UKRAINIAN MEDICAL DENTAL ACADEMY
NORMAL PHYSIOLOGY CHAIR

METHODICAL RECOMMENDATIONS
TO PRACTICAL CLASSES FOR STUDENTS OF MEDICAL AND DENTAL DEPARTMENTS

ON CHAPTER:

"BLOOD SYSTEM PHYSIOLOGY"

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MODULE 2 (CONTINUATION)
Content credit 8: "Blood system"

PRACTICAL WORK 20.
BLOOD PHYSICO-CHEMICAL INDEXES INVESTIGATION

"Blood reflects like in mirror
all processes taking place
in alive organism".

One part of these indexes belong to stable blood constants (pH – blood has weakly-alkaline reaction, its pH is equal to 7,35 in veins and 7,4 in arteries; osmotic tissues – 7,3 atm; oncotic pressure – 0,03-0,04 atm); others - to unstable (blood viscosity – relative is 4,5; VSR: in men- 6-12 mm/h; in women- 8-15 mm/h) et al.

Blood system is one of the most sensitive organism indicators which reflects organism state. Constants fluctuation may serve as diagnostic criterium of many diseases (sometimes – earlier than clinical criteriums or more exact then clinical indexes). Many blood constants are suffered at blood diseases. One can use unstable constants in laboratory and clinical practice more oftan than unstable ones. Especially it concerns such indexes as erythrocytes amount, haemoglobine concentration, velocity sedimentation rate and others. But several clinical situations needs stable constants determining. That's why this chapter, knowledge of normal blood indexes and primary skills of their interpretating is of very big importance for every doctor, of any medical profile.

It's necessary for work: scarificator, pipettes, blood dilutor, Panchenkov's device, test tubes, centrifuge, blood.

Object: rat.

Task 1. To get aquanted with blood taking technology for analysis performance.

The investigator must wipe investigated person left fourth finger with cotton wool washed in alcohol. To prick the cured finger with sterile scarificator. To dry first blood drop with cotton wool. To put the end of horizontally located pipette in the second blood drop and to fetch very carefully (without vesicles) blood till corresponding mark. To gain this goal it's necessary to immerse the pippette in complete blood drop. It's necessary to dry pipette end with cotton wool and to perform manipulations with blood according to investigation character.

Task 2. To determine erythrocytes osmotic resistance.

To put 20 test tubes in support and to number them. To pour hypotonic solutions in the dosage of 1 ml in corresponding test tubes (concentrations from 0,60 to 0,15 per cent with the difference in 0,05 per cent in every test tube). To add investigated blood in every test tube. To mix carefully the content and to stay them at room temperature for 15 minutes. To centrifugate after this at 1500 rotations per minute in course of 5 minutes. To determine the limits of erythrocytes maximal and minimal resistance.

Erythrocytic resistance - is red blood cells feature to resist injured actions (osmotic, chemical, mechanical and so on). But osmotic resistance to sodium chloridum hypotonic

solutions is the most-spread resistance index determined under clinical conditions (it is rather easily to be performed and to evaluated comparatively to other resistance types).

Under norma minimal resistance (haemolysis beginning) – at sodium chloridum content 0,42-0,48 per cent; maximal resistance (complete haemolysis) – at 0,30-0,34 per cent of it. In a fresh blood- 0,20-0,40 per cent of NaCl; in incubated one (in course of 24 hours) – 0,20-0,65 per cent of NaCl.

It is decreased at:

- congenital microspherocytic haemolytical jaundice;
- new-borns haemolytical disease;
- toxicoses;
- acute infections;
- leukemias;
- lymphogranulematoses;
- hepatic cirrhoses;
- ABO- and Rh- blood incompatibility.

It is increased at:

- drepanocytic anaemias;
- mechanical jaundices.

But this index is the most significant at anaemias differentiated diagnosis.

Task 3. Velocity sedimentation rate (VSR) determining.

To wash capillary pipette of Panchenkov with 5% solution of citrate sodium. To fetch this solution till the mark 75/25 mm^3 and to blow it to the clock glass.

To prepare the finger, to prick it and to fetch blood till the mark 100 mm^3. To blow blood to the clock glass and to mix it with citrate sodium in correlation of 1:4. To fill the pipette with this citric blood exactly till the mark "K" and to put it into support vertically for 1 hour. In 1 hour to determine the highland in mm of plasma column above formed elements.

Control questions.

1. Blood system.
2. Blood content, its amount.
3. Blood functions.
4. Blood constants and their significance in clinical practice.
5. Blood main components, haematocrit.
6. Blood buffer systems, acidosis, alkalosis.
7. Erythrocytic osmotic resistance.
8. Velocity sedimentation rate, factors, influencing on it, diagnostic value.
9. Osmotic pressure.
10. Oncotic pressure.
11. Blood viscosity.
12. Blood temperature, blood colour, factors they depend on.

PRACTICAL WORK 21.
ERYTHROCYTES PHYSIOLOGY
(ERYTHROCYTES AMOUNT, HAEMOGLOBINE CONCENTRATION AND COLOUR INDEX DETERMINING).

It's necessary for work: microscope, counter of Goryaev, scarificators, mixers, solutions, alcohol, cotton wool, photoelectrocalorymetr (PEC), apparatus for erythrocytes automatic count, haemometr of Sali, hydrochloric acid solution, alkaline solution, device for haemolysis assessment.

Task 1. To determine erythrocytes amount in blood.

In some clinics, unfortunately, this old method is still widely-used. Under modern conditions this method was changed on automatical one. Automatical devices have special instructions on their expluatation.

Erythrocytes number estimation in Goryaev's chamber.

To petch investigated blood in special mixer (melanger) till the mark 0,5 or 1,0 (it depends on blood dissolving). To wipe mixer end with cotton wool and to petch 3% NaCl in it till the mark "101". To mix carefully mixer content in course of 1 minute, to pour 1-2 blood drops on cotton wool and to fill up Goryaev's chamber with the next blood drop. But one should grind covering glass to Goryaev's chamber before this. Erythrocytes are estimated in 5 large squares (each of them is divided into 16 small squares) placed diagonally (obliquelly) to the net. It's necessary to estimate red blood cells located inside every small square as well as on its superior and left boundaries. One should put found erythrocytes amount under following formula:

$$X = (a \times 4000 \times 200 \text{ or } 100) : 80 \times 10^6,$$

where:

X – erythrocytes amount,
a - erythrocytes amount in 5 large (80 small) squares.
1/4000 mcl/mm^3 – one small square volume;
200 or 100 – blood dilution degree;
10^6 – coefficient for re-computation into SI.

Task 2. Haemoglobine content determining in blood.

Like in a case with erythrocytic amount, there are both rutine (old) measurements methods and new, automatical ones (on PEC, haemoglobinometers and others).

Haemoglobine content determination by Sali method.

To pour 0,1 normal (approximately 0,2 ml) hydrochloric acid solution in graduated pipette of Sali till inferior ring level. To petch exactly 0,02 ml of blood with pipette from haemometer and to blow it on the test tube floor. Shaking up the test tube, to mix its content carefully. The mixture must stay in course of 5-10 minutes at room temperature. Hydrochloric acid causes erythrocytic haemolysis and haemoglobine destruction. Releasing haem interacts with hydrochloric acid and is transformed into hydrochloric

haematin. Test tube content becomes dark brown as a result of this reaction. The investigator must add distillate water in 5-10 min till investigated liquid colour becomes equal to the standard solution colour. To mark on the scale at which level hydrochloric haemolytic is. Received ziphra multiple on 10. Result (product) will correspond to haemoglobine concentration in investigated blood in g/l.

Task 3. To estimate blood colour index.

Colour index characterizes erythrocytes satiation degree with haemoglobine. It is calculated on formula:

C.I.= (X heamoglob. x 5,0 x 10^{12}/l) : (167 g/l x X erythroc.), where:

X haemoglob. – found haemoglobine amount (g/l);

X erythroc.- found erythrocytes amount in 1 l of blood.

The second formula: Hb (g/l) x 3 : RBC (3 first ziphras). It is evaluated in conditional units.

Nowadays there are several additions to usual blood analysis. Such additions include some useful indexes the main of which we will describe now. At English-speaking countries all these indexes are automatically determined practically in every clinic.

1. MCV (Mean Cellular Volume) – average erythrocytic volume. MCV=HCT (%) : RBC (x 10^{12}/l) x 10, where: HCT- haematocrit; RBC- erythrocytic amount.

MCV (normocytes) - adults: 78-94 mcm^3 or fl (femptolitres)
new-borns: 95-105 mcm^3;
children: 76-90 mcm^3.

MCV↑ (macrocytosis):

- pregnancy;
- megaloblastic anaemia;
- myelodysplastic syndrom;
- liver diseases;
- hypothyreoidism;
- alcoholism;
- treatment with estrogens;
- treatment with barbiturates et al.

MCV↓ (microcytosis):

a) anaemias:
- hereditary microspherocytic;
- iron-deficient;
- syderoblastic;
- chronic anaemias;
- thalassaemia (hereditary haemoglobinopathy);

b) hypohydratation;

c) aluminum intoxication.

2. MCH (Mean Cellular Haemoglobine) – haemoglobine average content in erythrocytes.

MCH=Hb (g/l):RBC (x 10^{12}/l)

MCH (erythrocytic normochromy)- adults: 27-33 pg (picogram)
children: 24-30 pg

MCH↑(hyperchromy):

- new-borns;
- megaloblastic anaemia;
- liver cirrhosis.

MCH↓ (hypochromy):

- iron-deficient anaemia;
- thalassaemia;
- sideroblastic anaemia.

3. MCHC – Mean Cellular Haemoglobine Concentration – Mean haemoglobine concentration in erythrocyte – Hb (g/decaliter): Ht or HCT (l/l) x 100

MCHC (norma): 32-36 g/dl (320-360 g/l).

MCHC↑:

- new-borns;
- hereditary spherocytosis;
- long-termed hypohydratation.

MCHC↓ (absolute hypochromy):

- iron-deficient anaemia;
- thalassaemia;
- sideroblastic anaemia;
- hydraemia.

4. Reticulocytes amount in blood volume unit (this index is widely-estimated in Ukraine too often). As you know, reticulocytes are direct erythrocytes predecessors (precursors). They don't have any nucleus, but their rhibosomes containing is big. They circulate in blood up to 2 twenty-four-hours and become erythrocytes but reticulocytes maturation process depends on haematocrit.

Norma: adults and children: 0,2-2,0 % or 25-85 x 10^9/l;
new-borns : 2-6% or 85-250 x 10^9/l.

Reticulocytosis (increasing):

- anaemias (haemolytic, acute posthaemorrhagic),
- in initial period (6-10^{th} days) of effective anaemias treatment, caused by iron and folic acid, cyancobalamine and pyridoxine insufficiency;
- in course of exit from bone marrow hypoplasy after therapy with cytostatics;
- after splenectomy;
- at malaria.

Reticulopenia (decreasing):

- hypo- and aplastic anaemias;
- megaloblastic anaemias;
- acute leukemias;
- radiation disease;
- in course of cytostatic therapy;
- pre-regenerative crisises at haemolytic anaemias;
- kidney diseases;

- radiation disease anaemia.

5. Reticulocytic index (RI)= R (%) x Ht (of patient) : Ht (normal). It is used for more adequate bone marrow erythropoietic activity assessment with the haematocrit taking into account.

Norma: 1%

RI ↑:

- haematocrit decreasing;
- haemolytic anaemias (due to erythropoiesis activation);
- initial stage of effective anaemias treatment (due to the same reason).

6. Reticulocytes formation index – RFI=RI:t (reticulocytes maturation time in perypheral blood) x 10.

RFI (norm)=1 cond. un.

RFI (at anaemia)>3 indicates to erythropoietic cells prolypheration and maturation activating.

RFI (at anaemia)<3 indicates to erythropoiesis inhibition.

Control questions.

1. Erythrocytes structure and quantity, their amount changings under physiological conditions.
2. Erythrocytes functions.
3. Erythropoiesis regulation, specific and non-specific erythropoiesis regulative ways. Salivary glands role in this process.
4. Haemoglobine molecule structure, haemoglobines types.
5. Haemoglobine functions.
6. Haemoglobine chemicals in blood.
7. Colour index.
8. Erythrocytic haemolysis, its types.
9. Haemolysines.
10. Different environments and solutions influence on erythrocytic haemolysis.

PRACTICAL WORK 22.

LEUCOCYTES PHYSIOLOGY.

It's necessary for work: microscope, mixer (melanger less than erythrocytic one) for leucocytes counting, Goryaev's chamber, blood, haemocytometers.

Leucocytes amount as erythrocytes one may be estimated both rutine (non-automatical) method (estimation in Goryaev's chamber) and automatical methods.

Leucocytes estimation in Goryaev's chamber.

It's necessary to petch blood in melanger for leucocytes till the mark 0,5 or 1,0. To wipe melanger's end with cotton wool and then, putting it in dilutor – 5% acetic acid, coloured with methylenic blue, to petch melanger's content. To pour first 1-2 blood drops onto cotton wool, to fill up chamber with the third one. The investigator must estimate leucocytes under small increasing in 25 large (400 small) squares. Leucocytes amount is estimated on formula:

$X=(a \times 4000 \times 20 \text{ or } 10) : (25 \times 10) \times 10^6$, where:

X – leucocytes amount in 1 l of investigated blood;
a – leucocytes amount determined in course of count;
4000 mcl/mm^3 – small square volume;
20/10 - blood dilution degree;
400 – small squares amount;
10^6 – re-count co-efficient in international units system.

Control questions.

1. Leucocytes classification.
2. Leucocytic formula.
3. Separate leucocytes functions.
4. Leucopoiesis and its regulation.
5. Leucocytes functions significance in dentistry.

PRACTICAL WORK 24.

PLATELETS PHYSIOLOGY. VASCULAR-THROMBOCYTIC HAEMOSTASIS.

It's necessary for work: microscope, aggregatograms, Goryaev's chamber, covering glasses, plasma rich in platelets, aggregatograph.

Task 1. Bleeding duration determining (on Duke).

To prick the finger on the depth of scarificator point. To absorb flowing blood in every 30 sec in filter paper. Under norma bleeding duration is 2-4 minutes.

Task 2. Aggregatogram analysis principle.

Diagnostic value: it can give information about thrombocytes qualitative defects and platelelets dysfunctions, platelets quantitative changes at different pathologic processes. This platelets' function disturbance will be accompanied by haemorrhagias from gingivas, oral mucosa.

Main aggregatogram indexes:

1. Aggregation angle (α) – index which reflects aggregation coming velocity; it is determined by aggregatogram curve ascent steepness after aggregation inductor addition.

2. Aggregation time (t_1) – it is measured from aggregation initiating (beginning) till point on aggregatogram curve corresponding to maximal aggregation; it characterizes aggregation degree.
3. Aggregation latent peiod duration (t) – index which reflects processes not-registrated on photoelectrocalorymeter (PEC).
4. Desaggregation angle (β) – index which reflects desaggregation process velocity.
5. Aggregation height (h) – index reflecting aggregation degree; it corresponds to thrombocytic plasma optical density decreasing descent in course of aggregation.

Control questions.

1. Thrombocytes, amount, structure.
2. Platelets functions.
3. Vascular-platelet haemostasis mechanism.
4. Microcirculative haemostasis and its significance in dentistry.
5. Thrombocytopoiesis and its regulation.

Some normas:

- thrombocytes amount – 200-400 $x10^9$/l – at counting in chambers; (180-320 $x10^9$/l) – at counting in automatical analizators;
- width – 0,5-0,75 mcm;
- length – 1-4 mcm;
- juvenile thrombocytes – 0-0,8 %;
- mature thrombocytes – 90,3-95,1%;
- old thrombocytes – 2,2-5,6%;
- degenerative – 0-0,2%;
- irritational forms – 0,8-2,3%;
- thrombocytes life duration – 5-11 days.

Thrombocytopenias reasons (thrombocytes amount is less than 140 x 10^9/l):

- aplastic anaemias;
- megaloblastic anaemias;
- myelodysplastic syndrome;
- leukemias;
- lymphomas;
- malignant tumors metastases in bone marrow;
- accumulation diseases (Gausher's, Niemann-Pick's et al.);
- radiation disease;
- cytostatic therapy;
- immune thrombocytopenia;
- splenomegaly;
- disseminated intravascular coagulation (DIC) syndrom;
- haemolytical-uremical syndrom;
- viral infections;
- septicaemia;
- chronic hepatitis;

- liver cirrhoses;
- liver tumor.

Thrombocytosis reasons (thrombocytes amount is more than 500 x 10^9/l):

- thrombocytosis without any distinct reason;
- chronic myeloid leukemia;
- erythremia or Vakez' disease (or real polycythaemia);
- subleucaemic myelosis;
- iron-deficient anaemia;
- acute haemorrhagia;
- malignant tumors;
- state after splenectomy (up to 2 months) and other operations (up to 2 weeks);
- rheumatism;
- tuberculosis;
- haemolytic crisis and so on.

Indexes which are estimated mainly in English-speaking countries additionally to mentioned indexes:

1. ***Platelet average volume (PAV or MPV – mean platelet volume).***
   MPV (norm): 7-11 fl (femptolitres).
   MPV↑:
   - Bernard-Soulier's disease;
   - idiopathic thrombocytopenic purpura;
   - hyperthyreosis;
   - after splenectomy;
   - diabetes mellitus;
   - thalassaemia;
   - before labour;
   - systemic red lupus and so on.

   MPV↓:
   - splenomegaly;
   - liver cirrhosis;
   - megaloblastic anaemias;
   - aplastic anaemias;
   - myelodysplastic syndrom;
   - cytostatic therapy;
   - radiational therapy.
2. ***Thrombocytocrite or plateletcytocrite (PCT=MPVxPLT)*** i.e. average platelet's volume multiple on thrombocytes amount; the meaning of this index is the following: thrombocytic mass percentage in blood volume.
   PCT (norm): 0,15-0,35%
   PCT depends mainly on PLT (platelets amount) but while haemostasis platelet link assessment one uses both indexes.
3. ***Platelets distribution dyspersion on their volume (PDW).***
   PDW (norm): 10-15%.

PDW↑ (platelets anizocytosis):

- immune thrombocytopenias;
- some thrombocytopathies;
- myeloneoplastic diseases.

PDW↓:

- aplastic anaenia;
- myelodysplastic syndrome;
- leukemias;
- malignant tumors metastases in bone marrow;
- accumulational diseases (Gausher, Nimann-Pick's);
- radiational disease;
- cytostatics usage;
- immune thrombocytopenias;
- splenomegaly;
- DIC-syndrom;
- haemolytical-uraemic syndrome;
- viral infections;
- septicaemia;
- chronic hepatitis;
- liver cirrhosis;
- liver tumors;
- megaloblastic anaemia and other diseases.

Platelets functions assessment criteriums:

- platelets absolute quantity;
- capillary bleeding time;
- platelets aggregational activity;
- blood clot retraction;
- prostaglandines (thromboxanes, prostacyclines) metabolism.

PRACTICAL WORK 25.
BLOOD COAGULATION DISORDERS LABORATORY DIAGNOSTICS MODERN PRINCIPLES.

It's necessary for work: thromboelastograph, saliva, thromboelastogram, water bath, seconds-meter, plasma, 0,27% solution of $CaCl_2$, 5% solution of $CaCl_2$, capillaries, filter paper, centrifuge, thromboplastin, plasma.

Task 1. To study thromboelastogram.

Main thromboelastogram's indexes are the following:

- Reaction time (R) – is measured on direct line from record beginning till thromboelastogram curves dilation in 1 mm. Given segment corresponds to blood coagulation invisible phase, i.e. prothrombinase formation. Norma: 9-14 min.
- K-segment from R end till thromboelastogram dilation in 20 mm. It is blood coagulation visible phase, clot formation time; it depends on forming thrombine concentration and fibrinogen amount. Norma: 5-8 min.
- mA – thromboelastogram branches divergence maximal amplitude is linked mainly with fibrinogen concentration, thrombocytes amount and functional activity.

It's necessary to take into account for thromboelastogram analysis that diagram band velocity is 10 mm per 1 minute. Norma: 48-52 mm.

Task 2. Express-coagulogram.

It is laboratory tests set providing preliminarily but quite exactly to determine blood coagulation and fibrinolysis disorders. Doctor can prescribe differentiated coagulogram after its assessment in patient on its concrete nosologic forme.

Express-coagulogram (normal values):

1. Thromboelastogram: R	9-14 minutes
K	5-8 minutes
mA	48-52 mm
2. Thrombocytes	180-400 x 10^9/l
3. Thrombocytic aggregation: spontaneous	absent
on ADP	present
4. Recalcification time	180-400 sec
5. Prothrombine time	12-15 sec
6. Thrombine time	15-18 sec
7. Fibrinogen	2-4 g/l
8. Ethanole test	negative
9. Prothamine-sulphate test	negative
10. Fibrinogen "B"	negative
11. Fibrinolysis (probe on accelerated reaction)	120-240 min (10 min)

Thromboelastogram is registrated as blood coagulation process objective index. Platelets amount and their aggregation give us the information about microcirculative haemostasis.

Recalcification time – general coagulation test which reveals the most rude disorders in blood coagulation system. Factors deficiency participating in prothrombinase formation internal way influence in the biggest extent on test values.

Prothrombine time – this time prolongation at normal fibrinogen content and normal thrombine time testifies to defiviency of one or some prothrombine complex factors (II, V, VII and X). One should think about hypo- or dysfibrinogenemia or anticoagulants excessment (heparin, fibrinolysis products et al.) into blood at simultaneous thrombine time prolongation.

Thrombine time – characterizes antithrombine, fibrinogen, heparine content. It is prolonged at antithrombine excessment into blood, hypofibrinogenaemia, hypoheparinaemia.

Ethanole, prothamine-sulfate tests and probe to fibrinogen "B" allow to determine "paracoagulation" products – fibrin-monomeric complexes. They are formed as a result of

fibrinogen or fibrin decomposition at fibrinolysis activation. Positive probes testify to blood disseminated intravascular coagulation (DIC).

Probe to accelerated fibrinolysis gives the possibility to evaluate blood (plasma) lythic features.

Some express-coagulogram tests:

- Plasma recalcification time (it is not estimated in English-speaking countries but is still assessed in some Ukrainian clinics) – 0,1 ml of plasma + 0,1 ml of physiological solution, to stay test tube into water bath at 37°C; after 30 sec to add 0,2 ml of 0,277% $CaCl_2$. To determine plasma coagulation time by means of second arrow.
- Thrombine time – 0,1 ml of healthy person plasma + 0,1 ml of physiological solution and in 60 sec after test tube heating in water bath to add 0,1 ml of thrombine standard solution. To determine clot time formation time on stop-watch.
- Prothrombine time – 0,1 plasma of a healthy person + (after heating on water bath in course of 60 sec) 0,2 ml of thromboplastine-calcium mixture and to determine clot formation time on stop-watch.
- Fibrinogen content determining – 0,5 ml of plasma + 0,1 ml of 5% $CaCl_2$. To wait for solid clot appearence, to carry it to paper filter and to dry till dry-air state, to weigh it on torsion weight, to divide the result received into 2. We receive fibrinogen concentration in g/l.
- Reaction on ethanol (ethanol test) – 0,4 ml of plasma + 0,15 ml of 50% ethanol solution, to shake up the test tube and to switch on stop-watch. To determine in 10 min at room temperature whether gel clot occured in plasma. Probe is considered positive at clot existance.
- Fibrinogen "B" determining. To add 2 drops of beta-naphthol to 0,5 ml of investigated plasma. To shake up test tube and to leave it at room temperature for 10 minutes. Stock-taking is performed by the following way: plasma sediment (+), small granulose flakes falling (++), rude flakes falling (+++), clot formation (++++).

Control questions.

1. Coagulational factors.
2. Coagulation mechanism.
3. Fibrin and fibrinogen degradation products and their role in haemostasis.
4. Positive paracoagulational probes at dental diseases.

PRACTICAL WORK 26.
DIFFERENTIATED COAGULOGRAM
(HAEMORRHAGIAS TYPES DIFFERENTIATED DIAGNOSTICS)

It's necessary for work: coagulograms sets.

Task 1. Coagulogram for DIC-syndrom (disseminated intravascular coagulation) diagnostics

DIC-syndrom is rather widely-spread pathological state. Particularly, such reaction is observed at:

- shocks different forms;

- sepsis;
- acute intravascular haemolysis;
- massive haemotransfusions syndrom;
- therminal states;
- acute kidney insufficiency;
- malignant tumors;
- traumatical operations;
- oesophageus and stomach chemical burns;
- obstetric pathology;
- physiological labours;
- intoxications and so on.

Possible DIC-syndrom reasons in dentistry:

- after shock;
- facial-mandibular region traumatic injuries;
- malignant tumors;
- facial-mandibular region abscesses and phlegmones;
- jaws fractures and so on.

Next coagulogram (tests set) is essential for proper DIC-syndrom diagnostics:

	Norma:
1. Thromboelastogram: R	9-14 minutes
K	5-8 minutes
mA	48-52 mm
2. Thrombocytes	180-400 x 10^9/l
3. Thrombocytic aggregation: spontaneous	absent
on ADP	present
4. Thrombine time	15-18 sec
5. Fibrinogen_	2-4 g/l
6. Antithrombine III	80-100%
7. Ethanole test	negative
8. Prothamine-sulphate test	negative
9. Fibrinogen "B"	negative
10. Fibrinogen degradation (derivative) products	7,3±3,9 mg %
11. Euglobuline fibrinolysis	120-240 min

Acute DIC-syndrom determining is elicitated by the fact that it may be one haemostasis disorder at some pathology types. For example, at shocks, therminal states, sepsis hard forms, massive traumas and burns, acute intravascular haemolysis DIC is disease constant component, its inalienable part. DIC is diagnosted similtaneosely with main disease recognizing and its therapy is begun immediately.

First, for DIC-diagnosis one can perform simple methods: blood coagulation general time (norma: 5-8 min), prothrombine and thrombine time, paracoagulational tests indexes (ethanole, protamine-sulphate), platelet amount. Then one can add also other tests that prove DIC-syndrom picture.

In a whole, in clinic practice for DIC-syndrom dyagnostics one should perform next tests: platelet amount, platelet aggregation, fibrinogen content, APTT (activated partial thromboplastine time in plasma pour on thrombocytes), recalcification time, prothrombine

time, antithrombine III (in plasma pour on platelets), fibrinogen degradation products, soluble fibrin-monomeres determining – ethanole and prothamin-sulphate probes (in plasma pour on thrombocytes), fibrinolysis (in plasma pour on thrombocytes), fragmented erythrocytes determining.

Changes at acute DIC-syndrom form:

- platelets amount is up to 150 x 10^9/l and less;
- fibrinogen concentration is up to 1,5 g/l and less;
- activated partial thromboplastine time is up to 50 sec and more (norm: 30-40 sec) – hypocoagulation sign;
- recalcification time is up to 80 sec and more.

These changes are connected with plasma factors consumption as well as fibrin derivative products antithrombine action. Other disorders are the following:

- prothrombine time prolonging (due to platelets and blood coagulation factors consumption as as fibrin derivative products antithrombine action) – at acute and subacute forms;
- thrombine time prolonging on 10 seconds comparatively to normal indexes (it is linked with hypofibrinogenaemia and fibrin derivative products antithrombine action);
- at platelets aggregation investigation with main biological stimulators (ADP, adrenaline, collagen) in patients with acute and subacute DIC-syndrom forms expressed platelets hypoaggregation is observed as a result of hypothrombocyteaemia and transitory hypofunction caused by thrombine, ADP, adrenaline, prostaglandine and other proaggregants action to thrombocytes (it leads to platelets degranulation);
- positive probes on gel-formation with ethanol and prothamine-sulphate are very essential at DIC-syndrom diagnostics: intermediate products of fibrinogen transformation in fibrin are occured in blood in course of this syndrom; these substances form fibrin-like gel at presence of mentioned substances (ethanol, beta-naphtol and prothamine-sulfate); such phenomenon is named as paracoagulation; under norma concentration of these products is still small that ethanol and prothamine-sulphate don't cause gel formation (negative probe); fibrin degradation products content is more than 10 mcg/ml at DIC-syndrom that is delt with fibrinolysis secondary activation and plasmine occurence in blood in concentrations significantly more than under norma; sometimes it leads to decomposition of not only fibrinogen and fibrin but also other blood coagulation factors.

For secondary fibrinolysis fast diagnosis one of optimal test is the following: healthy person native blood, healthy and sick person native blood mixture + thrombin; then to observe formed blood clots dissolving. At high fibrinolysis level blood clot formed in healthy and sick blood mixture is dissolved before observant eyes (melt like sugar in a cup of tea); at the same time blood clot of a healthy person doesn't dissolve for many hours.

DIC-syndrom specific expression is microangiopathic haemolytic thrombotic anaemia, signs of which are observed at all this syndrom forms. Its essence is in following: fibrin fibres are accumulated in microcirculative vessels; these fibres injure erythrocytes stroma and retard their passage through capillaries. As a result of this erythrocytes haemolysis acceleration occurs; their osmotic resistance decreasing, plasma satiation with bilirubin (free, non-conjugated).

Proggressing antithrombine III content decreasing is one of the earliest DIC-syndrom sign. Being main physiological anticoagulant, antithrombin III reacts to any activation of haemostatic system procoagulant link. Given progress is the most expressed in DIC-syndrom patients in consumption coagulopathie stage. It explains heparin application ineffectiveness without simultaneous antithrombine III concentration introduction (it contains in fresh-frozen plasma or the "freshest" warm donor blood but in conserved blood antithrombin III is absent!)

If it's impossible to perform all mentioned tests under laboratory conditions then obligatory for DIC-syndrom diagnosis are the following methods: platelet number, prothrombine and thrombine time, probe with ethanole and prothamine-sulphate, antithrombine III and fibrinolysis. The others methods may be used as addictive and proving the diagnosis.

But every doctor must take into account that in every specialized clinics DIC-syndrom course (and its therapy correspondingly) are strongly differentiated.

Task 2. To assess haematomic haemorrhagia type.

Distinguishing signs for this haemorrhagia type are the following: deep, tensed, painful haemorrhagias in articular cavities, muscles, subcutaneous fat, in retroperitoneal space and other places. One can observe also spontaneous nasal, nephral, gastro-intestinal bleedings. Such haemorrhagias type may be at haemophily, at factor VIII or IX immune inhibitors occurence in blood (the most often in women after labour and in pregnant women with immune diseases), at indirect anticoagulants overdosage and others.

Bleedings from oral cavity are in dentistry at haemophilia.

Typical coagulogram necessary for this haemorrhagia type determining must include following tests:

	Norma:
1. Factor VIII	70-150%
2. Factor IX	70-150%
3. Prothrombine time	12-15 sec
4. Thrombine time	15-18 sec
5. Fibrinogen	2-4 g/l
6. Fibrinogen degradation products	7,3±3,9 mg%
7. Ethanole test	negative
8. Prothamine-sulphate test	negative
9. Fibrinogen "B"	negative

Task 3. To assess microcirculative (petekchio-spotted) haemorrhagia type

Distinguishing features are: capillary bleedings, petekchias on skin, painfulless ekchimoses, gingival, nasal bleedings, menometrorrhagias, bleedings at othorhinolaryngologic operations.

This bleeding type is observed at all forms of thrombocytopenias, platelets defects, fibrinogen hereditary deficiency, II, V and X coagulation factors genetic and aquired deficiency.

In dentistry any gingival bleedings may serve as suspicion to any of these states. Coagulogram in such a case must include such probes as:

Norma:

1. Thrombocytes	180-400 x 10^9/l
2. Thrombocytic aggregation: spontaneous	absent
on ADP	present
on adrenaline	present
3. Platelet adhesiveness	20-50%
4. Factor III activity	-
5. Factor 4 activity	-
6. Clot retraction	48-64%
7. Bleeding time	2-4 min
8. Prothrombine time	12-15 sec
9. Thrombine time	15-18 sec
10. Fibrinogen	2-4 g/l

Task 4. To assess mixed (microcirculative-haematomic) bleeding type

Distinguishing signs are the following: petekchio-spotted bleedings together with painful, tensed haematomas in subcutaneous and retroperitoneal fat, abdominal cavity, visceral organs. Described haemorrhagia type is observed at Willebrand's disease, VII and XIII factors deficiency, at complex deficiency of prothrombine complex factors (II, V, VII, X) and of factor XI which is also delt with liver disorders or vitamine K intestinal absorbtion disturbances, for example, at mechanical jaundice.

Main sign in dental practice is bleeding from oral cavity, gingival bleedings.

Coagulogram must be the following:

	Norma:
I. For Willebrand's disease exclusion:	
1. Thrombocytes	180-400 x 10^9/l
2. Thrombocytic aggregation: spontaneous	absent
on ADP	present
on adrenaline	present
3. Platelet adhesiveness	20-50%
4. Bleeding time	2-4 min
5. Willebrandt's factor	-
II. For II, V, VII, IX and X factors deficiency exclusion:	
1. Prothrombine time	12-15 sec
III. For factor XIII (fibrinase) deficiency exclusion:	
1. Fibrinase activity determining	70 sec (100%)

Task 5. To get aquanted to doctor tactics at vasculite-purpure and microangiomatose bleedings types

1. *Vasculite-purpure type* - is characterized by haemorrhagies caused by multiple local inflammatory processes in microvessels of skin, mucosa, inner organs (kidney, lungs, intestine), the most often of immune genesis. Such bleedings are observed at haemorrhagic vasculitis of Shenlein-Genoch's, viral fevers. Localization – gingival, nasal, uterine, pulmonal, gastro-intestinal bleedings. Doctor's tactics - to propose to laboratory to determine tests applied for DIC-syndrom diagnosis because this haemorrhagia type is a characteristic of DIC haemorrhagic phase and it is its expression.

2. *Microangiomatose type* – is characterized by strong, long-termed, repeated bleedings from nose, oral cavity, kidney, lungs, gastro-intestinal tract. It is observed at hereditary teleangioectasia different variants. Doctor tactics – blood mustn't been taken for analysis! It's necessary to perform only endoscopic investigation because vessels are not bleeded out of teleangioectases and all haemostatic probes will be under norm!

Control questions.

1. Haemostatic disorders types.
2. DIC-syndrom diagnosis.
3. How to evaluate microcirculative haemostasis state?
4. What is essential for such assessment?

PRACTICAL WORK 27.
FIBRINOLYSIS LABORATORY INVESTIGATIONS

It's necessary for work: watery bath, stop-watch, centrifuge, plasma, 1% solution of acetic acid, borate solution, fibrinolysin (plasmin) solution, 0,277% solution of calcium chloridum, distillate water, saliva.

Task 1. Blood fibrinolytic activity determining.

8,5 ml of distillate water + 0,15 ml of 1% solution of acetic acid and 0,5 ml of investigated plasma, mix and put in a fridge for 30 min. After this test tube is centrifugated at 1500 rotations per minute in course of 5 min. Then liquid is poured, test tube is turned over onto filter paper for several minutes to be dry. Investigator must add 0,5 ml of boric acid to sediment and to dissolve the sediment with glass stick, then it's necessary to add 0.1 ml of fibrinolysin solution and 0,1 ml of 0,277% of $CaCl_2$, content is mixed and is putted into bath at 37°C switching on the stop-watch. Norma: 120-240 min.

Task 2. Fibrinolytic bleeding laboratory diagnostics principles.

Blood fibrinolytic activity increasing as a rule is observed in a case of blood coagulation activating. That's why fibrinolysis as haemorrhagias primary reason is a very seldom phenomenon. But in a case of fibrinolysis expressed activation one can see complete degradation not only of fibrinogen but also other coagulational factors. Under these conditions real bleeding is developed which is only may be situated among fibrinolytic bleedings. Unfortunately, doctor in clinical practice without sufficient bases (without this process laboratory diagnostics) makes the diagnosis "fibrinolytic bleeding". Such situation may be with dentist too at alveolar bleedings after tooth extraction or other operations in oral cavity. To deny any suspicion (or to prove it, on the contrary) about possible fibrinolytic bleeding one must send to the laboratory following tests sets:

	Norm:
1. Natural clot lysis	10-20%
2. Probe on accelerated fibrinolysis	10 min
3. Fibrinogen derivative products	7,3± 3,9mg%

4. Ethanole test negative
5. Prothamine-sulphate test negative
6. Fibrinogen "B" negative

If natural clot lysis index is decreased but probe to accelerated fibrinolysis is increased with simultaneous fibrinogen derivative products reducing that it testifies to blood lytic features decreasing. In course of contrary change and positive paracoagulational probes existance – it is concluded about fibrinolytic system activation.

Control questions.

1. Fibrinolytic system factors.
2. Plasminogen external and internal activators.
3. Fibrinolysis scheme.
4. Fibrinolysis assessment methods.

PRACTICAL WORK 28.
BLOOD GROUPS

It's necessary for work: blood, china plate, scarificators, standard group-specific sera, subject glasses, glass sticks, antirhesus serum, Tsoliclones anti-A and anti-B.

Task 1. To determine human being blood group on ABO system:

A. By means of group-specific sera:

To pour blood sera on china plate correspondingly to blood groups designations. To process the finger and to prick it with scarificator. To place blood drop in a plate central nest. To add blood to the serum (in a correlation of 1:10) with clean subject glass separate angles. To get the plate rocking in course of 3-5 minutes. To mark the nets where agglutination reaction occured. To determine blood group.

B. By means of Tsoliclones anti-A and anti-B:

To pour Tsoliclones anti-A and anti-B on 1 big drop (0,1 ml) on the plate under corresponding writings. To pour investigated blood near drops in 10 times less than antibodies drop. To mix with glass stick (different in every drop). To get the plate rocking in course of 2-3 minutes. The result in every drop may be positive or negative. To determine blood group.

Results interpretation:

- if agglutination reaction is absent with all group-specific sera and with all (both) Tsoliclones, than given blood group doesn't contain antigenes A and B, thus it belongs to blood group 0(I);
- if agglutination reaction took place with I and III serum and Tsoliclone anti-A, then given group contains antigene A and belongs to A(II) group;
- if agglutination reaction occured with I and II sera and with Tsoliclone anti-B, then given blood group belongs to B(III) blood group;
- if agglutination reaction took place with sera of I, II, III groups and with both Tsoliclones, then investigated blood contains both antigenes A and B and blood belongs to the group AB (IV).

Task 2. To determine rhesus-factor while express-method usage.

To pour 1 drop (20 divisions of Panchencov's capillary pipette) of anti-rhesus serum to the investigated blood on test tube floor. To shake up the test tube and to turn over several times so that its content was flowing on the walls. To add 2-3 ml of 0,85% solution of NaCl solution in 3 min. To mix test tube content after its 1-2-folded turning over. Don't shake up!

To perform results assessment on agglutination absence or presence (large flakes on the background of enlighten liquid).

Task 3. To perform probe on individual compatibility.

To pour recipient blood plasma on subject glass. To add donor blood drop less in 10 times than plasma (in a correlation of 1:10) to this plasma. To evaluate their compatibility.

Control questions.

1. Blood groups discovery.
2. Representations about erythrocytic antigenes.
3. Data about blood groups systems.
4. Agglutinogenes and agglutinines. Main principles of blood division on groups.
5. Blood transfusion rules.
6. Rhesus-factor and its importance for clinics.
7. Knowledge about blood groups significance for doctors of different specialities and for any human being in their daily life.