Laboratory Practicals in Physiology

Contents

I. BLOOD

1. Methods of blood sampling

a) Capillary blood collection:

The ring-finger of the left hand is the most common source of capillary blood. Sometimes the earlobe or the heel is used (in newborns). To disinfect the skin wipe off the fingertip with a piece of cotton ball soaked in alcohol, then let it dry. While holding the subject's finger in one hand, prick the fingertip with a disposable needle in 2-3 mm depth with a firm movement of the other hand. After that the needle should be withdrawn and disposed of immediately.

Wipe off the first drop of blood with a piece of cotton ball because this drop is contaminated with tissue fluids. The subsequent blood drops are used for the laboratory tests. Squeezing the fingertip will result in a mixing of blood with tissue fluid which may cause error in the tests.

After blood sampling, a piece of cotton ball is pressed on the puncture site until the bleeding stops.

b) Venous blood collection:

Larger volume of blood in adults can be obtained from the cubital vein. The person taking the blood samples must wear gloves on both hands. When the procedure is terminated the gloves should be thrown into a container and they will be disposed of.

The subject should be in a lying or sitting position. A rubber sheet is placed under the subject's elbow, and a tourniquet is put on the upper arm. The fist is clenched. (The tourniquet has disadvantages: hypoxia in the arm may damage the red blood cells (RBC) resulting in hemolysis; pressure may alter the concentrations of substances (lipids, hormones, proteins) in the blood. Yet, the tourniquet makes venipuncture easier). The skin over the selected vein is disinfected with a piece of cotton ball soaked in alcohol. The subject's elbow is held in one hand with the thumb placed on the skin over the vein 3 cm below the venipuncture site. The thumb fixates the vein. With the sharp end of the needle pointing toward the skin, a sterile needle is inserted into the vein at an angle of approximately 35 degrees. The needle is pushed forward 1 cm in the lumen of the vein. The tourniquet is removed as soon as blood flow is established.

The blood is collected in special tubes. (By using laboratory test tubes the risk of accidental infections with microorganisms transmitted by blood is reduced). The blood collecting tubes are prepared with the required anticoagulants. Finally, a piece of cotton ball is placed on the site of the venipuncture and the needle is withdrawn. The cotton is pressed on the skin until the bleeding stops.

Direct contact with blood to be avoided!

2. Anticoagulants

Ethylenediamine tetraacetic acid (EDTA), Na-citrate, and heparin are used as anticoagulants in the laboratory practice.

c) EDTA forms chelate complexes with $Ca^{2}+$. The removal of free ionic $Ca^{2}+$ inhibits coagulation. Practically, EDTA-K_2 is dried in the test tubes. EDTA is used in the following tests: cytomorphological examinations, cell counts, hematocrit, haemoglobin measurements.

d) Na-citrate acts by removing free Ca^{2}+ from the blood by forming Ca-citrate. Na-citrate is used in blood clotting tests and during the determination of blood sedimentation rate.

e) Heparin is an injectable (i.e., also used in vitro) anticoagulant. It has antithrombin activity. The advantage of heparin is that it does not alter the size of the red blood cells and therefore it can be used in the hematocrit test. Since heparin does not induce remarkable hemolysis it is used when testing the osmotic fragility of RBCs.

3. Blood typing and compatibility tests

Blood group serology techniques are based upon antigen-antibody reactions. Blood group antigens are present on the membranes of the RBCs, whereas the antibodies are found in the serum. *In vitro* (i.e. within glass) the antigen-antibody reaction results in agglutination of the RBCs. This phenomenon is called hemagglutination.

ABO blood typing:
There are four major ABO groups (A, B, AB and O).

blood group	genotype	antigen	antibody (agglutinin)
A	AA or AO	A	anti-B
B	BB or BO	B	anti-A
AB	AB	A and B	-
O	OO	-	anti-A and anti-B

Blood typing is based on the **Landsteiner's principle:** the naturally occurring antibodies (agglutinins) in the serum of a subject (anti-A, anti-B) never correspond to the subject's antigens (A,B).

Before a recipient receives a transfusion, a compatibility test between donor and recipient blood must be done. The one-sided test determines the antigens on the RBCs. The two-sided test determines both the RBC antigens and the anti-A and anti-B agglutinin contents of the serum and therefore it is much more reliable than the one-sided test. In the one-sided test, RBCs with weak antigenic character may remain unnoticed. For example, a weak type A antigen may not agglutinate with antibodies in the O or B sera, and the blood is falsely typed as Group O. A two-sided test, however, will discover the anti-B agglutinins in the serum.

a) One-sided ABO blood typing:
Completely clotted venous blood is used. The blood is diluted with isotonic saline (0.9%); the suspension should contain 10 % blood. The test sera containing the agglutinins for the blood typing are supplied in glass capillaries or in 1 ml vials. **Remember: agglutinin against antigen A is coming from a person, having blood group B, agglutinin against antigen B is coming from a person having blood group A, etc.**

The tests are performed at room temperature. Different areas of a white tile are marked "O", "A", "B", and "S" ("S" for the serum of the subject = autoserum, for control).

One drop of test serum O (containing antibody against A and B) is placed in the area "O"; one drop of the test sera A (containing antibody against B) and B (containing antibody against A) are placed in the areas "A" and "B" of the tile, respectively.

One drop of the 10% suspension of the patient's blood (diluted with physiological saline) is added to each drop of test sera and to the autoserum. Each serum-blood suspension is mixed by using a glass rod or the corner of a glass slide. After each test site, the glass rod must be wiped off carefully. When using a slide change the corner before mixing the subsequent blood-serum suspension. This is important because mixing of the different antibodies carried by the rod or the slide from one place to the other leads to false positive reaction.

After about 0.5 min, the tile is tipped back and forth at 30 degrees. The blood group is determined only after a 5 min incubation period even if apparent agglutination is observed sooner than 5 min. The reason for the 5 min waiting period is to decide if agglutination between the patient's RBCs and serum (autoagglutination) develops in the "S" area. In the case of autoagglutination, the blood typing is likely to give false positive result and therefore the blood group cannot be determined with the method described here.

The agglutination is checked by the naked eye. Agglutinated RBCs form clusters, i.e., whenever there is a match between antigen and antibody, blood mixed with the antibody no longer forms a homogenous drop, but there will be an immunological reaction, an agglutination. With this method the blood group is determined as follows:

	antibody in the test serum			
	anti A&B	anti A	anti B	none (own serum)
blood type				
A	**agglutination**	**agglutination**	no reaction	no reaction
B	**agglutination**	no reaction	**agglutination**	no reaction
AB	**agglutination**	**agglutination**	**agglutination**	no reaction
0	no reaction	no reaction	no reaction	no reaction

ABO blood group determination

b) Rh blood typing:

Using the patient's serum (clotted blood) a 50% RBC suspension is produced.

The test is performed on a white tile at room temperature by using an anti-D test serum (monoclonal antibody). Different areas of the white tile are marked "anti-D" and "S" ("S" for the serum of the patient = autoserum)

One drop of anti-D test serum and one drop of the 50% suspension prepared from clotted blood are placed next to each other in the "anti-D" test area. The drops of are mixed by using a glass rod or the corner of a glass slide.

In the "S" area, one drop of the 50% suspension of the patient's blood is mixed with the autoserum. The tile is tipped back and forth for 3 min. Then the drops are examined. Clumped RBCs indicate agglutination. The Rh negative blood remains homogeneous. If agglutination is found in the "S" area then the Rh type cannot be determined using the method described here.

c) Cross-agglutination:

This test must be performed before transfusion because the ABO and Rh typing cannot determine all the possible mismatches between the blood of the donor and the blood of the recipient. The

"major" test checks whether the donor's RBCs are agglutinated by the serum of the recipient. The "minor" test uses the recipient's RBCs and the donor's serum. The antibodies of the donor blood are greatly diluted in the plasma of the recipient and therefore severe transfusion reaction is not likely. In contrast, the antibodies in the plasma of the recipient may cause massive agglutination of the RBCs in the donor's blood. Therefore, the "major" test is far more important than the "minor" test, and the "major" test must always be performed before transfusion.

The test is performed on a bottle filled with warm water (39-42 ^{o}C). Two test sites are selected on the surface of the bottle. One drop of the recipient's serum is placed in each test area. The serum is obtained by centrifugation of a blood sample.

One drop of the donor blood is mixed with the drop of the recipient's serum at one of the test sites.

The other test site is to study the possibility of autoagglutination. Therefore, a 50% RBC suspension is prepared from the recipient's RBCs and recipient's serum. One drop of this suspension is mixed with the serum sample at the second test site.

The bottle is tipped back and forth and the agglutination is examined after 5 min of incubation. If agglutination is noted on any of the test areas this donor's blood preparation should not be transfused. Hemolysis (varnish color) also indicates incompatibility; the blood should not be transfused.

The "minor" test follows the same procedure as the major test except that a suspension prepared from the RBCs of the recipient blood and a plasma sample of the donor blood are used. If agglutination occurs the blood should not be transfused.

Major test	Minor test	Control
donor RBC	recipient RBC	recipient RBC
+	+	+
recipient serum	donor plasma	recipient serum

d) Biological test before blood transfusion:

This is the final test to notice incompatibility. A volume of 25 ml of blood is infused to an adult patient at a high speed. Then the infusion is continued at a low speed and the patient is observed for 3-5 min (general condition, respiration, circulation). The infusion at high speed followed by observation is repeated until 75 ml of blood is infused. If pathological symptoms are not observed the test is negative; the transfusion can be continued at a normal speed.

Blood group serology with Serafol bedside card:

Serafol™ ABO and Serafol™ ABO+D are used to confirm patient blood type immediately before a blood transfusion (bedside test). The bedside test is used to confirm the recipient's ABO or D blood group antigens previously determined and thus ensures that there is a match between the recipient's blood group and the conserved product. This identifies any mix-ups that may occur.

With the Serafol bedside card the ABO identity can be checked in a very quick and simple way. Monoclonal test reagent are applied to the surface of the plastic card in four circular areas which are spatially separated from each other and dyed in order to avoid additional labelling mistakes. These monoclonal reagents provide strong and rapid agglutination.

Test procedure:

1. Place one drop of isotonic saline solution on each reaction field and auto-control field.
2. Add one drop of blood to each field of the card.

3. Stir each field with a stick for approx. 30 seconds. The reagents must dissolve completely.
4. Gently rock the card back and forth for approx. 30 to 60 seconds, then check each field for agglutination.
5. Dry the reaction mixtures and cover with self-adhesive film.

4. Bleeding time

The bleeding time is the most basic test to evaluate thrombocyte function.
DUKE method: A sterile disposable needle or a special lancet is used for pricking. The fingertip is wiped of with alcohol and pricked in 3-4 mm depth. The blood drops are wiped off with a piece of filter paper every 30 sec, until the blood no longer stains the paper.
Normal value: 1-3 min

IVY-method: A blood pressure cuff is inflated to 40 mmHg on the subjects upper arm. The volar surface of the lower arm is cleaned with alcohol and a superficial incision (10 mm long and 1 mm deep) is made with a sterile lancet avoiding visible blood vessels. The blood drops are soaked up every 30 seconds with a piece of filter paper, until the blood no longer stains the paper.
Normal value: 3-5 min.

A prolonged bleeding time indicates deficiency or decreased number of thrombocytes (thrompocytopenia) or impaired blood vessels. The depth of the puncture or incision can be a source of error.

5. Clotting time

LEE-WHITE method: Approximately 5-6 ml of venous blood is collected. The blood is immediately distributed in 3 test tubes that were prewarmed in a 37^{o}C waterbath. Blood clotting is tested by tipping the tube back and forth every 30 seconds. The clotting time is measured when the blood does not flow out of the test tubes when tilted horizontally. The clotting time is calculated by averaging the results obtained with the 3 test tubes. The normal value is 5-8 min.

Blood clotting time longer then 10 min is pathological. This test measures the endogenous way of blood coagulation. The sensitivity is low.

6. Prothrombin time (Quick-time)

Principle: this test determines the amount of prothrombin in the sample. The speed of coagulation depends on the concentration of prothrombin.

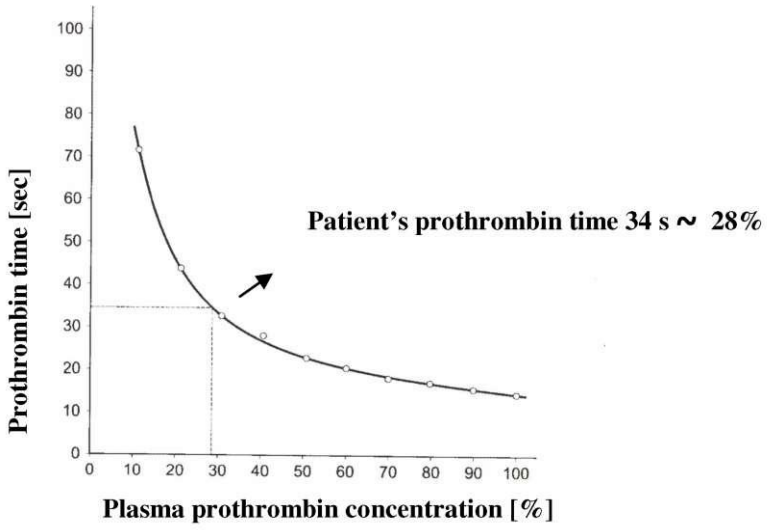
Venous blood is collected. First, free Ca ions in the blood are eliminated by means of Na-citrate to prevent clotting in the sample. Then calcium, thromboplastin and phospholipid containing thrombokinase, needed for coagulation is added in excess to the plasma, thus clotting only depends on the presence and amount of prothrombin. The prothrombin time is measured from the timepoint the reagents are added to the plasma until the fibrin clot develops. The prothrombin time tests the activities of fibrinogen, prothrombin and factors V, VII and X.

Perfomance: 4.5 ml of venous blood is drawn into a sterile syringe already containing 0.5 ml of 3.8% Na-citrate. The citrated blood is centrifuged as soon as possible, max. 2 hours after the blood collection, for 10 min at 1000 g. Then 0.1 ml plasma is pipetted onto a silicon/ wax covered watch glass and placed in a 37 ^{o}C water bath for 3 min. The thrombokinase reagent is also prewarmed in a test tube at 37 ^{o}C.

0.2 ml of prewarmed thrombokinase reagent (Simplastin D®) is added to the 0.1 ml of plasma in the watch glass and the timer is started. Approximately every second, the tip of an injection needle is pulled through the plasma. The prothrombin time is recorded when the first fibrin fiber appears at the tip of the needle. The normal value is: 18-20 seconds.

Prothrombin calibration curve:

To calculate the percentage of normal activity requires determination of prothrombin times in a dilution series of normal plasma. Blood is collected from 10 healthy subjects as described above. The prothrombin time is measured for each subject. Plasma samples with abnormal values are discarded. The plasma samples with normal prothrombin time are pooled and diluted with physiological saline so that the final plasma concentrations in the test tubes should be 10, 20, 30, 40, 60, 80% and 100% (undiluted plasma). The prothrombin time is measured for each tube. Using these values, the prothrombin time (sec) is plotted as the function of the plasma (prothrombin) concentration (%). When an unknown patient's prothrombin time is measured, this value can be compared to the curve and concentration of the normal plasma producing the same prothrombin time as that measured in the patient is read. . This value is the prothrombin time expressed as the percentage of the normal prothrombin activity. The normal value is between 70 and 130%.



Prothrombin calibration curve

International Normalized Ratio (INR)

In clinical practice INR was introduced to make the prothromin times determined by different reagents comparable. Every manufacturer determines the *International Sensitivity Index* (ISI) of the reagents they produce. This value is between 1.0 and 1.4 which shows how the activity of the reagent correlate to the international standard. The value of the INR is calculated by the following formula:

$$INR = \left(\frac{PT_{test}}{PT_{normal}} \right)^{ISI}$$

PT_{test} : prothrombin time of the patient PT_{normal} : prothombin time of a healthy subject
ISI: International Sensitivity Index, an individual value characteristic for the particular reagent.

7. Partial thromboplastin time (PTT, theoretically only)

Principle: the activity of plasma clotting factors is measured in the presence of calcium and a phospholipid reagent substituting thrombocyte factor 3.

Na-citrate is added to the blood sample in a ratio of Na-citrate : blood = 1 : 9, and the mixture is centrifuged. Te obtained serum is used. The time elapsed until the appearance of the first fibrin thread is measured after the addition of calcium and a phospholipid reagent (e.g. a cephalin-containing preparation) to the serum at 37 ^{o}C. This method is sensitive to all plasma clotting factors except factors VII and XIII.

The normal value of PTT is strongly dependent on the phospholipid reagent; it ranges from 40 to 90 seconds. Increased PTT indicates a deficiency of plasma clotting factor(s). The deficient plasma clotting factor(s) can be identified by adding plasma which lacks a particular clotting factor.

For example, if addition of plasma obtained from a patient with a known deficiency of factor VIII fails to correct the increased PTT, but addition of plasma deficient in factor IX does, then the plasma examined probably lacks factor VIII.

8. Thrombin time (theoretically only)

Priciple: the time needed for the coagulation of blood plasma, the clotting of which has been prevented by the addition of sodium citrate (citrate : blood = 1 : 9), is determined upon adding a solution of thrombin of known activity at 37 ^{o}C. The time interval elapsed between the addition of thrombin and the development of the fibrin clot is defined as thrombin time. Thrombin time is dependent on the plasma concentration of fibrinogen and on antithrombin effects (e.g. inhibition of the thrombin-fibrinogen reaction by degradation products generated from fibrinogen in response to plasmin; or the antithrombin action of heparin). Hence, determination of thrombin time can be used to follow the therapeutic efficacy of heparin. The normal value depends on the thrombin preparation used and is about 25 seconds.

9. Hematocrit

Hematocrit is the ratio of the volume of all cellular elements (the majority is made up by RBCs) to blood volume. Several methods may be used to determine hematocrit. Important: for inhibiting blood clotting, in both methods a chrystallized anticoagulant (heparin or EDTA-K2) is used, which does not change the ratio between the corpuscular elements and the plasma.

a) Macro-technique:
Venous blood is collected. Blood clotting is inhibited with heparin, EDTA-K_2 or sodium oxalate. A graded glass tube (Wintrobe tube or hematocrit tube) is filled with blood upto the "100" mark. The hematocrit tube is placed in a centrifuge tube, balanced on a scale against another tube or with a blank tube, both are put in the centrifuge and centrifuged for 5 min at 10000-20000 g. The hematocrit value (the level where the corpuscular elements and the overlying blood plasma are separated after centrifugation) is read directly from the scale of the Wintrobe-tube.
b) Micro-technique:
A glass capillary is used. The inner surface of the capillary is covered with heparin. The capillary is filled by capillary action with blood obtained from the finger tip. A 1-1.5 cm endportion of the glass capillary is left empty. The other end of the glass capillary is sealed with playdough. The capillary is placed with its sealed end pointing outwards into a hematocrit centrifuge and centrifuged for 5 min. The height of the centrifuged RBC mass is determined by means of a calibrated scale from which the hematocrit value can be directly read. Normal values: male 0.4-0.54
female 0.37-0.47

10. Erythrocyte sedimentation rate as measured by theWestergren method

The sedimentation rate depends on the volume, charge and surface tension of RBCs and on the composition of plasma. Elevated plasma fibrinogen and globulin levels (inflammation) and the

presence of pathological proteins (tumor) in the plasma may increase the sedimentation rate. Physiologically enhanced sedimentation rate can be measured after the third month of pregnancy.

Method: Venous blood (1.6 ml) is withdrawn into a syringe and mixed carefully with isotonic (3.8%) sodium citrate (0.4 ml) solution. The Westergren tube is 300 mm long and has a scale to 200 mm. (Length of the disposable vacuum tubes is different!) The tube is filled with blood upto the "0" mark without any air bubble and is placed in a special stand. After one hour the erythrocyte sedimentation value is read directly from the Westergren tube. The distance (in mm) of the top of the RBC column from the "0" mark provides the sedimentation value; the sedimentation rate is expressed as mm/h. Normal values: male 3-6 mm/h
female 8-10 mm/h

11. Blood cells counts

The number of RBCs, white blood cells (WBCs) and platelets is determined in a given volume of blood. The diluted blood sample is transferred into a Burker chamber and the number of blood cells are counted in several compartments of the same volume in the chamber under microscope (magnification: 120-200 x). An average value is then calculated, and multiplied with the dilution factor. The number of cells is referred to a given unit of blood volume (liter in the SI system).
The Burker chamber is a special counting chamber made of thick glass. Three glass stripes emerge in the middle part separated by thin gaps. The middle stripe is 0.1 mm lower than the neighboring ones. This part is divided into two scaled areas having a surface area of 9 mm each. This is the counting area of the Burker chamber. Since the cover slip used to seal the chamber is 0.1 mm above this area, the volume of the chamber is exactly 0.9 mm . Small springs serve to fix the cover slip. The springs have to be adjusted so that the so called Newton-rings appear on the side of the cover slips. The 9 mm area is subdivided by triple lines into a matrix of 3 x 3 squares, each having a side length of 1 mm. The squares are further

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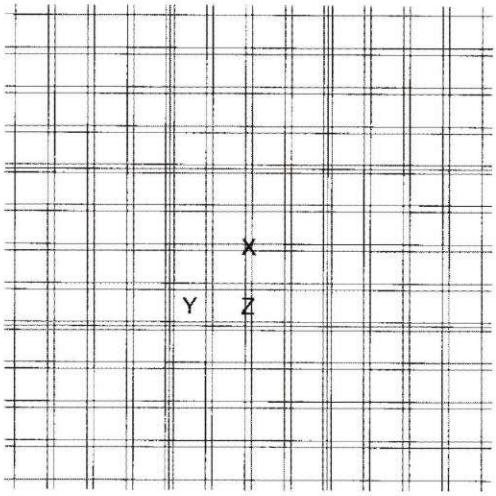


Bürker chamber

X A= 1/400 mm^2
Y A= 1/25 mm^2
Z A= 1/100 mm^2

a) Procedure for counting RBCs:

Wipe off the fingertip of the subject with a piece of cotton soaked with alcohol. Using a sterile, disposable needle, pierce the fingertip. Wipe off the first drop of blood with a piece of dry sterile cotton (the first drop of blood may contain alcohol and tissue fluid), and use the second drop for counting.

Attach the rubber tube to the thick end of the pipet with a red pearl in the container. Fill a dry and clean mixing pipet with blood up to mark "1", than add Hayem solution up to mark "101". Try to avoid bubbles in the blood and in the Hayem solution while filling the pipet. Composition of the Hayem solution: 2.5% Na2SO4, 0.5 % NaCl, 0.025 % HgCl2. This is a hypertonic salt solution which causes shrinking of the RBCs thus preventing the formation of sludges.

Remove the rubber tube, close both ends of the mixing pipet with your fingers and mix the content by gentle shaking for 1-2 minutes. Discard the first few drops from the pipet as this may contain only the Hayem solution using a piece of filter paper. Then fill the Burker chamber by touching the pipet's tip to the edge of the cover slip above the glass stripe in the middle. The capillary effect will draw the mixture of blood and Hayem solution into the chamber.

Cell counting is performed in the small squares (1/400 mm) by using a microscope with a magnification of 300xx to 400 x. Lower the condenser and use small aperture while counting. Count the RBCs in 40 small squares in both counting areas. Calculate the average

3 count per small square and multiple this value by 400.000. The number of RBCs in 1 mm (or 1 |l) of blood is obtained. Remember: supposing a normal RBC count of 5 million/|l means that during the procedure you can expect about 12 RBCs in the counting area. This value can be used to calculate the RBC number in 1 l of blood.

Counting error can result from the cells lying on the lines separating the squares. To reduce the error, count only those cells which lie on the upper and on the left hand edge of the square.

Normal values: male: 4.8-5.2 x 10^{12}/l female: 4.3-4.7 x 10^{12}/l

b) **Procedure for WBCs counting:**

The method for obtaining blood is described above. For the white blood cells, use the smaller mixing pipet with the white pearl in the container. Fill the pipet with blood up to mark "1" and add Türk solution up to mark "11".

Composition of the Türk solution: 0.5 % acetic acid, 1 % methylene blue in water, or gentian violet solution. The solution has an acidic pH and causes hemolysis of the RBCs. The nuclei of the white blood cells are stained.

The procedure for counting is similar to the one used for counting the RBCs except that this time the big squares are used. Multiply the average count obtained from 20-20 big squares by 2.500 to obtain the count of white blood cells in 1 mm (or 1 | l) of blood. Remember: supposing a normal WBC count of 6000/|l means that during the procedure you can expect about 3 WBCs in the counting area. This value can be used to calculate the amount in 1 l of blood. Normal value: 6-8 x 10^9/l.

c) Counting of thrombocytes (Fischer-Germer method):

Fill the pipet (with a red pearl in the container) with blood as described above up to mark "1". Than add Fischer-Germer solution up to mark "101". Composition of the Fischer-Germer solution: 3.5 g novocaine and 0.25 g NaCl in 100 ml distilled water (which will cause lysis of the corpuscular elements except for trombocytes).

Use a Bürker chamber and a phase-contrast microscope. If a phase-contrast microscope is not available, use a green color filter with a normal microscope. It is important that the Bürker chamber filled with the blood is placed in a wet chamber for 15 minutes; this makes it possible that the thrombocytes sink evenly to the same depth, they form a single layer, thus they will be easy to focus at.

The average of thrombocytes found in 10 large squares must be multiplied with 25000. Thus, the count of thrombocytes in 1 mm is obtained. Remember: supposing a normal thrombocyte count of 300000 /|l means that during the procedure you can expect about 10-12 thrombocytes in the counting area. Then the thrombocyte count per 1 l of blood can be calculated.

Normal value: 150-300 x 10^9/l

d) **Hematological counter:**

1) Fotoelectrical method: the RBC suspension is pushed through a glass capillary. A dark field condenser is focussed on the capillary. Each crossing particle flashes in front of the aperture of the condenser. The light impulses are converted into electrical impulses and counted by the instrument.

2) Method based on electrical resistance: The RBCs, which have high electrical resistance, are suspended in an electrolyte solution (plasma) with much higher electrical conductance. One of the electrodes is placed in the RBC suspension, the other is in an electrolyte solution. The RBC suspension enters the electrolyte solution through a capillary aperture. The electrical resistance is measured between the RBC suspension and the electrolyte solution. When an RBC closes the aperture the resistance increases. Thus, the variations in the electrical resistance are proportional to the number of cells. The variations in the electrical resistance are recorded by the instrument.

12. Osmotic fragility (resistance) of red blood cells

In hypotonic solutions water diffuses into the RBCs (osmosis). The RBCs swell and eventually hemolysis occurs because the cell membrane cannot withstand the increased intracellular pressure. The osmotic fragility is characterized by the highest concentration of the NaCl solution in which the hemolysis is noted.

Prepare a dilution series of NaCl (0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65%). In centrifuge tubes, one drop of blood is added to each dilution of 3 ml NaCl and the tubes are shaken. After a 10 min waiting period, the tubes are centrifuged.

In those tubes where hemolysis didn't occur, the RBCs sink to the bottom and are covered with a clear transparent liquid. In the tubes with hemolysis, the solution remains red. Normally, the highest NaCl concentrations in the tubes where the hemolysis starts and becomes complete are 0.45% and 0.3%, respectively.

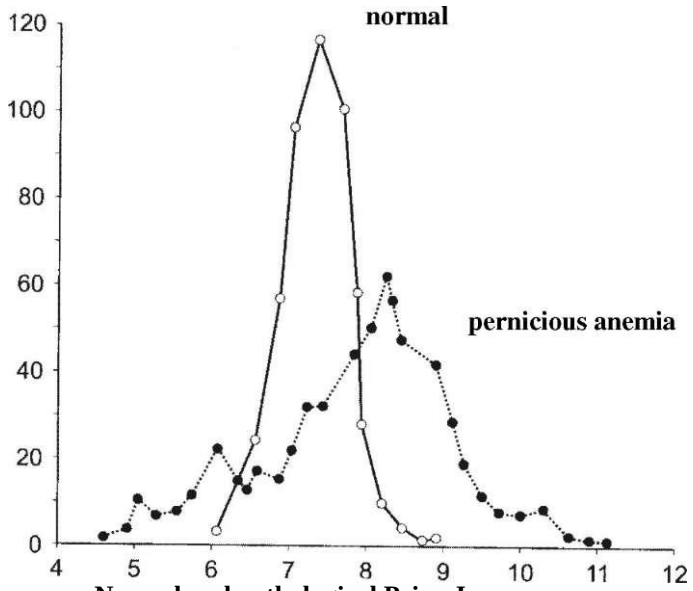
13. RBC's diameter (Price-Jones curve, in theory)

The ocular lens of the microscope is replaced with an ocular micrometer. The scale of the ocular micrometer is calibrated by means of the sideline of a small square (1/20 mm = 50 ^m) in a covered Burker chamber. Immersion magnification is used for the calibration.

Fingertip blood is diluted with physiological saline. One drop of this suspension is placed on a slide and covered with a cover slip. The diameter of the individual RBCs is measured under immersion magnification. The average diameter of 100 cells is calculated.

For the determination of the variations in the sizes of RBCs, 200-300 RBCs are measured. The percentage distribution of RBCs of various diameters are determined and plotted as a histogram.

The histogram should follow a Gaussian (normal) distribution with a mean value between 6 and 9 ^m, usually 7 ^m. In microcytosis, (anemia caused by iron deficiency) the peak of the histogram shifts to the left, towards the smaller values whereas in macrocytosis (pernicious anemia), the peak shifts to the right. A marked heterogeneity in the diameters of the RBCs is called anisocytosis. The presence of irregular shapes is called poikylocytosis.



Normal and pathological Price-Jones curves

14. Staining index (in theory)

The hemoglobin content of RBCs can be expressed by the staining index. It is calculated from the hemoglobin concentration of the blood and the RBC count as compared to their normal values. The normal value of the RBC count and Hb content is 5 million/mm and 158 g/l (2.5 mmol/l), respectively. These values are regarded as 100%. The staining index is (Hb content/RBC count) 100% per 100 % = 1.

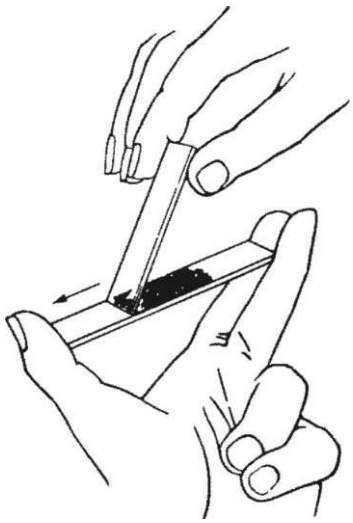
A staining index below 1 indicates that the Hb content is decreased relative to the RBC count though the RBC count might also be low. This occurs in hypochromic anemia, e.g., in iron deficiency. A staining index higher than 1 and low RBC count indicates hyperchromic anemia (e.g., pernicious anemia). The staining index is 1 in normocromic anemia.

15. The blood smear (differential leukocyte count)

The blood smear is used for the determination of the ratio of the various white blood cells in the sample, for the examination of their morphology, and for the identification of pathological cells in the blood. Alterations in the shapes or sizes of RBCs (anisocytosis, poikylocytosis) are also recognized in blood smears.

Touch one corner of a glass slide to the blood drop on the pricked fingertip. The slide with the blood drop is placed on an other clean slide at a angle of 45 degrees. After the blood drop has spread along the edge of the first slide push this slide slowly over the other. A thin blood film is produced. Keep the angle at 45 degrees between the slides throughout this procedure.

Let the smear dry. Then, fixate and stain the smear according to the method of Pappenheim. This method is a combination of the May-Grunwald and the Giemsa stainings. A cell can be identified by evaluating the size, the nucleus and the shape of the cell, and by examining the staining and the granulation of the cytoplasm. The percentages of the various cell types are determined.

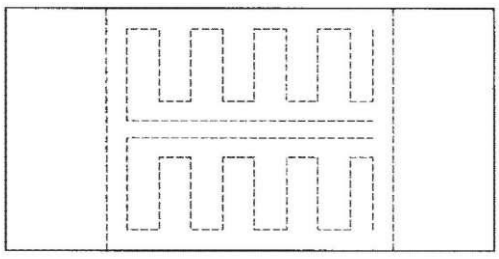


How to make a blood smear

Items needed for staining:
1. May-Grunwald solution (concentrated methyl blue and eosin in methanol-glycerin);
2. neutral distilled water;
3. Giemsa solution (azureosin in glycerin solution).

Place a dry smear on the staining stand. Cover the smear with concentrated May- Grunwald solution. The methyl alcohol in the solution fixates the smear. After 3 min, without removing the May-Grunwald solution, the dye is diluted with distilled water. The volume of distilled water should be the same as that of the dye. The eosin works only when diluted. After 1 min, let the dye flow off the slide. Do not wash the slide. Add freshly diluted Giemsa solution and wait for 15 min. Dilution of the Giemsa solution: mix 1 ml of the solution with 20 ml of distilled water.

After 15 minutes, repeatedly wash off the Giemsa solution with distilled water and soak up the remaining stain with a filter paper. Use immersion lens when examining the smear. Since the distribution of the cells in the smear is not homogeneous, counting should be performed along the so-called meander lines (these are imaginary lines along which the field of view is moved). At least 200 white blood cells should be identified and recorded.



(Imaginary) meander lines

Granulocytes

- neutrophil:

Nucleus: blue violet, varying shape (horseshoe, rod shaped or segmented); cytoplasm is pink, with irregularly distributed red/violet granules.

- eosinophil:

Nucleus: two purple lobes; cytoplasm: pink with regularly distributed yellowish red granules.

- basophil:

Nucleus: blue violet, varying shape; cytoplasm: pink with irregularly distributed blue granules.

Monocytes

Nucleus: purple/violet, varying shape e.g. lobular; cytoplasm: pale blue-grayish with no visible granules.

Lymphocytes

Nucleus: big, chromatin-rich, purple/violet; narrow pale blue cytoplasmic border. **Plasma cells**

Nucleus: situated laterally, chromatin-rich, purple/violet; there is only a narrow cytoplasmic border, cytoplasm: ultramarine blue with spoke-like chromatine.

Normal percentage distribution of white blood cells:

neutrophil granulocyte:

immature form (juvenile,band)	0-1 %
rod nucleus (stab)	0-3 %
mature (segmented)	60-70 %
eosinophil granulocyte:	1-5 %
basophil granulocyte:	0-1 %
monocyte:	2-6 %

lymphocyte: 20-30 %

II. CIRCULATION AND RESPIRATION

1. Experiments on the isolated rat heart preparation (Langendorf perfusion)

The preparation is suitable to examine the effect of several substances that act on the heart under physiological conditions. The principal of the Langendorf perfusion is that Krebs' solution diluted with carbogen (95% O2 and 5% CO2) is flowing through a cannula placed in the aorta. The solution fills the coronaries, gets into the right side of the heart through the sinus venosus and then leaves through the pulmonary artery. We register the frequency of contractions and their intensity during the experiment and it is also possible to determine the rate of coronary flow.

The course of the experiment:

1. Observe the activity of the heart under resting conditions, determine the heart rate (count for 10-20 sec) and the amplitude of the contractions.

2. Observe the effect of adrenaline: inject 0.1 ml adrenaline (dilution 1:10.000) into the perfusing solution. Wait 20-30 sec (the time needed by the fluid to reach the heart) and register the contractions for 10-20 sec (frequency, amplitude). Observe the time it takes for the contractions to return to their original rate. As a sympathomimetic, adrenaline increases the heart rate (positive chronotropic effect) and the force of the contractions (positive inotropic effect).

3. Repeat the previous observations now by adding 0.1 ml acetylcholine to the preparation. Compare the effects of sympathetic and parasympathetic stimulation, the time course of the events. Acetylcholine, as a parasymphatomimetic decreases the force (negative chronotropic) and frequency (negative inotropic effect) of contrations. The heart may stop in diastole. Remember: acetylcholine acts as a negative inotropic agent in this experiment because it can reach the ventricular mucles through the perfusion solution. When the heart is left in the body (in situ), parasympahetic stimulation does not result in a negative inotropic effect (in humans) since the parasympathetic innervation of the ventricular muscles is scarce.

4. Repeat the previous experiment: now inject ml atropine immediately before the injection of acetylcholine. Atropine inhibits the action of acethylcoline.

5. Examine the effect of potassium ions. Add 0.1 ml 5% KCl solution to the perfusing solution. The increased potassium ion concentration depolarizes the muscle fibers increasing initially the excitability and conductivity of the heart muscles. After reaching a critical potassium concentration, the excitability and conductivity decreases. The amplitude of the action potentials and the force of the contractions also decreases. The heart stops in diastole.

6. Examine the effect of calcium ions, following the injection of 0.1 ml 5% CaCl2. Increased extracellular calcium concentration has a positive inotropic effect on the heart muscle, the amplitude of the contractions increases. $CaCl_2$ administration via the cannula produces lower frequency. The heart stops in systole.

Save the fluid flowing through the coronary arteries into a measuring cup and determine its volume.

2. Electrocardiography

Electrocardiography is the interpretation of the electrical activity of the heart over a period of time as detected by electrodes attached to the skin surface. ECG device detects and amplifies the small electrical changes caused by the spreading electrical activity during each heart beat.

Conventionally, the electrodes are placed in the following manner: the patient lays down and the electrodes are attached to the wrists and above the ankles. These are metal plates which are covered with gauze, soaked with physiological saline in order to facilitate conduction. The colored plugs which are connected to the electrodes are as follows:

red = right arm
green = left leg
yellow = left arm

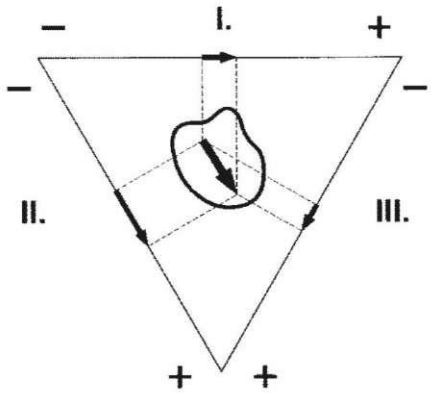
black = right leg (connected to the ground) The

following leads and electrode positions are used:

a) Einthoven's limb leads, standard bipolar (frontal plane):

I. right arm (- pole) and left arm (+)
II. right arm (-) and left leg (+)
III. between left arm (-) and left leg (+)

These are bipolar leads because the potential difference between the two (positive and negative) poles are registered. Since the distance of the leads from the heart is about the same, an equilateral triangle (Einthoven's triangle) can be formed connecting the location of the different leads. The current which is formed in the working heart has size, direction, and polarity which can be described as a vector. The projection of the vectors which are formed in the heart upon the sides of the triangle occur in the following way: potential difference within the triangle creates a potential difference on the sides of the triangle (in other words, in the different leads) which is as large as the right angle geometric projection of the original vector on the particular sides of the triangle. Einthoven's law is valid for standard leads: II = I + III
In other words, the sum of the amplitude of the projections of I and III is equal to the amplitude of the projection of II.

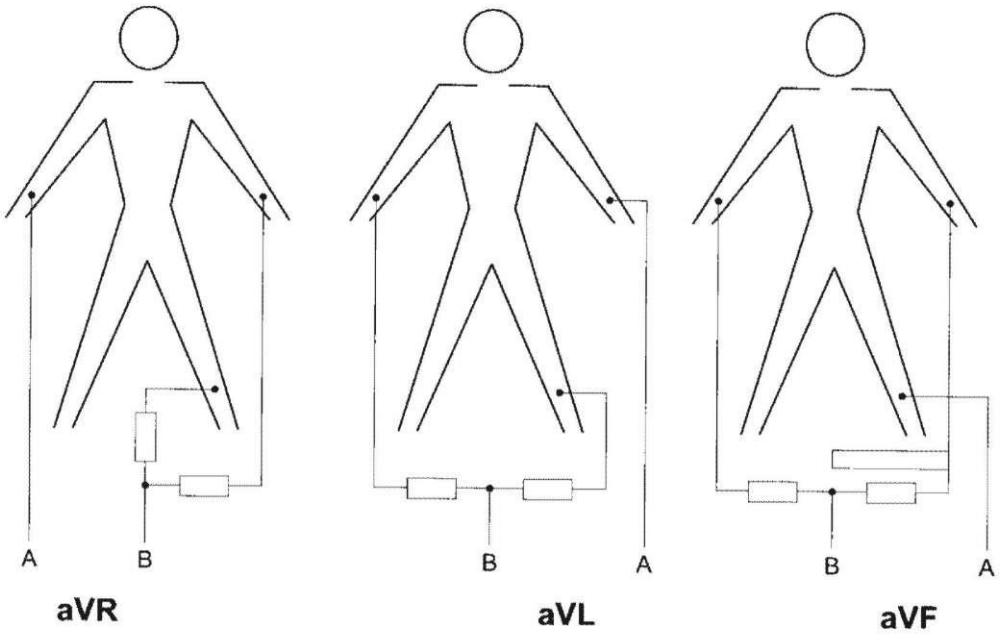


Einthoven's triangle

b) Golberger's unipolar limb leads (frontal plane):

Here the positive pole is one of the limbs: right arm (aVR), left arm (aVL) or left foot (aVF). The common potential lead of the other two limbs is the so called zero potential.

The "a" means "augmented" = enlarged. The limb from which the lead is being taken is disconnected from the common electrode in order to get a larger potential.

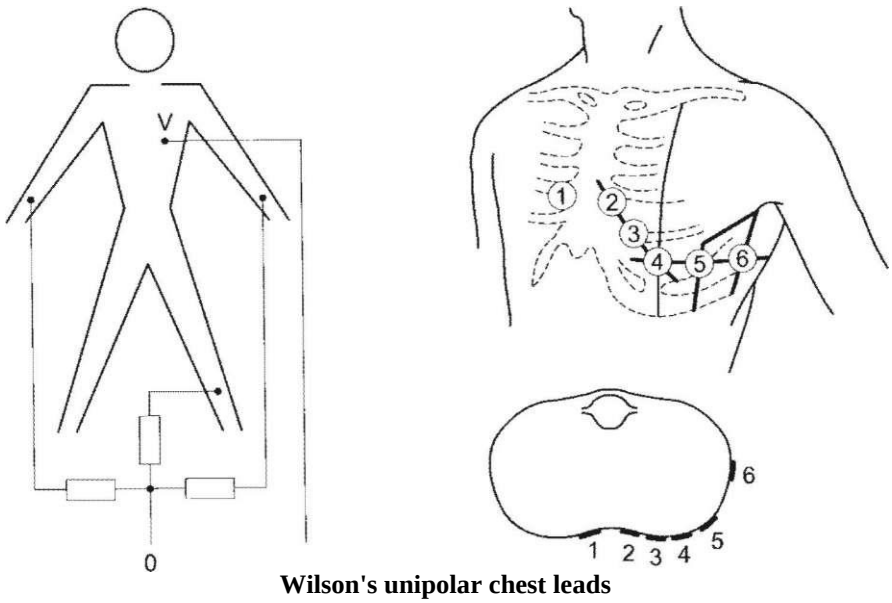


Goldberger's unipolar limb leads

c) Wilson's unipolar chest (precordial) leads:

The different electrodes are placed on pre set points on the chest (V1-V6). The indifferent electrode (Wilson's zero point) is created by connecting the limb electrodes through a large resistance.

Position of the electrodes: Vl 4th intercostal space, right edge of the sternum V2 4th intercostal space, left edge of the sternum V3 half-way between V2 and V4 V4 5th intercostal space in the medioclavicular line V5 at V4 height on the front axillary line Vg at V4 height on the middle axillary line



Wilson's unipolar chest leads

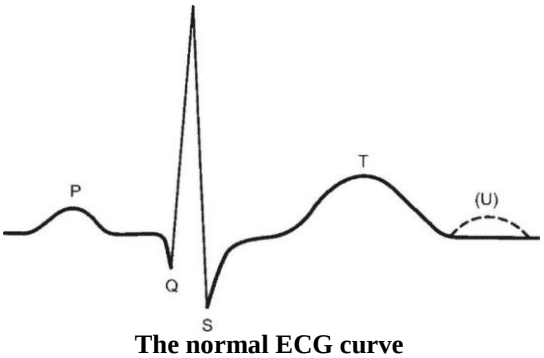
When recording the ECG, you must first calibrate the equipment. The writing arm should make a 10 mm deflection (this is equal to 1 mV) in a positive direction when the appropriate button is pushed. The speed of the paper is usually set to 25 mm/sec. In this case the small squares on the paper are equal to 0.04 sec and the large squares are equal to 0.2 sec.

The following intervals and waves are identified on the ECG:

P wave: occurs during the depolarization of the atrial muscles; its length is within 0.1 sec, amplitude is 0.1-0.15 mV.

PQ interval: the spread of the activation from the atria to the ventricular muscle fibers (atrio-ventricular conduction time); it is measured from the beginning of the P wave to the start of Q; length is 0.12-0.20 sec; the PQ distance decreases when the heart frequency increases.

QRS complex: occurs during the depolarization of the ventricular muscle; its duration is 0.06-0.11 sec.



The normal ECG curve

Q wave: occurs during the depolarization of the papillary muscles and septum; its size is usually compared to the size of R wave.

R wave: it occurs during the depolarization of the main mass of the ventricles; its average amplitude (in Einthoven II.) is 1 mV.

S wave: it indicates the depolarization of the back (basal part) of the left ventricle; its direction is usually negative; its size is approximately 0.1 mV.

ST interval: the slow repolarization of the ventricular muscle; it is between the end of the S wave and the beginning of the T wave. It is on the isoelectric line; it can physiologically deviate from this by 0.1 mV. If it is higher than 0.1 mV, it is called a pathological ST elevation and if it deviates in a negative direction it is called a pathological ST depression.

T wave: it shows the fast repolarization of the ventricular muscle; its amplitude is 0.1-0.5 mV.

QT interval: it is between the beginning of the Q wave and the end of the T wave; it is the depolarization and repolarization of the ventricles (the so-called electric systole). It's length is 0.35-0.45 sec. This interval decreases proportionally in case the heart frequency increases.

(U wave: it occurs after the T wave; it occurs in about 50% of the cases. Its origin is not clear yet.)

The evaluation of the ECG occurs as follows:

1. Determining the rhythmicity of the heart: we measure the length of the consecutive R-R intervals and compare them. In arrhythmia, the R-R intervals are irregular (different).

2. Measuring the frequency of the heart: the R-R interval is measured in seconds and we see how many times this value is present in 60 seconds (on 25mm/sec paper-speed, one small square = 0.04 sec).

3. Determining the electrical axis of the heart: in the standard I-III limb leads, we measure the amplitude of the R wave. The heart is centered (about 45 degrees), if the R wave in lead II is the largest and in lead I and III they are approximately the same. In left axis deviation, the R amplitude is the largest in lead I and is negative in lead III. In right axis deviation, the R wave amplitude is largest in lead III and negative in lead I. Consider the sides of the Einthoven's triangle and its vector projection.

4. Examining the other waves: measure the time of the PQ interval, measure duration of QRS complex and examine its contours. Give attention to the ST segment and its position on the isoelectric line.

3. Characteristics of the arterial pulse

The arterial wall is elastic therefore blood flow is continuous. With each systole, the stroke volume is ejected to the arterial system toward the periphery with a given speed, pressure and volume. The pressure change results in the pulse wave. The spreading speed of pulse waves in young men in muscular type arteries (external radial artery) is 8-12 m/s; the value depends on the age or distensibility of the walls.

Pulse can be felt in superficial arteries, for example the radial artery on the volar side of the wrist. Put your index, middle, and ring finger of the left hand on the radial artery and count the number of puls waves for one minute. Other palpation points in the arterial system are: temples, neck (carotid pulse), supraclavicular region, armpit, behind the knees, medial side of ankles, dorsum of the foot.

The resting pulse rate is 72 beats/min. In case of arrhythmia or an extrasystole, count for one minute to obtain the best results.

Pulse Qualities

a) Frequency:

- tachycardia (frequens)
- bradycardia (rarus)

Physiologically, the frequency of the heart reflects the pulse frequency, under pathological conditions some contractions of the heart can be so weak that the pulse wave does not reach the periphery. This is called a pulse deficit.

b) Rhythmic character of the cardiac activity:

- regular (regularis)
- irregular (irregularis)

The rate of the pulse wave fluctuates with respiration: during inspiration, the pulse is more frequent than in expiration. This is called physiological respiratory arrhythmia, which is pronounced in young people. Other forms of arrhythmia (extrasystole, absolute arrhythmia), are examined more precisely on the ECG.

c) Pulse wave amplitude (arterial wall deflection):

- high (altus)
- low (parvus)

The measure of the heart beat and the amount of blood flow during diastole depends on the elasticity of the arterial wall. During heartbeat the pulse amplitude is small, if the elasticity is great.

d) Steepness of pulse wave (arterial wall deflection speed):

- rapid rise (celer)
- slow rise (tardus)

The steepness of the pulse wave depends on the velocity of the pressure change. The given heart frequency with a strong pulse is accompanied by a steep pressure change and a weak pulse by a slow pressure change.

e) Pulse suppression:

- hard (difficult to suppress - durus)
- soft (easy to suppress - mollis)

When fullness, height, speed and hardness of the pulse waves are equal, this is called pulsus equalis. In absolute arrhythmia, the time intervals between beats are different and the fullness of the pulses are also different, this is called pulsus irregularis et inaequalis (irregular and unequal pulse). In hypovolemia (shock): parvus, frequent and mollis.

4. Observation of pulsation of the external jugular vein

During the cardiac cycle pressure waves are produced not only in the ventricles but also in the atria. These are simultaneous with the atrial systole, closing of the atrioventricular valves and (in case of closed atrioventricular valves) with the slow filling phase of the atria. The atrial pressure wave results in volume changes in the veins of the systemic circulation and eventually it increases the volume of the veins. If the subject is in a lying position, these volume changes can be observed with the naked eye.

5. Blood pressure measurement

Blood pressure can be measured with different types of invasive and noninvasive methods.

Measuring pressure invasively, involves an arterial cannule with a small transducer, inserted into an artery (mostly into the radial artery). The transducer detects the pressure changes and sends the detected signals to a computer. It is useful when rapid changes in blood pressure are anticipated or when non-invasive blood pressure monitoring is not possible or likely to be inaccurate (obesity, arrhythmias...). It is also used when long-term measurement in sick patients is required as it avoids the problem of repeated cuff inflation (causing localized tissue damage) and allows repetitive sampling for blood gases and laboratory analysis. This method usually is restricted to a hospital setting especially to the intensive care unit.
While measuring blood pressure noninvasively, the artery must be compressed in order to stop blood flow. During the procedure the surrounding tissues (skin, muscle) are also compressed, therefore the measured value is larger than the actual intravascular pressure. During the measurement, we obtain the highest (systole) and the lowest (diastole) blood pressure values.

The value of blood pressure depends upon:
- the intensity of the heart contractions;
- the peripheral resistance of the arterial system;
- elasticity of the arterial wall;
- volume and viscosity (hematocrit).

The indirect measurement of blood pressure can be obtained by using the Riva-Rocci sphygmomanometer (R-R in short) which consists of an:
- inflatable rubber- padded cuff. Standard width for adults is 12 cm;
- rubber balloon;
- mercury manometer.

a) **Palpation method:**

Wrap the cuff on the upper arm, inflate the balloon by pumping until the radial pulse is no longer felt. Then, pump a little more air into the cuff followed by opening the valve, slowly letting out the air until the radial pulse is just felt. At this time, read the value of the manometer. This is the systolic pressure. Due to the exterior pressure, the lumen is not totally open, therefore the pulse is weaker than normal. Continue expelling until the pulse resumes normality. The latter is only a rough estimation of the diastolic pressure.

b) **Auscultation method (Korotkoff type):**

This method is more precise than the one using palpation. Place the cuff on the upper arm so that the cubital artery can be detected and place the phonendoscope here. Pump the air until the pressure value exceeds the normal systolic pressure of 120 Hgmm (about 20-30 Hgmm greater). As a result, the brachial artery is compressed and the blood flow is stopped. Then, opening the valve, slowly decrease the pressure. At the point when the pressure drops below the systolic pressure, the blood flow resumes. At this time the cross section of the vessel is smaller than normally, the flow of blood is turbulent and each pulse is followed by a short, sharp, knocking sound. These are the Korotkoff sounds.

The systolic pressure is coincident with the first Korotkoff sound. As the cuff pressure decreases, the sounds are at first louder and then become weaker. When the sounds cease, the value on the scale is read and this will give the diastolic pressure. The result is given as systole/diastole (e.g., 120/80 Hgmm).

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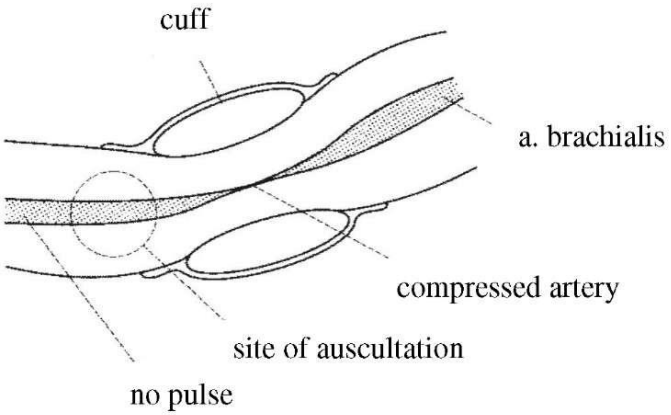

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The two goals of percussion are:

- information about the air content of different organs;
- determination of the borders of organs.

Technique: place the index or middle finger of the left hand onto the body surface which is to be percussed and tap your own finger with the middle finger of the right hand. The harder one presses on the surface and the stronger the percussion, the deeper lying organ's vibrations can be heard.

Organs which do not contain air (for example the liver), reflect a dull sound (high, quiet and short). If the organ contains air, then a louder, longer and deep sound is heard (lungs).

Percussion of the lungs:
When examining the chest, the patient should be laying on his back. When examining the back, the patient should be in a sitting position.

a) Comparative percussion: We compare the percussion sounds on the right side on the back to the sounds of the corresponding points of the left one. Also the scapular and axillary lines are examined.

b) Topographical percussion: used to determine the borders of the lungs. The organs under the diaphragm are airless, therefore dull sounds are heard over them. Over the lung, a resonant sound is heard. The borders are therefore determined at the point where the sound changes.

The inferior borders of the lungs:

- on the right side: in front of the midclavicular line in the 6th intercostal space (upper border of the liver);
- on both sides: midaxillary line in the 8th intercostal space; scapular line in the 9th intercostal; paravertebral at the 11th thoracic vertebra.

When percussing from top to down, a dull sound is heard when reaching the diaphragm, than we ask the patient to inhale. Since the lungs fill with air, as a result the resonant sound should be heard again at the place where the dull sound could be heard before. There is a decreased deflection of the diaphragm in different respiratory diseases.

Percussion of the heart:
The maximal frontal projection of the heart on the chest wall can be examined. The right border normally does not exceed the right side of the sternum. The left border is near the midclavicular line. For percussing the left boarder, start at the axillary line. For the upper border, start parasternally and move downwards (normally to the third intercostal space). The apex beat can normally be felt on the left side near the midclavicular line (1-2 cm) in the fifth intercostal space.

7. Auscultation over the heart and lung

Auscultation can be done in two ways:
- directly by placing one's ear on the chest;
- indirectly by a stethoscope.

Auscultation over the lungs:
In a normal lung, two types of respiratory sounds occur:

a) alveolar respiration: the sound that is heard during opening up the alveoli. Usually, this sound is heard only during inspiration because during expiration, the sound is short and soft.

b) tracheobronchial respiration: The sounds are heard where the larger bronchi lie near the chest wall, for example the right main bronchus in the interscapular region or in front, next to the manubrium sterni. Sound is heard during both inspiration and expiration. If alveolar respiration stops (i.e. fluid infiltration), only the bronchial breathing is heard.

Auscultation over the heart:
During one cycle, two heart sounds can be heard. The first is the systolic sound which is due to the closure of the cuspidal valves. This sound coincides with the radial pulse. The second is the diastolic sound which is due to the closure of the semilunar valves. A third heart sound is heard during diastolic filling when the blood flows from the atrium to the ventricle. This third heart sound is heard in children and young adults at the apex. The sounds generated by the various valves can be heard at distinct points on the chest surface: punctum maximum.

Auscultation with the stethoscope begins at the apex. We identify the first heart sound as being the longer which begins after diastole. It appears simultaneously with the carotid pulse. The first sound is longer and deeper; the second is shorter, higher and sharper.

The punctum maximum of the valves:
- aortic valve:
on the right side of the sternum in the 2nd intercostal space.
- pulmonary valve:
on the left side of the sternum in 2nd intercostal space
- mitral valve:
over the apex. about 2 cm medial to the midclavicular line in the 5th intercostal space.
- tricuspid valve:
on the right side of the sternum in the 4th or 5th intercostal space.

The first heart sound is heard louder over the cuspidal valves, while the second sound is best heard over the semilunar valves.

8. The effect of physical exercise on the circulation

The physical performance depends on the capacity of the cardiopulmonary system. Under resting condition, we measure the patient's blood pressure, pulse and breathing frequencies. After that, the patient must ride a bicycle-ergometer. We increase the load gradually. After 1, 2, 3 and 5 minutes exercise we measure the blood pressure, pulse and breathing frequency.

Under normal conditions, an increase of 30 watts in every 3 minutes results on average in an increased frequency of 10 beats/min, the systolic pressure increases by 10-20 Hgmm. Usually the parameters return to their original values within 2 minutes.

9. Spirometry

Spirometry is the most common of the Pulmonary Function Tests, measuring lung function, specifically the measurement of the amount (volume) and/or speed (flow) of air that can be inhaled and exhaled.The type generally used is the Hutchinson spirometer. This is a cylindrical container filled with water, in which a metal bell is submerged. A metal pipe is attached to the cylinder. The pipe is connected to a rubber tube, which connects the spirometer and the airway. During expiration, the gas volume in the spirometer increases and the bell rises; during inspiration it sinks. These movements are recorded and can be read on a calibrated scale, the spirogram. Nowadays the spirometers are computer based.

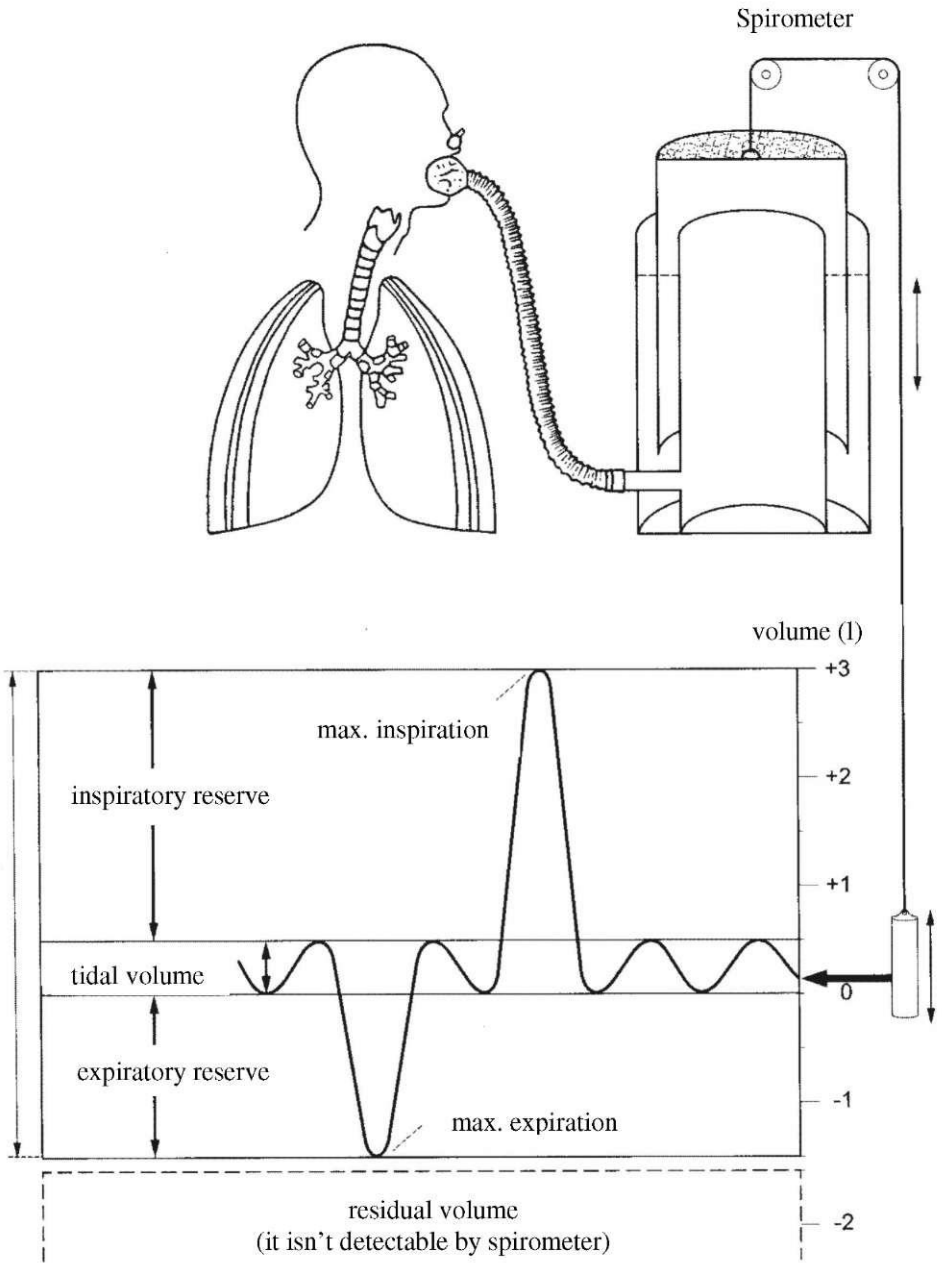
During measurement, the patient inspires and expires air into the spirometer. This volume is the tidal volume (500 ml). Inspiratory reserve volume is also measured which is the maximal

volume of gas that can be inspired after normal tidal volume inspiration (2500 ml). The expiratory reserve volume is the maximal volume of gas that can be expired after normal expiration (1000 ml).

Vital capacity is the maximal volume of gas that can be expired after maximal inspiration; thus, vital capacity = expiratory reserve vol. + tidal vol. + inspiratory reserve vol. The vital capacity depends on the elasticity of the lungs and chest which, in turn, depends on age, gender, chest size and physical condition. In females the value is 25% lower than males. Athletes have exceptionally high values, especially swimmers due to their well developed respiratory muscles (pectoralis major and minor). Vital capacity is small if the elasticity of the lung decreases or if volume capacity decreases.

A special recording paper is placed into the spirometer. During expiration, the machine draws a curve corresponding to the expired air volume. FEV_1 (forced expiratory volume) is the volume expired in the first second after maximal inspiration. Its value is 75% of the vital capacity, which decreases in airway obstruction (asthma).

Tiffeneau-index = $(FEV_1/VC) \times 100$

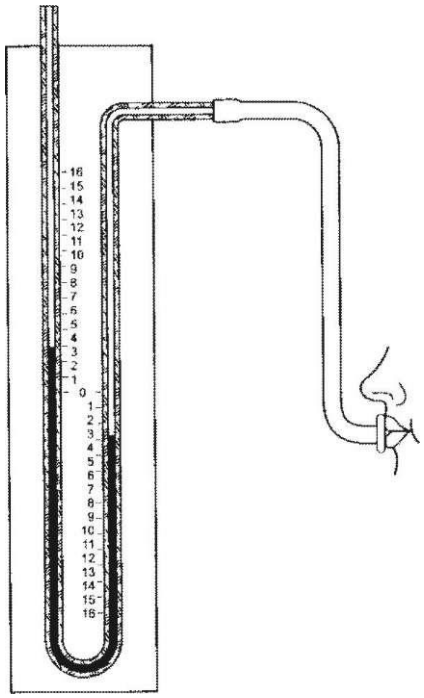


Lung volumes

10. Determination of the respiratory pressure

It reflects the function of respiratory muscles and achievement regarding pressure differences and maintenance ability.

The examination is performed by a pneumatometer, which is actually a mercury manometer. A rubber pipe is connected with a mouth piece to one end of the glass pipe. The patient holds his nose and expires with full force. We read the difference between the two mercury columns which gives the respiratory pressure value in Hgmm. In non-athletes the value is about 30 Hgmm; the value in an athlete can rise up to 300 Hgmm



Pneumatometer

11. Effect of the breathing cycle on the circulation

a) Valsalva-experiment:
a greater than normal pressure in the lungs compresses the intrathoracal vessels and impedes venous return.
The patient takes a deep breath. As a result, the respiratory and abdominal muscles contract, the intrathoracal and intraabdominal pressures increase and the venous return is impeded. The blood is pushed out of the pulmonary vessels. In the left heart the arterial pressure first increases then decreases. The left heart expels its content so there is almost no blood remaining so the radial pulse is barely felt.

b) Muller-experiment:
after expiration, the patient inspires forcefully with closed glottis.

The negative intrathoracic pressure is increased; the vessels of the heart and lungs are filled. The heart, due to the increased stretch, can not empty its content. In this condition the radial pulse is barely felt.

These two experiments should only be performed on healthy individuals and should be avoided in pathological conditions (e.g. heart disease).

12. The cold-pressor test

The effect of cold on the resistance and capacitance vessels of the skin is vasoconstriction (first in hands and feet, then on head and trunk). The frequency of the heart and the pulse volume decrease. Blood pressure rises; intensive cold stimuli cause change in blood pressure. This testis called the cold-pressor test, and is a diagnostic procedure, during which time blood pressure is measured, while one hand is placed in icy water.

The patient's blood pressure is measured several times until a value is maintained which will take from 1 to 5 minutes. Then loosen the cuff on one arm and place that hand in cold water for one minute. 30 to 60 seconds later, measure the blood pressure until it reaches a stable value. The results of the experiment should show about 20 to 30 mmHg increase in blood pressure.

13. Triple response of the skin

The response of the skin to firm stroking is composed of 3 phases:

1) a sharply demarcated red line at the site of the stroke (due to local vasodilation);
2) an intense red flare that extends beyond the margins of the line of pressure (due to the axon reflex, which is resulted in the release of vasodilator peptides i.e. CGRP release);
3) local oedema (substance P release resulted in increased permeability of blood vessels).

III. EXCRETION

1. The Color, Smell and Turbidity of Urine

The color of normal urine ranges from colorless through straw, yellow, amber and brown and depends on the concentration of various pigments (urochrome, uroerythrin, urobilin). The color of urine can be influenced by:

- pH: acidic urine usually darker.
- concentration: pale yellow urine is usually hypotonic, while dark urine is hypertonic (except in osmotic diuresis -e.g. in glucosuria- where the pale colored urine has high specific weight).

The color of urine might change due to different substances or under pathologic conditions. Interpretation of atypical urine color is as follows:

yellow/orange: concentrated urine, urobilinogen, bilirubin,
red/red-brown: red blood cells, hemoglobin, myoglobin, phenolphtalein (laxative), blackberries, menstrual contamination, porphyrin, amidazophen
brown-black/black: melanin, methemoglobin, homogentisic acid, (the homogentisic acid defect: homogentisic acid does not decompose, but rather is transformed into alkaptochrome while standing) fecal contamination
blue-green: pseudomonas infection, chlorophyll, biliverdin, methylene blue,
milky/cloudy: pyuria, lipids, mucus, radiographic dye, microorganisms

The observation of turbidity or lack of it in a urine specimen is made by the examination of a well mixed, uncentrifuged sample held against a good light source.
Terms: clear, hazy, cloudy, opaque.

2. The pH of the Urine

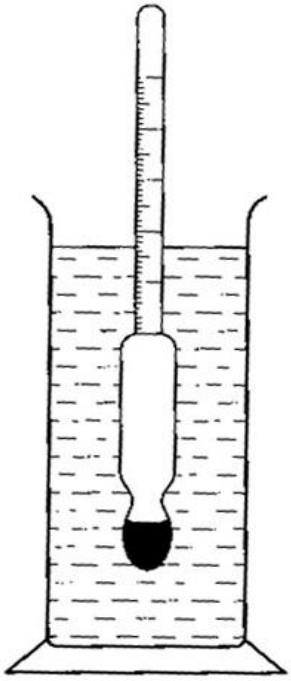
The pH of the urine is depending on the acid-base equilibrium of the organism and may vary between pH 4 and 8. In the case of a normal mixed diet it is slightly acidic. Protein-rich diets shift the pH into the acidic range, while vegetables shift it towards a basic value.

The pH of the urine can be determined simply with indicator paper: dip the reagent strip into the urine and remove immediately. Compare the reagent strip to the color chart and record the result.

3. The Specific Gravity of the Urine

The specific weight (gravity) of urine indicates the relative proportions of dissolved solid components to the total volume of the sample. Normal value is between 1015-1025 g/l (slightly hyperosmolar). Under extreme conditions it may vary between 1001-1030 g/l. Determination of specific weight may give information about concentrating or diluting processes in the kidney.
Determination is made with an urometer (hydrometer): it is a simple pyknometer or gravimeter calibrated at room temperature (20° Celsius) and having a measuring range of 1.000-1.060. The urometer is placed into the urine sample such a way that it does not touch the wall of the container. The value of the specific weight will be where the surface of urine touches the mark.

Correction is to be made if the temperature of the urine differs from the calibration temperature of the urometer: for every 3 °C 1 unit (0,001) should be withdrawn or added to the value seen. Glucose or protein in the urine may increase the specific gravity.



Urometer for the determination of the specific weight of the urine

4. Quantity of the Urine

The average volume of the urine excreted per day in an adult is 1000-1500 ml.
Terms: less than 500 ml/day: oliguria (in pyrexia, exsiccosis, shock)
More than 2000 ml/day: polyuria (polydipsia, diabetes insipidus, diabetes mellitus, and reduced concentrating capacity of the kidney)
Less than100 ml/day: anuria.
Under normal circumstances urine excretion is less at night than in daytime. If the amount of daytime and nocturnal excretion equals or the amount of the latter is higher we talk about nycturia (heart insufficiency, chronic kidney disease).

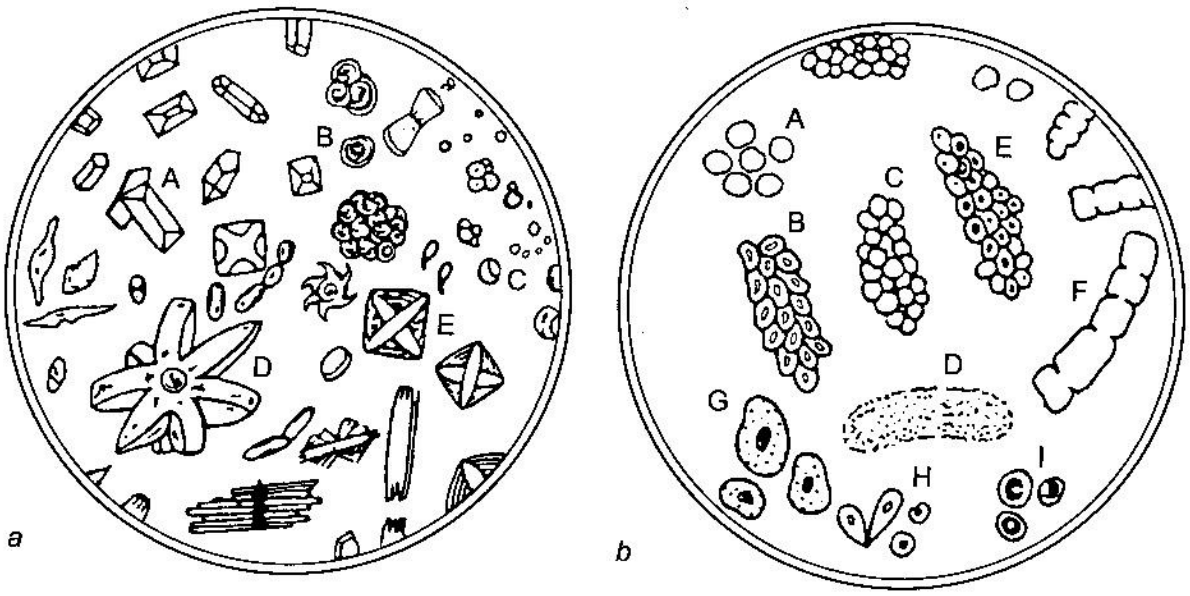
5. Microscopic Investigation of the Urine Sediment

First morning urine is the most suitable for this investigation since it is more concentrated than the one secreted during daytime. It is also more acidic and casts and other formed elements are more stable in acidic urine. If possible, the urine sample should not stay longer than one hour before the test.
Urine is first centrifuged for 5 minutes at 1000-2000 rpm. The supernatant is discarded from the sediment, stirred and one drop is smeared on a clean glass slide. It is covered with coverslip and viewed under the microscope (condenser in the lower position, narrow diaphragm).

Urine sediment may contain a few epithelial cells and white blood cells in normal conditions.

In acidic urine the following crystals can be found:
Normal (Fig.a): A) ammonium-magnesium (triple) phosphate (coffin-shaped), calcium sulfate, B,C) ammonium urate, D) uric acid, E) calcium oxalate,
abnormal: urea oxalate, tyrosine, bilirubin,

In alkaline urine: calcium phosphate, magnesium phosphate, calcium carbonate,



Urinary sediment crystals

In pathologic urine beside unorganic sediment organic ones also can be found:

a) Cells (Fig.b):
- A) red blood cells: pale, anucleate, biconcave refractive disks of 7 μm diameter, with sharp double contour. They shrink in concentrated urine and swell or burst in hypotonic urine to produce "ghost cells". One or two erythrocytes in 3-5 visual fields are considered normal.
- G, H) epithelial cells: renal tubular, bladder epithelial or squamous cells. Size and shape depends on the portion of urinary track from which they originate.
- I) leukocytes: segmented neutrophil or polymorphonuclear leukocyte is the predominant type. They are round cells with granulated surface and with a diameter of 10-12 μm. One or two per visual field is not pathologic but they are accumulated in pyuria.

b) Casts (cylindrical shaped aggregates of protein-like material) (Fig.b)**:**
They are protein-containing casts of tubuli and collecting tubules. The slightly alkaline pH of protein containing filtrate changes to acidic in the distal tubuli; protein of sol state turns into gel state: cast are formed which correspond to the form of the tubulus. Cylinders can be classified according to their shape and to the various substances or cellular elements deposited on them. Their refractive capacity differs only slightly from their surrounding, their recognition therefore is rather hard. Using narrow diaphragm and lowered condenser, cylinders should be detected at lower magnification and then identified at higher magnification.

- hyaline casts have a diameter of 10-15 μm, are transparent, have cylindrical shape. Few might occur in normal sediment, but generally they cannot be observed in routine tests.
- D) shape and size of granulated cylinders is similar to that of hyaline but their surface is granulated due to the sedimentated amorphous granules.
- F) waxy casts are pale yellow, more corpulent forms, sometime retractions can be observed on the surface.
- E) leukocyte casts: easy to recognize because of the presence of white blood cells attached to the surface of the hyaline matrix
- C) red blood cell casts: may be readily recognized because of the red blood cells on the surface of the casts. In time the red cells will undergo hemolyzis and give a golden-brown color of the cast.
- B) epithelial casts: these casts are made up of a hyaline cast matrix with the epithelial cells embedded in the casts. The cells have a large spherical central nucleus.

c) Microorganisms: bacteria, protozoa may also show up in the urine sediment.

6. Detection of UBG in Urine with Ehrlich's Aldehyde test.

Conjugated bilirubin secreted by the liver is reduced in the intestinal tract. These reduced products (mesobilirubin, stercobilinogen, urobilinogen) are partly excreted, partly reabsorbed and secreted again by the liver (enterohepatic recirculation).
Normally there is 0.5-2.5 mg UBG in the urine collected for 24 hours. UBG is secreted in higher amounts in pathologic cases, like in hemolytical and hepatocellular diseases. If bile flow into the intestine is obstructed neither UBG nor urobilin will be formed and they are not present in the urine.

UBG is always detected in fresh, cool urine (it oxidizes to urobiline if left standing in the air and urobiline cannot be detected with Ehrlich's reagent).

Few drops of Ehrlich's reagent (p-dimethyl-aminobenzaldehyde dissolved in 20% HCl) is added to 3 ml urine and the sample is inspected after 15-20 s:
- Normal UBG concentration: no significant change in color, urine is light pink if seen from above and colorless if held against the light but becomes red upon boiling.
- Slightly increased UBG: light red if held against the light.
- Markedly increased UBG: expressed red color if held against light.
- Decreased UBG: red color does not appear even upon boiling.

The reaction is not very specific, it can be induced by porphobilinogen and various medicines (e.g. p-amino salicylic acid).

7. Detection of Calcium According to Sulkowitsch

Equal amounts of Sulkowitsch's reagent (ammonium oxalate, oxalate, acetate) and urine are put in a test tube. Acetic acid, as an acidic medium accelerates the reaction and in the presence

of Ca-oxalate insoluble precipitates will be formed. This test is a semi-quantitative test and serves only for gross information.
Ca content of the urine might be:
normal: light turbidity (haze) develops
increased: immediate heavy turbidity (opaque) develops
decreased: a light opalescence develops after a while.

8. Detection of Sugar According to Nylander and Fehling:

Usually the urine sample is checked for glucose but sometimes the detection of fructose, galactose, lactose and pentoses might be necessary as well. Glucosuria physiologically does not exceed 130 mg / day which cannot be detected with routine tests. Glucose amounts in the urine exceeding this value are called glucosuria. It's most frequently cause is diabetes mellitus. It may also occur in healthy humans after excessive carbohydrate consumption or when the diet is containing mainly carbohydrates: this is called alimentary glucosuria.
Determination of glucose in the urine is based on the reducing capacity of glucose: reducing sugars in an alkaline medium are reducing Cu, Bi, Hg, Fe, Ag-ions from metal hydroxides.

a) Nylander 's test
Reagent: 100 g NaOH, 20 g bismuth-nitrate 40 g Seignette-salt (K-Na-tartarate) in 1000 ml distilled water).
Proteins, if there are any, should be eliminated from the urine sample before.
Few drops of Nylander reagent are added to 2 ml of urine in a test tube and boiled for 3 minutes. In the presence of glucose black precipitate develops: the precipitate is bismuth in metallic form. The test is fairly sensitive, positive results can be observed at the presence of as much as 0.08% glucose. Results should be read immediately after cooling, because after prolonged standing the boiled urine becomes black anyway even if glucose is not present. Compared to other reducing tests the advantage of the Nylander test is that in case of increased uric acid or creatinine content of the urine (they also have reducing capacity) it does not give misleading positive reaction.

b) Fehling's test:
Reagent: Fehling I = 3.5% $CuSO_4$ solution
Fehling II = 17% Seignette salt dissolved in 5% NaOH.
Equal volumes of the two reagent solutions are mixed. Boil it and add few drops of urine to the hot solution. The solution having azure blue color (resulting from mixing Fehling I and II solutions), turns to red (copper (I) oxide) when urine having glucose is added. The background of the reaction is that Cu^{++} ions bound in complex are reduced to Cu^{+} ions by glucose.
Both tests give positive results not only in the presence of glucose but also in the presence of other kinds of sugar (pentose, fructose, lactose, galactose) present in the urine.

9. Detection of Proteins

Protein content of the urine is 2-8 mg/100 ml under physiological conditions; upper limit of the daily protein excretion is 100-150 mg, on average 40-80 mg. Excretion higher than that is called proteinuria. The urine may contain serum albumin, globulins, paraproteins, or protein

degradation products. Albuminuria is the most frequent one. Generally materials of small molecular weight have higher clearance thus they are present in higher amounts in the urine.

Protein in the urine can be detected with precipitation tests. Since these protein tests might give positive result with the cellular elements in the urine it is suggested to make the tests with a centrifuged urine sample.

a) Sulphosalicylic acid test:
It is a fairly sensitive test; few drops of sulphosalicylic acid (20%) is added to about 3 ml of urine. In the presence of protein opalescence or precipitate can be observed (alkalic urine should be acidified previously). Content of test tube should be compared to reagent-free urine.
Evaluation:

- negative: no change can be observed
- protein in traces: just a slight, smoke-like turbidity can be observed against a black background.
  Protein content: 0.1-0.5 g/l.
- slightly positive: moderate turbidity
  + = 0.5-1 g/l
- positive: turbidity without precipitate
  ++ = 1-2 g/l
- positive: turbidity with fine precipitate
  +++ = 2-5 g/l
- positive: increased precipitate
  ++++ = above 5 g/l

b) Boiling test:
Urine proteins coagulate and form a precipitate upon boiling in slightly acidic medium. A few drops of acetic acid (1%) is added to 5 ml urine and the sample is boiled. In the presence of protein precipitate is formed. If precipitate cannot be dissolved with acetic acid, this indicates the presence of protein, if the precipitate dissolves, it indicates the presence of phosphates and carbonates.
Evaluation:

- opalescence (formation of sediment after few minutes): < 10 mg/100 ml urine
- turbidity: about l0 mg/100 ml
- precipitate: > l0 mg/100 ml

c) Heller's test:
The principle of the reaction is that proteins are denaturated by strong acids. HNO_3 is layered under urine in the test tube. (Caution! Urine has to be put first into the test tube, than put HNO_3 with a Pasteur pipette to the bottom of the tube.) A marked white disk develops at the interface of nitric acid and urine the thickness of which indicates the approximate amount of proteins.

10. Detection of Blood and its Decomposition Products with the Benzidine Test

Hemoglobin in the urine can be found usually in intact RBCs, which is called hematuria. If hemoglobin is released from the RBCs in the blood vessels it will show up in a dissolved state

in urine: this is called hemoglobinuria (the hemoglobin binding plasma-haptoglobin is saturated due to the marked intravascular hemolysis, and free hemoglobin is filtered into the urine).
In both cases tests for detection of Hb are positive. In case of hemoglobinuria, however, the characteristic color of the urine does not change even after centrifugation, while in hematuria RBCs sediment during centrifugation and the supernatant of the centrifuged urine remains clear.
Hb present in the urine releases atomic oxygen from H_2O_2 in the reagent solution which oxidizes the chromogen in the reagent (benzidine, o-toluidine, guayacol-resin) into a colored end product.
The reagent is usually fresh-made. A knife-tip amount of benzidine is dissolved in 2-3 ml acetic acid; 3% H_2O_2 solution is prepared separately, the reagent is the mixture of the two solutions. Add 3 ml of reagents to the same amount of urine. In the presence of Hb a blue-green color will appear.

11. Detection of Acetone

Ketone bodies (acetic acid, beta-hydroxy-butyric acid, acetone) are present in the urine in very small amounts only. They are produced in higher amounts even in healthy humans, if carbohydrates are missing from the diet. In this case the antiketogenic effect of carbohydrates is missing, fats are not burned completely to CO_2 and H_2O. Diabetes mellitus is the most frequent cause of ketonuria; it's presence indicates the marked disturbance of carbohydrate and fat metabolism which may lead to serious ketoacidosis. In addition to diabetes mellitus ketonuria might occur after repeated vomiting and during prolonged starvation.

a) Rothera's test
For the detection of ketones a color reaction given by sodium nitroprusside in alkalic medium is suitable. A small amount ("knife-tip") of the reagent powder is put on filter paper and few drops of urine is added. In the presence of ketones a violet color will appear which might become darker (purple) with time.

Preparation of the reagent (modified Rothera mixture): 10g of powdered sodium nitroprusside is added first, then 200 g of ammoniumsulphate and 200 g dehydrated sodium-carbonate are added, then mixed and stored in a glass stoppered flask.

b) Legal's test:
Principle: Urine containing acetone turns the mixture of sodium-hydroxide and sodium-nitroprusside solution to burgundy red; this color changes to purple upon adding concentrated acetic acid.
To 5 ml urine in a test tube about 5-10 drops of sodium nitroprusside solution (dissolved in water) is added, this is alkalized with NaOH until the color turns red (that means positive reaction). Then acetic acid is added to the tube and observed; if the reaction is due to creatinine the color will fade and turn into a light blue, but if the red color originates from acetone there is no change in the color observed or the red tint becomes darker.

12. Detection of Bile Pigment

Normal urine does not contain bilirubin. The kidney threshold is about 30 µmol/l. Bilirubinuria may be observed at serum levels higher than that. The color of the excreted urine is characteristic: from saffron yellow to dark brown (the foam of the urine is also yellow). The principle of detection is that bilirubin turns into biliverdin having green color when treated with strong oxidizers.

a) Gmelin's test:
Proteins are previously precipitated and HNO_3 is layered under the urine. First colored rings will be formed at the interface, finally the whole solution becomes yellow. The diferent steps are as follows:
biliverdin (green) $\Rightarrow$ bilicyanin (blue) $\Rightarrow$ biliprasin (violet) $\Rightarrow$ choletelin (yellow)

b) Rosin's test
1% alcoholic iodine solution (iodine tincture) is layered on about 3 ml of urine. In the presence of bilirubin a green ring develops at the interface. (With bovine bile used on the practical demonstrations a red-brownish reaction can be observed).

c) Rosenbach's test:
The urine to be tested is filtered through filter paper several times and a few drops of HNO_3 is dropped on the filter paper. The filter paper absorbs bilirubin and colored rings can be seen around the spot where the HNO_3 dropped which corresponds to oxidized products of bilirubin.

13. Detection of pus with Donne's Test (principle)

Pus in the urine (dead leukocytes, bacteria, tissue debris) might origin from kidney, bladder or urethra (pyelonephritis, pyelytis, urocystitis, urethritis). In women vaginal discharge (vaginal fluor) might be misleading.
First of all, 2 ml of 20% KOH is added to 5 ml of urine in a test tube and shaken (just once). In the presence of pus air bubbles in the fluid are floating or ascending very slowly. Explanation: Mucus passed into the leukocytes in the urine upon the effect of reagent increases the viscosity of urine which decreases the speed whit which air bubbles rise to the surface (+ = is when the ascent of the small bubbles is slow, ++++ = bubbles of a larger size do not move). Since the test might be positive in proteinuria too, it is important to perform a urine sediment test.

14. Rapid Strip Tests

Different reagent papers and pills might give instant, semiquantitative information concerning urine. Sticking to instructions found on most reagents' container is of high importance! Fresh, well mixed but not centrifuged urine of room temperature should be used. Test paper impregnated with the buffer and the special indicator is immersed into the urine for a short time (30 to 60 seconds) and evaluated by comparing the color developed to a color scale usually found on the container.
Do not touch the reagent area on the stripes!

a) Detection of protein

Bromphenol-blue indicator (yellow if adjusted to pH 3 with buffer) turns to greenish-blue depending on the kind and concentration of the protein present. The test is based on the so-called protein-error of some indicators: that is, the color change of the indicator is proportional to the concentration of the protein. The indicator is most sensitive to albumin. The cause of an occasionally false positive result can be due to the following: urine has high alkalic buffer capacity and the test paper cannot provide the acidic pH needed and bromphenol-blue becomes blue even in the absence of proteins. In this case few drops of 3% acetic acid is added to the urine and the test is repeated.

b) Detection of glucose
The test paper is impregnated with buffered glucose-oxidase, peroxidase and a chromogen indicator. Glucose is formed to gluconolactone by glucose-oxidase at the oxygen of the air, while H_2O_2 is released. This product is decomposed by the peroxidase present and the releasing atomic oxygen oxidizes the indicator to a colored compound. The intensity of violet color developed is approximately proportional to the concentration of glucose.

c) Detection of Ketonbodies
The test is based on the nitroprusside reaction of ketones (test paper is impregnated with sodium nitroprusside). In the case of a positive reaction a violet color can be observed, color intensity is approximately proportional to the ketone concentration. The reagent is more sensitive to acetacetic-acid than to acetone.

d) Detection of Urobilinogen
The test is based on Erlich's aldehyde reaction. The test paper becomes yellowish-brown or brownish in the presence of UBG; color intensity is proportional to the concentration.

e) Detection of Hemoglobin or Blood
Its principle is similar to that of benzidine test but the chromogene used is o-toluidine. The test is positive if the paper becomes blue. It is more sensitive to hemoglobin and myoglobin than to the intact RBCs.

15. The Effect of ADH on Rats

15 ml/kg of water is given to four rats through a gastric tube (with a syringe). Two groups of two animals are formed. 0.2 ml vasopressin (Adiuretin SD) is administered to one group ip. and urine is colllected for a 2 hour period. Compare the volume of urine in the two groups. Rats treated with ADH will excrete less urine than the controls.

16. Dilution and Concentration Tests in Humans (principle)

a) Concentration capacity is tested by inducing thirst; the person tested receives dry and salty supper and is asked not to consume any fluid afterwards. While collecting urine every second hour starting from next morning till noon (or, alternatively, until the patient is able to bear thirst), the volume and specific weight of urine samples is determined. If any of the specific weight values measured reaches 1025 g/l within 4 hours the test might be stopped since this value indicates a good tubular function. If cellular functions of the distal tubuli are impaired but they are still able to some adjustments of the urine concentration: this is called hyposthenuria. Asthenuria means that there is no controlling function (kidney insufficiency) and the specific

weight of urine equals with that of plasma-ultrafiltrate, that is 1010 g/l. No precise results can be obtained if the patient is edematous, drinks water secretly, or consumed a lot of water right before the test.

b) Diluting capacity of the kidney is investigated by giving 1.5 liters of water to the patient with empty stomach (it should be consumed during a 15 minute period) after the first urination in the morning. Urine is collected during the next 4 hours in 30 minute intervals and the quantity and specific weight of the urine fractions are measured. In case of good diluting capacity the 1.5 l of water consumed is excreted within 4 hours, and the specific weight of at least one of the fractions is around 1003. The test cannot be evaluated right after thirst induction, or if the patient is edematous. Dilution test is not used any more in diagnosis of kidney diseases; in kidney or tubulus diseases concentrating capacity impairs sooner than diluting capacity: the concentration test is more sensitive to impaired tubulus function than the dilution test.

17. Counting Cellular Elements in Urine According to Addis's method

The Addis's count expresses the number of cellular elements (RBCs, leukocytes, cylinders) excreted with urine in one minute. The procedure of cell number determination is cumbersome and gives just questionable qualitative information, though it is rarely applied in practice.

Urine collected for definite time is mixed and its quantity measured. After tenfold diluton of a small sample of collected urine the number of cellular elements are counted in Bürker's chamber. Knowing the time of urine collection and the volume of urine the number of excreted cells over one minute can be determined. Normally is less than 2000 erythrocyte/min or 4000 leukocyte/min.

18. Determination of Clearance

Clearance is concerns particular substances excreted by the kidney; it is the virtual amount of plasma cleared from a given substance in the kidney in a minute. Clearance of a particular substance can be calculated with the following equation:

$$C = U \times V/P$$

U = urine concentration of the substance
V = urine output/min
P = plasma concentration of the substance

Each endogenous and exogeneous substance has a particular clearance value, different substances thereby can be classified in the following manner:

- Zero is the clearance of substances which are not excreted under normal conditions. Their characteristics is that they are either not filtrated (plasma proteins), or if filtrated, they are actively reabsorbed (glucose).

- GFR is equal to C for those substances which are freely filtrated, but the amount once filtrated does not change in the tubuli, there is neither secretion or reabsoprtion. Examples include creatinine, which is a physiological component of plasma, or inulin which is an exogenous substance.

- The clearance is equal to the RPF of those substances which are filtrated and are secreted into the tubulus-lumen by an active transport; e.g. PAH.

- The clearance is between these two theoretically extreme values for those substances which are freely filtrated and undergo both reabsorption and secretion in the tubuli (e.g. potassium ion).

There are substances, the clearance values of which give information on kidney function, therefore, they are routinely checked in everyday clinical practice.

Clearance techniques:
A substance providing a relatively constant plasma concentration would be optimal for this investigation; it should be freely filtrated in the kidney and should not be toxic.

The great advantage of the clearance test of endogenous substances is that their plasma level is practically constant; opposed to exogenous substances the plasma level of which can be kept constant with continuous infusion only. Accurate collection of urine is important and the bladder should be completely emptied before the test. In testing the clearance of exogenous substances relatively frequent urine collection might be necessary. It can be achieved with a bladder catheter, which might cause some urethral infection, therefore catheterization should be performed under sterile conditions. In determination of clearance of endogenous substances a simple 24-hour-long urine collection is sufficient, thus the complications of catheterization can be avoided.

In clinical practice the clearance of endogenous creatinine is the most frequently used for the measurement of GFR.
Performance of the test:
- 24 hour urine collection
- taking blood sample from the cubital vein
- determination of creatinine concentration from urine and blood sample
- urine output/min is calculated: quantity of 24 hour collected urine is divided by 1440
- Clearance value is calculated by the equation: UxV/P.

Normal value is 120 ± 25 in men, and 110 ± 25 ml/min in women.

In clinical practice the value of **"osmotic clearance"** is also frequently used. The term is identical with the well known definition of the clearance: $C_{osm}=U_{osm}xV/P_{osm}$ and means that amount of plasma which will be cleaned from it's osmotically active particles in a minute.

The **"free-water clearance"** means a difference between minute diuresis and osmotic clearance.

$C_{H2O} = V-C_{osm} = V-U_{osm}xV/P_{osm} = Vx\ (1-U_{osm}/P_{osm})$

IV. ALIMENTARY CANAL

1. The pH of Saliva

The test is performed with universal indicator paper. The pH of fresh saliva is slightly acidic (pH 6.6-6.9), later CO_2 evaporates and the pH becomes slightly alkalic (pH 7.1).

2. The detection of proteins

Acetic acid of 1% is added to the saliva, mixed, and the precipitate developed is filtered.

a) The sulphosalicylic acid test is made with the filtrate.

b) If a sufficient amount of precipitate is seen in the acetic acid reaction, it is boiled with 20% HCl until it becomes brown. The HCl is then neutralized with Na_2CO_3 and the Fehling's test is performed.

Mucine precipitates in the form of flakes when reacting with acetic acid. Dropping sulfosalicylic acid to the filtrate results in precipitate again, which is albumin. Hydrolysis with HCl decomposes mucine into its components from which the glucose released from glycoprotein gives the Fehling reaction.

3. Investigation of saliva-amylase and maltase

Add 1 ml of amylase containing solution (saliva) to 20 ml of a starch-solution in a test tube and shake well. Then 3 ml of this solution is boiled in another test tube, both tubes are put into water bath of 40°C. Amounts of 3 ml are taken from the first test tube after 2, 5, 10 and 20 minutes, then iodine and Fehling's tests are performed. Both tests are performed also with the boiled content, too.

Amylase in the amylase containing solution (saliva) hydrolyzes starch, it is decomposed into amylodextrin, erythrodextrine, maltodextrine and finally into maltose. Maltose is further decomposed to glucose by maltase.

Erythrodextrin gives a red color when reacting with iodine (after 5 minutes), but does not give a reaction in the Fehling's test. Maltodextrin does not give an iodine reaction (after 10 minutes), but gives a Fehling's reaction. Maltose does not react with iodine, but the Fehling's reaction is rather strong (after 20 minutes). Thermolabile enzymes become inactivated in the test tube when boiled, thus a blue color reaction is seen with iodine (starch was not decomposed).

4. Obtaining Gastric Juice, investigation of the acidity of gatsric juice

A soft rubber tube of 4-6 mm in diameter is introduced into the stomach to a length of 45-60 cm (checked on the calibration of the tube). An olive shaped metal weight with aperture (“oliva”) is fixed on one end of the tube. Gastric juice can be extracted through the “oliva”. The gastric juice produced is extracted with a syringe in every 10-15 minutes, and the hydrochloric acid content of the samples is determined.

In the clinical practice gastric juice is collected with a tube, which is introduced to the antrum. At first, the gastric content is extracted completely then the basal gastric juice is collected during 2 (possibly 3 or 4) periods of 15 minutes. The extracted gastric juice fractions are measured with a graded measuring cylinder.

After fasting pentagastrin is administered sc. in a dose of 6 μg/kg bodyweight stimulating the gastric juice secretion. After stimulation, gastric juice is collected in four fractions every 15 minutes.
Hydrogen ions are titrated with 0.1 N NaOH until the pH is 7.4.
Calculation of the Hydrogen ion concentration: considering the factor of the applied NaOH, the titrated acidity of the gastric juice is:
H^+ concentration = [NaOH diminution (ml) x NaOH molarity (mmol/l) x factor] / the volume of the titrated gastric juice (ml).
Calculation of the acid secretion in each collecting intervals of 15 minutes:
H^+ secretion = volume of secretion x the calculated H^+ concentration

Evaluation of the results:
The most important parameters concerning gastric juice in these tests are as follows:
- BAO (basal acid output): The basic secretion, which is the amount of H^+ (<3 mmol/hour) produced in the stomach in the absence of any stimulating agent.
- MAO (maximum acid output): The total amount of hydrochloric acid produced in one hour after the application of the stimulating agent. In normal cases it is 15 to 30 mmol/hour.
- PAO (peak acid output): The highest secretion amount, that is, the sum of the two highest peaks on a graph showing the time curse of the acid secretion is multiplied by two. It's value should be greater than 50 mmol/hour.

5. Detection of lactic acid in the gastric juice (Uffelmann's reaction).

In the case of achlorhydria the gastric content should be tested for lactic acid. If HCl is missing from the gastric juice, bacteria may invade the stomach, the metabolism of which is resulting in the accumulation of lactic acid. This condition may indicate atrophic gastric mucosa as e.g. in chronic gastritis or in gastric cancer.
One drop of phenol is added to 1 drop of ferri-chloride solution, then it is diluted with 2-3 ml distilled water (color: light violet) and few drops of the gastric juice are added to the solution. An amethyst-blue ferri-phenolate solution develops upon the combination of $FeCl_3$ solution and phenol, from which a yellow colored ferrilactate is produced in the presence of lactic acid.

6. Investigation of the protein digesting function of pepsin in gastric content.

2 ml of pepsin is put in one test tube, 2 ml of hydrochloric acid into the second, and pepsin and hydrochloric acid of equal amounts are added in the third one. Small pieces of boiled egg-white are added to all the three test tubes which are placed into a water bath of 40°C for about 20-30 min. Since the action of pepsin requires acidic pH, egg-white will remain intact in the 1st and 2nd tubes, while protein-decomposition can be observed in the 3rd test tube.

V. CENTRAL NERVOUS SYSTEM

1. Investigation of Blood-brain barrier and neurogen inflammation in the rat

The animal is anesthetized with 5 % Chloral hydrate (400 mg/kg), then 1 ml of 1% Evans blue solution is injected into the tail vein. A blue tint of hair-free regions of the body (eyes, ears, tail, limbs) can be observed after few minutes. Thereafter, the dorsal surface of one of the hind paws is painted with 5% Mustard oil (allyl-isothiocyanate) and one of the ears is pinched with a forceps.
After 30 minutes the chest is opened, the pericardium is peeled and an incision is made on the right auricle by lifting the heart. Another incision is made on the left chamber of the heart through which a cannula is introduced. The animal is perfused with physiological saline until the fluid flowing out from the right auricle is free of the blue dye. Then perfusion is made with 4% formalin, which will fix the tissues. Removing the brain we can detect spots of blue coloration. Evans blue stain cannot penetrate through the blood-brain barrier, therefore, it does not stain the whole brain tissue, except for those areas where the blood-brain barrier is missing. These regions include the circumventricular organs e.g. the pineal body, hypophysis, area postrema.

Mustard oil has a strong stimulatory effect on the sensory nerves; therefore it releases vasoactive peptides from the chemosensitive nerve endings of the skin. Due to the action of substance P (vasodilator peptide) and CGRP (increases the permeability of the capillaries) the albumin-bound Evans blue dye penetrates into the tissues (the skin of the treated hind paw and the pinched ear).

2. Tendon Reflex (synonyms: proprioceptive reflex, stretch reflex, monosynaptic reflex)

The characteristic of these reflexes is that both the receptor and effector are in the same organ, stimulus induces response in the same muscle which was affected by the stimulus (proprioceptive-reflex). They can be induced with a reflex-hammer in a patient in laying or sitting position who holds the limb in a resting posture (app. halfway between flexion and extension). Apply a moderate hit on the tendon of the muscle checked. The latency time and intensity of the reflex response of the two sides are compared.

Reflexes observed can be classified:
- normal
- enhanced, increased (hyper-reflexia)
  The immediate, energetic reflex can be evoked on the usual location only. In case of enhanced reflexes the reflex zone is extended, e.g. patellar knee-jerk reflex can be evoked even with a hit on the tibia. Hyper-reflexia is the typical sign of the upper motorneuron lesion.
- no reflex can be observed (hyporeflexia or areflexia).
  Hyporeflexia can be detected in lower motorneuron lesion.

a) Patella (knee-jerk) reflex (L_{2-4})

The person to be tested is sitting or the examiner puts his left arm under the knees of the person being in laying position, with slightly relaxed muscles. A light hit is applied on the patellar tendon. Contraction of the quadriceps femoris muscle and the subsequent extension of the leg

will occur. The patient may not be able to relax muscles and thereby the reflex can not be evoked. In these cases or in case of pathologic decrease of reflex (hyporeflexia) various maneuvers (e.g. Jendrassik's maneuver is applied). The two hands of the patient are clasped in front of the chest, and the patient should try to pull them apart, while the knee-jerk reflex is evoked. A similar method is to ask the laying patient to lift the head. These procedures may help since during active contraction of some muscles the basal tone in the brain-stem reticular formation is increased, and the descending facilitation decreases the reflex threshold.

b) Achilles tendon reflex (S_{1-2})

The person to be tested is in kneeing position; if he is laying his knees are bent and the legs are slightly dorsiflexed. Hit the Achilles tendon. The triceps surae muscle will contract upon the hit, and plantar flexion can be observed. Plantar flexion, if not seen, can be felt with the examiners palm through the pushing movement of the leg.

c) Biceps-reflex (C_{5-6})

The elbow joint is bent, the lower arm is kept across the chest, the hand is in supinated position, and the tendon of the biceps is slightly pressed with the thumb. Hit on your thumb with the reflex hammer and the biceps will contract as a response.

d) Triceps-reflex (C_{6-7})

The upper arm is abducted and lifted up to horizontal position and is supported by the examiner. Let the lower arm hang and strike the tendon of the triceps (the tendon is very short!) right above the olecranon with the reflex hammer. Reflex response will be the extension of the elbow joint and that of the lower arm.

e) Radial reflex (C_{5-6})

Lower arm is bent transverse (semiflexed elbow joint) and the head of the radius or the brachioradial muscle tendon is tapped. Flexion of the forearm with sometimes the flexion of the fingers as well can be observed (the hand is slightly dorsiflexed).

f) Masseter (jaw jerk) reflex (n.V.-n. VII.)

The mandible—or lower jaw—is tapped at a downward angle just below the lips at the chin while the mouth is held slightly open. In response, the masseter muscles will jerk the mandible upwards. Normally this reflex is absent or very slight. However in individuals with upper motor neuron lesions the jaw jerk reflex can be quite pronounced.

3. Skin Reflexes (synonyms: flexor reflex, protective reflex, polysynaptic reflex)

Their characteristic feature is that the receptor and effector are not in the same organ. They can be induced with the handle of the reflex hammer or with a blunt needle.

a) Deep plantar reflex (L_5-S_2)

The medial side of the sole is stimulated which causes plantar-flexion.

b) Superficial plantar reflex (L_5-S_2)

Toes contract upon stimulation of the lateral side of the sole.

c) Abdominal skin reflexes:
- upper (Th_{7-8})

Handle of reflex hammer is drawn over the upper part of abdominal skin parallel with the costal arc (proceeding from lateral to medial).
Contraction of the abdominal muscles -movement of the navel towards the stimulated side- is the response.
- medial (Th_{9-10})

The abdominal skin is stimulated at the level of the navel. Contraction of the abdominal muscles can be observed; The oblique abdominal muscle is contracted, and navel moves towards the stimulated side.
- lower (Th_{11-12})

The lower portion of the abdominal skin is stimulated parallel with the inguinal ligament. The response is the same as above.

d) Cremaster reflex (L_{1-2})
Stimulation of the skin on the inner side of the thigh causes contraction of the ipsilateral cremaster muscle and lifts the testicle on the same side.

4. Investigation of Tremor

Tremor is a rhythmic hyperkinesis of low amplitude and varying frequency. Generally it appears in the limbs and in head-neck muscles, and it is consisting of involuntary motions. It is caused by the preponderance of alternating innervation of agonist and antagonist muscles.

a) Resting tremor:
It is the classic symptom of Parkinson's disease has a frequency of a 4- 6/sec and regular amplitude.

b) Intention tremor:
Characteristic of ponto-bulbar and cerebellar diseases, it appears as tremor with irregular amplitude and rhythm increasing when approaching the target.

c) Static tremor:
It can be observed in fatigue, or hyperparathyroidism, and exerted by a voluntary, fixed posture.

The above tests can be performed with simple observation, finger to nose-tip test, or with EMG. In a laboratory it is performed with the Tremometer. A plastic thimble is put on one (generally on index finger) with a magnet on its top. The finger with the thimble is put into the hole of the tremometer. The finger should not touch the wall of the hole! The magnet in the thimble reaches into the coil of the tremometer; as the finger with the magnet moves the magnetic field generates a potential in the coil of the tremometer, this can be visualized on the computer screen. Printing out the image on the screen the amplitude of the tremor can be measured.

5. Investigation of Reaction Time

The patient has to perform a task when receiving a signal. Reaction time is the time passed between signal onset and the completion of the task required. This time might depend upon

several factors. It might be influenced e.g. by the intensity and type of the stimulus, the task (complexity) and the attention evoked.
A positive (+) signal appears from time to time on the computer screen. The instrument shows the signal according to a preset average time but at intervals which may be shorter or longer than the average. This is necessary, because reaction time may decrease due to learning if the signal appears routinely (anticipation). The signal has to be eliminated from the screen as soon as possible with the proper keyboard key. Latency period of the responses are defined during the test. The test should be repeated ten-times, at least then the computer shows the average latency of the responses in milliseconds.

6. Electroencephalogram (EEG)

The EEG results from the activity of cortical neurons, has a magnitude in the μV range and has a frequency of 0.5 -70 Hz . Potential differences can be recorded over different regions of the brain with electrodes attached to certain standard points of the skull. In bipolar electroencephalography potential differences between two electrodes attached on the scalp are recorded (in clinical practice this is applied). In unipolar electroencephalography a differential electrode is attached on the scalp, while the indifferent electrode is placed on a neutral place, e.g. attached to the ear-lobe. Fluctuations in the potential differences are amplified with a differential amplifier and recorded on paper.
When analyzing EEG fluctuations in the electric potential between corresponding and non corresponding cortical regions are compared. Frequency and rhythmicity of the waves, their shape and amplitude are investigated.

Spectral composition of the EEG

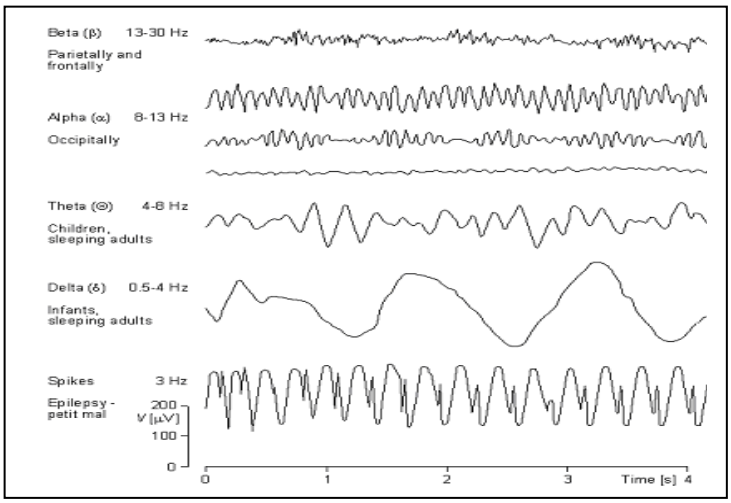
delta (δ) wave = 1-3/s

theta (θ) wave = 4-7/s

alpha (α) wave = 8-13/s

beta (ß) wave = 14-30/s

gamma (γ) wave = 30-50/s



EEG waves

Alpha rhythm dominates the EEG of the adults in a relaxed, awake state. Amplitudes are highest in the parieto-occipital region where it often forms series of waves with fluctuating amplitude in the 100 μV range - these are the so called spins. The EEG becomes desynchronized (i.e. frequency increases and amplitude decreases) upon opening of the eyes, focused attention or mental work. In these cases beta waves having lower amplitude with fronto-precentral dominancy can be recorded. Closing the eyes will induce alpha-activation

again. Theta rhythm can be observed in children or in sleeping adults under normal conditions. Delta waves are physiologic in infants and in adults during deep sleep.

A pathologic waveform is the so-called spike, which is the result of simultaneous excessive discharge of neurons. Short spike- potentials with steep peaks are characteristic of the excited state in epilepsy. Slow waves accompanying spike-potential (spike-wave complex) are also characteristic of certain types of epileptic diseases.

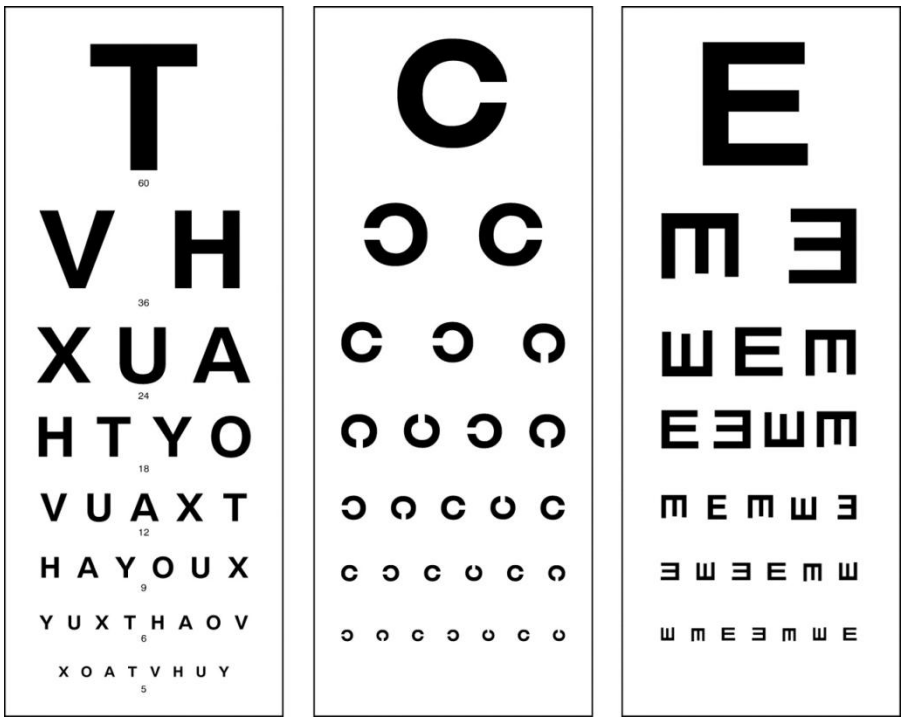
Different activation procedures are routinely applied in EEG diagnostics:
- hyperventilation
- intermittent light stimulus (especially in epileptic patients)
- sleep deprivation

EEG might provide information on the developmental state of the cerebral cortex, alertness, or on intracranial volume constricting processes (tumor, hematoma). It is applied in the diagnosis of epilepsy and in follow up investigation of the disease, unconscious states (coma), differential diagnosis of repeated loss of consciousness, and in the diagnosis of brain death.

VI. THE ORGANS OF SENSE

1. Visual acuity testing

To measure visual acuity an eye chart is used. Charts usually display several rows of test symbols (e.g. black Snellen letters and numbers or “Landolt C”s (incomplete rings) against a white background), each row in a different size. The person is asked to identify the numbers or letters on the chart, usually starting with large rows and continuing to smaller rows until the symbols cannot be reliably identified anymore. Each symbol row is associated with a charachteristic distance (it is represented on the chart beside the rows) from where the figures can be seen in a visual angle of 5 minutes of arc (5'), while details of the figures in a visual angle of 1'. The visual angle of the uppermost figure is 1' from 50 meters, while the figures in the penultimate row from 5 meters (figures of the last row are used for hyperacuity testing). Symbols on the chart are placed in a square distributed into 25 small squares. Each of the small squares gives a visual angle of 1', while the large square gives a visual angle of 5'.



a **b** **c**

Eye charts (a: Snellen, b: Landolt C and c: illiterate E)

The chart should be well illuminated during the test. The person to be tested is sitting 5 meters from the chart which is suspended at eye level. The patient covers an eye with the ipsilateral hand or holds an eye card over the eye. The patient is requested to read the letters, numbers or figures starting from the top. The examiner looks for the smallest symbol readily recognized. The degree of visual acuity determined is expressed as a fraction number:

$$V=d/D$$

d = is the distance (m) of viewing

D = is the distance from where the symbols are recognized at 5' visual angle, while its details at 1' visual angle.
Normal value: 1 (V = 5/5)
If the person can see the characters of the size that he/she should be able to see at 5 m, the subject is said to have 5/5 visus, that is normal. If the patient can only see characters that should be seen from 50 m, than the visus is 5/50. The characters are viewed in a visual angle of 5 minutes of arc (5') from the distance indicated, while details of the figures in visual angle of 1'. If the patient recognizes symbols giving 5' visual angle from 15 m (which is recognized at normal vision from 15 m), visual acuity will be V = 5/15.

If the patient tested cannot see the largest symbols, the chart should be placed closer. If the patient can view the topmost symbol from 2 m, then the visual acuity will be 2/50.

If visual test is unsuccessful, counting of fingers is made from a distance of 5 m. In these case the distance is given instead of visus from where the patient can count fingers shown at dark background (e.g. finger counting from 2 m). If the patient is unable to perform this task, light-dark perception is tested.

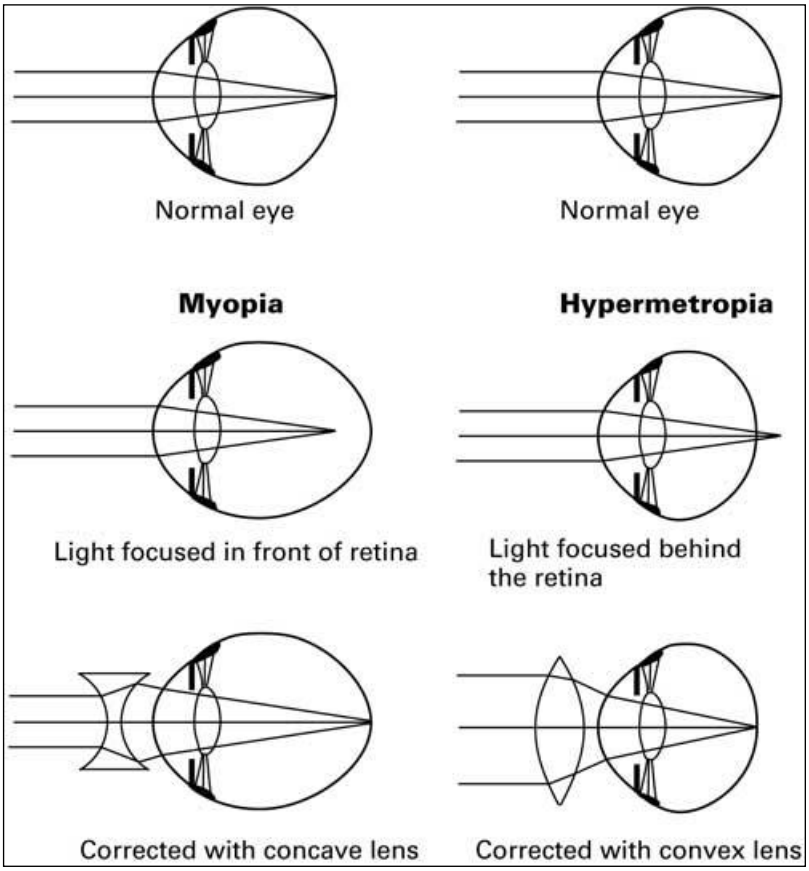
2. Refractive error correction (principle)

The patient is tested with an eye chart; if deviations are found, different lenses are placed before the eyes to find the lens correcting the error. The lens set contains biconvex, biconcave and cylindrical lenses of different refractive power which can be placed in a frame. Refractive error of one eye is corrected at a time, the other is covered with the black metal slip of the spectacle.

a) Normal, relaxed, not accommodating eyes refract parallel rays that way they project a sharp image on the fovea centralis of the retina: emmetropic eye.

b) Myopia (short-sightedness): the longitudinal axis of eye is longer than normal (24 mm), or its refractive power is too strong for the length of the bulbus, and the sharp image is formed infront of the retina. Myopia is corrected by concave (negative) lenses placed before the eye causing light rays to diverge and form sharp image at the retina.

c) Hypermetropia (far-sightedness): the longitudinal axis of the eye is shorter than the normal, or the refractive power is too weak related to the length of bulbus: parallel rays arriving at the eyes (would) form thze sharp image behind the retina. Placing convex (positive) lenses before the non-accommodating eyes cause light rays to converge and form sharp image at the retina.



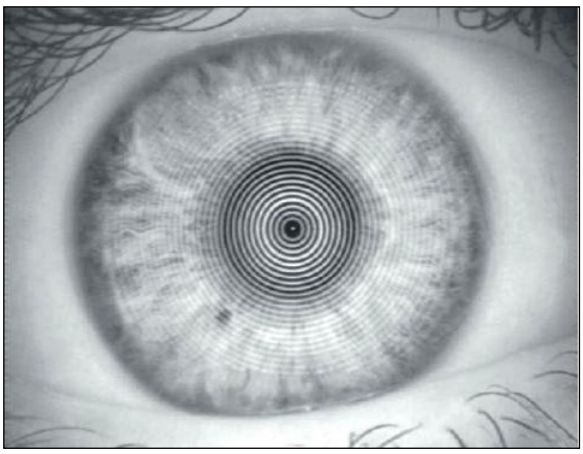
Refractive errors and their corrections

3. Detection of astigmatism

If the curvature of the cornea is different along the different meridians, the image will be distorted. This condition is called astigmatism. Normally the cornea has a 0.5 D (diopters) stronger refractive power in the vertical plane: this is called physiological astigmatism. Regular astigmatism: the curvature is regular within the same meridian and the meridians of the weakest and strongest refractive power of the eye meet at a right angle. Their correction is possible with cylindrical lenses having a convex or concave surface.
The cylinder acts like a plan-parallel sheet of glass plane along its axis, and it does not refract light, but its refraction is maximum perpendicularly to the axis and the transition between the two stages is not linear.

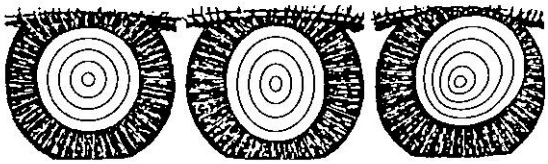
Detection of astigmatism can be performed by:

a) Placido's disk:
The disk having a diameter of 25 cm is equipped with a handle. On the surface there are concentric circles with a round hole in the middle. The person to be tested is standing with his/her back to the light, the examiner looks through the hole and checks the image of the concentric circles on the subject's cornea.



Placido's disk

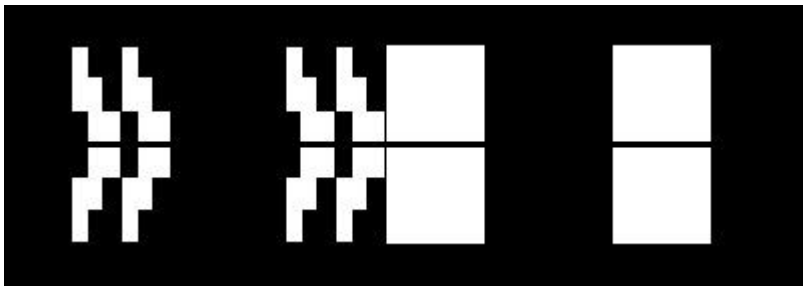
If the curvature of the cornea does not differ too much in the different meridians, then the reflected concentric circles are similar to those on the keratoscope. In the case of astigmatism, however, concentric circles reflected on the cornea are distorted and shifted (like altitude lines on a map).



Astigmatism

b) Javal-Schiötz ophthalmometer:
Refractive power and radius of curvature of the cornea can be determined with this instrument in any meridian. A chin stand is adjusted so that the eyes are at the correct level and one eye is covered. The test has to be performed in a dark room. The ophthalmometer projects two light rings that have to be focused onto the patient's cornea and the device has to be shifted until the rings fuse on the cornea. Afterwards this focusing light is covered. Through a telescope placed perpendicularly to the central point of cornea two images (a square-like pattern and a staircase-like figure) are projected on the cornea. Each figure can be seen doubled. Sharpen the image and by turning the ring on the telescope move the two medial figures that way that they touch each other. At this position the curvature radius and the refractory power in this meridian can be read in the smaller telescope on a scale of the device.
Normal Value = 42.75 - 43.40 D or 7.8 mm.
Now turn the telescope 90 degrees and check the pattern again. If the position related to each other did not change the refractory power and curvature radius in this meridian are the same as they were in the previous one. If the figures are shifted, try to set back the original situation (as it was 90 degrees before), and read the values on the smaller telescope. The difference between the values obtained in the original and in the new position will give the value of astigmatism.

Astigmatism is considered "physiological " if the value of it does not exceed 0.5 D. 1 step overlapping = 1 D refraction difference.



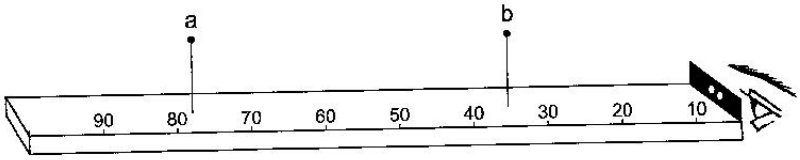
Figures of the Javal-Schiötz ophthalmometer

4. The accommodation test

Accommodation is the process by which the eye changes its refractive power to maintain a clear image on an object as its distance varies.

a) Scheiner's net test:
Printed text is read through the plain-net. The net is not seen while reading the text. If the net is fixed with the eyes, the text cannot be read, since it is blurred. However, we can choose which object to fixate on i.e. we can change the refractive power of our eyes.

b) Scheiner's test:
Two pins are fixed on a rod at about half a meter distance. At one end of the rod there is a metal piece with two round holes; the distance of the holes should not be larger than the diameter of the pupil. When looking through the holes and fixating the closer pin (b), the pin being farther (a) will be seen double and vice versa.



Scheiner's test

Human eyes are adjusted (accommodated) to view objects in one plane, objects inside and outside of the fixed plane do not fall on corresponding retina points and seem to be double.

5. Mariotte blind-spot test

There are spots within the normal visual space which are not perceived since light rays coming from them reach the "head of the optic nerve" the papilla where photoreceptors are not present.

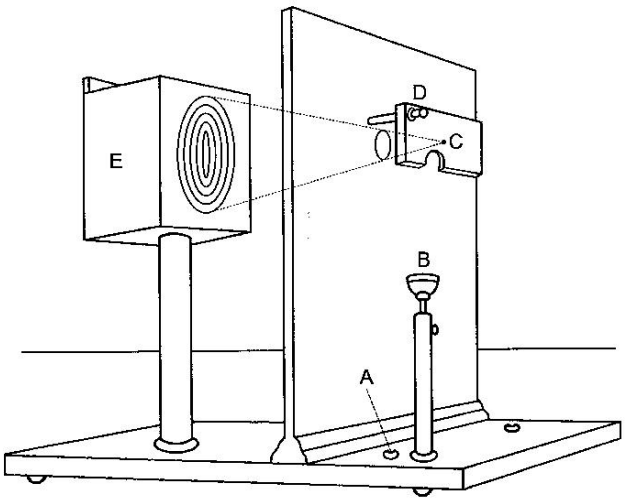
An image similar to the one represented below is held before the eyes at about a distance of 20 to 25 centimeters. The left eye is closed and the cross in the figure is fixated. Moving the picture slowly back and forth the distance is found from where the circle in the picture cannot be seen. The image of the cross (+) falls at the fovea centralis, while that of the circle on the blind spot which does not contain rods or cones, thus we cannot see it. However, it does not cause any problem in everyday life: we do not perceive a "hole" in our visual world, the visual system completes the image.

Mariotte blind spot experiment

6. Testing the light-response of the pupil

Inducing the direct light response: one of the eyes is covered, while the other one is illuminated with a medium or weak light; the pupil will constrict. Pupil will dilate to the original size if illumination is stopped.
Consensual reaction: Light shown in one eye will cause a constriction of the contralateral pupil.

The light reaction of the pupils can be tested even in our own eyes with the help of a pupilloscope. We place our chin on the chin-stand (B), through the hole (C) we can view the concentric circles (E) on the board and try to fixate on the middle.
Pushing the test button we switch on the light illuminating our left eye. The left pupil will constrict and due to the consensual light reaction so will the right pupil. Because the light beams from the periphery are closed out we shall see 2-3 circles less when the light is on. If the light is turned off both pupils will extend, and with our right eye we shall see all of the concentric circles again.

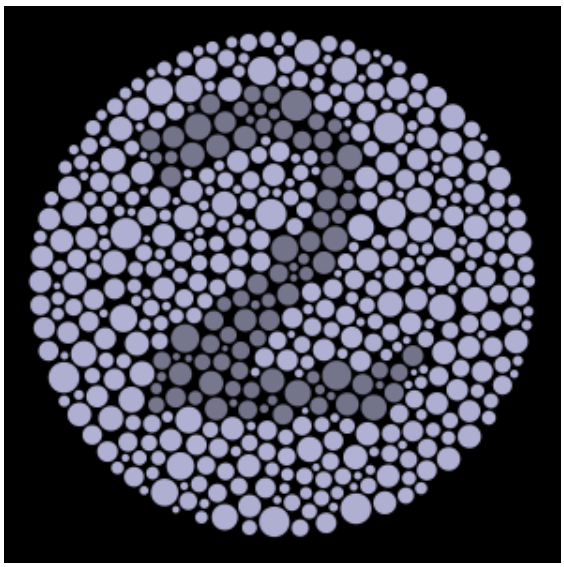


Pupilloscope

7. Testing color blindness

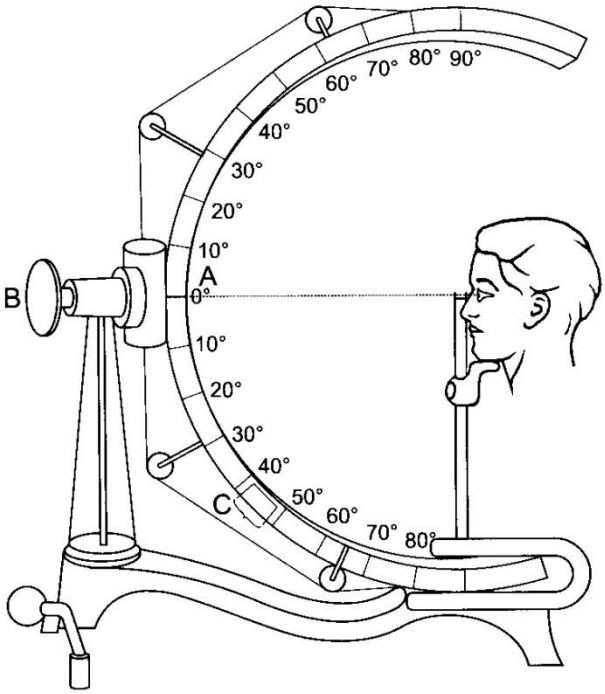
a) Holmgren's test:
A box of thread having different color and shade is used in this test. A sample is taken from the box and the patient is asked to choose the same color. In case of color blindness the person tested cannot perform the test quickly. The type of color blindness can also be estimated (e.g. red-green mixing people often choose green threads when they are told to select red ones). This is not a very reliable test, however, it is very simple, does not need instrumentation and can be performed on poorly educated subjects or on persons who can not cooperate well (e.g. children).

b) Pseudoisochromatic (Ishihara) plates:
The plates are viewed in diffuse light at a distance of 0.5 m. There are some marks, lines, numbers or letters different from the background color of the plate. The color of the mark to be recognized is readily distinguished by persons of normal color vision, whereas persons with color blindness will not find the mark. The colors are not homogeneous, they consist of spots, disks, or wavy lines, thereby the effect of contrast can be ruled out.



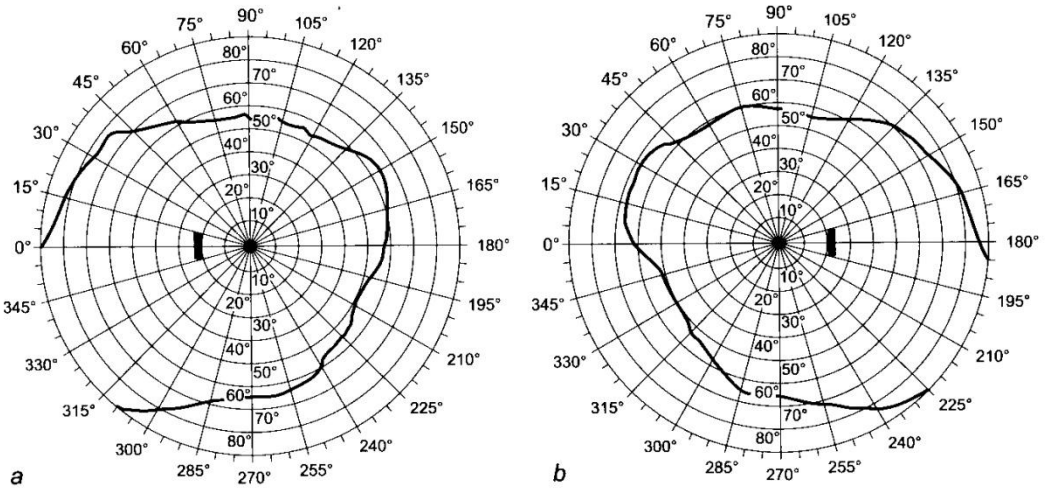
Ishihara plate

8. Testing the size of the visual field (perimetry)



Perimeter

The visual field is the a area of space viewed at with motionless eyes. The purpose of the test is to define the space viewed by the patient, the recognition of a possible scotoma, its localization and determination of its extent.
The most simple method of testing visual field is the “confronting test” of the visual field. The patient is seated opposite the screen at a distance of 1 meter. His right eye is covered and asked to look into our right eye with his/her left eye, while we cover our left eye. With the index finger we move from the periphery to the center from several directions and the patient gives a signal when he sees our finger. If both the patient and the examiner fixate each other's pupils and their visual fields are intact they will perceive the movement of the hand at the same time. The same examination should be repeated with the other eye.

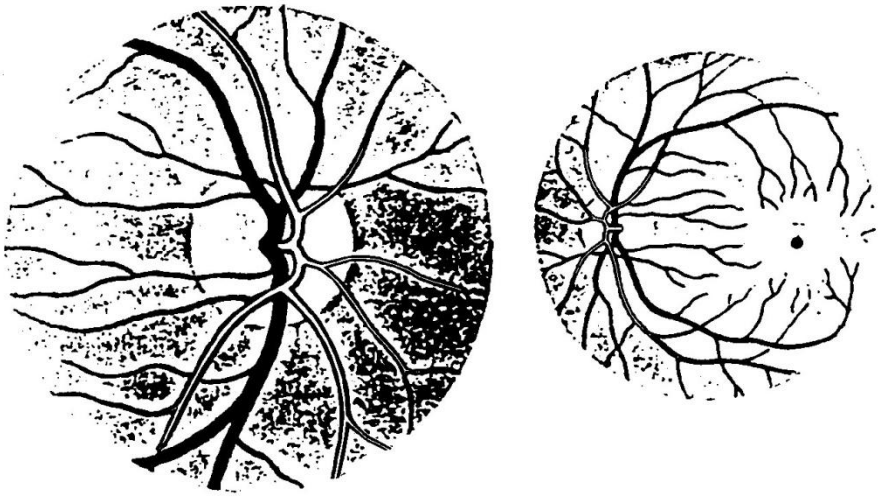


Visual field of the left (a) and the right (b) eye

This method is not sophisticated, it can detect a large scotoma only. For a more accurate testing a perimeter is required. It is an illuminated half-sphere or an arch having a radius of 0.33 m. The patient's eye is positioned in the geometrical center of the sphere/arch, he/she looks on the inner surface. The extent of visual field can be tested with a small blinking light along each meridian or a moving paper sign with this test.
The chin of the patient is placed on the stand in a position that the eye tested is at the level of the central point of the sphere and can be fixed with the eye tested. The other eye of the patient is covered. A white paper sheet (or paper of the desired color in case of color perimetry) is fixed in the groves of the arch. It is first located at the periphery then slowly proceeded towards the center. The patient is asked to give a signal when detecting the mark. The value obtained is recorded on the patient's perimeter diagram. Next the marker is moved slowly in the same meridian between the two extreme points to test any scotomas.
The test is repeated rotating the arch of the perimeter into different meridians of each eye. By joining the recorded points the outermost borders of the visual field can be recorded. Compare the results obtained with the physiological visual field (indicated in the diagram with dotted line). Deviations from the normal values can be given indicating the meridians where they were detected.
Visual fields of both eyes are 100-120 degrees vertically, 180 degrees horizontally when the eyes are stationary; with moving eyes it is 200 degrees vertically and 260 degrees horizontally. The two visual fields partially overlap.

The determination of the visual field for colors is performed in a similar way by applying the desired colored mark. The size of the visual field is different for different colors; it is the largest for white followed by blue and red and the smallest one is for green.

9. Ophthalmoscopy



The fundus of the eye as seen with an ophthalmoscope

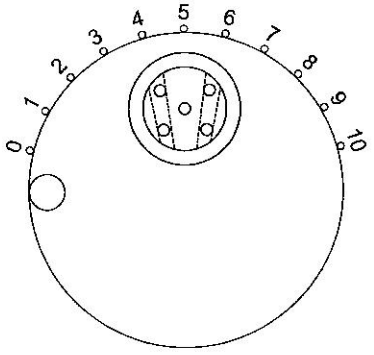
The test is based on the fact that the light that is projected through the pupil is not fully absorbed but a part of it is reflected back; light rays will exit from the eye at the same angle as they entered. Under normal circumstances the pupil seems to be black because our head prevents the entry of those very light rays that would be reflected back towards our eyes. If we use light of proper intensity and illuminate the eye of a person the light that is reflected will be sufficient for the examiner to view the back of the eye, the fundus. When performing ophthalmoscopy the examiner shines a parallel light ray into the eye. The eyes of the examiner and the examined have to be in the same optic line (axis) so that light that would be reflected from the fundus can be seen. For the test we use an ophthalmoscope which has a built in light source and has a series of lenses which can be used for making refractory corrections. The lenses help to create parallel light which can be used to look into the eye and visualize the fundus.

The examiner looks through a little hole above the mirror which reflects parallel light into the eye tested. Various lenses can be selected by turning a dial. Options include: 0, +10, -10, - 20, D or in the upper part of the equipment you can insert other lenses with lower D values; a range of +20 to -30 D can be obtained. In addition a green filter can also be used; this makes shades of red darker, increases the contrast and helps to differentiate between the items of the fundus.
The test is performed in a moderately dark room (it is easier with a dilated pupil). The examiner and the examined are sitting opposite to each other. The examination is always done with the right eye of the examiner examining the right eye of the examined, and like wise for the left.
If both the examiner & examined are emmetropic and neither of them are accommodating then the light rays coming from the examined eye will focus on the fovea centralis of the examiners retina, and the fundus will be seen sharply. If one of the two eyes (or both) is not emmetropic,

the fundus cannot be seen sharply. In this case both eyes have to be made emmetropic. To overcome this problem adjustments are made on the lens dial of the ophthalmoscope, first with a convex lens, then (if they do not help) with a concave lens, until the fundus can be seen sharply.
To exclude accommodation, both persons are fixing at a (imaginary) distant point. This way light beams coming from the fundus are made parallel by the lens, these beams are collected together again by the eye of the examiner on his/her retina.
A detailed image of the fundus can be seen if neither the eyes of examiner nor that of the person tested are accommodating, and none of them have any refractory errors. The retina will be seen as shining red. The bright red, thin lines are vessels in the fundus. If one of these vessels is followed proceeding in a central direction we can reach the papilla of the optic nerve (a light pink disk). Other arteries and veins can also be seen. Arteries are thinner and of lighter color, while veins are thicker and a bit darker. Moving temporal from the papilla the macula lutea can be seen as a darker gray-red region.

10. Testing dark-adaptation with Birch-Hirschfeld's adaptometer

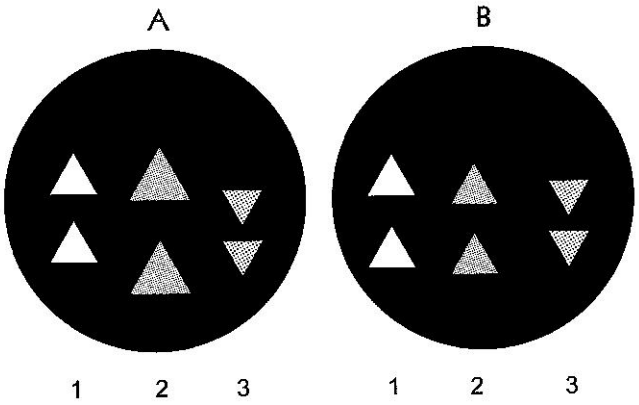
The instrument is made of two revolving disks, with a series of glasses with different light transmission ratios in the front disk. Light transmission of the consecutive glasses is 50 %. White, red and blue glasses are in the second disk, which serve for testing the adaptation to white, red and blue colors. Five light spots can be seen on the surface of the instrument. There is one in the middle, and the spots on the left are twice as bright while those on the right are twice as dark.
The testing procedure is as follows. The examiner and the person to be tested enter together the dark room and the examiner sits about 30 cm from the adaptometer. The frontal disk of the instrument is turned completely to left. First the light sensitivity of the examiner is tested. Turn the disk to the right until the light spot in the middle can be still perceived and the two lighter spots are also well visible. The patient is then asked about the number of spots he can see, and if he can see the spot in the middle, the initial light sensitivity of the two persons (examiner and the patient) is the same. If the patient cannot see anything, the disk is turned further to the right until the patient can perceive the spot in the middle. If, however, the patient can see all the five spots, his initial light sensitivity is greater than the examiner's.



Birch-Hirschfeld's adaptometer

11. Purkinje-Sanson mirror-images

The lens of a flashlight is covered with black paper on which two triangles are cut out. In a dark room the eye of the patient is illuminated with the flashlight from the side direction. Size and distance of the so called Purkinje-Sanson's images can be observed in the refraction surface of the eye during far and close accommodation.



Purkinje-Sanson's images

a) If the eye tested looks far in the distance (diagram A) we see three images reflected
1 - from the cornea
2. - from the frontal surface of the lens
3. – from the posterior surface of the lens

Of those seen 1 is the sharpest, this is coming from the fovea. 2 & 3 are coming from the anterior surface of the lens and of the posterior surface, respectively. These images are hazy because light is absorbed in the aqueous humor and in the lens. Images 1 and 2 (images of convex surfaces) are reduced and are in normal position. The 3rd image (reflected from the posterior -concave- surface of the lens) is reduced and up side down.

b) If the eye tested looks close (diagram B) the 2nd image (coming from the frontal surface of the lens) will be smaller and gets closer to the center of pupil. During accommodation the curvature changes on both lens surfaces but the frontal surface change dominates.

12. Investigation of the critical fusion frequency

A feature of the retina is that a light sensation can lasts longer than the actual light stimulus; even when the stimulus has ceased, light is still seen in the form of positive after-images. Short lasting stimuli of high frequency tend to fuse.
For the test we use an instrument emitting a series of light pulses with different frequencies. Set a high frequency value which is well above the critical fusion frequency. Next the frequency is gradually reduced until the patient does not see a continuous light but rather a vibrating one. The frequency value read at this point is the critical fusion frequency, generally seen to be

around 40-50/sec (Hz). This value may depend also on light intensity, with increasing light it can be as high as 60-70 Hz.

13. Investigation of nystagmus

Nystagmus (a rapid, involuntary, conjugated, cyclic and rhythmic movement of the eyeball) comes about during the excitation of the receptors in the ampulla upon both spontaneous and artificial stimuli. It can also be triggered by the stimulation of vestibular apparatus or the movement of visual field. It consists of a rapid and a slow element. The latter is of labyrinth origin, while the former is a central, correctional movement. The direction of the nystagmus is named traditionally after the direction of the fast component.

The phenomenon of optokinetic nystagmus can be observed if e.g. someone is looking out of the window of a train. Telegraph poles, or trees pass by, the eyes follow them until they disappear at the window's edge (slow component), then the eyeballs return back to the starting position (quick, "saccadic" eye movements) to catch a new (tree) object to follow and so on.
The process of investigation is as follows: the rotating drum of the kimograph is covered with paper having of vertical black and white stripes and looked at. An observer from behind the kimograph will be able to observe the optokinetic nystagmus.
Postrotatory nystagmus is triggered by rotation and can also be tested. The patient is seated into the revolving chair (Bárány's chair). To rule out visual fixation the patient is provided with glasses of 20D convex lenses (Frenzel's glasses), or the eyes are closed. The chair is than turned. If the chair is suddenly stopped, the endolymph in the horizontal semicircular canal will move due to its inertia and the cupula will bend opposite to the direction of rotation, thus the direction of the nystagmus will be the opposite of the direction of the rotation (postrotatory nystagmus).

14. Laryngoscopy

The patient is seated with a strong light-source above his/her shoulder. The examiner puts on a forehead mirror (a concave mirror with a hole in the middle). The examiner should be able to look through the hole and direct the reflected light to the desired location. The examined person opens his/her mouth while the tongue can be pressed down with a spatula. A small mirror is then introduced along the soft palate behind the uvula to reflect light down on the glottis. The mirror is pre-warmed to avoid developing condense water then checked on the hand to be sure it is not too hot. Touching the root of the tongue or the pharyngeal arches should be avoided since it might cause regurgitation. The image in the mirror can reveal the epiglottis and the plica aryepiglottica on both sides, the frontal part of the epiglottis can be seen in the top of the mirror, while the back part is seen at the bottom. The two white vocal cords can be seen in the middle surrounding a triangular opening. The space between the vocal cords dilates upon inhalation, while it constricts or completely closes during phonation.

15. Otoscopy

A light source is placed close to the head of the patient; and the examiner puts on a forehead mirror. For the examination, the curvatures of the external acoustic duct (meatus) have to be made straight by gently pulling the external ear (auricle). In adults the pulling direction is

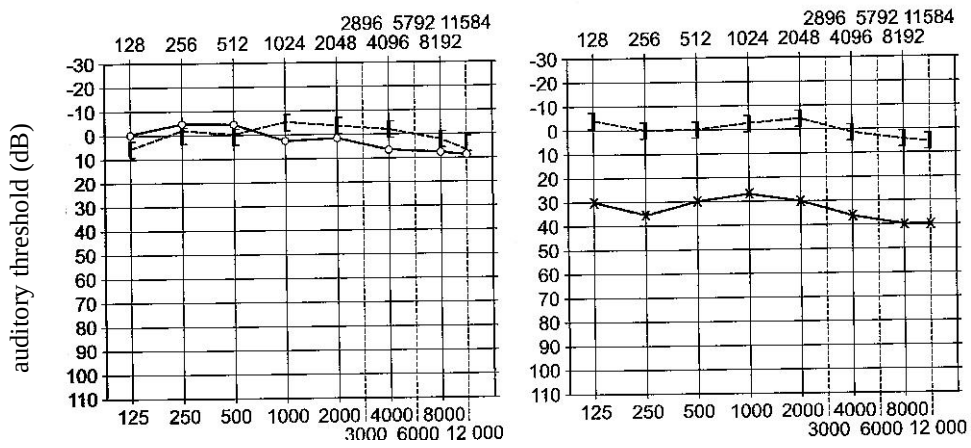
backwards and upwards while in children backwards and downwards. Next a metal ear-speculum is introduced into the meatus (one should progress carefully, the inner 1/3 of the meatus is running in the bone and pressing on the periosteum might be painful.
First the eardrum (tympanic membrane) is observed; it is a light grey, mother-of-pearl colored, oval shaped membrane around 8-10 mm in diameter. It's retracted part indicates the adhesion place of the malleal process.

16. Investigation of Acoustic Acuity

a) The examiner is standing at about 5 meters distance from the patient. The patient covers the ear on the opposite side, while short words of 2 or 3 syllables are whispered, which should be recited by the patient. If the patient cannot recognize them from 5 meters, then the test is continued by approaching the patient one meter each time. Finally the distance from which recognition is complete is given. E.g. in impaired hearing of the conductive type deeper sounds, such as "a" and "o" are not recognized.

b) Drop-test
The instrument is a vertical rod with 1 mm calibration, with a sliding holder and a felt lined box underneath. A metal ball is dropped into the box from gradually decreasing height. The patient is standing at a meter away with his back to it and indicates the recognition of the dropping of the ball. Acoustic acuity is expressed by the lowest height when the patient still hears the sound of the ball dropped.

c) With audiometer
During the so-called threshold audiometry the acoustic threshold of both ears is determined at different sound frequencies. The instrument, an audiometer, is an electric sound-generator, which generates sounds of different frequencies (60-20000 Hz). The intensity (auditory threshold) of the lowest detectable sound is defined and plotted as the function of the frequency. Values obtained in dB at the given frequency will show the deviation from the normal threshold. An important precondition of the test is a quiet (sound-proof) room. During testing the aerial conduction the two ears have to be isolated acoustically from each other. This is done by a headphone with built-in loud-speakers and noise-generators. The patient is sitting with the headphone on his head and he should not see the switchboard of the instrument.
The sound of required pitch is adjusted (frequency), a masking noise of 50 dB is given in the other ear, and starting from 10 dB the sound intensity is gradually increased. The patient indicates when the sound is detected.
(The first measurement usually has to be repeated until the patient gets the required experience in cooperating in the test).
Threshold values of all the frequencies available are defined and the values obtained in dB are plotted as the function of frequency. Then the other ear is tested, the results are plotted on separate diagrams.
In case of normal aerial conduction a curve closely fitting the 0 line should be obtained. A 10-15 dB deviation is still acceptable. In case of any disturbances in aerial conduction the threshold of sounds with lower frequencies (<2000 Hz) is increased.
Investigation of bone conduction is carried out with a special vibrator placed on the mastoid process after properly adjusting the audiometer. No masking noise is applied and the test is performed as described above. If bone conduction is disturbed, the sensation of higher sounds (>2000 Hz) is decreased.



Audiogram

17. Tests with tuning fork

Under physiological conditions sounds between 16-2000 Hz reach the organ of Corti through aerial conduction. In this range there is a combined bone and aerial conduction but the threshold for bone conduction is about 50-60 dB higher, thereby it's role is not significant. Conduction of sounds with a frequency higher than 2000 Hz occurs mainly by bone conduction (the middle ear ossicles -as a mechanical device- cannot follow the high frequencies).
Differentiation between aerial and bone conduction is performed with a tuning fork, through which the perceptive or conductive character of a possible hearing loss can be determined. In the case of a conductive (otogen) hearing loss, the aerial conduction is impaired, and sound waves do not reach the receptor cells. Perceptive hearing loss is associated with the lesion of receptors, auditory pathways or that of the auditory cortex.

a) Weber's tests:
A vibrating tuning fork is held against the forehead. Under physiological conditions the sound is heard equally by both ears of the patient. In case of a conductive hearing loss the sound is heard louder on the impaired side (lateralization). In case of impaired aerial conduction, weaker sound reaches the internal ear which has become adapted to a lower noise level and its receptors become more sensitive than those of the healthy side (e.g. in otitis media-inflammation of the middle ear). In case of perceptive deafness the sound is better heard with the intact ear.

b) Rinné's test:
In this test aerial and bone conduction of the same ear is compared. The handle of a vibrating tuning fork is placed on the mastoid process of the patient (bone conduction) until it is no longer heard. Next the still vibrating tuning fork is held near the ear of the patient (aerial conduction). Under normal conditions aerial conduction is better than bone conduction, therefore the sound can be heard by the ears i.e. the test is Rinné positive. In case of conductive hearing loss, the aerial conduction is shortened and after ceasing of bone conducted sound the patient does not hear the sound of the forks. In perceptive impairments the test is positive.

c) Schwabach's test:
Bone conduction of the examiner with intact hearing and that of the patient is compared. The shank of a vibrating tuning fork is placed on the mastoid process of the patient until its tone is

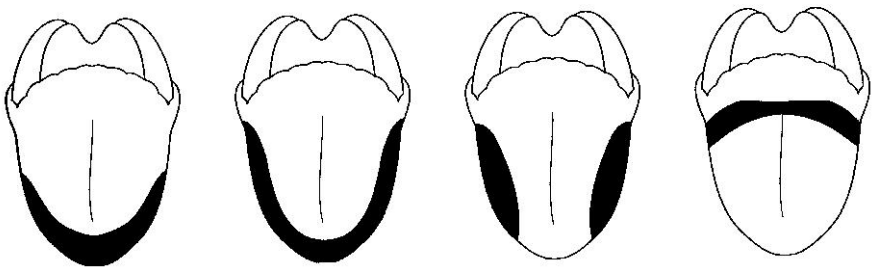
no longer heard, then transferred to the mastoid of the examiner (whose hearing should be normal). If the examiner does not hear the sound, the test should be repeated in the inverse sequence. In case of perceptive hearing loss the examiner hears the sound longer: the Schwabach test is shortened. In case of a conductive disturbance the patient will hear the sound longer: the Schwabach test is elongated.

18. Bárány's pointing test

During this test the coordination of motor activity is tested, usually together with other tests of cerebellar functions.
Our index finger is pointed forward. The patient is asked to look at it, lift his arms up and try to sink his/her fingers to our index finger's level with the eyes closed. In case of cerebellar lesion the arm on the ipsilateral side will deviate to the side.
Disturbance in coordination can be evoked experimentally, if the patient is turned around in a revolving chair for 1 minute or so and perform the test right after stopping.

19. Sense of taste

The test is performed for 4 basic tastes. The patient sticks out the tongue which is touched at different places with a glass rod that had been immersed in different solutions, diluted glucose, salt, acetic acid and quinine (the quinine solution is always the last). The patient rinses the mouth after each taste sample. On a prepared table the patient (with his tongue still out) points on the name of the different taste qualities. One should observe that sensation of different tastes cannot be evoked on the tongue on any locations. Different parts of the tongue react to different tastes, tip to sweet, sides to sour, root to bitter.

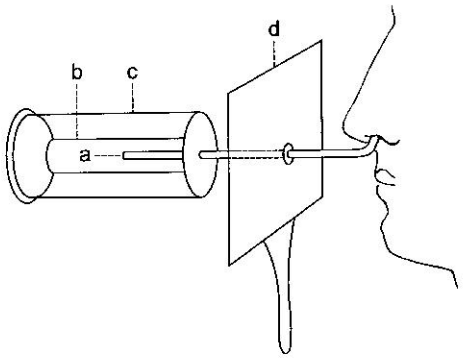


Localization of taste qualities

20. Testing the sense of smell with an olfactometer

During this test the individual sense of smell is measured. The instrument used is the olfactometer, which is a calibrated glass tube (a) the bent part of which is introduced into the nostril. The other end is placed into a porous clay cylinder (b) surrounded by a glass cylinder (c). There is a wooden plane for shading between the external end of the glass tube and the glass cylinder.

The clay cylinder is soaked with some odorous substance. If the cylinder is completely pulled over the glass tube the patient will not smell the odor because air is coming through the end of the glass tube. If, however, the glass tube is pulled out slightly the air mixing with the odorous substance will come through the cylinder. Position of the glass tube should be there where the patient still perceives the smell.



Olfactometer

21. Tests of somatic sensations

During this test corresponding symmetrical points on the body surface are compared; that is the injured part with the intact. Borderline between lesioned and healthy areas is determined with very light stimuli.
Anesthesia: lack of any sensation.
Hyperaesthesia: excessive sensitivity to stimuli.
Hypaesthesia: decreased sensitivity to stimuli.

a) Pressure sense:
The test is performed with an instrument having a blunt tip. The phenomenon of dermolexia can be observed i.e. the patient can normally recognize letters or numbers drawn on skin.

b) Sense of touch (tactile sense):
Skin surface to be tested is touched with a small cotton swab; the patient with eyes closed gives signal upon touching. A piece of hair (from a horse-tail, the so-called Frey's hair) can be used as well; the hair is pressed on the skin until it bends.

c) Pain:
Different points of the body surface are prickled with a blunt tipped needle, the patient has to give a sign when sensing the pain.

d) Spatial resolution of tactile stimuli:
Calipers are used for this test. The distance between the tips of the calipers is gradually decreased until the two tips cannot be sensed separatedly anymore. This threshold value ("minimum separabile") differs in different parts of the body (e.g. it is 2 mm on the fingertips, 70 mm on the back).

VII. THE ENDOCRINE SYSTEM

1. The Thorn Test (principle)

Glucocorticoid hormones (cortisol) of the adrenal cortex induce a decrease in number of lymphocytes and eosinophil granulocytes in the peripheral blood. In this test ACTH is used to mobilize cortisol and the test demonstrates the granulocyte count decreasing effect of cortisol. A blood sample is taken for control from a vein in the ear of a rabbit. The blood is sucked into a melangeur-pipette (with the white pearl), and diluted 10-fold with Türk solution and the total leukocyte count is calculated (in the practice general leukocyte count is defined). Then 40 units of ACTH are administered subcutaneously, and the blood count is repeated one hour intervals for 3 hours. Please note that in our test the total leukocyte count is calculated. The decrease is due to the decrease of the eosinophils and the lymphocytes, however, in the original Thorn test the number of eosinophils has to be counted.
Affected by ACTH, the leukocyte number (eosinophil granulocyte, lymphocyte) will decrease to half. Later it will increase again but usually it does not return to the initial value even by the 3rd hour.

2. The Effect of Insulin on Blood Glucose Level- Oral glucose tolerance test (OGTT)

A glucose tolerance test is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia and acromegaly, or rarer disorders of carbohydrate metabolism. A variant is often used in pregnancy to screen for gestational diabetes. In the most commonly performed version of the test, an oral glucose tolerance test (OGTT), a standard dose of glucose is ingested by mouth and blood levels are checked two hours later.
Procedure:
1. A zero time (baseline) blood sample is drawn.
2. The patient is then given a measured dose of glucose solution (75 g glucose in 0,5 L water) to drink within a 5 minute time frame.
3. Blood is drawn at 60 minute and 120 minute intervals for measurement of glucose (blood sugar), and sometimes insulin levels.
A 2 hour OGTT glucose level below 7.8 mmol/L (140 mg/dL) is normal, whereas higher glucose levels indicate hyperglycemia. Blood plasma glucose between 7.8 mmol/L (140 mg/dL) and 11.1 mmol/L (200 mg/dL) indicate "impaired glucose tolerance", and levels above 11.1 mmol/L (200 mg/dL) at 2 hours confirms a diagnosis of diabetes.

3. Pregnancy test

A pregnancy test attempts to determine whether a woman is pregnant. Most chemical tests for pregnancy which can give the quickest result after fertilisation look for the presence of the beta subunit of hCG, or human chorionic gonadotropin, in the blood or urine. This hormone is produced by the placenta shortly after the embryo attaches to the uterine lining and builds up

rapidly in your body in the first few days of pregnancy (six to twelve days after fertilization). hCG levels continue to rise through the first 20 weeks of pregnancy, so the chances of false test results diminish with time. It's best to do the home pregnancy test first thing in the morning, when your urine is most concentrated and hCG levels are highest. Most home pregnancy tests use something like a plus or minus sign, a coded color change, or the words "pregnant" or "not pregnant" on a digital display. If the results are negative: Wait another week and if you still haven't started your period you should do the test again. False negatives are fairly common, especially if you miscalculated your ovulation date and took the test too soon. This is why many home pregnancy tests come with two test sticks. If the second test comes back negative, make an appointment with your doctor to find out if there is some other problem affecting your menstruation or causing symptoms of pregnancy.