. Oil electrodes with the current-conducting pasta or moisten filter paper with 1,0% solution of NaCl. Apply electrodes according to the scheme: red — right hand, yellow — the left hand, green — left leg, black — indifferent, applied on the right leg.

B. Record ECG with all standard leads and attach it into the copy-book.

1. Note waves and intervals as appropriate.

2. Define HR and heart rhythm — by the duration of R-R intervals. Compare with the norm.

3. Measure voltage of P, Q, R, S, T waves.

4. Measure duration of waves and intervals (considering 1 mm = 0,04 sec, proceeding from the speed of tape — 25 mm/sec).

On the basis of ECG analysis characterize in conclusion the functional state of heart of the person.

Lab. work 2.2. Definition of cardiac cycle duration in person by pulse

The duration of cardiac cycle depends on the heart rate. Also, arrhythmia of cardiac activity connected with phases of respiration (respiratory arrhythmia) which can be caused by fractional calculation of pulse (every 5 sec) with subsequent calculation of duration of cardiac cycle for every 5 sec of the calculation.

Purpose of work to learn technique and to define the duration of cardiac cycle by pulse.

Necessary material stop-watch. Object of research — a person.

Course of work

Define pulsing of the radial artery, count heart rate (HR) for 5 sec some times within 3 minutes. Divide 5 by each HR value to calculate duration of one cardiac cycle. Define average duration of cardiac cycle in every 5 sec of the analysis.

Then count HR within 1 minutes. Divide 60 by HR value and determine the average duration of cycle.

Note if there is a difference in duration of cardiac cycle at different methods of analysis. In conclusion specify an advantage and disadvantage of both methods.

Lab. work 2.3. Auscultation of cardiac tones

Cardiac tones relate to external parameters of work of heart. When auscultating heart with stethoscope, two tones of different height and duration are heard. There are the first (systolic) and the second (diastolic) tones. Systolic tone

is dull, long and low, diastolic — short, high and clear. Systolic tone is caused by the contraction of ventricles, simultaneous closure of atrioventricular valves, strain of muscles of ventricles, papillary muscles and vibration of tendinous strands. The second tone depends exclusively on closure of semilunar valves of aorta and pulmonary artery. Both tones are auscultated on the surface of thorax in heart region, but best of all — at projections of heart valves.

To auscultate the tones, place stethoscope on the thorax in places where sounds of separate valves are conducted, then each tone is heard more clearly than another one. The place of mitral valve projection lays below the attachment of the third left costal cartilage to the thorax, it is best auscultated on the left in the fifth intercostal space, 1,0–1,5 cm towards inside from mammary line. The place of projection of the tricuspid valve is on medium line of the thorax, lower than place of the fourth costal cartilages are attached to the thorax; its tone is best auscultated in the region of the lower third of the thorax on the right. The semilunar valve of the pulmonary artery is projected in the second intercostal space but only on the right from the breast bone.

Purpose of work: to learn technique of auscultation and differentiate cardiac tone.

Necessary material phonendoscope. Object of research — a person.

Course of work

Systolic tone is best heard in the fifth intercostal space, one finger left from the nipple. Semilunar valves are projected on the right at the level of the second intercostal space at breast bone — the second sound is heard here. Hold phonendoscope without touching its membrane with finger to prevent side murmurs.

Define two separate tones and characterize them.

Lab. work 2.4. Definition of systolic and minute volumes of blood

With each contraction, heart outputs into arteries a certain amount of blood which is called systolic volume. The amount of blood output by the ventricle within a minute is called minute volume of blood. To define systolic and minute volumes of blood of the examined, one should know his diastolic (DP), pulse pressure (PP), heart rate and age. Starr formula is commonly applied to define systolic and minute volumes of blood:

$$SV = 101 + 0,5 \times PP – 0,6 \times DP – 0,6 \times A,$$

where: SV — systolic volume (ml);

PP — pulse pressure;

DP — diastolic pressure;

A — age of the examinee (years).

Purpose of work: to calculate systolic and minute volume of blood.

Necessary material: tonometer, phonendoscope, stop-watch. Object of research — a person.

Course of work

Measure arterial pressure (systolic, diastolic, pulse) and heart rate in a person for one minute at rest. Then the person does exercises — 15–20 curtseys. After the exercises, count pulse for the first 10 seconds and multiply by 6 (HR per minute). Measure systolic, diastolic and pulse pressure.

Calculate size of systolic volume by Starr formula.

Calculate minute volume of blood (MVB) at rest and after exertion by the formula:

$$MVB\ (ml/min) = SV \times HR$$

In conclusion compare the obtained size of SV and MVB with the norm, note changes SV and MVB after exercises.

Lab. work 2.5. Definition of reserve resources of hearts (Roufier's test)

Test is used to define the working capacity of heart at exertion.

Purpose of work: to define reserve resources of heart.

Necessary material stop-watch. Object of research — a person.

Course of work

Count pulse of a person for 15 sec at rest (P1), then let him do 30 curtseys for 1 minutes and count pulse for the first and last 15 sec (P_2, P_3) during the first minute after exercises. Calculate cardiac activity (PCA) with the formula:

$$PCA = \frac{4 \times (P1 + P2 + P3) - 200}{10}$$

Result of test:

At PCA from 0,1 up to 5 — excellent.

At PCA from 5,1 up to 10 — good.

At PCA from 10,1 up to 15 — satisfactory.

At PCA from 15,1 up to 20 — bad.

Evaluate reserve resources of heart of the person upon these results.

Lab. work 2.6. Danini-Achner's ocular-cardiac reflex

Heart rate (HR) frequency of a person is usually slowed down when pressing his eyeball. This phenomenon is explained by reflex excitation of the vagus nerve nuclei. The reflex path of this reflex consists of afferent fibers of third cranial nerve, neurons of medulla and vagus nerves which when excited inhibit heart work.

Purpose of work: to evaluate change of cardiac activity influenced by vagus nerve.

Necessary material stop-watch. Object of research — a person.

Course of work

Define HR of the person. Press his closed eyes for 10–20 sec (so that not to cause unpleasant sensations or pains) with your thumbs. Simultaneously, count his pulse rate. Then count pulse rate again 20 sec having stopped pressure.

Usually, HR decreases in 10 contraction per minute when eyeballs are pressed.

In conclusion characterize change of HR, list parts of reflex path of the oculocardiac reflex.

3. PHYSIOLOGY OF VASCULAR SYSTEM

Lab. work 3.1. Research of arterial pulse properties by palpation

Arterial pulse is easily palpated on the arteries located superficially, in practice — more often on the radial one.

Purpose of work: to learn the technique and evaluate properties of pulse by means of palpation.

Necessary material stop-watch. Object of research — a person.

Course of work

Grab the wrist of the surveyed person with your right hand in region of radiocarpal articulation so that your thumb were on the outside of the forearm, and anothers — on the inside. Having determined the radial artery, press it slightly with three fingers till pulse appear. Assess pulse by the following parameters:

1. HEART RATE: slow, rapid, normal. Count pulse within 1 minute (or within 10 seconds and multiply it by 6). In children at rest pulse is more rapid. In newborns on the average pulse is 140 beats per minute, influenced only by sympathetic nerve. In sportsmen pulse at rest is slower due to predominant influence of vagus nerve and increase of systolic volume of blood.

2. RHYTHM: rhythmic, arrhythmic. It is determined by the duration of the interval between the pulse beats or R-R interval on the ECG. Respiration (respiratory arrhythmia) influences the rhythm. At inspiration the pulse raises, at expiration it slows down.

3. FILLING (height): good, satisfactory, weak, thready pulse. Filling depends on systolic volume and volumetric rate of blood-flow in diastole, elasticity of walls of vessels. It is determined by the height the arterial wall rises, and palpated volume of the artery under the fingers during systole.

4. RATE: normal, rapid, slow pulse. It is determined by the rate of ascending and descending of the arterial wall. Rapid pulse can reflect failure of the aortal valve. The enlarged amount of blood is pumped out, part of blood returns into ventricle. Slow pulse can be observed at narrowing of the aortal opening when blood comes slower to the aorta.

5. STRAIN: moderate, firm, mild pulse. It is determined by squeezing of an artery till the pulse disappears under the fingers located next to the pressed spot.

Put the results of checkup into the table.

Properties of the pulse	Norm	Deviations	Result
Heart rate:	normal (60–80)	slow, rapid	
Rhythm:	rhythmic	arrhythmic	
Filling (height)	good	satisfactory, weak, thready pulse	
Strain	moderate	firm, mild pulse	
Rate	normal	rapid, slow pulse	

Lab. work 3.2. Measuring of arterial pressure in person

Arterial pressure changes depending on the phases of the cardiac cycle. In systole period it is increased (systolic, or maximal), in diastole period it is decreased (diastolic, or minimal). The difference between the value of systolic and diastolic pressure is called pulse pressure. There are different of methods of taking arterial pressure: auscultation (N.S. Korotkov's method), palpation (Riva-Roche's method) and with various devices.

A. Palpation method of Riva-Roche's

Purpose of work: to learn technique of definition of arterial pressure by *Riva-Roche's* method.

Necessary material tonometer. Object of research — a person.

Course of work

The method allows to define only the maximal (systolic) pressure. Place the cuff on the arm above the elbow, hand of the examined is on table in comfortable position. Pressure is created in the cuff till pulsing in the radial artery is not palpated. Decreasing pressure in the cuff, note indications of the manometer at moment when pulse appears. These indications correspond to maximal (systolic) pressure in radial artery.

B.N S. Korotkov's auscultation method.

Purpose of work: to learn technique of definition of arterial pressure by N.S. Korotkov's and to define its value in the individual.

Necessary material: tonometer, phonendoscope. Object of research — a person.

Course of work

With this method it is possible to measure both systolic and diastolic pressure. An individual is sitting on a chair, his relaxed hand on the table. Put the cuff onto the naked arm so that it were overlapped tightly but do not squeeze tissues. The lower edge of the cuff should be placed 1,5 cm higher of the elbow. In the antecubital fossa define the pulsating brachial artery and place the head of the phonendoscope above it (Fig. 7).

Pumping in the cuff, create a pressure in it exceeding the expected systolic in 20–25 mm Hg. Letting the air out, hear the tones in the phonendoscope (Korotkov’s tones) in the brachial artery. The moment tones appear corresponds to systolic pressure. Continue reducing the pressure in the cuff and hear the increasing tones which then weaken and disappear. The moment they disappear corresponds to diastolic pressure.

Make a comparative analysis of the results of taking the arterial pressure by the described methods and compare them with norms.

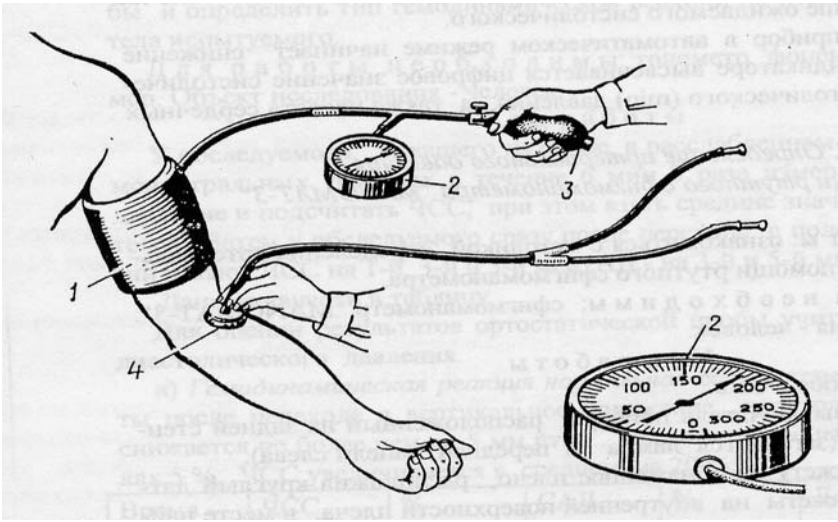

Fig. 7. Taking arterial pressure in the person by Korotkov's auscultation method: 1 — rubber cuff, 2 — tonometer, 3 — bulb, 4 — phonendoscope.

Lab. work 3.3. Orthostatic test

The test allows to characterize the functional appropriateness of the reflex mechanisms of hemodynamics regulation and evaluation of excitability of sympathetic innervation centers.

Purpose of work: to learn technique taking orthostatic test and to define the type of hemodynamic reactions to change of body posture of an individual.

Necessary material: tonometer, phonendoscope, stop-watch. Object of research — a person.

Course of work

The individual lays relaxed on his back in thermoneutral environment for 6 minutes. Measure his arterial pressure 3 times and calculate heart rate (HR), take average values of these parameters. After the individual has stood up, calculate immediately his HR during the 1st, 3rd and the 5th minutes, arterial pressure — during the 3rd and the 5th minutes.

Put the data into the table.

To evaluate the results of the orthostatic test, changes of diastolic pressure (DP) should be considered.

a) *Hemodynamic reaction is normal* if the first minute after the individual has stood up his diastolic pressure is decreased in no more than 5 mm Hg, systolic pressure (SP) changes within 5%, HR increases on the average in 20%.

b) *The hyperdiastolic type of reaction* — diastolic pressure increases in more than 5 mm hg, systolic decreases even bigger value, pulse pressure decreases significantly, HR increases more than in 20% (reaction is caused by the substantial increase of the tonus of sympathetic nervous system).

c*) Hypodynamic type of reaction* – both systolic and diastolic pressure decrease, pulse pressure decreases, HR practically does not change (due to the decreased tonus of sympathetic nervous system).

Time	Heat rate	% deviation	SP mm Hg	% deviation	DP mm Hg	% deviation
lying position (average)						
vertical position						
1st min						
3d min						
5th min						

By results of research define the type of hemodynamic reactions of the individual to the orthostatic test.

Lab. work 3.4. Functional test for reactivity of cardiovascular system

Synchronic registration of separate parameters of the cardiovascular system activity at change of the body posture and under the influence of the dosed exertion allows to estimate its reactivity that has the certain diagnostic value.

Purpose of work: with the help of functional test, define the reactivity of the cardiovascular system to the changes of body posture and exercises.

Necessary material: phonendoscope, sphygmomanometer, couch, stopwatch. Object of research — a person.

Course of work

At least 4 person participate in the test. Prepare the table. The individual sits on a couch. One of assistants measures his arterial pressure, another fills up the table, the third counts the pulse rate and puts it into the table too. The arterial pressure is taken simultaneously with the definition of HR. Measurement is carried out for some times till 2 identical (close) values of the arterial pressure and pulse are not received. Then the individual stands up and is measured pressure for several times successively with saying outloud the found parameters of the monometer. Simultaneously, each 15 sec data of pulse rates are said. Measuring proceeds till parameters do not return to the initial values.

Similar observation is made after exercises (20 curtseys).

Received data to be put into table.

Parameters	Rest	Stand up			After the work is completed in		
		1 min	2 min	3 min	1 min	2 min	3 min
HR							
SP							
DP							

In the adult healthy person hemodynamic parameters (pulse rate, arterial pressure) normalize within 3 minutes after the exercise is done.

Estimate the reactivity of cardiovascular system of the individual.

Lab. work 3.5. Influence of exercises on cardiovascular system

Functionalities of the cardiovascular system of a person can be defined with the help of special dosed loads. One of such functional tests is S.P. Letunova's test. It consist of three consecutive exercises — 20 curtseys within 30 seconds,

in 3 minutes — running with the maximal speed for 15 seconds, and in 4 minutes — 3 minutes running on one place with the rate of 180 steps per minute. Test allows to characterize completely the state of cardiovascular system since the high-speed load (15-seconds run) and the load for exercise tolerance (3-minutes run) assert different demands to the organism. Reaction of the organism to the load is defined by the changes of the pulse rate and values of arterial pressure.

Purpose of work: to learn technique of S.P. Letunov's test and investigate functional state of cardiovascular system in the individual.

Necessary material: tonometer, phonendoscope, stop-watch, metronome. Object of research — a person.

Course of work

In state of rest the individual sitting on a chair is counted HR and measured the arterial pressure. Then the individual should make 20 deep curtseys for 30 sec with his hands stretched forward. Do not remove the cuff of the tonometer. Once the exercise is done, the individual sits on the chair and is measured pulse and the arterial pressure every minute for 5 minutes. Run test is performed in similar way.

The dynamics of change of arterial pressure and pulse reveals the physical training of the individual. With good functional state of cardiovascular system after load HR increases in 50–70% from the initial level and systolic pressure in 20–40 mm Hg. Recovery of initial parameters comes to the end in 1–3 minutes.

Received data to be put into the table.

Parameters	At rest	Period of rehabilitation														
		After 20 curtseys					After 15-second of running					After 3-minute of running				
		Minutes					Minutes					Minutes				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Heart rate																
Systolic pressure																
Diastolic pressure																
Pulse pressure																

To draw a conclusion on functional state of cardiovascular system at the individual.

CONSTANTS OF SYSTEM CIRCULATION

Heart rate:	
at adults	60–80 in minutes
at newborn	135–140 in minutes
Systolic volume of blood	65–70 ml
Minute volume of blood:	
at rest	4,5–5 l
At exercise stress up	to 30 l
Duration of cardiac cycle	0.75–1,0 sec

Arterial pressure:	
Max (systolic)	110–125 mm Hg
Min (diastolic	60–85 mm Hg
At newborn:	
Max	50–60 mm Hg
Min	34–40 mm Hg
Pressure in capillaries	30–10 mm Hg
Average rate of blood flow:	
large arteries of	0,5 m/s
Veins of average calibre	60–140 mm / sec
Veins cava	200 mm / sec
The capillary	0,5–1,0 mm / sec
Time complete circulation of bloods	20–23 sec
Coronary circulation	200–250 ml / minutes
At an exercise stress	up to 3–4 l/minutes

4. PHYSIOLOGY OF RESPIRATION

Lab. work 4.1. Pneumotachometry

Pneumotachometry — method of definition of the maximal volumetric rate of air stream at the forced expiration and inspiration with the help of pneumotachometer. The pneumotachometer consist of differential monometer and a sensor which represents a tube with a narrowing inside. With the air passing in the tube, the difference of pressure is created in both sides of narrowing. The more is the flow rate, the higher is the difference. The monometer catches this difference of pressure and transforms it into the indications of volumetric rate in l/sec.

Purpose of work: to learn the technique of pneumotachometry and define the maximal flow rate of air at inspiration and expiration.

Necessary material: pneumotachometer, alcohol, cotton wool. Object of research — a person.

Course of work

The test is conducted with the individual standing. Wipe the mouthpiece of the tube with alcohol. To measure the expiratory rate, it is necessary to breathe out into the end of pneumotachometer tube marked «expiration». Having breathed fully in, the individual breathes out as fast as possible into the mouthpiece. To define inspiratory rate, invert the tube into the «inspiration» position and after complete expiration the individual makes the forced inspiration through the tube of pneumotachometer.

The maximal volumetric rate expiratory and inspiration is defined by the maximal parameters of the pneumotachometer.

The maximal volumetric expiratory rate in adults is 4–8 l/sec.

Due volumetric expiratory rate (DVER) is defined by the formula:

$$DVER = Vital\ capacity\ of\ lung \times 1{,}25\ (l/s)$$

Deviation from the estimated value in 15% is permitted. The size of the volumetric inspiratory rate is smaller but is not lower than 3 l/sec.

The pneumotachometry is valuable for revealing infringement of transit of air through the bronchi. At the marked infringement of transit of air through the bronchi the expiratory rate is lower than 1,5 l/sec.

In results note the maximal volumetric expiratory and inspiratory rate, due volumetric expiratory rate.

In conclusion compare the received data with the norm.

Lab. work 4.2. Spirometry

Spirometry — method of definition of vital capacity of lung and its composing volumes.

Purpose of work: to learn the technique and define vital capacity of lungs (VCL) and its composing volumes.

Necessary material: water or dry spirometer, nasal clamp, alcohol, cotton wool. Object of research — a person.

Course of work

Wipe the mouthpiece of the spirometer with cotton wool moistened with alcohol. The individual should make the maximal inspiration while standing and breathe it out in maximum into the spirometer (Fig. 8), define the VCL by the scale of the spirometer. Measure VCL for several times and calculate the average.

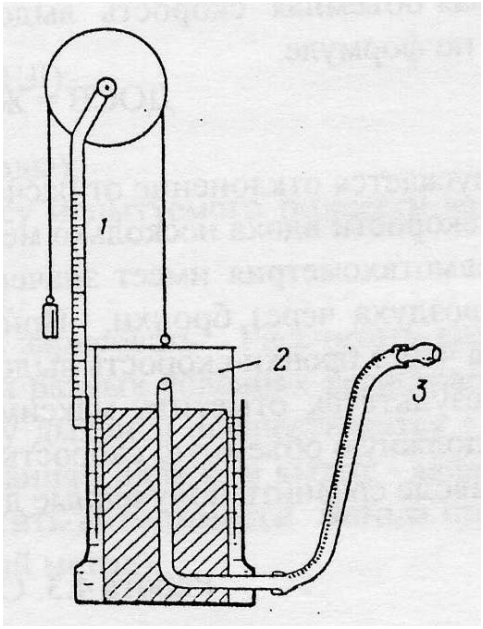

Fig. 8. Scheme of the spirometer
1 — scale, 2 — cupola of the spirometer, 3 — mouthpiece.

Define the VCL of the individual placed horizontally, and also upon exercise (20 curtseys).

Breathe out several times (5–7) into the spirometer as usual (breath in the open air). Define respiratory volume of air by dividing indications of the spirometer by the number of expirations into the spirometer.

To define the reserve volume of the expiration, make forced breathe out into the spirometer after you have made usual breathe out. Repeat measuring several times and calculate the average.

To measure reserve volume of inspiration, collect into the water spirometer 5 l of air (lift the cupola with hand) and make the maximal inspiration from the spirometer. The difference between the initial volume of air in the spirometer and the one left after deep inspiration have been made corresponds to reserve volume of inspiration. To calculate with another — calculation way — the reserve volume of inspiration, subtract the sum of respiratory volume and reserve volume of expiration from VCL.

Use indirect methods to define the residual volume of air. In norm the residual volume is 25–30% from the VCL amount.

By the nomogram (Fig. 9) calculate the necessary VCL (DVCL), in conclusion compare it with the obtained parameters of VCL (standing, lying, after exercise).

Votchel's test. In norm the difference between sizes of VCL measured at usual rate of expiration and at the maximum fast expiration does not exceed 300 ml. Increase of this difference signifies the narrowing (obstruction) of small bronchi.

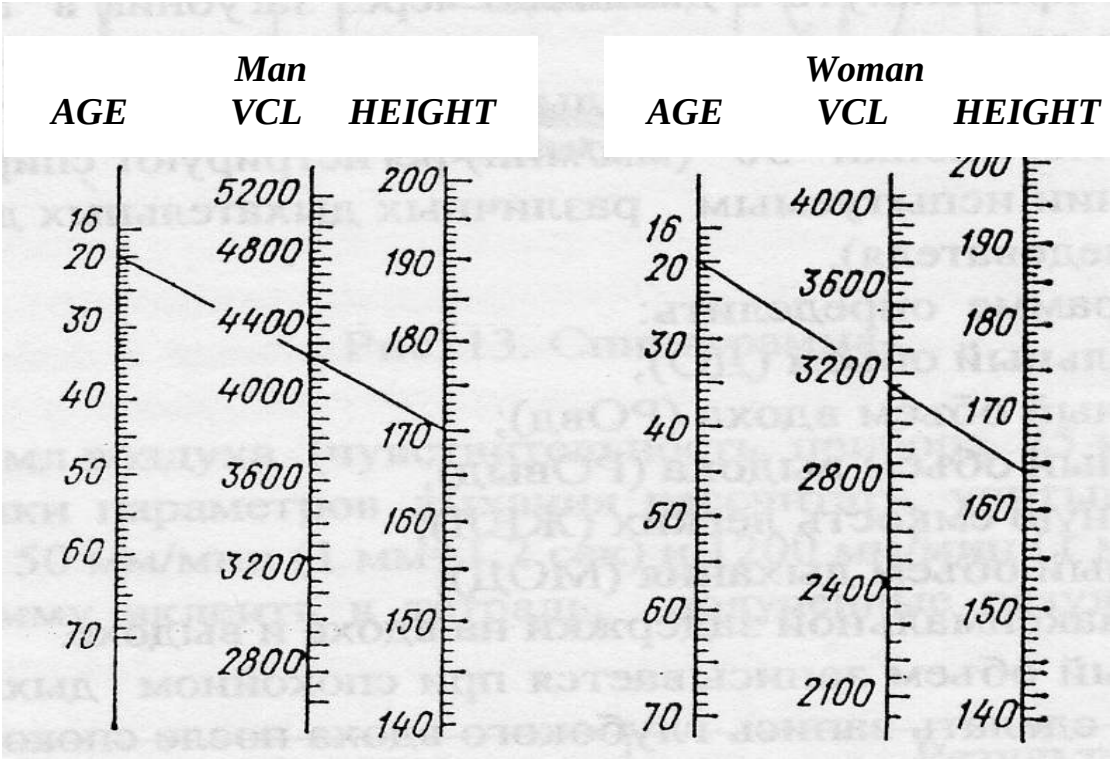

Fig. 9. Nomogram for definition of due size of the VCL

Write down VCL, FVCL, DVCL, parameter of Votchel's test and compare results with norm.

Lab. work 4.3. Spirography

It is possible to define in detail and more precisely all capacities and volumes of lung with the help of a spirograph.

Purpose of work: to learn technique of graphic registration of external respiration parameters.

Necessary material: spirograph, disinfected nasal clamp and mouth-piece. Object of research — a person.

Course of work

Prepare the device for work. Place the mast in the position suitable for the individual. Attach the device to the individual through the mouth-piece. Apply nasal clamp. Let the individual to get used to breathe through the mouth-piece.

Register spirogram (Fig. 10) at tape speed 50 mm/min. Individual is to make various respiratory motions by the request of the assistant.

Define on the spirogram:

a) Respiratory volume (RV);

b) Reserve volume of inspiration (RVI);

c) Reserve volume of expiration (RVE);

d) Vital capacity of lung (VCL);

e) Minute volume of respiration (MVR);

f) Time of the maximal breath-holding for inspiration and expiration.

The respiratory volume is registered at quiet breathing. To define RVI, make record of deep inspiration after quiet inspiration. To define RVE, make record of deep expiration after quiet expiration.

To define VCL, the individual should breathe deeply in, and then deeply breathe out. Make record of the maximal breath-holding of inspiration-expiration pause.

After finishing to record pulmonary volumes, record the spirogram at tape speed of 1200 mm/min and calculate the following parameters:

1) Duration of quiet inspiration and expiration (*t* of inspiration and *t* of expiration); by the received data considering RV calculate minute volume of respiration (MVR).

2) Volume of forced expiration (VFE) (measure the volume of deep and fast expiration for 1 sec).

3) Maximal ventilation of lungs MVL (respiration with the maximal frequency and amplitude, write down 3–4 cycles).

Calculate all lung volumes, based on that 1 mm of record (in height) corresponds to 40 ml of air. Calculate time characteristics of parameters of respiration, considering tape speed of 50 mm/min (1 mm=1,2 sec) and 1200 mm/min (1 mm=0,05 sec).

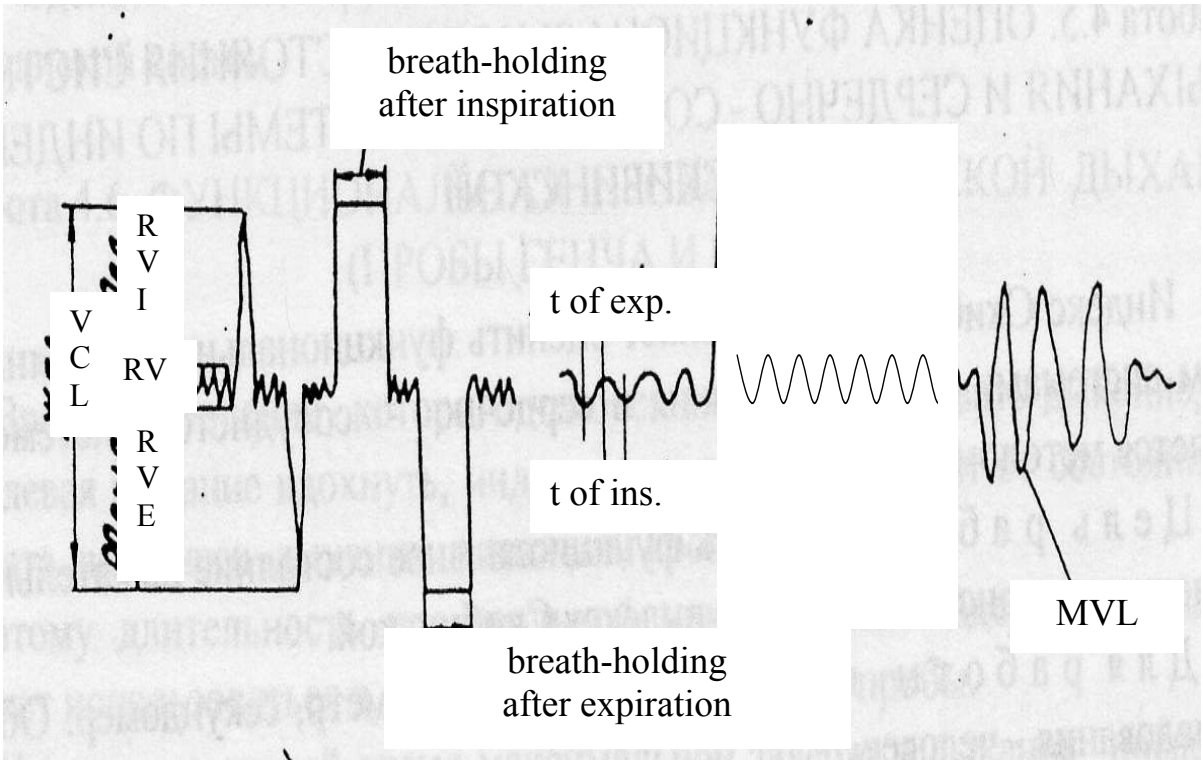

Fig. 10. Spirogram

Put down the obtained results into the table.

Recorded parameters	Result
Respiratory volume, l	
Reserve volume of inspiration, l	
Reserve volume of expiration, l	
Vital capacity of lung, l	
Minute volume of respiration, l/min	
Volume of forced expiration l/sec	
Maximal ventilation of lungs, l/min	
Time of quiet inspiration, sec	
Time of quiet expiration, sec	

Draw conclusion on the state of external respiration of the individual.

Lab. work 4.4. Evaluation of functional state of respiratory system and cardiovascular system by Skibinskaya's index

Skibinskaya's index allows to estimate functional state of the organism by the two systems: system of respiration and cardiovascular system. It is the method of self-control.

Purpose of work: to estimate functional state of respiratory and cardiovascular systems by Skibinskaya's index.

Necessary material: the spirometer, stop-watch. Object of research — a person.

Course of work

First, define pulse and respiration rate. Then define VCL by making 2–3 measurings and put down the largest result with the help of the spirometer. After 5–10 minutes' rest measure respiration rate for one minute, make deep breathe in and out, then deep breathe in and hold it for 1 time (measure in seconds).

Skibinskaya's index is calculate with the formula:

$$SI = \frac{VCL \times DBH(\text{sec})}{100 \times HR},$$

where: VCL — vital capacity of lungs;
DBH — duration of breath-holding;
HR — heart rate.

Trained people's respiration after its breath-holding should not become frequent, since the appeared oxygen deficiency is compensated due deepening of breathing.

Using the obtained VCL data, pulse rates and time of breath-holding calculate Skibinsk's index with the formula and compare it with the data of table.

Size of Skibinsk's index	Result
5	very bad
5–10	unsatisfactorily
10–30	satisfactory
30–60	good
60 and more	excellent

Make a conclusion on the functional state of respiratory and cardiovascular systems.

Lab. work 4.5. Functional with breath-holding (Genche's and Stange's tests)

Breath-holding time is individual in each person. It depends on state of the external respiration apparatus and system of blood circulation. So, the duration of voluntary maximal breath-holding can be used as functional test.

In healthy people maximal breath-holding time after quiet inspiration is 50–60 sec, after quiet expiration it is less than 30–40 sec. These indications change at forced ventilation.

Purpose of work: to define time of breath-holding and factors influencing the time of breath-holding.

Necessary material: stop-watch. Object of research — a person.

Course of work

Define the maximal breath-holding time at inspiration (Stange's test) and expiration (Genche's test) while breathing quietly. The individual should breathe quietly for 3–4 minutes, then, after usual expiration breathe in or breathe out deeply and hold up breathing as long as possible. Using stop-watch, define time from the moment of breath-holding till the moment it re-starts. In both cases to define the time of maximal breath-holding, use the data of 3 attempts and take simple average.

To define time of the maximal breath-holding for inspiration and expiration during voluntary forced breathing (after artificial hyperventilation of lung). For this purpose the individual should breath with the maximal depth (not frequency) for 1–2 minutes and then hold up breath at the maximal inspiration or expiration. Each time to define the size of the maximal breath-holding taking the simple average of 3 attempts.

Analyze the data obtained using the following:

Stange's test (after *deep inspiration*):

< 39 sec — unsatisfactory;

40–49 sec — satisfactory;

> 50 sec — good.

Genche's test (after *deep expiration*):

< 34 sec — unsatisfactory;

35–39 sec — satisfactory;

> 40 sec — good.

Write down the received data in the protocol.

In conclusion compare size of the maximal breath-holding at expiration and inspiration. Also, compare size of the maximal breath-holding at inspiration, after quiet and after forced breathing. Explain the reason for observed differences.

Lab. work 4.6. Definition of physical endurance in person by calculation of cardiorespiratory index

Parameters of the systems of blood circulation and respiration are widely used when testing physical endurance and training level of a person. For example, cardiorespiratory Samko's index (CRSI) is used which unites seven parameters and can be easily performed in a lab.

Purpose of work: using cardiorespiratory index, define the level of physical endurance of the individual.

Necessary material: sphygmomanometer, phonendoscope, alcohol, cotton wool, spirometer, stop-watch. Object of research — a person.

Course of work

Take the arterial pressure in the individual (systolic and diastolic). Then define maximal pressure of expiration. To do this, the individual should take rubber tube of sphygmomanometer into his mouth make a maximal breathe out. With spirometer define vital capacity of lung, and with stop-watch count heart rate for 10 sec and time of the maximal breath-holding. Calculate CRSI with formula:

$$CRIS = \frac{VCL + MPE + MBH + A}{SP + DP + HR},$$

where: VCL — vital capacity of lung (for unit accept 100 ml of volume);
MPE — maximal pressure of expiration, mm Hg;
MBH — maximal breath-holding after quiet inspiration, sec.;
A — age, amount of complete years;
SP — systolic pressure, in mm Hg;
DP — diastolic pressure, mm Hg;
HR — heart rate for 1 minute.

CRSI it is possible define during three phases of physical activity: adynamic, dynamic and recovery phase.

Adynamic phase corresponds to 10-minute rest, dynamic phase — to dosed exercise stress, size about 20 kilojoule (30 curtseys), and recovery phase is defined by time necessary returning of CRSI to initial level.

In well-trained athletes size of CRSI in adynamic phase is 1.000 and higher, in unexercised but practically healthy people — from 0.800 to 0.900, in patients with various cardiovascular and respiratory abnormality size of CRSI is within 0,300–0,400.

Measuring of CRSI made during dynamic phase have shown that in well trained athletes the decrease of size of CRSI up to 5% of the initial size is observed. In unexercised but practically healthy people the decrease of CRSI is 15–30%, and in patients with various cardiovascular and respiratory abnormality — 35–65%.

In recovery phase in unexercised but practically healthy people, recovery of the initial parameters takes 1–3 minutes, in patients with cardiovascular and respiratory abnormality — 10 and more minutes.

Calculate sizes of CRSI in the individual in adynamic, dynamic and recovery phases, draw conclusion on the level of physical endurance.

CONSTANTS OF RESPIRATORY SYSTEM

Respiration rate: in adults	12–18 / minute
in neonatal	40–55 / minute
in baby	30–40 / minute
General respiratory surface of lungs	50–90 m^2
Excursion of thorax: (m)	7–10 cm
(f)	5–8 cm
Negative pressure in pleural cavity: at inspiration	6–8 cm H_2O, or 4–5 mm Hg
at expiration	3–5 cm H_2O, or 2–3 mm Hg
Interrelation of duration inspiration-expiration	1:1.2
Thickness of lungs membrane	0,4–1,5 micrometer
Respiratory volume	0,3–0,9 l
Reserve volume of inspiration	1,5–2,0 l
Reserve volume of expiration	1,0–1,5 l
Vital capacity of lung	3,5–5,0 l
Residual volume	1,0–1,5 l
Functional residual capacity	2,5 l
Capacity of inspiration	2,0 l
Dead space	140–170 ml
Coefficient of lung ventilation	1/7
Minute volume of respiration: at rest	up to 7 l
at physical activity	Up to 120 l/minute
Alveolar ventilation	4,2–5,6 l/minute
Maximal ventilation lung	120–170 l/minute
pO_2 in alveolar air	110 mm Hg
pCO_2 in alveolar air	40 mm Hg
pO_2 in arterial blood	100 mm Hg
pCO_2 in arterial blood	39 mm Hg
pO_2 in venous blood	40 mm Hg
pCO_2 in venous blood	46 mm Hg
Volume of forced expiration	3 l
oxybinding ability of Hb	1,34 ml/g
Oxygen capacity of blood	19 percent by volume
Ventilation-perfusion coefficient	0,8–0,9
Consumption of oxygen: at rest	350 ml/min
at physical activity	under 5000 ml
Coefficient of use O_2: at rest	40%
at physical activity	under 50–60%
Critical limit of hypoxia (pO_2)	30–35 mm Hg in alveolar air

5. PHYSIOLOGY OF DIGESTION AND ABSORPTION

Lab. work 5.1. Examination of gastric juices acidity

The pH parameter reflects the activity of hydrogen ions, the value of the latter influences the hydrolytic action of pepsin.

Purpose of work: to learn the technique of potentiometric titration of gastric juice and define the concentration of H^+ in it.

Necessary material: ionomer, electrodes for pH instrumention, electromagnetic blender with a magnet, beaker, micropipet, distilled water with pH 7,0, automatic pipettes on 1,0 ml, measuring beaker, 0,1, normality solution of NaOH, filter paper, gastric juice.

Course of work

For make titration of the gastric juice, they use a device which consists of pH-meter, the electromagnetic blender, ionselective electrode with a reference electrode.

Bring distilled water to pH 7.0 with the alkali solution and pour 20 ml of water into the glass. Put the magnet into the glass and place it on the blender. Switch the blender on and put the electrodes into the glass carefully. In this case the arrow of the pH-meter shows pH 7,0 on the scale. Adding 2–3 ml of acidic gastric juice into the glass makes the arrow deviate towards lower pH values. The degree of arrow deviation depends on gastric juice acidity.

Collect 0,1 normal solution of NaOH into the micropipet. Carefully put it into the glass till its tip contacts the contents of the glass. Watch the indication of the pH-meter. Slowly add alkali from the micropipet into the titrate solution. In the result of neutralization of the hydrochloric acid of the gastric juice with the alkali solution arrow of the device moves towards higher pH. Once the arrow has reached pH 7,0, stop the addition of the alkali and mark the amount of the alkali needed for titration of the gastric juice in the glass.

Example of calculation. Assume that for titration 1 ml of gastric juice it was taken 0,5 ml of 0,1 n solution of NaOH. Then, for titration of 1000 ml of juice it is necessary 500 ml of the alkali solution. Hence, concentration of H^+ in the gastric juice is:

$$\frac{5000 \times 0{,}1}{1000} = 0{,}05 mi\lim ole/l = 50 mi\lim ole/l$$

Make titration of test of the gastric juice, write down the results into the report.

Lab. work 5.2. Digestion of starch with enzymes of the person's saliva

Chemical treatment of food begins in the oral cavity with the participation of enzymes of saliva: alpha-amylases, dextrinases and maltases. Alpha-amylase splits up starch and glycogen to dextrins. Under the action of dextrinase dextrins split up to maltose. Maltose under the influence of maltase is hydrolyzed to glucose. Optimal conditions for the action of amylolytic enzymes of saliva is the alkalescent medium and temperature 37–38°C.

Purpose of work: examine conditions of action of enzymes of saliva on starch.

Necessary material: thermostat, spirit-lamp, matches, support with tubes, pipettes, saliva of the person, solutions: 1% of cooked starch, 1% of crude starch, Lugol's iodine solutions, Pheling's solutions, 0,5% of HCI), litmus paper, ice, tube carrier, thermometers. Object of research — a person.

Course of work

To make the test, it is necessary about 10 ml of saliva. Saliva is collected by splitting out into the tube through the funnel. Put 1 ml of saliva to each of the 5 numbered tubes. In the 1st tube add 3 ml of 1% solution of the cooked starch. Saliva in the 2nd tube is boiled on the spirit-lamp and, after cooling, add 3 ml of cooked starch (mind safety rules). In the 3rd tube add 0,5% of HCI solution till litmus paper stains, and after that 3 ml of 1% solution of cooked starch.

№ of tubes	Contents of tubes	Color of contents tubes after addition		Test results
		Lugol's iodine solution	Feling's solution	
1.	1 ml of saliva + 3 ml of cooked starch			
2.	1 ml of boiled saliva + 3 ml of cooked starch			
3.	1 ml of saliva + 0,5% solution of HCl + 3 ml of cooked starch			
4.	1 ml of saliva + 3 ml of uncooked starch			
5.	1 ml of saliva + 3 ml of cooked starch + cold			

In the 4th add 3 ml of 1%. solution of crude starch. In the 5th tube, once it's cold, add 3 ml of cooked starch solution. Place tubes 1, 2, 3, 4 for 30 minutes into the bain-marine or thermostat (37–38°C), tube 5 is put into the pot with snow.

In a while, divide the contents of tubes into two equal parts. Check the first group of tubes for starch, the second one — for sugars. For this, add 2–3 drops of Lugol's iodine solution into each of the first group tubes. Intensive staining of the content into in dark blue color testifies presence of starch. Add 1 ml of Feling's solution to the contents of the second group tubes. This solution is prepared by students beforehand. For this, mix Rochelle salt and $CuSO_4$, 10 drops each.

Contents of tubes is heated on the spirit-lamp till boiling. At splitting of starch to glucose the contents of tubes are stained into orange-red color.

Note in what tubes starch has split up to sugars (in full or partially), in what it has remained unchanged. Conclude upon how various factors influence enzymatic properties of saliva.

Lab. work 5.3. Digestion of protein by gastric juice. Role of hydrochloric acid

Gastric juice has some forms of proteoclastic enzymes characterized by various spectrum of pH of their optimal effect that determines high proteoclastic activity in stomach in wide diapason of pH.

Purpose of work: investigate conditions of action of enzymes of gastric juice and role of HCl in this process.

Necessary material: gastric juice, 0,5% solution of HCl, 10% solution of sodium of hydrate natrium, 0,1% solution of cuprous sulphate, baking soda, tubes, support, thermostat, spirit-lamp, tube carrier.

Course of work

Number 4 tubes. Add 2 ml of gastric juice to each 3 tubes, to the 4th–2 ml of HCl. Add baking soda into the 2nd tube till complete neutralization of HCl (stop of bubbling). Boil slowly gastric juice in the 3rd tube (inactivate enzymes). Put small piece of egg-white into all 4 tubes. Place tubes into thermostat at 38°C for 15 minutes. Cool them afterwards. Make biuret reaction (add 4 drops of solution of sodium hydroxide and 2 drops of solution of cuprous sulphate into each tube).

Write down the results of the test into the table:

№ of tubes	Components of tube	Absence or presence of peptones
1.	2 ml gastric juices + egg-white.	
2.	2 ml of gastric juice + baking soda + egg-white	
3.	2 ml of gastric juice. Boil thoroughly + egg-white	
4.	2 ml HCI + egg-white	

In tubes with unsplit protein the contents stains blue-violet, with hydrolysates of protein peptones — pinky-violet.

In conclusion note optimal conditions of proteopepsis by gastric juice and role of HCI in the given process.

Lab. work 5.4. Influence of bile on fats

Bile has an ability to emulsify fats which come in gastrointestinal tract with food. It promotes an increase of interaction of substrate (fat) with the enzyme (lipase) resulting in acceleration of hydrolysis.

Purpose of work: to study emulsifying action of the bile on fats.

Necessary material: fresh bile, vegetable oil, water, pipettes, slide, glass rod, magnification glass or microscope.

Course of work

Place drop of bile on the clean slide and drop of water next to it. To each drop add small amount of vegetable oil. Carefully mix with glass rod. Examine the contents of drops immediately and in 10–15 minutes with the magnifier or microscope.

Describe the obtained distribution of fats, explain different results for drops of bile and water.

Lab. work 5.5. Examination of gastro-intestinal absorbtion and excretion

To examine gastro-intestinal absorption, test of potassium iodide absorption is applied in clinical laboratory diagnostics. This test is based on the ability of salivary glands to excrete iodine. Iodine very quickly appears in saliva after intake. Amount of iodine is determined by potentiometric measurements

Purpose of work: to learn the technique of definition of gastro-intestinal absorption by method of iodometry and to assess the absorbing function in a person.

Necessary material: ionomer, potassium iodide, distilled water, beaker, filter paper, pipettes, electrode membranous. Object of research — a person.

Course of work

The individual rinses oral cavity with distilled water. Take a sample of his saliva in the volume of 0,5–1,0 ml. Further, the individual is given to drink of potassium iodide, the dose of 0,06 mg / kg of body mass dissolved in 5–10 ml of water, and 100 ml of distilled water to drink after. Test a sample of saliva in 10 minutes.

Measuring of iodine concentration in saliva samples is made in microcell. Gastrointestinal absorption is evaluate by the concentration of iodine in saliva (mV) prior to the intake of potassium iodide and after.

Write down the results of investigation into the writing-book and make conclusions.

CONSTANTS OF THE DIGESTIVE SYSTEM

Saliva: amount of excreted saliva daily	1,5 l/day
pH	7,4–8,0
Gastric juice: daily volume	2,0–2,5 l
pH	1,5–1,8; (0,3–0,5% HCI)
Intestinal juice: pH of juice of duodenal glands	7,0–8,0
pH of juice of small intestines	5,05–7,07
Pancreas juice: daily volume	1,5–2,0 l
pH	7,8–8,4
Bile	
Hepatic bile: water	97%
solid residual	3%
pH	7,5–8,0
cystic bile: water	87–90%
solid residual	10–13%
pH	7,8–8,4
daily volume	500–1500 ml

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Заика Эдуард Михайлович
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для иностранных студентов, обучающихся на английском языке

Под ред. Э.С. Питкевича

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