State Budgetary Educational Institution of Higher Professional Education "Krasnoyarsk State Medical University named after Professor V. F. Voino-Yasenetsky" of the Ministry of Health and the Russian Federation V. F. Voino-Yasenetsky State Medical University of the Ministry of Health of the Russian Federation

Department of Physiology

named after Prof. A. T. Pshonik.

**METHODOLOGICAL GUIDELINES**

**FOR STUDENTS # 11**

**for extracurricular (independent) work**

**"Normal physiology"**

**for students in the specialty Specialty 31.05.01 – General medicine**

**FOR PRACTICAL CLASS № 11**

**10.11.2021 - 16.11.2021**

**Topic:**

**Physiology of the coagulation and anti-coagulation systems.**

**Antigenic properties of blood (blood groups)**

**LESSON 11**

1. Topic: "Physiology of the blood coagulation and anti-coagulation systems, antigenic properties and blood groups".

2. Form of organization of the educational process: preparation for practical training

3. Questions for self-training on the topic of the lesson.

1. The concept of hemostasis. The process of blood clotting and its phases.

2. The role of plasma and cellular coagulation factors in the formation of fibrin.

3. Anticoagulation system of the blood. A functional system that maintains the fluid state of the blood.

4. Blood group systems. Rh factor.

5. Methods for determining blood group membership

6. Physiological basis of blood transfusion.

7. Physiological justification of ways to prevent and stop bleeding during operations in the oral cavity.

**Practical work No. 1. DETERMINATION OF THE RATE OF BLOOD CLOTTING.**

**Work progress**. A drop of blood from a finger is applied to the slide and a stopwatch is started. After 2 minutes, every 20-30 seconds, the needle tries to prick the drop, tracking the time of the appearance of fibrin threads trailing behind the needle.

***Interpretation of the results.*** The rate of blood clotting is taken into account in minutes from the time of application of the drop on the slide to the appearance of fibrin filaments. Normally, the rate of blood clotting by this method is 5-7 minutes.

**Practical work no.2. DETERMINATION OF BLEEDING TIME.**

**Progress and interpretation of the results.** Make a puncture of the skin of the finger with a scarifier with a depth of at least 3.5 mm. Immediately after the puncture, turn on the stopwatch. Protruding drops of blood are blotted every 30 seconds with filter paper, without touching it to the wound. The stopwatch is stopped when the blood flow stops (oozing lesioin is not taken into account).

The width of the linear puncture is 3 mm. Immediately after the puncture, turn on the stopwatch. Protruding drops of blood are blotted every 15-30 minutes with filter paper, without touching the wound with it. The stopwatch is stopped at the moment of stopping the flow of blood (oozing lesioin is not taken into account). For greater accuracy, the test is recommended to be performed twice (on both lobes).

Reading the results. The normal bleeding time does not exceed 4 minutes, most often it is within 2-4 minutes. With pronounced thrombocytopenia and severe forms of qualitative inferiority of platelets, the bleeding time is sharply prolonged. With blood clotting disorders, hemophilia, etc. it usually remains normal or lengthens only slightly, since the bleeding in the microcirculation zone is mainly stopped by platelets, and not by hemocoagulation, And only in severe thrombo-hemorrhagic syndromes, significant hypergeparinemia, the bleeding time is also prolonged.

**Practical work No. 3. DETERMINATION OF BLOOD GROUP AND RHESUS**

**ACCESSORIES WITH TSOLIKLONS**

Approved by the Order No. 2 of the Ministry of Health of the Russian Federation of 09.01.1998.

**3. Technique for determining human blood groups of the ABO system using Tsoliklons**

Monoclonal Anti-A and Anti-B antibodies are produced by two mouse hybrids and belong to class M immunoglobulins. Tsoliklons are made from the ascitic fluid of mice carrying anti-A and anti-B hybrids. Tsoliklon Anti-AB is a mixture of monoclonal anti-A and anti-B antibodies.

There are also Anti-D and Anti-C tsoliklons for determining Rh blood affiliation.

The blood group is determined in a room with good lighting at a temperature of 15-25°C.

3.1. Apply the Anti-A, Anti-B and Anti-AB Tsoliklons to the tablet or plate with individual pipettes, one large drop (0.1 ml) under the appropriate labels.

3.2. Next to the antibody drops, apply one small drop of the test blood (0.01-0.03 ml).

3.3. Mix the blood with the reagent.

3.4. Observe the course of the reaction with the Tsoliklons visually with a slight rocking of the plate or tablet for 3 minutes. Agglutination of red blood cells with Tsoliklons usually occurs in the first 3-5 seconds, but observation should be carried out for 3 minutes due to the later appearance of agglutination with red blood cells containing weak varieties of antigens A or B.

3.5. The reaction result in each drop can be positive or negative. A positive result is expressed in agglutination (gluing) of red blood cells. Agglutinates are visible to the naked eye as small red aggregates that quickly merge into large flakes. In case of a negative reaction, the drop remains uniformly colored red, agglutinates are not detected in it.

3.6 Interpretation of the results of the agglutination reaction of the test blood with Tsoliklons are presented in the table:

\* The plus sign ( + ) indicates the presence of agglutination, the minus sign ( - ) indicates the absence of agglutination.

\*\* Finally, the ABO affiliation is established by the results of cross-determination of: antigens A and B on red blood cells and isohemagglutinins in serum.

|  |  |
| --- | --- |
| **The result of the reaction\* with Tsoliklon:** | The test blood belongs to the group\* |
| Anti-A | Anti-B | Anti-AB |
| - | - | - | О(I) |
| + | - | + | А(II) |
| - | + | + | В(III) |
| + | + | + | АВ(IV) |

The plus sign (+) indicates the presence of agglutination, the minus sign ( - ) indicates the absence of agglutination.

The presence of agglutination with Anti-D tsoliklon indicates that the blood is Rh-positive, and the absence of agglutination indicates that the blood is Rh-negative.

**1. Abstract.**

If the blood released from the blood vessel is left for a while, then it first turns into jelly from the liquid, and then a dense clot is organized in the blood, which, contracting, squeezes out a liquid called blood serum. This is a plasma devoid of fibrin. The described process is called blood clotting (hemocoagulation). Its essence lies in the fact that the protein fibrinogen dissolved in plasma under certain conditions passes into an insoluble state and precipitates in the form of long strands of fibrin. In the cells of these threads, as in a grid, cells get stuck and the colloidal state of the blood as a whole changes. The significance of this process is that the clotted blood does not flow out of the injured vessel, preventing the body from dying from blood loss. The modern scheme of blood clotting includes a number of phases that successively replace each other. These phases are as follows: prothrombinase formation, thrombin formation, fibrin formation, fibrin polymerization and clot organization, fibrinolysis. The anti-clotting system should be understood as a set of organs and tissues that synthesize and utilize a group of factors that ensure the liquid state of the blood, that is, prevent blood clotting in the vessels. In 1901, Landsteiner discovered substances in the blood of healthy people that can cause agglutination (gluing) of red blood cells of other people. It turned out that human red blood cells (as well as other blood cells) are carriers of numerous antigens that have a certain specificity and cause the formation of antibodies of the same name against themselves. More than 15 blood group systems are already known, but practically the most important of them are the AB0 system and Rh (Rh). The group properties of the AB0 system appear in humans in the early stages of embryonic development (already in the 5-7-week-old embryo, the tissues have antigenic differentiation). A person's blood type is a constant sign of it and does not change throughout life. The isolation of four blood groups marked the beginning of a new era in the history of blood transfusion, eliminating the main cause of post-transfusion reactions. In the AB0 system, two agglutinable factors are detected in red blood cells - agglutinogens A and B, and in plasma - two agglutinins-a and b (alpha and beta), respectively. In the blood of a person, there are never simultaneous factors of the same name, so there is no agglutination in the body. Severe consequences of blood transfusion occur when the red blood cells of the blood donor (blood giver) are agglutinated by the blood plasma of the recipient (blood recipient). This happens when the red blood cells of the injected blood contain agglutinogen that coincides (with the same name) with plasma agglutinin, and the concentration of the latter is sufficient for the adhesion of agglutinogens. As a result of the gluing of red blood cells and their subsequent hemolysis, a so-called blood transfusion shock occurs, which can lead to death. It is established that all people can be divided into 4 groups according to the presence or absence of these factors. In people of group 1, red blood cells do not contain agglutinogens - 0(I), and in plasma both agglutinogens. In people of the second group-A (II), agglutinogen A is found in red blood cells, and beta - agglutinin is found in plasma. In individuals with the third group-B (III), there is agglutinogen B in red blood cells, and agglutinin alpha in plasma. And people with the fourth blood group-AB (IV) in red blood cells have both agglutinogens, but in plasma there are neither alpha nor beta agglutinins. Rh factor. In the red blood cells of most people (85%), there is another factor found by Landsteiner and Wiener in 1940 in the blood of macaques and called Rh factor (Rh). This factor was discovered using serum obtained from rabbits immunized with monkey red blood cells. The immune serum of such a rabbit showed the ability to agglutinate the red blood cells of monkeys and the red blood cells of most people, regardless of their AV0 affiliation. Rh factor is found in the red blood cells of 85% of people, regardless of age and gender, and is not associated with agglutinogens of other systems. With the help of standard antiresus sera, it is possible to determine the Rh-belonging of people (the presence of the factor is designated as Rh+, the absence-Rh -). The Rh factor is inherited and is evenly distributed across all blood groups. Being in human red blood cells, Rh-agglutinogen does not have the corresponding antiresus-agglutinins in the serum, but they can be produced in persons with Rh-negative blood under the influence of ingestion of Rh-antigens in the body of such persons. At the same time, Rh-immunization occurs. Rh-immunization can occur under two conditions: if a patient with Rh - blood was transfused with Rh+ blood; if a Rh - woman is pregnant with a Rh + fetus. In the latter case, the Rh factor of the fetus diffuses through the placenta and immunizes the mother, in whose blood antiresus-agglutinins begin to accumulate. During the first pregnancy, their titer usually does not reach a critical value, and the first child is born normally.

4. Self-control on the test tasks of the topic "Physiology of the blood clotting and anti-clotting systems, antigenic properties and blood groups".

1. IN THE PROCESS OF HEMOSTASIS, ... TAKE PART

1) plasma, cellular and tissue coagulation factors\*

2) only plasma clotting factors

3) blood buffer systems

4) only tissue clotting factors

2. CORRECT SEQUENCE OF FIBRINOLYSIS PROCESSES:

1) prothrombinase formation, thrombin formation, fibrin formation

2) plasminogen formation, plasmin formation, fibrin formation

3) activation of plasminogen activator, plasmin formation, fibrin cleavage\*

4) thrombin formation and fibrin formation

3. "HYPOCOAGULATION" IS

1) acceleration of blood clotting

2) increased content of clotting factors

3) slowing blood clotting

4) reducing the content of clotting factors

7. IN THE FIRST PHASE OF BLOOD CLOTTING, THE FOLLOWING IS FORMED:

1) thrombin

2) plasmin

3) fibrin

4) thromboplastin

5) blood and tissue prothrombinase\*

8. HEPARIN

1) prevents the transfer of fibrinogen to fibrin

2) inactivates prothrombinase

3) prevents the transition of prothrombin to thrombin\*

4) activates the plasminogen

9. SUBSTANCES THAT BLOCK VARIOUS PHASES OF BLOOD COAGULATION ARE CALLED

1) coagulants

2) anticoagulants\*

3) hematopoietins

4) antibodies

10. THE FIRST PLASMA COAGULATION FACTOR

1) fibrinogen\*

2) prothrombin

3) thromboplastin

4) calcium

11. SECOND PLASMA CLOTTING FACTOR

1) calcium

2) fibrinogen

3) prothrombin\*

4) thromboplastin

12. THE THIRD PLASMA COAGULATION FACTOR

1) fibrinogen

2) prothrombin

3) thromboplastin\*

4) calcium

13. THE FOURTH PLASMA COAGULATION FACTOR

1) fibrinogen

2) prothrombin

3) thromboplastin

4) calcium\*

14. THE FIFTH PLASMA COAGULATION FACTOR

1) proaccelerin\*

2) proconvertin

3) antihemophilic globulin A

4) antihemophilic globulin B

15. EIGHTH PLASMA COAGULATION FACTOR

1) proaccelerin

2) proconvertin

3) antihemophilic globulin A\*

4) antihemophilic globulin B

16. THE THIRTEENTH PLASMA COAGULATION FACTOR

1) the Rosenthal factor

2) antihemophilic globulin C (Koller)

3) Hageman factor

4) Fibrin-stabilizing\*

17. WHEN TRANSFUSING RED BLOOD CELL MASS FROM A RH-NEGATIVE DONOR TO A RH-POSITIVE RECIPIENT, THERE IS no RH-CONFLICT:

1) \*

2) it can be used for transfusion of large amounts of red blood cell mass

3) it may be if the recipient is a woman with a history of multiple pregnancies

4) it may be if the donor is a woman with a history of multiple pregnancies

5) there is no correct answer

18. THE FOURTEENTH FACTOR OF BLOOD CLOTTING IS

1) the Rosenthal factor

2) antihemophilic globulin C (Koller)

3) Hageman factor

4) fibrin-stabilizing

5) the Fletcher factor\*

19. THE RETRACTION OF A CLOT IS ITS

1) dissolution

2) seal\*

3) inactivation

4) stabilisation

20. THE THIRD STAGE OF ENZYMATIC FIBRINOLYSIS ENDS WITH THE FORMATION OF

1) plasminogen

2) Prothrombinases

3) fibrinolysin (plasmin)

4) peptides and amino acids\*

21. THE FIFTEENTH PLASMA COAGULATION FACTOR IS

1) the Rosenthal factor

2) antihemophilic globulin C (Koller)

3) Hageman factor

4) the Fitzgerald factor\*

5) the Fletcher factor

22. AGGLUTINATION OF RED BLOOD CELLS WILL OCCUR AT THE MEETING

1) agglutinogen A and agglutinin β

2) agglutinogen B and agglutinin α

3) agglutinogens AB with blood group IV plasma

4) agglutinogen A and agglutinin a\*

23. IF THE BLOOD CONTAINING THE RH FACTOR IS TRANSFUSED FOR THE FIRST TIME TO A PERSON WHOSE BLOOD DOES NOT CONTAIN IT, THEN

1) no changes will occur

2) immune antiresus-agglutinins are formed\*

3) there will be blood transfusion complications

4) there will be a decrease in the BCC

24. RHESUS CONFLICT OCCURS WHEN:

1) high concentration of antiresus-agglutinins

2) sufficient concentration of antiresus-agglutinins\*

3) the first pregnancy of a rh-positive woman with a rh-negative fetus

4) the first pregnancy of a rh-negative woman with a rh-negative fetus

25. IN PEOPLE OF THE WHITE RACE, THE RH FACTOR OCCURS

1) in 65% of cases

2) in 75% of cases

3) in 85% of cases\*

4) in 50% of cases

26. THE BLOOD OF THE FIRST GROUP CONTAINS AGGLUTINOGENS OF THE ABO SYSTEM

1) A and B

2) A

3) In

4) 0\*

27. THE BLOOD OF THE SECOND GROUP CONTAINS AGGLUTINOGENS

1) A and B

2) A\*

3) In

4) 0

28. THE BLOOD OF THE THIRD GROUP CONTAINS AGGLUTINOGENS

1) A and B

2) A

3) In\*

4) About

29. THE BLOOD OF THE FOURTH GROUP CONTAINS AGGLUTINOGENS

1) A and B\*

2) A

3) In

4) 0

30. THE BLOOD PLASMA OF THE FIRST GROUP CONTAINS AGGLUTININS

1) alpha

2) beta

3) alpha and beta\*

4) 0

31. THE BLOOD PLASMA OF THE SECOND GROUP CONTAINS AGGLUTININS:

1) alpha

2) beta\*

3) alpha and beta

4) 0

32. THE BLOOD PLASMA OF THE THIRD GROUP CONTAINS AGGLUTININS:

1) Alpha\*

2) beta

3) alpha and beta

4) 0

**Questions for self-control**

1. Name the 1st plasma clotting factor.

2. When determining the blood groups, it turned out that agglutination occurred with standard serum 0(I) and A(II). What is the blood type of the subject?

3. How is the individual blood compatibility test performed in the subject?

4. What mechanism is able to independently stop bleeding from the most frequently injured microcirculatory vessels with low blood pressure?

5. What substances can be used to preserve the liquid state of the blood?

6. What is the 2nd phase of enzymatic blood clotting?

7. Which blood group can be transfused to the patient if the agglutination of his red blood cells occurred in the standard serum of groups 0(I) and B(III)?

8. What are anticoagulants?

9. In a mother with Rh-negative blood, the first pregnancy led to Rh-conflict. Why would this happen?

10. What is non-enzymatic fibrinolysis?

11. Why does the blood not clot when adding citric acid sodium?

12. With what process does vascular-platelet hemostasis begin?

13. How is the biological compatibility test performed for blood transfusions?

14. When determining the blood group, agglutination occurred in 0(I), and(II), in(III) standard sera. What is the blood type of the subject?

15. What is the essence of blood clotting?

16. What happens in the first phase of enzymatic blood clotting (list)?

17. What conditions are necessary to maintain the liquid state of the blood (list)?

18. What state of the body develops when transfusions of incompatible blood group are carried out?

19. How long (on average) does the retraction of a blood clot last?

20. What groups are the anticoagulants available in the body divided into?

21. What happens in the 3rd phase of enzymatic blood clotting?

22. What is the Rh-immunization?

23. What is the importance of vitamin K for blood clotting?

24. When determining the blood group, agglutination was not observed in any of the sera. What is the blood type of the subject?

25. In what cases is there a rhesus conflict?

26. Name the active primary anticoagulant produced by basophils and mast cells of connective tissue.

27. By what mechanism does the formation of blood prothrombinase occurs and how long does this process last?

28. What physical factors influence the polymerization of fibrin?

29. When determining the blood group, agglutination occurred in B (III) standard serum and was not observed with others. What is your conclusion?

30. In what cases does a blood transfusion shock develop?

31. What is the name of the 5th phase of enzymatic blood clotting?

32. What is an anti-clotting system?

33. The father has Rh-positive blood, the mother - Rh-positive. The fetus has no Rh factor. Is there a risk of rhesus conflict between the mother and the fetus?

34. Name the 2nd plasma coagulation factor.

35. What happens to red blood cells after their agglutination as a result of group incompatibility?

36. How many blood groups are there in the AB0 system?

37. What is the name of the 4th phase of enzymatic fibrinolysis?

38. What is the seventh plasma coagulation factor its active form?

39. What blood can be transfused to a recipient with A (II) Rh blood?

40. What is prothrombin time?

41. What changes will occur in the blood clotting process if aminocapronic acid, which is a plasmin inhibitor, is added to the system?

Answers to questions for self-control:

1. Fibrinogen.

2. In (W).

3. A drop of blood from the donor and recipient are mixed on the glass.

4. Vascular-platelet hemostasis.

5. Heparin, citric acid sodium.

6. Formation of thrombin (from prothrombin under the influence of prothrombinase).

7. A (II).

8.Substances that prevent blood clotting.

9. She probably once had a Rh-positive blood transfusion and has anti-resus antibodies in her body.

10. Inactivation of blood clotting factors with heparin.

11. Citric acid sodium binds calcium ions, which are necessary for blood clotting.

12. With reflex spasm of damaged blood vessels.

13. First pour 10-15 ml of donor blood and then for 3-5 minutes. monitor the patient's condition, if it has not changed, continue the

transfusion.

14. AB(IV).

15. The transition of the soluble plasma protein fibrinogen to the insoluble form-fibrin.

16. Formation of blood and tissue prothrombinase.

17. a) the smooth surface of the vascular endothelium, b) the negative charge of the vascular wall and cellular elements, c) the speed of blood flow, d) the walls of the vessels are covered with a thin layer of soluble fibrin that adsorbs active blood clotting factors, e) the natural anticoagulants available in the blood.

18. Hematransfusion shock.

19. 2-3 hours.

20. Previous (primary) and formed in the process of blood clotting and fibrinolysis (secondary).

21. Transition of fibrinogen to fibrin.

22. The process of production and accumulation of antiresus agglutinins.

23. In the liver, in the presence of vitamin K, some plasma clotting factors are formed.

24. 0(I).

25. When the Rh factor meets with antiresus agglutinins, for example, during repeated transfusion of Rh ( + ) blood to a Rh-negative recipient.

26. Heparin.

27. On the inside, 3-4 min.

28. pH and temperature.

29. It is impossible to give a conclusion. The analysis must be repeated.

30. When transfusing incompatible blood groups.

31. Fibrinolysis.

32. A set of organs and tissues that synthesize, produce and utilize factors that prevent blood clotting.

33. There is no danger. If the Rh antigen of the mother gets into the blood of the fetus, then immunization will not occur, because the fetus has not yet developed immunocomponent organs.

34. Prothrombin.

35. Hemolysis.

36. Four.

37. Phases of fibrin stabilization and blood clot retraction.

38. Proconvertin (active form-convertin).

39. A (II) Rh ( -).

40. Blood clotting time in excess of thromboplastic substances.

41. Delayed hydrolysis of fibrin.

**4. Self-control on situational tasks on the topic** "Physiology of the blood clotting and anti-clotting systems, antigenic properties and blood groups" **with keys**.

1. The number of platelets in the test blood is 100 \* 109 /L.

1. What changes in the coagulation system should be expected in this patient and why?

2. How many platelets does the blood normally contain?

3. What properties of platelets ensure their participation in vascular-platelet hemostasis?

4. Which platelet protein provides a blood clot seal?

**Keys:**

1. The patient has thrombocytopenia. Since platelets are actively involved in all phases of blood clotting, an increase in clotting time should be expected.

2. A liter of blood normally contains 2-3 \* 1011 platelets

3. Platelets are capable of adhesion and aggregation

4. Retractozyme (thrombostenin).

2. When determining the blood group, red blood cell agglutination occurred with anti-B and anti-AB coliclones.

1. What group of blood can be transfused to the patient?

2. Under what condition does red blood cell agglutination occur?

3. What is tsoliklon?

4. In what ratio is tsoliklon and blood taken when determining the blood group?

**Keys:**

1. The patient's blood type is B (III) and he can be transfused with blood of the third group

2. At the meeting of the same agglutinogens and agglutinins (A with α, B with β)

3. Monoclonal anti-A and anti-B antibodies are produced by two mouse hybrids and belong to class M immunoglobulins. Tsoliklons are made from the ascitic fluid of mice carrying anti-A and anti-B hybrids. Tsoliklon anti-AB is a mixture of monoclonal anti-A and anti-B antibodies.

4. 10:1

3. When determining the blood group, red blood cell agglutination occurred with anti-A, anti-B, and anti-AB coliclones.

1. What is the patient's blood type?

2. Which blood group can be transfused to this patient?

3. How is the individual compatibility test performed before the blood transfusion?

1. The patient has blood of the AB (IV) group, since it contains both agglutinogens-A and B.

2. Blood of group IV only

3. The blood of the donor and the recipient is mixed on the glass

4. To eliminate incompatibility across the rest of the blood group systems

4.The blood of the father Rh+, the mother Rh -. First pregnancy.

1. Is there a risk of Rh conflict between the mother and the fetus if the fetus has Rh+ blood?

2. What is Rh immunization?

3. In what percentage of cases do white people have Rh antigen?

4. What blood is transfused, if necessary, to a Rh-positive child born with hemolytic disease as a result of Rh-conflict

**Keys:**

1. The risk of Rh-conflict exists if the mother was transfused with Rh-positive blood before pregnancy and Rh-immunization occurred before the first pregnancy. Then antiresus antibodies from the mother's blood can enter the fetal blood and cause hemolysis of its Rh-positive red blood cells. The fetus may die, or be born with a hemolytic disease.

2. Rhesus immunization is the process of producing antiresus-agglutinins

3. In 85% of cases.

4. Rh negative.

5.A plasmin inhibitor, epsilonaminocaproic acid (EACC), was added to the blood.

1. What changes will occur in the process of blood clotting?

2. What is the process called enzymatic fibrinolysis?

3. How long does fibrinolysis last?

4. How does enzymatic fibrinolysis begin?

**Keys:**

1. Since plasmin is the main active agent of the fibrinolytic system, the addition of EACC will cause a delay in the dissolution of fibrin, which will promote thrombosis.

2. Dissolution of fibrin filaments under the influence of plamin (fibrinolysin).

3. From a few minutes to several hours, depending on the size of the blood clot.

4. Fibrinolysis begins with the activation of the plasminogen activator (profibrinolysin).

6. A woman with Rh-blood is pregnant with a Rh + fetus. Pregnancy is the first. The baby was born healthy. A few months after the birth, for vital reasons, the woman was transfused with a single group of blood, but the patient died in the phenomena of hemotransfusion shock.

1. What could be the cause of the blood transfusion shock in this case?

2. In what case does a blood transfusion shock develop?

3. What happens to the red blood cells of the donor when transfusing incompatible blood groups?

4. Is it possible to transfuse a Rh-positive recipient with a single group of Rh-negative blood?

**Keys:**

1. The cause of death in this case was the transfusion of Rh-positive blood to the woman, although the same group according to the ABO system. As a result of Rh-immunization, which occurred during pregnancy with a Rh-positive fetus, a Rh-conflict arose during transfusion, which ended with hemolysis of the donor's red blood cells and the death of the woman from hemotransfusion shock.

2. When transfusing incompatible blood group.

3. Agglutination of red blood cells followed by hemolysis.

4. It is possible only if the donor was not immunized with Rh-antigen (he was not transfused with Rh-positive blood).

**6. List and standards of practical skills.**

1. Determination of blood groups according to the ABO and Rh system.

**INDEPENDENT EXTRACURRICULAR WORK OF STUDENTS**

Use the program" Virtual Physiology " to perform the following experiments on the physiology of the blood system and record their results in a protocol notebook:

Work # 4. Study of blood group affiliation

**INDEPENDENT CLASSROOM WORK OF STUDENTS**

**Practical work No. 1. DETERMINATION OF THE RATE OF BLOOD CLOTTING.**

**Work progress.** A drop of blood from a finger is applied to the slide and a stopwatch is started. After 2 minutes, every 20-30 seconds, the needle tries to prick the drop, tracking the time of the appearance of fibrin threads trailing behind the needle.

Interpretation of the results. The rate of blood clotting is taken into account in minutes from the time of application of the drop on the slide to the appearance of fibrin filaments. Normally, the rate of blood clotting by this method is 5-7 minutes.

**Practical work no.2. DETERMINATION OF BLEEDING TIME**

**Progress and interpretation of the results. Practical work No. 1. DETERMINATION OF THE RATE OF BLOOD CLOTTING.**

**Work progress**. A drop of blood from a finger is applied to the slide and a stopwatch is started. After 2 minutes, every 20-30 seconds, the needle tries to prick the drop, tracking the time of the appearance of fibrin threads trailing behind the needle.

Interpretation of the results. The rate of blood clotting is taken into account in minutes from the time of application of the drop on the slide to the appearance of fibrin filaments. Normally, the rate of blood clotting by this method is 3-5 minutes.

**Practical work no.2. DETERMINATION OF BLEEDING TIME BY DUKE.**

**Progress and interpretation of the results.** The earlobe is warmed between the fingers for 1 min, wiped with alcohol and warmed again with a table lamp with a reflector until the alcohol dries completely. A sterile lancet is used to puncture the skin at the lower-outer edge of the earlobe with a depth of 3.5 mm (make sure that the depth of the puncture always corresponds to this value). The width of the linear puncture is 3 mm. Immediately after the puncture, turn on the stopwatch. Protruding drops of blood are blotted every 15-30 minutes with filter paper, without touching the wound with it. The stopwatch is stopped at the moment of stopping the flow of blood (oozing lesion is not taken into account). For greater accuracy, the test is recommended to be performed twice (on both lobes).

Reading the results. The normal bleeding time does not exceed 5 minutes, most often it is within 2-4 minutes, With pronounced thrombocytopenia and severe forms of qualitative inferiority of platelets, the bleeding time is sharply prolonged. With blood clotting disorders, hemophilia, etc. it usually remains normal or extends only slightly, since the bleeding in the microcirculation zone is mainly stopped by platelets, and not by hemocoagulation, and only in severe thrombo-hemorrhagic syndromes, significant hypergeparinemia, the bleeding time is also prolonged.

It should be borne in mind that the Duke method is not sensitive enough and often gives erroneous results due to early gluing of the wound edges and other reasons.

**Practical work No. 3. DETERMINATION OF BLOOD GROUP AND RHESUS**

**ACCESSORIES WITH TSOLIKLONS**

Approved by Order No. 2 of the Ministry of Health of the Russian Federation of 09.01.1998.

**3. Technique for determining human blood groups of the ABO system using Tsoliklons**

The determination is made in native blood taken in a preservative; and blood taken without a preservative; in blood taken from a finger. The method of direct hemagglutination on a plane is used: on a plate or tablet. The blood group is determined in a room with good lighting at a temperature of 15-25°C.

3.1. Apply the Anti-A, Anti-B and Anti-AB Tsoliklons to the tablet or plate with individual pipettes, one large drop (0.1 ml) under the appropriate labels.

3.2. Next to the antibody drops, apply one small drop of the test blood (0.01-0.03 ml).

3.3. Mix the blood with the reagent.

3.4. Observe the course of the reaction with the Tsoliklons visually with a slight rocking of the plate or tablet for 3 minutes. Agglutination of red blood cells with Tsoliklons usually occurs in the first 3-5 seconds, but monitoring should be carried out only due to the later appearance of agglutination with red blood cells containing weak varieties of antigens A or B.

3.5. The reaction result in each drop can be positive or negative. A positive result is expressed in agglutination (gluing) of red blood cells. Agglutinates are visible to the naked eye as small red aggregates that quickly merge into large flakes. In case of a negative reaction, the drop remains uniformly colored red, agglutinates are not detected in it.

3.6 Interpretation of the results of the agglutination reaction of the test blood with Tsoliklons are presented in the table:

\* The plus sign ( + ) indicates the presence of agglutination, the minus sign ( - ) indicates the absence of agglutination.

\*\* Finally, the ABO affiliation is established by the results of cross-determination of: antigens A and B on red blood cells and isohemagglutinins in serum.

|  |  |
| --- | --- |
| The result of the reaction\* with Tsoliklon: | The test blood belongs to the group\* |
| Anti-A | Anti-B | Anti-AB |
| - | - | - | O (I) |
| + | - | + | A (II) |
| - | + | + | B (III) |
| + | + | + | AB(IV) |

The plus sign (+) indicates the presence of agglutination, the minus sign ( - ) indicates the absence of agglutination.

The presence of agglutination with Anti-D tsoliklon indicates that the blood is Rh-positive, and the absence of agglutination indicates that the blood is Rh-negative.

To better master the skill of reading the results of determining the blood group membership, interested students can use the computer program "Simulator for determining blood groups".

**Practical work No. 3. DETERMINATION OF BLOOD GROUP AND RHESUS**

**ACCESSORIES WITH THE HELP OF TSOLIKLONS**. Approved by Order No. 2 of the Ministry of Health of the Russian Federation of 09.01.1998.

**3. Technique for determining human blood groups of the ABO system using Tsoliklons**

The determination is made in native blood taken in a preservative; and blood taken without a preservative; in blood taken from a finger. The method of direct hemagglutination on a plane is used: on a plate or tablet. The blood group is determined in a room with good lighting at a temperature of 15-25°C.

**3.1.** Apply the Anti-A, Anti-B and Anti-AB Tsoliklons to the tablet or plate with individual pipettes, one large drop (0.1 ml) under the appropriate labels.

**3.2.** Next to the antibody drops, apply one small drop of the test blood (0.01-0.03 ml).

**3.3.** Mix the blood with the reagent.

**3.4.** Observe the course of the reaction with the Tsoliklons visually with a slight rocking of the plate or tablet for 3 minutes. Agglutination of red blood cells with Tsoliklons usually occurs in the first 3-5 seconds, but monitoring should be carried out only due to the later appearance of agglutination with red blood cells containing weak varieties of antigens A or B.

**3.5.** The reaction result in each drop can be positive or negative. A positive result is expressed in agglutination (gluing) of red blood cells. Agglutinates are visible to the naked eye as small red aggregates that quickly merge into large flakes. In case of a negative reaction, the drop remains uniformly colored red, agglutinates are not detected in it.

**3.6** Interpretation of the results of the agglutination reaction of the test blood with Tsoliklons are presented in the table:

\* The plus sign ( + ) indicates the presence of agglutination, the minus sign ( - ) indicates the absence of agglutination.

\*\* Finally, the ABO affiliation is established by the results of cross-determination of: antigens A and B on red blood cells and isohemagglutinins in serum.

|  |  |
| --- | --- |
| The result of the reaction\* with Tsoliklon: | The test blood belongs to the group\* |
| Anti-A | Anti-B | Anti-AB |
| - | - | - | O (I) |
| + | - | + | A (II) |
| - | + | + | B (III) |
| + | + | + | AB(IV) |

The plus sign (+) indicates the presence of agglutination, the minus sign ( - ) indicates the absence of agglutination.

The presence of agglutination with Anti-D tsoliklon indicates that the blood is Rh-positive, and the absence of agglutination indicates that the blood is Rh-negative.

To better master the skill of reading the results of determining the blood group membership, interested students can use the computer program "Simulator for determining blood groups".

**7. The approximate subject of the students' scientific research on the topic:**

1. Rhesus conflict.

2. Diet by blood group: myths and reality.

**8. Recommended literature on the topic of the lesson:**

**REFERENCES ON THE TOPIC OF THE LESSON:**

**Mandatory**

**REFERENCES OF THE LESSON**

**Main**

**Course textbooks and manuals**

1. Dunn, R. B. USMLE Step 1. Lecture Notes. Physiology / R. B. Dunn ; ed. D. E. Fitzovich. - [S. l.] : Kaplan, 2006. - 576 p.

2. Hall, J. E. Guyton and Hall Textbook of Medical Physiology / J. E. Hall. - 13th ed., Int. ed. - Philadelphia : Elsevier, 2016. - 1145 p.

3. Sherwood, L. Fundamentals of Human Physiology / L. Sherwood. – 4th ed. – Belmont, CA, USA: Brooks/Cole, 2012. – 764 p.

4. Silbernagl, S. Color Atlas of Phisiology / S. Silbernagl, A. Despopoulos. - 7th ed. - Stuttgart : Thieme, 2015. - 458 p.

5. Wilson, L.B. USMLE Step 1. Lecture Notes. Physiology / L.B. Wilson. - Kaplan, 2013. - 423 p.

**Additional**

1. Praktikum po normal'noj fiziologii: metodicheskie ukazaniya dlya studentov po spec.- stomatologiya /pod red. YU.I.Savchenkova. 2009

2. Savchenkov YU.I. Metody issledovaniya fiziologicheskih funkcij: uchebnoe posobie.

3. Smirnov V.M. Normal'naya fiziologiya: uchebnik.

4. Testovye zadaniya po normal'noj fiziologii: v 2-h tomah /red. YU.I.Savchenkov.

5. Savchenkov YU.I., Pac YU.S. Stomatologicheskaya fiziologiya: uchebnoe posobie. Feniks, 2007

**URL references on the University's website:**

[https://krasgmu.ru/index.php?page[common]=content&id=189233](https://krasgmu.ru/index.php?page%5bcommon%5d=content&id=189233)