PAVOL JOZEF ŠAFÁRIK UNIVERSITY IN KOŠICE
Faculty of Medicine
DEPARTMENT OF MEDICAL BIOLOGY

PRACTICAL LESSONS IN MEDICAL BIOLOGY

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University textbook “Practical Lessons in Medical Biology” are intended for practical exercises in the courses Biology I and Biology II for first-year students in the field of General Medicine, and in the courses Medical Biology and Genetics and Regenerative Medicine in Dentistry for first- and second-year students in the field of Dental Medicine at Pavol Jozef Šafárik University in Košice – Faculty of Medicine.

Practical Lessons in Medical Biology

University textbook

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CONTENTS

Safety and Health Protection at Work

Introductory training for students of Pavol Jozef Šafárik University in Košice on occupational safety and health regulations and fire protection

A. OCCUPATIONAL SAFETY AND PROTECTION OF HEALTH

1. School injury, reporting its occurrence, investigation

1.1 A registered school injury is a student's health injury that has occurred:
a) During educational or training activity at UPJŠ or during an optional activity organized by UPJŠ or in direct connection with the same,
b) In the case of activities not covered by point (a) above, if the student has performed them on the instruction or with the consent of a teaching staff member - a UPJŠ teacher or tutor.
The registered school injury is an injury that is a reason for the student's absence from the classes, the absence being based on an attending physician's opinion as lasting for longer than three days or resulting in the death of the student.

1.2 An injury of a student suffered during practitioner teaching, field training performed with a natural person - entrepreneur or legal entity under an agreement, training or any other activity shall not be considered a registered school injury. Such an injury shall be considered a registered occupational injury. The investigation and the record of the same shall be taken by the physical entity – an entrepreneur or a legal entity in whose premises the occupational injury of an UPJŠ student has occurred.

1.3 An unregistered school injury is an injury that is the reason for the absence of a student at training based on the opinion of the attending physician and lasting for less than four days, or, eventually, if the student fails to attend the classes, but his/her health is impaired due to the injury.

1.4 Investigation of an injury shall mean the detection of all of the circumstances and causes of the occurrence of an injury, the site of injury, witnesses of the injury, the injury site documentation in the event of a grave injury, giving the name and surname of the teacher present at the time of the injury, or the name and surname of the employee who instructed the student to perform the activity that had led to the injury.

1.5 Every student is obliged to report the occurrence of a school injury and the emergence of a dangerous event to the relevant teacher present at the time of the event.

1.6 The obligation to report the occurrence of a school injury and of a dangerous occurrence shall also be vested on a person who was witness to the occurrence of the event.

1.7 Records of a registered school injury and of an unregistered school injury shall be taken immediately upon the receipt of the notification of its occurrence, but no later than within four days of the day of the receipt of the notification of its emergence. The record shall be taken by a competent teacher who, in cooperation with the senior staff member, finds the cause and any other circumstances required for determining the necessary measures to prevent the injury recurrence.

1.8 Students taking part in field trips shall be demonstrably instructed and familiarized in advance by their teachers with the entire schedule, with the safety and organizational measures and on how to follow the rules of behaviour at the field trip site. While staying on their field trips, the students shall be required to unconditionally follow the instructions of their field trip instructors. They shall not be permitted to leave, break free, and violate the agenda without permission. During field trips, it is strictly forbidden to use any alcoholic beverages, narcotics, and/or psychotropic substances.

1.9 A dangerous event is an event in which safety or health of an employee, or a student is compromised, but no harm to his or her health has been done.

1.10 Each student is obliged to immediately notify the occurrence of a dangerous event to the relevant pedagogical staff present at the time of the occurrence of this event.

2. Obligations of UPJŠ students in the field of occupational safety and protection of health

2.1 Observe the ban on the use of alcoholic beverages, narcotics, and psychotropic substances at the workplaces and in the premises of UPJŠ. Ingestion of these substances during teaching or appearing for the classes under the influence of these substances, as well as the refusal to undergo a check on the use of these substances carried out or provided by the UPJŠ or the State Inspection authority shall be considered a disciplinary offense. Any positive finding of the abuse of these substances will be resolved according to the UPJŠ Disciplinary Rules of Procedure.

2.2 Abide by the ban on smoking in all indoor and outdoor premises of UPJŠ and its units, on external roads, as well as at the place of entry into the premises or buildings. A breach of this prohibition shall be considered a disciplinary offense.

2.3 Obey and comply with the applicable safety and health protection labels relating to a specific object, activity or situation and providing instructions or information necessary to ensure OSPH or FP, where appropriate, by a label, pictogram, symbol, and colour. Labels are used to indicate the ban, alert, instruction, to identify emergency exits and escape routes and for placing and identification of the first aid tools and devices, as well as the location of fire extinguishing agents. It is forbidden to damage or misrepresent those signs in any way.

2.4 Observe the principles of safe work on electrical machines and equipment:

A. Students are prohibited from:
a) interfering with the internal parts of any electrical equipment in both switched-on and switched-off state,
b) working on uncovered live parts of electrical equipment and touching these parts directly or with any object,
c) using and repairing defective and damaged electrical equipment.

B. It is the duty of students to:
a) follow the instructions and guidelines when operating the work equipment as issued by the manufacturer and as instructed by the teacher,
b) ensure that work equipment is not overloaded or damaged, that electrical equipment and wiring are always freely accessible and unladen, to remove from their vicinity any easily and

heavily flammable substances and objects that are harmful to the working medium and electrical wiring by mechanical, thermal, chemical, and any other damage,
c) immediately disconnect the faulty working tool and report the fault to the teacher (the faulty device is considered, for example, the one with damaged insulation, smelling of burning, smoking, unusually noisy, with impact operation, strong groping, jerky start, sparkling, grinding, shaking, etc.),
d) after the work has finished, reliably disconnect the power supply from the mains by means of a switch, and on the instruction of the teacher, also by pulling out the fork of the electric power supply socket,
e) ensure that the work device is not plugged into an electrical outlet, which is dirty, wet, damaged or without a protective pin,
f) before starting work with the operating equipment, make sure that the equipment, including the leads, and extension cords, and cables is not wet or sprayed with water or other liquid.

B. FIRE PROTECTION

3. Acquaintance with general fire protection requirements in UPJŠ buildings and premises

3.1 Fire is any undesired burning that causes damage to property, life, the environment or the result of which is a killed or injured individual. Fire or smoke occurring shall be reported by telephone or in person.

3.2 The Fire Reporting Office is marked with the inscription "OHLASOVŇA POŽIAROV" and is set up at the relevant gatehouse of the UPJŠ building, where the service is provided by employees of gatekeepers - informants.

3.3 Obligations of students in case of fire are defined in the Fire Alarm Directives. Organization of the evacuation of persons from objects hit or threatened by fire is regulated by the Fire Evacuation Plan. These documents, as well as the current list of the firefighting patrol members, are located at suitable locations of the buildings so as to be visible and permanently accessible to all the persons.

3.4 The obligations of employees, students, and any persons present in UPJŠ buildings with the university's knowledge include:
a) acting in a way that prevents fire when storing, placing, and using flammable substances or handling open flames,
b) adhering to the indicated prohibitions, executing the orders and instructions regarding protection against fires,
c) notifying without undue delay the occurrence of a smoke abatement or fire at the Porter's - Fire Reporting Office, in the event of a fire during the training, reporting the accident to the relevant teacher,
d) allowing responsible employees and state fire inspection authorities to carry out necessary actions when determining the cause of a fire.

3.5 It is prohibited to:
a) smoke and use open flames in all premises of UPJŠ as specified in point 2.2,
b) burn vegetation of herbs, shrubs and trees,
c) damage, abuse or hinder access to firefighting equipment, such as portable fire extinguishers, fire hydrants, fire closures, electrical fire alarms,

d) cause a fire alarm for no reason, call the fire brigade for no reason, or misuse the emergency line.

4. Fire hazard, fire precautions

4.1 The term combustion is a chemical oxidation-reduction reaction in which a combustible substance reacts with an oxidizer, it is accompanied by the release of heat and the emission of light. In order for the combustion to begin and continue, the presence of a combustible substance, an oxidizing agent (e.g., air oxygen) and a sufficient heat source (e.g., spark, flame, glowing body, etc.) is inevitable. Removing at least one of these conditions for burning will result in extinguishing the fire. The sooner it is achieved, the smaller the damage caused, and the risk of a major fire will significantly drop.

4.2 Types of fires:
Class A – Fires of solid combustible materials burning with flame or smoldering (e.g., wood, paper, straw, coal, textiles)
Class B – Fires of flammable liquids burning with flame (e.g., gasoline, oil, varnishes, alcohol)
Class C – Fires of flammable gases burning with flame (e.g., methane, propane, coal gas, hydrogen)
Class D – Fires of light combustible metals (especially aluminum and magnesium)

4.3 Fire protection equipment for rapid fire suppression includes portable fire extinguishers, fire hydrants, fire doors, and fire alarm systems.

4.4. A fire extinguisher is a device containing an extinguishing agent expelled under internal pressure when activated. The extinguisher is labeled with instructions for use and the fire class it is intended for (A, B, C, D). It is placed at a designated location marked with a red safety sign featuring a white extinguisher pictogram. The location must be visible and permanently accessible.

4.5 The main switches of electrical appliances and water and gas closures are marked by security labels with a green background and the inscription: HLAVNÝ VYPÍNAČ, HLAVNÝ UZÁVER VODY, HLAVNÝ UZÁVER PLYNU (MAIN SWITCH, MAIN WATER CONNECTION, MAIN GAS CONNECTION).

5. Fire alarm announcement

5.1 An employee or student who notices a fire is obliged to:
a) take the necessary measures to rescue people,
b) declare a fire alarm for people in endangered areas by loudly calling H O R Í (F I R E),
c) switch off electrical equipment,
d) remove flammable materials to a safe distance,
e) report the occurrence of a fire to the fire reporting station to summon the fire patrol or the fire and rescue unit,
f) until help arrives, attempt to extinguish the fire using portable extinguishers or fire hydrants,
g) quickly leave the workplace if it contains materials that could explode and cannot be removed,
h) when calling a fire and rescue service, provide:
- the telephone number from which the fire is reported,

- the name of the person reporting the fire,
- the name and address of the building in which the fire started,
- specification of the type of fire,

and remain on the line for the dispatcher's follow-up questions to verify the report.

5.2 Important telephone numbers for the Integrated Rescue System (112), Fire Fighting and Rescue Service (150), Police (158), Emergency Health Service (155) and the Heads of the staff are listed in the Fire Alarm Directives on the building corridors.

1. Microscopy

1.1. Light microscope

Microscopes are optical instruments that magnify microscopic structures invisible to the naked eye, typically in the range of 10x to 2 000x. Biological microscopes equipped with built-in illumination allow observation of thin and transparent biological material in transmitted light (e.g., onion cells, muscle tissue, bacteria, microbes, blood cells, DNA).

Microscope (Fig. 1-1) is a centered optical system composed of an objective lens and an eyepiece. Both the objective and the eyepiece form converging optical systems. In light optical microscopes, two sets of lenses are used: **the objective** – (placed near the specimen) produces a magnified, real image of the object, and **the eyepiece** – (viewed through by the observer) acts like a magnifying glass to further enlarge this image. The eyepiece is located in the upper part of the microscope, and the objective is attached to the lower part of the metal **tube** via **the revolving nosepiece turret**. Both optical systems can be often changed, depending on what overall magnification is required.

Microscopy is the basic method of observation in all biological fields that study cells (animal and plant tissue, microorganism), because its resolving power matches the size of cells and many organelles. A light microscope uses light rays for imaging, while an electron microscope uses a beam of electrons. According to this, two basic types of microscopes can be distinguished:

1. light microscopes – observe objects in visible light. They use so-called white or ultraviolet light. The light source is an incandescent lamp or discharge lamp. The optical parts are made of precision-ground glass.

2. electron microscopes – use a beam of electrons instead of light, equivalent to radiation with a very short wavelength. Magnetic lenses replace optical lenses. Specimens are specially prepared and fixed using precise technical procedures. According to the way the specimen is examined, we can distinguish between:

a) Transmission Electron Microscopy (TEM) – a beam of electrons passes through a specimen treated with electron-dense (electro-condensing) agents,

b) Scanning Electron Microscopy (SEM) – a beam of electrons scans the surface of a metal-coated specimen, and the image is recorded.

According to the object illumination method, we can distinguish:

1. transmitted light microscopy – light passes through the specimen. Includes bright-field, dark-field, phase-contrast, interference, polarization, and fluorescence microscopes. Under normal conditions, slides (fresh or fixed) are viewed in transmitted light.

2. reflected light microscopy – light is directed from above onto the specimen's surface. Used mainly in fluorescence and inverted microscopes.

According to the field illumination, we can distinguish:

1. bright-field microscopy – the object appears dark against a bright background. This method is one of the basic and most commonly used,

2. dark-field microscopy – the object appears bright against a dark background.

According to the type of microscope construction, we can distinguish:

1. simple magnifying glasses and **stereomicroscopes**, consisting of a single lens or a single lens system,

2. compound microscopes consisting of at least two lenses or at least two lens systems, with one or more (interchangeable) objective lenses.

According to the number of eyepieces, we can distinguish:
1. monocular microscopes – they have single eyepiece for viewing,
2. binocular microscopes – feature two eyepieces, allowing for simultaneous viewing with both eyes.

A light microscope consists of three basic parts (Fig. 1-1, 1-2, 1-3):
a) mechanical part: base, arm, tube, stage, coarse and fine focus knobs, X-Y translational control knobs (stage adjustment knobs, stage controls), revolving nosepiece,
b) optical part: objectives, eyepieces,
c) illumination system: microscopic built-in lamp, condenser with iris diaphragm.

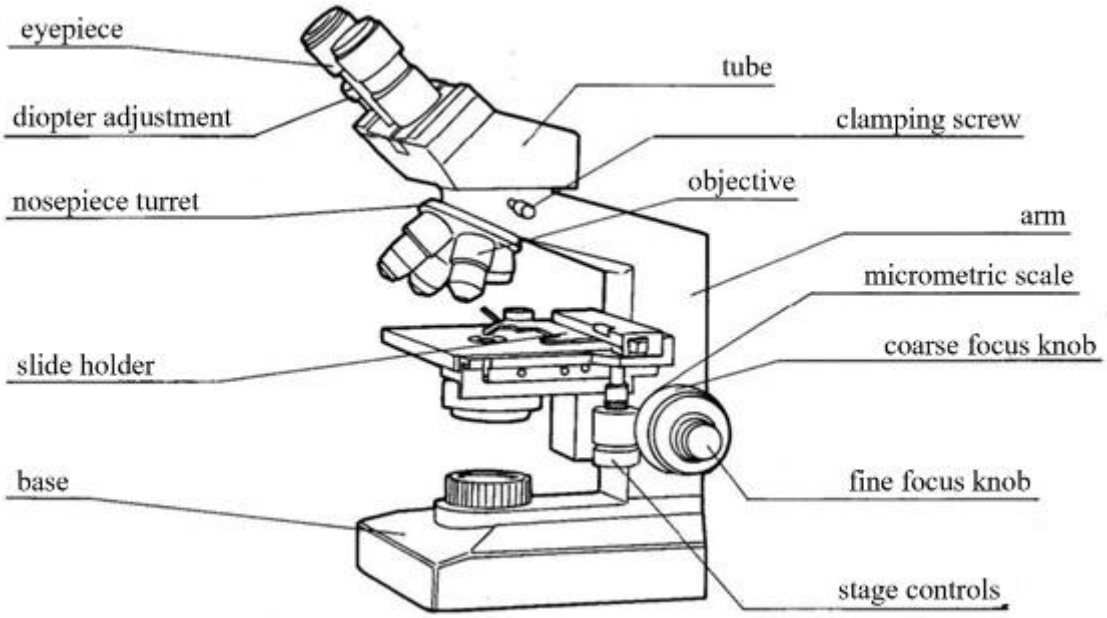


Figure 1-1. Light microscope

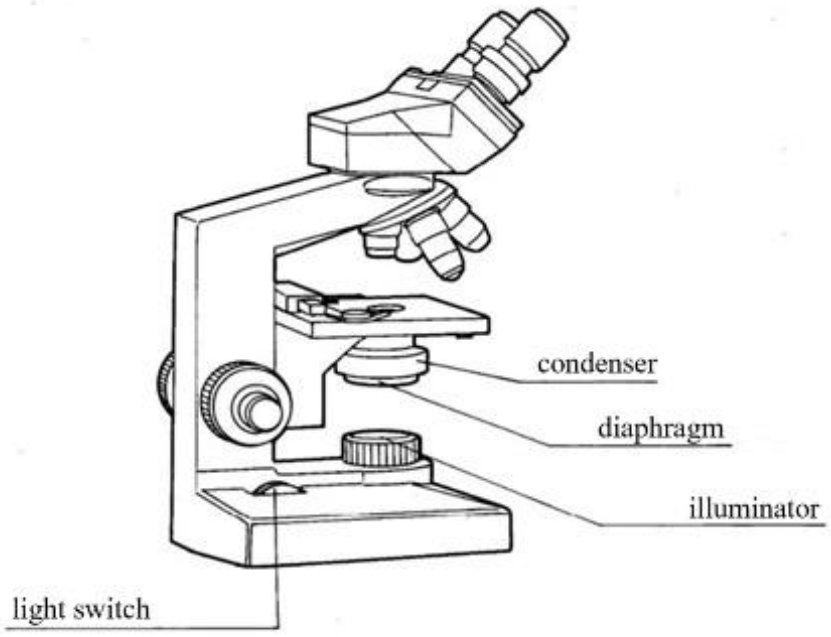


Figure 1-2. Illumination part of an optical microscope

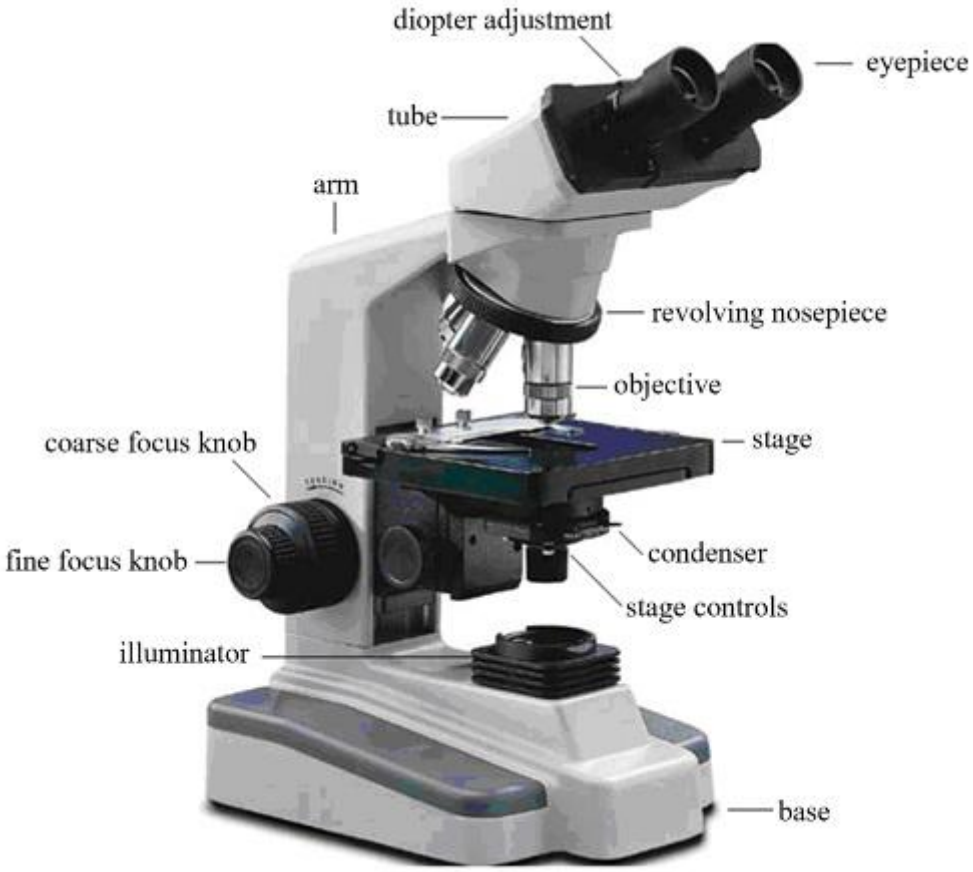


Figure 1-3. Binocular optical microscope

The **mechanical part of the** microscope includes the ***base***, to which the ***arm*** (tube carrier) is attached. At the top is the ***tube*** or ***binocular head*** mounted in a reduction sleeve. The lower part of the reduction sleeve holds the interchangeable ***revolving nosepiece*** for objectives. The binocular head is angled and has adjustable ***interpupillary distance.*** The left eyepiece tube is fixed, while the right has ***diopter adjustment.*** The ***tube*** allows light rays from the objective to pass to the eyepiece and sets the distance and position between them. Eyepieces are mechanically interchangeable. Objectives are mounted on a rotating disc ***(revolving nosepiece)*** that allows easy switching and precise alignment with the optical axis.

Focusing is carried out by vertically moving the microscope stage toward the light source or away from the fixed objective. The design of the adjustment mechanism allows both coarse and fine movement (up and down) using the ***coarse-focus and fine-focus knobs***, thereby changing the distance between the objective and the specimen to achieve the **optimal working distance.** The ***coarse-focus knob*** moves the stage or the tube carrier vertically, providing rapid movement and coarse focus of the observed object. The ***fine-focus knob*** is used exclusively for very precise focusing. The circumference of the fine-focus knob has divisions (a micrometric scale) that indicate, when turned, how far the microscope stage has moved up or down. This is used for measuring the thickness of microscopic objects. A movement of one division on the micrometric scale corresponds to a stage movement of 2.5 μm. Turning the coarse-focus and fine-focus knobs away from the observer raises the stage; turning them toward the observer lowers it.

The microscope specimen is placed on the ***stage.*** The microscope stage is a platform with a central opening through which light rays pass to the specimen, and it can be moved

horizontally (front-to-back and left-to-right) using two positioning screws ***(X-Y translational control knobs)***. Graduated locator markings on the side of the stage are used to find the same spot on the specimen during the examination. The specimen (slide) is held in place by the stage clip of the ***mechanical specimen holder.***

The **optical part** of a light microscope consists of two sets of lenses: those housed in the ***eyepiece (ocular)*** and those in the ***objective.*** In modern instruments, neither the objective nor the eyepiece is a single simple lens; each is a complex optical assembly. For example, an apochromatic immersion objective may contain up to ten individual lenses. The eyepiece is positioned at the upper end of the microscope's metal tube (the tube), while the objective is attached to the lower end. Both optical systems are frequently interchangeable, depending on the desired magnification. The objective, facing the specimen, produces a real, magnified, and inverted image. The second lens system, the eyepiece, faces the observer's eye and functions like a magnifying glass, enlarging the real image formed by the objective. The total magnification is calculated by multiplying the magnification of the objective (e.g., 100x) by that of the eyepiece (e.g., 10x), i.e. 100 x 10 = 1 000x total magnification. Special-purpose microscopes, such as demonstration or surgical microscopes, may be equipped with multiple eyepiece sets. Depending on the application, the image can be projected onto a screen, recorded on photographic paper, captured with a video camera, or digitalized.

The ***eyepiece*** is an optical system usually composed of multiple lenses that magnify the image produced by the objective. In practice, Huygens eyepieces are most commonly used. These consist of two plano-convex lenses mounted in a metal housing, marked with the letter H and a number indicating the magnification (e.g., H 4x, H 10x, H 12x). The upper lens (closer to the eye) is called the **eye lens.** The lower lens (closer to the specimen) is the **field lens.** For special purposes, various types of eyepieces are used: *measuring micrometer eyepieces* – contain a scale placed between the field and eye lenses, *pointer eyepieces* – have a built-in pointer needle for demonstration purposes, *projective lenses* – used for projection and microphotography, *wide-field eyepieces* – provide a large field of view. The image formed by the objective can be significantly enlarged by the eyepiece.

Objectives are attached to the lower end of the microscope's metal tube on a revolving nosepiece. Rotating the nosepiece allows for quick and easy exchange of objectives. They are marked with a number indicating magnification and another indicating the numerical aperture. An objective typically consists of 4 to 6 lenses of various shapes and types of optical glass, while the highest-quality objectives may contain up to 14 lenses. The lens closest to the specimen is called the **front lens.** Laboratory microscopes usually have 3 to 5 objectives mounted on the revolving nosepiece, allowing rapid switching between magnifications. Each objective is labeled with the manufacturer's name, magnification, and other specifications, as well as a colour code in the form of a thin coloured ring (red, yellow, blue, white). The objective is the component that provides the initial primary magnification of the image.
We distinguish between two types of objectives:

a) dry objectives – where there is air between the specimen and the objective,

b) immersion objectives – where the air is replaced by a special immersion oil.

Dry objectives do not require immersion oil. In practical exercises, we will use dry objectives with magnifications of 10x and 40x.

When using immersion objectives, a drop of immersion oil – whose refractive index is almost identical to that of glass (1.51) – must be placed between the specimen and the objective (for example, with a 100x objective). This increases the numerical aperture of the objective and, with it, both image brightness and resolving power. Immersion objectives are used for the highest magnifications and cannot be used without immersion oil, as the image would appear "foggy".

On the objective, below the magnification value, the ***numerical aperture*** (NA) is indicated. This value is directly proportional to the refractive index of the medium between the objective and the specimen and to the sine of the half-angle of the maximum cone of light that can enter or exit the lens. If the numerical aperture value is multiplied by 1 000, we obtain the **maximum useful magnification** for that objective. For example, a 40x objective with NA = 0.65 has a maximum useful magnification of 650x.

The numerical aperture of an objective has great practical significance:

1) it determines the resolving power and the limits of useful magnification.

2) it affects the brightness of the microscopic image.

3) it influences the depth of field. Reducing the numerical aperture increases the thickness of the specimen layer that appears in focus. Objectives with a high numerical aperture have low penetration depth and display only a very thin optical section.

The quality of an image in a light microscope is determined by the magnification of the object, the resolving power of the microscope, and image contrast.

The **total magnification of a microscope** is the product of the magnification of the objective and the eyepiece. With the best objectives, a useful magnification of 1 000x to 2 000x can be achieved. The total magnification can be further increased by using stronger eyepieces, but only up to the limit defined by the useful magnification of the objective, which equals one thousand times the numerical aperture of that objective. Beyond this limit, the objective will not reveal more detail, and only so-called "empty magnification" is obtained. The total magnification of the light microscopes used in practical exercises ranges from 100x to 1 000x.

The **resolving power of a microscope** – that is, its ability to distinguish two adjacent points – depends on the numerical aperture of the objective: the higher the NA, the greater the resolving power. It also depends on the wavelength of the light used; shorter wavelengths theoretically provide higher resolution. Objects smaller than 0.2 μm cannot be observed with a light microscope.

The **working distance of an objective** is the perpendicular distance from the observed object to the front lens of the objective when focused on the specimen. This distance decreases as the magnification of the objective increases. For magnifying glasses and the smallest objectives (4x, 10x), it is several centimeters, whereas for commonly used 40x objectives it is practically less than the combined thickness of the slide, specimen, and coverslip. This is especially pronounced with a 100x objective, where the front lens rests against the coverslip of the specimen (this objective is used only with immersion oil). Since specimens are usually observed under a coverslip, we also distinguish the **free working distance** – the distance from the surface of the front lens to the surface of the coverslip.

The **illumination system** (Fig. 1-2) is located beneath the microscope stage. It directs and concentrates the light rays emitted from the light source toward the specimen, into the optical part of the microscope, and then to the eye. It consists of a ***condenser,*** an ***iris diaphragm,*** and a ***microscope lamp.*** Microscopic specimens are most often observed in transmitted light. To make full use of the optical properties of the microscope, the observed object must be well illuminated.

The ***condenser*** is composed of several lenses. It focuses the light rays from the light source, passing them through the opening in the microscope stage toward the specimen so that the entire field of view is evenly illuminated. Light from the condenser passes through the microscopic specimen and enters the objective (Fig. 1-4). The amount and intensity of light reaching the specimen can be regulated by the ***iris diaphragm***, located beneath the condenser. By opening or closing it, we control the intensity of light falling on the specimen. The diaphragm is made of fine metal blades arranged in a fan shape, similar to the concentric diaphragms in cameras.

The ***microscope lamp*** is built into the microscope base. Whether the studied objects are visible

also depends on the difference in brightness between them and their surroundings – that is, on **contrast.**

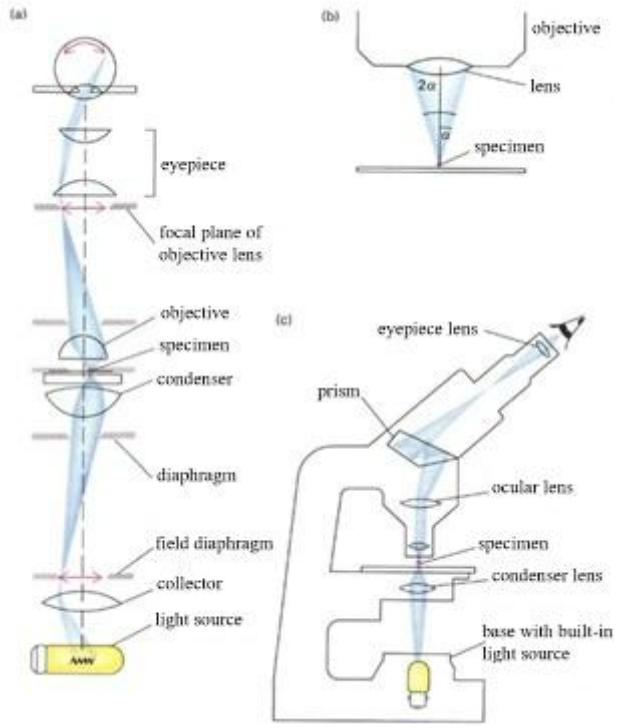


Figure 1-4. Diagram of the pathway of light rays from a light source to an eyepiece

The microscope generates image in two stages:

1) The objective lens creates a real, magnified, and inverted image of the object in the front focal plane of the eyepiece.
2) We observe this image through the eyepiece as if using a magnifying glass; the eyepiece further magnifies it. The magnified image is registered by the retina of the eye (or photographic film). The final image we see in the microscope is therefore virtual, inverted, and even more magnified.

Special Methods of Light Microscopy

A light microscope can be modified for certain special observation methods. According to this, we distinguish phase-contrast, interference, fluorescence, polarization microscopes, etc.

1) Phase-contrast microscope

If light passing through an object changes its wavelength and amplitude, we perceive this as differences in the object's colour and in light intensity. This is common in stained microscopic specimens (histological preparations). Unstained objects (e.g., living cells) do not change the amplitude of the light passing through them but only shift its phase in those parts of the object that differ in refractive index. Small phase differences, however, cannot be detected by the human eye, so we perceive these objects as transparent and without structure. The principle of the phase-contrast device is that it converts invisible phase differences into amplitude differences, which we can perceive. The phase-contrast microscope is used to observe unstained objects, especially structures in living cells such as the nucleus, nucleolus, chromosomes, and vacuoles.

2) Interference microscope
It uses light interference similarly to the phase-contrast microscope but in a different way. In an interference microscope, the light rays passing through the object are split and then recombined independently of the object. This method allows for contrast-rich to almost three-dimensional imaging of transparent and unstained objects and their components that differ in refractive index. The method has similar applications as phase contrast.
3) Fluorescence microscope
It takes advantage of the fact that some substances, when exposed to light of shorter wavelength, emit light of a longer wavelength – thus of a different colour. This phenomenon is called fluorescence. It is the result of intramolecular energy changes caused by radiation absorbed in the substance. To induce fluorescence, blue and ultraviolet radiation from high-pressure mercury lamps is most often used.

The usual procedure is to stain certain structures in the object with fluorescent dye or fluorochrome. The object is then illuminated with light of a specific wavelength (e.g., blue), which passes through an excitation filter and induces fluorescence of the dye used. The object is observed through the eyepiece via a barrier filter, which absorbs the light passing through the excitation filter (blue) and transmits only the light produced by fluorescence (yellow). The result: in a dark field of view, (yellow) structures bound to the fluorochrome glow. This method is used to visualize cell nuclei, chromosomes, nucleoli, cytoskeleton, cell walls, membrane antigens, and other structures.
4) Dark-field microscopy
The principle of the method is that only peripheral, very oblique rays (not central ones, which are absorbed) from the light source enter the plane of the object. The object is thus illuminated only from the sides (laterally). The rays are reflected, refracted, and diffracted by the object. Only those rays reflected by the object enter the objective lens. Therefore, in the dark field, the object appears to glow.
5) Polarization microscope
In a polarization microscope using transmitted light, linearly polarized light is used. This light oscillates in one plane and, when passing through birefringent (birefractive) materials, is split due to birefringence (double refraction) into two components taking slightly different paths. The polarization device has two parts with the same physical function: the polarizer and the analyzer. Both contain polarizing filters. Light passing through the filters is polarized. The polarizer is in or below the condenser, the analyzer is above or below the eyepiece. If the polarization planes of both filters are parallel, light passes through them, and the microscope's field of view is bright. If they are perpendicular, light does not pass through the analyzer, and the field of view is dark. Uniaxial (isotropic) substances remain dark with crossed filters (not displayed), whereas biaxial (anisotropic) substances appear bright. In biology, polarization microscopy is used to display structures with linear orientation or crystalline nature, such as striated myofibrils, tonofibrils, crystalline inclusions, cellulose structures in plants, tooth and bone sections, crystals produced by microorganisms, and others.

1.1.1. Light microscope observation

To study a specimen means to visually analyze, identify, and describe microscopic objects – cells, tissues, microorganisms, and their components – prepared and displayed by various methods, to compare their images, and to draw conclusions based on these observations. The microscope can provide a more or less high-quality image of objects. We must form our knowledge about them ourselves by thinking about the displayed structures, the relationship between the observed microscopic image and its description in textbooks, between a biological structure and its function, between changes in the shape of a structure and the factors acting

upon it, and between a disease and its cytological, histological, or microbiological basis. We always strive to explain microscopic findings. Working correctly with a microscope means obtaining a quick image of the object, achieving proper illumination, promptly changing magnification, and observing a high-quality image.

Place the microscope on the workbench so that it is comfortable to use and connect it to the power supply. Before actual microscopy, check that the mechanical parts of the microscope are not damaged and that the optical parts are clean.

Procedure for light microscopy:

1. First, switch on the light source and set the objective lens on the revolving nosepiece that will be used to observe the microscopic object. To locate the object, first use the 10x objective.

2. Place the specimen on the microscope stage, ensuring that the glass slide is underneath and the cover slip is on top. Secure the specimen in the stage's slide holder and move it into the optical axis of the microscope using the centering positioning screws.

3. Locate the object using the coarse focus knob and refine the image with the fine focus knob. By turning the coarse focus knob "away from yourself," move the stage with the specimen close to the objective lens. Beginners are advised to check this movement from the side, watching the front lens area of the microscope to avoid hitting the specimen with the objective, whose working distance is very small, thus preventing damage to the specimen and the lens.

4. Adjust the illumination of the specimen using the iris diaphragm and the lamp's aperture.

5. Focus on the specimen by looking into the eyepieces, turning the coarse focus knob slowly toward yourself, and lowering the stage until the image appears in the field of view. Fine-tune using the fine focus knob.

6. Then, by rotating the nosepiece, replace the 10x objective with one of 40x magnification. Objectives on the nosepiece are usually parfocal, meaning they are focused on the same distance, so when changing objectives, coarse focusing is unnecessary – only fine focusing is needed. Adjust the illumination of the field of view using the iris diaphragm and the lamp's aperture.

7. By moving the specimen or using the positioning knobs, locate the part of the specimen to be observed at higher magnification and place it in the center of the field of view. When focusing on higher magnifications (from 40x upward), work especially carefully to avoid damaging the specimen or scratching the front lens of the objective.

8. Adjust the interpupillary distance of the eyepieces. On one of them, the focus (diopter correction) can be changed by rotation to suit your eyes. Close your left eye and observe the microscopic object only through the right eyepiece, fine-tuning the image with the fine focus knob. Then close your right eye, look through the focusing eyepiece with your left eye, and fine-tune the image by rotating the diopter correction ring.

9. During actual observation, gently move the fine focus knob with one hand to focus on different planes of the specimen and observe its spatial structure. Use both eyes for microscopy. At the same time, use your other hand to draw the image of microscopic objects.

10. If observing very small objects or their details, it is necessary to use an oil immersion objective (100x) and immersion oil. Rotate the nosepiece to move the dry objective (40x) out of the optical axis. Fully open the iris diaphragm and the lamp's aperture and place a drop of immersion oil on the best-illuminated area of the specimen. Rotate the nosepiece to the immersion objective (100x), which must be immersed in the drop of oil and brought into the optical axis of the microscope. Fine-tune the image gently with the fine focus knob.

11. Draw the observed object. Label the drawing with the title, magnification, and descriptions of the individual observed structures.

12. When done with microscopy, rotate the nosepiece to move the immersion (100x) or dry (40x) objective out of the optical axis. Turn the coarse focus knob toward yourself to lower the

stage to its lowest position. Wipe the objectives with gauze moistened with benzine-alcohol to remove immersion oil residues, then use dry gauze to remove the film left by the benzine-alcohol on the lens.
Always keep the microscope clean, protect it from dust, from harmful vapors (acids, chlorine, ammonia), and from other chemical agents.

Drawing a microscopic image

Although a drawn image is often influenced to a considerable extent by the observer's subjective perception of the microscopic view and by the author's artistic skills, in some cases it is the most suitable way to represent a microscopic object. A good drawing requires, as far as possible, preserving the proportions of the observed specimen (object) – its width, length, and, where applicable, depth. It is important that the object be depicted at an appropriate and sufficiently large scale. Suitable explanatory notes should be written at the margins of the drawing for individual details, and, beneath the image, technical information should be provided as needed (title of the drawing, its magnification, the type of specimen from which it was made, and, if appropriate, a brief description of the findings). Compared to photography, drawing has certain advantages as well as disadvantages. Among the advantages are the ability to capture details from several optical planes of the object, to deliberately emphasize certain features, and the fact that a drawn image can be easily reproduced and is often "more readable" than a photograph, which shows everything, including many irrelevant details. The disadvantages include the influence of the subjective factor and a relatively high degree of inaccuracy.

Questions and Tasks

Task 1: Name the parts of the light microscope (Fig. 1-5) using the following keywords and explain the role of each part of the microscope

Base, Arm, Illumination System, Iris Diaphragm, Mechanical Stage, Objectives, Nosepiece Turret, Eyepieces, Interpupillary Adjustment, Slide Holder, Coarse and Fine Focus Knobs, X-Y Translational Control Knobs, Stage Opening, Graduated Locator Markings, Tube, Power ON/OFF switch, Condenser

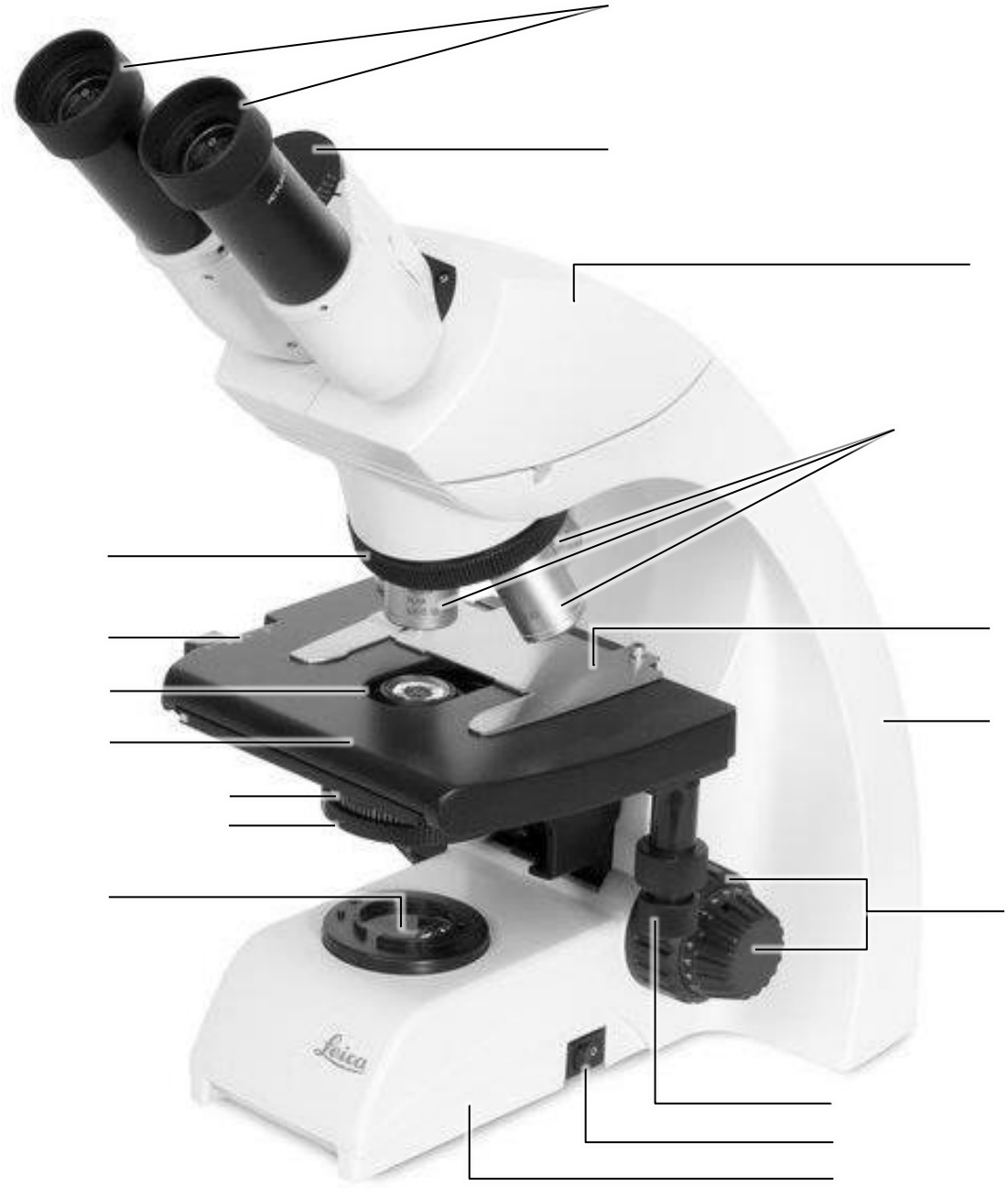


Figure 1-5. Light Microscope

Task 2: Microscopic examination of printed text

Material and Equipment: Binocular microscope, glass slide, paper with small, printed letters.
Procedure: Choose one specific printed letter for observation. First, observe it with the naked eye and then draw it in your lab protocol.
Next, position the glass slide on the microscope stage with the printed text facing the correct reading orientation, and locate the letter you previously observed with the naked eye. Now, observe it using the 10x objective (resulting in 100x total magnification) and then the 40x objective (resulting in 400x total magnification). Draw the observed letter again.
With this specimen, practice using the condenser's iris diaphragm and the microscope lamp's aperture, which allow you to increase or decrease illumination.
Results: The letter appears in the microscope's field of view in an inverted orientation relative to its actual position. When the specimen is moved, the image shifts in the opposite direction. Features that appear on the right in the field of view are in fact located on the left side of the specimen, and those appearing at the top are at the bottom, and vice versa. This reversal must be considered when locating specific areas on the specimen. Draw the letter (or part of it), including any incomplete print areas or printing defects. This observation serves to confirm that the image seen through the microscope is a magnified, laterally reversed, and inverted representation of the object.
Drawing:
Observation with the naked eye: **Observation with the microscope:**

Task 3: Microscopic examination of air bubbles

Material and Equipment: Glycerol, glass slides and cover slips, test tube, pipette, microscope.
Procedure: Aerate the glycerol in the test tube using a pipette, creating air bubbles. Transfer a drop of glycerol onto a glass slide, cover it with a cover slip, and observe using the 10x and 40x objectives.
Results: When focusing on a small bubble, the observed image changes depending on whether the focus is on the bubble's center, lower edge, or upper edge. Parallel rays from the light source strike the bubble from below. Rays passing through the center are not refracted, whereas those passing through other regions are refracted. As a result, peripheral rays do not reach the objective, leaving the bubble's edges unilluminated and creating the appearance of a dark ring around its surface. Because the bubble has a lower refractive index than the surrounding medium, it acts as a diverging lens.
Drawing:

Task 4: Microscopic examination of a permanent slide at a different magnification and with an oil-immersion objective

Material and Equipment: Permanent blood smear slide, immersion oil, gauze.
Procedure: Begin by using the 10x objective to locate the specimen and adjust the illumination. Switch to the 40x objective, center the specimen in the field of view, focus, and readjust the lighting as needed. Using the revolving nosepiece, gently rotate the 40x objective slightly out of position and place a drop of immersion oil on the brightest area of the specimen. Engage the 100x oil-immersion objective, achieving a total magnification of 1 000x. Finally, fine-tune the focus with the fine adjustment knob and optimize the illumination for clear viewing.
Results: To build skill in using the oil-immersion objective, repeat the procedure several times. Create a detailed drawing of the observed specimen, ensuring that all structures are depicted in correct proportion.
After Observation: Lower the microscope stage by turning the coarse focus knob towards yourself. Carefully clean both the oil-immersion objective and the specimen to remove all traces of oil. Wipe the immersion oil from the permanent slide using dry gauze. Clean the objective first with dry gauze, then with gauze moistened in benzine–alcohol. Finally, wipe all glass surfaces again with dry gauze, as benzine–alcohol can leave a dull residue.
Drawing:

Task 5: Microscopic examination of the fingerprint

Material and Equipment: Glass slide, microscope.
Procedure: Make a fingerprint on a clean glass slide and observe it under the microscope using the 10x and 40x objectives.
Results: This observation shows how dirt and fingerprints on the slide can interfere with and distort the image, compromising the accuracy of the entire examination. To prevent such issues, slides and specimens should be handled only by their edges.
Drawing:

2. Preparation of Microscopic Slides

A **microscopic specimen** is an object of microbial, plant, or animal origin, enclosed in a suitable medium between a glass slide and a cover slip, prepared for microscopic examination. Slides and cover slips must be clean, of appropriate thickness, and of standard size. They are an essential part of microscopic technique. Always hold the preparation by its edges to avoid leaving fingerprints. Blood smears preparations can be observed without a cover slip.

Standard **microscope slides** measure 76x26 mm and have a thickness of 0.9-1.2 mm, with ground or cut edges. Only slides that are clean and free from manufacturing defects, bubbles, or scratches should be used.

A **cover slip** is placed over the microscopic object on the slide to protect it and improve image quality. Cover slips may be square, rectangular, or circular in shape, with common sizes including 18x18 mm, 22x22 mm, 24x24 mm, and 22x32 mm. Their thickness typically ranges from 0.10 to 0.25 mm.

Microscopic preparations can be classified as:

- **native** (fresh) – used to observe living objects in their natural state, without preparation,
- **permanent** – allow repeated, long-term observation, but only of non-living organisms or their parts.

According to the preparation method, microscopic specimens may be:

- **section (cut)**
- **smear or spread**
- **squash (press) preparation**
- **prints, imprints**

2.1. Native preparations

Native preparations are among the simplest methods for studying living specimens that have not been exposed to any fixative solution. In biology and medicine, they are particularly useful when the diagnostically important features are the vital activities of cells – such as movement (e.g., *Trichomonas vaginalis*) – or their characteristic morphology (e.g., *Treponema pallidum* observed under dark-field microscopy). However, this form of microscopy has limitations: due to the similarity in the refractive index between cellular structures and their surroundings, fine intracellular details are difficult to differentiate. These structures can, nevertheless, be made more visible through techniques such as phase-contrast microscopy.

Native preparations contain a living specimen that has not been damaged during preparation. Their main advantage is that they allow direct observation of living organisms in a simple manner, revealing microscopic structures in an almost unchanged state, as they occur naturally. In such preparations, only the specimen's naturally pigmented structures are visible. However, native preparations have limitations. They cannot be stored for extended periods, and many structural details remain invisible unless stained, as they are otherwise undetectable under a light microscope. Another drawback is their tendency to dry out quickly. During prolonged observation, the medium must be replenished using the soaking technique. Typical specimens for native preparations include protozoa, bacteria, sperm cells, small aquatic invertebrates, and cells from surviving isolated tissues. They are also ideal for studying osmotic processes – changes in cell shape caused by variations in water concentration and osmotically active substances in the surrounding medium. The medium used is typically the liquid in which the organisms naturally occur, such as water, blood serum, plasma, or culture medium. In some cases – particularly when examining parts of organs, tissues, or individual cells of multicellular

organisms – isotonic solutions are used, including physiological solution (0.9% NaCl), Ringer's solution, PBS (phosphate-buffered saline), and similar formulations.

Native preparation is made as follows: Hold the microscope slide by its edges (Fig. 2-1) and place a drop of water in the center. Cut a small sample such as a moss leaf and transfer it with tweezers into the drop. Hold the cover slip by its upper corners with two fingers and position it at an angle of approximately 45° to the drop. Allow the water to spread by capillary action along the lower edge of the cover slip, then slowly lower it into a horizontal position (Fig. 2-2). A dissecting needle may be used to assist with this step. Remove any excess water by touching the edge of the cover slip with filter paper. If the preparation appears too dry, add a small amount of water from the side toward the cover slip's edge. If air bubbles form, gently lift the cover slip with two dissecting needles and lower it again slowly.

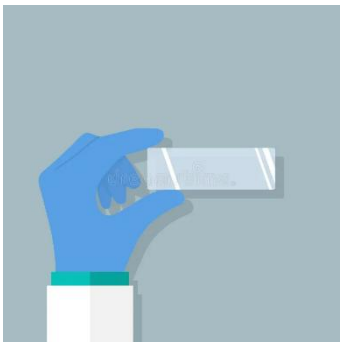


Figure 2-1. Proper holding of the slide when cleaning it or making a native slide.

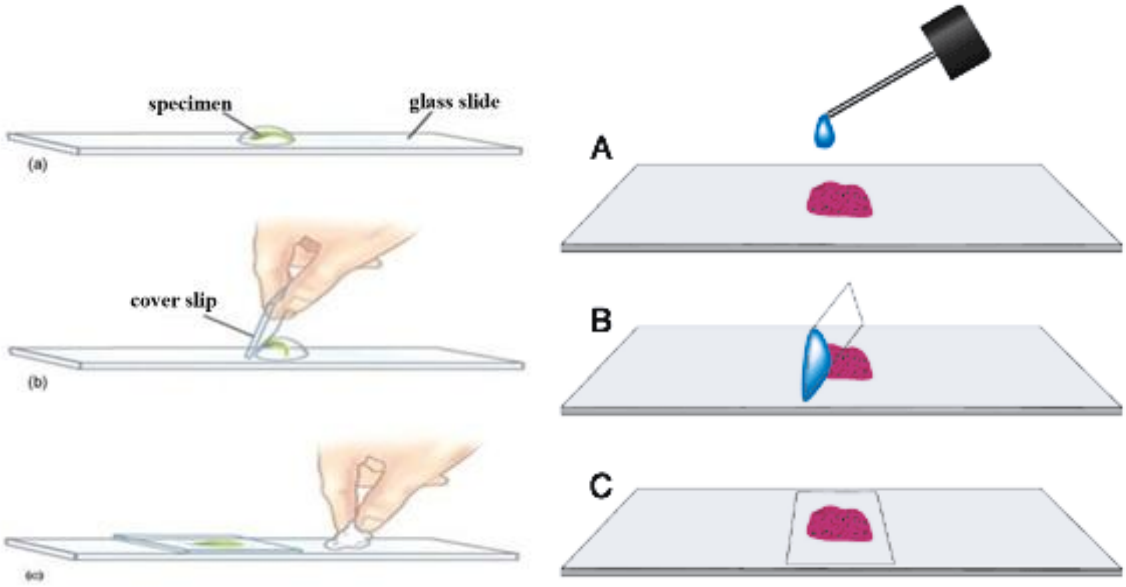


Figure 2-2. Procedure for the preparation of the native preparation**.**

A correctly prepared native slide contains no air bubbles and has an appropriate amount of water; that is, the cover slip neither floats nor presses too firmly on the specimen. The cover slip is centered on the microscope slide, and the specimen lies in the middle of the cover slip. The preparation is first observed under low magnification (10x objective), and then under higher magnification (40x objective).

Small aquatic organisms can be briefly and roughly examined in a drop of water without a cover slip. Since water on a slide dries out during prolonged observation, a so-called hanging drop is used when we wish to observe living specimens for a longer period. To prepare a hanging drop, a depression slide is required. Place a small drop of the examined medium in the center of a cover slip, then quickly invert the cover slip to create the hanging drop and position it over the depression in the slide.

If we wish to add to or replace the liquid medium in a native preparation, or possibly use a vital stain, we use the so-called soaking technique. Place a drop of the new medium at one edge of the cover slip and hold a strip of filter paper against the opposite edge. The paper draws out the original medium while simultaneously pulling the new liquid under the cover slip from the other side.

To enhance the contrast of certain structures that are not visible in living cells in their native state, **vital staining** is employed. This technique does not significantly compromise cell viability. It is particularly useful when a native preparation provides sufficient detail and when the characteristic features of the observed cells would be lost in a fixed preparation. Vital staining enables the observation of various manifestations of cellular activity, including movement (e.g., amoeboid movement in amoebas, characteristic locomotion in flagellates and ciliates), phagocytic activity (uptake of pigments), osmotic phenomena, cell migration (e.g., cancer cells within tissue), assessment of cell vitality (e.g., using trypan blue) etc.

Types of Vital Staining:

1. *intravital* – observation of intact, living cells,
2. *supravital* – observation of excised cells that are still viable,
3. *postvital* – observation of dying cells.

Vital dyes used for native preparations are typically organic compounds that, at the applied concentrations, are non-toxic to cells. The principle of vital staining lies in the ability of plants, tissues, or individual cells to absorb certain dyes from their environment by diffusion without noticeably altering their physiological state or life processes, thus preserving cell vitality. These dyes do not stain the entire cell; instead, they are selectively taken up by specific cellular structures, producing strong contrast. For example: vacuoles absorb neutral red, mitochondria absorb Janus green. Common synthetic dyes suitable for vital staining include neutral red, Janus green, methylene blue, toluidine blue, orange G, and others.

A certain disadvantage of native preparations is that the liquid medium tends to dry out quickly, particularly due to heat generated during prolonged microscopy. To counter this, the medium can be replenished using the soaking technique. As the medium dries, various artifacts may form within the cells, which can easily be mistaken for genuine structures of living specimens. Artifacts are foreign elements that distort the microscopic image and do not belong to it, such as dust particles, dye crystals, cotton fibers, fingerprints, or damage caused during tissue and cell preparation.

When preparing native slides, it is important to ensure that:

a) the specimen is not so thick that it obscures fine structural details,
b) the amount of liquid under the cover slip is balanced, neither excessive (which causes the cover slip to float and tremble) nor insufficient (which leads to drying, cell death, and artifact formation),
c) air bubbles are avoided when placing the cover slip. Air bubbles appear as sharply outlined, round structures with a bright center and no internal detail,
d) fragile specimens are not damaged by excessive pressure.

2.2. Permanent preparations

Permanent preparations show cells, parts of tissues, or plant structures that have first been killed and then, after specific processing, are examined. The principle of permanent preparations is the drying of the specimen or the replacement of water in it with another medium. Permanent preparations can be made as:

a) whole mounts
b) sections, cuts
c) prints, imprints (e.g., imprint of human bone marrow, liver, spleen)
d) squash preparations (e.g., root meristems, salivary glands of midge larvae)
e) smears (e.g., blood)
f) spreads – (dry spreads: the sample e.g., sperm suspension is transferred onto a slide and spread out; wet spreads: the sample is mixed with a drop of glycerol and egg white, or serum e.g., amoebas and the drop is spread out)

There are numerous methods for preparing permanent slides, which can be divided into:
a) simple preparation
b) complex preparation

In the simpler methods of preparing permanent slides, fixation and staining are omitted. Specimens are mounted directly in a medium such as gelatin, Canada balsam, or synthetically produced substances (e.g., Solacryl, Entellan). As the medium solidifies, it both secures the specimen and firmly bonds the slide to the cover glass. This approach is best suited for specimens that require no special treatment and, after partial clearing in the mounting medium, are ready for observation (e.g., arthropod parts, worm eggs, blood smears, chromosomes).

Permanent preparations may be **whole mounts,** containing the entire organism or at least a complete organ, or **partial mounts**, representing only part of an organ. They are designed for repeated use and long-term storage without significant alteration or damage to the specimen. Virtually any fresh biological material can be prepared in this way, including microorganisms, organ fragments, tissues, and isolated cells. In some cases, particularly in medical diagnostics, tissue samples are taken from living organisms (biopsy). The key advantage of permanent preparations is their ability to reveal structures that cannot be observed in fresh (native) specimens. Fixation also prevents the degradation of biological material and preserves staining, which selectively enhances specific details. The final step in preparing a permanent slide is mounting it in a medium that ensures optimal light transmission, improves specimen clarity, and seals out air, thereby preventing oxidative damage.

Complex preparation is required primarily for specimens that need to be processed into thin sections (histological or histochemical preparations). The preparation of a permanent slide consists of the following steps:

a) collection of histological material
b) fixation
c) preparation of samples for sectioning with a microtome
d) staining
e) embedding

Complex preparation of a permanent slide

An example is a histological preparation with a tissue section stained, for instance, with hematoxylin–eosin. The preparation process includes the following basic steps:

1. Fixation – rapid killing of cells or tissue samples, e.g., with a formaldehyde solution.
2. Rinsing – removal of the fixative solution with water.

3. Dehydration – removal of water from the sample in containers with alcohol of increasing concentration, ending with 100% alcohol.
4. Saturation of the sample with benzene or xylene.
5. Embedding the sample in paraffin.
6. Preparation of paraffin blocks containing the tissue sample.
7. Sectioning the paraffin blocks with a microtome into slices 5 micrometers thick.
8. Mounting the sections onto microscope slides.
9. Removal of paraffin with benzene or xylene, leaving the tissue section on the slide.
10. Rehydration – immersing the section in containers with alcohol (starting from 100%) of decreasing concentration down to water.
11. Staining the section in hematoxylin and eosin solution.
12. Rinsing – removing excess stain with water.
13. Dehydration and clearing – transferring the sample through a series of containers with alcohol of increasing concentration and then into xylene (or benzene).
14. Sealing the preparation – a drop of balsam (resin) or another medium miscible with xylene is placed on the stained section saturated with xylene (or benzene), covered with a cover glass, and allowed to harden.

The complexity of the procedure lies in the fact that benzene and xylene do not mix with water but do mix with 100% alcohol. Balsam mixes with xylene and benzene but not with alcohol or water. There are many fixation, staining, and mounting techniques, described in manuals of cytological and histological methods.

Permanent preparations expand the possibilities for observing cells and their structures. Fixation and subsequent staining of cells (microscopic preparations) are standard methods for preparing cells, especially tissues, for microscopic observation. Fixation stops vital and autolytic processes in cells.

Two basic types of fixations are used:

1. physical (by heat and drying) – used mainly for smears (e.g., blood smears).
2. chemical – by the action of liquid fixatives (methanol, ethanol, formaldehyde, etc.). Some dyes also have fixing properties (e.g., Lugol's solution, orcein).

Staining is carried out in solutions of various compositions, and according to the affinity of their components for specific cell structures, they can be classified as: general (screening) stains – e.g., Nile blue, or specific stains – e.g., Giemsa stain for visualizing chromatin.

From a chemical standpoint, dyes are divided into:

1. acid (plasmatic) dyes – stain the cytoplasm of cells, e.g., eosin, acid fuchsin, orange G.
2. basic (nuclear) dyes – stain the cell nucleus, e.g., hematoxylin, methyl green, basic fuchsin.
3. neutral dyes – salts of a coloured base and a coloured acid, e.g., eosinate, methylene blue.

Smear preparations

Accurate identification of different blood cell types requires a technically flawless blood smear. Poor-quality smears can lead to misinterpretation or missed pathological findings. Either capillary or venous blood may be used for preparation.

Collect a small sample of blood (2–3 drops) by pricking the pad of the fourth finger with a sterile needle. Ensure the puncture site is clean, dry, and well perfused. After pricking, touch one end of a microscope slide with a formed drop of blood. Hold the slide between the index finger and thumb of the left hand, positioning the drop near the index finger. Take a second slide, which will be used to make the smear, and place it in front of the drop of blood (never directly into the drop). Position it at a 45° angle and touch the drop so that, upon contact with the slide edge, the blood spreads along the entire length of that edge. Holding the slide between the thumb and index finger in this position, make the smear with a smooth, even motion toward the opposite end of the base slide (Fig. 23).

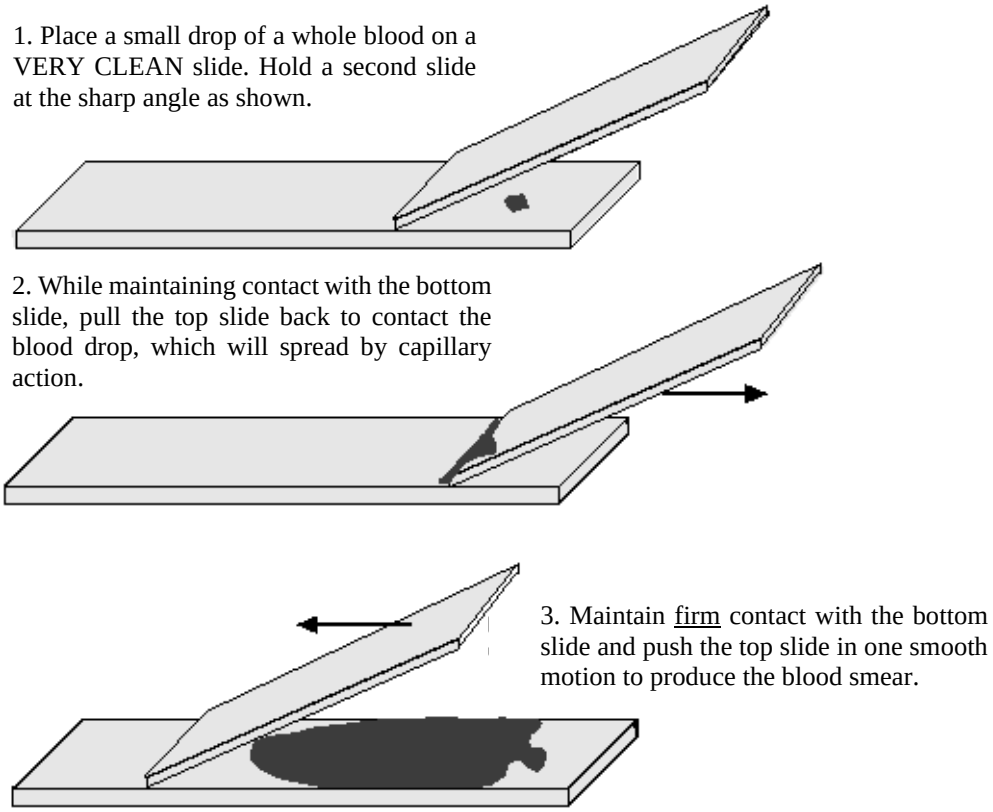


Figure 2-3. Proper blood smear**.**

A properly prepared blood smear is even and, in the final third of the microscope slide, ends with feathered edges (Fig. 2-4).

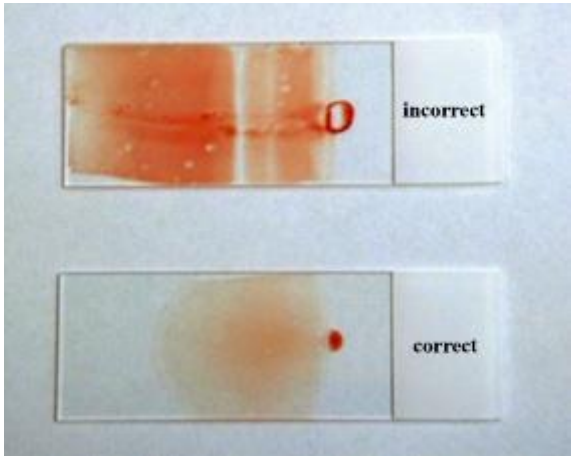


Figure 2-4. Improperly and correctly made blood smear.

Questions and Tasks

Task 1: Preparation of the tongue print

Material and Equipment: Microscope slide and cover slip, methylene blue (0.005%), Pasteur pipette, filter paper.
Procedure: On a clean microscope slide, prepare an imprint specimen from the surface of the tongue, first gently roughened with the teeth. Apply methylene blue stain to the specimen, then place a cover slip over it. Carefully remove any excess stain by touching the edge of the cover slip with a piece of filter paper.
Results: Under the microscope, large polygonal cells with nuclei distinctly stained blue should be visible. Record an accurate drawing of these cells in your notebook and note the presence of bacteria within the preparation.
Drawing:

Task 2: Microscopic examination of Protozoa

Material and Equipment: Protozoa obtained from a hay or cabbage infusion, microscope slide and cover slip, Pasteur pipette, filter paper, dissecting needle.
Procedure: Place a small drop of the protozoan culture onto a clean microscope slide. Gently lower a cover slip over the drop, taking care to avoid trapping air bubbles. Observe the specimen under a 10x or 40x objective lens, as appropriate.
Results: To reduce the rapid movement of the protozoa, carefully draw off a small amount of liquid from the edge of the cover slip using filter paper. Record your observations by making an accurate, labeled drawing of the specimen.
Drawing:

Task 3: Preparation of the blood smear

Material and Equipment: Freshly collected blood, microscope slides.
Procedure: Place a drop of fresh, non-coagulated blood near one end of a clean microscope slide. Using another slide held at an appropriate angle, smear the blood across the specimen slide in a single, smooth motion. Avoid repeated passes, as these can disrupt the smear and damage the cells. Because blood coagulates rapidly, work quickly. Allow the smear to air-dry completely before applying the stain.
Results: In the stained blood smear, the various cellular components of the blood can be clearly distinguished. A well-prepared smear is uniform in thickness and tapers into fine, feathered edges in the final third of the slide (Fig. 2–4).

Task 4: Making simple permanent preparation

Material and Equipment: Fly or bee wing, microscope slide and cover slip, Canada balsam, dissecting needle, scissors, tweezers, burner.
Procedure: Place a drop of mounting medium (Canada balsam) in the center of a slightly warmed microscope slide. Using a dissecting needle, insert the cut-off wing of a fly or bee into the medium, then carefully place a cover slip over it. Avoid creating air bubbles. The amount of mounting medium used should be sufficient but not excessive, so that it does not spread excessively when the cover slip is applied.
Results: Draw the observed object under low magnification and note any possible presence of air bubbles.
Drawing:

3. Structure and Function of Biomacromolecules. DNA Isolation.

Biomacromolecules, including biopolymers, are large molecules (100 000 – 1 000 000 Da) that occur naturally in living organisms and participate in biological processes. Biopolymers are formed by linking smaller building blocks – monomers. They may be composed of identical monomers (homopolymers) or different monomers (heteropolymers). Biopolymers include:

1. **Polysaccharides (carbohydrates)** – e.g., starch, cellulose, glycogen.
   Composed of **monosaccharides** (e.g., glucose) linked together by glycosidic bonds. Their functions are storage (energy) and structural (cell wall). They are polyhydroxyaldehydes or polyhydroxyketones.
2. **Proteins** – e.g., hemoglobin, enzymes.
   Composed of one or more polypeptide chains made up of **amino acids.** Their functions include enzymatic catalysis, transport, structural support, and defense.
3. **Nucleic acids** – DNA and RNA.
   Composed of **nucleotides** (e.g., DNA from dAMP, dGMP, dTMP, dCMP; RNA from UMP, CMP, AMP, GMP). Their function is the storage and transmission of genetic information.
4. **Polyhydroxyalkanoates** – biodegradable plastics produced by microorganisms.

Lipids are not true polymers. They are composed of glycerol and fatty acids (fats, oils, waxes, phospholipids, steroids). Their functions are diverse, including energy storage, biomembranes formation, and acting as regulatory molecules.

Macromolecules adopt a spatial arrangement (conformation) that is energetically most favorable under given conditions. They can be chemically modified (e.g., hydroxylation, carboxylation, methylation, phosphorylation, thiolation, acetylation), which alters their physical and biological properties.

Informational biomacromolecules ensure the transmission of genetic information. The sequence of nucleotides in the primary structure of DNA and RNA determines the sequence of amino acids in a polypeptide chain.

Questions and Tasks

1. Which biological molecules are classified as biopolymers?

2. List the monomers of all known biomacromolecules.

3. Which biopolymers belong to the informational macromolecules?

4. The atoms C, H, N, O, P, and S are the basic elements of organic molecules. Which of them occur in DNA molecules but are not commonly present in proteins? Which, on the other hand, occur in proteins but are normally not found in DNA molecules?

5. How are amino acids classified from a physicochemical point of view?

6. Describe the structure of the α-helix and the β-pleated sheet.

7. Compare the secondary and tertiary structure of a protein.

8. Explain the importance of disulfide bridges in the structure of a protein.

9. Explain the term protein denaturation.

10. Describe the key biological functions of proteins.

11. Describe the structure and properties of monosaccharides and disaccharides.

12. Provide examples of polysaccharides and compare their biological roles.

13. Compare the structure and functions of triglycerides and phospholipids.

14. What is the difference between saturated and unsaturated fats?

15. Describe the basic structure of a nucleotide and explain the type of chemical bond that links individual nucleotides together.

16. Explain the differences between the secondary and tertiary structure of DNA.

17. Compare the structure and function of DNA and RNA.

18. List the main types of RNA molecules and describe their functions.

19. Determine the nucleotide sequence of the DNA strand oriented in the 5'→3' direction, given that the complementary strand has the sequence 3'-ATGGGCTCCGGAT-5' in a double-stranded DNA molecule.

20. Which of the following ratios equals 1 for double-stranded DNA?
(A + T) / (G + C)
(A + G) / (C + T)
C / G
(G + T) / (A + C)

21. The following table lists the relative percentages of bases of nucleic acids isolated from various species. For each species, state what type of nucleic acid it is and whether it is single-stranded or double-stranded. Explain your answer.

Species	Adenine	Guanine	Thymine	Cytosine	Uracil
I.	21	29	21	29	0
II.	29	21	29	21	0
III.	21	21	29	29	0
IV.	21	29	0	29	21
V.	21	29	0	21	29

22. The percentage of cytosine in double-stranded DNA is 17%. What is the percentage of adenine in this DNA?

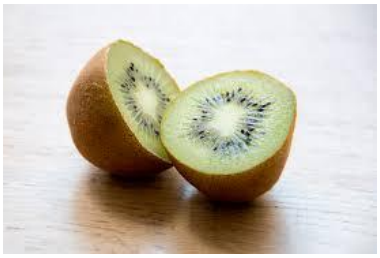
23. Analysis of the DNA of a bacterial virus revealed that it contains 33% A, 26% T, 18% G, and 23% C. What can you say about this DNA based on the data obtained?

24. Double-stranded DNA contains 90 adenines (A) and 110 cytosines (C). What is the total number of nucleotides in this DNA molecule?

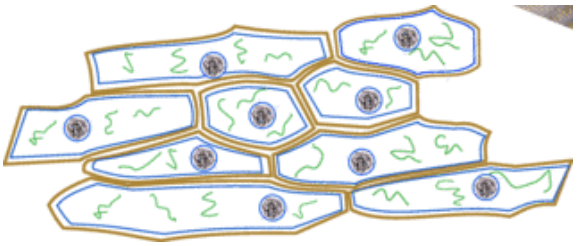
25. DNA in the nucleus of a human somatic cell consists of approximately $6x10^9$ base pairs. What is the length of this DNA if each nucleotide occupies approximately 0.34 nm?

Task 1: Isolation of DNA from fruit

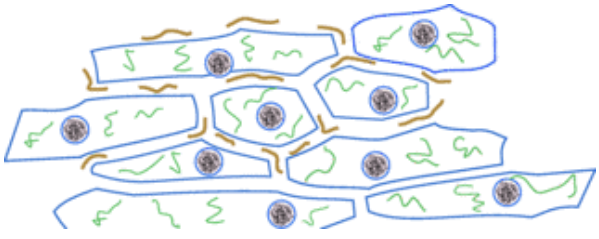
Material and Equipment: Kiwi (or banana), 5 g detergent or soap, 2 g salt, 100 ml water, 10 ml chilled absolute alcohol, knife, Petri dishes, beakers, measuring cylinder, funnel, glass stirring rod, water bath, filter paper, gauze, pipette, pipette tips.




Procedure:
1. Peel the kiwi (or banana) and cut it into four parts. The skin is removed because most of the cells in it are not alive and therefore contain little DNA. Plant cells (see diagram) have, in addition to the cytoplasmic membrane (blue), a cell wall (brown) on their surface.



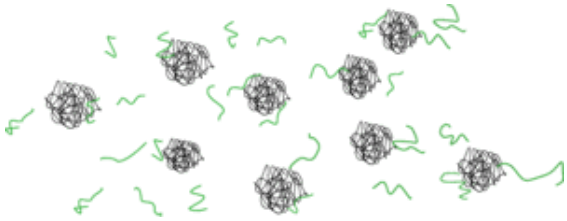
2. Place the kiwi pieces on Petri dishes, cut them into small pieces, and crush them as much as possible (homogenize). Homogenization helps break down the cell walls (see figure).



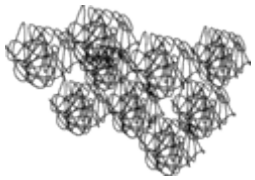
3. Mix the detergent (soap), salt, and water. Salt is added to maintain the osmotic pressure of the solution. Stir slowly until the salt dissolves. This mixture is called the extraction buffer.

4. Add the prepared extraction buffer to the crushed kiwi pieces and continue homogenizing. The amount of DNA isolated will be greater if the kiwi is thoroughly crushed and mixed with

the extraction buffer. The detergent (soap) dissolves cell membranes and some proteins (see figure).



5. Incubate the blended kiwi in a water bath for 15 minutes at 60 °C. Incubation helps to break down the remaining cells and degrade some proteins (green) in the cell. Proteins are also broken down by the kiwi's own proteases.



6. After removing the beaker from the water bath, filter the kiwi through filter paper (or banana through gauze) into a clean beaker. Filtration removes substances that are insoluble in water. The resulting filtrate is a green liquid containing DNA from the kiwi (in the case of banana, the filtrate is yellowish). The filtrate also contains salt and various other substances from the original cells that are soluble in water.

7. Finally, precipitate the negatively charged DNA molecules from the aqueous solution using chilled alcohol. Slowly pour the alcohol down the inner wall of the beaker. The alcohol will form a transparent layer on the surface of the mixture.

Result: In the upper alcohol layer, a gelatinous mass containing DNA will begin to form. The isolated DNA comes from a large number of cells, making it easily visible to the naked eye.

4. Cell Structure

All living organisms are composed of one or more cells. The cell is the basic structural and functional unit of living organisms. All cells arise from pre-existing cells through division.

Genetic information is transmitted from the parent cell to the daughter cell via DNA. Energy flow occurs within cells, for example, through respiration and photosynthesis. The chemical composition of all cells is similar.

The development of cell theory has been marked by several key milestones. In 1665, Robert Hooke, while observing cork tissue, introduced the term **cell**. With improvements to the microscope in 1674, Anton van Leeuwenhoek observed and described bacteria and protozoa, which he called **animalcules.** The nucleus of the plant cell was discovered in 1831 by Robert Brown. Matthias Schleiden's 1838 observation that all plants are composed of cells was extended by Theodor Schwann in 1839, who concluded that all living organisms are made up of cells. Rudolf Virchow's statement in 1855, "*Omnis cellula e cellula"* ("Every cell arises from another cell."), became an important addition to cell theory in the 19th century.

Advances in electron microscopy, molecular biology, cell engineering, regenerative medicine, stem cell research, and more recently the development of CRISPR technology (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) for precise DNA editing, have greatly enhanced our understanding of the structures and functions of both prokaryotic and eukaryotic cells.

Questions and Tasks

1. What is the fundamental principle of cell theory?

2. Identify and explain the key differences between prokaryotic and eukaryotic cells.

3. What is a nucleoid, and what role does it play in the cell?

4. Describe the molecular structure of biomembranes and explain their primary functions.

5. Compare and contrast the structure and functions of plant and animal cells.

6. Explain the evolutionary origin of membrane-bound organelles and provide examples.

7. Describe the structure and functions of the nucleus in a eukaryotic cell.

8. Which types of molecules can pass through nuclear pores?

9. Define ribosomes and explain how prokaryotic ribosomes differ from eukaryotic ribosomes.

10. Describe the structure and functions of mitochondria.

11. Which membrane-bound organelles are considered the main synthetic centers of the cell?

12. Compare the structure and functions of smooth and rough endoplasmic reticulum.

13. Outline the main processes that occur in the Golgi apparatus.

14. Which enzymes are present in lysosomes, and what are their functions?

15. What is the role of peroxisomes in eukaryotic cells?

16. Explain the importance of cytoskeleton in maintaining cell structure and function.

17. Compare the structure and functions of microtubules, microfilaments, and intermediate filaments.

Task 1: Microscopic examination of the nucleus and nucleolus

Material and Equipment: Onion, methylene green and pyronine solution, microscopic utensils.
Procedure: Carefully peel one fleshy scale leaf from the onion. On its inner surface, use a razor blade to make four cuts forming a 3 mm × 3 mm square. Using tweezers, gently remove the transparent, single-layer epidermis from the marked area and place it on a clean microscope slide. Add a drop of methylene green and pyronin solution, cover with a coverslip, and prepare a wet mount. Examine the specimen under low magnification first, then switch to high magnification for detailed observation.
Results: The nucleus appears blue when stained with methylene green, indicating the presence of DNA, while the nucleolus appears pink with pyronin staining, indicating the presence of RNA.
Drawing:

Task 2: Vital staining of mitochondria and cytoplasmic streaming

Material and Equipment: Onion (or red onion), Janus green solution (1:10 000 in water), water, microscopic utensils.
Procedure: Using tweezers, carefully peel the epidermis from the inner surface of the onion. Cut a 5 × 5 mm section and place it into a drop of Janus green dye on a microscope slide. Allow the tissue to stain for 15–20 minutes. Transfer the stained sample into a drop of water on a clean slide and, under low magnification, locate an area containing intact cells. Alternatively, the outer epidermis of a red onion can be examined directly in a drop of water without staining. Observe the cells at 400x and 1 000x magnification for detailed visualization.
Results: Within the cells, numerous small, oval structures with a pale green colour – mitochondria – are visible. These organelles often appear to move, a result of being carried along by the streaming cytoplasm.
Drawing:

Task 3: Microscopic examination of chloroplasts in the leaf cells of the moss or the aquatic plant *Brazilian waterweed (Egeria densa)*

Material and Equipment: Moss, microscopic utensils.
Procedure: Prepare a fresh mount from a moss leaf. Place a drop of water on a microscope slide, insert a small piece of green leaf into the drop, and cover it with a cover slip. First, observe the preparation under low magnification. The surface of the observed part of the leaf appears divided into small compartments – individual cells. The entire leaf consists of only a single layer of cells. However, the cells are not uniform in shape across the whole leaf. At the edges and along the main vein, the cells are elongated, whereas in the rest of the leaf they are smaller and usually polygonal. Under high magnification (400x), the individual cells can be examined in more detail. Inside them, green plastids – chloroplasts – are visible. Note the number of chloroplasts in each cell, their arrangement, and their shape. Here, chloroplasts are most often polygonal with rounded corners. Also present are elongated chloroplasts, sometimes constricted in the middle – these are chloroplasts in various stages of division. In damaged cells, usually at the cut edge, there may also be round chloroplasts showing signs of shrinkage. With careful observation, small starch grains can be seen inside them, which are almost absent in chloroplasts of undamaged cells. The rounding and shrinkage of chloroplasts, as well as the presence of starch grains, are often indicators of slight cell damage.
Results: Draw the shapes of chloroplasts in individual cells.
Drawing:

Task 4: Microscopic examination of calcium oxalate crystals in onion scale cells

Material and Equipment: Dry onion skin, glycerol, alcohol, microscopic utensils.
Procedure: Prepare the specimen for observation several days in advance. Cut the dry onion skin into small squares with sides of 3-4 mm and place them for 3-5 days in a glycerol-alcohol mixture prepared in a 1:1 ratio. The onion squares can be kept in this mixture for a longer period if needed. This mixture removes air bubbles from the cells, which are undesirable during microscopic observation, and acts as a preservative for the specimen. To prepare the slide, take one onion square from the glycerol-alcohol mixture and place it in a drop of pure glycerol on a microscope slide, then cover it with a cover slip.
Results: When examined under the microscope, individual cells reveal crystals of tetragonal calcium oxalate, $Ca(COO)_2.3H_2O$. These crystals vary in size and may appear as elongated, prismatic tetragonal forms or as aggregates joined in a cross-like arrangement.
Drawing:

Task 5: Leucoplasts in the epidermal cells of *Tradescantia* leaves

Material and Equipment: *Tradescantia* leaves, glycerol, filter paper, microscope utensils.
Procedure: On the underside of a *Tradescantia* leaf, make a shallow incision approximately 4-5 mm long using a razor blade. Using tweezers, grasp the cut section of epidermis and gently peel it away. Place a drop of glycerol on the small piece of epidermis and prepare a fresh mount. Begin the observation under low magnification. The leaf epidermis is composed of irregular pentagonal or hexagonal cells that fit closely together. At regular intervals, pairs of guard cells are present. Each pair is surrounded by four smaller cells of a different shape. Together, the guard cell pair and the four surrounding cells form the stomatal apparatus.
Results: Under higher magnification (400x), colourless granules arranged in a circular pattern can be observed within the epidermal cells, particularly in those adjacent to the stomata. These granules are leucoplasts of the epidermal cells. At the center of this ring of leucoplasts lies the cell nucleus, which is not easily visible in unstained preparation; however, its position is indicated by the circular arrangement of the leucoplasts.
Drawing:

Task 6: Microscopic examination of starch grains in potato tuber cells

Material and Equipment: Potato (*Solanum tuberosum*), water, Lugol's solution, Petri dishes, razor blade, tweezers, scalpel, microscopic utensils.
Procedure: Prepare a fresh mount from a very thin slice of potato flesh (or from a small portion of the flesh scraped from a cut potato using a scalpel) and a drop of water. Observe the large starch grains, then confirm their presence using Lugol's solution with the soaking technique. The starch grains will stain blue to purple. Examine the specimen under all magnifications, including with the oil immersion objective.
Results: Note the characteristic layered structure of the starch grains and make a drawing of the observed cells.
Drawing:

Task 7: Microscopic examination of cells and their nuclei in a blood smear

Material and Equipment: Permanent blood smear slide, immersion oil, microscopic utensils.
Procedure: At 400x magnification, locate the individual types of cells using the method illustrated in Fig. 4-3.

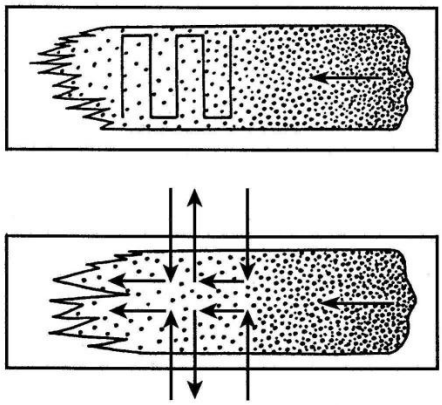


Figure 4-3. Scheme of examining and evaluating properly prepared blood smear.

Results: Using the oil immersion objective, draw the observed cells and their nuclei (Fig. 4-4).

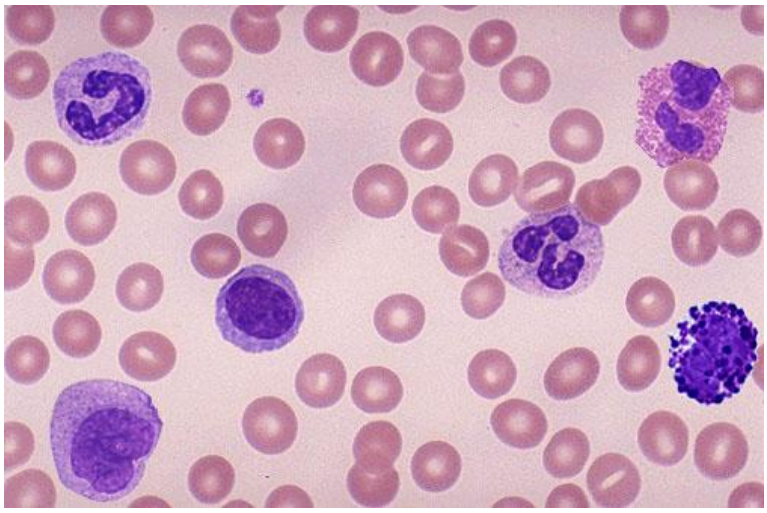
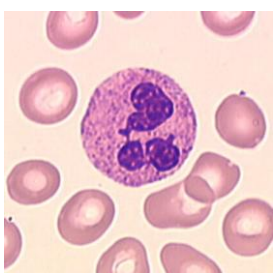


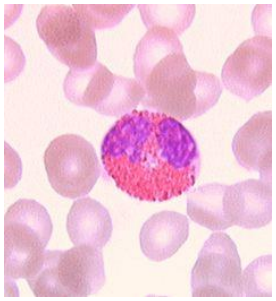
Figure 4-4. Blood smear.

Drawing:

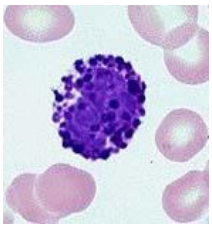
Neutrophil



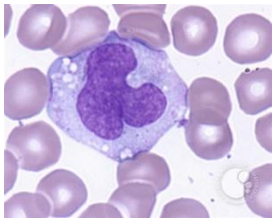
Eosinophil



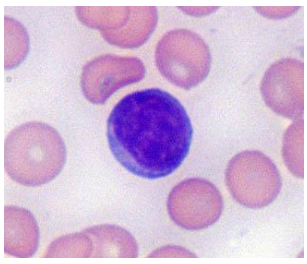
Basophil



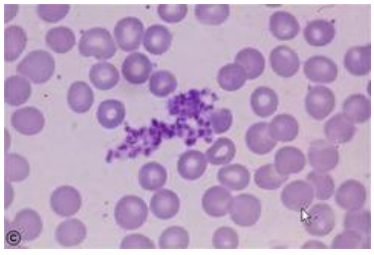
Monocyte



Lymphocyte



Platelets



5. Cell physiology

Cell physiology constitutes a comprehensive field of study focused on the functions, processes, and interactions occurring within the cell. It integrates interdisciplinary knowledge from biochemistry, molecular biology, biophysics, and cell biology. The principal domains of cell physiology encompass:

- Cellular environment and homeostasis, with particular emphasis on the role of the plasma membrane and mechanisms that ensure the maintenance of a stable intracellular milieu.
- Membrane transport mechanisms, including passive, active, and vesicular transport.
- Cellular signaling, encompassing signaling cascades, receptor-mediated processes, and cellular responses.
- Cell cycle regulation and cell division.
- Gene expression and protein biosynthesis.
- Cell death modalities—such as apoptosis, necrosis, necroptosis, pyroptosis, ferroptosis, and autophagy—together with less common, specialized forms including anoikis (apoptosis induced by the loss of cell–extracellular matrix interactions), entosis (cell-in-cell invasion, frequently observed in cancer cells), and mitotic catastrophe (cell death resulting from mitotic failure).
- Cell-type–specific functions, for example, those characteristics of neuronal, muscular, or immune cells.

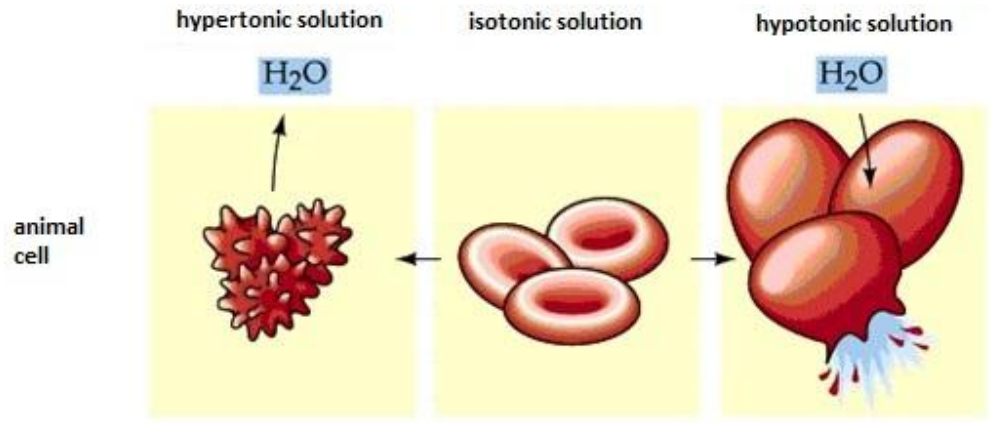


Figure 5-1. Animal cell in hypertonic, isotonic and hypotonic solution.

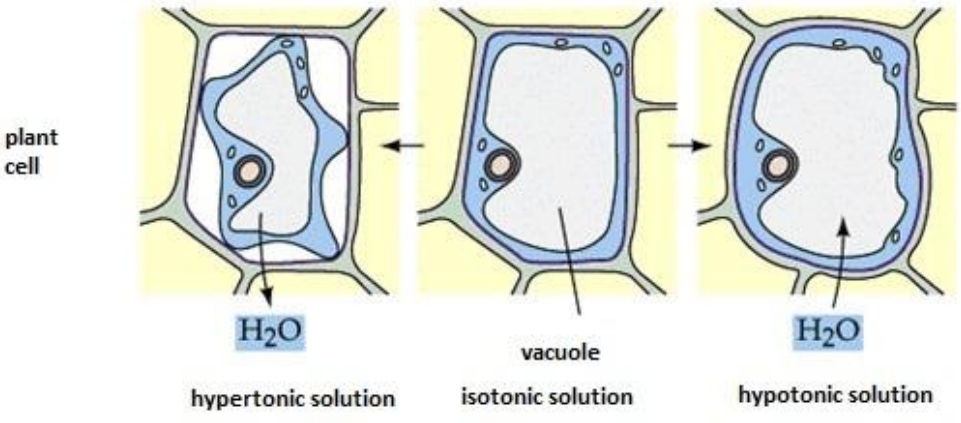


Figure 5-2. Plant cell in hypertonic, isotonic and hypotonic solution.

Questions and Tasks

Task 1: Match the numbered terms to the following descriptions:

1. Symport	7. Phagocytosis	13. Plasmalemma
2. Diffusion	8. Antiport	14. Shrinks
3. Plasmolysis	9. Hypotonic	15. Bursts
4. Osmosis	10. Cell wall	16. Plasmoptysis
5. Pinocytosis	11. Plasma membrane	17. Isotonic
6. Secondary active transport	12. Receptor-mediated endocytosis	18. Facilitated diffusion

..... a) Spontaneous movement of substances from a region of higher concentration to a region of lower concentration.
..... b) Uptake of solid particles by the cell.
..... c) Transport of one ligand can be coupled with the transport of another ligand in the same direction.
..... d) An environment that has the same osmotic value as the cell.
..... e) What happens to an animal cell in a hypertonic solution?
..... f) Which cell surface structure determines whether or not substances enter the cell?
..... g) When a plant cell is placed in a hypertonic environment, it loses water and decreases in volume.
..... h) Which cellular structure forms the osmotic barrier of the cell?
..... i) If an animal cell is placed in a hypotonic environment, water flows into the cell, and it subsequently increases in volume.
..... j) Movement of water from an area of lower concentration to an area of higher concentration across a semipermeable membrane.
..... k) Passive transport of molecules via membrane channels and carrier proteins.
..... l) Continuous uptake of substances by the cell.
..... m) Active transport of molecules in vesicles coated with clathrin.

Task 2: Change in the shape of red blood cells in a hypertonic environment

Materials and Equipment: Fresh blood, 1.5% NaCl solution (hypertonic solution), 0.9% NaCl solution – physiological saline, pipettes, test tubes.
Procedure: Place 0.2 ml of blood into two test tubes. Add 2 ml of physiological saline to the first test tube and 2 ml of 1.5% NaCl solution to the second test tube. Mix gently and prepare a wet mount from both samples. Observe under 400× magnification.
Results: In the physiological saline, which is isotonic for red blood cells, the cells maintain their normal shape. While the red blood cells in the physiological solution retain their original form, the red blood cells in the hypertonic NaCl solution become rounded, shrink, and acquire a spiky (crenated) appearance. Draw the normal and altered erythrocytes.
Drawing:
Cell in an isotonic environment: Cell in a hypertonic environment:

Task 3: Hemolysis of erythrocyte plasma membrane

Materials and Equipment: Fresh blood, 1.5% NaCl solution, 0.9% NaCl solution – physiological saline, pipettes, test tubes.
Procedure: Place a drop of physiological saline on a microscope slide. Using a needle, add a small amount of blood until the drop turns slightly pink. Mix gently and cover with a cover slip. Observe the individual floating erythrocytes under low and high magnification.
Without lifting the microscope tube, add a drop of Septonex solution to the edge of the cover slip. Facilitate the penetration of the solution under the cover slip by using filter paper to draw the liquid from the opposite edge. Identify the boundary where erythrocyte lysis occurs. Select an intact erythrocyte and observe it until it disintegrates. Repeat the observation and record the results with drawings in the protocol.
Results: Septonex is a quaternary ammonium compound with a strong effect on the surface tension of liquids. In its presence, the bimolecular phospholipid film of the erythrocyte plasma membrane loses stability and disintegrates into spherical micelles. Erythrocytes are particularly sensitive to such compounds. They undergo lysis, releasing their contents, including dissolved hemoglobin, into the surrounding medium (**chemical hemolysis**). With proper illumination, the residual, colourless shadow of the erythrocyte can be seen briefly at the site, corresponding to remaining cellular structures, mainly the cytoskeleton. A similar disintegration of erythrocytes can also be observed when cells are placed in a hypotonic solution. In this case, the cause is water uptake due to osmotic pressure (osmotic hemolysis). The plasma membrane is not chemically disrupted but bursts mechanically. Record your observations with drawings.
Drawing:

Task 4: Dye transport into living cells

Materials and Equipment: Kitchen onion, 0.2% neutral red solution, watch glass, dish, forceps, razor blade, needles.
Procedure: Peel a fleshy leaf from the onion and make four cuts with a razor blade on the inner surface, forming a square with 3 mm sides. Using forceps, remove the square of transparent, single-layered epidermis and immerse it in 0.2% neutral red solution on a slide for 15 minutes, ensuring it does not float on the surface. Rinse the epidermis with clean water, prepare a wet mount, and observe under low and high magnification.
Results: The epidermis consists of typical plant cells with prominent cell walls, a thin layer of cytoplasm along the walls, and usually a clearly visible nucleus and large central vacuole. In living cells, the vacuolar contents are stained red, while the cytoplasm and nucleus remain unstained. In dead cells, all structures are faintly stained. Neutral red is a non-toxic dye. This type of staining is referred to as vital or intravital staining. In this case, neutral red penetrates the cytoplasmic membrane into the cytoplasm, from where it is actively transported into the vacuole, where it accumulates. Neutral red is a pH indicator: above pH 7.4 it appears honey-yellow, while below pH 7.0 it appears crimson red.

Drawing:

Task 5: The course of plasmolysis and deplasmolysis

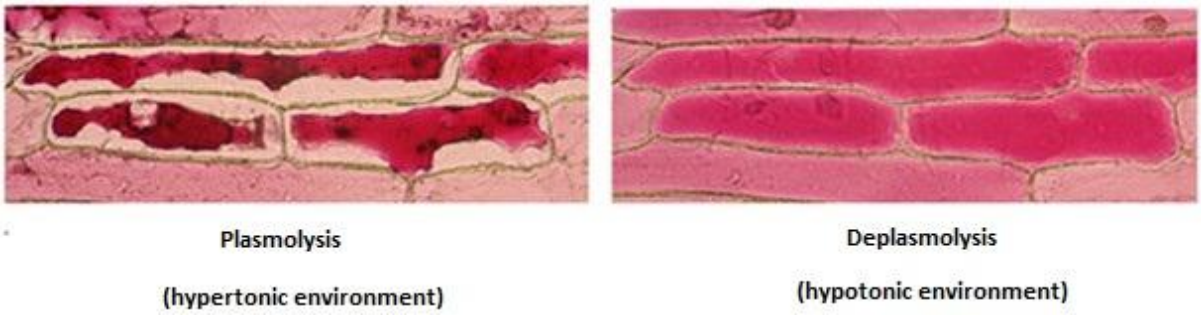


Figure 5-3. The course of plasmolysis and deplasmolysis.

Materials and Equipment: Outer epidermis from the red onion scale, 1 M sucrose solution, standard microscopy equipment.
Procedure: Place the onion epidermis into a drop of sucrose solution and observe the wet mount first under low magnification and then under higher magnification.
Results: Exposure to a hypertonic environment causes plasmolysis of the cells. Plasmolysis is the reduction of the protoplast volume due to the exosmotic loss of water from the cell in a hypertonic solution. Morphologically, it is observed as the detachment of the cytoplasm from the cell wall. Under higher magnification, draw one plasmolyzed cell and one non-plasmolyzed cell. When distilled water is applied to the preparation, deplasmolysis occurs: the cytoplasm returns to its original position, and the plasma membrane re-adheres to the cell wall.
Drawing:
Plant cell in a hypertonic environment:

Plant cell in a hypotonic environment:

Task 6: Traube's osmotic cell

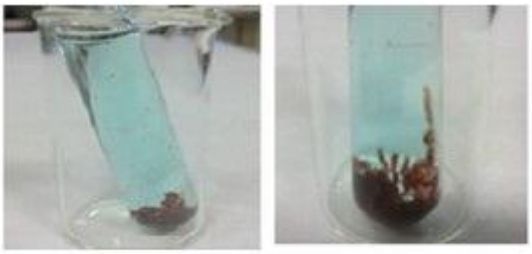


Figure 5-4. The shape of Traube's osmotic cell after two and five minutes.

Materials and Equipment: Test tube, stand, forceps, 2% aqueous $CuSO_4$ solution, potassium ferrocyanide crystals.
Procedure: Fill a test tube approximately halfway with the $CuSO_4$ solution. Using forceps, carefully add a small fresh piece of potassium ferrocyanide crystal. Place the test tube in a stand and observe.
Results: A reddish-brown film forms on the surface of the crystal in the shape of a vesicle, which gradually increases in size. Draw the shape of the vesicle at 2, 5, and 10 minutes. The potassium ferrocyanide crystal dissolves, and in reaction with Cu^{2+} ions, forms a semipermeable membrane of copper ferrocyanide, giving the vesicle its characteristic shape.

$$2CuSO_4 + K_4[Fe(CN)_6] \rightarrow Cu_2[Fe(CN)_6] + 2K_2SO_4$$

The concentrated solution inside the vesicle osmotically draws water from the surrounding $CuSO_4$ solution via endoosmosis, causing the vesicle to increase in volume until it eventually bursts. The opening allows the potassium ferrocyanide solution to escape, which immediately reacts upon contact with $CuSO_4$, forming a new membrane that closes the opening and restores water endosmosis. The growth of the entire structure appears as the formation of small vesicles in succession. Traube's osmotic cell serves as a chemical model of osmosis. Vesicles form, grow, change shape, and exhibit movement due to physicochemical causes: through a substitution-type chemical reaction, copper ferrocyanide molecules spontaneously organize into planar layers, forming the semipermeable vesicle membrane. This membrane allows only water to pass, establishing an osmotic system: inside the vesicle is a hypertonic solution, which expands the volume via endosmosis, while the surrounding solution is hypotonic. The interaction of these two simply organized systems (solutions of inorganic compounds) spontaneously gives rise to a higher-order complex system with new structures and properties.

6. Chromosomal basis of heredity. Human karyotype

A chromosome is the functional and structural carrier of genetic information in a eukaryotic cell. It forms through the condensation of chromatin and plays a key role in heredity, gene expression, and the proper progression of cell division. Chromatin is a complex of DNA and proteins, including histone and non-histone proteins. Various structural regions can be identified on a chromosome, such as the centromere, telomeres, chromosomal arms, and sister chromatids after replication. Chromosomes are classified into four groups based on the position of the centromere.

A karyotype is an organized set of all chromosomes in an individual's cell, arranged by size, shape, and type within the nucleus of a somatic cell in metaphase of mitosis, and displayed in homologous pairs. It represents the cytogenetic profile of an organism's genome and serves as a fundamental tool for studying chromosomal abnormalities. A karyotype is typically visualized as a karyogram, which is the arrangement of chromosomes into pairs (1–22 + XX/XY).

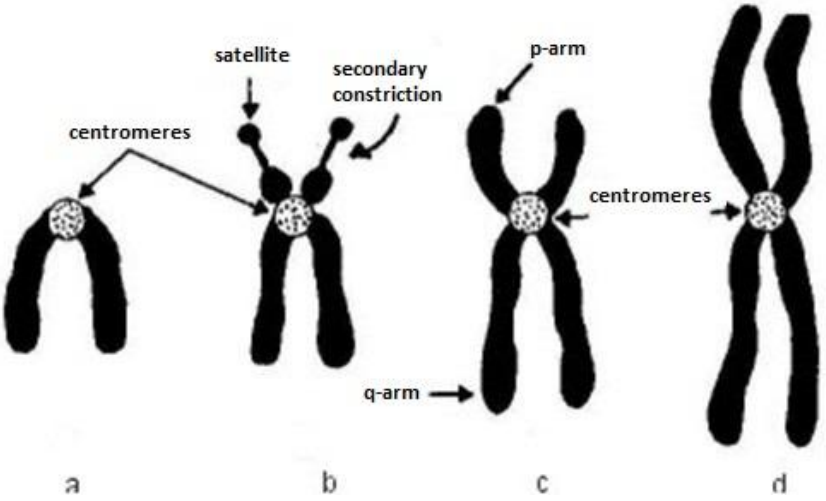


Figure 6-1. Chromosome types: a) telocentric, b) acrocentric, c) submetacentric, d) metacentric.

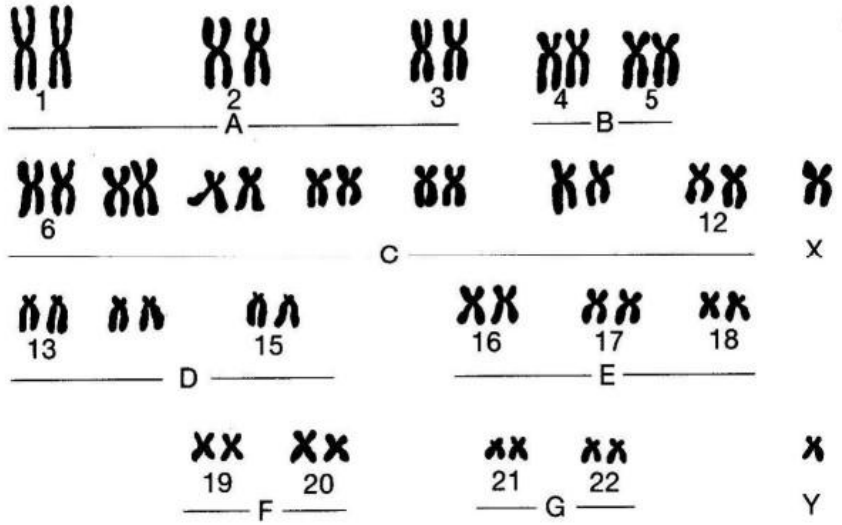


Figure 6-2. Normal male karyotype (46, XY, conventional method, Giemsa stain).

Table 6-1. Sorting human chromosomes.

Group	Autosomes	Gonosomes	Characteristics of chromosomes	Number of chromosomes	
				man	woman
A	1 – 3	-	largest metacentric	6	6
B	4 – 5	-	largest submetacentric	4	4
C	6 – 12	X	middle submetacentric	15	16
D	13 – 15	-	large acrocentric	6	6
E	16 – 18	-	smallest submetacentric	6	6
F	19 – 20	-	smallest metacentric	4	4
G	21 – 22	Y	smallest acrocentric	5	4
Total	44	2		46	46

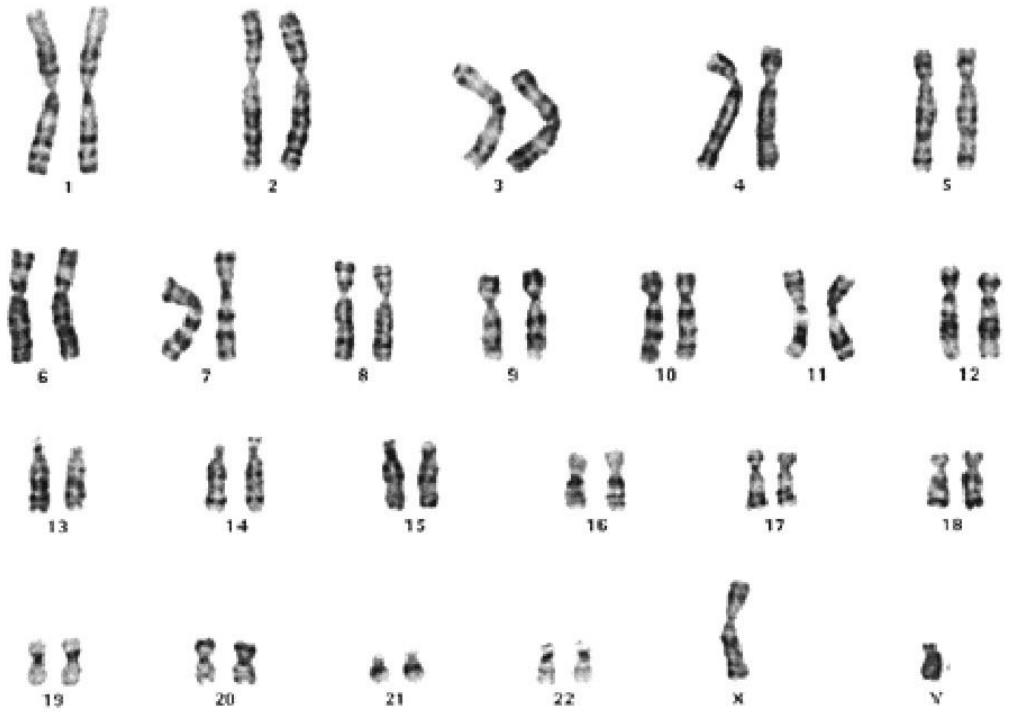


Figure 6-3. G-banding method – male karyotype (46,XY).

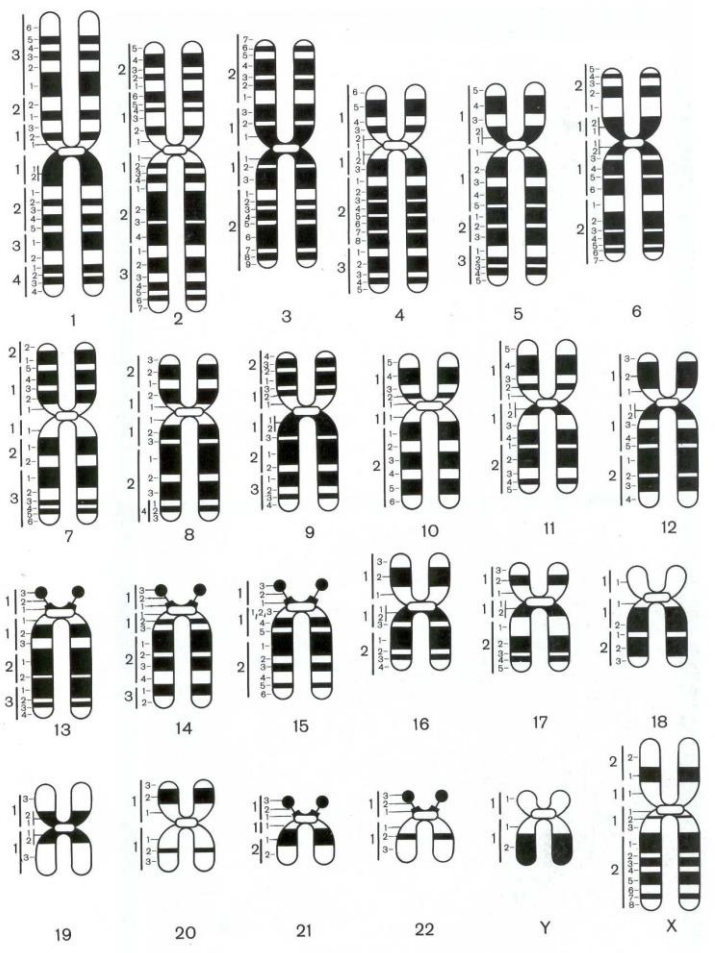


Figure 6-4. Paris nomenclature: idiogram of human chromosomes – diagram of G-bands on human chromosomes.

Questions and Tasks

1. Describe the molecular structure of chromosomes, their morphology, and explain why we can observe them during mitosis and meiosis.

Draw the individual types of chromosomes in metaphase and anaphase in the table. Describe the morphological differences.

chromosome type	metaphase	anaphase
metacentric		
submetacentric		
acrocentric		
telocentric		

2. Why is the centromere an essential part of chromosome structure?

Task 1: Evaluation of human chromosomes under a microscope

Materials and Equipment: Permanent preparation obtained from peripheral blood lymphocytes after short-term in vitro culture, stained using a conventional method.
Procedure: On the prepared slide, made from human peripheral lymphocytes after short-term culture and stained conventionally, locate a mitotic figure under low magnification (100×) and then under higher magnification (400×) with well-spread chromosomes.
Results: Chromosome analysis is performed at 1000× magnification. Only well-spread mitotic figures, where individual chromosomes do not overlap, are suitable for evaluation. Select an appropriate mitosis, draw it schematically in the protocol, determine the total chromosome number, and identify the chromosomal sex of the individual.
Drawing:

Task 2: Determining the sex chromosomes of an individual

Materials and Equipment: Microphotograph of human chromosomes in metaphase (conventional staining).
Procedure:
a) Count the chromosomes on the microphotograph of human chromosomes (conventionally stained).
b) Determine the sex chromosomes of the individual.

Figure 6-5. Photomicrograph of human chromosomes in metaphase (conventional staining). Determining the sex chromosomes of an individual:

Task 3: Construction of a normal human karyotype from conventionally stained chromosomes and G-banded chromosomes

Materials and Equipment: Microphotograph of human chromosomes in c-metaphase of mitosis from short-term cultured lymphocytes, scissors, glue.
Procedure: On the microphotograph of human chromosomes in c-metaphase, first count the chromosomes. Cut out the individual chromosomes and arrange them into homologous pairs according to centromere position and size, from largest to smallest, and into groups A–G. Accurately arrange the chromosomes from pairs 1 to 22 in the karyotype based on the evaluation of their banding patterns. After verification by the assistant, glue the chromosomes into the protocol. Determine the chromosomal sex of the individual based on the completed karyotype.
Results:

7. Cell cycle - DNA replication

DNA replication is an accurate, regulated, and complex process of copying genetic information. Replication is semiconservative, precise, and controlled, proceeding through the action of enzymes and proteins during the S-phase of the cell cycle. It occurs in three stages: initiation, elongation, and termination.

During the initiation phase, replication begins at the origin of replication (in prokaryotes, a single origin – oriC; in eukaryotes, multiple origins forming replicons). The unwinding of the double helix and formation of the replication fork is mediated by helicase, while single-strand binding (SSB) proteins stabilize the open DNA. Topoisomerases (in prokaryotes, DNA gyrase) relieve the torsional stress ahead of the replication fork.

The elongation phase begins with the synthesis of a short RNA primer by primase, which provides a starting point for DNA polymerase, adding nucleotides in the $5' \rightarrow 3'$ direction. The leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously as Okazaki fragments. Multiple types of DNA polymerases are involved in this process. DNA polymerase I (in prokaryotes) or RNase H and DNA polymerase δ (in eukaryotes) remove RNA primers and replace them with DNA. Okazaki fragments are joined together by DNA ligase.

The termination phase occurs when replication forks meet or at specific termination sequences. In eukaryotic cells, these are the telomeres.

Questions and Tasks

1. Describe the individual phases of the cell cycle.

2. What changes does the nuclear genetic material undergo during the different phases of the cell cycle and the stages of mitosis?

3. How does DNA replication and bacterial cell division occur?

4. How does chromosomal DNA replication in eukaryotic cells differ from DNA replication in prokaryotic cells?

5. List the enzymes involved in DNA replication and describe their functions.

6. How is the leading strand of the DNA duplex replicated?

7. How is the lagging strand of DNA replicated?

8. Describe the semiconservative model of DNA replication.

9. Explain what (a) Okazaki fragments, (b) DNA ligase, and (c) RNA primer represent and their functions during DNA replication.

10. What is meant by (a) unidirectional and bidirectional DNA replication and (b) continuous and discontinuous DNA synthesis?

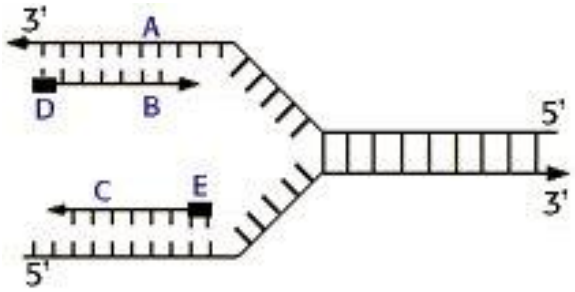
11. Examine the replication fork diagram:

a) A represents the DNA strand that is _____.

b) B represents the DNA strand that is _____.

c) The newly synthesized DNA strand labeled C is _____.

d) The black rectangles labeled D and E are _____.



12. Why is it important for chromosomes to duplicate from single-chromatid to double-chromatid before mitosis?

13. A diploid organism contains 4.5×10^8 base pairs (bp) in its DNA. The entire DNA is replicated in 3 minutes. Assuming the replication rate is 10^4 bp per minute, how many replication origins (replicons, replication bubbles) are present in the genome of this organism?

14. The rate of DNA replication at each replication fork in a human cell (in one direction) is approximately 100 nucleotides per second. What is the minimum number of replication origins in a human cell if the entire DNA must be replicated within 24 hours? A human cell contains two copies of the genome, one inherited from the mother and one from the father, each with 3×10^9 base pairs.

15. Assume that DNA synthesis in *Escherichia coli* occurs at a rate of 100,000 base pairs (bp) per minute, and replication of its chromosome takes 40 minutes.
a) How many base pairs are present in the entire *E. coli* chromosome?
b) What is the total length of the *E. coli* chromosome DNA if each nucleotide in the DNA strand has a length of 0.34 nm?

16. How long would it take *E. coli* to replicate its entire genome (4.2×10^6 bp) assuming a replication rate of 1000 base pairs per second at each replication fork?

17. How long would it take to replicate a human metacentric chromosome containing 1.5×10^6 kb, if replication proceeds bidirectionally from a single origin at the centromere and the replication rate at one replication fork is 3000 bp per minute?
And how long would it take if replication proceeds bidirectionally from 1000 origins of replication?

18. The entire genome of the fruit fly (*Drosophila melanogaster*) contains 1.65×10^8 bp. Replication at each replication fork proceeds at a rate of 30 bp/s. Calculate the minimum time required to replicate the entire genome if replication starts:
a) From a single origin of replication and proceeds bidirectionally.
b) From 2000 origins of replication and proceeds bidirectionally.
In early embryogenesis, replication is completed in 5 minutes. What is the minimum number of origins of replication required at this replication rate?

8. Cell cycle – Mitosis

Mitosis occurs continuously during the interphase of somatic cells. It is divided into five phases, which can be described as follows: Prophase: Chromatin condenses into visible chromosomes, and the nucleolus disappears. Centrosomes move to opposite poles of the cell, and the mitotic spindle forms. Chromosomes begin moving toward the equatorial plane. Prometaphase: The nuclear envelope breaks down, and microtubules attach to the kinetochores of the chromosomes. Metaphase: Chromosomes align along the metaphase plate in the equatorial plane. The attachment of chromosomes to the mitotic spindle is checked. Anaphase: Sister chromatids separate at the centromere and move toward the poles as microtubules shorten. Telophase: Nuclear envelopes reform, chromosomes decondense, and nucleoli reappear.

Cytokinesis, the division of the cytoplasm, begins during anaphase or telophase. In animal cells, it occurs via a contractile ring, while in plant cells, it proceeds through the formation of a cell plate.

Mitosis is a tightly regulated process, with key checkpoints at: G_2/M: Checks for DNA damage and cell size. Metaphase: Checks proper attachment of chromosomes to the spindle. G_1/S: Controls the initiation of DNA replication.

These regulatory processes involve cyclins, cyclin-dependent kinases (CDKs), and the anaphase-promoting complex/cyclosome (APC/C), which triggers the onset of anaphase.

Questions and Tasks

1. Describe the processes that occur in the individual stages of mitosis.

2. Describe the components of the mitotic apparatus. During which phase of the cell cycle is it formed, from what material, and by what mechanism?

3. What is the difference between cytokinesis in animal and plant cells?

4. What changes does the nuclear genetic material undergo during the different stages of mitosis?

5. Describe all the cell cycle checkpoints.

Task 1: Observation of individual stages of mitosis on a crushed preparation from the tip of an onion root.

Materials and Equipment: Germinated onion (*Allium cepa*) with roots approximately 5 mm long, fixative solution, maceration solution, distilled water, aceto-orcein, watch glass, microscope slide and cover slip, forceps, small squares of cotton wool.

Procedure: Cut the tip of the onion root to a length of 3–5 mm. Fix the root tip for 10 minutes in the fixative solution, then transfer it to the maceration solution for another 10 minutes. Rinse the roots in water and stain for 15 minutes with aceto-orcein. Transfer the root tip onto a microscope slide, cover it with a cover slip, and press with your thumb through a small square of cotton wool so that the cells of the root tissue spread evenly across the slide.

Results: Onion cells are suitable for observing mitotic nuclear division because they are large, and their chromosomes are also large and clearly visible under a microscope (2n = 16). Examine the smear preparation first under low magnification (100×), and draw the individual stages of mitosis under higher magnification (400×).
Drawing:

Interphase Prophase Metaphase

Anaphase Telophase

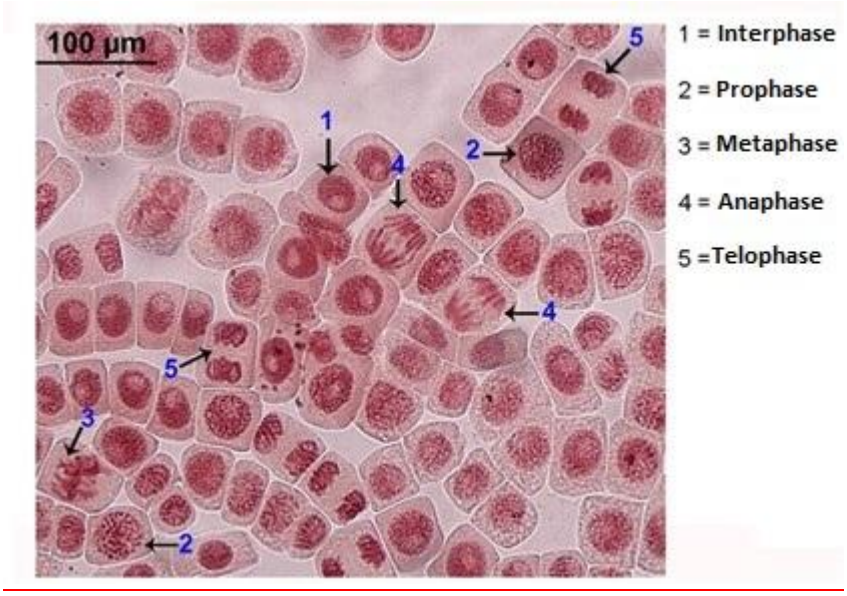


Figure 8-1. Individual phases of mitosis in onion root tip cells.

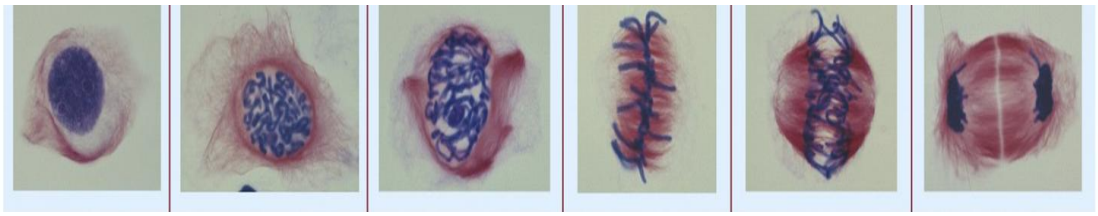


Figure 8-2. Individual stages of mitosis.

Task 2: Observation of human chromosomes at the metaphase stage.

Materials and Equipment: Permanent preparation of human peripheral lymphocytes after in vitro culture, standard microscopy equipment.
Procedure: Examine the preparation under 100× magnification, locate c-metaphases, and draw the set of 46 metaphase chromosomes at 1000× magnification.
Drawing:

Task 3: Determination of the mitotic index from 100 evaluated human peripheral lymphocyte cells after culture *in vitro*.

Materials and Equipment: Permanent preparation of human peripheral lymphocytes after in vitro culture, standard microscopy equipment.
Procedure: Record the number of cells in mitosis from 100 analyzed cells in a table and calculate the mitotic index.

Number of cells in mitosis (N mit)	Number of cells evaluated (Nt)	MI = N mit / Nt

MI = mitotic index; N mit = number of cells in mitosis; Nt = number of cells evaluated

Task 4: Observation of individual stages of mitosis in mouse and rat bone marrow.

Materials and Equipment: Permanent preparation of mouse and rat bone marrow, standard microscopy equipment.
Procedure: Under 400× magnification, identify the individual stages of mitosis and draw them.
Drawing:

9. Cell Cycle – Meiosis

Meiosis produces haploid gametes (n) from diploid cells (2n) in the gonads. It is the basis of sexual reproduction and ensures genetic diversity through recombination and the random segregation of chromosomes during two consecutive divisions.

Reductional division (Meiosis I): Prophase I proceeds through five sub-stages: leptotene, zygotene (synapsis, bivalents), pachytene (crossing-over), diplotene (chiasmata), and diakinesis. In Metaphase I, bivalents align at the equatorial plane, and microtubules attach to the kinetochores of homologous chromosomes. During Anaphase I, homologous chromosomes move to opposite poles of the cell. Telophase I and cytokinesis result in two cells, each with chromosomes composed of two chromatids. An interphase may follow, but DNA replication does not occur.

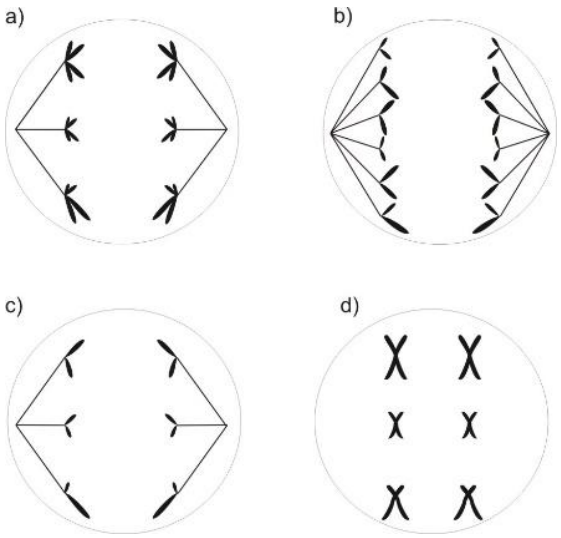
Equational division (Meiosis II): Prophase II: The spindle apparatus reforms and the nuclear envelope breaks down. Metaphase II: Chromosomes align at the equatorial plane. Anaphase II: Sister chromatids separate and move toward opposite poles. Telophase II and cytokinesis produce four genetically distinct haploid cells.

Meiosis in males and females differs in location, timing from zygote formation, duration, the number of haploid cells produced, and arrest points. The process is hormonally regulated.

Questions and Tasks

1. Compare the final products of meiosis and mitosis.

2. In the image, cells are obtained from the same individual:
   a) Identify the phases of mitotic or meiotic division.
   b) What is the diploid chromosome number?
   c) If these are mammalian cells, what is the sex of the individual?



3. Explain the significance of the prolonged prophase I of meiotic division and why meiosis contributes significantly to genetic variability, whereas mitosis does not.

4. Explain the following terms:

a) Synapsis
b) Bivalents
c) Chiasmata
d) Crossing-over
e) Sister chromatids
f) Tetrads
g) Dyads
h) Monads

5. If a certain organism has a diploid chromosome number of 2n = 16 in the oocyte:
   a) How many tetrads are present in prophase I?
   b) How many dyads are present in prophase II?
   c) How many monads migrate to each pole during anaphase II?

6. What are the differences in gametogenesis in males and females? Complete the table. Can you explain the significance of their differences?

	Man	Woman
The beginning of gametogenesis		
Duration of gametogenesis		
Number of mitoses during gamete formation		
The number of gametes per meiosis		
Number of gametes		

7. How many chromosomes and chromatids are present at these stages of meiosis in humans:
   a) in spermatogonia before S phase?
   b) in primary spermatocytes in metaphase I?
   c) in secondary spermatocytes in metaphase?
   d) in spermatids?
   e) in spermatozoa?

8. How many sperm are produced:
   a) from one spermatogonia?
   b) from one primary spermatocyte?
   c) from one secondary spermatocyte?
   d) from one spermatid?

9. How many human eggs are produced:
   a) from one oogonia?
   b) from an ootid?
   c) from one primary oocyte?
   d) from a polar body?

10. What is the main cause of numerical chromosome abnormalities in humans? Explain the consequences of the long duration of the dictyotenic stage associated with increasing maternal age.

11. How many chromosomes does a mule have, which comes from a cross between a horse (64 chromosomes) and a donkey (62 chromosomes)?

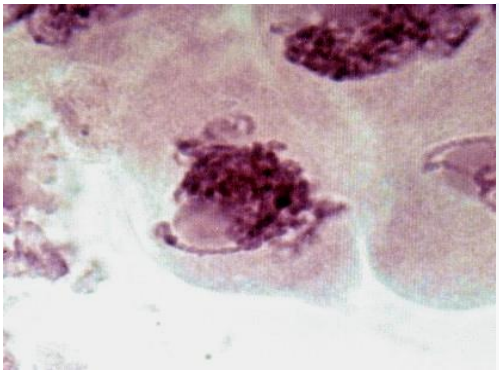
12. There are 40 chromosomes in the somatic cells of mice.
    a) How many chromosomes did a mouse inherit from its father?
    b) How many autosomes are present in mouse gametes?
    c) How many sex chromosomes are in a mouse egg?
    d) How many autosomes are in the somatic cells of a female?

13. During oogenesis in an organism with a haploid number of n = 6, nondisjunction occurred during the second meiotic division, with 1 undivided dyad entering the egg?
    a) How many chromosomes are in a mature egg?
    b) How many chromosomes are in a secondary pole cell?
    c) What is formed after fertilization of the above egg with a normal sperm?
    d) How do we call this condition?

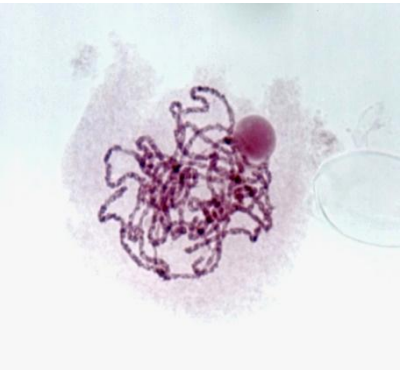
14. A diploid cell contains three pairs of chromosomes labeled A, B, and C. Each pair consists of one maternal and one paternal chromosome (A^m, A^p, etc.).
    a) Which chromatid (chromosome) combinations are possible in metaphase of mitosis? Which combinations are at each pole of the cell at the end of anaphase?
    b) Which chromatid (chromosome) combinations are possible at the end of prophase I (without crossing-over)?
    c) Draw all possible chromatid combinations at the end of anaphase II.
    d) If nondisjunction of the chromatids of chromosome C occurred during meiosis II, what would be the consequences and what would be produced?

Task 1: Observation of meiosis stages on a permanent preparation of mouse testes.

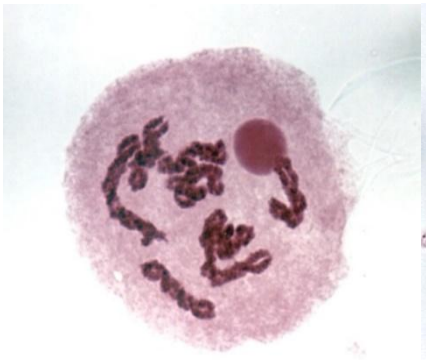
Materials and Equipment: Permanent preparations of mouse testes stained with aceto-orcein.
Procedure: Using the permanent preparations under 400× magnification, identify the individual stages of the first meiotic division (Fig. 9-1) and record them in the protocol by drawing. Differentiation between the leptotene and zygotene stages is usually difficult. Locate a cell in the diakinesis stage and determine the number of chromosomes or bivalents. In the diplotene stage, observe the presence of chiasmata on the chromosomes.



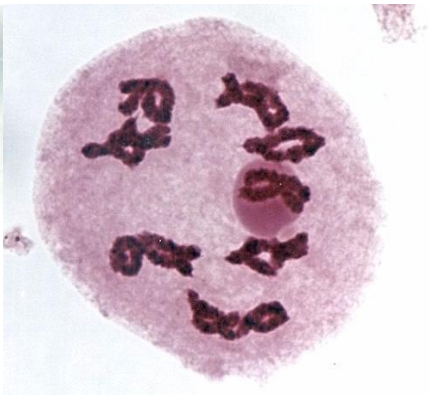
a) leptotene or zygotene stage



b) pachytene stage



c) diplotene stage



d) diakinesis

Figure 9-1. Individual stages of prophase I of meiotic division.

Drawing:

a) leptotene or zygotene stage

b) pachytene stage

c) diplotene stage

d) diakinesis

Task 2: Observation of individual stages of meiosis on a smear of lily anthers (*Lilium sp.*)

Materials and Equipment: Permanent preparations of lily anthers.
Procedure:
Using the preparations under 400× magnification, identify the individual stages of the first and second meiotic divisions (Fig. 9-2) and record them in the protocol by drawing.

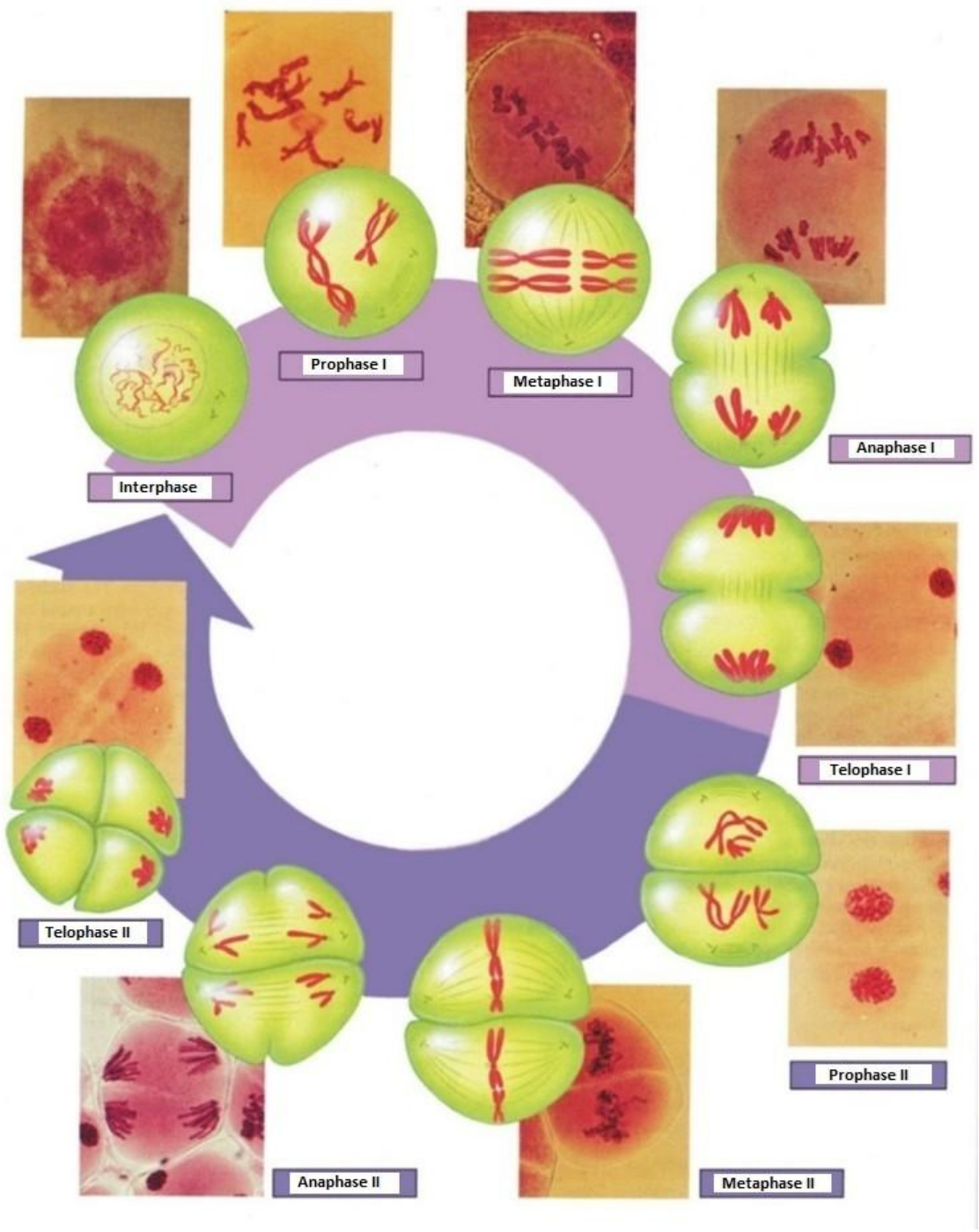


Figure 9-2. Individual stages of meiotic division I and II.

Drawing:

Prophase I

Metaphase I

Anaphase I

Telophase I

Prophase II

Metaphase II

Anaphase II

Telophase II

10. Microscopic Measurement Methods – Measuring the Size of Microscopic Objects

10.1. Measuring the size of microscopic objects with a micrometric device

Using a microscope, it is possible to measure the size of an object in the plane perpendicular to the optical axis, i.e., in the horizontal plane (the length and width of the object), as well as along the optical axis, i.e., in the vertical plane (the thickness of the object).

For measurements in the horizontal plane, an **ocular micrometer** is used — a circular glass disk with divisions of varying values. The value of one division in micrometers for a given magnification is determined using a stage micrometer — a calibrated slide with a 1 mm scale divided into 100 divisions, each corresponding to 10 micrometers. With this, the actual value of one division on the ocular micrometer can be determined. This value represents the micrometric coefficient of the corresponding objective and tube length. After calibration with the stage micrometer, the preparation is inserted, and the length and width of the object are measured in ocular micrometer divisions. These values are then multiplied by the micrometric coefficient to obtain the actual dimensions of the object in micrometers

The **ocular micrometer** is a special eyepiece that contains, in the focal plane of the front lens, a glass plate with a scale from 0 to 8 and a movable glass plate with an index mark, i.e., a double line and a horizontal cross. The movement of the index mark is controlled by a precise micrometer screw (divided into 100 increments), allowing the reading of tenths of screw rotations. A displacement of one division on the 0–8 scale corresponds to one full rotation of the micrometer screw. Since the exact value of one division at a given magnification is not known, it must be determined by comparison with the scale of a stage micrometer with precise calibration.

There are also **measuring grids** that can be inserted directly into the eyepiece. These grids may have different divisions in millimeters. In order to achieve sharpness with instruments equipped with measuring grids, two adjustable eyepieces are required. The eyepiece is adjusted precisely to the observer's eyes until both the grid and the specimen are clearly visible (without parallax). For measuring lengths, for example, grids with different divisions such as 12 mm : 120 or 5 mm : 100 are used. The grid itself is magnified only by the eyepiece. However, the total magnification of the specimen depends on the magnification of the objective, the eyepiece, and any intermediate tube used, and it changes whenever any of these factors is altered. When calibrating with a stage micrometer, the value of each grid interval is defined relative to the magnification of the specimen. The actual dimensions of the specimen can only be determined once the calibration constant has been established. Calibration should be performed after every change of magnification, especially when high measurement accuracy is required.

The **objective micrometer** has the form of a slide on which one millimeter is engraved into 100 equal divisions (one division = 10 μm). Using this scale, we can determine the actual value of the eyepiece micrometer scale division for a given magnification, the so-called **micrometric coefficient**.

10.2. Calibration using a measuring grid

Place the objective micrometer on the microscope stage and adjust the focus. Select the magnification with which you want to measure later. Set the objective micrometer (X) parallel

to the eyepiece grid (Y) so that they do not have parallax. Align the common point of both scales (lines opposite each other). According to the example given 0 and 10, calculate how many mm of the objective micrometer (X) corresponds to a certain number of divisions on the eyepiece grid (Y). Using the calibration formula, calculate the calibration constant = micrometric coefficient (the value of one division on the eyepiece grid) and write it down.

Calibration formula:
X objective micrometer - number of mm (")
Y eyepiece raster - number of divisions
Calibration constant mm (") = X/Y mm (")

Measurement of an object: Replace the objective micrometer with the specimen on which we want to measure the cell. Focus on the cell and count the number of divisions of the object we want to measure. Multiply the result by the calibration constant and thus find the actual width (length) of the given cell.

Example:

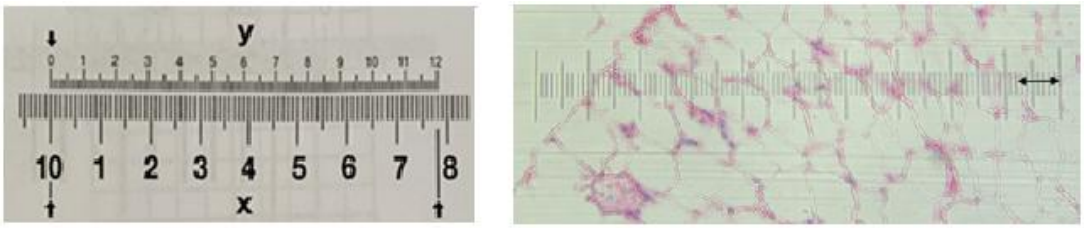


Figure 10-1. Measuring grid 12 mm: 120 and objective micrometer with 0.1 mm division, Y – scale of eyepiece micrometer and X – scale of objective micrometer and object measurement.

Measuring grid 12 mm: 120 and objective micrometer with 0.1 mm graduation (Fig. 10-1)**:**
Calibration: 7.8 mm on the objective micrometer (X) corresponds to 120 divisions on the eyepiece grid (Y).
Calculating the calibration constant: 7,8 mm/120 mm = 0,065 mm
Measurement: interval on the eyepiece grid: 7 divisions
Result of measured length: 7 divisions x 0.065 mm (calibration constant) = 0,455 mm.

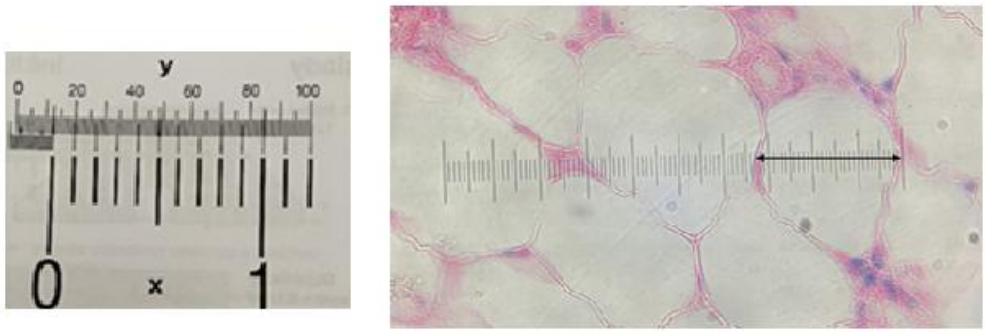


Figure 10-2. Measuring grid 5 mm: 100 and objective micrometer with 0.1 mm division, Y – scale of the eyepiece micrometer and X – scale of the objective micrometer and measurement of the object.

Measuring grid 5 mm:100 and objective micrometer with 0.1 mm graduation (Fig. 10-2)**:** Calibration: 1.37 mm on the objective micrometer (X) corresponds to 100 divisions on the eyepiece grid (Y).
Calculating the calibration constant: 1.37 mm/100 mm = 0.0137 mm

Measurement: interval on the eyepiece grid: 32 divisions
Result of measured length: 32 divisions x 0.0137 mm (calibration constant) = 0,4384 mm

10.3. Measurement of the thickness of microscopic objects

Each objective has a certain penetrating ability, which decreases with increasing numerical aperture. Objectives with a high numerical aperture display a very thin layer – the specimen plane. Therefore, they are used to measure the height of microscopic objects. By focusing, move the plane of focus vertically – as if slicing the object into several optical planes. In this way, we obtain information about structures at different depths of the object.

The thickness of the object is measured with the micrometer screw of the microscope by focusing on the upper surface of the object and its center with the displacement read on the micrometer screw. The head of the micrometer screw has a scale on the circumference, with one scale division representing a value of 2.5 μm (Olympus microscope). When measuring the thickness of the object, we first focus on the upper optical plane (we see the center of the object sharply and the edges are out of focus) and with this setting we note the number of divisions on the micrometer screw scale (e.g. 20). Then we focus on the middle optical plane of the measured object (we see the edges of the object sharply and the center is out of focus) by turning the micrometer screw. We find the displacement in the number of divisions on the micrometer screw scale (e.g. 47). We subtract the values: 47 – 20 = 27 and multiply this result by the value of one division of the micrometer screw (27 x 2.5 = 67.5 μm) and by two more (67.5 x 2 = 130 μm) to find the thickness of the entire object. This result gives the height, or thickness, of the object in micrometers. The measurement is not absolutely accurate, because sometimes it is difficult to determine the exact upper and middle surface of the section or object.

Questions and Tasks

Task 1: Calculation of the calibration constant for lenses 10x and 40x

According to the instructions in chapter 10.2, we calculate the calibration constant for the 10x and 40x magnifying objective.

Calibration constant for the objective **10x** **Calibration constant** for the objective **40x**

Task 2. Measurement of the fat cell size with a micrometer device

Materials and Equipment: Permanent preparation of adipose tissue, microscope with ocular and objective micrometers, and other standard microscopic supplies.
Procedure: Measure the size (length and width) of 10 fat cells at 100× and 400× magnification.
Results: Record the micrometric coefficient for the 10× and 40× objectives, and then enter the measured values into a table.

Micrometric coefficient for the objective **10x:** **F_{10x} =**

Length	**Cell size in tiles (D)**	**Cell size in µm ($D \times F_{10x}$)**	**Width**	**Cell size in tiles (D)**	**Cell size in µm ($D \times F_{10x}$)**
1.			**1.**		
2.			**2.**		
3.			**3.**		
4			**4.**		
5.			**5.**		
6.			**6.**		
7.			**7.**		
8.			**8.**		
9.			**9.**		
10.			**10.**		
Average:			**Average:**		

Micrometric coefficient for the objective **40x:** **F_{40x} =**

Lenght	**Cell size in tiles (D)**	**Cell size in µm ($D \times F_{40x}$)**	**Width**	**Cell size in tiles (D)**	**Cell size in µm ($D \times F_{40x}$)**
1.			**1.**		
2.			**2.**		
3.			**3.**		
4			**4.**		
5.			**5.**		
6.			**6.**		
7.			**7.**		
8.			**8.**		
9.			**9.**		
10.			**10.**		
Average:			**Average:**		

Task 3: Measurement of the blood cell size with a micrometer device

Materials and Equipment: permanent human blood smear preparation, microscope and microscopy supplies, ocular and objective micrometer
Procedure: Measure the size of 10 erythrocytes and 10 different types of leukocytes at 400x magnification. Compare them with the given average size of each type of blood cell. The measurement is performed on the peripheral parts of the preparation, where the blood cells are not very dense.
Results: Write down the values of the micrometric coefficient for the 40x magnifying lens and then write the measurement result in the table.

Micrometric coefficient for the objective **40x:** **F_{40x} =**

Erythrocytes	**Cell size in tiles (D)**	**Cell size in µm (D x F_{10x})**	**Leukocytes**	**Cell size in tiles (D)**	**Cell size in µm (D x F_{10x})**
1.			**1.**		
2.			**2.**		
3.			**3.**		
4			**4.**		
5.			**5.**		
6.			**6.**		
7.			**7.**		
8.			**8.**		
9.			**9.**		
10.			**10.**		
Average:			**Average:**		

Task 4. Measurement of the epithelial cell size with a micrometer device

Materials and Equipment: native preparation (tongue impression), methylene blue, microscope and microscopy supplies, eyepiece and objective micrometer
Procedure: Prepare a native tongue impression preparation. We will measure the length and width of at least 5 cells.
Result: Write down the values of the micrometric coefficients for the 10x and 40x magnifying objective lenses and write the measurement result in the table.

Micrometric coefficient for the objective **10x:** **F_{10x} =**

Micrometric coefficient for the objective **40x:** **F_{40x} =**

Length	Cell size in tiles (D)	Cell size in µm ($D \times F_{10x}$)	Cell size in µm ($D \times F_{40x}$)	Width	Cell size in tiles (D)	Cell size in µm ($D \times F_{10x}$)	Cell size in µm ($D \times F_{40x}$)
1.				**1.**			
2.				**2.**			
3.				**3.**			
4				**4.**			
5.				**5.**			
Average:				**Average:**			

Task 5: Measurement of the width of a human hair with a micrometer device

Materials and Equipment: human hair, scissors, water, slide and coverslip, microscope and microscopy supplies
Procedure: Place a drop of water on a glass slide, add a cut hair about 5 mm long, and cover with a coverslip. At 100x magnification, find a hair and place it in the center of the field of view. Rotate the objective to 400x magnification, place the object in the center of the field of view, and adjust the position of the eyepiece micrometer so that you can measure the thickness of the hair (Fig. 10-3). Measure the thickness of the hair at five locations. From the five hair thickness measurements, calculate the average value and write it in the protocol.
Results: The average thickness of a hair ranges from 42 to 95 micrometers. Women's hair is thicker and stronger than men's hair.

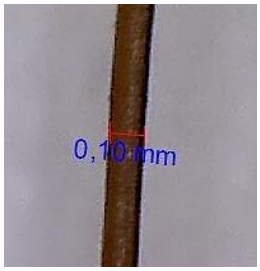


Figure 10-3. How to measure human hair.

Micrometric coefficient for the objective 40x: **F_{40x} =**

Hair width	Cell size in tiles (D)	Cell size in µm ($D \times F_{40x}$)
1.		
2.		
3.		
4		
5.		
Average:		

11. Microscopic Measurement Methods – Microscopic Objects Counting

Microscopic objects (e.g. blood cells, sperm, dissociated cells) are counted using a microscope in counting chambers or using special counting devices.

Counting devices are based on the principle of measuring light scattering caused by, for example, blood cells, or on the principle of measuring changes in conductivity (so-called Counter) or other physicochemical properties (flow cytometer).

Counting chambers in the form of a glass slide are used to determine the number of objects in a precise volume of suspension. A suspension of objects diluted in a certain ratio is added to a chamber of known height with a Pasteur pipette, the bottom of which is divided by a network of lines into fields of known dimensions. By counting the objects over several fields, the average number of particles in a certain volume is determined.

11.1 Bürker counting chamber

The most commonly used chamber method is counting microscopic objects in a **Bűrker counting chamber**. Blood elements can be used as microscopic objects. Determining the number of blood elements, especially in hematology, is a basic, undemanding examination for both patients and healthy individuals as part of preventive examinations. The number of cells in tissue culture or bone marrow is determined in a similar way. The number of cells can also be determined in other fields, such as in microbiology in samples after cultivation, when the number of grown colonies is counted. We can also count algae, yeasts and other unicellular organisms, when an aqueous suspension of the culture is dropped directly into the chamber and these organisms can be counted directly without staining.

Bürker counting chamber (Fig. 11-1) consists of ground glass about 0.5 cm thick, about 7 cm long and about 3 cm wide. Its upper surface is divided in the middle by two thick transverse grooves and one short longitudinal groove into two large fields lying opposite each other. These two fields are at the same time 0.1 mm lower than the surroundings. By covering both fields with a ground coverslip, a special space is created in which blood cells are counted. It is 0.1 mm high and a very precise grid of known dimensions is drawn on its lower surface with fine scratches. Using this grid and the height of the chamber, a small microscopic space is determined in which blood cells in diluted blood can be counted.

Figure 11-1. Bűrker counting chamber

Bürker chamber counting grid (Fig. 11-2) is divided by triple lines into **9 large square fields** and double lines into **144 medium and 169 small squares**. The large square

fields have an area of 1 mm2 (side length 1 mm), the medium squares have an area of 1/25 mm2 (side length 0.2 mm) and the small squares 1/400 mm2 (side length 0.05 mm) (Fig. 11-3). Between the double lines, rectangles are formed, the area of which is equal to the area of 4 small squares, i.e. 1/100 mm2. If we take into account the height of the chamber (0.1 mm), there is a space with a volume of 1/250 µl above the medium square, 1/4000 µl above the small square and 1/1000 µl above the rectangle (Table 11-1).

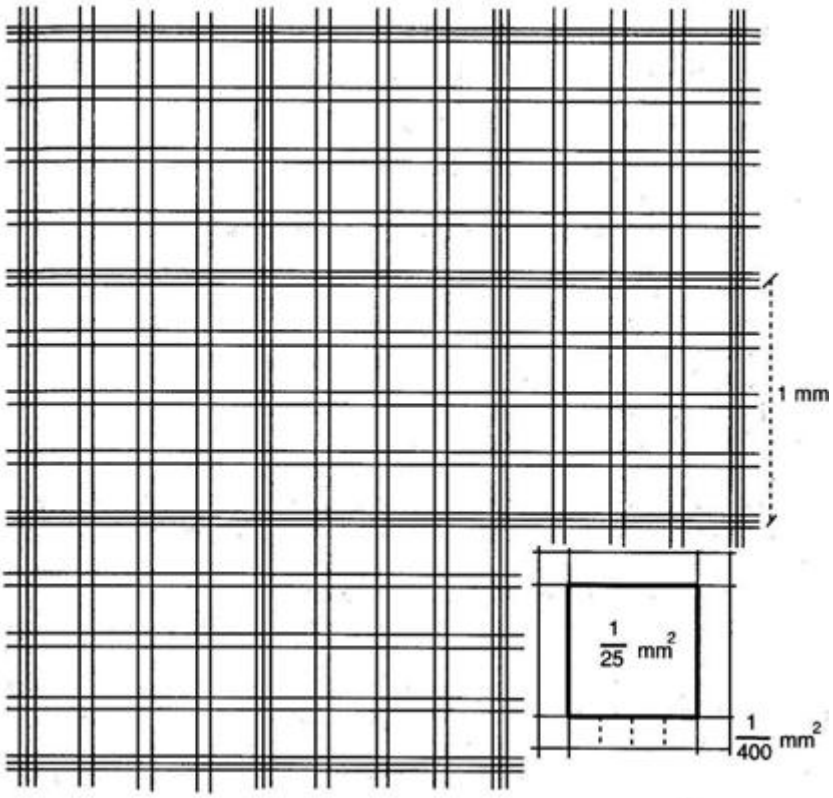


Figure 11-2. Bűrker chamber counting grid.

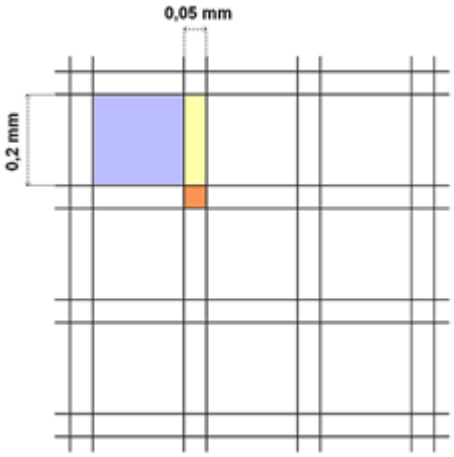


Figure 11-3. Scheme of Bűrker chamber.

Table 11-1. Dimensions of Bűrker chamber

	DIMENSIONS (mm)	AREA (mm^2)	HIGH (mm)	VOLUME (mm^3)
Medium square	**0.2 x 0.2**	**0.04 = 1/25**	**0.1 = 1/10**	**0.004 = 1/250**
Rectangle	**0.05 x 0.2**	**0.01 = 1/100**	**0.1 = 1/10**	**0.001 = 1/1000**
Small square	**0.05 x 0.05**	**0.0025 = 1/400**	**0.1 = 1/10**	**0.00025 = 1/4000**

11.2 Blood elements counting

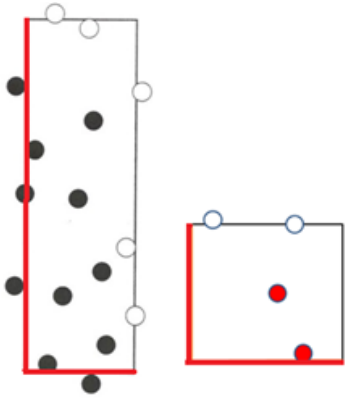
The number of blood cells in the blood is very large, so they cannot be counted in native blood. The blood is diluted with a suitable diluent. In addition to dilution, the solution also highlights the type of blood cells that we intend to count and removes other blood elements. Dilution is necessary both when counting in chambers and when counting with blood cell counters.

Hayem's solution is most often used as a diluent for **counting erythrocytes**. It highlights the erythrocytes and disrupts the other elements, so they do not make counting difficult. Hayem's solution must be used with caution, as it also contains a strong poison - mercuric chloride. In older solutions, erythrocytes may stick together or clump, which can make counting difficult.

In **leukocyte counting, Türk's solution** is generally used as a diluent. The acetic acid present in the solution disrupts erythrocytes and platelets, but does not disrupt leukocytes. Methyl violet stains the nuclei of leukocytes, so that leukocytes that are then in the field of view of the microscope are prominent and can be easily counted.

Blood cells are counted on a certain selected area of known dimensions, at a stable height, i.e. in a certain selected volume..

For counting blood cells (erythrocytes and leukocytes), the rule according to Bűrker applies: we count all blood cells that lie inside the rectangle or square and also those that touch the two selected sides from the inside and outside. We choose either the lower and left or the upper and right side. We do not count the blood cells on the other two opposite sides, even if they touch them from the inside (Fig. 11-4).



Figrue 11-4. Diagram of the Bürker chamber and the method of counting blood cells (in this particular case, we count 11 blood cells in the rectangle, 2 blood cells in the square).

When counting blood cells, take into account the entire area of the counting grid and it is necessary to include the edge, corner and central squares in the counting plan. They should not be counted exclusively in the edge, corner or explicitly only in the central parts of the grid.

A diagonal procedure through the grid is recommended, but counting can also be done in a transverse direction through the central part of the grid, from left to right or from top to bottom (Fig. 11-5). It is necessary to observe the number of rectangles or squares when counting, their number should not be simplified, because any simplification and adaptation of the prescribed working procedures may be the cause of inaccurate results.

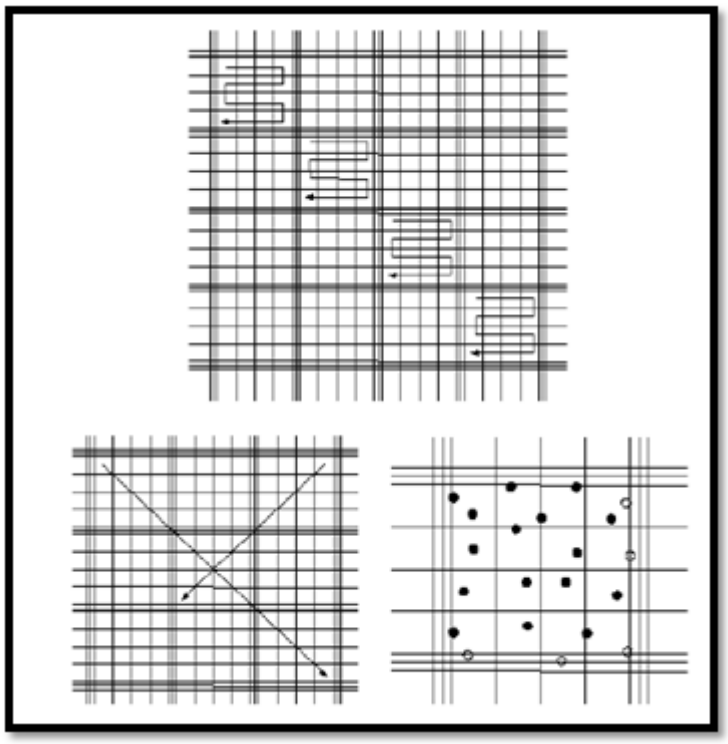


Figure 11-5. Methods of cells counting.

Before counting, we make sure that the chamber is dry, clean and that the ground cover glass precisely and firmly covers the counting fields. For this purpose, counting chambers are equipped with two pen catches of the cover glass.

After counting blood elements, we must disassemble the Bűrker chamber. Using the two pen catches, we release the cover glass and rinse both the cover glass and the counting grids of the chamber in running water or distilled water. We carefully and gently wipe the chamber dry with soft clean gauze and then polish it with gauze moistened in benzine. Finally, we wipe everything again with dry gauze. Care must be taken not to scratch the grid of the chamber when wiping. We reassemble the Bűrker chamber and put it away.

Questions and Tasks

Task 1: Erythrocyte counting in a Bürker counting chamber

Materials and Equipment: venous blood with EDTA (EDTA - ethylenediaminetetraacetic acid) or capillary blood, Bürker counting chamber, blood dilution bottle, Hayem's dilution solution, 25 µl and 5 ml pipettes, Pasteur pipette, microscopy supplies
Procedure: Pipette exactly 4975 µl of Hayem's diluent into the blood dilution bottle and add 25 µl of blood (this will achieve a 200-fold dilution of the blood). Rinse the pipette with the blood several times with the diluent. Then close the bottle with a rubber stopper and mix thoroughly to create a uniform deposit of erythrocytes. Then transfer a drop of suspension from the deposit to the Bűrker counting chamber with a Pasteur pipette.
Fill the chamber with diluted blood only just before counting. Before that, make sure that it is dry, clean and that the ground cover slide precisely and firmly covers the counting fields. After the counting space has been properly filled, proceed to the actual counting of blood cells.
Red blood cells are counted at 100x magnification in 20 rectangles. Bűrker's rule applies. Make sure that the number of 20 rectangles includes rectangles from the edge and central parts of the counting grid approximately equally. The count can be done diagonally across the grid, which determines the number of erythrocytes in 18 rectangles and adds the number of erythrocytes from 2 random rectangles (Fig. 11-6) or also transversely across the central part of the grid. The counted erythrocytes are recorded in a table.

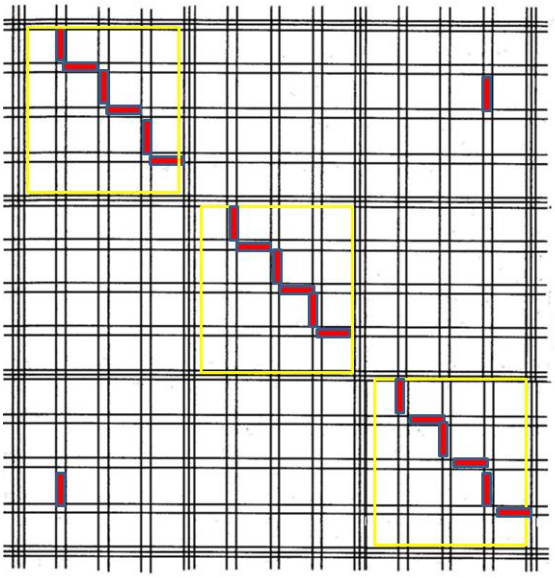


Figure 11-6. Method of counting red blood cells in 20 rectangles.

Calculation: Taking into account the height of the counting chamber, **count erythrocytes in 1/50 µl (volume of 20 rectangles). To find out the actual number of erythrocytes in 1 µl of the examined blood, you have to multiply the determined number by 50 times and, due to the dilution of the blood, by another 200 times. In this case, multiply the sum of the counted blood cells by 10,000.**
Example: if counted 460 erythrocytes in 20 rectangles, the examined person has 460 x 10,000 = 4,600,000 erythrocytes in 1 µl of blood (4.6 x 106/ µl, Er), or 4.60 x 1012 in 1 liter of blood.

Red blood cell count:

Rectangle	Red blood cell count	Rectangle	Red blood cell count
1.		**11.**	
2.		**12.**	
3.		**13.**	
4.		**14.**	
5.		**15.**	
6.		**16.**	
7.		**17.**	
8.		**18.**	
9.		**19.**	
10.		**20.**	
Total erythrocyte count:			

Calculation of the number of erythrocytes:

Number of erythrocytes in 1 μl of blood tested:

Number of erythrocytes in 1 l of blood tested:

The normal number of erythrocytes in the blood of an adult is 4.3 - 5.3 x 1012/l in men, 3.8 - 4.7 x 1012/l in women. In some pathological conditions, the number of erythrocytes increases (polyglobulia, polycythemia) or, conversely, decreases (anemia). The ability of blood to carry oxygen depends on the number of erythrocytes and thus the hemoglobin content.

Task 2: Leukocyte counting in a Bűrker counting chamber

Materials and Equipment: venous blood with EDTA or capillary blood, Bürker counting chamber, Türk's solution diluent, 25 μl and 0.5 ml pipettes, blood dilution bottle, microscopy supplies

Procedure: Pipette 475 μl of Türk's solution into a blood dilution bottle, to which add 25 μl of blood (this will achieve a 20-fold dilution of the blood). Rinse the micropipette with which we added the blood several times in the bottle with the dilution solution. Then close the bottle with a rubber stopper and mix the contents well to create a uniform influx of leukocytes. Transfer a drop of the suspension into the counting space of the chamber with a Pasteur pipette.

To obtain a reliable and sufficiently accurate data on the number of leukocytes, **we count them at 100x magnification in 50 central squares of the counting grid. The rule according to Bűrker applies.**

A diagonal procedure is recommended, which determines the number of leukocytes in the 48 central squares and adds to them the number of 2 random squares placed in the middle (Fig. 11-7). It can also be counted transversely across the center of the grid, from left to right or from top to bottom. The leukocyte counts are recorded in a table.

Calculation: Above each middle square there is a space with a volume of 1/250 μl. In 50 middle squares the volume is 1/5 μl. If we want to find out how many leukocytes are in 1 μl of blood of the examined person, we must multiply the number of leukocytes from the 50 middle squares by 5 times and, due to the dilution of the blood (1:20), by another 20 times. In this case, we **multiply the sum of the counted blood cells by 100**.

Example: if counted 65 leukocytes in 50 mean squares, there are 65 x 100 = 6500 leukocytes in 1 μl of examined blood, or 6.5 x 109 in 1 liter of blood.

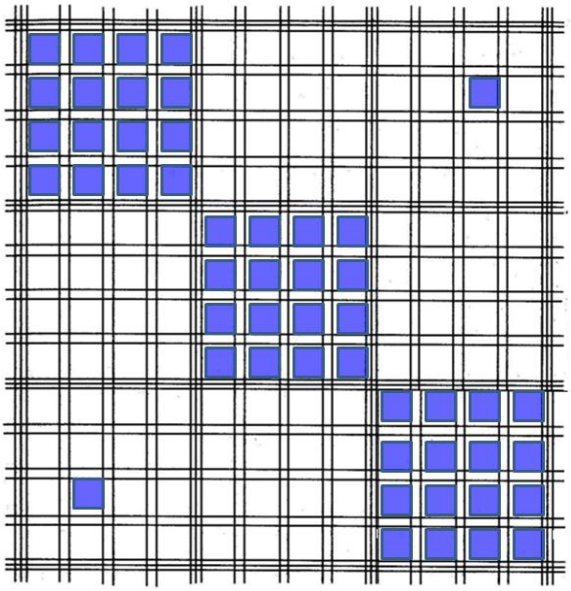


Figure 11-7. Method of counting white blood cells in 50 medium squares.

White blood cells counting:

Rectangle	Leukocyte count	Rectangle	Leukocyte count	Rectangle	Leukocyte count	Rectangle	Leukocyte count	Rectangle	Leukocyte count
1.		**11.**		**21.**		**31.**		**41.**	
2.		**12.**		**22.**		**32.**		**42.**	
3.		**13.**		**23.**		**33.**		**43.**	
4.		**14.**		**24.**		**34.**		**44.**	
5.		**15.**		**25.**		**35.**		**45.**	
6.		**16.**		**26.**		**36.**		**46.**	
7.		**17.**		**27.**		**37.**		**47.**	
8.		**18.**		**28.**		**38.**		**48.**	
9.		**19.**		**29.**		**39.**		**49.**	
10.		**20.**		**30.**		**40.**		**50.**	
Total leukocyte count:									

Calculation of the number of leukocytes:

Number of leukocytes in 1 μl of blood tested:

Number of leukocytes in 1 l of blood tested:

The normal leukocyte count in an adult ranges between 4.0 – 10.5 x 109/l. Higher values indicate leukocytosis and lower values indicate leukopenia. The leukocyte count is one of the important indicators of the body's defenses and at the same time an indicator of the functional state of the active bone marrow.

12. Self-organization Processes - Natural and Artificial Coacervates

The spontaneous formation of ordered structures without external control plays an important role in the formation of cells, organelles, as well as in the early stages of life. Spontaneous phase separation in an aqueous environment produces coacervates, droplets of a colloidal mixture of macromolecules. Other mechanisms for the formation of coacervates are electrostatic interactions between the charges of macromolecules, changes in pH, concentration, ionic strength and temperature.

Natural coacervates are formed in living systems. In eukaryotic cells, they are e.g. nucleoli, stress granules, P-bodies. They have various functions, such as controlling gene expression, storing RNA and proteins, regulating the response to stress conditions.

Artificial coacervates are formed in vitro as models of pre-cellular structures, protocells. They were first used as a model for the origin of life by A. Oparin in 1924 (primordial soup). A similar conclusion was reached in 1929-1930 by the British biologist J. B. S. Haldane. They have a semipermeable surface, concentrate molecules inside the droplet and allow enzyme reactions to take place inside. Currently, they are used not only in the study of abiogenesis, but also as reactors, sensors or drug carriers.

Questions and Tasks

Task 1: The emergence of molecular structures through self-organization

Materials and Equipment: lecithin, 0.1% aqueous KOH solution, pipette, dissection needle, microscopy supplies

Procedure: Using the tip of a dissecting needle, transfer a small amount (about 1/2 mm3) of lecithin to a slide and spread it a little on the glass. Cover with a coverslip, pressing it slightly onto the material so that it sticks. At low magnification, look for the edge of the lecithin material. Observe at high magnification (40x objective). The material is amorphous. Its edge is smooth, refractive and dark. Add a drop of aqueous potassium hydroxide solution to the edge of the coverslip. At the same time, observe the edge of the material.

Results: When amorphous lecithin comes into contact with water, it forms tubes that bend and intertwine in various ways. They are called myelin structures. Lecithins are phospholipids, the chain molecules of which contain hydrophobic groups (terminal - CH3 groups of fatty acids) at one end (apolar) and hydrophilic groups (glycerol, phosphoric acid and choline) at the other end (polar). The molecules are therefore amphiphilic. They exhibit surface activity when in contact with a polar liquid - water: they orient themselves at the phase boundary. They reduce the surface tension of water. When in contact with water, they assemble into regular structures, since only the non-polar ends connect to each other or only the polar ends, which also connect to water. As a result, a parallel arrangement of lecithin molecules is formed into a relatively stable molecular bilayer (bimolecular film) about 5 nm thick. Other bilayers are added to it at a high speed in parallel, which are separated from each other by a layer of water molecules. A large number of such bilayers eventually form a tube-shaped supramolecular structure that we see under a microscope.

Drawing:

Task 2: Protein-lipid coacervate

Materials and Equipment: egg yolk, saline solution, dissection needle, pipette, slide and coverslip, microscopy supplies
Procedure: Place a drop of saline solution on a slide, add a drop of egg yolk to it. Mix thoroughly with a dissecting needle, cover with a coverslip and observe first at low and then at higher magnification.
Results: Yolk balls represent yolk coacervates with different structures and sizes. Draw the structure of yolk coacervates in the protocol at higher magnification. Notice that the structure of the coacervate is different from the structure of the surrounding medium. The nutrient yolk (deutoplasm) is a colloidal system containing many proteins and fats. Coacervate droplets in the shape of balls have a membrane on the surface, which forms the interface of two phases: the contents of the coacervate (which is more viscous and granular) and the surroundings (which is less viscous). The membrane is formed by phospholipid molecules arranged in layers. Coacervate droplets are sensitive to pressure, therefore the coverslip must not be pressed against the observed object. On the preparation, we will notice Brownian molecular motion of small particles, which is caused by collisions of water molecules.
Drawing:

Task 3: Protein-polysaccharide coacervate (artificial coacervate)

Materials and Equipment: test tubes, pipettes, water bath, test tube stand, microscopy supplies, 1% gelatin solution (pH 3.5), 1% gum arabic solution (pH 3.5), 5% acetic acid solution
Procedure: Provide the experiments in test tubes. Keep the gelatin and gum arabic solution in a water bath at a temperature of 40ºC. Both solutions are transparent. Pipette 2 ml of gelatin, 2 ml of gum arabic into the test tube and add a few drops of acetic acid (the test tube must not be shaken). A white cloud appears in the test tube. Prepare a native preparation from the contents of the test tube.
Results: Gum arabic and gelatin molecules aggregate in an acidic environment and form a droplet coacervate. Draw several coacervate structures (spheres) in the protocol.
Drawing:

Task 4: Dye uptake by coacervates

Materials and Equipment: artificial coacervate, slide and coverslip, pipette, 1% neutral red, microscopy supplies
Procedure: Add a small amount of 1% neutral red with a pipette on the slide with the drop containing coacervates, mix, cover with a coverslip and observe.
Results: Observe small droplets representing coacervates and at the same time selective absorption of the dye by the coacervates in the field of view of the microscope.
Drawing:

13. Expression of Genetic Information

It is the transcription of genetic information from DNA into RNA and, in the case of structural genes, the subsequent translation into protein. Transcription takes place in the nucleus, translation in the cytoplasm. Both processes occur in a sequence of specific steps. Gene expression is regulated at multiple levels: epigenetic, transcriptional, post-transcriptional, translational, and post-translational, and it also influences cell differentiation.

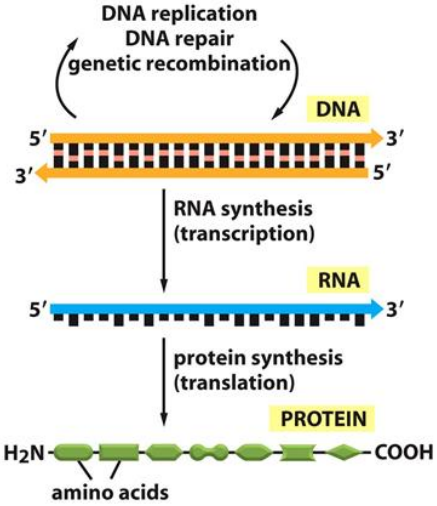


Figure 13-1. The flow of genetic information – from gene (DNA) to protein.

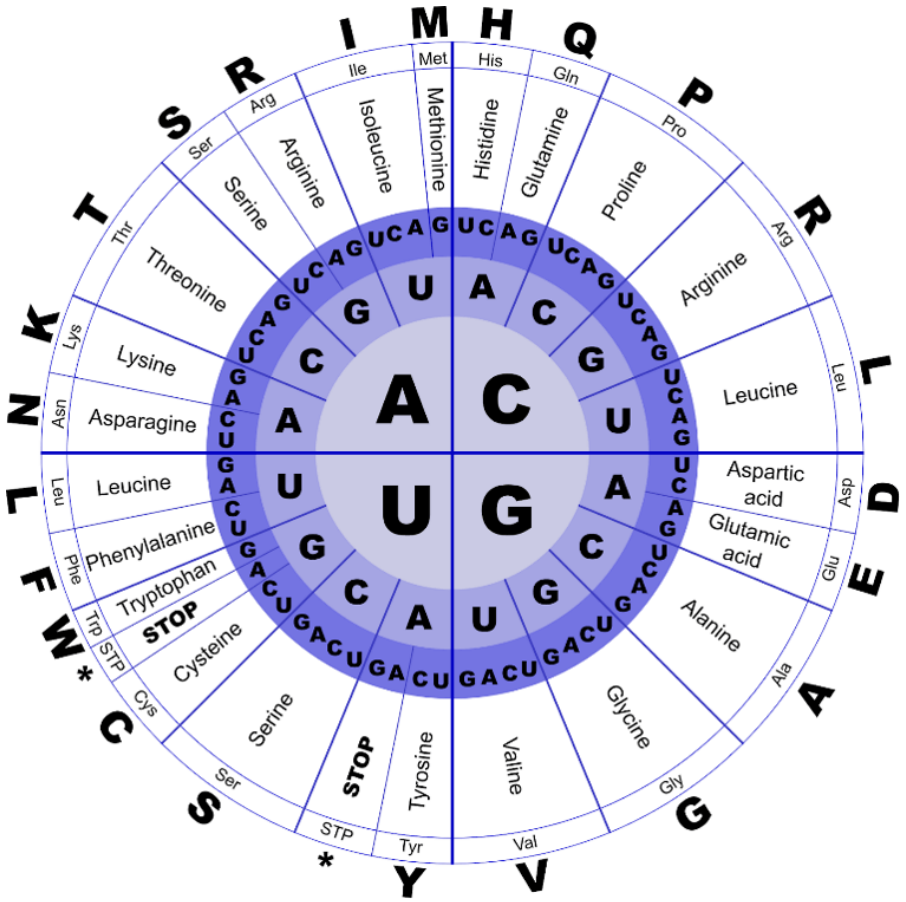


Figure 13-2. Circular diagram of the genetic code.

Questions and Tasks

1. Explain the central dogma of molecular biology (see Fig. 13-1).

2. Describe the basic structure of a gene in a eukaryotic cell and state its function.

3. Explain the main function of RNA polymerase and compare it with DNA polymerase.

4. Which sub-processes are characteristic of transcription?

5. What is a promoter and what is its function?

6. Characterize transcription factors and their role in the process of transcription.

7. Compare the primary transcript in a prokaryotic and a eukaryotic cell.

8. How do exons and introns differ?

9. Which post-transcriptional modifications do you know?

10. Which sub-processes are characteristic of translation?

11. Explain the significance of the codon and anticodon in the process of translation.

12. Describe the function and basic properties of the genetic code (see Fig. 13-2).

13. How are the beginning and end of translation defined?

14. Describe the structure and function of the ribosome. What is the significance of polyribosomes?

15. Which post-translational modifications do you know?

16. Compare gene expression in a prokaryotic and a eukaryotic cell.

17. During transcription from double-stranded DNA, the so-called antisense (noncoding) DNA strand serves as the template for RNA synthesis. The strand complementary to it in the given segment is called the sense (coding) strand. For the coding DNA strand with the following nucleotide sequence 5′ T T A A C G C G A T G G T A A 3′, construct the noncoding DNA strand, determine the sequence of bases in the mRNA, and the sequence of amino acids in the oligopeptide.

18. The mRNA molecule has the following composition of bases: 21% A, 33% U, 28% G, and 18% C. What is the base composition in the coding and noncoding DNA strands from which this mRNA was transcribed? Assume the gene contains no introns.

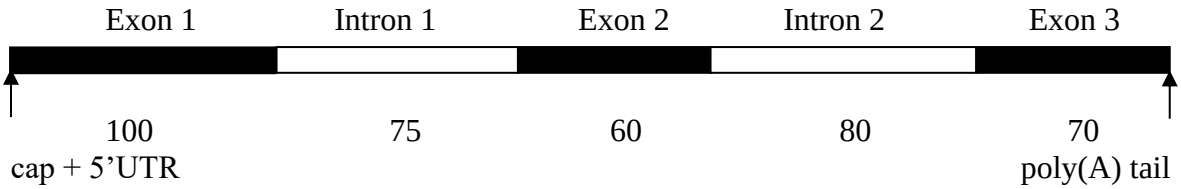
19. A part of a protein molecule contains a sequence of amino acids, which are listed together with the corresponding codons and anticodons in the table. Complete the triplets in the mRNA, tRNA, and in both strands of the DNA molecule.

	mRNA	tRNA	DNA strand	
			template	coding
amino acids	5′- 3′	3′- 5′	3′- 5′	5′- 3′
leucine	CUU			
tyrosine		AUA		
valine				GTA
cysteine			ACA	
arginine		GCA		
glycine	GGC			

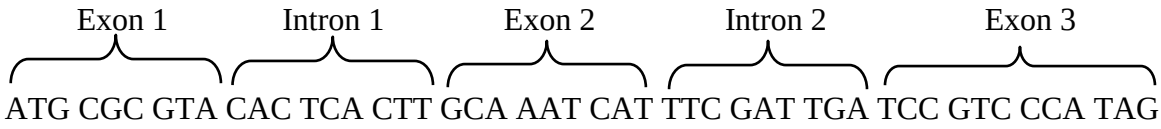
20. The following table contains incomplete data for triplets in mRNA, tRNA, both DNA strands, and the amino acids they encode. Complete it and indicate the correct polarity of the DNA strands.

DNA	A A A	_ _ _	_ _ A	T _ _	_ _ T
DNA	_ _ _	_ _ _	_ G _	_ _ _	G _ _
mRNA	_ U _	A _ _	A _ _	_ U _	_ U _
tRNA	_ _ _	_ _ U	_ _ _	_ _ A	_ _ _
amino acid		Arg			

21. The figure shows a transcribed DNA segment representing a typical eukaryotic structural gene. What will be the size (in bases) of the mature (processed) mRNA after all modifications? Assume that the poly(A) tail is 200 A's and that the cap together with the 5′UTR (UTR = UnTranslated Region) accounts for 100 bases.



22. The polypeptide chain is encoded by the following DNA segment (the given nucleotide sequence is within the nontemplate or coding strand).



a) Write the sequence of bases in the template (noncoding) DNA strand.
b) Write the sequence of bases in the mRNA.
c) How many amino acids does the given polypeptide chain have?
d) Write the sequence of amino acids in the polypeptide chain.

23. The bacterial DNA of *E. coli* consists of 4.2×10^6 nucleotide pairs, and approximately 1,500 nucleotide pairs correspond to one gene. How many genes does the complete *E. coli* DNA contain?

24. One nucleotide in a DNA molecule is approximately 0.34 nm long.
a) How long is the DNA segment encoding the insulin molecule in cattle, given that insulin contains 51 amino acids?
b) How long is the DNA segment encoding the hemoglobin molecule, given that hemoglobin contains 287 amino acids?

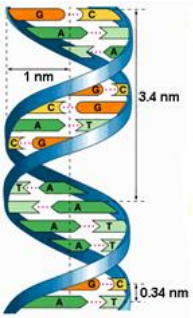


Figure 13-3. DNA structure diagram.

25. The nucleic acid of a bacteriophage has about 1.2×10^5 nucleotide pairs. How many proteins with a molecular weight of 48 kDa can it encode, assuming that an amino acid has an average mass of 120 Da?

26. The largest known protein to date is called titin (it is produced in muscle cells) and its molecular weight is 3 MDa. How long will it take a muscle cell to translate the mRNA for titin (assume that the average molecular weight of an amino acid is 120 Da and that in a eukaryotic cell an average of two amino acids are incorporated into the polypeptide chain per second)?

27. Based on the given data, calculate the average number of amino acids in each of the 13 proteins encoded within the 16.61-kilobase-long mitochondrial DNA. Of the total number of bases, about 587 bases do not code for either proteins or RNA molecules. Two rRNA molecules are encoded by 950 and 1,560 bases, and the average length of each of the 22 tRNAs is approximately 70 bases. The remainder serves to encode proteins.

28. In an imaginary world, the genetic code is based only on pairs of nucleotides (with the four known RNA nucleotides - A, U, C, G). How many amino acids could it specify? In another imaginary world, a triplet genetic code is used, but the order of nucleotides does not matter; it is sufficient to know which kinds of nucleotides make up the triplet to determine the correct amino acid. How many different amino acids could such a genetic code specify?

29. If codons were not triplets but consisted of four bases, how many codons could exist in the genetic code?

30. According to the base-pairing rule, complete the table with the missing complementary nucleotide sequences. Underline the first initiation codon. Then count how many amino acids the peptide encoded by the mRNA nucleotide sequence would contain, starting from the initiation codon.

+DNA	5′GAAACAGCTATGACC	TAATGT
-DNA	3′CTTTG TACCTTTCGCCCGTCACT	ATTACA
mRNA	5′GAAACA	GCGCAACGCAAUUAAUGU
Anticodon tRNA	3′CUU	ACA

31. A part of the polypeptide chain consists of the amino acids Arg–Gly–Ser–Phe–Val–Asp–Arg. It is encoded by the following DNA segment:

```
G G C T A G C T G C T T C C T T G G G G A
| | | | | | | | | | | | | | | | | | | |
C C G A T C G A C G A A G G A A C C C C T
```

Which of the DNA strands is the template (nonsense) strand? Indicate the correct 5′ and 3′ polarity of the strands.

32. The human hormone somatostatin was the first product obtained using recombinant DNA technology. The bacterium synthesized this hormone after biochemists synthesized and introduced the following DNA sequence into its genome:

3′ CGA CCA ACA TTC TTG AAG AAA ACC TTC TGA AAG TGA AGC ACA 5′

Determine the order of amino acids in somatostatin.

33. The β-globin gene family spans about 50 kb but contains only 5 structural genes for the β, δ, Aγ, Gγ, and ε chains of hemoglobin. Each of these polypeptide chains contains 146 amino acids. What percentage of this DNA segment encodes amino acids?

14. Mutations

Mutations are permanent changes in the DNA sequence of an organism, forming the basis of genetic variability. They may affect a single nucleotide or larger sections of DNA. According to their extent, they are classified as gene, chromosomal, and genomic mutations. Mutations can arise spontaneously or they can be induced by chemical, physical, or biological mutagens. Their consequences may be neutral, harmful, or even beneficial. Depending on various contexts, mutations are divided into several groups.

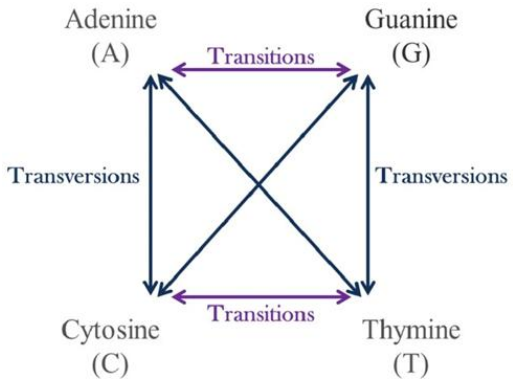


Figure 14-1. Transition versus transversion mutations.

- **Transition** → substitution of a purine base for another purine (A↔G) or a pyrimidine for another pyrimidine (C↔T).
- **Transversion** → substitution of a purine base for a pyrimidine and vice versa (e.g., A↔C, A↔T, G↔C, G↔T).

Table 14-1. Below are a few of the most commonly used cytogenetic symbols in the standard nomenclature.

add = addition material of unknown origin
del = deletion
der = derivative chromosome
dic = dicentric
dup = duplication
fra = fragile Site
ins = insertion
inv = inversion
i = isochromosome
mar = marker chromosome
mat = maternal origin
minus sign (-) = loss
p = short arm of chromosome
pat = paternal origin
plus sign (+) = gain
q = long arm of chromosome
r = ring chromosome
rcp = reciprocal
rob = robertsonian translocation
t = translocation
tel = telomere (end of chromosome arm)
ter = terminal end of chromosome

International Standard of Cytogenetic Nomenclature **(ISCN)** is a system describing chromosomes and chromosome abnormalities that allows one lab to "write out" the chromosome findings. Any other lab will know what they have found without looking at the karyotype.

Here are some examples:

46,XX - normal female karyotype

46,XY - normal male karyotype
These descriptions say there are 46 chromosomes and that it is a male or female.

46,XX,del(14)(q23)
Female with 46 chromosomes with a deletion of chromosome 14 on the long arm (q) at band 23.

46,XY,dup(14)(q22q25)
Male with 46 chromosomes with a duplication of chromosome 14 on the long arm (q) involving bands 22 to 25.

46,XX,r(7)(p22q36)
Female with 46 chromosomes with a 7 chromosome ring. The end of the short arm (p22) has fused to the end of the long arm (q36) forming a circle or 'ring'.

47,XY,+21
Male with 47 instead of 46 chromosomes and the extra chromosome is a 21. (Down syndrome)

Questions and Tasks

1. There is following sequence of deoxyribonucleotides in the coding or sense strand of DNA molecule and some type of mutations. Describe this point mutations and state the difference in amino acid sequence after transcription and translation of given DNA strands:

original strand 5′ **C G C A A T T C G A G G G G G A C C** 3′

mutation 1 5′ **C A C A A T T C G A G G G G G A C C** 3′

mutation 2 5′ **C G A A A T T C G A G G G G G A C C** 3′

mutation 3 5′ **C G C C A A T T C G A G G G G G A C C** 3′

mutation 4 5′ **C G C A A T T - G A G G G G G A C C** 3′

2. The original DNA strand is:
5′-GGACTAGATACG-3′

Which of the following point mutations is a transition, transversion, addition, or deletion?

a) 5′-**GAACTAGATACG**-3′

b) 5′-**GGACTAGAGACG**-3′

c) 5′- **GGACTAGTACG**-3′

d) 5′-**GGAGTAGATACG**-3′

3. The original DNA strand is:
5′-ATGGGACTAGATACC-3′

Which of the following point mutations is a silent, missense, nonsense, or frameshift mutation?

a) 5′-**ATGGGTCTAGATACC**-3′

b) 5′-**ATGCGACTAGATACC**-3′

c) 5′-**ATGGGACTAGTTACC**-3′

d) 5′-**ATGGGACTAAGATACC**-3′

4. In a DNA strand, the following nucleotide sequence of a short structural gene is present:
5′-ATGGGTCGTACGACCGGTAGTTACTGGTTCAGTTAA-3′

a) Write the amino acid sequence of the polypeptide chain encoded by this gene.

b) Show a silent (no-sense-changing) mutation and its effect on the polypeptide.

c) Show a nonsense mutation and its effect on the polypeptide.

d) Show a frameshift mutation and its effect on the polypeptide.

e) Show a missense (sense-changing) mutation and its effect on the polypeptide.

5. Explain what type of gene mutation occurs in the change of the following codons:
a) the codon for glycine changes to the codon for alanine
b) the codon for tryptophan changes to a termination (stop) codon
c) the codon for cysteine changes to the codon for arginine
d) the codon for serine changes to the codon for isoleucine
e) the codon for serine (AGT or AGC) changes to the codon for threonine

6. The following amino acid sequence is part of a certain protein. The normal sequence and its two mutated forms are shown. Using the genetic code table, determine the corresponding nucleotide sequence in the double-stranded DNA that corresponds to the normal gene. Which strand is the coding strand? What would be the resulting mRNA sequence? What is the most probable type of mutation that caused the changes in the individual mutated forms?

a) normal order: –lys–arg–his–his–tyr–leu–

b) mutation 1: –lys–arg–his–his–cys–leu–

c) mutation 2: –lys–arg–ile–ile–ile–

d) mutation 3: –lys–glu–thr–ser–leu–ser–

7. Determine the type of mutation from the following amino acid sequence:

normal order: –phe–asn–pro–thr–arg–

a) mutation 1: –phe–asn–pro–

b) mutation 2: –phe–asn–ala–his–thr–

c) mutation 3: –phe–his–pro–thr–arg–

Which of the mutations could theoretically cause the mildest disease symptoms?

8. A deletion of one nucleotide in DNA changed the amino acid sequence in the protein

...lys-ser-phe-cys-asn-leu-ala-ala-lys...

to the sequence:

...lys-val-phe-ala-ile...

Using the genetic code table, write the corresponding mRNA for the normal protein, for the altered protein, and determine the deletion that caused this change.

9. The centromere is represented by • and the original order of genes on the chromosome was **A B C D E F • G H I J K L**. State the types of chromosomal mutations for the following gene sequences and indicate which change represents a balanced and which an unbalanced chromosomal mutation.

a) **A B E F • G H I J K L**

b) **A B B C D E F • G H I J K L**

c) **A D C B E F • G H I J K L**

d) **A B C D H G • F E I J K L**

e) **D E F • G H I J K L**

f) **A B C D E F • F E D C B A**

g) **A B C D E F • G H I X Y Z J K L**

10. When the centromere is represented by • and the original order of genes on the chromosome was **A B C D E • F G H I J**, which of the following sequences is not an interstitial deletion:

a) **A B E • F G H I J**

b) **A B C D G H I J**

c) **A B C D E • H I J**

d) **A B C D E • F G H**

11. What is the difference between a chromosomal mutation and a gene mutation?

12. Compare somatic and gametic mutations and their consequences for the individual.

13. What is the difference between a gene mutation of the transition type and the transversion type? Using DNA bases (A, T, C, G), write the four types of transitions and the eight types of transversions.

14. Which of the given karyotypes correctly represents the notation of an unbalanced translocation?

a) 46,XX,t(5;12)(p14;q23)

b) 46,XX,der(5)t(5;12)(p14;q23)

c) 46,XX,t(5;12)(p14q23)

d) 46,XX,-5,+der(5)t(5;12)(p14;23)

15. How many chromosomes are present in the somatic cells of individuals (2n = 46), who are:

a) monosomic
b) trisomic
c) monoploid
d) tetrasomic
e) triploid

16. How do you justify the statement that Down syndrome is more often the result of nondisjunction during oogenesis than during spermatogenesis?

17. Illustrate schematically the types of gametes, with respect to trisomy of chromosome 21, that can be produced by a woman with Down syndrome.

18. Diagram nondisjunction of one chromosome pair, occurring:

a) in meiosis I of oogenesis
b) in meiosis II of oogenesis

Compare it with the same nondisjunction during spermatogenesis.

19. What zygote results from the fertilization of an egg with disomy of chromosome 21 by a sperm with nullisomy of chromosome 21?

20. In a certain family, several children were born with Down syndrome. How can this be explained?

21. A married couple, as part of family planning, comes to a genetic counseling center, since on the husband's side, in the previous three generations, there have been several stillbirths, or several children were born with congenital anomalies who died in early childhood. Cytogenetic examination of the husband revealed that he has a normal number of chromosomes 46, XY, but the banding method showed that one chromosome no. 1 has a pericentric inversion that involves about 70% of the length of this chromosome. The other chromosome no. 1 is normal.

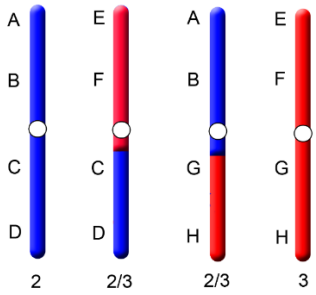
a) How do you explain the high incidence of stillbirths in the husband's family?
b) What is the risk of giving birth to a child with congenital malformations in the given family?
c) What advice can you give to these spouses who want to prevent the mentioned complications?

22. In a woman, a chromosomal rearrangement between the second and third chromosome was found during cytogenetic examination.

a) What type of chromosomal aberration is shown in the picture?

b) How can these chromosomes pair during meiosis?

c) The woman is phenotypically healthy. However, she has had two spontaneous miscarriages. She asks about the causes of the miscarriages and the probability that she can have a viable child.



23. By what type of mutation can a metacentric chromosome be changed into an acrocentric one?

24. Write two ways in which a triploid individual can arise.

25. Explain the difference between vital, sublethal, semilethal, and lethal mutations. How do they manifest?

26. Diagram nondisjunction of one autosomal chromosome pair and its consequences:
a) during oogenesis in the first meiotic division
b) during oogenesis in the second meiotic division

27. Some women with Down syndrome can have children.

a) Illustrate schematically meiosis I and II with respect to free trisomy of chromosome 21.

b) What is the theoretical risk that the child will be affected by the same syndrome as the mother?

28. A child with Down syndrome was born to healthy parents. The mother was found to have a Robertsonian translocation of chromosomes 21 and 14.

a) Write the mother's karyotype and schematically depict the given mutation.

b) Schematically illustrate the types of gametes the woman produces with respect to this translocation.

c) Express the theoretical probabilities of the occurrence of individual karyotypes in newborns after the fusion of these gametes with a normal male gamete.

d) What is the probability that their next child will also be affected by Down syndrome?

29. A child with Down syndrome was born to healthy parents, and the father was found to have a Robertsonian translocation of chromosomes 13 and 21.

a) Write the father's karyotype and schematically depict the given mutation.

b) Schematically illustrate the types of gametes the man produces, and in what

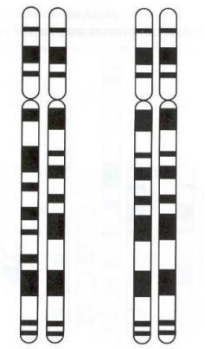
proportions, with respect to this translocation.
c) What is the probability that their next child will also be affected by Down syndrome?

30. In healthy spouses, it was found that the man is a carrier of a balanced Robertsonian translocation of chromosomes 14 and 15.
a) Write the karyotype of the man and schematically depict the given mutation.
b) Schematically illustrate the types of gametes the man produces with respect to this translocation.
c) What is the probability that this couple will have an affected child in view of the man's translocation?

31. What is the theoretical risk of having affected children in the offspring of an individual with a Robertsonian balanced translocation of chromosomes 15–21?

32. What is the theoretical risk of having affected children in the offspring of an individual with a Robertsonian balanced translocation of chromosomes 21–21?

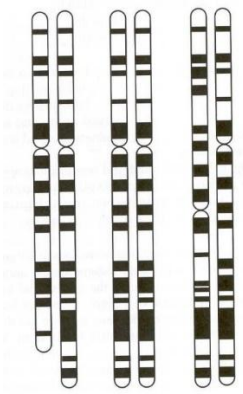
33. A married couple has been trying to have a child for several years. They consulted a doctor, and cytogenetic examination revealed that the husband has an abnormal chromosome number 12.



husband wife

a) What type of chromosomal abnormality does the husband have?
b) What consequences does this have for the offspring of the mentioned couple?

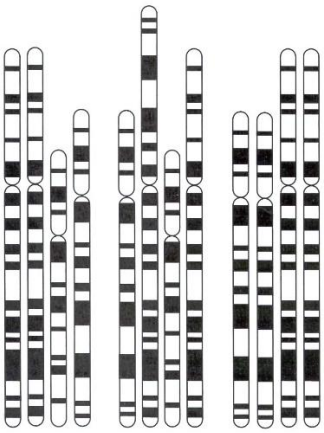
34. The parental couple cannot have a viable child. The woman had two miscarriages and one severely impaired child, who died soon after birth. Cytogenetic analysis of banded chromosomes of the father, mother, and child showed that all chromosomes are normal except for chromosome pair number 6 in the father and the child.



child mother father

a) Which parent has the abnormal chromosome no. 6?
b) Determine the type of chromosomal mutation.
c) Why is the child not phenotypically normal?
d) What is the prognosis for the couple's future offspring?

35. A married couple has been trying to have a child for several years. The woman has had several miscarriages, and last year she gave birth to a baby with multiple congenital defects. The baby died within a few days after birth. The results of cytogenetic analysis showed that all chromosomes are normal except chromosomes no.6 and no.12. The banded chromosomes of the individual family members are shown in the figure (chromosomes no. 6 are longer).



child mother father

a) Which parent has an abnormal karyotype?
b) Determine the type of chromosomal aberration.
c) Why is the child phenotypically affected?
d) What is the prognosis for the offspring of the given parental couple?

36. Explain what the following karyotypes indicate:

- 47,XXX
- 47,XXY
- 47,XYY
- 45,X
- 48,XXXY
- 47,XY,+13
- 48,XX,+13,+21
- 47,XY,+21/46,XY
- 45,X/46,XX/47,XXX
- 46,XX,del(5)(p13)
- 46,XY,del(5)(q13q33)
- 46,Y,del(X)(p11p21)
- 46,XY,inv(3)(p13q21)
- 46,XX,inv(3)(q21q26)
- 46,XX,t(2;18)(p13;q21)
- 46,XX,t(2;5)(q21;q31)
- 46, X,t(X;13)(q27;q12)
- 46,XY, r(9)(p24q34)
- 46,XX, r(18)(p11q22)

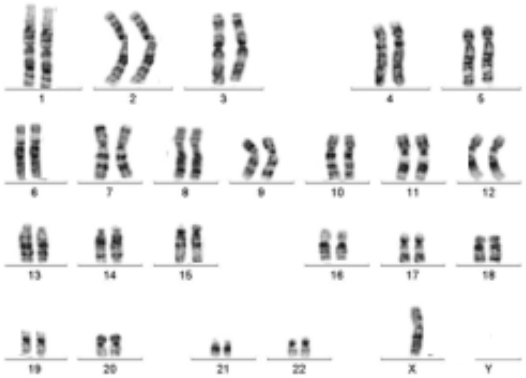
- 46, XX,dup(1)(q22q25)
- 46, XY,dup(1)(q25q22)
- 46, XX,ins(5;2)(p14;q22q32)
- 45,XY,der(21;21)(q10;q10)
- 46,XY,+21,der(21;21)(q10;q10)
- 45,XX,der(14;21)(q10;q10)
- 46,XY,+21,der(14; 21)(q10;q10)
- 46,XX,+13,der(13;14)(q10;q10)
- 46,XY,t(9;10)(p22;q24)
- 46,XX,del(13)(q22)
- 46,X,i(X)(q10)
- 46,XX,i(17)(q10)
- 46,Y,fra(X)(q27)
- 46,X,fra(X)(q27)
- 45,XY,dic(13;15)(q22;q24)

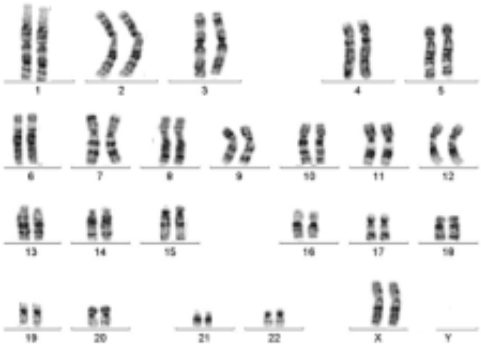
37. Write down the karyotypes of the following persons:

- man with trisomy 22
- man with terminal deletion of short arm of chromosome 4
- woman with Edwards syndrome
- man with balanced Robertsonian translocation 13 – 14
- women with nonbalanced Robertsonian translocation 14 – 21
- women with reciprocal translocation of chromosomes 4 and 9
- man with intersticial deletion of short arm chromosome 3
- person with Turner syndrome
- man with paracentric inversion of long arm of chromosome 10
- woman with pericentric inversion of chromosome 6
- person with Klinefelter syndrome
- person with trisomy X

- woman with terminal deletion of short arms chromosome X
- man with monosomy 21
- woman with mosaic form of Down syndrome
- woman with isochromosome X for q arms
- man with isochromosome for long arms of 21
- man with reciprocal translocation of chromosomes 2 and 5
- man with ring chromosome 2
- woman with Patau syndrome

38. In the newborn's karyotype, two cell lines were identified. 28% of the cells had karyotype 1 and 72% of the cells had karyotype 2. Write the karyotypes and answer the following questions.





1..... **2**.....

a) **Karyotype of the newborn:**

b) **The cause of the condition is:**

- nondisjunction during the first meiotic division in one of the parents?
- nondisjunction during the second meiotic division in one of the parents?
- nondisjunction during intrauterine development of the child (mitotic nondisjunction)?

c) **What is the prognosis for the newborn?**

15. Mendelian Genetics

Mendel's laws:

1. **Law of Uniformity (dominance and recessivity)** – the offspring of a cross between two homozygotes are all uniform heterozygotes in the F1 generation and express the dominant trait. When two heterozygotes are crossed, the traits of the parents segregate in the offspring in ratios of simple whole numbers.
2. **Law of Segregation** – each individual has 2 alleles for each trait, and these alleles are randomly separated during gamete formation, so that each gamete carries only one allele.
3. **Law of Independent Assortment** – alleles of different genes located on different chromosomes are inherited independently of each other and assort randomly.

Mendel's laws do not apply, or do not fully apply, when the inheritance of traits does not correspond to the model of dominant and recessive alleles, such as in incomplete dominance, codominance, lethal alleles, sex-linked genes, gene linkage, epistasis, polygenic inheritance, and multifactorial inheritance.

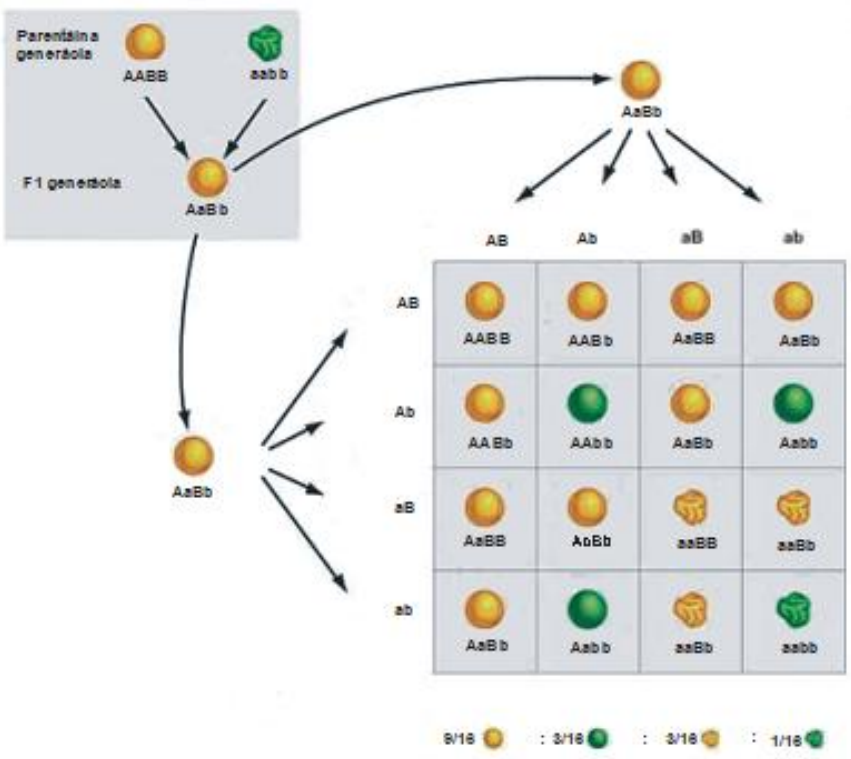


Figure 15-1. Dihybrid cross.

Questions and Tasks

1. Explain the concepts of allele, gene, locus, trait, genotype, phenotype.

2. Explain the difference between a homozygote and a heterozygote.

3. Explain the terms expressivity and penetrance.

4. What are allelic interactions and non-allelic interactions? What is pleiotropy?

5. Explain the terms heterogeneity and phenocopy.

6. Write down the types of gametes produced by an individual with the following genotypes:
a) AABB

b) AABb

c) Aabb

d) AaBb

e) AABbCc

f) AaBBCcee

g) AaBbCc

h) aaBBccDdeeff

7. Write down how many types of gametes are produced by:
a) a monohybrid
b) a dihybrid
c) a trihybrid
d) an n-hybrid

8. Assume that gene B has two alleles, the dominant B and the recessive b. Write down all the crosses (parental genotypes) that can produce a heterozygous child. From which cross will heterozygous offspring be the most frequent? From which cross will we get only one genotype in the offspring? Write down all the crosses from which we get only two genotypes in the offspring.

9. Galactosemia (a hereditary disorder of galactose metabolism) is an autosomal recessive disease (AR). What is the probability of the birth of an affected child if one parent is a recessive homozygote whose symptoms of the disease were eliminated by diet and the other parent is a heterozygote for the disease?

10. Right-handedness is dominant over left-handedness. A right-handed man, whose mother was left-handed, married a right-handed woman who has three brothers. Two of them are left-handed. Determine the genotype of the woman and state the probability that the couple will have a left-handed child.

11. In humans, eye colour is the result of the interaction of alleles of several genes that determine the production and deposition of pigment in the iris. For simplification, let us assume that the production and deposition of pigment in the iris are determined by a single gene, where the allele for brown eye colour **B** is dominant over the allele for blue eye colour **b**.

a) A brown-eyed man marries a blue-eyed woman and they have 8 brown-eyed children. What are the most probable genotypes of all family members?

b) A brown-eyed man, whose both parents were brown-eyed, marries a brown-eyed woman whose father was brown-eyed and mother was blue-eyed. They have one blue-eyed child. What are the genotypes of all family members?

12. Polydactyly is the medical term for having extra fingers or toes (digits). It is one of the most common birth defects that affects babies' hands and feet and it is an autosomal dominant (AD) malformation.

a) Determine the probability of the birth of an affected child in a family where both parents are heterozygotes for this condition.

b) One parent has a normal number of fingers and the other has six fingers. They have one child with a normal number of fingers. What is the probability that their second child will also be healthy?

13. Dentinogenesis imperfecta is an autosomal dominant disorder that affects dental tissues of mesodermal origin (dentin, pulp, periodontium, and cementum). A man with this condition, whose father was also affected and whose mother had normal dentition, married an

unaffected woman. What is the probability that their first child will be a boy affected with dentinogenesis imperfecta?

14. Albinism in humans is an autosomal recessive (AR) disorder caused by the absence of an enzyme required for the synthesis of the pigment melanin. The synthesis of this enzyme is determined by a dominant allele, meaning that individuals with genotypes **C/C** and **C/c** have normal pigmentation, and recessive homozygotes **c/c** are albino. Phenotypically normal parents had an albino child. What is the probability that:
a) their next child will be albino
b) their next two children will be albino
c) they will have two children, one albino and the other with normal pigmentation?

15. What offspring, and in what percentage proportions, can be expected in a family where the woman is normally pigmented and the man is an albino?

16. a) A woman with normal skin pigmentation and an albino man have nine normally pigmented children and one albino. What is the most probable genotype of the woman?
b) If a normally pigmented woman and an albino man have ten normally pigmented children and no albino, what is the most probable genotype of the woman?

17. Jane has normal skin pigmentation, but her brother and her husband are albino.
a) What is the probability that her first child will be an albino?
b) If her first child is an albino, what is the probability that the second child will also be an albino?

18. Both parents are carriers of a recessive allele for congenital deafness that is autosomal recessive disorder. What is the probability that:
a) the first child will be affected by deafness
b) their five children will all be healthy
c) two out of five children will be affected by deafness?

19. For a rare autosomal dominant disease, it is the rule that dominant homozygotes never occur among the offspring. One reason may be that this genotype is lethal in the early period of fetal development and is eliminated by spontaneous abortion. Assume a situation where the genotype **B/B** is lethal during intrauterine development, **B/b** survives but the individual is affected, and **b/b** survives and the individual is healthy. What genotypes, and in what percentage proportions, can be expected among live-born children from the cross **B/b** × **B/b**?

20. Hemeralopia (night blindness) is an autosomal dominant disorder. A healthy man married an affected woman. They have one healthy and one affected child.
a) What are the genotypes of all family members?
b) What is the probability that the next child will be healthy?

21. Phenylketonuria (a disorder of phenylalanine metabolism) and one of the rare forms of Swiss-type agammaglobulinemia (usually resulting in death within 6 months after birth) are autosomal recessive disorders. What is the probability of healthy children births in a family in which both parents are heterozygous for both genes?

22. In a family where both parents had normal hearing and one had straight hair while the other had curly hair, a deaf son with straight hair was born. Their second son had normal hearing and curly hair. What is the probability of the birth of a deaf, curly-haired child in this family, given that the allele for curly hair is dominant over the allele for straight hair, deafness is genetically determined by a recessive allele, and these genes are located on different autosomes?

23. For simplicity, assume that in humans brown eye colour is dominant over blue and right-handedness over left-handedness.
a) A blue-eyed right-handed man, whose father was left-handed, married a brown-eyed left-handed woman whose relatives were all brown-eyed for multiple generations. What children can be expected from this marriage?
b) A brown-eyed man married a blue-eyed woman. Both were right-handed. Their first child had blue eyes and was left-handed. What children can be born in this marriage, and with what probabilities?

24. Cataract in humans is autosomal dominant disorder (C/–), and albinism in an autosomal recessive disorder (aa). The genes are located on different chromosomes. From the marriage of a normally pigmented mother and a father with cataract, an albino daughter was born. If you consider this family situation, write the probable genotypes of the parents. With what probability will the couple have a child affected by at least one of the mentioned diseases?

16. Gene Linkage

Gene linkage is a phenomenon in which two or more genes are located in close proximity on the same chromosome. Such genes constitute a *linkage group* and tend to be inherited together as a unit, since they do not assort independently during meiosis. Crossing-over may occur during meiosis if the genes are farther apart. The closer two genes are, the lower the probability of their separation. The genetic distance between genes is determined based on the frequency of recombination between them during meiosis. Recombination frequency is expressed in centimorgans (cM), where 1% probability of recombination corresponds to 1 cM.

Genetic (map) distance (in cM) = (number of recombinants/total number of offspring) x 100

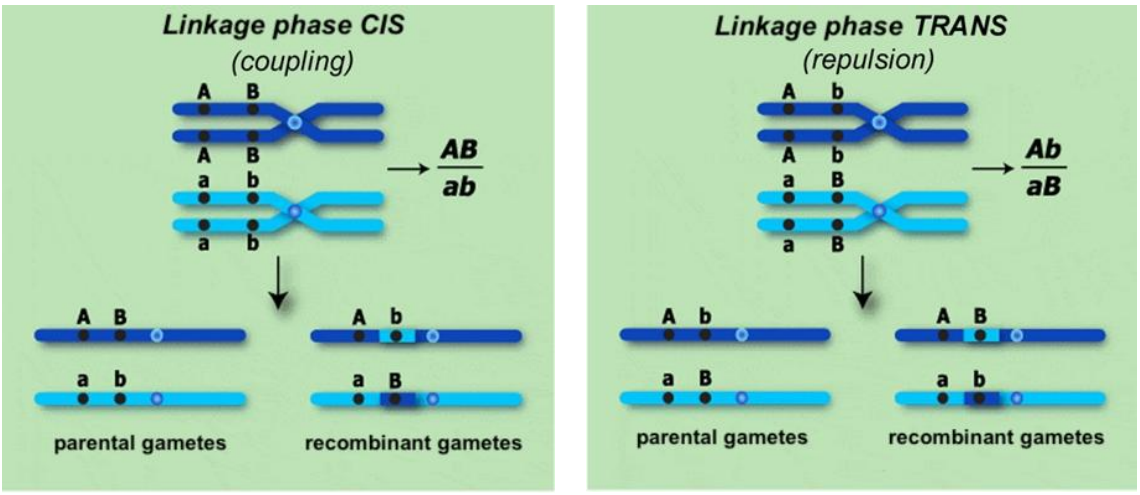


Figure 16-1. Cis configuration – dihybrid AB/ab (left) and trans configuration – dihybrid Ab/aB (right).

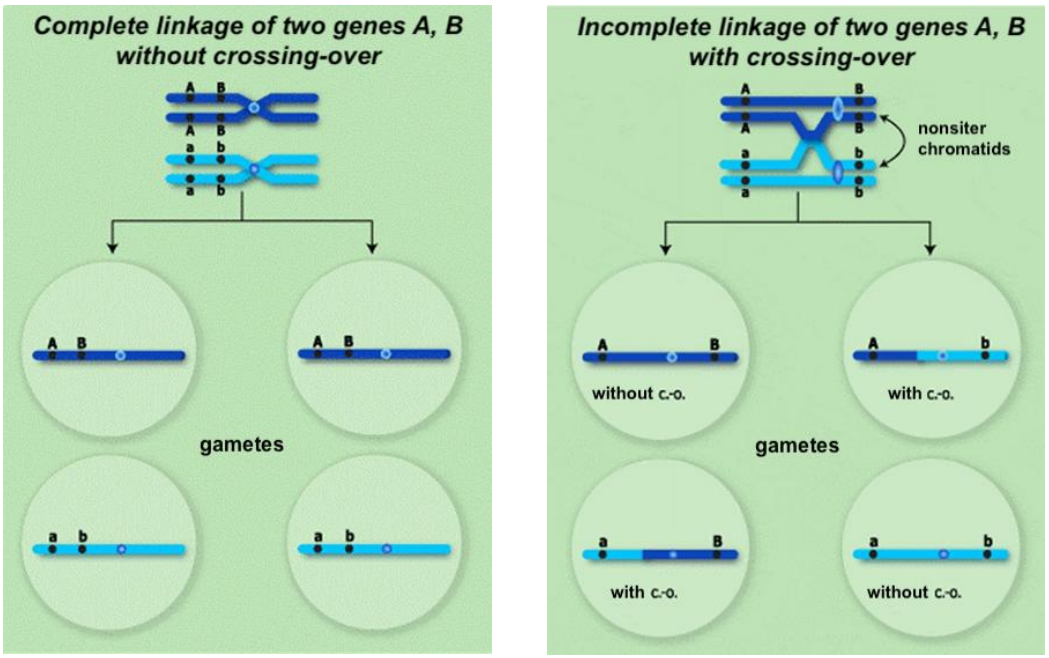


Figure 16-2. Complete linkage of genes A and B – crossing-over does not occur (p = 0 cM) and recombinant gametes are not produced (left), and incomplete linkage of genes A and B – crossing-over occurs, p = (0, 50 cM), recombinant gametes Ab and aB are produced (right).

Questions and Tasks

1. Indicate the method by which you can demonstrate that two allelic pairs A/a and B/b are:
a) assorting independently (AaBb)
b) completely linked (AB/ab, Ab/aB)
c) incompletely linked (AB/ab, Ab/aB)

2. Perform the cross AaBb × aabb assuming the genes are linked and both allelic pairs are:
a) incompletely linked, in cis configuration, p = 5 cM
b) incompletely linked, in trans configuration, p = 5 cM
What will be the percentage distribution of genotypes in the offspring from both crosses?

3. Perform the cross AaBb × aabb assuming the genes are linked and both allelic pairs are:
a) completely linked, in cis configuration,
b) completely linked, in trans configuration,
What will be the percentage distribution of genotypes in the offspring from both crosses?

4. If an individual arose from a cross of parents Ab/Ab × aB/aB and is backcrossed with a double recessive homozygote, how does the offspring consisting of 1850 individuals segregate if the linkage between genes A and B corresponds to a 40% recombination frequency?

5. In humans, the locus for the Rh factor is located on chromosome 1 and is linked to the locus determining erythrocyte shape, at a distance of 3 cM. Rh positivity and elliptocytosis (elliptically shaped erythrocytes) are autosomal dominant (AD) traits. A man is heterozygous for both genes, having inherited the Rh+ factor from one parent and elliptocytosis from the other. His wife is Rh− and has normal erythrocyte shape. Determine the percentage distribution of the probable genotypes and phenotypes among the children in this family.

6. A man with elliptocytosis, whose mother had normal erythrocyte shape and was homozygous dominant at the Rh factor locus and whose father was Rh− and heterozygous at the elliptocytosis locus, married a healthy Rh− woman (the map distance between the respective genes is 3 cM). Both traits are autosomal dominant (AD).
a) What is the probability that their first child will be Rh− with elliptocytosis?
b) If the first child is Rh+, what is the probability that it will also be affected with elliptocytosis?

7. Andersen disease (a hereditary disorder of glycogen metabolism) and alkaptonuria are autosomal recessive diseases and are caused by linked genes. Their distance on the chromosome is 6 cM. A healthy woman homozygous at both loci married a healthy man whose mother was affected with Andersen disease and whose father had alkaptonuria.
a) Determine the phase of linkage and write the genotypes of the parents.
b) Determine all possible genotypes and phenotypes of the offspring.
c) What is the probability of a healthy child being born in this marriage?
d) What is the probability of a child being born affected by at least one of the diseases?

8. Pigmentary retinopathy is a progressive degenerative disease of the retina. The gene for this disease is linked to the gene for familial hypercalcemia, with a map distance of 4 cM. Both conditions are autosomal dominant (AD). The woman is affected by both anomalies, while her mother is healthy. She married a healthy man.
a) Determine the linkage phase and write the genotypes of the parents.
b) List all expected genotypes in the offspring.
c) What is the probability that a child born in this marriage will be affected only by pigmentary retinopathy?
d) What is the probability that a child born in this marriage will be affected only by familial hypercalcemia?

9. Cataract and polydactyly in humans are autosomal dominant (AD) traits determined by genes which are in complete linkage.
a) A woman inherited the allele for cataract from her mother and the allele for polydactyly from her father. Her husband is healthy. What offspring can be expected in this family?
b) What offspring can be expected in a family where the man is healthy and the woman is a heterozygote for both genes, given that her mother was affected with both anomalies and her father was healthy?
c) What offspring can be expected in a family where both parents are heterozygotes for both traits and it is known that their mothers were affected with cataract and their fathers with polydactyly?

10. Congenital adrenal hyperplasia and congenital hypothyroidism in humans are determined by autosomal recessive inheritance (both genes are in complete linkage). Both parents are healthy, but their mothers were affected with both anomalies.
a) Determine the linkage phase and write the genotypes of the parents.
b) List all expected genotypes in the offspring.
c) What is the probability that healthy children will be born in this marriage?
d) What is the probability that a child will be born affected with both anomalies?

11. Nail-patella syndrome (unguis–patella syndrome) is an autosomal dominant (N) hereditary malformation characterized by a variable degree of nail dystrophy, absence or hypoplasia of the patella, other skeletal dysplasias and occasionally nephropathy. This gene is located 10 cM from the ABO locus on chromosome 9. A man with nail-patella syndrome and AB blood group married a healthy woman (n/n) with blood group O. Their three children are healthy and have blood group A; one child with blood group B is affected.
a) Determine the most likely linkage phase in the man—a double heterozygote.
b) The woman is pregnant again, and fetal red blood cells indicate blood group A. What is the probability that the child will be unaffected by the malformation (assume the same linkage phase as in part a)?
c) What is the probability that the child will be unaffected if it has blood group B?

12. Idiopathic cardiomyopathy is inherited in an autosomal dominant (AD) manner. This gene is located 10 cM from the ABO gene on chromosome 9. A man with the disease and blood group B, whose father was healthy and had blood group A, married a healthy woman with blood group O.
a) State and correctly write the genotypes (linkage phase) of the parents.
b) Determine the distribution of all possible genotypes (with the correct linkage phase) in the offspring.
c) Determine the distribution of individual phenotypes (disease status, blood group) in the offspring.
d) The woman is pregnant, and the fetus has been found to have blood group O. What is the probability that the child will be born healthy?

13. Clasical hemophilia and colour blindness are determined by X-linked recessive (XR) pattern of inheritance (both genes are located on the X chromosome at a distance of 3 cM).
a) A woman whose father was affected with both hemophilia and colour blindness and whose mother was healthy married a healthy man. Determine the probable genotypes and phenotypes of the children from this marriage.
b) A woman whose mother was affected with colour blindness and whose father had hemophilia is marrying a man affected with both anomalies. Determine the probability that their children will be born with both hemophilia and colour blindness.

14. A woman with normal colour vision is a heterozygote in the cis phase for the X-linked genes for deuteranomaly (deuteranopia, G/g) and protanomaly (protanopia, R/r). The loci are 6 map units apart. What is the probability that her sons will have normal colour vision? Solve this situation for the case in which the woman is a double heterozygote in the trans (repulsion) phase.

15. Determine the order of genes on the chromosome based on the following recombination frequencies: A–B, 8 map units; A–C, 28 map units; A–D, 25 map units; B–C, 20 map units; B–D, 33 map units.

17. Sex and Heredity

It is a type of inheritance in which genes are located on the sex chromosomes – most often on the X chromosome, and rarely on the Y chromosome. Genes on the X chromosome are expressed differently in males and females, since females have two X chromosomes (with one undergoing inactivation) while males have only one. X-linked inheritance can be recessive (e.g., hemophilia, colour blindness, Duchenne muscular dystrophy) or dominant (e.g., Rett syndrome, vitamin D–resistant rickets). In Y-linked inheritance, genes are passed only through the male line (e.g., SRY, mutations in genes of the AZF region). Mutations in genes in the AZF region (azoospermia factor) can cause disorders of spermatogenesis; however, since they result in infertility, they are not transmitted to offspring. If the SRY gene is mutated or absent, an individual with an XY genotype but female phenotype may arise — Swyer syndrome.

a) X-linked recessive inheritance

P: X_HY x X_HX_h

	X_H	X_h
X_H	X_HX_H	X_HX_h
Y	X_HY	X_hY

Daughters: 1/2 healthy, 1/2 healthy carriers
Sons: 1/2 healthy, 1/2 affected

P: X_HY x X_hX_h

	X_h	X_h
X_H	X_HX_h	X_HX_h
Y	X_hY	X_hY

Daughters: healthy carriers
Sons: affected

P: X_hY x X_HX_H

	X_H	X_H
X_h	X_HX_h	X_HX_h
Y	X_HY	X_HY

Daughters: healthy carriers
Sons: healthy

P: X_hY x X_HX_h

	X_H	X_h
X_h	X_HX_h	X_hX_h
Y	X_HY	X_hY

Daughters: 1/2 healthy carriers, 1/2 affected
Sons: 1/2 healthy, 1/2 affected

b) X-linked dominant inheritance

P: X_aY x X_AX_a

	X_A	X_a
X_a	X_AX_a	X_aX_a
Y	X_AY	X_aY

Daughters: 1/2 healthy, 1/2 affected
Sons: 1/2 healthy, 1/2 affected

P: X_AY x X_aX_a

	X_a	X_a
X_A	X_AX_a	X_AX_a
Y	X_aY	X_aY

Daughters: affected
Sons: healthy

Questions and Tasks

1. Explain the concept of heterogametic and homogametic sex.

2. Explain Lyon's hypothesis.

3. What is a Barr body?

4. What number of Barr bodies are found in interphase cells in individuals with:
a) Klinefelter syndrome
b) Turner syndrome
c) karyotype 47, XYY
d) karyotype 48, XXXX
e) karyotype 47, XXX

5. According to Lyon's hypothesis, what will the retina of a woman heterozygous for colour blindness look like?

6. What are the possible causes of the female-to-male sex ratio of 1.40 : 1.60 at birth in humans?

7. Illustrate nondisjunction during oogenesis in a woman that leads to the occurrence of Klinefelter and Turner syndromes after fertilization with a normal sperm.

8. Compare the inheritance of sex-limited traits and sex-influenced traits.

9. In humans, the inability to distinguish red and green is a congenital X-linked recessive (XR) disorder. A woman with normal colour vision, whose father was colour-blind, married a man with normal red–green colour vision. What is the risk of affected children being born?

10. Colour blindness (daltonism) is an X-linked recessive disorder of colour vision. A colour-blind man marries a woman with normal vision whose father was also colour-blind.
a) What is the probability that these parents will have a colour-blind daughter?
b) What is the probability that their first son will be colour-blind? (Note: these two questions are formulated slightly differently).

11. A man and a woman have normal colour vision, although both of their fathers are colour-blind. What is the probability that their first child will be:
a) a son who can distinguish red and green,
b) a daughter with normal colour vision,
c) a son who is affected,
d) a daughter who is affected?

12. If parents, both with blood group A and normal colour vision, have a colour-blind son with blood group O, what is the probability that their next child will be a daughter with normal colour vision and blood group O?

13. The Xg antigen is present on the surface of cells and is determined by a gene on the X chromosome. There is no equivalent form of this gene on the Y chromosome. This gene has two alternative forms—the dominant allele $\mathbf{Xg^a}$ and the recessive **Xg**. A woman with genotype $\mathbf{Xg^a/Xg^a}$ married a man with genotype **Xg/Y**. They had a son with Klinefelter syndrome, genotype $\mathbf{Xg^a/Xg/Y}$. From which parent did the extra X chromosome originate, and during which meiotic division did the nondisjunction occur?

14. Daltonism (red–green colour blindness) is determined by a recessive allele located on the nonhomologous segment of the X chromosome.
a) A father with normal vision had a colour-blind son. State the genotypes of both parents.
b) A couple had one colour-blind son and a daughter with normal vision, who married a healthy man. They had seven sons, four of whom were colour-blind and three had normal vision. What were the genotypes of their parents and grandparents?
c) What is the probability that a son whose father is colour-blind will be affected by the same colour-vision defect, assuming the mother is not a carrier of the recessive allele?
d) What is the probability that a colour-blind son will be born to a mother who is a carrier (X^DX^d) if the father is not colour-blind?

15. A man with hemophilia (X-linked recessive inheritance) has a daughter with a normal phenotype. The daughter marries a man who also has a normal phenotype.
a) What is the probability that their daughter will have hemophilia?
b) What is the probability for a son?
c) If this couple had four sons, what is the probability that all four would be born with hemophilia?

16. Classical hemophilia (hemophilia A) is determined by an X-linked recessive pattern of inheritance.
a) A man with hemophilia married a healthy woman. They had healthy daughters and sons, who in turn married unaffected partners. Determine the probability that hemophilia will appear among the grandchildren.
b) A man affected with hemophilia marries a healthy woman whose father was a hemophiliac. Determine the probability that the children in this family will be healthy.

17. A woman who had normal blood clotting, but her father was a hemophiliac (XR), married a healthy man.
a) What is the probability that their children will have hemophilia?
b) If a daughter from this marriage marries a healthy man, what is the probability that their children will have hemophilia?
c) Suppose these parents wish to have two sons. What is the probability that both sons will have normal blood clotting?

18. Duchenne muscular dystrophy is an X-linked recessive (XR) lethal disease that primarily affects muscles and occurs in approximately 1 in 3500 newborn boys. A healthy woman with the heterozygous genotype married a healthy man. What is the probability that they will have:
a) a healthy son?
b) a healthy daughter or son?
c) three children who are all affected by the disease?

19. Hereditary hypophosphatemic rickets resistant to vitamin D is determined by a dominant allele located on the nonhomologous segment of the X chromosome (XD).
a) Affected woman marries a healthy man. Can they have a healthy son?
b) Healthy woman marries an affected man. What will be the sex of the healthy children? If a child from this marriage later marries a healthy partner, can they have a healthy child?

20. Albinism (a defect in melanin pigment production) is an autosomal recessive (AR) trait. Anhydrotic ectodermal dysplasia (a congenital dryness of the skin) is an infrequent X-linked recessive disorder with an almost complete absence of sweat glands in affected males. In a family where both parents are healthy, a son was born with both anomalies.
a) What is the probability that their next child will be a daughter and will be healthy?
b) What is the probability that their next child will be a son and will be healthy?

21. A colour-blind man is also affected by scapulohumeral muscular dystrophy (autosomal recessive inheritance). With his wife, who has normal colour vision and normal muscle function, he has a colour-blind daughter with muscular dystrophy.
a) Determine the genotypes of the parents and the child.
b) What is the probability that the couple will have a child affected with both anomalies?

22. Enamel hypoplasia is genetically determined by a dominant allele probably linked to the nonhomologous region of the X chromosome (XD). A healthy man, whose mother was affected and father healthy, married a woman affected with this defect. Her mother was healthy and her father was affected. Determine the genotypes of all family members. What offspring can be expected in this marriage?

23. Primary enamel matrix disorder is inherited as an X-linked dominant (XD) trait. Teeth are yellow to brown, sometimes semi-translucent.
a) A woman with healthy dentition married a man affected by this defect. What is the probability that their daughter will be healthy? What is the probability that their son will be healthy?
b) A healthy man married a woman with primary enamel matrix disorder. They want to have four sons and one daughter. What is the probability that none of the children will have the dental defect present in the mother?

24. Complete anodontia (absence of nearly all teeth) is a rare X-linked recessive disorder.
a) An engaged couple planning to marry both have healthy dentition, but the woman's father had complete anodontia. What is the probability that they will have a son with this defect?
b) An engaged couple planning to marry both have healthy dentition, but the woman's maternal grandfather had complete anodontia. What is the probability that they will have a son who is affected?

25. In humans, hairiness of the auricle is determined by gene **D**, which is located in the nonhomologous region of the Y chromosome. The **D** gene can therefore occur only in males. Males are always hemizygous, and a single dominant allele of **D** is sufficient for the phenotypic manifestation, causing excessive auricular hairiness in males. A single recessive allele of **D** is sufficient for the phenotypic manifestation of normal auricular hairiness in males.
a) Write the genotypes of the parents whose son had excessively hairy auricles.
b) With what probability will their second son be affected in the same way? With what probability will their next child not be affected by excessive auricular hairiness?

26. The gene **T** is located on homologous regions of the sex chromosomes, and its recessive homozygous combination causes blindness in humans. In the parental generation, the following parental genotypes were crossed:
P: mother XX × father XY
Tt Tt
Determine:
a) the phenotypes of both parents;
b) the probability that a blind individual will appear in the F_1 generation;
c) the sex of the blind individuals in the F_1 generation.

27. Hypospadias—cleft of the urethra—occurs predominantly in males as a sex-limited trait. One form of hypospadias is inherited as follows: males with genotypes HH and Hh are healthy, whereas males with genotype hh are affected with hypospadias (sex-limited inheritance).
a) A healthy man has two sons affected with hypospadias and one daughter. Determine the genotypes of all family members. What offspring can the daughter expect if she marries a healthy man from a family in which hypospadias has not occurred for several generations?
b) A man affected with hypospadias has two daughters. Both marry healthy men. One has two sons; the other has one son. All the sons from both marriages suffer from hypospadias. Determine the genotypes of their parents and grandparents. Can the sons have healthy children?

28. An example of a sex-influenced pattern of inheritance in humans is baldness. In men, premature baldness is determined by the dominant allele P. For baldness to manifest in women, the P allele must be in the homozygous state (PP). In men, baldness appears even in the heterozygous state (Pp), likely due to the presence of sex hormones.
a) A bald man whose father had normal hair married a woman with normal hair, but her mother and all her brothers were bald. What children can be born from this marriage?
b) A healthy man and a healthy woman, whose father was colour-blind and mother was bald, married. What children can be born from this marriage?
c) A brown-eyed, bald man whose father had normal hair and blue eyes married a woman whose father and all her brothers were bald. What children can be born from this marriage?

29. A blue-eyed woman with thalassemia minor (Tt) who is a carrier of hemophilia married a brown-eyed man whose father was blue-eyed and who also has thalassemia minor, but is not affected by hemophilia. Brown eye colour is dominant (A) over blue (a). Thalassemia is an inherited disease characterized by insufficient hemoglobin function. The severe form, thalassemia major, has the genotype tt.
a) What is the probability that this couple will have a blue-eyed son who is a hemophiliac with thalassemia major?
b) What is the probability that they will have a completely healthy son (without hemophilia and thalassemia major)?

Task 1: Examination of X-chromatin in buccal mucosa cells stained with aceto-orcein

Materials and Equipment: cover slip and slide, Pasteur pipette, 2% aceto-orcein solution, filter paper.

Procedure: Rinse the mouth with water. Using the shorter edge of a clean slide, make a smear from the buccal mucosa. Do not use the material from the first smear. Transfer the material from the second smear onto another slide and prepare a smear in the same way as a blood smear. Leave the smear in the air for 30 seconds, but do not let it dry completely. Add two drops of aceto-orcein stain and stain for one minute. Carefully place a cover slip. Remove excess stain with a piece of filter paper. Observe under 400× magnification.

On a separately prepared slide, examine 20 cell nuclei for the presence of X-chromatin.

Results: X-chromatin occurs in the interphase nuclei of female individuals. In female mammals, including women, one of the two X chromosomes is genetically inactive, remains condensed, and is well stained even in interphase (lyonization). It can be observed as a plano-convex body (chromatin mass) about 1 μm in size located on the inner side of the nuclear membrane. In healthy adult women, an average of 20–30% of cells are positive for the presence of X-chromatin. When 6–10% of nuclei are positive, this is considered proof of the gonosomal complement XX. For diagnostic evaluation, at least 100 cell nuclei must be assessed. Draw a cell with a positive and a negative finding. Evaluation of X-chromatin (Barr bodies) allows for

an approximate determination of chromosomal sex in interphase cells. It is used as a so-called sex test in women in top-level sports competitions. The examination can also indicate abnormalities in the number of X heterochromosomes.

Drawing:

Determining the presence of the Barr body

1.	5.	9.	13.	17.
2.	6.	10.	14.	18.
3.	7.	11.	15.	19.
4.	8.	12.	16.	20.

18. Inheritance of blood group systems

Blood group systems represent genetically determined traits—glycoproteins on the surface of erythrocytes. The genes of blood group systems encode enzymes or proteins that:

1. form or modify antigens on the surface of erythrocytes,
2. determine the structure and type of carbohydrate chains or proteins,
3. decide the presence or absence of a specific antigen, thereby defining a particular blood group.

Among the most important, in the **ABO system**, the **ABO gene** encodes the enzyme glycosyltransferase, which adds specific carbohydrate units to a precursor chain, forming antigen A or B. If the enzyme is non-functional, the antigen is not produced, resulting in blood group O. The **Rh system** encodes membrane proteins. The **MN system** encodes membrane sialoglycoproteins. The **Lewis system** encodes fucosyltransferase. The **Kell system** encodes an endopeptidase. Other systems also encode functional proteins. For example: **Kidd** encodes a urea transporter (gene *SLC14A1*), **Duffy** encodes a chemokine receptor (*ACKR1/DARC*) and **Lutheran** encodes the adhesion protein *BCAM*.

Questions and Tasks

1. A man has blood group B, and his mother had blood group O. A woman has blood group A, and her father had blood group B. What blood groups can be expected in their children, and in what percentages?

2. A man with blood group A marries a woman with blood group B. Their child has blood group O. What are the genotypes of the parents? What other genotypes, and with what probabilities, can be expected in the offspring of these parents?

3. A boy has blood group O, and his sister has blood group AB. What are the blood groups of their parents?

4. A woman with blood group B, N gave birth to a child with blood group O, MN. A man with blood group A, M was named as the biological father of the child.
a) Based on this information, can the man be excluded from paternity?
b) Which of the following men can be excluded from paternity?

1. A man with blood group B, N
2. A man with blood group AB, MN
3. A man with blood group O, M
4. A man with blood group A, N

c) Which alleles must the child have inherited from the father?

5. The task lists the parents' phenotypes (1–5) for the ABO, MN, and Rh blood group systems. Each cross produces one of the five offspring (a–e). Correctly match one offspring to each parental pair.

Parents' phenotypes:			Childrens' phenotypes::
1. A, M, Rh^-	x	A, N, Rh^-	a) A, N, Rh^-
2. B, M, Rh^-	x	B, M, Rh^+	b) O, N, Rh^+
3. O, N, Rh^+	x	B, N, Rh^+	c) O, MN, Rh^-
4. AB, M, Rh^-	x	O, N, Rh^+	d) B, M, Rh^+
5. AB, MN, Rh^-	x	AB, MN, Rh^-	e) B, MN, Rh^+

6. Fill in the children's blood groups in the table:

Parents' blood groups		**Children's possible blood groups**	**Children's impossible blood groups:**
Phenotype	Genotype	Phenotypes	Phenotypes
O x O			
O x A			
A x A			
B x B			
O x B			
A x B			
A x AB			
AB x AB			

7. Fill in the fathers' blood groups (phenotypes) in the table:

Mother	**Child**	**Possible father**	**Impossible father**
O	A		
O	AB		
A	A		
A	O		
B	A		
B	AB		
B	O		
AB	A		
AB	O		
AB	AB		

8. Antigens A and B in individuals with genotypes Se/Se and Se/se can be present in soluble form in body fluids (e.g., saliva), but are not present in soluble form in se/se homozygotes. In the population, we therefore distinguish "secretors" and "non-secretors." Write the possible genotypes and phenotypes from a cross between individuals with blood groups AB and O, with both being Se/se. How will this result be influenced if both parents are heterozygous (Hh) for the gene determining the formation of the H antigen?

9. A man with blood group A_1 had, with a woman of blood group O, two sons with blood group A_1B and a daughter with blood group O.
Complete:
1. genotype of the man with phenotype A_1:
2. genotype of the woman with phenotype O:
3. genotype of the sons with phenotype A_1B:
4. genotype of the daughter with phenotype O:

10. Fill in the missing genotypes of the Xg blood group system:
a) for the son
b) for the daughter

Mother	**Father**	**Child**
Xg^aXg	XgY	a) b)
Xg^aXg^a	Xg^aY	a) b)

XgXg	Xg^aY	a) b)
	Xg	a) Xg^aY b) XgXg, Xg^aXg
Xg^aXg		a) Xg^aY, XgY b) Xg^aXg^a, Xg^aXg

11. Parents with blood group B and normal colour vision had a colour-blind son (XR) with blood group O. What is the probability that their next child will have blood group B and will also be affected by this colour-vision disorder?

12. The parents of 61 infants with hemolytic disease of the newborn (caused by incompatibility in the Rh system) had blood groups O and AB. However, only in two cases did the mother have blood group O and the father AB. Explain this finding.

13. What blood groups can be born in the following marriages?
a) A MN Rh^+ x AB M Rh^-
b) AB MN Rh^- x B N Rh^+
c) O MN Rh^+ x AB M Rh^-
d) A MN Rh^+ x O N Rh^-

14. Hypocalcification is a disorder of tooth mineralization. Enamel is soft and brittle, and dentin gradually becomes exposed. The defect is inherited in an autosomal dominant manner. The marriage is between: a woman with healthy dentition and blood group O, MN, Rh+, and a man with hypocalcification, AB, N, Rh−. What children can be expected from this marriage?

15. The child has blood group A, N; the mother has blood group O, MN.
a) Write the genotypes of the mother and the child for the ABO and MN blood group systems.
b) List the combinations of ABO and MN blood groups for men who can be excluded from paternity.
c) List the combinations of ABO and MN blood groups for men who could be the biological father of the given child.

16. A marriage was contracted between a woman with blood group O and a man with blood group A. They had a child with blood group AB. The woman's parents (a consanguineous marriage): the father had blood group O (genotype OO) and the mother had blood group B (genotype BB). Draw the pedigree and write the possible genotypes of all family members.

17. Determine the HLA genotypes and HLA haplotypes of the given family members, based on the following phenotypes.

	HLA phenotypes	**HLA genotypes**	**HLA haplotypes**
father	HLA-A1,2; B8,9		
mother	HLA-A3,7; B5,6		
daughter	HLA-A1,7; B5,8		
son	HLA-A1,3; B6,8		

18. Fill in the table with the HLA haplotypes of the **HLA-A, HLA-B,** and **HLA-C** genes that are in complete linkage.

HLA genotypy	**HLA haplotypy**
Father: A1 A3 B8 B15 C1 C1 **Mother**: A1 A23 B7 B8 C2 C4 **Child:** A1 A23 B7 B15 C1 C4	
Father: A3 A24 B7 B12 C2 C4 **Mother**:A2 A32 B7 B35 C4 C5 **Child**:A2 A3 B7 B7 C4 C4	
Father: A2 A2 B5 B38 C3 C4 **Mother**: A11 A26 B12 B18 C1 C2 **Child**: A2 A26 B18 B38 C2 C3	
Father: A28 Aw33 B14 Bw40 C3 C3 **Mother**: A11 A29 B15 Bw40 C3 C5 **Child**: A11 A28 B15 Bw40 C3 C3	

19. The HLA system is genetically determined by genes that are in complete linkage, so HLA haplotypes are transmitted from parents to offspring as a unit. Therefore, HLA antigen typing is used in paternity disputes. In this task, only HLA-A and HLA-B antigens were determined serologically. Complete the HLA genotypes and haplotypes and decide whether the indicated man can be the biological father of the child or not.

Phenotypes	HLA genotypes	HLA haplotypes
Mother: A3,7; B7 Child: A1,3; B7,8 Indicated man: A1,24; B8,12		

Conclusion:

Mother: A2,32; B13,35 Child: A1,32; B8,35 Indicated man: A2; B7,13		

Conclusion:

Mother: A11,36; B8 Child: A3,11; B5,8 Indicated man: A1,3; B5		

Conclusion:

Mother: A1,2; B7 Child: A1,2; B7 Indicated man: A2; B7,8		

Conclusion:

20. The parents' HLA haplotypes are HLA-A2B9/A4B7 and HLA-A3B14/A9B5. Determine all possible HLA haplotypes of their children.

21. A defect in enamel formation is determined by a dominant allele (D) with a locus on an autosome. Individuals with this defect have yellow-brown teeth with thin, soft enamel. A marriage was contracted between a man with blood group AB, N who was found to have this defect, and a healthy woman whose father had blood group AB, M and mother O, N, with both parents having normal enamel. What children can be expected from this marriage?

22. A marriage was contracted between a man with blood group O (genotype OO) and a woman with blood group B (genotype BB). They had monozygotic twin girls with blood group O. Write the possible genotypes of all family members and explain.

23. What is a possible explanation for the fact that fetal erythroblastosis caused by incompatibility in the Rh system occurs more frequently when the mother and child are compatible in the ABO system (e.g., father O, mother AB) than when there is incompatibility in the ABO system (e.g., father AB, mother O)?

24. A marriage was contracted between a non-secretor man, Le(a+b−), and a secretor woman, Le(a−b−). They had three children:

a) Le(a+b-)
b) Le(a-b-)
c) Le(a-b+)
d) Write the genotypes of all family members for the secretor status of ABO blood group antigens and for the Lewis blood group system.

25. Which of the listed genotypic combinations of the Rh system in married couples can cause incompatibility between mother and fetus?

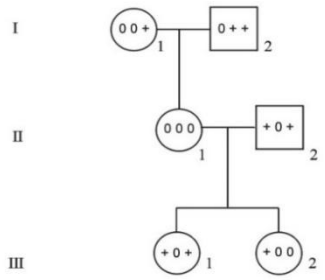
Rh haplotype of the woman		Rh haplotype of the man	Result
1.	cDe/Cde	cDe/cde	
2	CDE/cDe	cde/cde	
3.	CdE/Cde	CDe/CDe	
4.	cdE/Cde	cDE/cde	

26. A marriage was contracted between two secretors, both with the same phenotype **Le(a−b+)**. They had two children:

1. Le(a+b-)
2. Le(a-b-)

a) Write the genotypes of all family members for the secretor status of ABO blood group antigens and the Lewis blood group system.
b) Determine and list all expected genotypes in the offspring of the given spouses for the secretor status of ABO blood group antigens and the Lewis blood group system.
c) List all expected phenotypes in the offspring of the given spouses.
d) What is the probability that their next child will be Le(a−b+)?

27. In the pedigree symbols, the reaction of each person with antibodies is indicated in the following order:
anti-A
anti-B
anti-D
Write the genotypes and phenotypes of the individuals for the ABO and Rh blood group systems. (0 = negative reaction, + = positive reaction).



19. Population Genetics

Population genetics deals with the distribution and frequency of alleles and genotypes in a population, as well as factors such as selection, migration, mutations, chance, and inbreeding that influence these frequencies. The Hardy–Weinberg law describes an equilibrium population without external influences, i.e., without mutations, selection, or migration, under random mating and a large effective population size without genetic drift. In reality, these conditions are rarely fully met, but the law is useful as a model. It has applications, for example, in monitoring changes over time—evolution, epidemiology, forensic genetics, and pharmacogenetics.

Questions and Tasks

1. The incidence of albinism (autosomal recessive, AR) in the Caucasoid–Europoid population is 1 : 33,000. Calculate the frequency of:
a) the recessive mutant allele
b) the normal dominant allele
c) heterozygotes in the population
d) a cross between two heterozygotes.

2. Based on the incidence of rare autosomal recessive diseases in the population, calculate the percentage of heterozygous carriers of mutant alleles in the given populations.
a) 0,0064
b) 0,01
c) 0,09
d) 0,10
e) 0,000081

3. If 4% of individuals in a certain population in genetic equilibrium express a recessive trait, what is the probability that the trait will appear in the offspring of two individuals who do not show the trait?

4. In a certain population, three genotypes occur with the following frequencies: AA 0.81; Aa 0.18; aa 0.01.
a) What are the frequencies of alleles A and a?
b) What will their frequencies be in the next generation?
c) What proportion of all matings in this population will be Aa × Aa?

5. Cystic fibrosis is a relatively common autosomal recessive disease with a frequency of 1/2000 newborns. What is the approximate frequency of heterozygotes (carriers) for cystic fibrosis in this population?

6. Thalassemia, a malignant anemia, also occurs in a relatively closed Southern Italian immigrant population in the USA. In how many members of this population (%) can we expect the mild form of the disease (genotype Tt), if 4% of newborns die from the severe form (tt)?

7. The ability to taste the bitter compound PTC (phenylthiocarbamide) is determined by the dominant allele T, while recessive homozygotes are "non-tasters." Among 125 students, 88 were PTC tasters and 37 did not perceive the bitter taste of PTC. What are the allele frequencies of T and t in this population? What are the frequencies of the individual genotypes?

8. Tay–Sachs disease is caused by a gene mutation on chromosome 15 leading to a deficiency of the lysosomal enzyme hexosaminidase A and is inherited in an autosomal recessive manner. While in the general population the incidence of this disease is 1 : 360,000, in the Ashkenazi Jewish population (mainly of Central and Eastern European origin) the incidence is 1 : 3,600. What is the frequency of carriers of the mutant allele in this population?

9. Achondroplasia is an autosomal dominant disorder that results in dwarfism in affected individuals. In a population of 50,000 individuals, 5 cases of achondroplasia were identified. Three of the affected individuals also had affected parents, while two of the affected individuals had parents of normal stature. Calculate the frequency of the dominant allele for achondroplasia in this population.

10. Calculate the genotype frequencies of AA, Aa, and aa after one generation of random mating, if in the current population the frequencies are AA = 0.2; Aa = 0.6; aa = 0.2. What will be the frequencies of the individual genotypes after two generations of random mating?

11. a) The incidence of brachydactyly in a population is 1 in 10,000. What is the frequency of the allele for brachydactyly (an autosomal dominant disorder) in this population?
b) The incidence of phenylketonuria (an autosomal recessive disorder) in the same population is 1 in 10,000. What is the frequency of the allele for phenylketonuria and the frequency of heterozygous carriers of this disease in the given population?
c) The frequency of classical hemophilia (X-linked recessive inheritance) in men is 1 in 10,000. What is the frequency of the allele for hemophilia in the population? What is the frequency of heterozygous women?

12. Hereditary methemoglobinemia is genetically determined in an autosomal recessive manner. Among the indigenous inhabitants of Alaska, it occurs at a frequency of 0.09%. Determine the genetic structure of the analyzed population with respect to the gene for methemoglobinemia.

13. In a study of the inhabitants of southern Poland, 11,163 individuals were found with blood group M, 15,267 with blood group MN, and 5,134 with blood group N. Determine the allele frequencies of M and N in the population of southern Poland.

14. The Kidd blood group system is determined by two alleles, K^a and K^b. The K^a allele is dominant over K^b, and individuals who carry it in their genotype are designated as Kidd-positive. The frequency of the K^a allele among the inhabitants of Krakow is 0.458. The frequency of Kidd-positive individuals among Black populations is 80%. What is the genetic structure of both populations for the Kidd blood group system?

15. In a population of 376 Navajo Indians, 305 individuals with blood group M, 67 with MN, and 4 with N were found.
a) Is this population in genetic equilibrium?
b) Calculate the allele frequencies of M and N.
c) What is the probability that a child of a mother with blood group N will have the same phenotype?

16. The Duffy blood group system is determined by three alleles at a single locus: Fy^a, Fy^b, and Fy. The Fy allele is recessive and occurs only in Black populations. Fy^a is codominant with Fy^b. The frequency of individuals with the Fy^a antigen is 74.53% in the Russian population, 66.46% in Italians, and 69.9% in Poles. What are the allele frequencies of Fy^a and Fy^b in these populations?

17. The type of earlobe is a monogenic trait with complete dominance between the dominant and recessive alleles. Dominant homozygotes and heterozygotes have an earlobe separated by a cleft—free (non-attached) type. Recessive homozygotes have an attached (convergent) earlobe. In the population of Prague, which can be considered panmictic, 1,000 individuals with the attached type and 11,500 with the free type were found. Calculate:
a) the frequency of the recessive and dominant alleles in the given population,
b) the frequencies of the individual genotypes in the given population.

18. For simplification, assume that congenital hip dislocation is determined in an autosomal dominant manner with 25% penetrance. The disease occurs with a frequency of 6 : 10,000. Determine the number of recessive homozygotes.

19. Podagra (gout, arthritis urica) occurs in 2% of people and is assumed to be autosomal dominant trait. In women, the gout gene is not expressed; in men, its penetrance is 20%.
a) Determine the genetic structure of the population for the analyzed trait based on the given information.
b) What is the frequency of gout in another population in which the frequency of the dominant allele is 60%? The penetrance remains unchanged in this population.

20. In a region with 500,000 inhabitants, 4 cases of alkaptonuria (autosomal recessive disease) were registered. Calculate the number of heterozygotes for the analyzed trait in this population.

21. In a population, one individual with a rare type of skin albinism (spotted depigmentation of the skin) occurs among 15,000 normally pigmented people. This is an autosomal dominant disorder. Determine the frequency of the dominant allele (A) and the frequency of heterozygotes (Aa).

22. Assume that ocular albinism (depigmentation of the ocular fundus) is an X-linked recessive disorder. Calculate the frequency of female carriers of the pathological allele in a population where there is one man with ocular albinism for every 1,000 men with normal ocular fundus.

23. Gout is determined in an autosomal dominant manner. The penetrance of the dominant allele is 20% in men and 0% in women.
a) What is the probability that a child affected by gout will be born in a family where both parents are heterozygotes?
b) What is the probability that a child affected by gout will be born in a family where one parent is a heterozygote and the other one is a recessive homozygote for this trait?

24. Arachnodactyly is inherited in an autosomal dominant manner with 30% penetrance, while left-handedness is autosomal recessive with complete penetrance. What is the probability that both anomalies will occur simultaneously in the children of a family where both parents are heterozygous for both genes?

25. According to data from Swedish geneticists, some forms of schizophrenia are determined by an autosomal dominant pattern of inheritance. The penetrance of the dominant allele is 100% in homozygotes and 20% in heterozygotes.
a) Determine the probability that affected child will be born in a family where one parent is a heterozygote and the other is a recessive homozygote.
b) Determine the probability that affected child will be born in a marriage between two heterozygotes.

26. In a certain isolated population, all 800 members have blood group O. In another population, all members have blood group A (genotype AA). What will be the frequency of these blood groups after one generation of random mating, if 200 members of the second population are added to the first population?

27. If approximately 10% of men are affected by colour blindness (daltonism - XR), how many women with impaired colour vision can be expected in the population?

28. Which of these populations is in Hardy–Weinberg equilibrium?
a) AA – 0,70, Aa – 0,21, aa – 0,09
b) M – 0,33, MN – 0,34, N – 0,33
c) 100 % MN
d) AA – 0,32, Aa – 0,64, aa – 0,04
e) AA – 0,64, Aa – 0,32, aa – 0,04

For each population, calculate the expected genotype distribution after one generation of random mating.

29. The incidence of colon polyposis in our population is approximately 1 in 8,000. The disease is inherited in an autosomal dominant manner. What is the frequency of the dominant allele in the population?

30. Torus palatinus is among anomalies in the shape of the palate. It is a benign tumor on the oral side of the hard palate that can cause speech disorders. This autosomal dominant anomaly occurs in 9% of the population.
a) What will be the average frequency of marriages between individuals in whom torus palatinus occurs?
b) If two individuals with a normal palate marry, what is the probability that they will have a child with the anomaly?

31. Palatoschisis (isolated cleft palate) has an incidence of 1 in 2,500 healthy individuals in the population. It is inherited in an autosomal dominant manner. Calculate the frequency of the mutant allele in this population, and the frequencies of heterozygotes and dominant homozygotes in this population.

32. Diastema (a gap between the canine and incisor) is inherited in an autosomal dominant manner. In the population, 10% of individuals have this trait. Calculate the frequency of the dominant allele in the population and the frequencies of heterozygotes and dominant homozygotes in this population.

33. One form of dwarfism is known as Ellis–van Creveld syndrome. Affected individuals exhibit chondrodysplastic short stature, shortening of the long bones on X-ray, bilateral polydactyly, polymetacarpia, hypoplasia of teeth and nails, congenital heart defects, etc. In a certain community, 5 affected individuals were found per 1,000 newborns, while in the entire population consisting of 8,000 individuals the incidence was 2 : 1,000. All affected individuals had parents of normal stature. What is the most likely mode of inheritance? Calculate the frequency of the mutant allele in the population and in the given community, and the carrier frequency assuming the conditions of the Hardy–Weinberg law are met.

34. The Rh blood group system is primarily determined by two alleles, D and d. Allele D is dominant over d, and individuals who carry it in their genotype are designated Rh positive. In a certain population, 16% of citizens are Rh negative.
a) Determine the genetic structure of the given population.
b) Calculate the frequency of marriages between an Rh-negative woman and an Rh-positive man in which every child is at risk of erythroblastosis fetalis.
c) In another population, 90.1% of individuals are Rh positive. Calculate the same characteristics as in the previous population and compare the risk of children being affected by erythroblastosis fetalis.

20. Genealogy

Genealogy (Pedigree Analysis) is the science that studies the origins of individuals, families, and their mutual relationships. Its goal is to compile a family tree (**pedigree**) – that is, to map bloodlines, determine the geographical or ethnic origin of a family, clarify family history, or find relatives and the occurrence of hereditary diseases. The basis of genealogical research is the collection of data from various sources, such as parish registers; supplementary sources may include land registers, censuses, lists of serfs, documents, old photographs, letters, or chronicles, as well as online genealogical databases (e.g., FamilySearch, Geni, MyHeritage), which allow searching records and creating family trees.

By means of mitochondrial DNA (mtDNA) tests, the maternal line can be traced, while Y-DNA tests (available only for men) serve to track the paternal line. Autosomal DNA is used to search for current relatives going back 5–7 generations. Genealogical research can take different forms. An **ascending family tree** traces the ancestors of a given individual, while a **descending tree** focuses on the descendants of a particular person. The results can be presented graphically (as a tree) or in textual or tabular form.

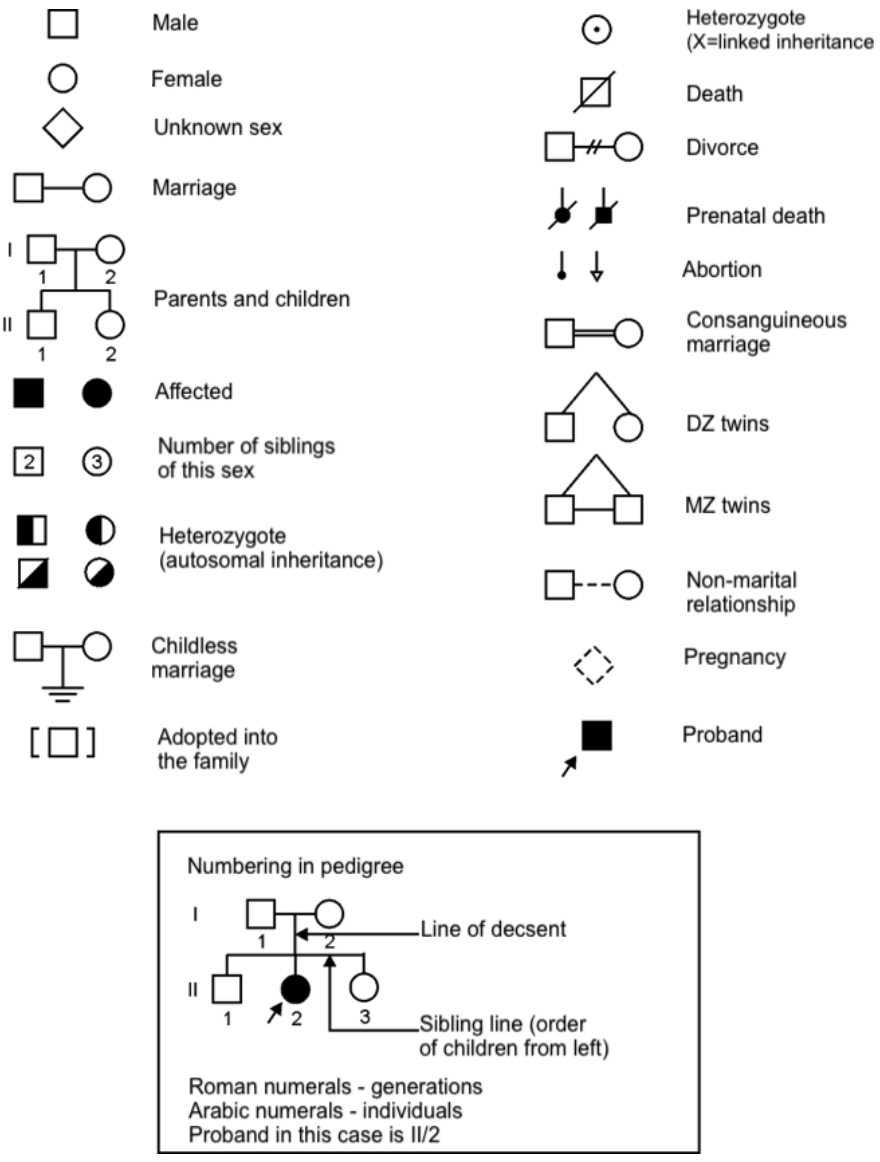
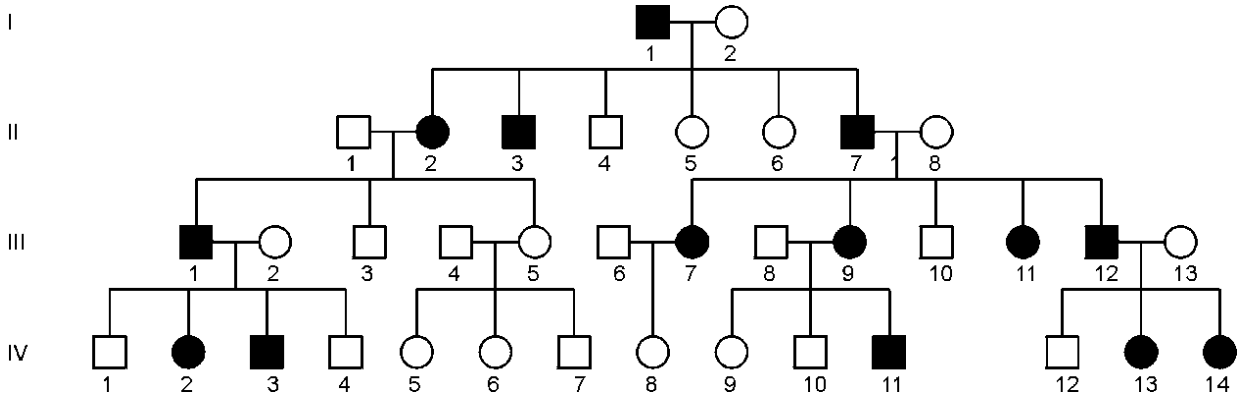
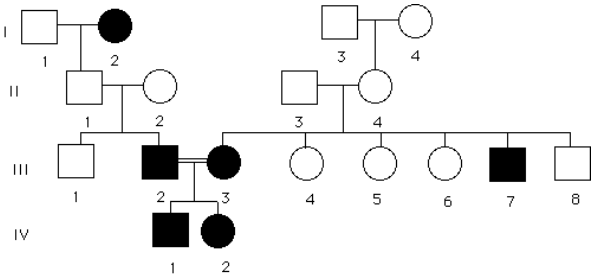


Figure 20-1. Standard genealogical symbols.

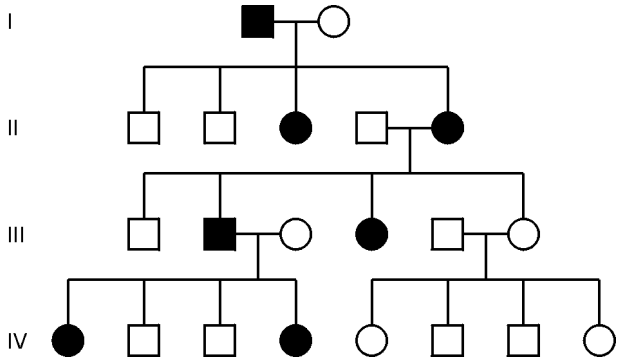
Example of a pedigree with an autosomal dominant inheritance (AD)



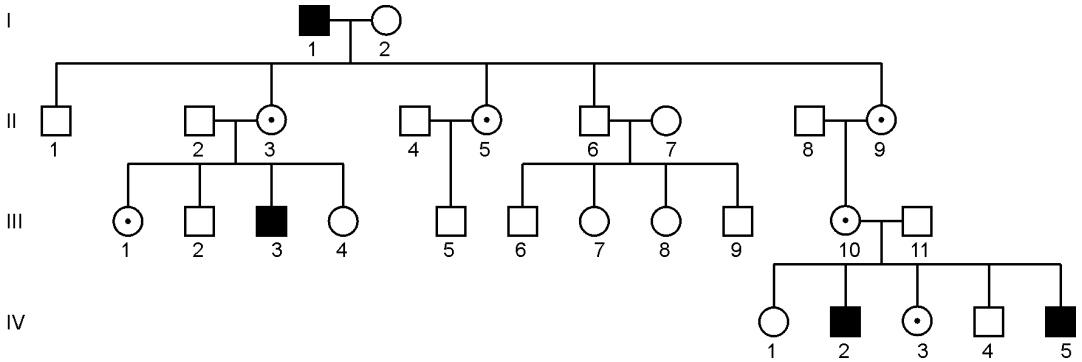
Example of a pedigree with an autosomal recessive inheritance (AR)



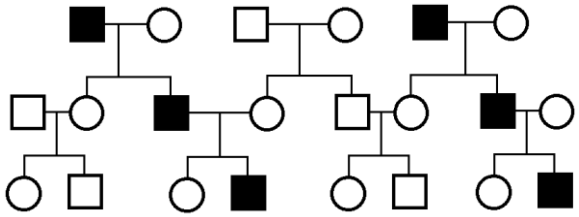
Example of a pedigree with an X-linked dominant inheritance (XD):



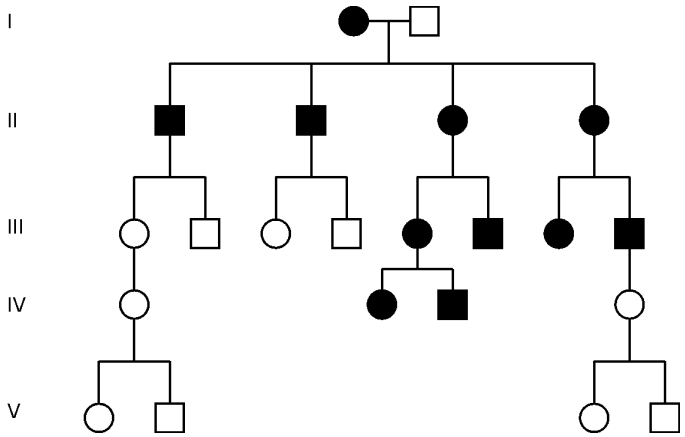
Example of a pedigree with an X-linked recessive inheritance (XR)



Example of a pedigree with holandric (Y-linked) inheritance



Example of a pedigree with mitochondrial (maternal) inheritance



Questions and Tasks

1. Create a genealogical chart (pedigree) of your own family covering at least three generations, capturing as many relatives as possible.

2. Analyze the pedigrees of families with the occurrence of the following diseases and determine the type of inheritance for each pathological condition:

a) Galactosemia

b) Dentinogenesis imperfecta

c) Ocular albinism (type I)

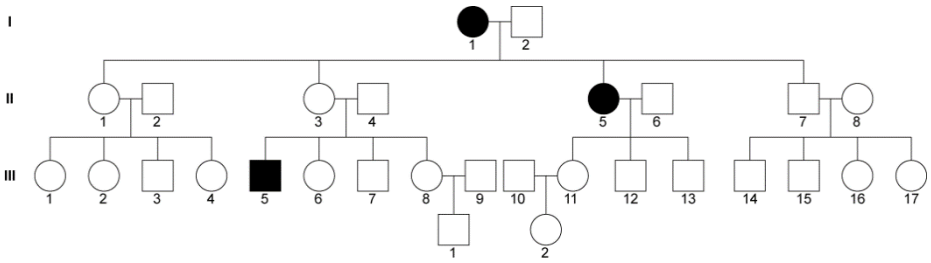
d) Huntington's chorea

e) Leber's hereditary optic neuropathy (blindness)

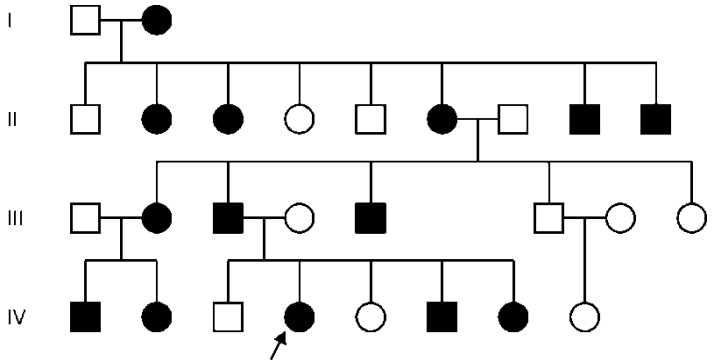
f) Phenylketonuria

g) Hypercholesterolemia

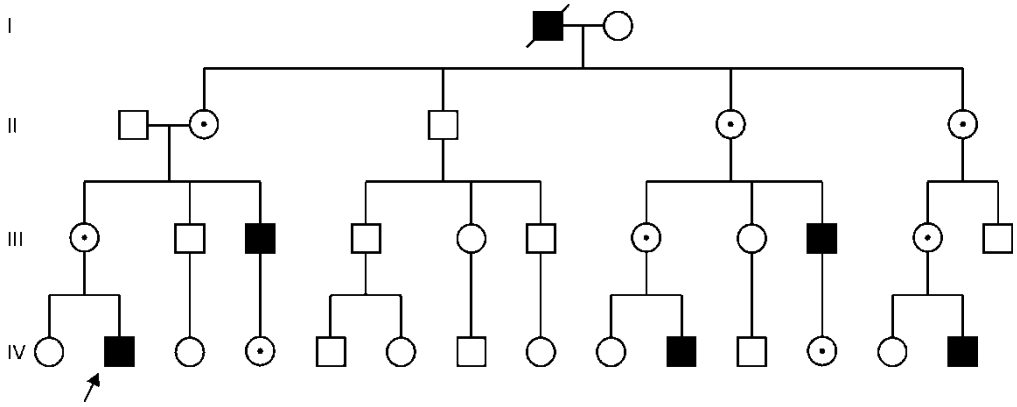
a)



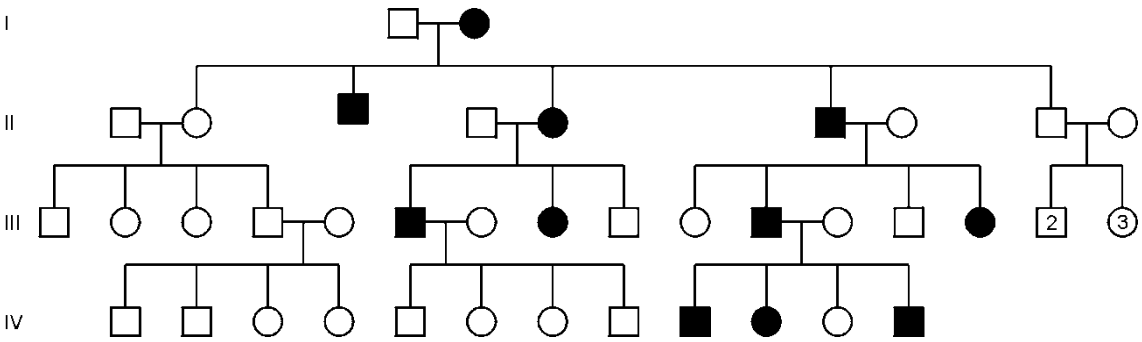
b)



c)



d)



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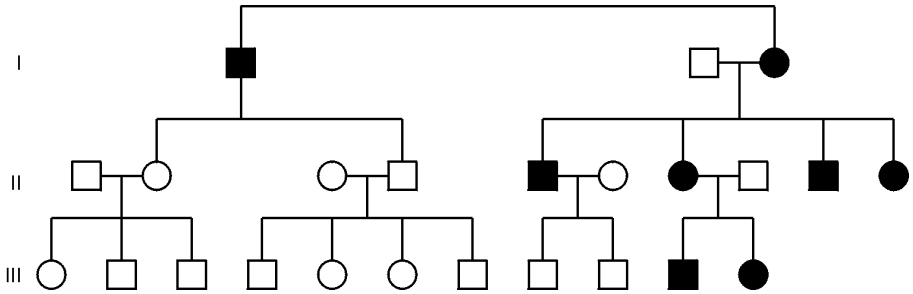
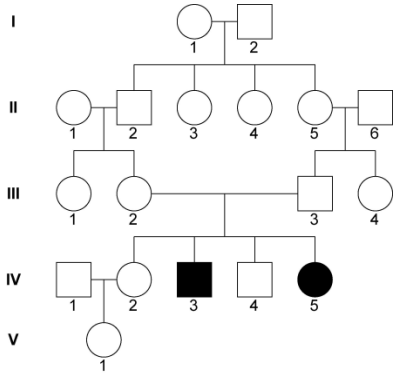
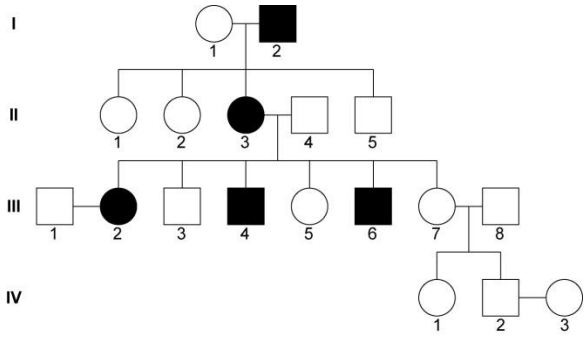


f)

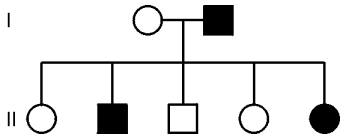


g)



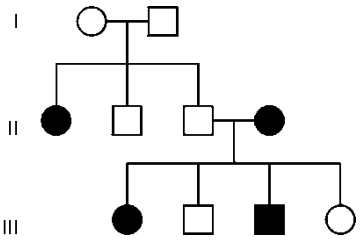
3. Draw a pedigree of a healthy boy, who has healthy parents and a brother who died from Tay–Sachs disease (autosomal recessive trait). What is the probability that he is a carrier of the recessive allele (heterozygote)?

4. In the following pedigree we study an autosomal recessive trait.
a) What is the genotype of the mother?
b) What is the genotype of the father?
c) What are the genotypes of the children?
d) What is the risk of having affected children?

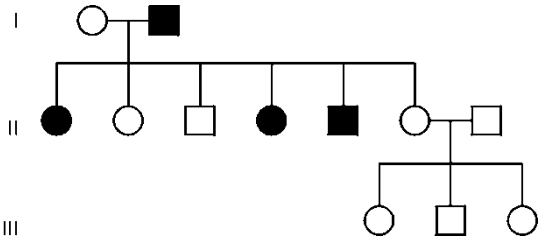


5. Analyze the following two pedigrees and determine whether the given trait is recessive or dominant.

a)



b)



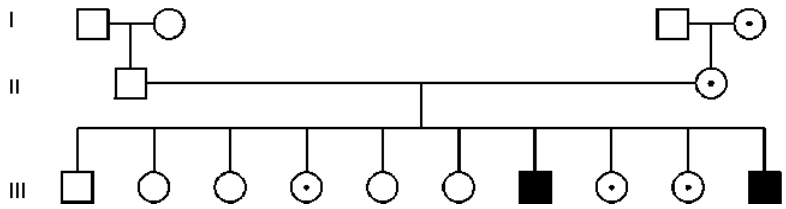
6. A woman whose brother was affected with cystic fibrosis (mucoviscidosis, a disorder of exocrine gland function, AR) married her maternal cousin whose sister is also affected with cystic fibrosis.
a) Draw the pedigree of this family.
b) State the probability that an affected child will be born.
c) What are the genotypes of the parents of both mother and father with respect to cystic fibrosis?

7. The proband is a man who plans to marry his cousin. The man's father and the woman's mother are siblings and have shortened finger phalanges (brachydactyly, AD). Their mother was also affected. The engaged couple have normal finger length. Draw the pedigree of this family and determine the risk of having children with brachydactyly in this marriage.

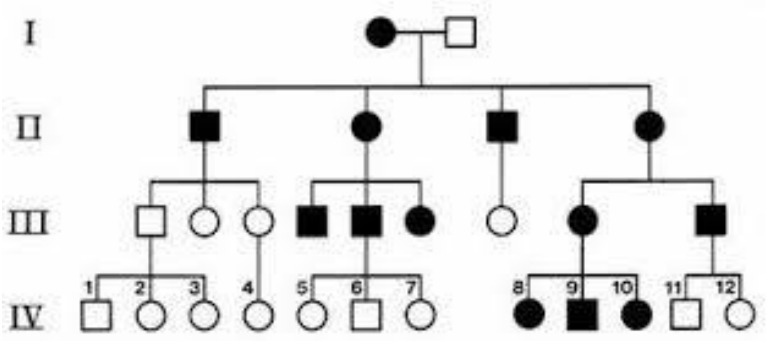
8. Draw the pedigree of a woman whose maternal cousin (their mothers are siblings) died in childhood from Lesch–Nyhan syndrome (X-linked recessive inheritance).
a) What is the probability that this woman is a carrier of the recessive allele for this disease?
b) What is the probability that, in a marriage with a healthy man, she will have an affected child?

9. Analyze the given pedigrees and determine the probable modes of inheritance of the traits under study.

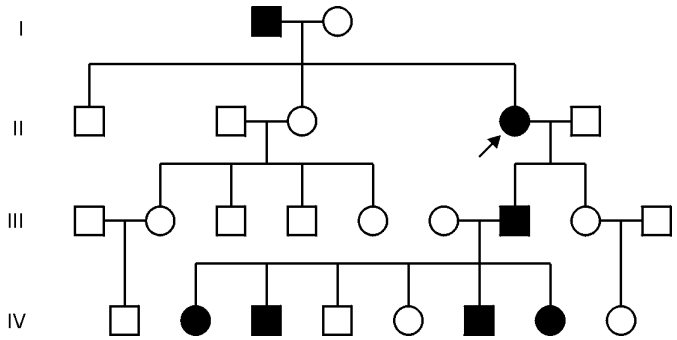
pedigree 1



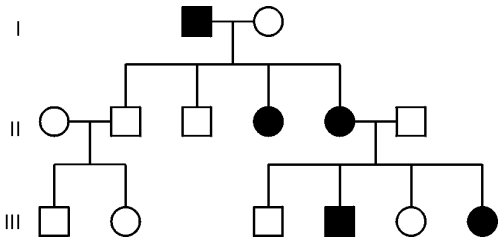
pedigree 2



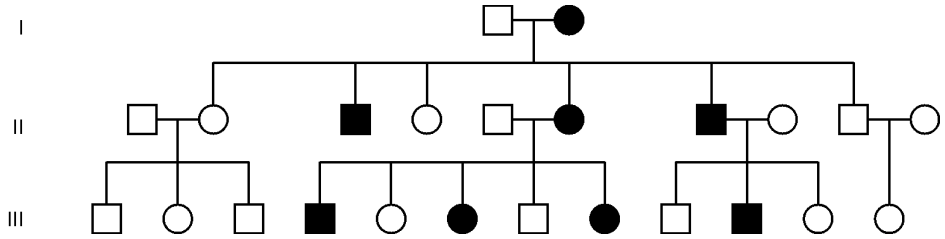
pedigree 3



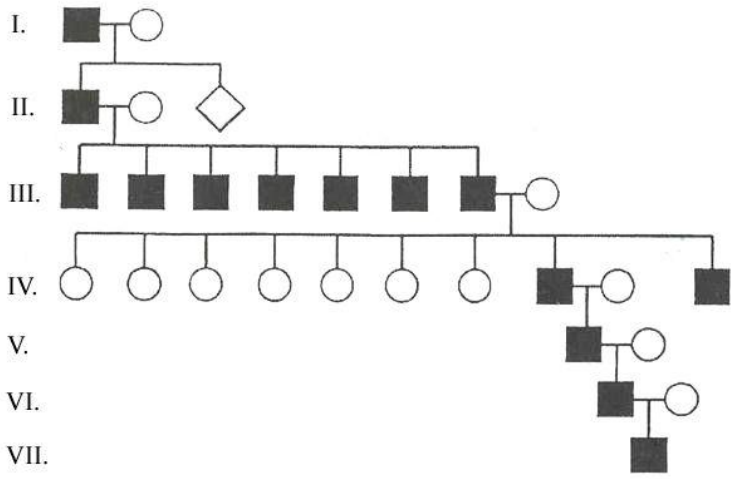
pedigree 4



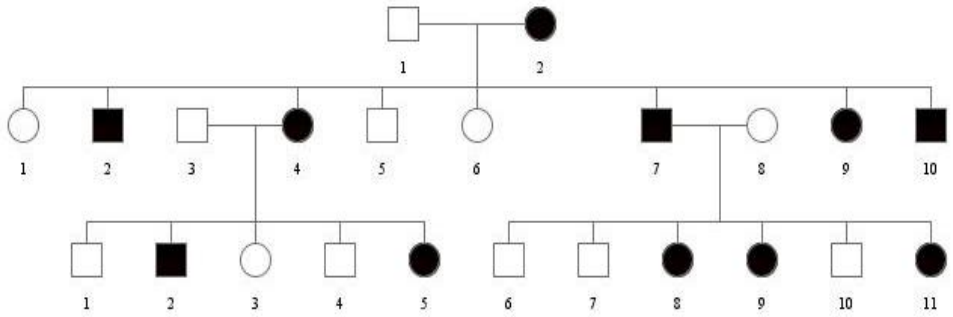
pedigree 5



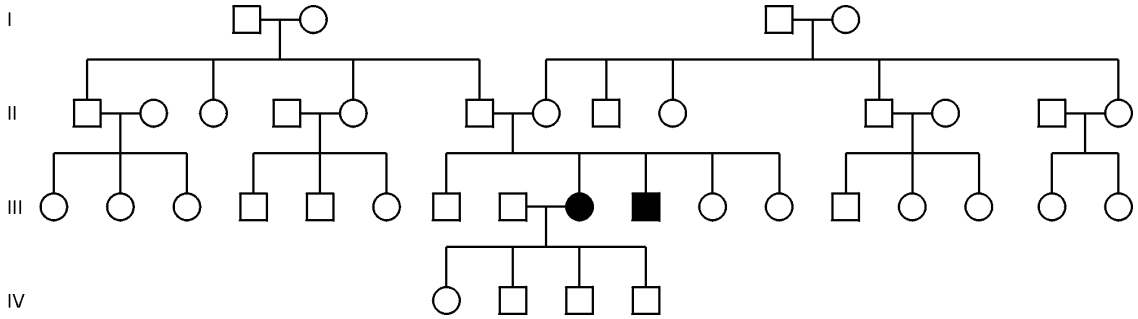
pedigree 6



pedigree 7



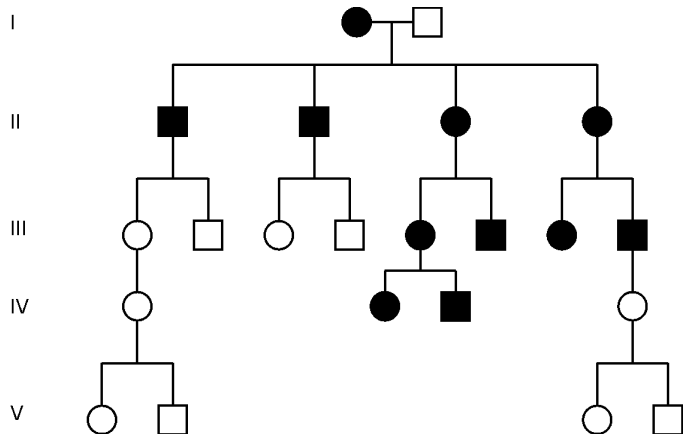
pedigree 8



10. Tay–Sachs disease is an autosomal recessive metabolic disorder that results in the death of the affected individual by the age of two. Phenotypically normal parents ask about the risk of this disease occurring in their children because the man had a first cousin (on paternal side) who died of the disease, and the woman had an affected uncle (on maternal side).
a) Draw the pedigree of this family.
b) What is the probability that both parents are carriers of the mutant allele?
c) What is the probability that neither of them is a carrier?
d) What is the probability that they will have an affected child?
e) What is the probability that one parent is a carrier and the other is not?

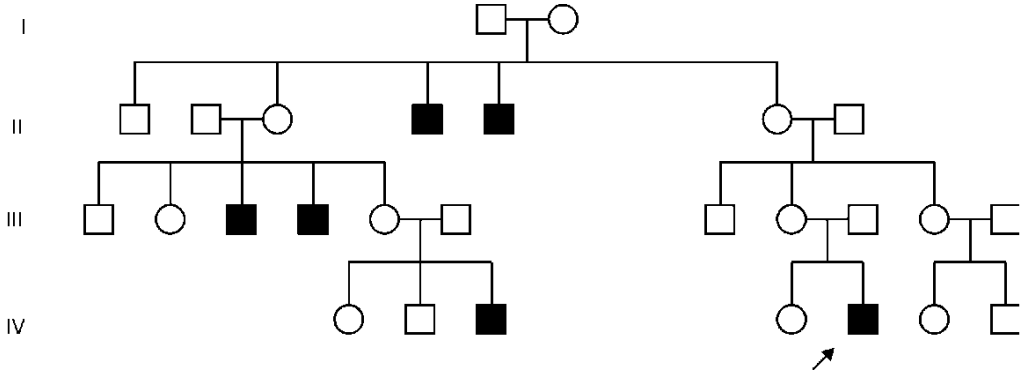
11. Perceptive (sensorineural) hearing loss is an autosomal dominant (AD) disease which arise from an inner ear defect. Two healthy individuals were married. The woman's mother is affected and her father is healthy. Both of the woman's brothers are affected. The man's father has this defect, his mother is healthy, and his sister is affected.
a) Draw the pedigree of this family.
b) What is the probability that the couple will have an affected child?
c) If the affected sister of the man marries a healthy man, what is the probability that they will have an affected child?

12. The following pedigree illustrates the inheritance of Leber's hereditary optic neuropathy, which causes blindness in adulthood.



a) What type of inheritance is involved in this rare disease? Justify your answer.
b) Suppose woman V-1 marries an affected man. With what probability will their offspring be affected by this disease?
c) Suppose woman IV-2 marries a healthy man. With what probability will their sons be affected by this disease? What is the probability that their daughters will be affected?

13. The figure shows a pedigree of a family with the occurrence of Duchenne muscular dystrophy. Determine the type of inheritance.

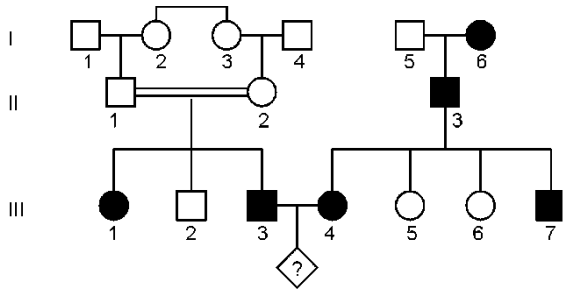


14. Marfan syndrome is caused by an autosomal dominant allele on chromosome 15 with 80% penetrance. It is a hereditary connective tissue disorder resulting in skeletal (mainly thoracic), CNS, cardiovascular, ocular, pulmonary, and other abnormalities. An affected woman and a healthy man married. Relatives on the woman's side: mother healthy, father affected, brother affected. Relatives on the man's side: father healthy, mother affected, both of the man's brothers are affected. Illustrate this situation in a pedigree.
a) What is the probability that this couple will have an affected child?
b) If the woman's brother marries a healthy woman, what is the risk that their child will be affected?

15. A healthy woman married a healthy man. The woman's parents are healthy, but her maternal grandfather had daltonism (an X-linked recessive colour-vision disorder). The man's mother is healthy, but his father and grandmother are affected with daltonism.
a) What is the probability that this couple will have an affected son?
b) If they already have one colour-blind son, what is the probability that the next child will also be affected?

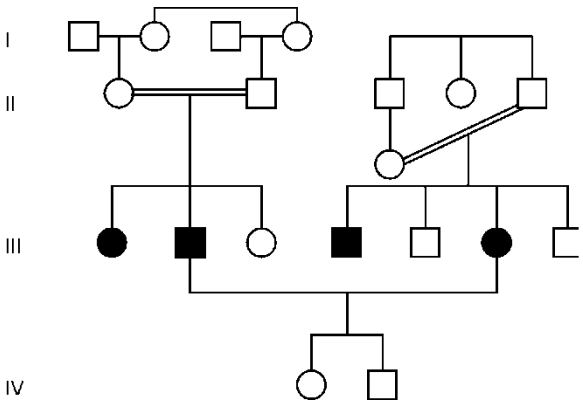
16. In galactosemia, the enzyme (galactose-1-phosphate uridyltransferase) which is required for galactose digestion is missing, causing galactose to be metabolized to galactitol, being toxic to the liver, brain, kidneys and eyes. If untreated, the disease is lethal. A healthy man whose brother and both parents are healthy, but whose maternal grandfather was affected by galactosemia, married a healthy woman whose parents were also healthy, but whose brother and sister were affected by this disease.
a) Draw the pedigree and determine the mode of inheritance.
b) What is the probability that the couple will have a child with galactosemia?
c) If the first child was born affected by this disease, what is the probability that the second child will be affected as well?

17. The pedigree shows a marriage between two individuals affected with hereditary deafness. What type of inheritance can be assumed for the husband III/3 and the wife III/4, and what risk does this entail for their offspring?

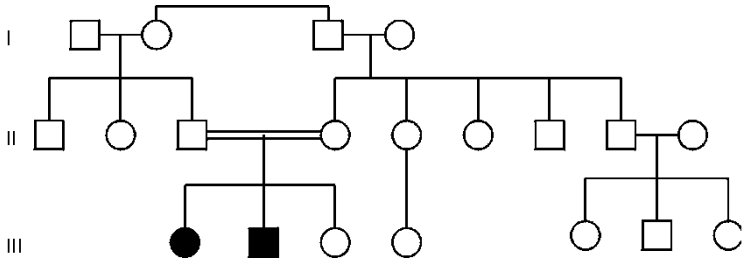


18. Determine the types of inheritance based on the analysis of the given pedigrees.

a)



b)



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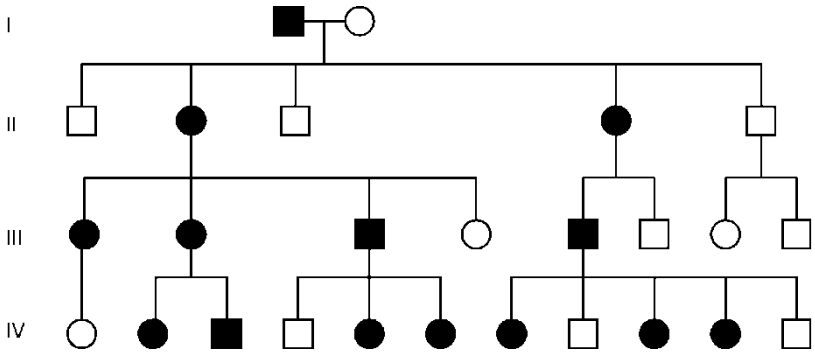

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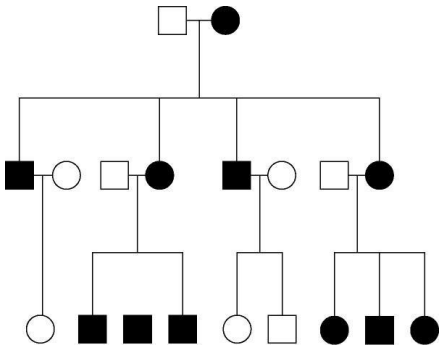
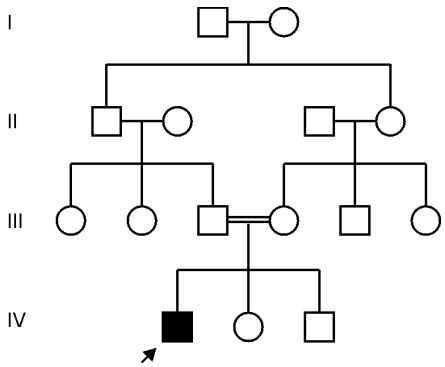
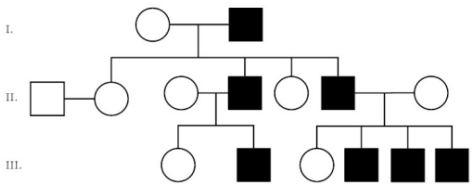


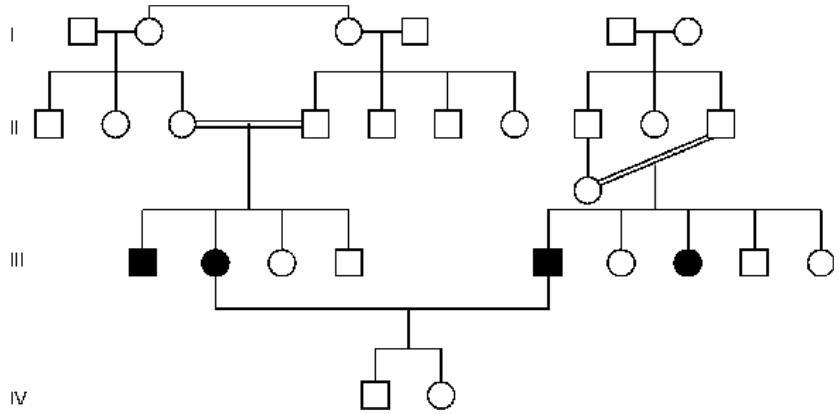
e)



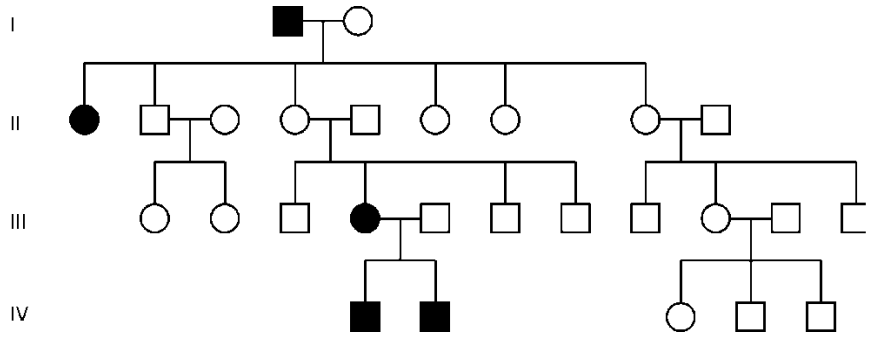
f)



19. Write the genotypes of individuals II/3, II/4, II/10, III/2, III/5, IV/1, and IV/2 for congenital deafness. How can the birth of normal-hearing children to two deaf-mute parents be explained?



20. In the picture, a pedigree is shown with the occurrence of polydactyly in humans. What type of inheritance is it and how can we explain the irregularities in the transmission of the gene in the given pedigree?



21. In the family of a man who wishes to marry a healthy woman, there is a defect in enamel formation and related health problems. The man is affected by this defect, as well as his brother, sister, and mother. Another sister is healthy and, with a healthy husband, has a healthy daughter and son. The affected sister has, with a healthy husband, one healthy and one affected daughter. The man's maternal grandfather is affected, while the grandmother is healthy and comes from a family in which this defect has never occurred. Both of the mother's brothers are also healthy.

a) Draw the pedigree and determine the mode of inheritance.

b) What is the probability that the affected man, who wishes to marry a healthy woman from a family without this defect, will have a healthy daughter?

c) What is the probability of having a healthy son?

22. A woman whose mother and brother are affected with albinism (AR) married an unrelated man whose sister is also affected by this disease.
a) Draw the pedigree.
b) What is the probability that the child of these parents will be affected with albinism?

23. A healthy woman and her affected husband have two daughters (affected Ann and healthy Beata) and two sons (healthy Cyril and affected Daniel). Ann and her affected husband have a healthy son Emil and an affected daughter Gabriela. Daniel married a healthy woman, with whom they have an affected daughter Hana and a healthy son Ivan.
a) Draw the pedigree.
b) Determine the most probable mode of inheritance of the disease.
c) Write the genotypes of the individual members of the pedigree.
d) What is the probability that Gabriela will have a healthy child with a healthy man?
e) What is the probability that Hana will have an affected daughter with a healthy man?

21. Genetic Counselling

Genetic counselling provides information, support and recommendations to people and families at risk of hereditary genetic disorders. Counselling is carried out by a clinical geneticist, but also by a genetic counsellor, with the aim of determining the risk of a genetic disorder in an individual or offspring, by explaining the mechanism of inheritance, proposing further action and providing psychosocial support. Counselling can be preconception, prenatal, postnatal, oncogenetic, and population based. It is recommended in cases of a family history of genetic disease, suspicion of a genetic disease, the occurrence of a congenital developmental defect, infertility, repeated miscarriages, consanguineous marriages and family planning after the age of 35. Methods or tools include clinical examination of the patient, construction of a pedigree, genetic tests (karyotyping, molecular biology methods, amniocentesis, methods of non-invasive prenatal testing without intervention in the fetus) and risk calculation.

Questions and Tasks

1. A couple planning to start a family comes to a genetic counseling center for information. Karol has been married once and had a child affected by cystic fibrosis (AR) with his first wife. His current fiancée Elena had a brother who died of cystic fibrosis. What is the probability that Karol and Elena will have a child with cystic fibrosis (neither of them has the disease)?

2. Galactosemia is an autosomal recessive (AR) metabolic disorder with a heterozygote frequency of 1:100 in the population. A man whose sister is affected by galactosemia is married to a healthy woman. What is the risk of this couple having an affected child?

3. Albinism is inherited in an autosomal recessive (AR) manner, with a heterozygote frequency of 1/50 in the population. A woman with normal pigmentation whose father had albinism wants to know the risk of albinism in her children. Her husband does not have pigment disorder.

4. A healthy couple wants to know the probability of having a son who will be colourblind. The wife's mother and the wife's brother have this colour vision disorder (X-linked recessive inheritance, XR).

5. Cystic fibrosis (CF) is an autosomal recessive disease (AR) of the pancreas and other exocrine glands with a heterozygote frequency (Aa) in the population of 1:25. A woman is about to marry a healthy man. Her brother died of cystic fibrosis; her parents are healthy. What is the probability that she will have an affected child?

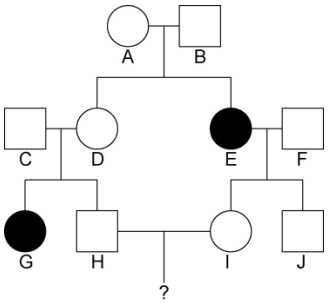
6. A woman with cataract (AD) married a healthy man whose parents and two sisters are also healthy. Draw a pedigree of this family and determine the prognosis for the children of the couple. Cataract has a penetrance of 80%.

7. Anna has oculocutaneous albinism (AR). Jana, the daughter of Anna's sister, married Rob, the son of Anna's brother. Jana and Rob have a son, Karol.
a) Draw a family tree for the above family.
b) What is the probability that Karol has albinism?
c) If Karol has albinism, what is the risk that Jana and Rob's next child will also have albinism?

8. What is the probability that a woman whose brother died in childhood from cystic fibrosis (an exocrine gland disorder, AR) will have an affected child if the frequency of healthy heterozygotes in the population is 1:25?

9. A healthy sister of an albino married an unrelated man and asks about the probability of having an albino child the frequency of the recessive allele for albinism in the population is 1%.

10. The following pedigree shows the occurrence of an autosomal recessive trait. If individuals H and I, who are first cousins, marry and have a child, what is the probability that the child will have the recessive trait?



11. Kate is pregnant for the second time. Her first child, Dan, has cystic fibrosis (CF). Kate has three healthy siblings, brothers Karol and Peter, and a sister Zuzana. Peter and Zuzana are single. Karol is married to Beth, and they have a healthy two-year-old daughter, Danka. Kate's mother has a sister, Barbora, who is the mother of Kate's husband Hugo (Kate and Hugo are first cousins). Hugo's family did not have CF.
a) Draw a pedigree for the above family.
b) What is the risk of cystic fibrosis for Kate and Hugo's next child?
c) Which individuals in the pedigree are obligate heterozygotes?

12. Karin and Noro are engaged, and both have siblings with sickle cell anemia. Neither of them has been tested for the sickle cell trait. Based on this incomplete information, calculate the probability that Karin and Noro's child will have sickle cell anemia.

13. What is the probability that albinism will occur in a child of parents with normal pigmentation if the grandfather of the child (father's father) had albinism? The recessive allele for albinism occurs in the general population in one in 50 healthy individuals (Aa). Draw the pedigree of this family.

14. A woman whose brother was affected by alkaptonuria (AR, a disorder of the enzyme that catalyzes the oxidation of homogentisic acid) married a man who is affected by the same disease. What is the probability that their child will have alkaptonuria? Draw this situation with a genealogical diagram.

15. The screening program to detect carriers for Tay-Sachs disease (AR, lack of the lysosomal enzyme hexosaminidase A) in the Ashkenazi Jewish population has found an incidence of about 0.035. Calculate:
a) The frequency of marriages that can produce an affected child.
b) The incidence of Tay-Sachs disease in the Ashkenazi Jewish population (the incidence in other populations is 1:360,000).

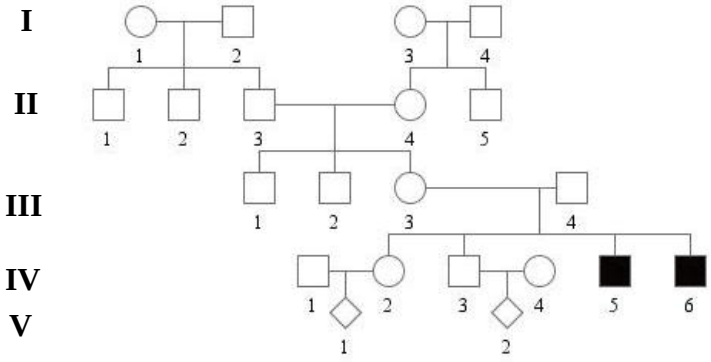
16. George, who has muscular dystrophy, married his cousin Dana (their mothers are sisters). The type of muscular dystrophy is not specified; the disease began to manifest itself at the age of 16 and is slowly progressing. There are no other known cases of muscular dystrophy in the family, but in the offspring of George and Dana, two sons are affected, one son is healthy, and two daughters are also healthy.
a) Draw a pedigree for this family.
b) What type of inheritance can we assume for this type of muscular dystrophy?
c) Now you have information that Dana's two brothers have the same disease; her three sisters are healthy. What is the most likely type of inheritance?
d) If George's affected son Peter asks about the risk of having an affected child, what can we tell him?

17. The fiancé and his fiancée are first cousins (their mothers are siblings). Since the mother of the fiancée had phenylketonuria (a disorder of the metabolism of the amino acid phenylalanine, AR), and so did the sister of the fiancé, the couple is concerned that their child may be affected by the same disease. Draw a genealogical diagram of this family and determine the risk of having an affected child.

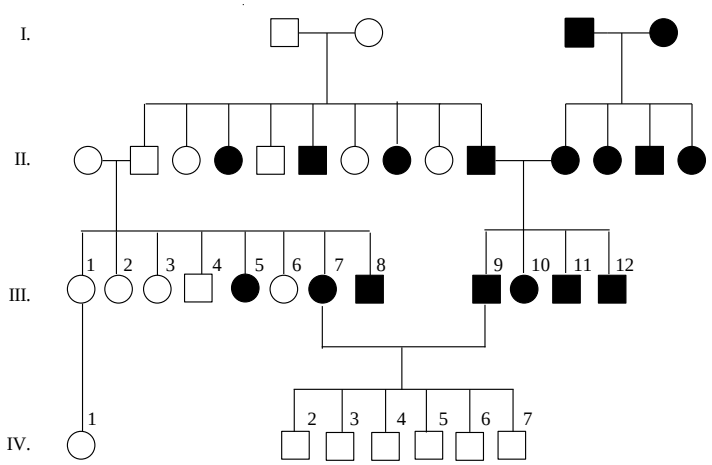
18. What is the probability that cousins' child will have hepatolenticular degeneration (Wilson's disease, copper deposition in the liver, AR) if the grandfather of cousins was affected? Make a genealogic scheme of the family.

19. A woman has a brother and maternal uncle who are affected by hemophilia A. She has two healthy sons, but since she plans to have more children, she is wondering about the risk of having affected offspring. Draw a pedigree for this family.

20. The Lesh-Nyhan (LN) syndrome is a serious metabolic disorder in which purines, metabolic biochemical precursors of DNA, accumulate in the nervous tissues and joints of people with the LN syndrome. This metabolic abnormality is caused by a deficiency for the enzyme hypoxanthine phosphoribosyltransferase (HPRT) which is encoded by a gene located on the X chromosome. Individuals deficient in this enzyme are unable to control their movement and unwillingly engage in self-destructive behaviour such as biting and scratching themselves. The males, labelled IV-5 and IV-6 in the following pedigree, have the LN syndrome. What are the risks that V-1 and V-2 will inherit this disorder?



21. In the following pedigree of individuals with congenital deafness, the marriage of I-3 with I-4 results in only deaf children, as does the marriage of II-10 with II-11. Conversely, the marriage of III-7 with III-9 produces only hearing children. Determine the probable genotypes of the children of the fourth generation. If one of these boys (IV-2) marries the daughter of woman III-1, what is the probability that their child will be deaf?



22. Daniel and his maternal grandfather, Boris, have classic hemophilia. Daniel's wife, Diana, is his cousin (their mothers are sisters). Daniel and Diana have one son, Ed, who has hemophilia, two daughters, Elena and Emilia, who also have hemophilia, and one healthy daughter, Erika.

a) Draw the family tree.

b) What is the probability that Elena's son will have hemophilia if she marries a healthy man?

c) What is the risk that Erika's son will have hemophilia if she marries a healthy man?

23. The affected woman and her healthy husband have two sons (healthy John and affected William) and a healthy daughter Lucia. John and his healthy wife have an affected daughter and a healthy son Matthew. Lucia and her healthy husband have an affected daughter Vera.

a) Draw the family tree.

b) Determine the most likely type of inheritance for the disease.

c) Determine the genotypes of the individual members of the family tree.

d) If Matthew marries Vera, what is the probability that they will have an affected child?

22. Molecular Biology Methods

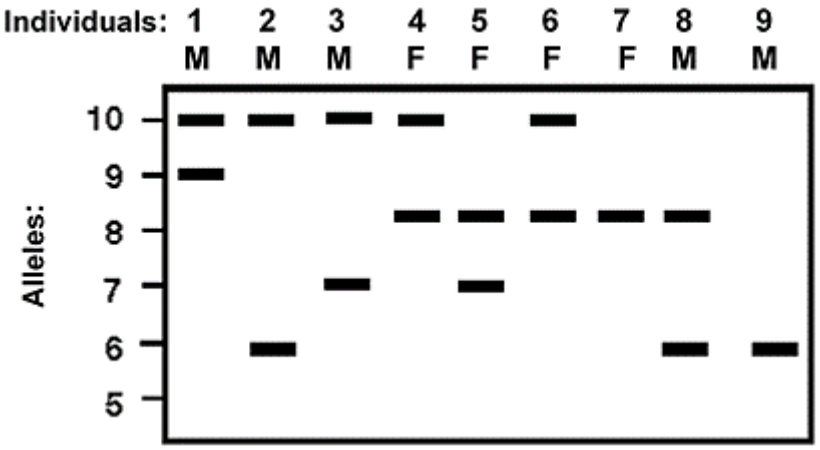
Methods of molecular biology are techniques for isolating, analyzing, manipulating, and modifying nucleic acids and proteins. Basic methods include extraction and purification of DNA, RNA, and proteins, PCR, sequencing, hybridization methods, electrophoretic separation of PCR products, cloning, genetic transformation and transfection, CRISPR genome editing (Clustered Regularly Interspaced Short Palindromic Repeats - a natural bacterial immune system capable of very precisely cutting DNA at a selected location), DNA chips, and gene expression analysis.

Questions and Tasks

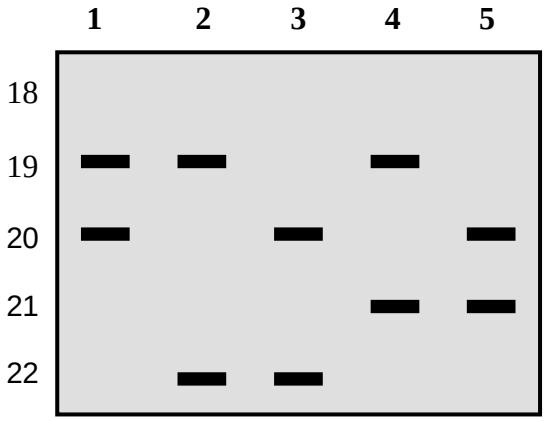
An example of the use of repetitive sections of nuclear DNA in forensic practice is the classic determination of paternity. Within the human population, a large number of different alleles can occur in the relevant gene loci, which arise as a result of different numbers of repetitions of certain sequences in them: in the case of STR loci, these are repetitive sequences up to 6 bp long, in the case of VNTR loci longer than 6 bp. This creates a huge number of different (differently long) alleles within the human population, which is a source of interindividual variability, since there is a high probability that the individuals being compared will differ from each other for a given locus. These polymorphisms are therefore widely used in the identification of individuals (in forensic practice as well as in other areas).

Each individual inherits one allele from the mother and one from the father, so the parent and child must have one identical allele. No one can have an allele that is not found in either parent – except for the very rare possibility of *de novo* mutation. In paternity disputes, the genotype at the relevant variable locus is determined simultaneously in the mother, the child and the designated man. If the marked man is to be the biological father of the child, he must meet the condition that the child has inherited one allele of the relevant gene from him. If the child does not have an allele in his genotype that the marked man, if he were the biological father, would have to pass on to him, then the marked man is excluded as a possible biological father. Confirmation or exclusion of paternity is carried out using several polymorphic systems (loci). For VNTR loci, this is usually 4 to 5, while for STR loci, which are less variable, it is usually more than 5. If none of the polymorphic loci excludes the paternity of the marked man, the probability with which the given man is the biological father of the child is determined. For this, it is necessary to know the frequencies (occurrence) of the given alleles in the population and then, by summing the probabilities for the individual examined loci, determine the overall probability of paternity. The rarer the allele in which the child matches the marked man and at the same time differs from his mother, the more it confirms the paternity of the marked man.

1. In 1992, two Russian amateur historians discovered a grave near Yekaterinburg in the Central Urals that contained the remains of the last Russian Tsar and his family. The grave contained a total of nine skeletons, three of which were children. A sex-determination test identified all three child skeletons as female. At the same time, only one of the adult skeletons (No. 7) was identified as female – the one that anthropological expertise identified as a possible Tsarina. Based on the presence of individual alleles of the variable STR locus TH01 (repeat of the AATG motif), determine which of the found skeletons may belong to the Tsar and which belong to unrelated persons – servants.

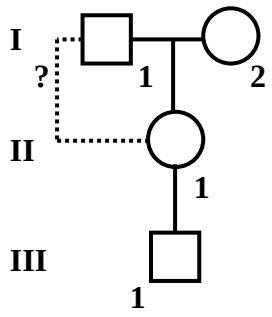


2. Family members (parents and three children) were examined for the VNTR polymorphism MCT118 (alleles arise as a different number of tandem repeats of a 16 bp sequence). Determine the genotypes of individual family members. Can family relationships be unambiguously determined based on the results given, i.e. is it possible to determine the parents and their children?



3. Based on the analysis of genotypes for VNTR and STR loci of individual members of the studied family, confirm or exclude the suspicion of incest (father - daughter).

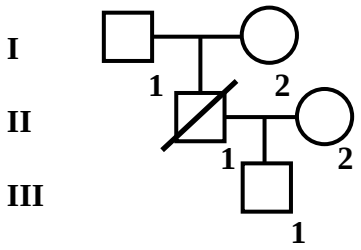
Pedigree of the studied family:



	Designation of family member			
Genotype	**I.1**	**I.2**	**II.1**	**III.1**
ApoB	35/45	37/45	35/37	35/35
D16S539	9/12	12/12	12/12	9/12
D7S820	11/11	8/11	8/11	11/11
D13S317	10/11	11/12	10/12	10/10
vWA	15/16	14/17	14/16	14/15
CSF1PO	11/11	10/11	10/11	11/11
TPOX	8/11	8/8	8/11	11/11
TH01	7/9	7/6	9/6	7/6

4. Another specific case of using repetitive sequences of the human genome is determining the kinship of a child of a deceased father from the genotypes of the father's parents. Since we do not have biological material of the deceased father (II.1), in order to determine paternity, it is necessary to determine the kinship of the child (III.1) from the genetic traits (alleles) of the grandparents (father's parents - I.1 and I.2).

The pedigree of the above family:



	Designation of family member			
Genotype	**I.1**	**I.2**	**II.2**	**III.1**
CSF1PO	10/10	10/11	10/12	12/11
TPOX	8/10	8/8	8/10	10/11
TH01	7/9	6/9	7/9	9/9
F13A01	5/6	5/7	6/6	6/7
vWA	17/18	14/18	15/16	15/18
D16S539	9/11	10/12	9/14	9/12
D7S820	10/11	8/12	8/11	8/11
D13S317	8/12	10/11	11/12	9/12

5. The DNA fragment sequence below contains a target site for a restriction endonuclease. How can you identify it? The table shows some restriction endonucleases and the sequences they

cleave. Find out which restriction enzyme it is and how exactly the DNA sequence will be cleaved.

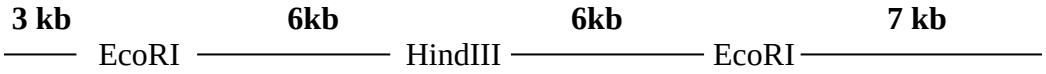
5′ G A A C T G G A A T T C C T G 3′
3′ C T T G A C C T T A A G G A C 5′

Microorganism	**Symbol**	**Sequence**
Haemophilus aegyptius	HaeIII	5′ G G/C C 3′ 3′ C C/G G 5′
Haemophilus haemolyticus	HhaII	G C G/C C/G C G
Escherichia coli	EcoRI	G/A A T T C C T T A A/G
Bacillus amyloliquefaciens	BamHI	G/GA T C G C C T A G/C
Providencia stuartii	PstI	C T G C A/G G/A C G T C
Brevibacterium albidum	BalI	T G G/C C A A C C/G G T

6. After digestion of a certain DNA with the restriction enzymes EcoRI, HindIII, and both together, DNA fragments with the following lengths were revealed on the electrophoretogram:
- after digestion with EcoRI alone, three bands with lengths of 3, 7, and 12 kb were detected;
- after digestion with HindIII alone, two bands with lengths of 9 and 13 kb were formed;
- after digestion with both enzymes, four bands with lengths of 1, 2, 7, and 12 kb appeared.

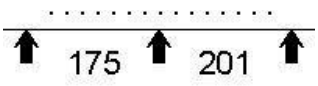
Determine and schematically sketch the restriction map of the given DNA.

7. On the examined DNA fragment with a length of 22 kb, assume the presence of restriction sites for EcoRI and HindIII in the following order:

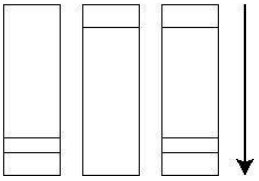


What bands (DNA fragments of what size) will be present on the gel after electrophoresis if the given DNA is digested with the enzyme EcoRI alone, HindIII alone, or both together?

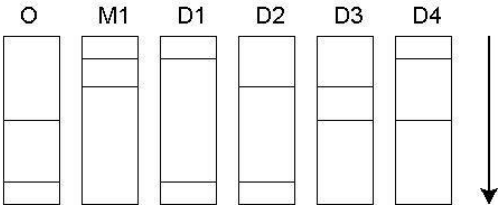
8. We have a probe that binds to a region of the beta globin gene that contains three target sites for the DdeI restriction enzyme (arrows in the picture).



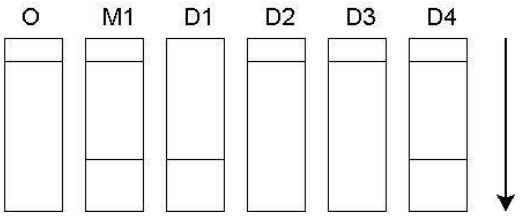
The probe therefore binds to two fragments of 175 and 201 bp. In sickle cell disease, a mutation (HbS) occurs in the beta globin gene that disrupts the middle target site. What change occurs? Match the electrophoretic separation patterns in the following figure to the three possible phenotypes (AA, AS, SS).



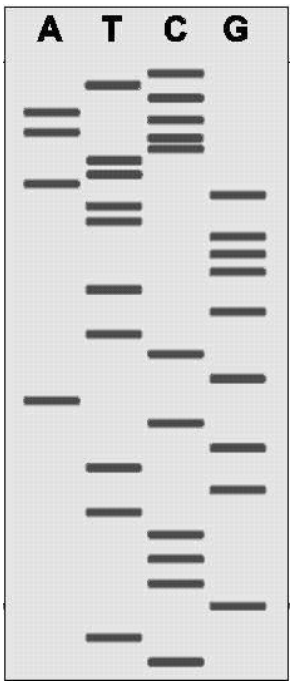
9. The result of the indirect DNA diagnostic analysis is presented as an autoradiogram of electrophoretic separation, hybridizing fragments are indicated by transverse lines. The shortest fragments travel furthest in the direction of separation (arrow).
a) This is an autosomal dominant inheritance. The father (O) and the first child (D1) are affected. Assess the situation and state the prognosis of the disability for children D2-D4.



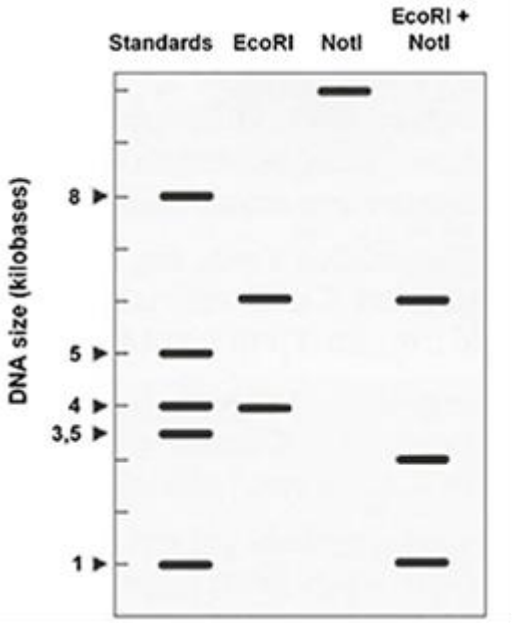
b) This is a gonosomal recessive inheritance. The parents are healthy, the first child (son) is affected. Assess the situation and state the prognosis of the disease for the next son D2 and for the daughters D3 and D4.



10. According to the electrophoretogram shown (picture on the right), determine the DNA sequence that was used for sequencing analysis. The four columns show the products in which ddATP (column 1), ddTTP (column 2), ddCTP (column 3) and ddGTP (column 4) were used.



11. A DNA molecule from E. coli was digested with two restriction endonucleases (EcoRI and NotI) and the products were separated electrophoretically. DNA fragments of known sizes were separated in the same gel and used as size standards (first lane from the left). The size of the standards is given in kilobases (kb). Determine the sizes of the obtained restriction fragments, construct a map of the original sequence and show the relative positions of all restriction sites.

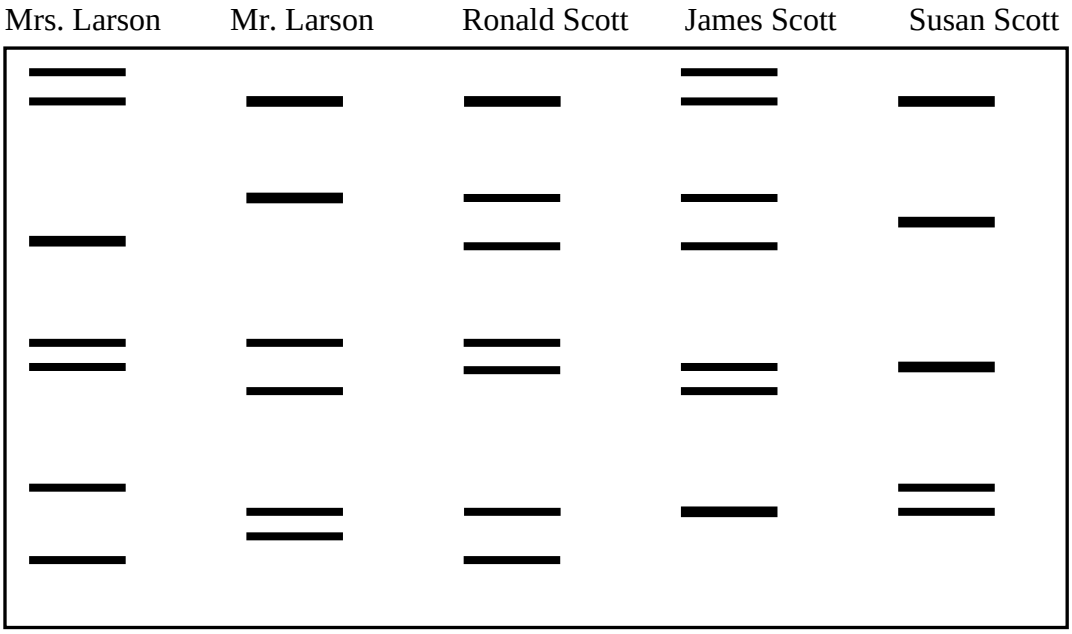


12. ROC is a hypothetical polymorphic STR locus in humans with the CAGA repeat sequence. There are four known alleles with 15, 12, 10 and 7 copies of the sequence.

a) Determine how many possible genotypes there are for these four alleles and list them.
b) If one parent is heterozygous for 15/10 and the other is heterozygous for 10/7, what possible genotypes can we expect in their children and in what proportions?
c) Three children with genotypes 10/10, 15/10 and 12/7 lived in the same house with the parents (b). What can you conclude from this?

13. Suppose that human DNA ($3x10^9$ bp) contains the same number of all four bases and that they are arranged in random order along the entire DNA molecule. We will cut it with a restriction endonuclease that recognizes a specific 6 bp sequence (e.g. GAATTC). What is the average length of the DNA fragments produced by the enzyme?

14. Bobby Larson was kidnapped at the age of 4 from a parked car in front of a supermarket in New Jersey in 1978. In 1990, a 16-year-old boy named Ronald Scott was found in California, presumed to be the kidnapped child. His parents, Susan and James Scott, were suspected of the kidnapping. Based on a court order, DNA analysis (determination and comparison of VNTR polymorphic loci) was performed on Ronald Scott, his current parents (the Scotts) and Bobby Larson's parents. After the forensic DNA analysis of the samples, a verdict was rendered. What do you think was the verdict based on the DNA analysis of the polymorphic VNTR loci shown in the picture?



15. The recognition site for the restriction endonuclease MstII is the sequence GGANTCC, where N can be any base. MstII also cleaves human DNA in the region encoding the 5th, 6th and 7th amino acids of the ß globin chain of normal hemoglobin:

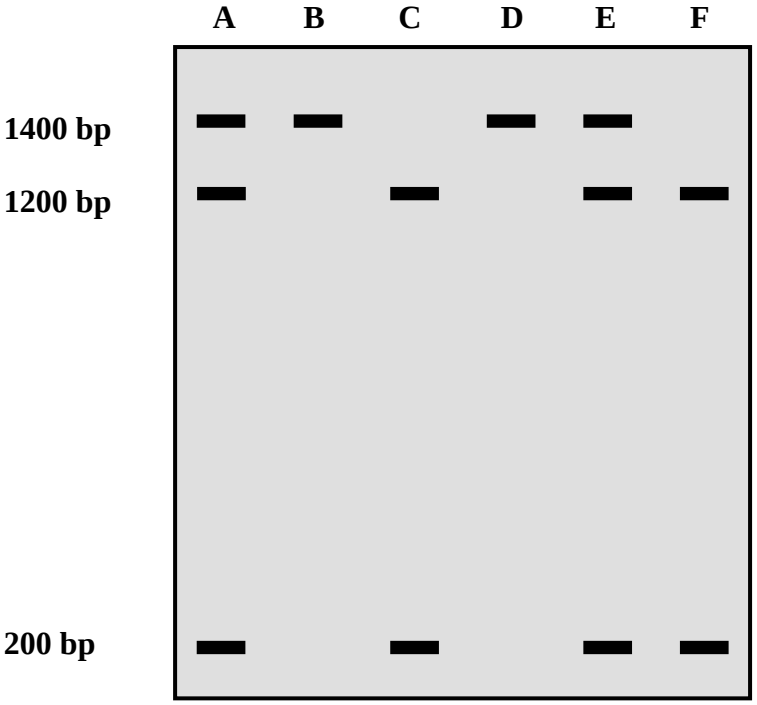
***MstII* cleavage site**

... G G A C **T** C C T C ...

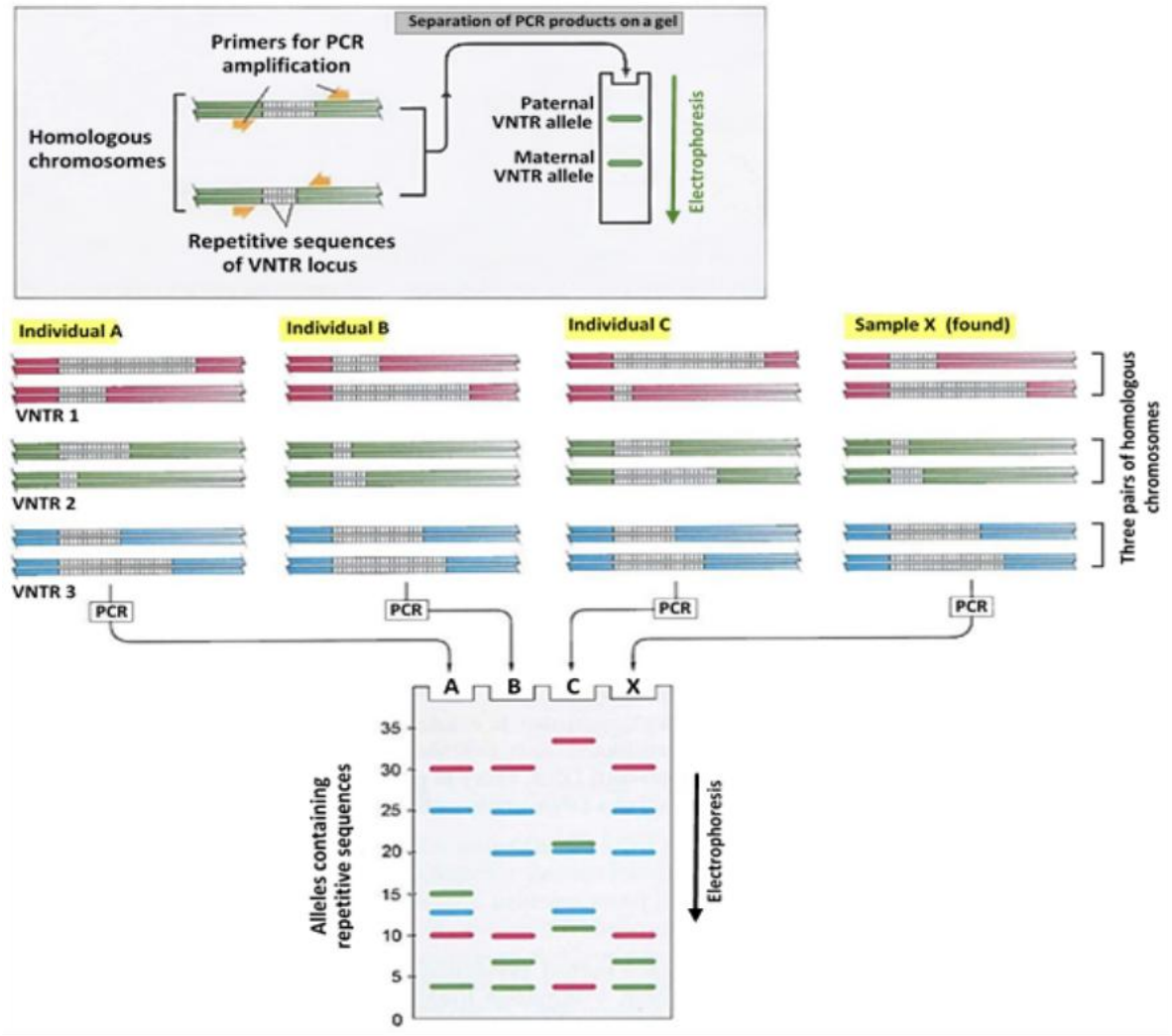
Pro Glu Glu

5. 6. 7.

Based on RFLP analysis, it is possible to diagnose sickle cell anemia, where there is a substitution of A instead of T (highlighted T), because after the substitution of bases, MstII no longer recognizes the changed site and does not cleave human DNA in the given region. After amplifying the relevant DNA segment (gene for the ß globin chain) by PCR, cleavage with MstII restriction enzyme was performed on DNA samples from 6 unknown individuals. Based on the electrophoretogram of the given RFLP analysis, identify the individuals who are healthy, those who are carriers (heterozygotes) and those who are affected by sickle cell anemia.



16. At the scene of a violent crime, traces of blood were found, left by the offender when he was injured by the defending victim. The investigation revealed three suspects who could be the potential offender. After taking samples of their blood, subsequent DNA analysis and comparison with the sample found at the crime scene (X), the real offender finally confessed. Which of the suspects (A, B, C) was the offender?



Task 1: Preparation of the PCR (polymerase chain reaction)

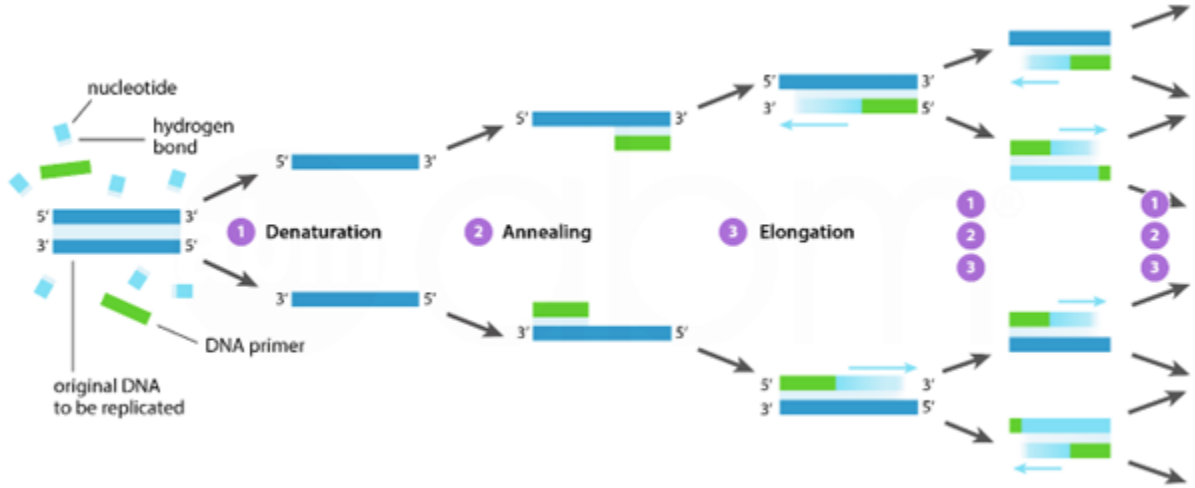


Figure 22-1. Polymerase chain reaction (PCR) – three main steps – denaturation of DNA, hybridization of primers and synthesis of DNA are repeated in cycles (approximately 20-30x).

Materials and Equipment: 0.5-10 µl and 10-100 µl pipettes, 10 µl and 200 µl tips without filter, racks, 1.5 ml plastic tubes with coloured screw caps, 0.2 ml eppendorf tubes, PCR reagents (water, buffer, dNTPs, primers, $MgCl_2$, Taq DNA polymerase, DNA sample), thermal cycler, disposable gloves).

Figure 22-2. Eppendorf test tubes.

Procedure:

1. First, label three 0.2 ml PCR tubes with numbers corresponding to the numbers of the individual DNA samples. Place the tubes in a rack.

2. Mix and briefly spin all stock solutions (PCR reagents).

3. Prepare a master mix – pipette all PCR reagents into a 1.5 ml tube except for DNA for five samples (see the right column of the table):

PCR reagents	**Volume for 20 µl reaction (1 sample)**	**Volume for 3 samples plus 2 samples excess (total of 5 samples)**
Water	14.4 µl	**72 µl**
12.5 mM dNTP mix	2 µl	**10 µl**
10x Taq buffer	0.4 µl	**2 µl**
Primer 1 (forward)	1 µl	**5 µl**
Primer 2 (reverse)	1 µl	**5 µl**
Taq DNA polymerase	0.2 µl	**1 µl**
$MgCl_2$	component of PCR buffer	component of PCR buffer
DNA	1 µl (100 ng)	5 µl

4. Pipette 19 µl of the master mix prepared in the previous step into each of the three 0.2 ml PCR tubes (total PCR reaction volume will be 20 µl).

5. Finally, add 1 µl of DNA into each of the three 0.2 ml PCR tubes (the number on the PCR tube must match the number of the DNA sample). Close the lid on each PCR tube tightly.

6. Mix and briefly swirl all 0.2 ml PCR tubes.

7. Place the PCR tubes in the thermocycler (see picture) and start the appropriate PCR program.

Figure 22-3. PCR thermocycler.

Task 2: Preparation of agarose gel for electrophoresis

Materials and Equipment: gel preparation tray, combs, agarose, electrophoresis buffer.

Procedure: Prepare a tray for pouring the gel. Place a suitable comb/combs in the grooves on the tray. To prepare a 2% agarose gel, mix 2 g of powdered agarose and 100 ml of electrophoresis buffer. Then heat the solution in a microwave oven until the agarose is completely dissolved. After the solution has cooled to approximately 60°C, carefully pour it onto the gel preparation tray and let it solidify at room temperature. After it has solidified completely, remove the comb/combs from the gel.

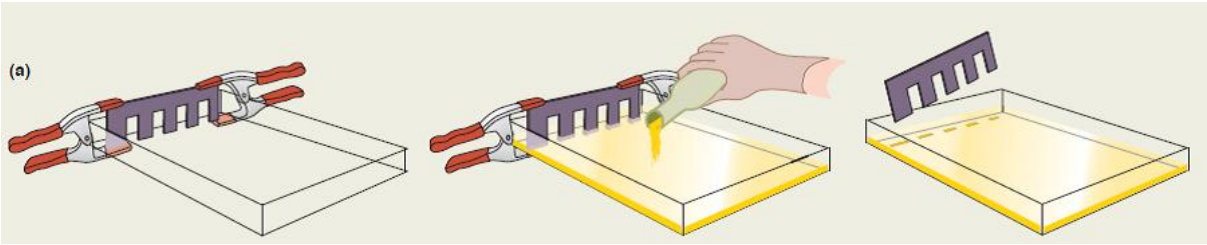


Figure 22-4. Agarose gel preparation.

Task 3: Using agarose gel for electrophoretic analysis

Materials and Equipment: electrophoresis apparatus (see image) and power supply, gel preparation tray, agarose gel, electrophoresis buffer (electrophoresis buffer), staining dye (mixture), PCR product, ethidium bromide, UV transilluminator.

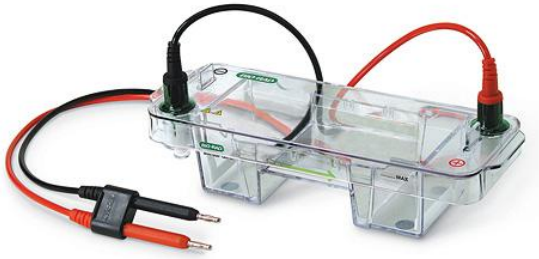


Figure 22-5. Electrophoretic apparatus.

Procedure:
1. Place the agarose gel on the tray horizontally in the electrophoresis apparatus and fill it with electrophoresis buffer.
2. Mix 8 µl of PCR product with 2 µl of loading dye and pipette the mixture into one of the wells in the agarose gel. Continue in the same way until all samples have been loaded.
3. Close the electrophoresis apparatus with a lid and connect the electrical cables to the power source. Verify that the current is flowing by observing the bubbles forming on the individual electrodes. Negatively charged DNA molecules move towards the positively charged electrode (anode), which is usually marked red.
4. After electrophoresis is complete, remove the gel from the apparatus and immerse it in ethidium bromide solution for approximately 5 minutes.
5. Use a UV transilluminator to visualize the PCR products (DNA fragments). We observe them as discrete fluorescent bands.

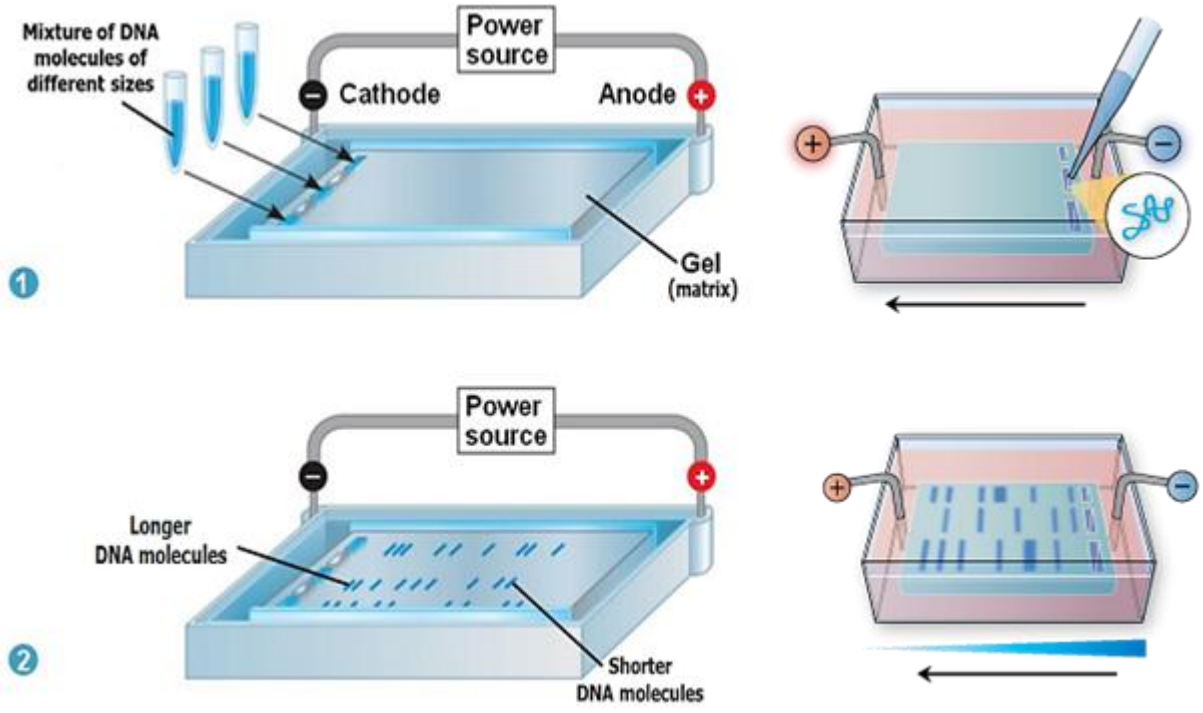


Figure 22-6. Electrophoretic analysis.

23. Dermatoglyphic examination

Dermatoglyphic examination analyzes the so-called *papillary terrain* – that is, the patterns created by the arrangement of skin ridges (papillary lines) on the palms, soles of the feet and on the ventral part of the fingers and toes, and the course of the main palm lines. There are no hairs or sebaceous glands in these places, but there are a large number of sweat gland ducts. **Dermatoglyphics** deals with the variability of papillary relief and the analysis of papillary terrain in relation to various genetic syndromes and diseases. Papillary lines on the fingers are derived from tactile papillae in suede and form patterns, called dermatoglyphics, which are characteristic for each person.

Papillary lines are functionally structures associated with the tactile properties of the limbs. They create continuous raised reliefs with a height of 0.1–0.4 mm and a width of 0.2–0.7 mm, which are arranged in permanent patterns - dermatoglyphs (loop, whorl). These lines cross and intersect, branch, connect and interrupt, thereby creating characteristic features, the so-called minutiae. They occur on the palms, fingers and soles.

Dermatoglyphs are features (patterns, patterns) that create papillary skin ridges on the pads of the fingers, palms (Fig. 23-1) and soles (Fig. 23-8). For clinical genetics, they are currently only of importance as an auxiliary diagnostic method for some dysmorphic syndromes, especially Down syndrome. They are also a useful tool in determining the zygosity of twins.

Skin features begin to develop early in the development of the hands and feet, with dermal ridges on the thickening skin differentiating around the 13th week of gestation. By the end of the 4th month, their development is essentially complete. Any change that would affect dermatoglyphics must be made within this time frame, as dermatoglyphics do not change later in life. Dermatoglyphics on the feet develop somewhat later. Dermal ridges generally appear in mammals only where increased friction is required between the surface of the limb and the substrate, or between the surface of the limb and the object being grasped, as a result of a change in limb function.

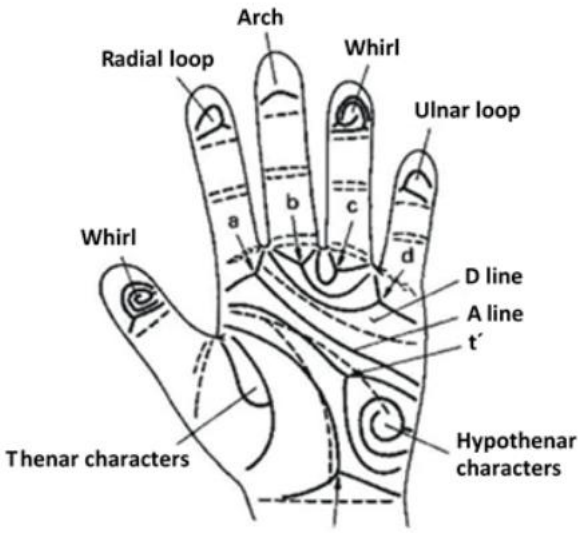


Figure 23-1. The most important areas of the palm. Four digital triradii (a, b, c, d) and the axial triradius t (t′ indicates its distal displacement). The main lines A and D originate from the corresponding triradii. Features on the thenar, hypothenar, and fingers are also shown.

The following applies to dermatoglyphics:

- the number of papillary lines and their patterns are not affected by age;
- in the postnatal period they are not influenced by the environment, they are constant throughout the life of the individual, only their dimension changes with overall growth;
- they are one of the most variable features in humans. The variability of dermatoglyphic patterns is so great that no two individuals ever have a completely identical arrangement of papillary lines, even the prints of two fingers of the same individual are never completely identical;
- despite this variability, the patterns can be classified into several basic types.

23.1. Classification of dermatoglyphic patterns

1. Fingerprints of the hands
On the fingers, dermatoglyphic patterns are evaluated almost exclusively on the distal joints. We distinguish 4 basic formations:

a) **arch** (A)
b) **loop** (L)
c) **whorl** (W- whorl)
d) **twin loop** (TL) **(**Fig. 23-2)

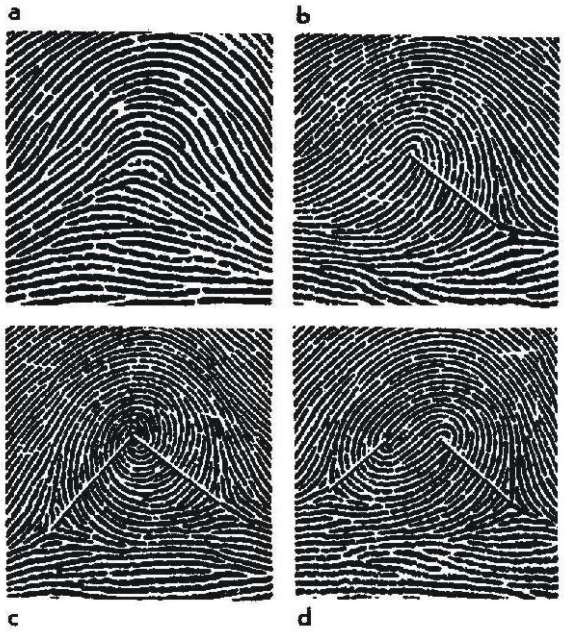


Figure 23-2. Basic dermatoglyphic patterns on the fingers.
A – arch, **B** – loop, **C** – whorl, **D** – twin loop.

The whorl and the double loop are sometimes combined into one category. The patterns that are formed depend to some extent on the thickness of the epidermis. Thick skin is characterized by arches, thinner skin by loops or whorls. In most populations, the most common pattern on the fingers is loops (L) and the rarest is arches (A).

The following is the representation of individual departments in the Slovak population:

- ***loops*** – 62%
- ***whorls and double loops*** – 34%
- ***arches*** – 4%

Ulnar loops are much more common than radial ones.

An important reference point for classifying patterns is the **triradius** (Fig. 3). It is the point where three systems of differently running papillary lines meet and three radiants emanate from it, and the **core** – the inner part of the pattern, its center. It can be a single point, an island or a line (Fig. 23-3).

Radiants are papillary lines that meet in a triradius.

Minuets are small changes and irregularities in the course of the papillary lines (branching of lines, creation of islands and broken lines), which actually make the print a unique original and allow the use of the papillary terrain for personal identification.

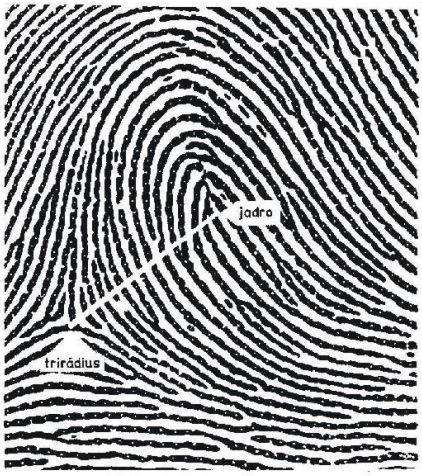


Figure 23-3. Dermatoglyphic pattern (loop) – the triradius and the core of the pattern are shown.

Arches (A) – are characterized by a simple course of papillary lines across the entire surface of the ventral surface of the distal finger joint, with the papillary lines being only slightly curved in an arch.

We distinguish the following arches:

a) *simple* – do not have a triradius

b) *tent-like* – have an indicated triradius usually located in the middle of the finger pad in the form of a tent

Loops (L) – are formed after bending at least 2-3 papillary lines by 180 degrees. They have one triradius. Depending on which direction the loop opens, we distinguish loops:

a) *ulnar (Lu)* – the loop opens on the ulnar side of the finger

b) *radial (Lr)* – the loop opens on the radial side of the finger. The triradius is located on the opposite side.

Whorls (W) – are characterized by a closed circular, elliptical or spiral pattern. They have two triradii.

Double loops (TL) – are formed by two hooked loops. They have two triradii and therefore two quantitative values.

Within the population, there are bilateral and intersex differences in the frequency of individual formations. The right hand has a higher incidence of whorls and radial loops than the left, and vice versa, there are more ulnar loops and arches on the left hand. Intersex differences consist in the fact that men have more whorls and fewer arches than women, and they also have more radial loops and fewer ulnar loops than women.

In dermatoglyphic examination, in addition to **qualitative assessment**, i.e. determining the type of drawings, a quantitative assessment is also made, i.e. the size of the sign is expressed by the number of papillary lines. The quantitative value of the pattern is a value defined as the number of papillary lines intersecting the line between the triradius and the core of the formation without these two extreme points (Fig. 23-4). An arch has zero lines (zero quantitative value) because it does not have a triradius, a loop has one quantitative value. A vortex and a double loop each have two quantitative values, which we give in a fraction with the higher value in the numerator.

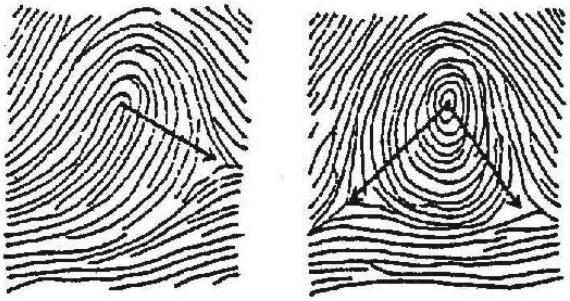


Figure 23-4. Counting of papillary lines. Quantitative values:L = 10, W = 12/10

An important quantitative indicator is the **total number of ridge lines** on the prints of all ten fingers – the **TRC value** (total ridge count). It is therefore the sum of the quantitative values of all ten fingers (in the case of a whorl and a double loop, we include the higher value, which is in the numerator). It ranges from 0 to 300. In our population, the average TRC value is 152 papillary lines in men and 130 in women.

2. Palm print

On the palms, the classification of features is more complex (Fig. 23-5)

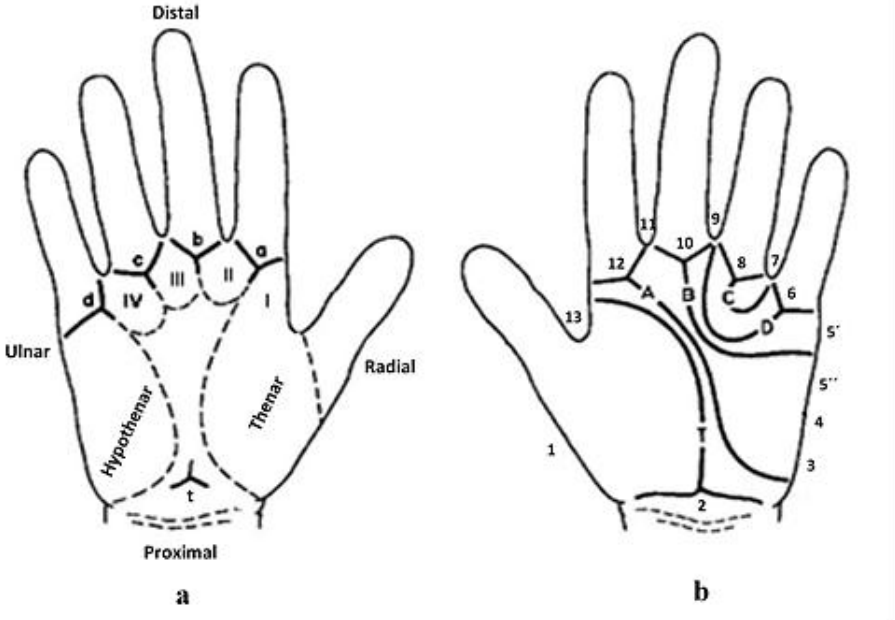


Figure 23-5. Scheme of the palm print

a – palmar triradii *a, b, c, d, t* and dermatoglyphic areas on the palm – thenar, hypothenar, and interdigital areas I–IV.
b – the main palmar lines *A, B, C, D, T* and the numerical values 1–13 assigned to individual areas along the palm margin, which are used to indicate the termination of the main lines.

At least 5 triradii can be identified on a palm print:

- four **digital triradii**, which are located at the base of the 2nd to 5th fingers and are designated by the letters a, b, c, d in radial-ulnar order,
- the fifth **palmar triradius (axial)** lies near the axis of the palm, near the proximal edge and is designated by the letter **t** or **t´** if it is shifted more distally (if it has an angle *-atd-* up to 45°, we designate the palmar triradius as ***t***, from 46° to 57° as ***t'***, from 58° to 81° as ***t''*** and at a size of 82° and more as ***t'''***).

Radiants that emanate from these triradii toward the center of the palm are called **main lines** and are designated by the corresponding capital letters **A, B, C, D, T**. The main lines leave the palm print at a place called **the end of the main line**, which is assigned a numerical value from 1 to 13 according to the established classification. When marking the main lines, we proceed as follows: first, we mark the digital triradii and the palmar triradius with a coloured pencil and then we draw the course of the main lines (Fig. 23-6). Sometimes a triradius is formed, but the main line emerging from it suddenly ends blindly or turns distally so that it merges with itself or merges with the triradius.

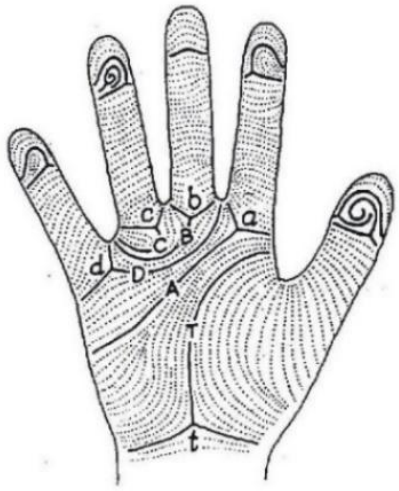


Figure 23-6. Example of evaluating the main palmar lines.

The writing of the endings of the main lines in the order D, C, B, A forms the so-called **palm pattern** (e.g. 11.9.7.5.). There are several ways to evaluate and interpret the palm pattern. One of them is its expression by the **Valšík number (P)**. This quantity represents the sum of the endings of the main lines of the palm, e.g. in the above case of the endings of the lines 11.9.7.5. the value P = 32. Comparing the palm pattern of the mother, child and the marked man was used as one of the dermatoglyphic criteria in determining disputed paternity.

From the point of view of evaluating the palm print, the *position of the axial triradius* is important, because in many hereditary pathological conditions it is shifted distally. Its shift can be expressed in relation to the total length of the palm or by the size of the *angle **atd*** (Fig. 23-7).

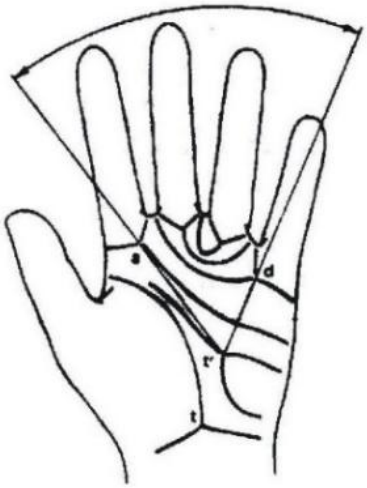


Figure 23-7. Measuring the ***atd*** angle on the palm. In the case shown, the *atd* angle is 60°. If there are multiple palmar triradii on the palm, the one located most distally is taken into account.

The **flexion furrows** (the so-called heart, head and life lines) are not true papillary lines. However, they arise at the same time and are partly shaped by influences that also condition the arrangement of the papillary lines. The monkey furrow (a single transverse furrow) instead of the usual two furrows occurs on at least one hand in about 1% of healthy people. However, **monkey furrows** are very common in cases of Down syndrome and other chromosomal aberrations, as well as in children with congenital malformations.

3. Plantar features

Plantar features (Fig. 23-8) have been studied to a lesser extent than palmar features. Perhaps because footprints are more difficult to capture and classify. Footprint classification follows similar criteria to those used in evaluating palm prints.

A prominent and highly variable configuration area on the foot is the **hallucal** area - the area proximal to the big toe. In the Caucasian population, loops and whorls are most common at this location, and arches are very rare (0.3%). The **tibial arch** (A^t), on the other hand, is found in about 50% of patients with Down syndrome (Fig. 23-9). This feature can be used in the diagnosis of this syndrome.

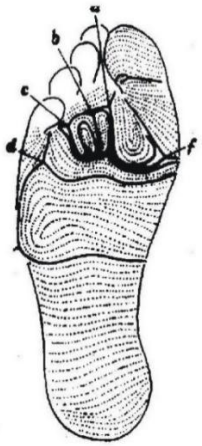


Figure 23-8. Digital triradii of the foot.

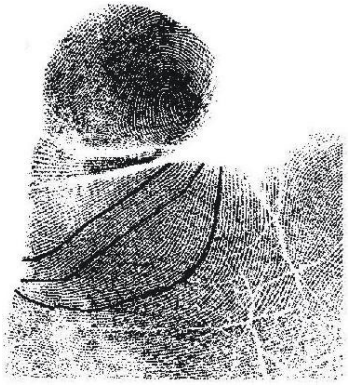


Figure 23-9. Tibial hallucal arch (At) in Down syndrome. In cases of this disorder, it is frequent, whereas otherwise it is very rare.

Variability in basic dermatoglyphic formations is largely determined genetically. All exogenous factors that influence it are limited in time to the first trimester of intrauterine life, because no changes occur later. However, the configuration of small formations visible under a magnifying glass along individual papillary lines – the so-called minutiae – is influenced by non-genetic factors. Even monozygotic twins differ in minutiae, although there is a high concordance between them in the basic types of patterns. Given that the arrangement of papillary lines is about 90% genetically determined and 10% dependent on external conditions, even monozygotic twins do not have identical fingerprints. Their prints match in basic patterns, but they differ in small details.

The exact mechanism of inheritance of individual features is mostly unknown. No single dermatoglyphic feature has been demonstrated to be monogenic. Most dermatoglyphic features are polygenic. We find a significantly higher correlation between monozygotic (0.95) than dizygotic (0.5) twins.

Dermatoglyphs have some importance in the grasping function of the limbs and are to some extent related to tactile acuity. They can also develop on the surface of the stump of an amputated hand when the latter performs long-term activities that require touch.

23.2. Use of dermatoglyphics

In practice, dermatoglyphics have been or are currently used in the following areas:

1) identification of persons in criminalistics (dactyloscopy)
2) determination of zygosity of twins
3) hereditary-biological tests in resolving cases of disputed paternity
4) medical diagnostics – since dermatoglyphics are established in early embryonic development, they can be an indicator of growth disorders that occurred during this period. Unusual dermatoglyphic signs occur in all cases of numerical chromosome aberrations. The described differences relate to:

- TRC values – in individual pathological conditions it is either increased or decreased compared to healthy individuals;

- flexion grooves on the palms, which are often reduced to a single groove (monkey groove);
- position of the axial (palmar) triradius – it is usually shifted more distally.

It should be emphasized, however, that dermatoglyphic features are extremely variable and none of the described features is abnormal in itself. However, the different frequency of a feature in healthy and affected individuals, as well as the combination of several unusual findings in one patient, may be of diagnostic significance.

Changes are typical in chromosomal anomalies (Down syndrome, Fig. 23-10), but various deviations are also found in thalidomide and rubella embryopathies.

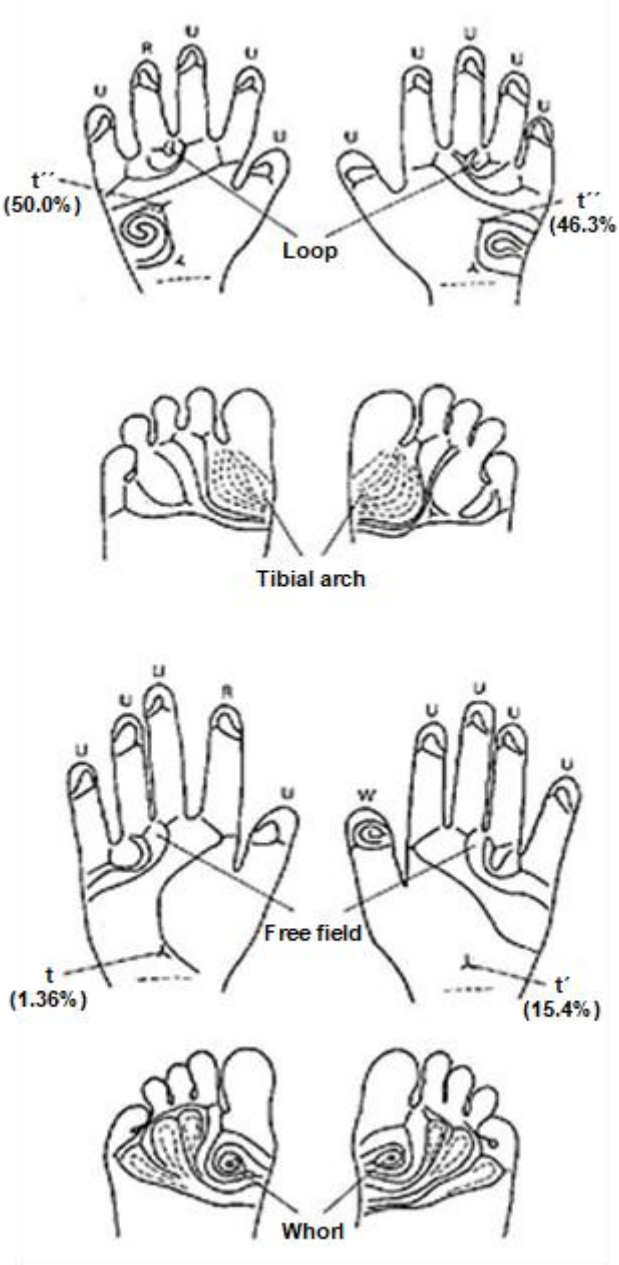


Figure 23-10. Typical dermatoglyphic features in a patient with Down syndrome (above) compared with a healthy control individual (below). The axial triradius is displaced distally (t´´); the hypothenar pattern is visible only in the patient but not in the control; a radial loop on the fourth finger is typical for patients with Down syndrome but otherwise occurs rarely. Plantar patterns are also shown.

Significant deviations in TRC values are observed in heterochromosome anomalies, with the highest number in monosomy X and the lowest numbers in all cases with supernumerary X and Y chromosomes.

Dermatoglyphics can be significantly deformed due to limb malformations that occurred early in embryonic development. If dermatoglyphic findings are present, this indicates that the disease originated or developed at the time of papillary line differentiation.

23.3. Changes in dermatoglyphic formations in the most common clinical syndromes

There is a connection between some dermatoglyphics and chromosomal aberrations. Examples include typical dermatoglyphics in women with Turner syndrome, and the "monkey groove" in people with Down syndrome. Dermatoglyphics can therefore also be used as an auxiliary method in establishing a diagnosis.

Trisomy 21

a) tendency to simple dermatoglyphic formations on the fingers - predominance of ulnar loops, low TRC value

b) single flexion groove (monkey groove) on the palm (50%)

c) axial triradius in the middle of the palm (85%)

d) *atd* angle 81° (Fig. 23-11)

e) tibial arch (50%) or small distal loop on the foot (35%)

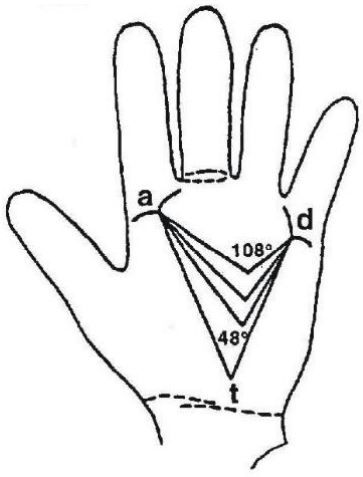


Figure 23-11. Changes of *atd* angle, in certain clinical syndromes:
108° - t´´´ - Patau syndrome (trisomy 13)
81°- t´´ - Downov syndrome (trisomy 21)
66°- t´- Turner syndrome (monosomy X)
48°- t – normal individual

Trisomy 13

a) increased number of arches on fingers
b) low TRC value
c) single flexion groove (monkey groove)
d) axial triradius shifted distally
e) *atd* angle 108° (Fig. 23-11)

Trisomy 18

a) increased number of arches on fingers
b) very low TRC value
c) single flexion groove (monkey groove)

Turner syndrome (45, X)

a) large number of vortices and loops on fingers with high quantitative value
b) very high TRC value
c) transverse (monkey) groove
d) axial triradius shifted slightly more distally than in control subjects (t′= 66°)

Klinefelter syndrome (47, XXY)

a) predominance of arches on the fingers
b) low TRC value – the more gonosomes, the lower the TRC value
c) axial triradius located lower than in the normal population

Cri-du-chat syndrome (5p-)

a) many arches
b) low TRC value
c) single flexion groove

Questions and Tasks

Task 1: Determining the basic types of patterns on the fingers of the hands

Material and Equipment: printing ink (or stamping ink and pad), rubber roller, glass plate for spreading the ink, smooth white paper, magnifying glass.

Procedure and Results: For dermatoglyphic analysis, it is essential to have a well-readable, sharp and complete print of the relevant terrain area. The most commonly used method of

obtaining prints is using printer's ink. We apply an appropriate amount of paint to a glass plate, which we spread evenly over the entire surface of the plate with rubber roller movements so that it creates an evenly thin film. We rub the surface of the fingers and their lateral edges with the roller. We distinguish between contact prints and rolling prints. Each student makes rolling fingerprints from both hands so that the entire papillary relief of the last finger joints is captured. Place the ulnar edge of the finger on the paper and gradually rotate until the entire desired area is imprinted, i.e. up to the radial edge to the nail bed.

a) Determine the types of patterns on all fingers – determine the qualitative value of each feature.
b) Mark the triradius and center of the feature.
c) Determine the quantitative value of each pattern and the TRC value of all ten fingers. Fingerprints are numbered 1 – 5 in radial-ulnar order.

Task 2: Evaluation of the papillary terrain of the palm

Material and Equipment: as in the previous task.

Procedure and Results: Make palm prints. Mark:

- digital triradii and palm triradii (a, b, c, d, t)
- course of main lines A, B, C, D, T
- termination of main lines (1 – 13)
- *atd* angle
- write down the palm formula
- calculate Valšík's number

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