MINISTRY OF THE PUBLIC HEALTH OF UKRAINE
ZAPOROZHYE STATE MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL BIOLOGY

TEXT-BOOK FOR MODULE I
MOLECULAR AND CELLULAR
LEVELS ORGANIZATION OF LIVING THINGS.

ZAPOROZHYE – 2015

“Text-Book” deals with topics mentioned in the Academic Curriculum for the Medical University. The textbook pays attention to the molecular and cellular levels organization and to some problems of Genetics. The questions of each topic act as test-pieces and help to fix the knowledge acquired.
The book will contribute to examination success while engendering a genuine love of biology which will stimulate the students to delve further and deeper into the subject.

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Topic 1. Parts of the microscope and their function. Preparing a wet-mount slides

Key concepts:

1. Microscopic techniques significance in the studying biological objects;
2. Parts of the microscope and their functions;
3. Use of the microscope;
4. Preparing a wet-mount slides.

If almost any structure from a plant or an animal is examined, it will be seen to consist of more or less distinct units - cell, which is too small to be seen with the naked eye. So they must be magnified in order to get any idea about their structure. This may be achieved by using the microscope. Tools play a major role in science. This means that many great discoveries lie in wait for the tools needed to make them.

For laboratory examinations available light microscope that produce magnified images by focusing visible light rays. Electron microscopes produce magnified images by focusing beams of electrons.

Learning the name, function and location of the microscope's parts is necessary for proper use.

Parts of the microscope. How to use them.

1) ***Eyepiece*** — contains a magnifying lens.

2) ***Arm*** - supports the body's tube.

3) ***Stage*** - supports the slide being observed.

4) ***Opening of the stage*** - permits light to pass up to the eyepiece.

5) ***Fine adjustment knob*** — moves the body tube slightly to sharpen the image.

6) ***Coarse adjustment knob*** - moves the body tube to focus the image.

7) ***Base*** **-** supports the microscope.

8) ***Illuminator*** **-** produces light or reflects light up toward the eyepiece.

9) ***Condenser and diaphragm*** - regulates the amount of light passing toward the eyepiece.

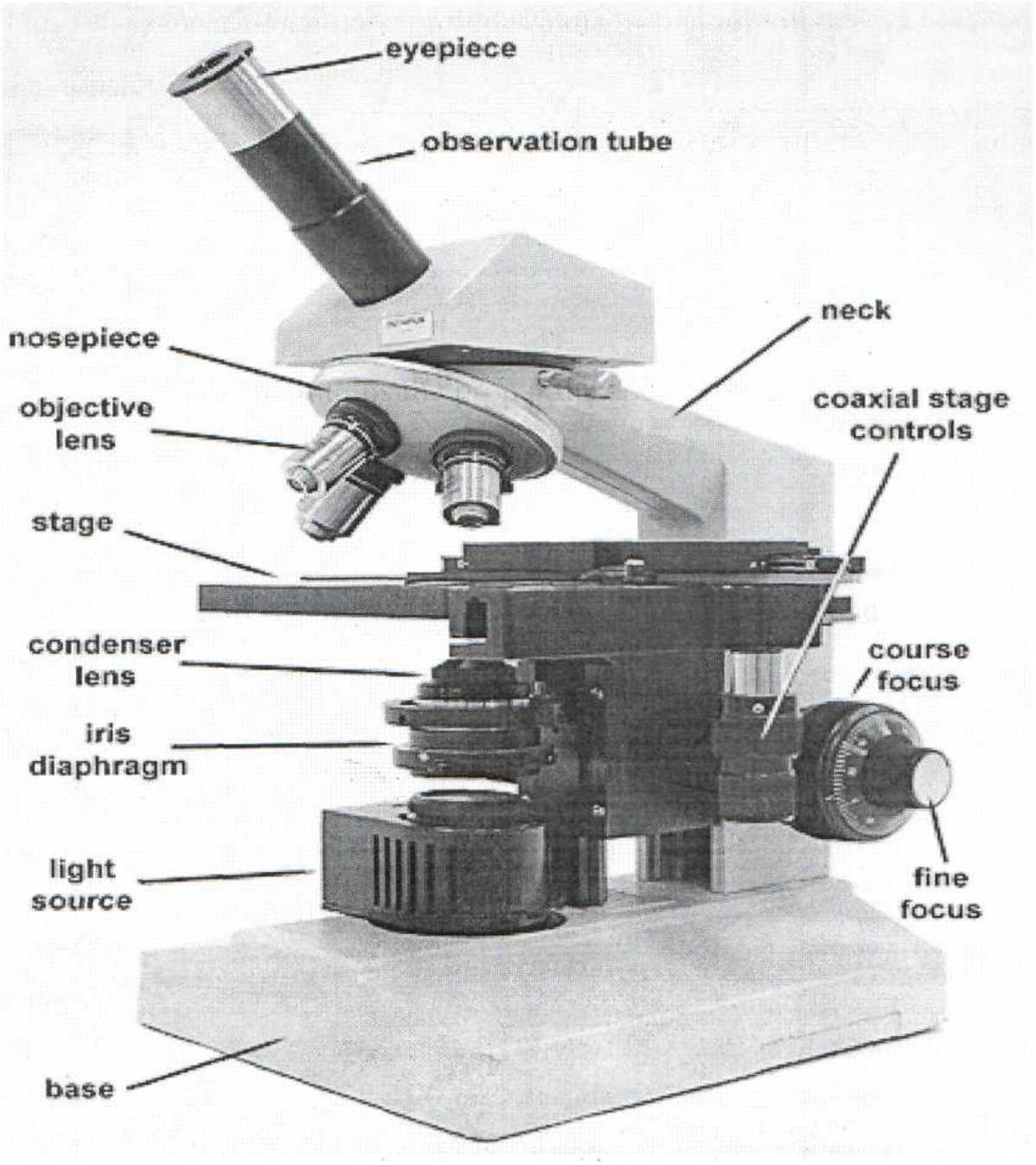
10) ***Stage clips*** - hold the slide in the place.

11) ***Low-power objective*** - provides a magnification of 10 X and is the shortest objective.

12) ***High-power objective*** — provides a magnification of 40 X and is the longest objective.

13) ***Nosepiece*** - holds the objectives and can be rotated to change the magnification.

14) ***Body tube*** - maintains the proper distance between the eyepiece and the objective.



Use of the microscope.

Working with the microscope use the following procedures:

1) Carry the microscope by placing one hand beneath the base and grasping the arm of the microscope with the other hand.

2) Gently place the microscope on the lab table with the arm facing you.

3) Raise the body tube by turning the coarse adjustment knob until the objective lens is about 2 cm above the opening of the stage.

4) Rotate the nosepiece so that the low power objective is directly in line with the body tube.

5) Look through the eyepiece and switch on the lamp or adjust the mirror so that a circle of light can be seen. This is the field of view.

6) Place a prepared slide on the stage so that the specimen is over the center of the opening.

7) Look at the microscope from the side. Carefully turn the coarse adjustment knob to lower the body tube until the low-power objective almost touches the slide. Do not allow the objective to touch the slide.

8) Look through the eyepiece and observe the specimen. Focus the image as best you can with the coarse adjustment knob. Then use the fine-adjustment knob to focus the image more sharply.

9) Adjust the lever of the diaphragm to allow the right amount of light to enter.

10) After every use, remove the slide. Return the low-power objective into place in line with the body tube.

Practice:

Assignment 1. Preparing a wet-mount slides.

1. Obtain a clean microscope slide and a cover slip. A cover slip is very thin, permitting the objective lens to be lowered very close to the specimen.

2. Place the specimen in the middle of the microscope side. The specimen must be thin enough for light to pass through it.

3. Using a dropper pipette, place a drop of water on the specimen.

4. Lower one edge of the cover slip so that it touches the side of the drop of water at about a 45^0 angle. Slowly lover the cover slip over the specimen and water. Try not to trap any air bubbles under the cover slip.

5. Remove any excess water at the edge of the cover slip with a paper towel. If the specimen begins to dry out, add a drop of water at the edge of the cover slip.

a) Slide 1. Cotton fibers.

Take a small piece of the cotton fiber and put it in the center of glass slide. Add a drop of water and cover it with a cover slip. Examine you slide under low-power objective of the microscope and sketch the specimen.

b) Slide 2. Plant cells.

Using the same method prepare slide of Onion cells. Examine your slide under low-power and high power objectives of microscope. Sketch 2-3 cells and label any structures you recognize.

Literature:

1. Lazarev K.L. "Medical biology" Simferopol, 2003.
2. T.V. Bihunyak “Medical Biology”. – Ternopil, 2010.

Topic 2. Cell structure (cell wall, plasma membrane, cytoplasm, organelles and inclusions).

Key concepts:

1. What is a cell?
2. The main statements of the cell theory.
3. Basic cell structure: organelles, inclusions.
4. What are the characteristics of prokaryotes and eukaryotes?
5. How are plant and animal cells similar in structure? How are they different?

Cellular organization.

In 1665, Robert Hooke, using a compound microscope discovered that cork was composed of numerous small units. He called these units cells. In the years that followed Hooke and other researchers discovered that many other types of material were similarly composed of cells. In 1838, a German botanist Matthias Schleiden

concluded that all plants were made up of cells. The following year, Theodor Schwann reached the same conclusion about the organization of animals. Their point finding became known as the cell theory, which makes the cell the fundamental unit of structure and function in living organisms.

The development of the electron microscope revolutionized our understanding of cell structure. The electron microscope revealed the fine structure of cells including many new organelles. This detail is called the ultrastracture of the cell.

Biologists divide cells into two categories: ***prokaryotes and eukaryotes.*** The cells of prokaryotes are generally smaller and simpler than the cells of eukaryotes. Prokaryotes have cells membranes and cytoplasm but do not contain nuclei. All bacteria are prokaryotes. Examples of prokaryotes include Escherichia coli, which live in the intestine, and Staphylococcus auras, which can cause skin infections. Prokaryotes carry out every activity associated with life. They grow, reproduce and change in the environment. Some even move by gliding along surfaces or swimming through liquids.

Unlike the cells of prokaryotes, the cells of eukaryotes do contain nuclei. In addition to a nucleus, a cell membrane and cytoplasm, most of cells of eukaryotes contain other specialized structures, called organelles, that perform important cellular functions Although some eukaryotes live solitary as single - cells organisms, many are large, multicellular organisms. All plants, animals and fungi, and many microorganisms, are eukaryotes. The differences between the cells of prokaryotes and those of eukaryotes are shown in the table.

Table. ***Differences between prokaryotic and eukaryotic cells.***

#	**Feature**	**Prokaryotic cell**	**Eukaryotic cell**
1	***Cell wall***	Present in most but not in all cells	Present in plant and fungal cells only
2	***Plasma membrane***	Present	Present
3	***Nucleus***	Absent	Present
4	***Nuclear membrane***	Absent	Present

5	***Genetic material***	Circular or linear, double – stranded DNA: genes are not interrupted by introns∗	Linear, double – stranded DNA: genes frequently interrupted by intron sequences, especially in higher eukaryotes
6	***Nucleoli and mitotic apparatus***	Absent	Present
7	***Cellular organelles***	Absent, except ribosomes	Present
8	***Respiration***	Many strict anaerobes (oxygen fatal)	All aerobic, but some facultative anaerobes by secondary modifications

∗Intron is an intervening sequence of nucleotides in DNA, located within a gene that is not included in the mature mRNA.

CELL STRUCTURE

The number of cells present in an organism varies from a single cell in a unicellular organism (Protozoa) to many cells in multicellular organisms.

A eukaryotic cell consists of the following components:

- Cell wall and plasma membrane;
- Cytoplasm;
- Nucleus.

Cell wall. The outer structure of most plant cells is a dead and rigid layer called ***cell wall.*** It is mainly composed of carbohydrates such as cellulose, pectin, hemicelluloses and lignin and certain fatty substances like waxes. Ultra structurally cell wall is found to consist of a microfibrillar network lying in a gel - like matrix. The microfibrils are mostly made up of cellulose. There is a pectin-rich cementing substance between the walls of adjacent cells which is called middle lamella.

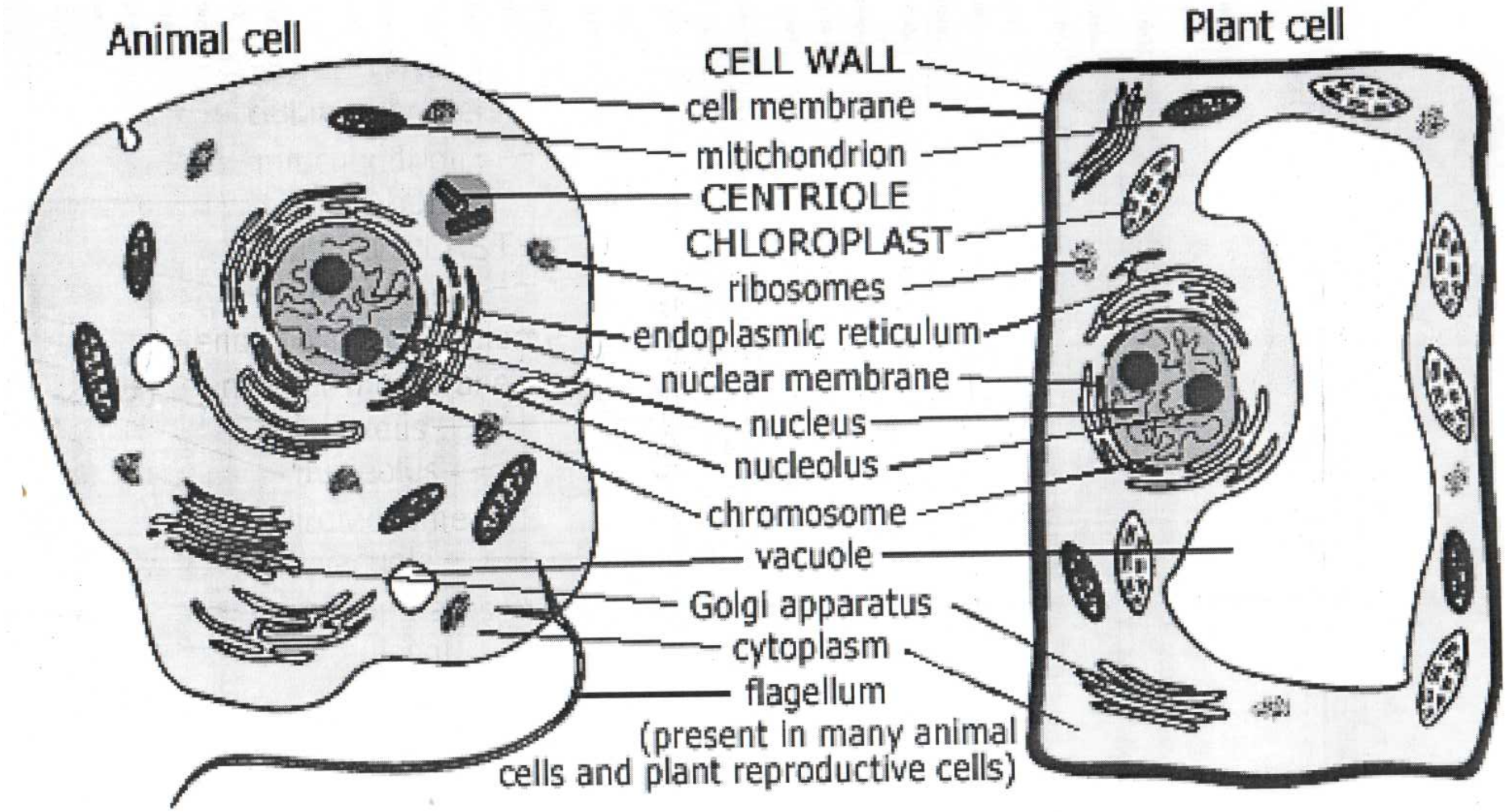
The cell wall constitutes a kind of exoskeleton that provides protection and mechanical support to the plant cell. It determines the shape of plant cell and prevents it from desiccation.

Plasma membrane. Every kind of animal cell is bounded by a living, extremely thin and delicate membrane called plasmolemma, cell membrane or plasma membrane.

In plant cells, plasma membrane occurs just inner to cell wall, bounding the cytoplasm. At molecular level, it consists of a continuous bilayer of lipid molecules (i.e., phospholipids and cholesterol) with protein molecules embedded in it or adherent to its both surfaces. Some carbohydrate molecules may also be attached to the external surface of the plasma membrane, they remain attached either to protein molecules to form glycoproteins or to lipids to form glycolipids. The plasma membrane is a selectively permeable.

Cytosol. The plasma membrane is followed by the colloidal organic fluid called ***matrix*** or ***cytosol.*** The cytosol is the aqueous portion of the cytoplasm (the extranuclear protoplasm) and of the nucleoplasm (the nuclear protoplasm). It fills all the spaces of the cell and constitutes its true internal milieu. Cytosol is particular rich in differentiating cells and many fundamental properties of cell are because of this part of the cytoplasm. The cytosol serves to dissolve or suspend the great variety of small molecules concerned with cellular metabolism, e.g., glucose, amino acids, nucleotides, vitamins, minerals, oxygen and ions. In all type of cells, cytosol contains the soluble proteins and enzymes which form 20 to 25 per cent of the total protein contents of the cell. Among the important soluble enzymes present in the matrix are those involved in glycolysis and in the activation of amino acids for the protein synthesis. In many types of cells, the cytosol is differentiated into following two parts: (1) Ectoplasm or cell cortex is the peripheral layer of cytosol which is relatively non-granular, viscous, clear and rigid. (2) Endoplasm is the inner portion of cytosol which granular and less viscous.

Cytoplasmic structure. In the cytoplasmic matrix certain non-living and living structures remain suspended. The non-living structures are called paraplasm or inclusions, while the living structures are membrane bounded and are called organelles.


Animal cell
Plant cell
CELL WALL
cell membrane
mitichondrion
CENTRIOLE
CHLOROPLAST
ribosomes
endoplasmic reticulum
nuclear membrane
nucleus
nucleolus
chromosome
vacuole
Golgi apparatus
cytoplasm
flagellum
(present in many animal cells and plant reproductive cells)

a) ***Cytoplasmic inclusions.*** The stored food and secretory substances of the cell remain suspended in the cytoplasmic matrix in the form of refractive granules forming the inclusions. The cytoplasmic inclusions include oil drops, fat cells of adipose tissue, yolk granules, secretory granules, glycogen granules (muscle cells and hepatocytes of liver) and starch grains (in the plant).

b) ***Cytoplasmic organelles.*** Besides the separate fibrous systems, cytoplasm is coursed by a multitude of internal membranous structures, the organelles (literally the word organelle means a tiny organ). Membranes close off specific regions of the eukaryotic cells performing specialized tasks: oxidative phosphorylation and generation of energy in the form of ATP molecules in mitochondria; formation and storage of carbohydrates in plastids; protein synthesis in rough endoplasmic reticulum, etc. The structure and function of some important organelles are as follows:

1. Endoplasmic reticulum (ER). Within cytoplasm of most animal cells is an extensive network (reticulum) of membrane-limited channels, collectively called endoplasmic reticulum. Some portion of ER membranes remains continuous with plasma membrane and the nuclear envelope. The membranes of the ER may be loosely organized or tightly packed. Where the membranes are lined with ribosomes they are called ***rough ER.*** The rough ER is concerned with protein synthesis and is most abundant in those cells which are rapidly growing or secrete enzymes. Damage to a cell often results in increased formation of ER in order to produce the proteins necessary for the cell's repair.

Where the membranes lack ribosomes they are called ***smooth endoplasmic reticulum.*** The smooth ER is concerned with lipid synthesis and is most abundant in those cells producing lipid-related secretions, e.g. the sebaceous glands of mammalian skin and cells secreting steroids.

The function of the ER may thus be summarized as:

1) Providing a large surface area for chemical reactions.
2) Providing a pathway for the transport of materials through the cell.
3) Producing protein, especially enzymes (rough ER).
4) Producing lipids and steroids (smooth ER).

5) Collecting and storing synthesized material.

6) Providing a structural skeleton to maintain cellular shape (e.g. the smooth ER of a rod cell from the retina of the eye).

2. Golgi apparatus. It is a cup-shaped organelle which is located near the nucleus in many types of cells.

The Golgi apparatus consists of flattened membranous sacs-cisternae, looking like a stack of pita bread. The membrane of each cisterna in a stack separates its internal space from the cytosol. Vesicles concentrated in the vicinity of the Golgi apparatus are engaged in the transfer of material between the Golgi and other structures.

A Golgi stack has a distinct polarity. The two poles of a Golgi stack are referred to as the ***cis face*** and the ***trans face;*** these act, respectively, as the receiving and shipping departments of the Golgi apparatus. The cis face is usually located near the ER. Transport vesicles move material from the ER to the Golgi. A vesicle that buds from the ER will add its membrane and the contents of its lumen (cavity) to the cis face by fusing with a Golgi membrane. The trans face gives rise to vesicles, which pinch off and travel to other sites.

The Golgi apparatus or Golgi body of eukaryotic cells plays a variety of functions:

- it packs and transports certain materials like proteins and polysaccharides out of the cell;
- it produces glycolipids and glycoproteins;
- it forms acrosome of animal sperms and primary lysosomes;
- it syntheses hemicellulose, cellulose and pectin compounds during cell wall formation in plants.

3. Lysosomes The cytoplasm of animal cells contains many tiny, irregular-shaped, membrane-bounded vesicles known as lysosomes. The lysosomes are originated from Golgi apparatus and contain numerous (about 50) hydrolytic enzymes for intracellular and extracellular digestion. They digest the material taken in by endocytosis (such as phagocytosis and pinocytosis), parts of the cell (by autophagy) and extracellular substances.

4. Cytoplasmic vacuoles. The cytoplasm of many plants and some animal cells (ciliate protozoa's) contains numerous small or large-sized, hollow, liquid-filled structures, the vacuoles. The vacuoles of animal cells are bounded by a lipoproteinous membrane and their function is the storage, transmission of the materials and the maintenance of internal pressure of the cell.

The vacuoles of the plant cells are bounded by a single, semipermeable membrane known as tonoplast. These vacuoles contain water, phenol, flavones, anthocyanins (blue and red pigment), alkaloids and storage product, such as sugars and proteins.

5. *Peroxisomes*. These are tiny circular membrane-bounded organelles containing a crystal-core of enzymes (such as urate oxidase, peroxidase, D-amino oxidase and catalase). These enzymes are required by peroxisomes in detoxification activity, i.e. in the metabolism and decomposition, of hydrogen peroxide or H_2O_2 molecules which are produced during neutralization of certain superoxides-the end product of mitochondrial or cytosolic reactions. Peroxisomes are also related with b-oxidation of fatty acids and thermogenesis like the mitochondria and also in degradation of the amino acids. In green leaves of plants, peroxisomes carry out the process of photorespiration.

6. Mitochondria. Mitochondria are oxygen-consuming ribbon-shaped cellular organelles of immense importance. Each mitochondrion is bounded by two unit membranes. The outer mitochondrial membrane resembles more with the plasma membrane in structure and chemical composition. It contains pores, proteins that render the membrane permeable to molecules having molecular weight as high as 10,000. Inner mitochondrial membrane is rich in many enzymes, coenzymes and other components of electron transport chain. It also contains proton pumps and many permease proteins for the transport of various molecules such as citrates, ADP, phosphate and ATP. Inner mitochondrial membrane gives out finger-like outgrowths (cristae) towards the lumen of mitochondrion and contains tennis - racket shaped particles which contain ATP-ase enzyme for synthesis. Mitochondria also contain in their matrix single or double circular and double stranded DNA molecules, called mt DNA and also the 55 S ribosomes. Since mitochondria can synthesize 10 per cent of their proteins in their own protein-synthetic machinery,

they are considered as semi-autonomous organelles. Mitochondria may also produce heat (brown fat), and accumulate iron-containing pigments.

7. *Plastids* occur only in the plant cells. They contain pigments and may synthesize and accumulate various substances.

Plastids are of the following types:

❖ ***Leucoplasts, Amyloplasts, Proteinoplasts, Chromoplasts*** contain pigment molecules and are coloured organelles. Chromoplasts impart a variety of colours to plant cells, such as red colour in tomatoes, various colours to petals of flowers and green colour to many plant cells. The green coloured chromoplasts are called ***chloroplasts.*** They have chlorophyll pigment and are involved in the photosynthesis of food.

8. Ribosomes. Ribosomes are tiny spheroidal dense particles (of 150 to 200 A^0 diameters) that contain approximately equal amounts of RNA and proteins. They are primarily found in all cells and serve as a scaffold for the ordered interaction of the numerous molecules involved in protein synthesis.

9. Microtubules. Microfilaments The cytosol of cells also contains fibers that help to maintain cell shape and mobility and that probably provide anchoring points for the other cellular structures. Collectively, these fibers are termed as the cytoskeleton. The following types of such fibers have been identified:

1. The thickest are the microtubules (20 run in diameter) which consist primarily of the tubulin protein. The function of microtubules is the transportation of water, ions or small molecules, cytoplasmic streaming (cyclosis), and the formation of fibers or asters of the mitotic or meiotic spindle during cell division. Moreover, they form the structural units of the centrioles, basal granules, cilia and flagella.

2. The thinnest fibers are the microfilaments (7 nm in diameter) which are solid and are principally formed of actin protein. They maintain the shape of cell and form contractile component of cells, mainly of the muscle cells.

3. The fibers of middle order are called the intermediate filaments (IFs) having a diameter of 10 nm. They have been classified according to their constituent protein such as desman filaments, keratin filaments, neurofilaments, and glial filaments.

10. Basal bodies and centrioles. Basal bodies and centrioles are similar in structure and function; both act as nucleating centers from which microtubules grow. Centrioles are cylinders. This cylinder is open on both ends; unless it carries a cilium or flagellum (then it is called ***basal body*** or ***kinetosome).*** The wall of a centriole has nine groups of microtubules arranged in a circle.

The ***procentriole*** (or daughter centriole) is formed at right angles to the centriole and is located near the proximal end of the centriole. Both centrioles are found in specially differentiated region the ***centrosome, cell centre*** or ***centrosphere.*** The centrosome firmly attached to the nuclear envelope. At the time of cell division two pairs of centrioles are formed and form the spindle of microtubules which help in the separation and movement of chromosomes during concluding stage of cell divisions.

Practice:

Assignment 1. Plant cell structure.

Prepare a wet-mount slide using onion or Elodea leaf. Observe the plant cells under the microscope under both low power and high power. Sketch 2-3 cells and label any structures you recognize.

Assignment 2. Animal cell.

Observe the slide "Frog's blood" under the microscope. Draw 2-3 cells and label: cell membrane, cytoplasm and nucleus.

Assignment 3. Comparison plant and animal cells.

Study the diagrams of the plant cell (A) and animal cell (B). Make a list of main similarities and differences between plant and animal cells.

Literature:

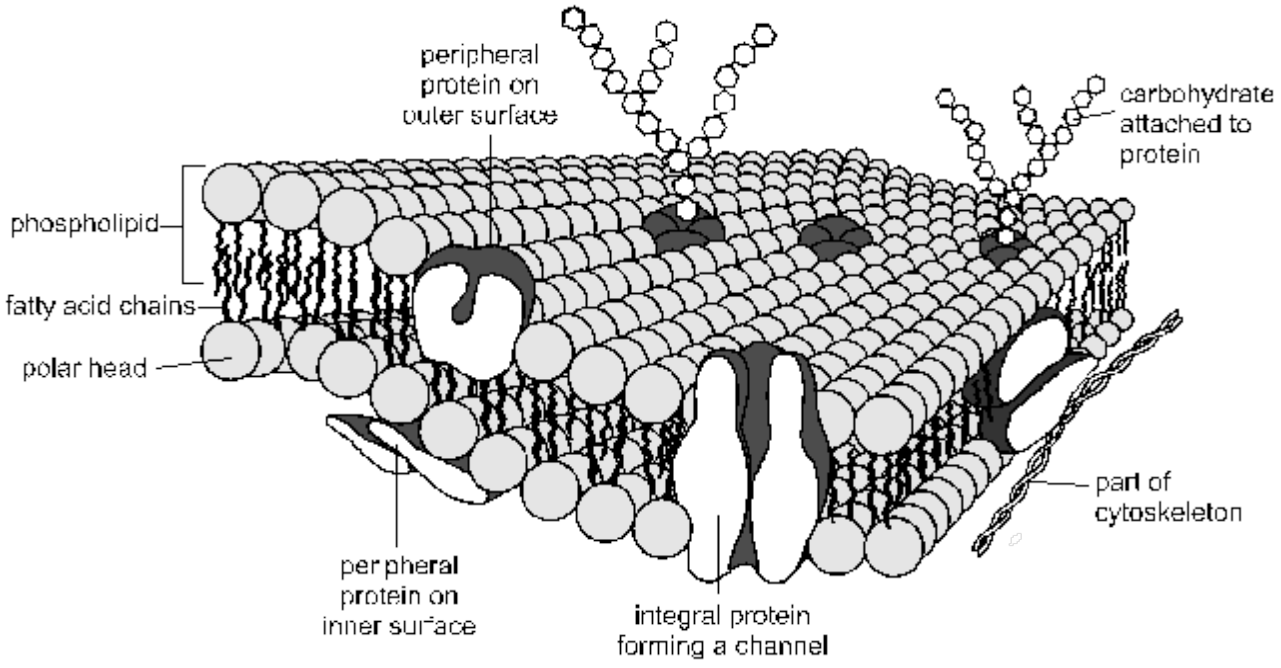
1. Lazarev K.L. "Medical biology" Simferopol, 2003.
2. Lecture's notes.
3. T.V. Bihunyak “Medical Biology”. – Ternopil, 2010.

Topic 3. Movement through the Membrane

Key concepts:

1. The Structure of the Cell Membrane
2. What is the passive transport?
3. How does diffusion affect cells?
4. What happens during diffusion?
5. What is osmosis? How does water move during osmosis
6. What is facilitated diffusion?
7. How does active transport differ from diffusion?
8. Describe phagocytosis and pinocytosis.

The Structure of the Cell Membrane The cell membrane (or plasma membrane) surrounds all living cells, and is the cell's most important organelle. It controls how substances can move in and out of the cell and is responsible for many other properties of the cell as well. The membranes that surround the nucleus and other organelles are almost identical to the cell membrane. Membranes are composed of phospholipids, proteins and carbohydrates arranged in a fluid mosaic structure, as shown in this diagram. The phospholipids form a thin, flexible sheet, while the proteins "float" in the phospholipid sheet like icebergs, and the carbohydrates extend out from the proteins.



The phospholipids are arranged in a bilayer, with their polar, hydrophilic phosphate heads facing outwards, and their non-polar, hydrophobic fatty acid tails facing each other in the middle of the bilayer. This hydrophobic layer acts as a barrier to all but the smallest molecules, effectively isolating the two sides of the membrane. Different kinds of membranes can contain phospholipids with different fatty acids, affecting the strength and flexibility of the membrane, and animal cell membranes also contain cholesterol linking the fatty acids together and so stabilising and strengthening the membrane.

The proteins usually span from one side of the phospholipid bilayer to the other (integral proteins), but can also sit on one of the surfaces (peripheral proteins). They can slide around the membrane very quickly and collide with each other, but can never flip from one side to the other. The proteins have hydrophilic amino acids in contact with the water on the outside of membranes, and hydrophobic amino acids in contact with the fatty chains inside the membrane. Proteins comprise about 50% of the mass of membranes, and are responsible for most of the membrane's properties.

- Proteins that span the membrane are usually involved in transporting substances across the membrane (more details below).
- Proteins on the inside surface of cell membranes are often attached to the cytoskeleton and are involved in maintaining the cell's shape, or in cell motility. They may also be enzymes, catalysing reactions in the cytoplasm.
- Proteins on the outside surface of cell membranes can act as receptors by having a specific binding site where hormones or other chemicals can bind. This binding then triggers other events in the cell. They may also be involved in cell signalling and cell recognition, or they may be enzymes, such as maltase in the small intestine (more in digestion).

The carbohydrates are found on the outer surface of all eukaryotic cell membranes, and are attached to the membrane proteins or sometimes to the phospholipids. Proteins with carbohydrates attached are called glycoproteins, while phospholipids with carbohydrates attached are called glycolipids. The carbohydrates are short polysaccharides composed of a variety of different

monosaccharides, and form a cell coat or glycocalyx outside the cell membrane. The glycocalyx is involved in protection and cell recognition, and antigens such as the ABO antigens on blood cells are usually cell-surface glycoproteins.

Remember that a membrane is not just a lipid bilayer, but comprises the lipid, protein and carbohydrate parts.

The various organelles and structures within a cell require a variety of substances in order to carry out their functions. In turn they form products, some useful and some wastes. Most of these substances must pass in and out of the cell.

This they do by: 1) diffusion, 2) osmosis, 3) active transport (K-N pump,endo- and exocytosis).

Passive transport

Diffusion. Diffusion is a process by which a substance moves from a region of high concentration of that substance to the region of low concentration of the same substance.

Diffusion occurs because the molecules, of which substances are made, are in random motion (kinetic theory). The rate of diffusion depends upon: the concentration gradient, the distance and the area over which diffusion takes place, the nature of any structure across which diffusion occurs, the size and nature of the diffusion molecule.

Facilitated diffusion. This special form of diffusion allows more rapid exchange. It may involve channels within a membrane which make diffusion of specific substances easier. These channels form water - filled connections across the lipid bilayer which allow water - soluble substances to move across. They are important therefore in transporting ions. The channels are selective in that they will open or close in response to certain signals such as a change in voltage or the binding of another molecule. In this way the cell can control the entry and exit of molecules and ions. An alternative form of facilitated diffusion, involves different protein molecules in the membrane called ***carrier proteins.*** These bind molecules to them and then change shape as a result of this binding in such a way that the molecules are released to the inside of the membrane.

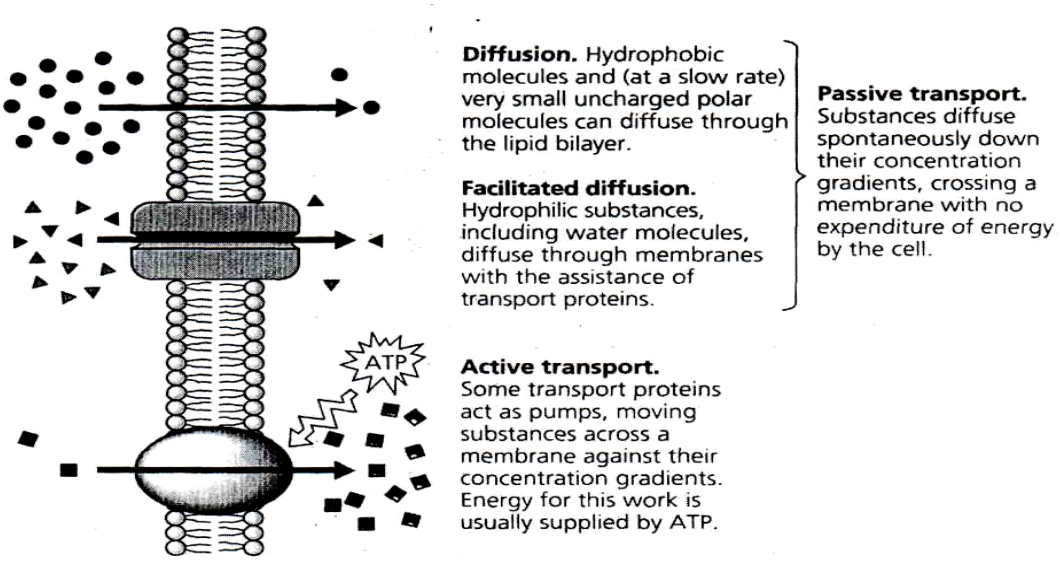
In all cases facilitated diffusion does not involve the use of energy (i.e. it is passive) and hence material is moved along a concentration gradient (i.e. from high to low concentration).

Osmosis. Osmosis is a special form of diffusion which involves the movement of solvent molecules. The solvent in biological system is invariably water. Most cell membranes are permeable to water and certain solutes only. Such membranes are termed partially permeable. Osmosis in living organisms can be defined as: the passage of water from a region where it is highly concentrated to a region where its concentration is lower, through a partially permeable membrane.

If a solution is separated from its pure solvent, the pressure which must be applied to stop water entering that solution and so prevent osmosis is called ***osmotic pressure.*** The more concentrated a solution the greater is its osmotic pressure.

Osmosis occurs not only when a solution is separated from its pure solvent by a partially permeable membrane but also when such a membrane separates two solutions of different concentrations. In this case, water moves from the more dilute or ***hypotonic,*** solution, to the more concentrated, or ***hypertonic*** solution. When a dynamic equilibrium is established and both solutions are of equal concentration they are said to be ***isotonic.***

Types of transport through membrane



Active transport

Some molecules are transported in and out cells by active means, i.e. energy is required to drive the process. The energy is necessary because molecules are transported against a ***concentration gradient,*** i.e. from a region of low concentration to one of high concentration. It is though that the process occurs through the proteins that span the membrane. These accept the molecule to be transported on one side of the membrane and by a change in the structure of the protein, convey it to the other side. A good example of active transport is the ***sodium - potassium pump which exits*** in most cell membranes. This actively removes sodium ions from their surroundings.

These pumps (an integral protein) require energy in order to function. In fact, ATP molecules are broken into ADP + P + energy which are utilized by the pump.

Transport of Na^+ and K^+ is believed to occur in the following stages. Sodium ions and ATP molecule inside the cell are bound to specific sites on the enzyme carrier, while potassium ions are bound to a site on the same enzyme facing the exterior of the cell. Binding of the substrate results in and is followed by a change in the conformation (the tertiary structure) of the enzyme carrier in such a way that the bound sodium potassium ions are translocated across the membrane.

Translocation is followed by an alteration of the binding sites so that, sodium ions are released outside and the potassium ions are released inside. Once the ions and ADP released, the enzyme carrier undergoes another change in its tertiary structure and comes back to its initial conformation and restart another round of transport cycle.

Due to the energy expenditure necessary to move molecules against a concentration gradient, cell and tissues carrying out active transport are characterized by:

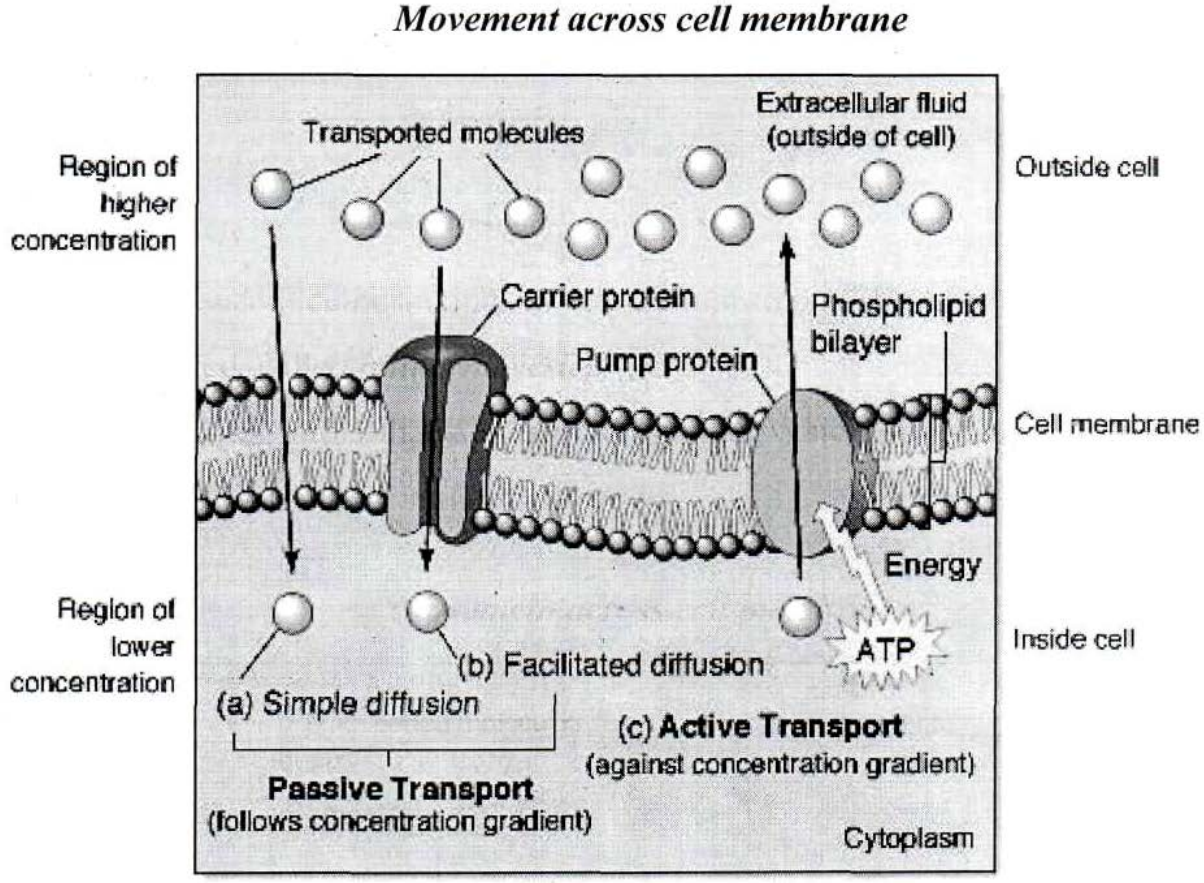
1. The presence of numerous mitochondria.
2. A high concentration of ATP.
3. A high respiratory rate.

Any factor which increases the rate of respiration, e.g. a higher temperature or increased concentration of oxygen, will increase the role of active transport.

Any factor reducing the rate of respiration or causing it to cease, e.g. the presence of cyanide, will cause active transport to slow or stop altogether.

Bulk transport

Carriers and channels located in membranes are not the only actors involved in transportation across membranes. Macromolecules and large substrates can not pass through the membrane in the same manner as ions, monosaccharide and amino acids. Bulk transport is a transport of material (ranging from ions to macromolecules and microorganisms) through cell membranes, in both directions by vesicles. It is divided into two classes: ***endocytosis*** and ***exocytosis.***



Endocytosis is a formation of cytoplasmic vesicles from the plasma membrane and the consequent entrapment within these vesicles of substances formerly located in the cell surroundings. There are following types of endocytosis: ***pinocytosis*** and ***phagocytosis.*** On the contrary, movement of material from the cytoplasm outward by fusion of cytoplasmic vesicles with the plasma membrane is called **exocytosis.**

Phagocytosis is the process by which the cell can obtain particles that are too large to be absorbed by diffusion or active transport. The cell invaginates to form a cup - shaped depression in which the particle is contained. The depression is then pinched off to form a vacuole. Lysosomes fuse with the vacuole and their enzymes break down the particle, the useful contents of which may be absorbed. The process only occurs in a few specialized cells (called phagocytes), such as white blood cells where harmful bacteria can be ingested, or Amoeba where it is a means of feeding.

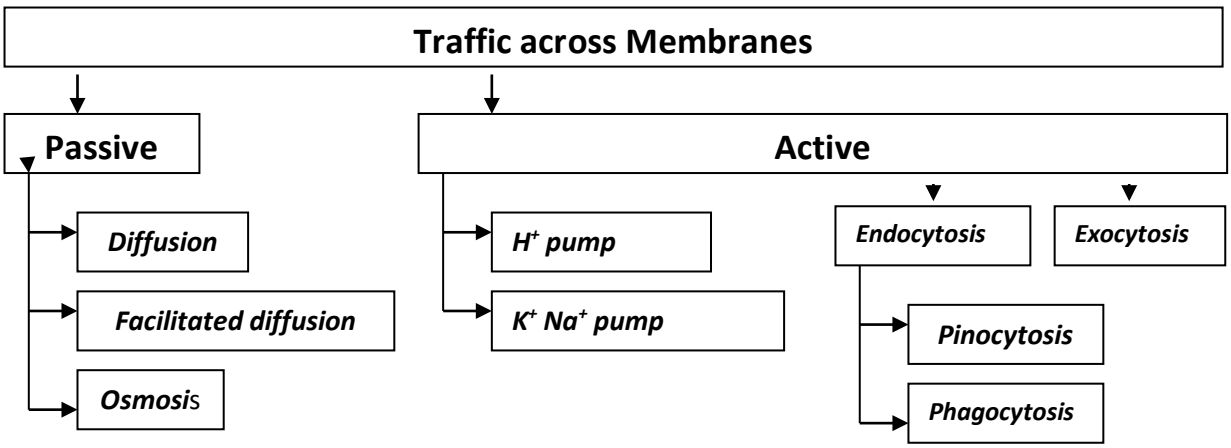
Pinocytosis or "cell drinking" is very similar to phagocytosis except that the vesicles produced, called ***pinocytic vesicles,*** are smaller. The process is used for the intake of liquids rather than solids. Even smaller vesicles called ***micropinocytic vesicles*** may be pinched off in the same way.

Both pinocytosis and phagocytosis are methods by which materials are taken into the cell in bulk. This process is called ***endocytosis.*** By contrast, the reverse process, in which materials are removed from the cell in bulk, is caused ***exocytosis.***

Practice:

Assignment 1. Movement through the membrane.

Copy the diagram and give an example of each type of transport through membrane.



Assignment 2. Plasmolysis and desplasmolysis in the cells.

Prepare a wet-mount slide using Elodea leaf (or thin section other plant cell). Add a drop of concentrated salt solution to the slide. If the cells are watched, the cytoplasm will be seen to shrink away from the cell wall as the solution withdraws

water by osmosis. There is now no pressure outwards of the cell wall and the cell is flaccid. This condition is called ***plasmolysis.*** Draw it. Mount the plasmolysed section in distilled water. As a result it is observed that the protoplasmic layer recovers its position. This condition is called ***desplasmolysis.*** Draw it.

Literature:

1. Lazarev K.L. "Medical biology" Simferopol, 2003.
2. Lecture's notes.
3. T.V. Bihunyak “Medical Biology”. – Ternopil, 2010.

Topic 4. Chemical Nature of Genetic Material. Gene structure.

Key concepts:

1. What is the overall structure of the DNA molecule?
2. What is a nucleotide? Describe it structure.
3. How are the nucleotides joined together to form the DNA chain?
4. Were and in what form is eukaryotic DNA found?
5. How are the long DNA molecules found in eukaryotes packed into short chromosomes?
6. What happens during DNA replication,reparation?
7. What are the tree main types of RNA? What function do they perform?
8. What are introns and exons?
9. Describe the main differences between DNA and RNA.
10. What is a codon (anticodon)?
11. Describe the main characteristics of Genetic Code.
12. What is a gene? Types of Genes.
13. Gene structure in prokaryotic cell and eukaryotic cell.

Nucleic Acids

Nucleic acids are biopolymers consisting of smaller units (monomers) called ***nucleotides.*** Each nucleotide consists of:

- a sugar molecule (ribose or deoxyribose)
- a mole of phosphate

- a molecule of a nitrogenous base: adenine (A), guanine (G), cytosine (C), thymine (T) and uracile (U). Covalent bonds that link the three nucleotide - constitutive - molecules always involve the same atom. The nitrogenous base is covalently bonded to the number 1 carbon atom of one pentose molecule. The phosphoric acid molecule is covalently bonded to the fifth carbon atom of the pentose molecule.

There are two classes of nucleic acids:

- Deoxyribonucleic acid (DNA)
- Ribonucleic acid (RNA)

DNA

Two forms of nitrogenous base are found in the DNA molecule: double-ring purines and single-ring pyrimidines. The two purines of DNA are ***adenine*** and ***guanine,*** and the two pyrimidines of DNA are the ***cytosine*** and ***thymine.*** A phosphate and a deoxyribose sugar (pentose) molecule are common to all the four kinds of nitrogenous bases.

The DNA molecule consists of two helical polynucleotide chains wound around each other. Its purine nitrogenous base always faces a pyrimidine one because the diametric distance between two helical polynucleotide chains is too small to accommodate two juxtaposed purine molecules. In DNA A is complementary to T and C is complementary to G. Complementary within each of these two couples is based on the possibility of establishing hydrogen bonds that maintain the double-helical structure. The two complementary polymers of nucleotides (called strands) that make up a double helix are antiparallel (5^1 end faces 3' end and vice versa). That is, beginning at one end of the molecule and progressing toward the other, successive nucleotides of the same chain are joined together by **3'—>5'** ***phosphodiester bonds,*** whereas the complementary nucleotides of the other chain are joined by **5'—>3'** ***phosphodiester bonds.***

The exact arrangement of purine-pyrimidine matches is specific for every species of organism. This means that there are many patterns of DNA molecules as there are kinds of animals and plants. The same specific pattern is duplicated at the time of cell division, so that the daughter cells receive the same kind of DNA not

only qualitatively, but also quantitatively. This principle automatically leads to another principle - that before a cell can divide, its DNA must be duplicated exactly. This process is carried out during interphase before cell division (mitosis and meiosis) and is responsible for transmission of genetic information from one generation to another. It involves enzymes (such as DNA polymerase) and expenditure of energy. Because the sequence of bases in one polynucleotide chain automatically determines the sequence of bases in the other, it is clear that one-half of DNA molecules contain all the information necessary for constructing a whole molecule.

DNA ***replication*** (=duplication) is *semi-conservative* because each of the two daughter DNA molecules (the two sister chromatids) contains one old strand and it's complementary, which is newly synthesized. Replication starts by a local separation of the two strands forming the DNA double helix, then DNA polymerases start polymerizing nucleotides on both strands. Selection of the sequence in which nucleotides must be polymerized is based on complementary: A - T and C - G; the old strand serves as a template.

DNA Reparation

Since many mutations are deleterious, DNA repair systems are vital to the survival of all organisms.

Living cells contain several DNA repair systems that can fix different type of DNA alterations.

DNA repair mechanisms fall into 2 categories

– Repair off damaged basses

– Repair off incorrectly base paired basses during replication

In most cases, DNA repair is a multi-step process

1. An irregularity in DNA structure is detected
2. The abnormal DNA is removed
3. Normal DNA is synthesized

DNA is resistant to heating. In fact when a DNA solution is heated at 100°C, the two strands of the double helix untwist after cutting of hydrogen bonds (weak

bonds). Therefore, strands separate from each other but the nucleotide sequence is not affected. This process is called DNA denaturation. It is reversible because if temperature of the same solution is decreased progressively the two strands rejoin again (hydrogen bonds reestablished between complementary sequences) and reform the double helix.

DNA condensation

In a single human cell the length of the DNA helix that constitutes the 46 chromosomes is about two meters. This very long molecule can't fit in the nucleus without the interaction of proteins, which will allow many levels of condensation. The highest level of condensation is attained by the metaphase chromosomes whereas the lowest level is observed in euchromatin.

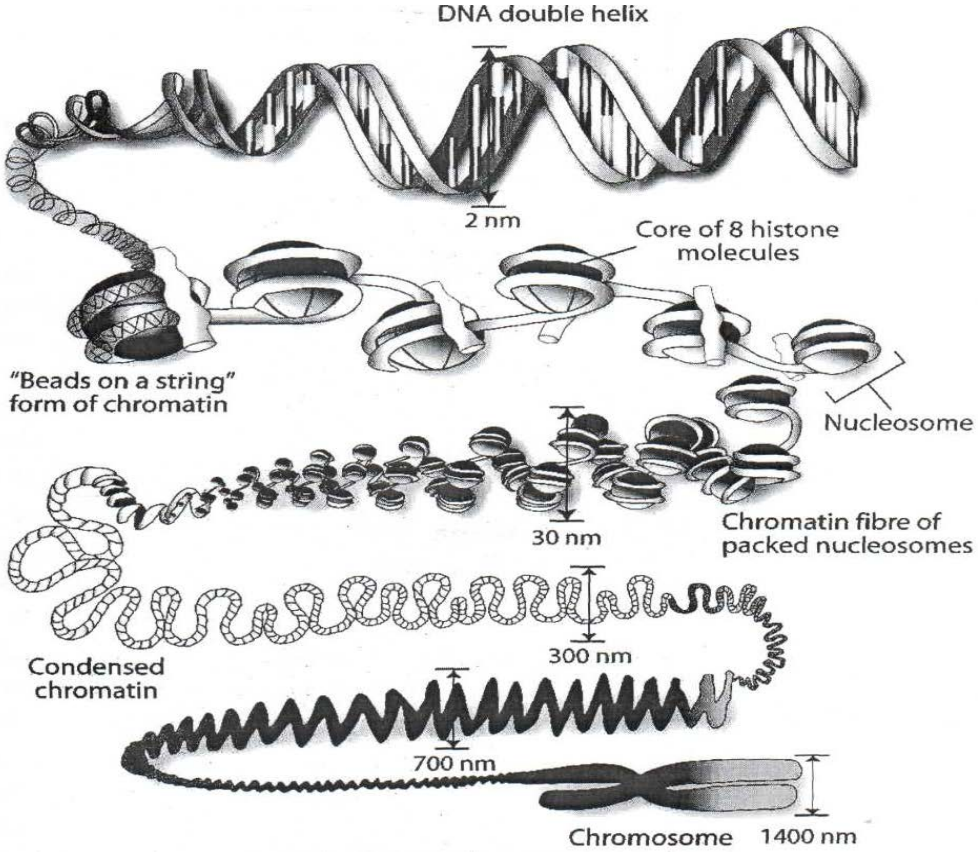
Observation of chromatin reveals the presence of chromatin fibers or ***nucleofilaments*** that consists of a filament of 11 nm of diameter having "beads on a string" morphology. This first level of condensation results from winding of the DNA helix around nucleosomes that are composed of 8 molecules of histones proteins. This ***octamer*** which is cylindrical is also called *histone core*. 146 base pairs of the DNA helix make one turn and three quarters around the histone core (about 80 base pairs per turn). The distance separating two nucleosomes is called DNA linker, which is about several tens of base pairs. This first level of condensation causes two to three-fold reduction of the genetic material length.

The nucleofilament undergoes an additional level of condensation that requires the intervention of the fifth histone protein called histone H1. H1 molecules bind to the DNA helix at the level of nucleosomes. The successive H1 molecules interact with one another to produce a 30 nm chromatin fiber also called **solenoid shape.** In this level nucleosomes are helically distributed at six nucleosomes per solenoid turn. The genetic material length is now reduced by 40 to 50-fold so that the average length of a chromosome is about 1 mm (about 200 times larger then the nucleus diameter).

Further condensation levels are required to permit fitting of chromosomes in the nucleus. These additional levels necessitate involvement of non histone proteins (or chromosomal protein). These proteins allow formation of a 300 nm

fiber that result from looping and attaching the 30-nm chromatin fiber. The last level of condensation is produced by specific proteins that cause spiralization of the 300 nm fiber into a chromosome having 700 nm of diameter.

DNA CONDENSATION



RNA. They differ from DNA by the fact that the pentose molecule is ribose instead of deoxyribose in DNA and the nitrogenous base uracil instead of thymine. RNA generally occurs as a single polynucleotide chains although some viral RNA are double-stranded. Nevertheless, the single stranded chain often looks back upon itself to make double-helical regions stabilized by hydrogen bonds, as is the DNA double helix. RNA synthesis is directed by specific segments of DNA (genes). Transcription of genes into RNA is carried out by a certain number of specific enzymes; among them are the RNA polymerases. Transcription is similar to sequence complementary to DNA. Generally only one strand (not chosen randomly) is read in transcription.

RNA is divided into several types:

- Ribosomal RNA (r-RNA, representing up to 85 % of total RNA);

- Messenger RNA (m-RNA, representing up to 5 % of total RNA);
- Transfer RNA (t-RNA, representing about to 10 % of total RNA).

M-RNA is much more heterogeneous with respect to size and sequence of nucleotides since they result from transcription of several ten thousands of genes. R-RNA is only several types and result from transcription of two genes existing in many copies.

Regarding t-RNA, there are about thirty three types having the same trefoil structure with three loops. This secondary structure is obtained by pairing between portions of the same single stranded molecule. However, the diverse t-RNAs differ by the anticodon and the aminoacyl-binding site. That is certain t-RNA is characterized by its anticodon and the corresponding site that binds a specific amino acid. R-RNA is components of ribosome, which, in collaboration with t-RNA and many enzymes translate the m-RNA sequence into protein in the cytoplasm.

Genetic Code

DNA carries genetic information from cell to cell and from generation to generation. ***Genetic Code*** is a system of nucleotides placed in DNA molecule that controls amino-acids position sequence in protein molecule.

The group of nucleotides that specifies one amino acid is a code word or ***codon.*** The codon words of DNA would be complementary to the m-RNA cord words (i. e., DNA codes run in 3'—>5' direction and m-RNA code words ran in 5'—3' direction) and so thereby the three bases forming the anticodon of t-RNA (i. e., bases of anticodons run in 3'->5'direction). Three bases of anticodon pair with m-RNA on the ribosome at the time of aligning the amino acids during protein synthesis. The codon and anticodon pairing is antiparallel.

The genetic code has the following general properties:

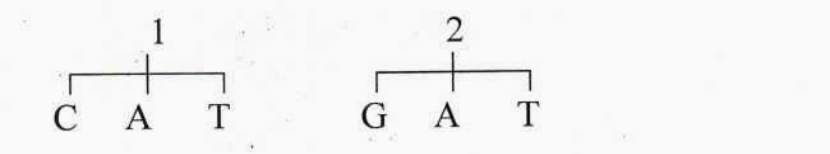
1. The code is a triplet codon.

The nucleotides of m-RNA are arranged as a linear sequence of codons, each codon consisting of three successive nitrogenous bases, i. e., and the code is a triplet codon. ***Triplet*** is a name for three nucleotides which code one amino acid. Four nucleotides combined by three make 64 different codones. There are 61

informational triplets and three triplets that code no amino acids (UAG, UAA, and UGA). A direct evidence for a triplet code came from the finding that a piece of m-RNA containing 90 nucleotides, corresponded to a polypeptide chain of 30 amino acids of a growing hemoglobin molecule. Similarly, 1200 nucleotides of "satellite" tobacco necrosis viruses direct the synthesis of coat protein molecules which have 372 amino acids.

2. The code is non-overlapping.

It means that a base in an m-RNA is not used for different codons.



3. The code is commaless. It means that no codon is reserved for punctuations: after one amino acid is coded, the second amino acid will be automatically coded by the next three letters and that no letters are wasted as the punctuation marks.

4. The code is non-ambiguous. Non-ambiguous code means that a particular codon will always code for the same amino acid. In case of ambiguous code, the same codon could have different meanings or in other words, the same codon could code two or more than two different amino acids.

5. The code has polarity. The code is always read in a fixed direction, i. e., in the 5'->3' direction.

Codon: UUG, AUC, GUC, UCG, CCA, ACA, AGG

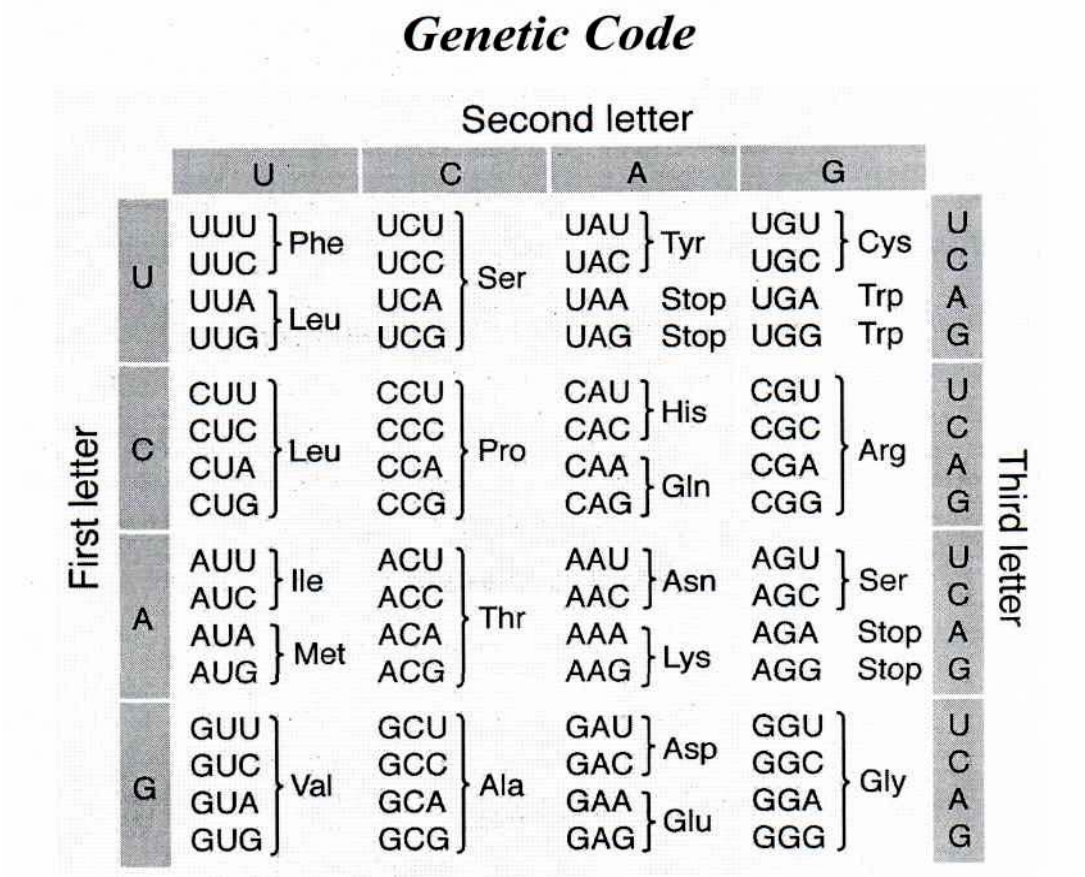
Polypeptide: Leu, Ile, Val, Ser, Pro, Thr, Arg

6. The code is degenerate. More than one codon may specify the same amino acid; this is so called ***degeneracy*** of the code. For example, except for ***tryptophan*** and ***methionine,*** which have a single codon, each, all other 18 amino acids have more than one codon.

The code degeneracy is basically of two types: partial and complete. ***Partial degeneracy*** occurs when first two nucleotides are identical but the third nucleotide of the degenerate codons differs (e. g. CUU and CUC code for leucine). *Complete* ***degeneracy*** occurs when any of the four bases can take third position and still code for the same amino acid (e. g. UCU, UCC, UCA code for serine).

Degeneracy of genetic code has certain biological advantages. For example, it permits essentially the same complement of enzymes and other proteins to be specified by microorganisms varying widely in their DNA base composition.

Degeneracy also provides a mechanism of minimizing mutational lethality.



Some codes act as start codons. In most organisms AUG codon is the ***start*** or ***initiation codon,*** i. e., the polypeptide chain starts either with methionine.

7. ***Some codes act as stop codons.*** Three codons UAG, UAA and UGA are the chain ***stop*** or ***termination codons.*** They do not code for any of the amino acids. These codons are not read by any t-RNA molecules, but are read by some specific proteins, called **release factors.** These codons are also called **nonsense codons** since they do not specify any amino acid.

9. The code is universal. Same genetic code is found valid for all organisms ranging from bacteria to man.

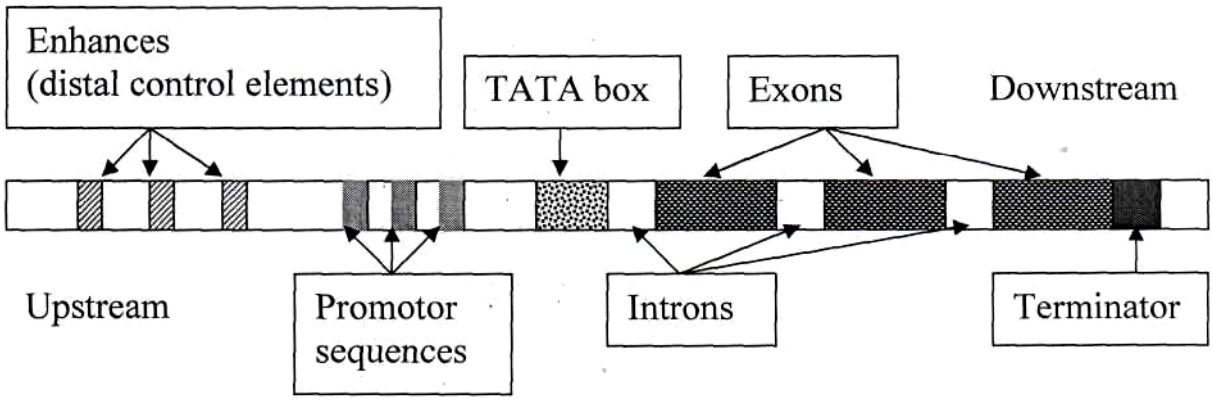
10. The code is collinear - the sequences of nitrogen bases of a given segment of DNA molecule, actually, has been found to be identical to linear sequence of amino acids in a protein molecule.

Gene.

Gene is a segment of DNA that codes for one polypeptide, one t-RNA or one ribosomal RNA (r-RNA) molecule.

Genes are components of genetic material and are thus units of inheritance. They control the morphology or phenotype of individuals, the metabolism of the body. Reshuffling of genes at the time of sexual reproduction produces variations. Differentiation or formation of different types of cells, tissues and organs in various parts of the body is controlled by expression of certain genes and no expression of others. Development of different stages in the life history is controlled by genes.

The organization of the eukaryotic gene.



Control elements - simply segments of noncoding DNA that help regulate transcription of a gene by binding proteins - transcription factors.

Promoter - the DNA sequence where RNA polymerase attaches and initiates transcription.

'TATA' box - helps position RNA polymerase by making a point just before the point at which transcription begins.

Exons - segments of the gene which contribute to encoding the amino acid sequence.

Introns - meaningless sequence which not translated into amino-acid chains.

Terminator - the sequence of nucleotides that signals the end of transcription.

Types of Genes

1. ***Structural Genes*** are those genes which have encoded information for the synthesis of chemical substances required for cellular machinery: proteins, enzymes, hormones, antibodies, non-translated RNAs (t-RNA, r-RNA).

2. ***Regulatory Genes.*** They are meant for controlling the functions of structural genes. The important regulatory genes are: promoters, terminators, operators, and repressor.

3. ***House Keeping Genes (Constitutive Genes).*** They are those genes which are constantly expressing themselves in a cell because their products are required for the normal cellular activities, e.g., genes for glycolysis .

4. ***Non-constitutive Genes.*** The genes are not always expressing themselves in a cell. They are switched on or off according to the requirement of cellular activities. Non-constitutive Genes are of further two types: inducible and repressible.

Practice;

Assignment 1. DNA structure and replication.

- Model a nucleotide taping together a phosphate group, a sugar and a nitrogenous base. Assemble some nucleotides molecule (4) and make a single strand of DNA. Label the main structures of a chain. Construct a segment of DNA molecule. Draw DNA replication.

Assignment 2. Genetic code.

Use genetic code and solve the problems.

1. One strand of DNA molecule has the following sequence of nucleotides: GAC-AAG-TCC-ACA-ATC. What is the sequence of nucleotides of the other strand? Define the percentage of each nucleotide in this fragment of DNA molecule.

2. The fragment of DNA molecule contains 440 guanine nucleotides that are 22% of the total amount. How many other nucleotides contain the fragment of DNA molecule? Define the length and mass of this fragment, if the nucleotide length is 0, 34 nm and its mass is 345.

3. In one fragment of DNA chain the nucleotides are placed in the following order: 5' GGC-CCC-CAA-GGA-TGC 3'. Find out the nucleotide sequence in the

complementary DNA chain. With an arrow show the direction of new DNA chains synthesis during its replication.

4. The percentage of nitrogenous bases in one chain of a DNA double helix is as follows: thymine - 40 % and cytosine - 22 %. What is the percentage of nitrogenous bases adenine and guanine in the same DNA chain? What is the percentage of nitrogenous bases adenine and guanine in the complementary DNA chain?

5. The analysis of a DNA sample extracted from a tissue showed that 38 % of nitrogenous bases were adenine. What percentage of the DNA nitrogenous bases would be guanine? Show how you arrive at your answer.

Assignment 3. A comparison of DNA and RNA.

Copy and complete the following table.

Features	**DNA**	**RNA**
Localization		
Molecule structure		
Composition of nucleotides		
Functions		

Literature:

1. Lazarev K.L. "Medical biology" Simferopol, 2003.
2. Lecture's notes.
3. T.V. Bihunyak “Medical Biology”. – Ternopil, 2010.

Topic 5. Gene Expression. Protein synthesis.

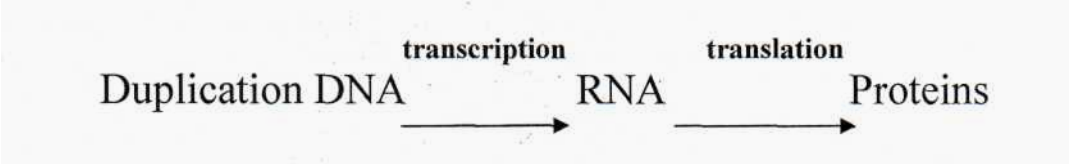
Key concepts:

1. What is "Central Dogma", transcription and translation?
2. Describe the main processes of translation and transcription.

DNA serves to carry genetic information from cell to cell and from generation to generation. This information is translated into proteins that determine the phenotype. All the phenotypes examined so far are the result of the biochemical reactions that occur in the cell. All of these reactions require enzymes and enzymes are proteins. Each enzyme is a unique molecule catalyzing a specific chemical reaction. Other phenotypes are due primary to the kinds and amounts of non-enzymatic proteins (including the structural proteins) present, for example, hemoglobin, myoglobin, gamma globulin, insulin, fibroin (silk protein) or collagen. Proteins are composed of one or more, long linear polymers of amino acid residues (polypeptide chains) that are synthesized almost exclusively in the cytoplasm.

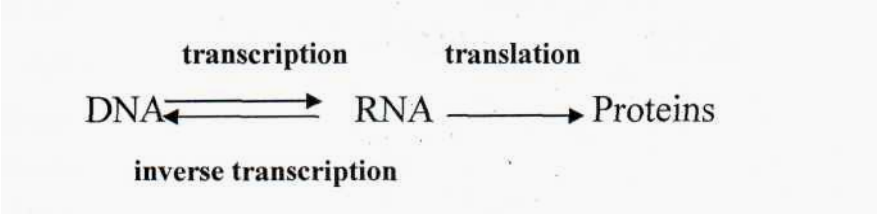
How does the information present in the sequence of bases (= triplet codons) of the m-RNA is translated into a sequence of amino acids in proteins.

The process of synthesis of protein involves one of the central dogma of molecular biology, which postulates that genetic information flows from nucleic acids to protein. The first step of this central dogma is known as *transcription* and does not involve a change of code since DNA and m-RNA are complementary. The second step involves a change of code from nucleotide sequences to amino acid sequences and is called ***translation.*** It can be illustrated as follows:



Later on it was found an enzyme "RNA depend DNA polymerase" ***(inverse transcriptase)*** which could synthesize DNA from a single stranded RNA template. It gave rise to the concept of ***"central dogma reverse"*** or ***teminizm***

suggesting that the sequence of information flow is not necessary from DNA to RNA to protein but can also take place from RNA to DNA. The central dogma reverse can be illustrated as follows:



In fact, the step of translation of this central dogma is the time in the flow of information between genes and proteins to change the language being used. In going from DNA to RNA the language (nucleotide sequences) remained the same. However, in going from RNA to protein the language is changed from a nucleotide sequence to an amino acid sequence.

Mechanism of protein synthesis.

Expression of genetic information is passage from the nucleic acid language (built up of a succession of four types of nucleotides) to the protein language (built up of 20 amino acids). The biological reactions are grouped into two major steps: ***transcription*** and ***translation.***

Transcription is a process that occurs in the nucleus (on chromosomal DNA), mitochondria and chloroplasts. During transcription the sequence of nucleotides of gene (a segment of DNA) is copied into the complementary sequence of nucleotides in RNA molecules.

Only one DNA strand of the double helix is transcribed into RNA. It is called the ***template strand,*** which, in a certain gene, is always the same and is not chosen randomly. The enzymes carrying out transcription are called ***DNA-dependent RNA polymerases,*** a large complex composed of several different subunits. While in prokaryotic cells only one type of RNA polymerase is found and is responsible for transcription of all of the genes, in eukaryotic cells there are three types of RNA polymerases, each producing a different class of RNA.

The process of transcription starts and terminates at specific sites (within the gene) called ***promoter*** and ***termination site,*** respectively. At the promoter level specific enzymes do a local separation of the two strands of the DNA double helix. Then the RNA polymerase recognizes the starting site and begins to polymerize triphosphate ribonucleotides in the 5'—»3' direction, where as the template strand is read in the 3'—»5' direction (antiparallel way).the energy necessary to

polymerization is supplied by hydrolysis of the ATP into AMP. The RNA-polymerase moves along the DNA helix parallel to movement of enzymes that locally unwind the DNA helix. When the transcriptional complex reaches the termination site, the synthesized RNA molecule is released, enzymes are recycled and the DNA helix comes back to its initial state.

Transcription principle is the same in eukaryotes and prokaryotes. However, because of genes of eukaryotes and prokaryotes are not similarly organized, the process of gene expression presents certain differences. The genes of prokaryotes are continuous while those of eukaryotes are discontinuous. That is genes of eukaryotes contain ***exons*** and ***introns.***

Exons are segments of the genes which contribute to encoding the amino-acids sequence, whereas ***introns*** are meaningless sequences (not translated into amino-acid chains). Consequently, in prokaryotes ***m-RNAs are primary transcripts*** and are ready to be translated as soon as the 5' extremity of the m-RNA is separated from the template. In prokaryotes translation starts on the 5' end of the m-RNA while transcription is still in progress because m-RNA does not require ***maturation*** and is directly accessible to ribosomes.

On the contrary, in eukaryotes, RNA polymerases produce ***primary transcripts*** that are not ready for translation. All RNA types require ***maturation.*** It occurs in the nucleus before the m-RNA is in contact with ribosomes. During maturation all introns are removed from the primary transcripts (the precursors of m-RNAs). The introns must not be translated. The primary transcript of almost all eukaryotes are spliced, capped and polyagenylated.

Splicing is removing of introns that must not be translated.

Capping is a modification of the 5'-end of the m-RNA molecule, consisting of the binding of a modified guanine molecule by a specific enzyme. The cap plays a certain role in continuation of maturation, but also in translation by helping recognition of mature m-RNA by ribosomes.

Polyadenylation is the addition of a polyadenine (poly A) tail at the 3'-by a specific enzyme called poly-A-polymerase.

There is another difference between m-RNAs of prokaryotes and eukaryotes. m-RNA of prokaryotes may be either polycistronic (polygenic) or monocistronic (monogenic) while those of eukaryotes are monocistronic (monogenic). That is, in prokaryotes two or three genes are transcripted into the same m-RNA because they are located between a promoter and a termination site. However, in eukaryotes, each gene has its promoter and termination site.

Translation. Translation is a polymerization of amino-acids in a sequence that depends on the sequence of codons (triplet of nucleotides) in the m-RNA molecule. It takes place in the cytoplasm of all cells and requires the collaboration between ribosomal particles, m-RNA and t-RNAs.

The process of translation can be divided into the following three main steps:

- initiation
- elongation
- termination

Initiation. Translation starts at an initiator codon (usually 5' AUG 3'). The small ribosomal subunit recognizes certain signals on the m-RNA molecule and binds to it at the level of the initiator codon. Subsequently, the initiator codon is recognized by a specific initiator t-RNA. The following action is the binding between the large ribosomal subunit and the complex (m-RNA, the small subunit and the initiator t-RNA) in such a way that the methionine-t-RNA attaches to the "A" binding site.

Then the methionine-t-RNA is translocated into the "P" site so that the "A" one becomes free (empty) and able to receive the convenient t-RNA that has an anticodon complementary to the codon - triplet occurring immediately after the initiator one and charged with its specific amino acid. The two t-RNAs are now occupying the two ribosomal sites and their respective amino acids are very close to each other. Specific enzymes, which are component of ribosomes, intervene to make a peptide (covalent) bond between the methionine COOH group and the NH_2 group of the amino acid carried by the t-RNA in the "A" site. During formation of peptide bond, methionine is separated from its initiator t-RNA so that the two linked amino acids are now carried by the t-RNA that is in the "A" site.

The main features of the ***initiation*** step are:

- the binding of m-RNA to the ribosome;
- selection of the initiation codon;
- amino acid activation;
- transfer of activated amino acid to t-RNA and binding of acylated t-RNA bearing the first amino acid.

Elongation following initiation. The "A" site is emptied after formation of the peptide bond and translocation of the t-RNA, carrying the growing amino-acid chain to the "P" site. So the growing chain, added by one amino-acid is transferred to the t-RNA located in the "A" site. The ribosome moves, relative to m-RNA, by three nucleotides in the 5'->3' directions in such a way that "P" site releases the t-RNA and receives the t-RNA that carries the growing chain. Thus, the "A" site is again free and can accept another charged t-RNA having an anticodon complementary to the following codon. These processes are repeated cyclically at each codon.

Termination. The ribosome moves along the m-RNA codon by codon and the polypeptide grows amino-acid by amino-acid until the ribosomes reaches one of the three terminating codons. Protein factors called ***terminating factors*** recognize the stop codon and destabilize the translational complex so that ribosomes are dissociated and separated from the m-RNA and the finished polypeptide chain is released (in the cytoplasm or the lumen of the ER).

A certain m-RNA molecule may be translated by many ribosomes in a successive manner. A polysome or polyribosome is a m-RNA molecule being translated by many ribosomes. The rate of translation which is the number of ribosomes that translate each m-RNA molecule depends on the m-RNA type (that is the gene which was transcribed).

Regulations of gene activities in eukaryotes are considered to occur at the following levels involving diverse mechanisms:

1) Regulation at the level of DNA.
2) Regulation at the level of transcription.
3) Regulation at the level of translation.

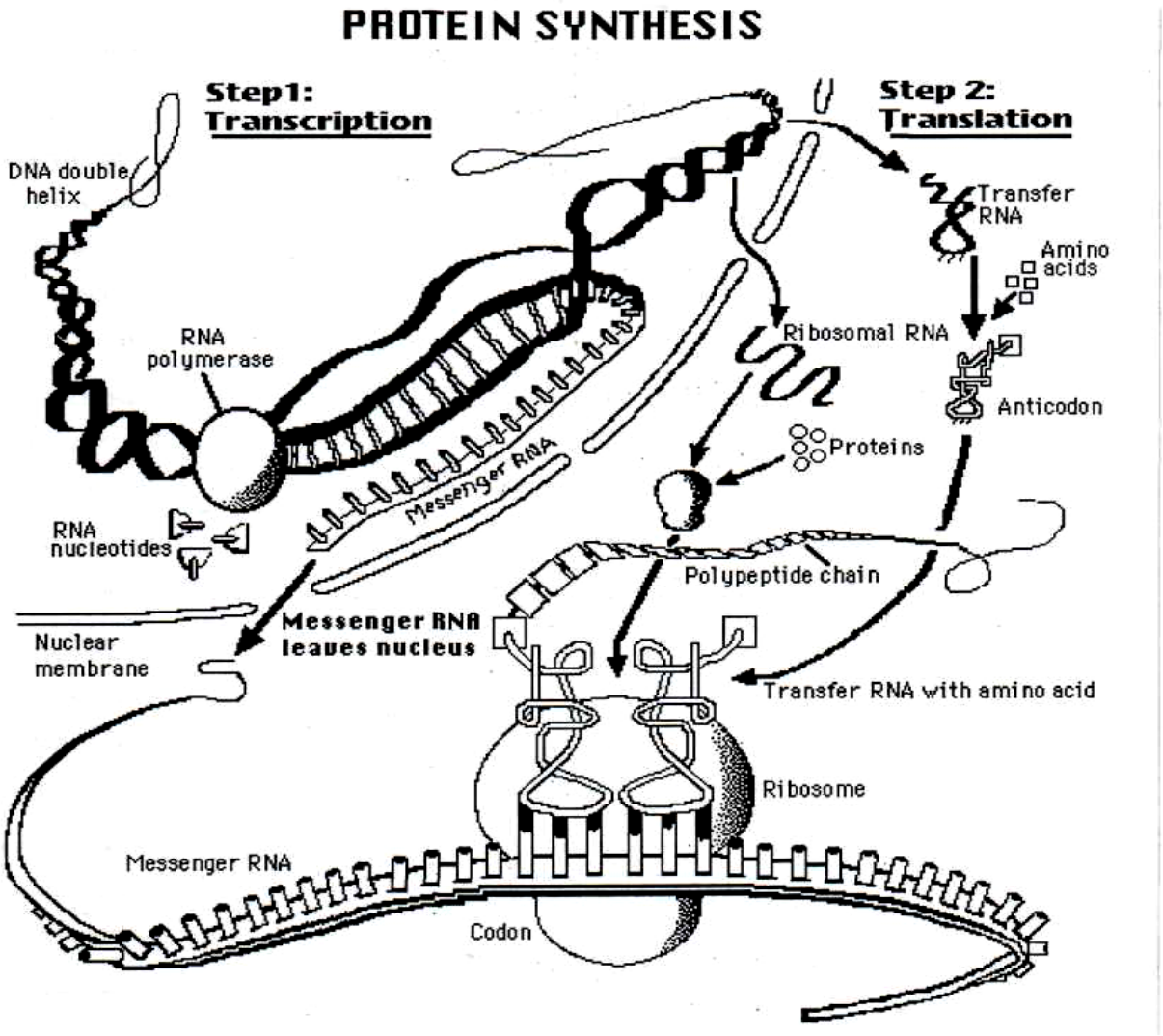
4) Regulation at the level of post-translation.

Practice:

Assignment 1. Solving problems.

- One template strand of a gene has the following sequence of nucleotides: GAC-AAG-TCC-ACA-ATC. Write the sequence of the mRNA molecule trasricribed from this gene and amino-acid sequence of the polypeptide translated from the mRNA.
- How many different mRNAs could specify the amino-acid sequence: Met-Phe-Ser-Pro?
- The starting section of protein molecule has the following structure: Ala-Ser-Lei-Val. Define the structure of DNA which codes this protein.

Assignment 2. Protein synthesis.



Exam the diagram "Protein synthesis" and write explanatory notes of the following:

1. Transcription — 2. Processing – 3.Splicing-4.Pre-mRNA-5.Translation - 6.Initiation- 7.Elongation-8.Termination-

Literature:

1. Lazarev K.L. "Medical biology" Simferopol, 2003.
2. Lecture's notes.
3. T.V. Bihunyak “Medical Biology”. – Ternopil, 2010.

Topic 6. Chromosomes. Human karyotype. Cell cycle

Key concepts:

1. Describe the structure and role of the nucleus in the cell.
2. What are chromosomes made of? Describe four types of chromosomes.
3. What are the levels of DNA condensation in the nucleus?
4. What is karyotype, idiogram?
5. What are the main events of the cell cycle?
6. Describe what happens during interphase?
7. Describe what happens during each of the four phases of mitosis.
8. What is cytokinesis?

Nucleus.

The ***nucleus*** is the most significant component of the cell which controls various metabolic activities of the cell and contains the genetic material - DNA. The nucleus was discovered by Robert Brown in 1831. Since its discovery the cytologists become interested in it more and more because of its extraordinary participation in the cellular metabolism and hereditary.

The nucleus is found in all the eukaryotic cells of the plants and animals. However, certain eukaryotic cells such as the mature sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. In such cells the nucleus are present during the early stages of development. The prokaryotic cells of bacteria do not have true nucleus, i.e., the single, circular and large DNA molecule remains in direct contact with the cytoplasm. The location of the nucleus in a cell is often

variable. Usually nucleus remains located in the center. But its position may change from time to time according to the metabolic states of the cell.

The nucleus is composed of the following structures:

1. The nuclear membrane.
2. The nuclear sap or nucleoplasm.
3. The chromosomes.
4. The nucleolus.

Nuclear membrane. The nuclear membrane forms an envelope-like structure around the nuclear contents and is called the nuclear envelope. The nuclear envelope separates the nucleoplasm from the cytoplasm.

The electron microscopic studies of the nuclear envelope have shown that it is composed of 2 unit membranes, viz., an outer membrane and an inner membrane. Each membrane is about 75 to 90 A^0 thick and lipoproteinous in nature. The intermembranous space is called the perinuclear space or cistema. The outer nuclear membrane often remains rough due to attached ribosomes. Sometimes it remains connected with the membranes of the endoplasmic reticulum, Golgi complex, mitochondria, etc. The inner nuclear membrane contains no ribosomes and sometimes it also remains associated with the chromatin.

The nuclear membranes are not altogether continuous but at several places they are pierced by nuclear openings or pores. The number of the pores for a particular nucleus is variable and often depends on the species and the type of the cell. The nuclear pores are the pathways for the exchange of the macromolecules. The ribonucleoproteins (RNP), granules and gold particles, etc., have been reported to pass through the nuclear pores from the nucleoplasm to the cytoplasm. The pores regulate the exchange of the macromolecules through the pore complex according to their chemical nature and size.

Nucleoplasm. The space between the nuclear envelope and the nucleolus is filled by the transparent, semi-solid, granular and slightly acidophilic substance or the matrix known as the nucleoplasm or karyolymph. The nuclear components such as the chromatin threads and the nucleolus remain suspended in the nucleoplasm.

The nucleoplasm has a complex chemical composition. It is composed of the nucleoproteins but it also contains other inorganic and organic substances, viz., nucleic acids, proteins, enzymes and minerals. The nucleoplasm contains many thread-like, coiled and elongated structures which absorb the basic stains such as the basic Fuxin. These thread-like structures are known as the chromatin substance or ***chromatin fibers.*** Chromatin represents the genetic material during interphase (life cycle of a cell is composed of interphase and mitotic phase). Chromatin consists mainly of ***DNA*** and ***proteins*** but it also contains a low proportion (up to 5%) of *RNA.* DNA molecules of chromatin are the 46 dispersed chromosomes.
Depending on their staining properties two different types of chromatin may be distinguished in the interphase nucleus. Portions of DNA (chromosomes) that stain lightly are only partially condensed and are called ***euchromatin.*** The dark staining regions are called ***heterochromatin*** and correspond to chromosome portions that are highly condensed. Euchromatin contains active genes, whereas heterochromatin contains inactive genes. Barr bodies (inactivated X chromosome in female mammals) are example of heterochromatin.

There are two major types of proteins associated with DNA in chromatin: ***histories*** and ***nonhistones.*** Histones and nonhistones play an important role in determining the physical structure of the chromosome. The DNA is wrapped around a core of histone molecules and the nonhistones are somehow associated with that complex. If the histones are removed from the chromosome the DNA unravels and is displaced from the complex but a skeleton of nonhistone proteins in the shape of the chromosome remains.

Chromosomes. The chromosomes are the nuclear components of a special organization, individuality and function. They are capable of self-reproduction and play a vital role in heredity and variation. The number or set of the chromosomes of the gametes such as sperms and ova is known as reduced or ***haploid set (n)*** of chromosomes. The haploid set of the chromosomes is also known as the ***genomes.*** The somatic or body cells of most organisms contain two haploid set or genomes and are known as the ***diploid*** cells. The diploid cells achieve the ***diploid set (2n)*** of

the chromosomes by the union of the haploid male and female gametes in the sexual reproduction.

In diploids, the members of a chromosome pair are called ***homologous chromosomes;*** each individual member of a pair is called a ***homolog.*** Homologous chromosomes are usually identical with respect to the arrangement of genes they contain and with respect to their visible structure; they are inherited one from each parent. Chromosomes of different pairs that are unpaired are called ***nonhomologous chromosomes.***

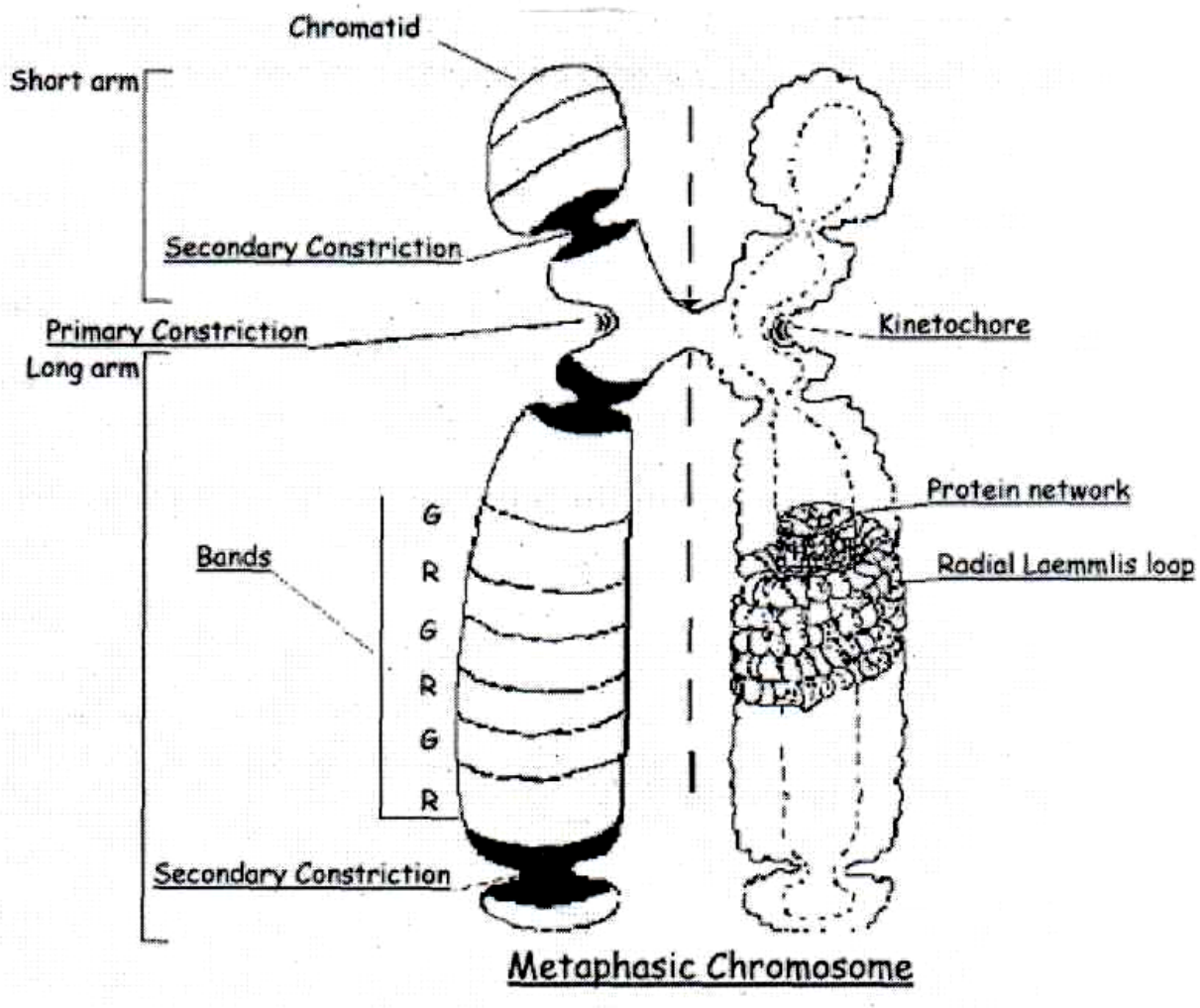
In animals and in some plants there are differences in the chromosome complement of male and female cells. One sex has a matched pair of sex chromosomes; the other sex has an unmatched pair or an unpaired chromosomes. For example, human males have one X and one Y (XY) while human females have two X chromosomes. The chromosomes related to the sex of the organism (X and Y chromosomes) are called ***sex chromosomes.*** Chromosomes other than sex chromosomes are called ***autosomes.***

The chromosomes differ in their size and morphology within and between species. Each chromosome has a specialized region somewhere along its length that is often seen as a constriction under the microscope. This constriction is called ***centromere.*** The centromere divides the chromosomes into two parts, each part is called ***chromosome arm.*** The position of centromere varies from chromosome to chromosome and it provides different shapes to the latter, which are following. A ***metacentric chromosome*** has the centromere in the center of the chromosome so that it appears to have two approximately equal arms. ***Submetacentric chromosomes*** have one arm longer than the other. ***Acrocentric chromosomes*** have one arm with a stalk and often with a "bulb" (called a satellite) on it. ***Telocentric chromosomes*** have only one arm since the centromere is at the end. Chromosome length and centromere position is constant for each chromosome and help in the identification of individual chromosomes.

While describing the structure of the chromosomes during various phases of Cell cycle it is necessary to determine their various components.

Chromatid. At mitotic metaphase each chromosome consists of two symmetrical structures, called **chromatids.** Each chromatid contains a single DNA molecule. Both chromatids are attached to each other only by the centromere and become separated at the beginning of anaphase, when the sister chromatids of a chromosome migrate to the opposite poles.

Chromonema. During mitotic prophase the chromosomal material becomes visible as very thin filaments, called **chromonems.** A chromonema represents a chromatid in the early stages of condensation. It contains a single linear DNA molecule with its associated proteins.



Chromonema. During mitotic prophase the chromosomal material becomes visible as very thin filaments, called **chromonems.** A chromonema represents a chromatid in the early stages of condensation. It contains a single linear DNA molecule with its associated proteins.

Chromomeres. The chromomeres are bead-like accumulations of chromatin

material (regions of tightly folded DNA.) that are sometimes visible along interphase chromosomes. The chromomere - bearing chromatin has an appearance of a necklace in which several beads occur on a string.

Centromere. Most chromosomes have two arms, "linked" at the level of side primary constriction called ***centromere*** (or kinetochore). The centromere is the site of attachment of the chromosome to the spindle of division which is responsible for migration of chromosomes toward the two opposite poles of the cell at anaphase.

Telomere. Each extremity of the chromosome has a polarity and therefore it prevents other chromosomal segments to be fused with it. The chromosomal ends are known as the **telomeres.** If a chromosome breaks, the broken ends can fuse with each other due to lack of telomeres.

Secondary constriction. The chromosomes besides having the primary constriction or the centromere possess secondary constriction at any point of the chromosome. Constant in their position and extent, these constrictions are useful in identifying particular chromosomes in a set. Secondary constrictions can be distinguished from primary constriction or centromere, because chromosome bends only at the position of centromere during anaphase.

Nucleoli organizers. These areas are certain secondary constrictions that contain the genes coding for ribosomal RNA and that induce the formation of nucleoli. The secondary constriction may arise because the r-RNA genes are transcribed very actively and thus interfering with chromosomal condensation. In human beings the nucleoli organizers are located in the secondary constrictions of chromosomes 13, 14, 15, 21, 22, all of which are acrocentric and have satellites.

Satellite. Sometimes the chromosomes bear round elongated or knob-like appendages known as ***satellites.*** The satellite remains connected with the rest of the chromosome by a thin chromatin filament. The chromosomes with the satellite are designated as the **sat chromosomes.** The shape and size of the satellite remain constant.

The characteristics of a certain species set of chromosomes are known as their ***karyotype.*** The diagram of the karyotype is called ***ideogram.*** Generally, in an

ideogram the chromosomes of a haploid set of an organism are ordered in a series of decreasing size. Sometimes an ideogram is prepared for the diploid set of chromosomes, in which the pairs of homologues are ordered in a series of decreasing size.

The nucleolus. Nucleolus which is not separated from the remaining nucleoplasm by membrane is mainly composed of RNA and proteins. It is the site of production of ribosomal constituents. The nucleolus is the site of transcription of r-RNA encoding genes which are located on five individual chromosomes (in human cells). Parts of the different chromosomes, that carry r-RNA encoding genes are called "nucleoli organizing regions" and, therefore, looped in such a way that they are located in the nucleolus.

The number and size of nucleoli depend on cell's activity. In cells engaged in active protein synthesis and which, therefore, need large number of ribosomes, nucleoli are larger and numerous. These nucleoli start to disappear when the cell starts division because during mitosis and meiosis protein synthesis is stopped and the need of ribosome is decreased.

Cell Cycle

All cells are produced by divisions of pre-existing cell. Continuity of life depends on cell division. A cell born after a division, proceeds to grow by macromolecular synthesis, reaches a species-determined division size and divides. This cycle defines life history of a cell. ***Cell cycle*** can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next.

The cell cycle involves the following four phases or stages: G_1 S, G_2 and Mitotic phase. The *G_1 phase*, S phase and G_2 phase are combined to form the ***interphase.***

G_1 is a resting phase. It is called ***first gap phase,*** since no DNA synthesis takes place during this stage, G1 is also called ***first growth phase.*** It involves synthesis of RNA, proteins and membranes which leads to the growth of nucleus and cytoplasm of each daughter cell towards their mature size. Proteins synthesized during G_1 phase:

- regulatory proteins which control various events of mitosis;
- enzymes (e.g., DNA polymerase) necessary for DNA synthesis of the next stage;
- tubulin and other mitotic apparatus proteins.

G_1 phase occupies 30 to 50 percent of the total time of the cell cycle or lacks entirely in rapidly dividing cells (e.g., blastomeres of early embryo of mammals). Terminally differentiated somatic cells (such as neurons and striated muscle cells) that no longer divide are arrested usually in the G1 stage.

S phase. During the S phase or synthetic phase, replication of DNA and synthesis of histone proteins occur. At the end of S phase each chromosome has two DNA molecules and a duplicate set of genes. S phase occupies 35 to 45 percent of cell cycle.

G_2 ***phase.*** This is a ***second gap*** or ***growth phase.*** During G2 phase synthesis of RNA and proteins continues which is required for cell growth. It may occupy 10 to 20 percent time of cell cycle. As the G_2 phase draws to a close, the cell enters the mitotic phase.

Thus the following events occur during interphase:

- the nuclear envelope remains intact;
- the chromosomes occur in the form of diffused, long, coiled and indistinctly visible chromatin fibers;
- the DNA amount becomes double;
- due to accumulation of ribosomal RNA (r-RNA) and ribosomal proteins in the nucleolus, the size of the latter is greatly increased;
- in animal cells a daughter pair of centrioles originates near the already existing centriole and, thus, an interphase cell has two pairs of centrioles.

Mitotic phase

The mitosis occurs in the somatic cells and it is meant for the multiplication of cell number during embryogenesis and blastogenesis of plants and animals. Fundamentally, it remains related with the growth of an individual from zygote to adult stage. Mitosis starts at the culmination point of interphase (i.e., G_2 phase). It is a short period of chromosome condensation, segregation and cytoplasmic

division. Mitosis is important for replacement of cells lost to natural friction (attrition), wear and tear and for wound healing. It is divided into following stages or phases.

Prophase

The appearance of thin-thread like condensing chromosomes marks the first phase of mitosis, called ***prophase.*** The cell becomes spheroid, more refractive and viscous. Each prophase chromosome is composed of two coiled filaments, the ***chromatids,*** which are the result of the replication of DNA during the S phase. As the prophase progresses, the chromatids become shorter and thicker and two sister chromatids of each chromosome are held together by a special DNA - containing region called the ***centromere*** or ***primary constriction.*** During early prophase the chromosomes are evenly distributed in the nuclear cavity; as prophase progresses, the chromosomes approach the nuclear envelope, causing the central space of the nucleus to become empty.

In the cytoplasm the formation of the spindle or mitotic apparatus takes place. In the early prophase there are two pairs of centrioles, each one surrounded by the so-called **aster,** which is composed of microtubules radiating in all directions. The two pairs of centrioles migrate to opposite poles of the cell along with the asters and become situated in antipodal positions. Between the separating centrioles forms a spindle. The microtubules of the spindle are arranged like two cones base to base, broad at the center or equator of the cell and narrowing to a point at either end or pole. The nucleolus gradually disintegrates. Degeneration and disappearance of the nuclear envelope marks the end of prophase

Metaphase

During metaphase the chromosomes are shortest and thickest. The chromosomes occupy the plane of the equator of the mitotic apparatus (a region known as the equatorial or metaphase plate), although the chromosomal arms may extend in any direction. At this stage the sister chromatids are still held together by centromere. Some of the fibers of the spindle attack with the centromere of each chromosome and are known as the chromosomal fibers. Fibers appear between the chromosomes are known as interchromosomal (interzonal) fibers.

Anaphase

At the anaphase the following changes occur in the cell:

1. The centromere of each chromosome divides in two.
2. The chromatids of the each chromosome are separated and form two daughter chromosomes.
3. The chromosomes become shorter and thicker and migrate towards the opposite poles of the cell.
4. The migration of the daughter chromosomes towards the opposite poles is achieved by the contraction of chromosomal fibers and the stretching of interchromosomal or interzonal fibers. The interzonal fibers push the daughter chromosomes towards the opposite poles.

Telophase.

The telophase is the final stage of mitosis and during this phase the following events occur:

1. The chromosomes that reach the opposite poles of the cell now elongate, the coils of DNA protein fibers loosen and the chromosomes become thread-like.
2. The nucleolus reappears.
3. The endoplasmic reticulum forms the new nuclear envelope around the chromosomes and the nucleolus.
4. The microtubules of the aster and mitotic spindle rearrange and disappear. Thus, after the telophase two daughter nuclei are formed due to the karyokinesis. The karyokinesis is followed by the cytokinesis.

Cytokinesis. At the process of cytokinesis the cytoplasm splits from the equatorial region and the two daughter halves of the cytoplasm are separated. A membrane of lipoproteins develops between the two daughter cells. The cytokinesis of animal cells involves the cyclosis of the cytoplasm, formation of a contractile ring, the expansion of the plasma membrane, ATP and interaction of the spindle and asters with the cell surface, while in the plant cells the cytokinesis involves the movement of the endoplasmic reticulum and dictyosomes to the equator where they fills to form the primary cell wall.

Significance of mitosis. Chromosomes contain the information that controls the development and activity of any cell. Mitosis is a mean of duplication and separation of chromosomes and thus the community of the hereditary information is ensured. Each daughter cell gets a complete set of chromosomes identical to that of the parent cell. Thus mitosis provides a mean of unchanged information transmission to the daughter cells. With few exceptions, all kinds of asexual reproduction are carried out by mitosis.

Amitosis. The amitosis or direct cell division is the mean of asexual reproduction in unicellular organisms like bacteria and protozoans and also a method of multiplication or growth in fetal membranes of some vertebrates. At amitosis type of cell division the splitting of nucleus is followed by cytoplasmic constriction. During amitosis the nucleus elongates first and then assumes dumbbell- shaped appearance. The depression or constriction increases in size and ultimately divides the nucleus into two nuclei. The division of nucleus is followed by the constriction of cytoplasm which divides the cell into two equal or approximately similar halves. Therefore, without the occurrence of any nucleus two daughter cells are formed.

Practice:

Assignment 1. Chromosomal structure. Types of chromosomes.

Study the chromosomes silhouettes from the photomicrograph or chart and draw different types of human chromosomes. Make a clear-labeled drawing to illustrate the chief features of the chromosomes.

Assignment 2. Mitosis in the plant cells.

Examine the cell of the growing point of a root on a slide under the high power magnification. Look for cell in process of division and make out as many stage of mitosis as you can. Draw each stage and label its main components.

Literature:

1. Lazarev K.L. "Medical biology" Simferopol, 2003.
2. Lecture's notes.
3. T.V. Bihunyak “Medical Biology”. – Ternopil, 2010.

Topic 7. Reproduction is the main characteristic of living things. Meiosis.

Key concepts:

1. What is a reproduction? Its main types?
2. What are the main events of the meiosis? Crossing-over?
3. Significance of meiosis.

Reproduction is one of the most important properties of living things to reproduce their kinds. For every organism, there comes a time when its powers of metabolism, growth and responsiveness are insufficient to maintain its complex organization against other forces: attack by predators, parasites, starvation etc. This results ultimately in the death of the organism. However the species survives for periods far greater than the life time of any individuals in it. This is accomplished with the production of new individuals by the olds before they die.

Two types of reproduction are known in living things:

- asexual reproduction and
- sexual reproduction.

Asexual reproduction and its types.

Asexual reproduction does not involve the union of two individuals. There are many types of asexual reproduction in organisms:

- ***Budding:*** a portion of the parent's body grows by repeated cell division to form an appendage or region that will eventually become another organism. A bud may be formed inside or outside the parent body. For example: in a hydra a portion of the body of an adult grows out and develops a mouth and tentacles. This offspring now resembles the adult and eventually breaks free to pursue its own fate. In some cases buds are always linked to the main individual and so form a colony. Some Protozoa and water sponges reproduced by budding.
- ***Fission*** is the division of an organism into two or more equal sized parts. There are two kinds of fission.

1) Binary fission:

The division of an organism into two daughter cells, ex. in Protozoa – Paramecium divides into two new daughter cells.

2) Multiple fission:

This is common in parasitic protozoa, as the nucleus of cell divides a number of times and each daughter nucleus breaks away together with a small portion of the cytoplasm. This division is termed *schizogony* when the products develop directly into adults and a cell that does it is called a *schizont;* when the products are sex cells the division is called gamogony and the cells are called *gametes.*

In the malarial parasite (Plasmodium) multiple fission produces as many as 1000 cryptozoites from a single schizont during the parasite's asexual cycle when it invades the liver cells. Multiple fission occurs again later when merozoites invade the red blood cells. In this case not more than 24 daughter cells being formed from a single parent.

- ***Fragmentation***

In one sense fragmentation is no more than a form of regeneration. If certain organisms are divided into sections, each portion will regenerate the missing parts thus giving rise to new individuals. If the division occurs as a result of injury, then the process is regeneration. However if an organism regularly and spontaneously divides itself up in this way, the process is fragmentation.

Organism exhibiting fragmentation must have relatively undifferentiated tissues and it is therefore limited to certain algae, sponges, cnidarians and flatworms. IN spirogyra, for example, portions of the filamentous alga break off when the filament reaches a certain length.

- ***Sporulation***

Sporulation is the formation of small unicellular bodies called ***spores***, which detach from the parent and, in given favorable conditions, grow into new organisms. Spores are usually small, light and easily dispersed. They are produced in vast numbers. Sporulation occurs in bacteria, protozoan, algae, fungi, mosses and ferns.

- ***Vegetative propagation***

It involves the separation of a part of the parent plant which then develops into a new individual. Almost any part – root, stem, leaf or bud – may serve the purpose. These parts are often highly specialized for the task and bear little resemblance to the original plant organ from which they evolved; the potato, for instance, is actually a modified stem.

Sexual reproduction

It is a type of reproduction that involves the fusion of gametes to from a ***fertilized egg*** or ***zygote.*** The sexual reproduction is the most common type of reproduction among the plants and animals. It may be of the following types:

- ***Syngamy*** The syngamy is the most common type of sexual reproduction in the plants and animals in which the fusion of two gametes takes place completely and permanently. Following kinds of syngamy are prevalent among the organisms:

1) Isogamy. In this case the fusion of morphologically and physiologically identical gametes (isogametes) takes place.

2) Anisogamy. *Same* organisms produce two types of gametes. Both types of gametes differ from each other in their shape, size and behavior and are collectively known as the heterogametes. The male gametes are mobile, have small size and known as the ***microgametes.***The female gametes are passive and have comparatively large size and are known as the ***macro-*** or ***mega gametes.*** The union of micro- and macrogametes is known as fertilization.

- ***Conjugation***

It is a temporary union of the two individuals of same species. Both individuals known as ***conjugants.*** Conjugants exchange certain amount of nuclear (DNA) material and then they are separated. Such type of reproduction is most common among the ciliates, e.g. Paramecium and Bacteria.

- ***Parthenogenesis***

The parthenogenesis is the special type of sexual reproduction. In this case the eggs of an organism develop into the young individuals without the fertilization of the eggs by the sperms. The parthenogenesis occurs in certain insects (wasps, bees) and in rotifers.

• ***Polyembryony***

Is the process of development a few embryos from a single fertilized egg. It is quite common in animals such as ciliated worms, annelids, arthropods.

In humans, during cleavage the blastomeres may separate; each one produces an independent individual with the same genetic traits. Such individuals are known as ***monozygotic twins.***

Meiosis

The term meiosis was suggested by J.B.Farmer in 1905. The meiotic division is of almost important for those organisms in which the union of the haploid gametes takes place during the sexual reproduction. By reducing the number of chromosomes of the diploid germ cells into the haploid gametes the meiosis maintains a constant number of the chromosomes in the species. Thus, meiosis helps in alternation of generations of haploidic and diploidic generations of plants and animals. In other words meiosis maintains the regularity of reproductive cycle of sexually reproducing micro-organism (e.g. Chlamydomonas, etc.) and macro-organism (e.g., Bryophytes and higher plants and animals).

In the process of meiosis the chromosomes divide once and the nucleus and cytoplasm divide twice. Due to the meiosis four haploid cells are formed from the single diploid cell.

Heterotypic Division or First Meiotic Division.

At the beginning of the first meiotic division the nucleus starts to swell up by absorbing the water from the cytoplasm and the nuclear volume increases about three times. This increase of nucleus volume causes modification of nuclear components. After these changes the cell passes to the first stage of first meiotic division which is known as prophase.

The ***first prophase*** is the longest stage of the meiotic division. It includes the following sub stages:

1. Proleptotene* or *Proleptonema

The proleptotene stage closely resembles the early mitotic prophase. At this stage the chromosomes are extremely thin, long, uncoiled, longitudinally single and slender thread-like structures.

2. Leptotene or *Leptonema*

At the leptotene stage the chromosomes become more uncoiled, long and thread-like. The chromosomes at this stage take up a specific orientation inside the nucleus; the ends of the chromosomes converge toward one side of the nucleus, that side where the centrosome lies (the bouquet stage). The centriole duplicates and each daughter centriole migrates towards the opposite poles of the cell. On reaching the poles, each centriole duplicates and, thus, each pole of cell possesses two centrioles of a single diplosome.

3. Zygotene or *Zygonema*

`At the zygotene stage, the pairing of homologous chromosomes takes place. The homologous chromosomes which come from the mother (by ova) and father (by sperm) are attracted towards each other and their pairing takes place. The pairing of the homologous chromosomes is known as *synapsis.* The synapsis begins at one or more points along the length of the homologous chromosomes. The pairing of the homologous chromosomes is very exact and specific.

4. Pachytene or *Pachynema*

At the pachynema stage the pairs of chromosomes become twisted spirally around each other and cannot be distinguished separately. In the middle of the pachynema stage each homologous chromosome splits lengthwise to form two chromatids. Actually, the doubling of the DNA molecule strands which is necessary for the subsequent duplication of chromosomes occurs earlier, before the beginning of meiotic prophase. Through the earlier part of the meiotic prophase, however, the DNA molecule in each chromosome behaves as a single body. At the pachynema stage, this is now changed, the two chromatids of each chromosome containing half of the DNA present in the chromosome at the beginning, become partially independent, although they still are linked together by their common centromere. The pachynema chromosome, thus, consists of four chromatids closely joined together in one complex unit called a **bivalent,** because it actually contains a pair of chromosomes.

During pachynema stage an important genetic phenomenon called "crossing-over" takes place. The crossing-over involves reshuffling, redistribution and

mutual exchange of hereditary material of two parents between two homologous chromosomes. After the division of chromatids, the exchange of chromatid segments takes place between the non-sister chromatids of the homologous chromosomes. This process of exchange of chromatin material between one non-sister chromatids of each homologous chromosome is known as the crossing over which is accompanied by the chiasma formation. The nucleolus remains prominent up to this stage and it is found to be associated with the nucleolar organizer region of the chromosome.

5. *Diplotene* or *Diplonema*

At diplonema stage, the homologous chromosomes repel each other because the force of attraction between the two homologous chromosomes decreases. The two homologous chromosomes thus separate from each other, however not completely because both remain united at the point of exchange or ***chiasmata.***

6. Diakinesis

At the diakinesis stage the bivalent chromosomes become more condensed and evenly distributed in the nucleus. The nucleolus detaches from the nucleolar organizer and ultimately disappears. During diakinesis the chiasma moves from the centromere towards the ends of the chromosomes and the intermediate chiasmatas diminish. This type of movement of the chiasmata is known as ***terminalisation.*** The chromatids still remain connected by the terminal chiasma and these exist up to the metaphase.

At the ***prometaphase*** the nuclear envelope disintegrates and the microtubules get arranged in the .form of spindle between the two centrioles which occupy the position of two opposite poles of the cell. The chromosomes become greatly coiled in the spiral manner and get arranged on the equator of the spindle.

Metaphase 1

At the metaphase I, the microtubules of the spindle are attached with the centromeres of the homologous chromosomes of each tetrad. The centromere of each chromosome is directed towards the opposite poles. The repulsive forces between the homologous chromosomes increase greatly and chromosomes become ready to separate.

Anaphase 1

Due to the contraction of chromosomal fibers or microtubules each homologous chromosome with its two chromatids and undivided centromere moves towards the opposite poles of the cell. The chromosomes with single or few terminal chiasma usually separate more frequently than the longer chromosomes containing many chiasma.

The homologous chromosomes which move towards the opposite poles are the chromosomes of either paternal or maternal origin. Moreover, because during the chiasma formation out of two chromatids of a chromosome, one has changed its counterpart, therefore, the two chromatids of a chromosome do not resemble with each other in the genetically terms.

Telophase 1

At the telophase I, the endoplasmic reticulum forms the nuclear envelope around the chromosomes which become uncoiled. The nucleolus reappears and, thus, the daughter chromosomes are formed. After the karyokinesis, cytokinesis occurs and two haploid cells are formed. Both cells pass through a short resting phase or interphase. During interphase, no DNA replication occurs, so that chromosomes at the second prophase are the same double-stranded structures that disappeared at the first telophase.

The ***homotypic or second meiotic division*** is actually the mitotic division which divides each haploid meiotic cell into two haploid cells. The second meiotic division includes following four stages:

Prophase 2

At the prophase 2 each centriole divides into two and thus, two pairs of centrioles are formed. Each pair of centrioles migrates to the opposite pole. The microtubules get arranged in the form of spindle at the right angle of the spindle of first meiosis. The nuclear membrane and the nucleolus disappear. The chromosomes with two chromatids become short and thick.

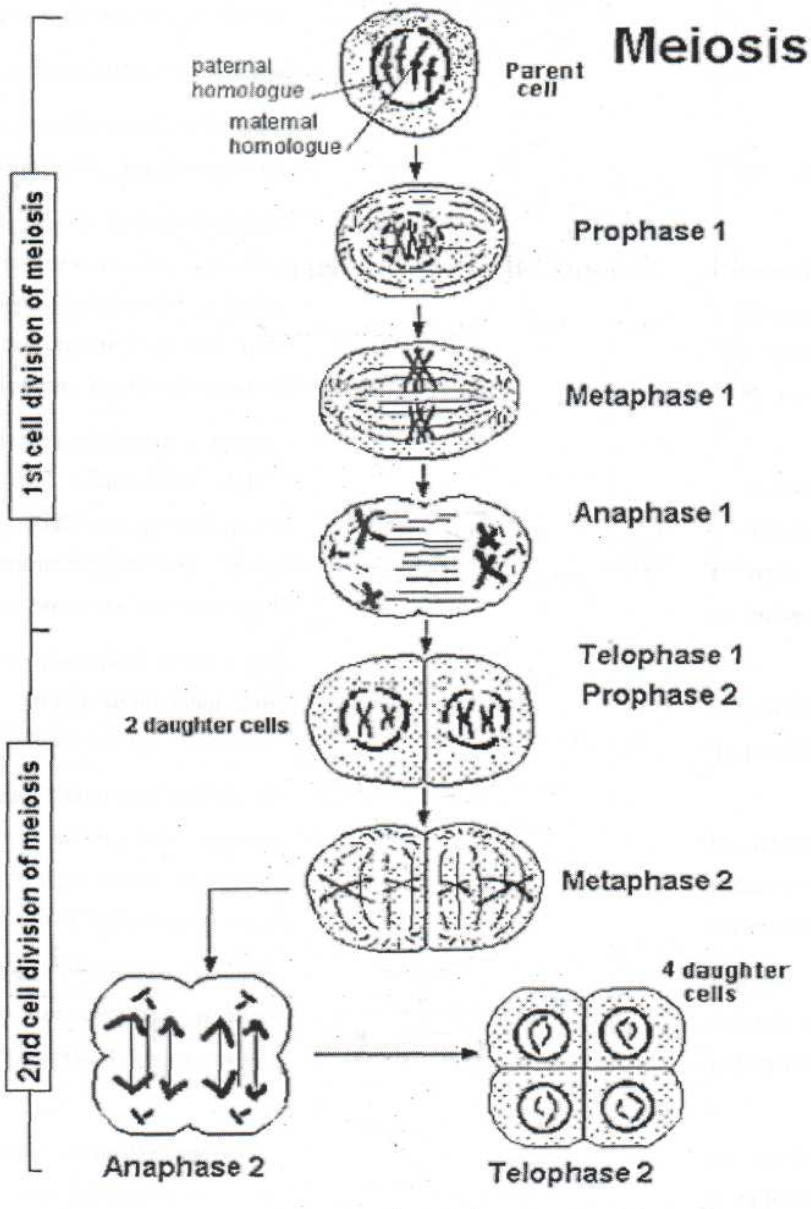
Metaphase 2

During metaphase 2, the chromosomes get arranged on the equator of the spindle. The centromere divides into two and thus each chromosome produces two

daughter chromosomes. The microtubules of the spindle are attached with the centromere of the chromosomes.

Anaphase 2

The daughter chromosomes move towards the opposite poles due to the contraction of chromosomal microtubules and stretching of interzonal microtubules of the spindle.



Telophase 2.

The chromatids migrate to the opposite poles and now are known as chromosomes. The endoplasmic reticulum forms the nuclear envelope around the

chromosomes and the nucleolus reappears due to synthesis of ribosomal RNA (r-RNA) by DNA and also due to accumulation of ribosomal proteins.

After the karyokinesis, in each haploid meiotic cell, the cytokinesis occurs and thus, four haploid cells appear. These cells have different types of chromosomes due to the crossing-over at the prophase **I.**

Significance of Meiosis. The meiosis has the greatest significance for the biological world because of its following features:

1. The meiosis maintains a definite and constant number of the chromosomes in the organism.
2. By crossing-over, the meiosis provides an opportunity for the exchange of the genes and thus causes the genetically variations among the species. The variations are the raw materials of the evolutionary process.

Practice:

Assignment 1. Types of reproduction.

Copy and complete the table.

Types of reproduction.		Examples
Asexual reproduction.		
Sexual reproduction.		

Assignment 2. Meiosis. Prophase I.

Examine the diagram "Meiosis" and draw Prophase I. Label the main substages of Prophase I.

Assignment 3. A comparison of mitosis and meiosis.

Event	**Mitosis**	**Meiosis**
DNA replication		
Number of division		

Synapsis of homologous chromosomes		
Number of daughter cells and genetic composition		
Role in the animal body		

Topic 8. Gametogenesis. Sex cells.

Key concepts:

1. What is the difference between a somatic and a sex cell?
2. From which cells do sex cells develop?
3. What is gametogenesis, spermatogenesis, ovogenesis?
4. What are the development stages of male and female gametes?
5. The structure of sex cells, their difference.
6. What is fertilization? Its main steps and significance.

Gametogenesis

The sexually reproducing animals have two types of cells in their body, e.g. ***somatic cells*** and ***germinal cells***. Both types of cells have diploid number of chromosomes but each type has its different destiny.

The somatic cells form various organs of the body and provide the condition for maturation, development and formation of the germinal cells. The somatic cells always multiply by mitotic division.

The germinal cells form the gonads (testes and ovaries) in the animal body. These cells produce gamete cells by successive mitotic and meiotic divisions. The male gamete is known as ***spermatozoon*** or ***sperm*** and the female gamete is known as ***ovum*** or ***egg***.

Gametogenesis is a process of gamete formation. It is known spermatogenesis (the process of sperm production) and oogenesis (formation of ovum).

Spermatogenesis

It occurs in male gonads or testes. The testes are composed of many ***seminiferous tubules*** which are lined by the cells of germinal epithelium which produce sperms. The stages of spermatogenesis are:

1. Formation of spermatids
2. Spermiogenesis

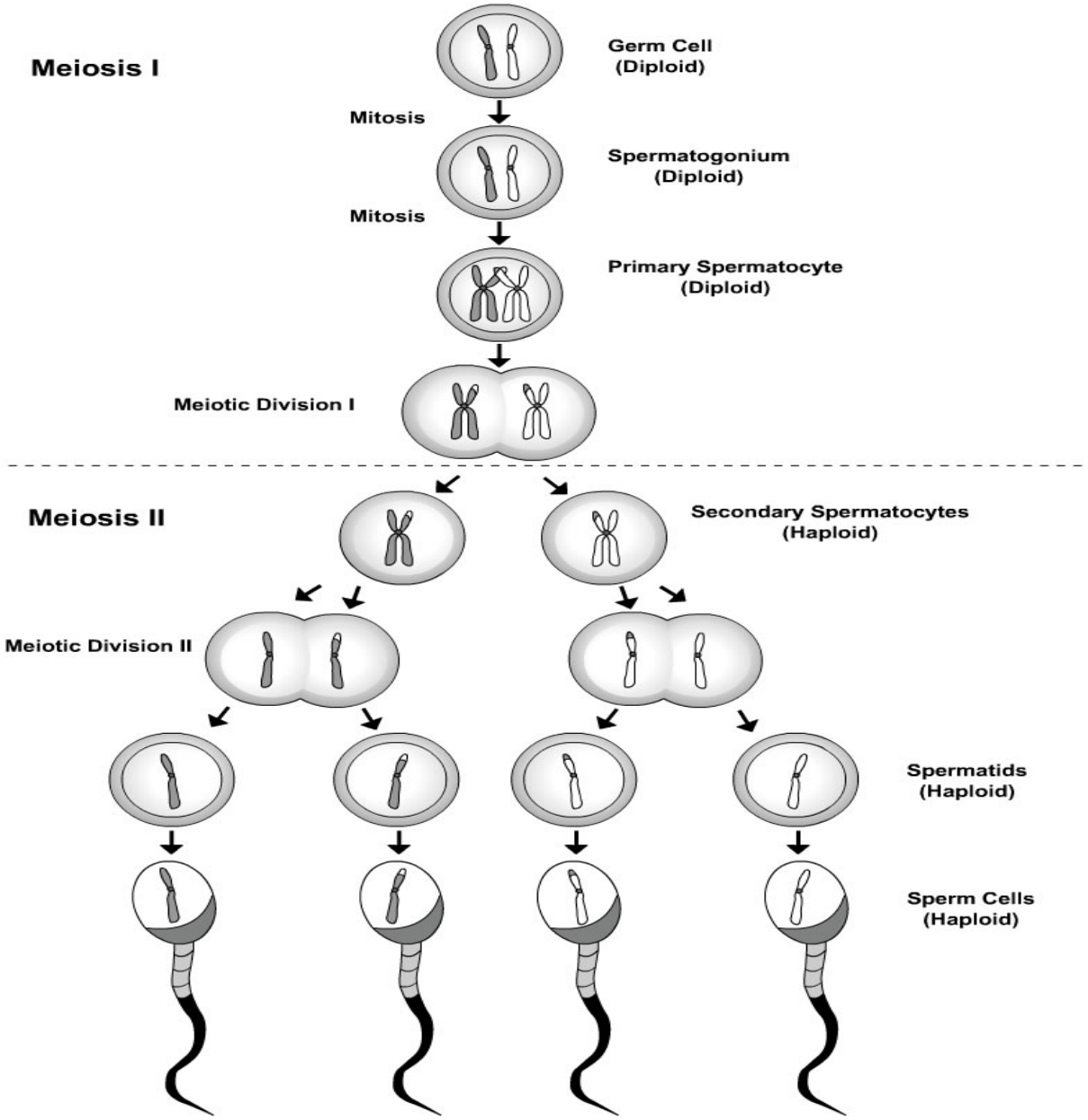
Formation of spermatids

Primary germinal cells or ***primordial cells*** which produce the sperms, pass through following three phases before the formation of spermatids:

- ***Multiplication phase:*** primordial cells multiply by repeated mitotic divisions and produce the cells which are known as the ***spermatogonia.*** Each spermatogonium is diploid and contains **2n** number of chromosomes.
- ***Growth phase:*** the spermatogonial cells accumulate large amount of nutrition and chromatin material. Now each spermatogonial cell is known as ***primary spermatocyte.***
- ***Maturation phase:*** the primary spermatocytes pass through the first meiotic division. Due to division two ***secondary spermatocytes*** are formed from each primary spermatocyte. The secondary spermatocyte is haploid and contains **n** number of chromosomes. It passes through the second meiotic division and produces two ***spermatids***. Thus, by a meiotic division a diploid spermatogonium produces 4 haploid spermatids. These spermatids cannot act directly as the gametes so they have to pass through the next stage, the spermiogenesis.

Spermatogenesis

It is the differentiation of the spermatids into the sperm. Because the sperm are very active and mobile cells the superfluous material of the developing sperms is discarded to provide the future cell mobility.

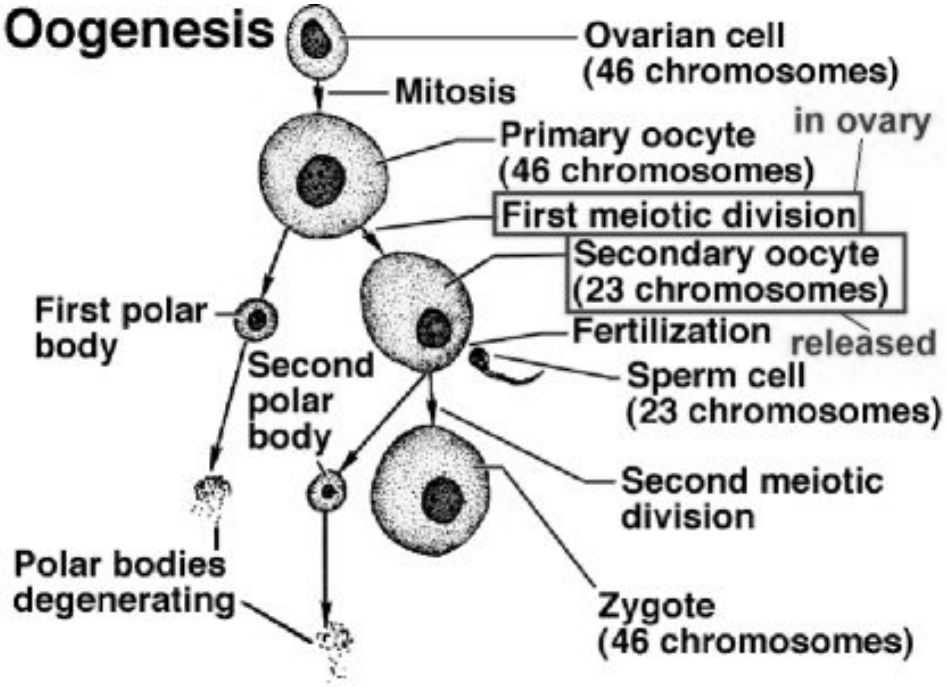


Oogenesis

It takes place in the germinal epithelium of the ovary and has got the same three successive stages as spermatogenesis:

- ***Multiplication phase:*** the primordial germinal cells divide to form the ***oogonia.*** The oogonia multiply by the mitotic division and form the ***primary oocytes***.
- ***Growth phase.*** The size of the primary oocyte increases enormously. Tremendous changes occur in the nucleus and the cytoplasm of the primary oocyte. The nucleus becomes large; the chromosomes change their shape and become giant lamp brush-like chromosomes which are directly related with increased transcription of m-RNA molecules and active protein synthesis.

➢ ***Maturation phase.*** The cytoplasm of the primary oocytes divides unequally to form a single large-sized haploid ***secondary oocyte*** and haploid ***first polar body***. During second meiotic division the secondary oocyte forms a ***mature egg*** and a ***second polar body*** and the first polar body also divides into two secondary polar bodies. The haploid egg becomes ready for the fertilization and the polar bodies degenerate.

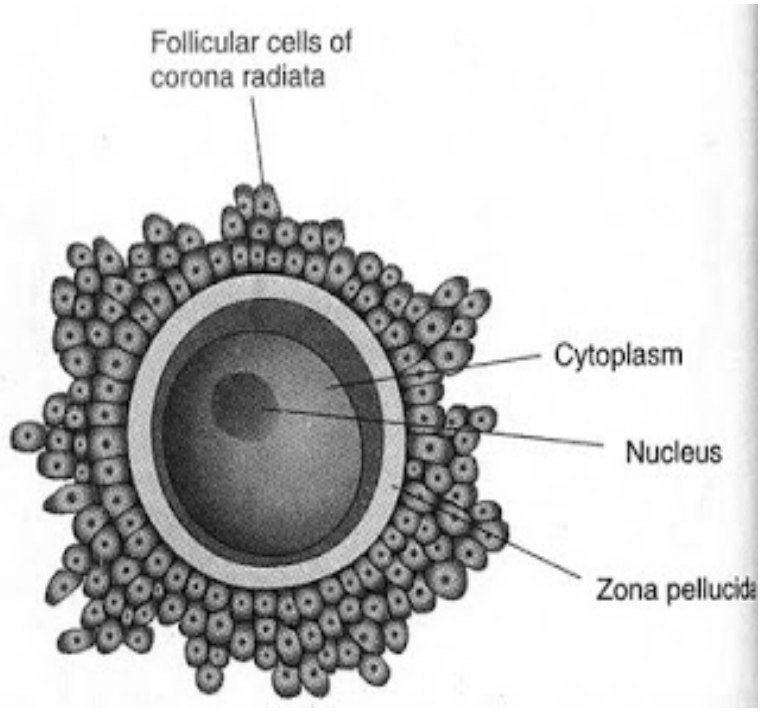


Gametes

1. The ovum

The mature egg is released from the ovary at the secondary oocyte stage. Its nucleus is large and eccentrically situated. The ovum is covered by a ***plasma membrane*** (vitelline membrane) which is the unit membrane composing of an outer and an inner layers of protein. Between the protein layers there occurs a lepidus layer.

In addition to the plasma membrane there are other membranes which are known as the ***primary*** and ***secondary egg membranes.*** The primary egg membrane is secreted around the plasma membrane by the oocyte itself and it is known as ***zona pellucida***. The secondary egg membrane is secreted by the ovarian tissues around the primary egg membrane. The number of follicle cells, still attached to the ovum, are located outside the zona pellucida and constitute the ***corona radiata***.



Scattered throughout the egg, are minute granules of yolk. The nutrient material is especially important, because it provides the development and body height of a blastema. According to the amount of yolk in the cytoplasm following types of ovum have been recognized:

Izolecital ovum contains a little amount of yolk. It is distributed in all cytoplasm of an ovum and nucleus occupies the central position of the cell (mollusks, mammalian).

Telolecital ovum has much yolk grains which are collected on the vegetal pole The cytoplasm without yolk and nucleus are located on the animal pole (fishes, amphibians, reptiles)

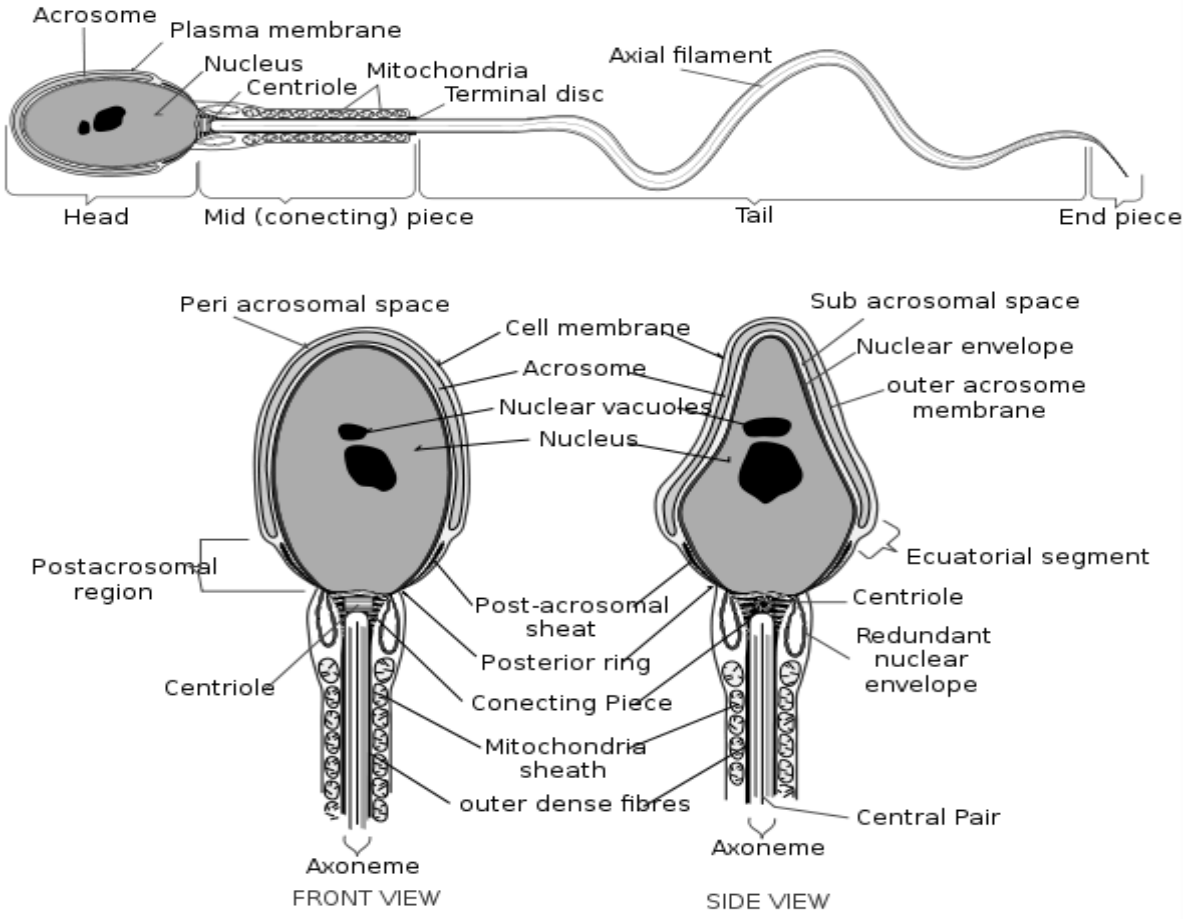
Centrolecital ovum has the yolk grains around nucleus, which locates in the central part of the cell (arthropods).

2. The sperm

The sperm consists of ***a head-piece, middle- piece*** and ***tail.*** The head piece contains the nucleus, sheathed in a minute quantity of cytoplasm over the nucleus and partly covering it, is the ***acrosome***, which is flattened and tapes to a rounded edge at its apex. The middle-piece is short and cylindrical. It contains centrosomes and a number of rod- like mitochondria embedded in a small quantity of

cytoplasm. The tail is long and tapering, its axial filament being continued posteriorly as a short flagellum.

Spermatozoa are produced in very large number. The sperm contains Y-chromosome or X- chromosome. The speed of the spermatozoa is 1, 2 to 3,6 mm per minute. In man the sperm lives for a few days.



Fertilization

Fertilization is the union of a sperm and an ovum to produce a ***zygote*** or ***fertilized egg.***Fertilization involves several steps. The first one is ***capacitation*** of the spermatozoon.

Capacitation is a stage of activation of sperm by enzymes of a wall of the uterus. During this period there is change in the cholesterol-phospholipids ratio in the sperm membrane. These changes are thought to assist the membrane fusion of the acrosome reaction.

The second stage of fertilization involves ***contact*** and ***recognition***. Before the sperm can enter the egg an ***acrosome reaction*** must occur. When the sperm

touches the egg, the sperm's acrosomal membrane breaks down and releases enzymes which will break down the jelly and provide a path for the sperm to the egg membrane. At the same time, actins molecules within the acrosome assemble to produce microfilaments. This is called the ***acrosomal process*** and it extends outwards, fusing the acrosome with the egg membrane.

The third phase is the ***gametes fusion***. Once the correct sperm has been recognized by the zona pellucida, enzymes of acrosome dissolve a bit of the zona pellucida in the area of the sperm head. The egg surface is covered with small projection – microvilli. Several microvilli elongate to surround the head of the sperm, forming a ***fertilization cone.*** The sperm is then drawn into the egg by contraction of the fertilization cone. As this occurs, the plasma membranes of sperm and egg fuse.

As soon as one sperm enters the egg, reactions occur that prevent additional sperm from entering.

The fourth phase of fertilization is ***fusion of the genetic material***. Fertilization is completed when the genetic material of the sperm and egg combine to form a diploid nucleus.

At the end of fertilization a process which can take about 20 hours in mammals, the genetic material from the egg and sperm has fused, proteins are being synthesized, DNA is replicating and the cytoplasm has been reordered. The next stage in development is to move from a single cell to a multicellular embryo.

Practice.

Assignment 1. **Comparative characteristics of oogenesis and spermatogenesis.**

Copy and complete the table.

Feature	Oogenesis	Spermatogenesis
In what glands the process takes place		
The successive steps of gamete development		
Process duration		
Distinction of the first meiosis division prophase		
The quantity of fertile gametes formed from the primary sex cell as a result of the process		

***Assignment 2.*Distinguishing characters of female and male mature mammalian gametes.**

Copy and complete the table.

Character	Ovum	Spermatozoon
Size		
Organelles of special purpose		
Morphological distinctions		
Nutrients storage		

Literature:

1. Lazarev K.L. “Medical biology” Simferopol, 2003.
2. Cell Biology. Module 1. Zaporozhye, 2009.
3. Lecture’s notes.
4. T.V. Bihunyak “Medical Biology”. – Ternopil, 2010

Topic 9. Sub module. “Cell – Reproduction”

Revise the topics 1-8.

GENETICS.

Topic 10. Genetics. Mendel's Laws.

Key concepts:

1. What is the subject of genetics?
2. Genetic terminology: gene, genotype, phenotype, heterozygote, homozygote, allele, heredity, variation, genome.
3. Mendel’s laws of heredity.

Individuals of any species are generally like the parents that produced them. All children tend to resemble their parents. But children are not exact duplicate of

his parents. Thus here we encounter two entirely different phenomena that are called **heredity** and **variation**.

By heredity is meant the transmission of characters from one generation to the next. It is the genetic continuity between successive generations.

By variation is meant all departures from a complete similarity between individuals of the same species, the differences that permit us to distinguish between two individuals of the same race, or between the offspring's of same parents. Characters that pass from the parents to the offspring are called **traits** or hereditary characters.

Genetics is the biological science which studies heredity and variation. It begins with experiments made during the last century, by the Abbot Mendel at Brain. They were published by him in 1865-1866.

He had performed the experiments of crossing of pea plants with different features and worked out the hybridological method. The terms of this method are:

- Paternal individualities must have distinct difference in signs studied. Such signs are called alternative within one pair.
- The exact mathematical calculation of signs shows in every generation for revealing of the regularities of these sings manifestation.
- Sign manifestation should be observed for several generations.

For better understanding Mendel's laws and other genetic laws definitions of specialized terms are given below:

Allele (Allelomorph). Alleles are genes controlling the same characteristic (e. g hair control) but producing different effects (e.g. black or red), and occupying corresponding positions on homologous chromosomes.

Dominance. A phenomenon in which one member of a pair of allelic genes expresses itself as a whole (complete dominance) or in part (incomplete dominance).

Gene. The fundamental physical and functional unit of heredity, which carries information from one generation to the next; a segment of DNA, composed of a transcribed region and a regulatory sequence that makes possible transcription.

Genome. A complete set of chromosomes inherited as a unit from one parent, or the entire genotype of a cell or individual.

Genotype. The genetic constitution of an individual, with reference to the traits under consideration, usually expressed by a symbol, e.g. DD (tall), dd (short), etc.

Haploid. An individual or cell containing a single complete set of chromosomes is known as haploid.

Heterozygote. An individual containing both dominant and recessive genes or traits or characters of an allelic pair is known as heterozygous or hybrid.

Hybrid. An individual from cross the parents of different genotypes.

Karyotype. The entire chromosome complement of an individual or cell, as seen during mitotic metaphase.

Locus. The position or place on a chromosome occupied by a particular gene or one of its alleles.

Phenotype. The characters of an individual, which dependent on his genes, e.g. "tall", "dwarf", "albino".

Pleiotropy. The multiple effect of a gene.

Pure line (pure breeding line). A succession of generations of organisms homozygous for all genes.

Monohybrid cross

A monohybrid cross is that in which the parents differ an only one pair of alleles or genes. Mendel started the examination of one unknown component. He crossed tall and short plants of the true breeding species of garden pea which is normally self-pollinating, these he called the parental generation which is indicated now by symbol "P". By "true breeding" was meant that the pea plants selected by Mendel for experimentation were producing the same traits of hereditary characters under study for much generation. Mendel made sure that the members of the parental generations were "pure" and not hybrids or of mixed breed. He self-fertilized two races of pea plants: a tall race and a short race. The offspring that resulted from this cross were all tall, that is they all resembled only one of the

parent plants. It seemed that the characteristic of shortness had disappeared altogether.

He called these offspring hybrids because they were produced from crossing two unlike parent varieties. The hybrid offspring are referred to us as the F_1 or first filial generation. Mendel found that if two contrasting pure forms are crossed they produce hybrids in which only one of the two contrasting characters appears. One of the two characters which appear in F_1 generation is called dominant, while the other which is hidden and does not show is recessive. We can show symbolically the results of Mendel's experiments, representing the dominant factor for tallness as - A, and the recessive factor for shortness as – a.

P ♀ AA x ♂aa

G (A) (a)

F_1 Aa

According to these results Mendel described the phenomenon of dominance (**Law of dominance**): in crossing between pure (homozygous) organisms for contrasting characters of a pair, only one character of the pair appears in the first filial generation.

Mendel studied the results of his experiments and it became evident to him that there must be something which represents the characters and is responsible for their production. This something of Mendel is now called gene. The gene can be considered as the unit of inheritance that is transmitted in a gamete and determines or controls the development of a character by interaction with the other genes, the cytoplasm and the environment. The cytological investigations have now established that the genes are the units of deoxyribonucleic acid (DNA) which along with ribonucleic acid (RNA) and nucleoproteins constitute the thread-like stainable structures - the chromosomes.

A diploid cell has two sets of chromosomes which come from two different parents (male and female). The chromosomes of similar size and nature often form pairs during meiotic cell division and such identical chromosomes are known homologous chromosomes. Each character of a pair of contrasting characters is

represented by an allele. All alleles of a gene are produced due to mutation of wild gene (or normal gene). When a gene for a unit character contains two or more alternative forms, they are called alleles. Thus, homozygous tall pea plant has two identical alleles (AA) on both gene loci of the homologous chromosomes, like-wise; homozygous short pea plant contains aa alleles.

During the gametogenesis the homologous chromosomes with AA or aa genes are separated and each chromosome with A or a gene is passed to the gamete. The gametes of both parents unite during the process of fertilization and produce a new-individual containing both tall (A) and short (a) characters. This new individual of first generation (F_1) contains two different genes (i.e., alleles) of a contrasting pair of characters, there fore, it is known as heterozygote or hybrid.

Mendel carried on his experiments by allowing self-pollination in the hybrid F_1 generation. This is known as hybridization, or the crossing of two hybrids. From the seeds of F_1 generation he obtained the second filial generation (F_2), and of these plants, most were again tall but some were short and the ratio of tall ones to short ones was approximately as three is to one. The short factor had not been totally lost after all but had only been temporarily masked for a generation. If the experiment is continued through further generations it is found that short plants always breed true, that tall plants, if themselves derived from true-breeding tall plants, always breed true, but that tall plants, whose parent plants did not breed true, themselves breed true and not true in the ratio 1:2; tall plants which did not breed true produced tails and shorts in the ratio 3:1. In F_2 the plants are of two: tall and short phenotypes (external appearance), but they belong to three genotypes (hereditary constitution): aa, Aa and AA.

Mendel concluded that in the crossing of two heterozygote organisms in the second generation the cleavage rationed 1:2:1 is observed in genotype and 3:1 in phenotype.

P ♀ Aa x ♂ Aa

G (A) (a) (A) (a)

F_2 AA Aa Aa aa

How can we explain the reappearance of short plants in the second generation? Mendel thought that must be something in the gametes of a short plant which makes for shortness. This "something" is called a gene or factor, for shortness. Similarly, since it is handled on from generation to generation, there must be a corresponding gene for tallness. And, in the F_1 plants, both genes must be present together, but the gene for tallness in some way suppresses that for shortness. All plants carry two genes: true breeding tall plants having two genes for tallness, and short plants two genes for shortness. When the gametes are formed the genes separate from one another and go into different gametes. Then each gamete of a true-breeding tall plant will contain one gene making for tallness, which we call - A and each gamete of a short plant one gene for shortness which we may call - a. When the gametes unite at fertilization, the zygote will contain the two genes - Aa and it will produce a tall plant because tallness is dominant over shortness. Now, when Aa -plant forms gametes, the two genes - A and a - will again separate from one another and go into separate gametes so that the gametes will be of two kinds: A and - a in equal numbers. There is another conclusion to be drawn: when two different genes come together in one individual, they do not contaminate one another but each retains its identity and qualities unimpaired and is handed on unimpaired to the next generation. Mendel drew one of his laws, the **Law of Segregation:** characters are controlled by pairs of genes of which only one can be represented in a single gamete (each gamete contains one but not both members of a pair).

Lethal Genes.

Lethal genes are mutant genes and result in the death of the individual which carries them. A fully (completely) dominant lethal allele kills both in homozygous and heterozygous state. Individuals with a dominant lethal allele die before they can leave progeny. There fore, the mutant dominant lethal is removed from the population in the same generation in which it arose. Recessive lethal genes kill only when they are in homozygous state.

In humans several hereditary diseases have lethal effects. For example, a recessive allele in homozygous condition causes a fatal disease called infantile amourotic idiocy in juvenile stage. Bearers of this genotype begin to lose their eye sight between the ages of four to seven years. The complete blindness is followed by mental degeneration and finally death before adolescence.

Dihybrid cross

Dihybrid cross is the cross between individuals which do not differ except in two characters. Mendel's experiments were not ended by the investigation of the behavior of one pair of contrasting characters, for he went further and included experiments to test the assortment of two pairs of such characters. He chose seed form and seed colour as his two subjects of study, using a pure - breeding plant with round and yellow seeds as one parent, and a pure - breeding plant with wrinkled and green seeds as the other. His results showed that in the first filial generation all the plants had round and yellow seeds, including the dominance of round over wrinkled and yellow over green characters respectively. Since round is dominant over wrinkled we can use as symbols of the corresponding genes - A and - a, while for yellow and green we can use - B and - b. Then:

P ♀ AABB × ♂ aabb

G AB ab

F_1 AaBb – 100%

F_2 obtained by self-fertilization of F_1 is made up of four kinds of plants in the following ratios: 9 round yellow: 3 round green: 3 wrinkled yellow: 1 wrinkled green

This result can be explained if we postulate: (1) that the members of gene pairs separate from one another in gamete formation; (2) that the members of each pair separate from one another independently of the members of the other pair.

When the F_1 plant forms gametes it has to obey singe rule: A and a must be in different gametes, and so must B and b, but A and B, or A and b, or a and B, or a and b may go together. The possible gametes are: AB, Ab, aB, ab. These four

types of gametes of F_1 hybrid unite at random in the process of fertilization and produce sixteen types of individuals in F_2 generation. The following table shows the possible zygotes and the relative numbers in which they occur.

Inspection of the 16 groups of the F2 generation shows that there are zygotes of nine different genotypes and the phenotypic character corresponding with each genotype is known if it is remembered that when the gene A is present the plant will produce round seeds, but wrinkled ones when it is absent, and yellow seeds when B is present but green when it is absent. The following table gives the result.

P ♀ AaBb× ♂ AaBb

G AB Ab AB Ab

aB ab aB ab

F_2 9AB; 3Ab; 3aB; 1ab

Гаметы♂ / Гаметы♀	*AB*	*Ab*	*aB*	*ab*
AB	*AABB*	*AABb*	*AaBB*	*AaBb*
Ab	*AABb*	*AAbb*	*AaBb*	*Aabb*
aB	*AaBB*	*AaBb*	*aaBb*	*aaBb*
ab	*AaBb*	*Aabb*	*aaBb*	*aabb*

Phenotypes F_2 9 round yellow: 3 round green : 3 wrinkel yellow : 1 wrinkled green

Thus the relative numbers and kinds of plants found by experiments are the same as the relative numbers and kinds which are theoretically expected. This correspondence is between ratios and is only expected if there are large numbers of

plants in F_2. These experiments show that rounded form does not remain always with yellowness, but it can combine with greenness as well, similarly, wrinkled form can go with yellowness. From this Mendel deduced his third law - the **Law of Independent Assortment** states that each one of a pair of contrasted characters may be combined with either of another pair.

In other words there are 9+3=12 round, 3+1=4 wrinkled, 9+3=12 yellow and 3+1=4 green. The ratio of 3 dominant to 1 recessive persists according to Mendel's law.

The same regularity is observed in three and tetra hybrid crossings too: while crossing two homozygosis individualities which differ by two or more pairs of alternative signs in the second generation the independent cleavage in every pair of signs rationed $(3:l)^n$, where "n" is a number of signs studied.

Practice.

Assignment 1. Formation the gametes in the organisms with the following genotypes.

Complete the table and determine the types of gametes.

Genotype	AABB	Aabb	AaBb	AABbCC	AaBbCc
Number of the types of gametes	1				
Gametes	AB				
The distribution of genes on the chromosomes					

Assignment 2. Solving problems.

1. A purple-flowered pea plant is crossed with a white-flowered pea plant. All the F_1 plants produced purple flowers. When the F_1 plants are allowed to self-pollinate, 401 of the F_2 have purple flowers and 131 have white flowers. What are the genotypes of the parents, F_1 and F_2 generations?

2. In cocker spaniel solid colour is dominant over spotted coat. Suppose a true-breeding, solid-coloured dog is crossed with a spotted dog and F_1 dogs are interbred.

What is the probability that the first puppy born will have a spotted coat?

What is the probability that if four puppies are born, all of them will have a solid coat?

3. In chickens the white plumage of the Leghorn breed is dominant over coloured plumage, feathered shanks are dominant over clean shanks, and pea comb is dominant over single comb. Each of the gene pair segregates independently.

If a homozygous white, feathered, pea-combed chicken is crossed with a homozygous coloured, clean, single combed chicken and the F_1 are allowed to interbreed, what proportion of the birds in the F_2 will produce only white, feathered, pea-combed progeny?

Topic 11. Genes interactions. Multiple alleles. Inheritance of blood groups.

Key concepts:

1. Allelic genes interactions (full dominance; incomplete dominance; co dominance).
2. Multiple alleles. Inheritance of blood groups.
3. Non-allelic genes interactions (complementary genes; epistasis; polygenic inheritance.
4. Pleiotropy: major and secondary effects.

Genetic Interaction

During the discussion of Mendel's monohybrid and dihybrid crosses, we encountered with the fact that for the determination of single phenotypic trait of an organism, two alleles of a single gene interacted in various ways. Such as out of two alleles of a single gene, one allele might show simple (complete) dominance over the action of other which was recessive; or both alleles might have partial or

incomplete dominant relationship or both alleles might have equal expression or co dominant relationship. These kinds of gene or genetic interactions occur in between the two alleles of a single type of gene are usually referred to as intra-allelic or **allelic genetic** interactions. These kinds of genetic interactions give the classical ratios of 3: 1 and 9:3:3:1. So, intra-allelic genetic interactions are the following ones: full dominance, incomplete dominance, co dominance.

Full dominance - is a case when one of the alleles is dominant and the other recessive. The dominant gene is responsible for the active form of the protein-enzyme; the recessive one controls the inactive protein enzymes.

Incomplete dominance. Sometimes in a heterozygote dominant allele does not completely mask the phenotypic expression of the recessive allele and there occurs an intermediate phenotype in the heterozygote. This is called incomplete dominance.

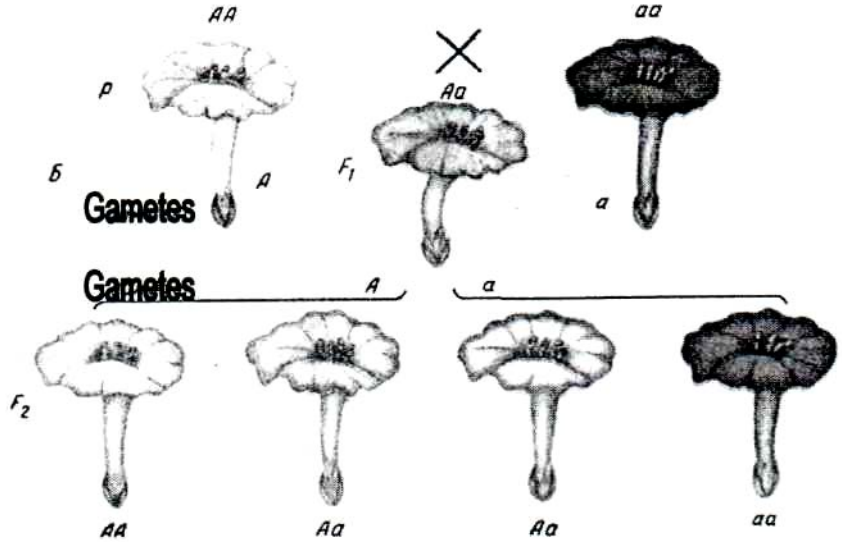
Examples:

1. When a red flowered pea plant (RR) is crossed with white flowered pea plant (rr) then the F1 hybrid pea plants are found to have pink flowers (Rr). It shows that gene for red colour could not completely dominate the gene for white colour. In such a case F_2 phenotypic ratio and genotypic ratio are the same, as follows:

F_2 phenotypic ratio = 1 red : 2 pink : 1 white

F_2 genotypic ratio = 1 RR : 2 Rr : 1 rr

2. in four o'clock plants (Mirabilis jalap) or snapdragons (Antirrhinum majors), when a pure line or homozygous with red petals (AA) is crossed to a pure line with white petals (aa), the *F1* progeny has no red petals but pink petals (Aa). It shows incomplete



dominance, in F_2 the progeny exhibits the following results: 0,25 red petals; 0,5 pink petals; 0,25 white petals.

Many other examples of incomplete dominance occur. Roan shorthorn cattle are heterozygous for genes in which the corresponding homozygotes are red and white. A rare allele in man producing a very severe anemia (thalassemia major) in the homozygous condition causes death. In the heterozygous condition it causes only mild anemia (thalassemia minor).

Co dominance. Sometimes both alleles of a gene in a heterozygote lack of dominant and recessive relationship, i.e. each allele is capable of some degree of phenotypic expression. In a sense, co dominance is no dominance at all, the heterozygote showing the phenotypes of both homozygotes. Hence, heterozygous genotype gives rise to a phenotype distinctly different from either of the homozygous genotypes. *Example:*

The alleles governing the AB blood group in humans are co dominants and may be represented by the symbols I^A and I^B (the genotype I^AI^B).

Multiple Alleles.

It has been observed that a given phenotypic trait (character) of an individual depends on a single pair of genes, each of which occupies a specific position called the gene locus, on a homologous chromosome. A particular gene has been found to occur in two alternative forms or allelomorphs, one being dominant and other recessive; one being wild form and the other mutant form. The wild form of allele can mutate in more than one way. The mutant form of allele too can mutate once

again to give rise to another mutant form of allele. It is possible to have more than two allelic forms, i.e. multiple alleles of one kind of gene. Although only two actual alleles of a gene can exist in a diploid cell (and only one in a haploid cell), the total number of possible different allelic forms that might exist in a population of individuals is often quite large. This situation is termed as multiple allelism and the set of alleles itself is called a multiple allelic series. The best examples of multiple alleles have been observed in blood groups of man.

ABO Blood Groups in Humans.

Landsteiner in 1900 and 1902 discovered two kinds of antigens, called A and B antigens from the surface of red blood cells of human blood. He found that out of A and B antigens, a person may contain either one (i.e., A or B antigen) or neither of them. Accordingly, he recognized three kinds of blood types or blood groups: type A, type B and type O. The fourth and the rarest, the AB blood group or type, was discovered in 1902 by two of Landsteiner's students, Von Donatello and Starlit. For A and B antigens, there occur two agglutinins or antibodies: anti-A (or a) and anti-B (or (3). Recent, chemical investigations have shown that A and B antigens are not proteins but are mucopolysacharides (sugars + amino acids) of 300,000 molecular weights.

Each person, therefore, can use the blood of its own blood group in emergency; otherwise, clumping of red blood cells may take place, if blood group of different type is transfused in him. The clumped red blood cells occlude capillaries and, thus, deprive vital organs of normal blood and may lead to death. The characteristics of blood groups and the types of transfusions can be summarized in the following table.

Human blood groups.

Blood groups (phenotype)	Antigen in red blood cells	Antibodies in plasma	Can give blood to groups	Can receive blood from group	Genotype
O	None	Anti-A Anti-B	O,A,B,AB	O	ii
A	A	Anti-B	A,AB	O,A	I^AI^A or I^AI^O
B	B	Anti-A	B,AB	O,B	I^BI^B or I^BI^O
AB	A and B	None	AB	O,A,B,AB	I^AI^B

Bernstein (1925) proposed that inheritance of A, B, AB and O blood types of man is determined by a series of three allelic genes. The gene controlling blood types has been labeled as L (after the name of discoverer, Landsteiner) or I (from is agglutination). The gene I exist in three different allelic forms: I^A, I^B and 1°. The first two alleles produce characteristic antigens on the surface of erythrocytes. Thus, I^A allele specifies A antigen, I^B allele determines B antigen and 1 allele specifies no antigen.

The pedigree analysis has shown that alleles I and I have dominance over I allele. Likewise the pedigree analysis of A and B parents revealed that their children have both A and B antigens on the erythrocytes, showing co dominance between I^A allele and I allele. The dominance hierarchy of this allelic series can be depicted as follows: $I^A=I^B>I°$

The H Antigen and Bombay Phenotype. Antigens A and B of A, B and O blood phenotype are synthesized from a precursor mucopolysacharide in the presence of the dominant allele of another pair designated as H and h. With genotypes HH or Hh the precursor is converted to an H antigen which, in turn, in the presence of I^A and/or I^B allele is partly converted to antigen A and/or antigen B. Gene h is termed amorphous because it is producing no demonstrable product. So long as persons are of genotype H- (i.e., HH or Hh), persons of group A produce antigens A and H, group B persons produce antigens B and H and group AB persons produce antigens A, B and H. However, group O persons produce only antigen H if they are of the genotype ii H-. On the other hand, blood of person of genotype —hh does not react with anti-A, anti-B or anti-H. This is very rare Bombay phenotype (i.e., one case in 13,000 persons); it is so named because it was first described in a family from the Bombay metropolitan. The allele h is found to be epistatic to the multiple alleles at the A-B-0 locus. Erythrocytes of person having —hh genotype gives no reaction with anti-A or anti-B sera (even though they possess I^A or I^B genes); in fact, they ontain no antigen of this multiple allelic series.

In addition to intra-allelic genetic interactions, non-allelic or inter-allelic genetic (genie) interactions also occur. In inter-allelic genetic interactions, the

independent (non homologous) genes located on the same or on different chromosomes interact with one another for the expression of single phenotypic trait of an organism. The discovery of the inter-allelic genetic interactions has been made after Mendel and they can be best understood by considering the way by which a phenotypic trait is governed by a gene. The interactions of nonallelic genes are: complementary genes; epistasis; polymery.

Complementary Genes.

Some genes appear to operate in a manner complementary to one — another, i.e. one must be present for the other to have an operative effect. There is a factor (C), which appears to control the production of a basic substance in flower pigment in wild peas. Another gene (R) controls the conversion of this basic substance into a purple pigment. If either of these factors is absent, no colour can be developed. This condition was first shown in a breeding experiment carried out by Bateson who crossed two true-breeding white strains of pea and produced coloured F1 offspring; in the F_2 he obtained coloured and white flowered peas in the ratio 9:7. The colour is produced only when the two different dominant genes C and R are present together in the same pea. One of the parents white strains lacked colour because it lacked the gene C, that is, had the constitution ccRR. The other parent was CCrr and also lacked colour. In the F1 the offspring were CcRr and thus were coloured. In the F_2 those containing C and R were also coloured but others were all white because they were without one or both of the complementary genes.

In certain cases, two pairs of genes determine a same phenotype but assorted independently, produce new phenotypes by mutual non-epistatic interactions and the F_2 phenotypic ratio 9:3:3:1 remains unaltered. Two pairs of genes which interact to affect size and shape of comb but are independently transmitted exist in chicken.

Examples:

Combs in fowl (9:3:3:1). The classical case of genetic interaction of two genes is discovered by Bateson and Punnett (1905-1908) in fowls. There are many different breeds of domestic chicken. Each breed possesses a characteristic type of

comb. The Wyandotte breed has a comb called "rose", the Brahmas breed has a comb called "pea", and the Leghorns have a comb called "single". Each of these types can be bred true. A cross of chicken with a rose comb to one with a single comb produces *A* rose and a single, showing dominance of rose over single. Another cross between pea combed and single combed chickens produces pea and single combed chickens in the ratio 3:1, showing dominance of pea over single. But, when a rose combed chicken crossed with that of pea combed, the F_1 progeny was found with a different type of comb known as "walnut" (Malay breed). When the F_1 walnut combed chickens were bred together, in F_2 all four types of combs were obtained, i.e., 9/16 walnut, 3/16 rose, 3/16 pea and 1/16 single.

These peculiar results were interpreted by Bateson and Punnett as follows: The rose comb is caused by the combination of homozygous recessive genes "pp" and homozygous and heterozygous dominant genes "RR" or "Rr". The pea comb is supposed to be produced by combination of a homozygous recessive condition (rr) and homozygous and heterozygous dominant condition (PP or Pp). While the single type comb is produced by the double recessive, rrpp genes. Thus, R gene determines the shape of rose comb and P gene determines the shape of pea comb, but when both genes happen to come together in single individual due to cross between rose and pea combed chickens, they interact to produce a walnut comb in F1. In the cross of two walnut chickens, two genes interact variously and produce four types of offspring in F_2.

In mice two independent pairs of genes (i.e., C-c and A-a) have interacted in the production of the phenotypic trait (i.e., coat colour) in such a way that one dominant (C) produces its effect whether or not the second (A) is present. But the second (A) gene can produce its effect only in the presence of the first. The common house mouse occurs in a number of coat colours, i.e., agouti, black and albino. The agouti (grey colour) patter is commonly occurred one (wild type)and is characterized by colour banded hairs in which the part nearest the skin is grey, then a yellow band and finally the distal part is either black or brown. The albino mouse lacks totally in pigments and has white hairs. When a homozygous black (CCaa) is crossed with a homozygous albino (ccAA) in F_1 all agouti (CcAa) offspring

appear. When the F_1 agoutis are crossed among themselves in F_2 agouti, black and albino off springs appear in the ratio of 9: 3: 4.

Epistasis

Epistasis is a form of gene interaction in which one gene interferes with the phenotypic expression of another non-allelic gene so that the phenotype is governed by the former gene and not by the latter gene, when both genes are present in the genotype. Epistasis may be caused by the presence of homozygous recessives of one gene pair, so that aa mask the effect of the B allele, or epistasis may result from the presence of one dominant allele in a gene pair. For example, the A allele might masks the effect of the B allele. Epistasis can occur in both directions between two gene pairs. Epistasis is the suppression of one gene by another non-allelic gene. All these possibilities can produce quite a number of modifications of the 9:3:3:1 ratio.

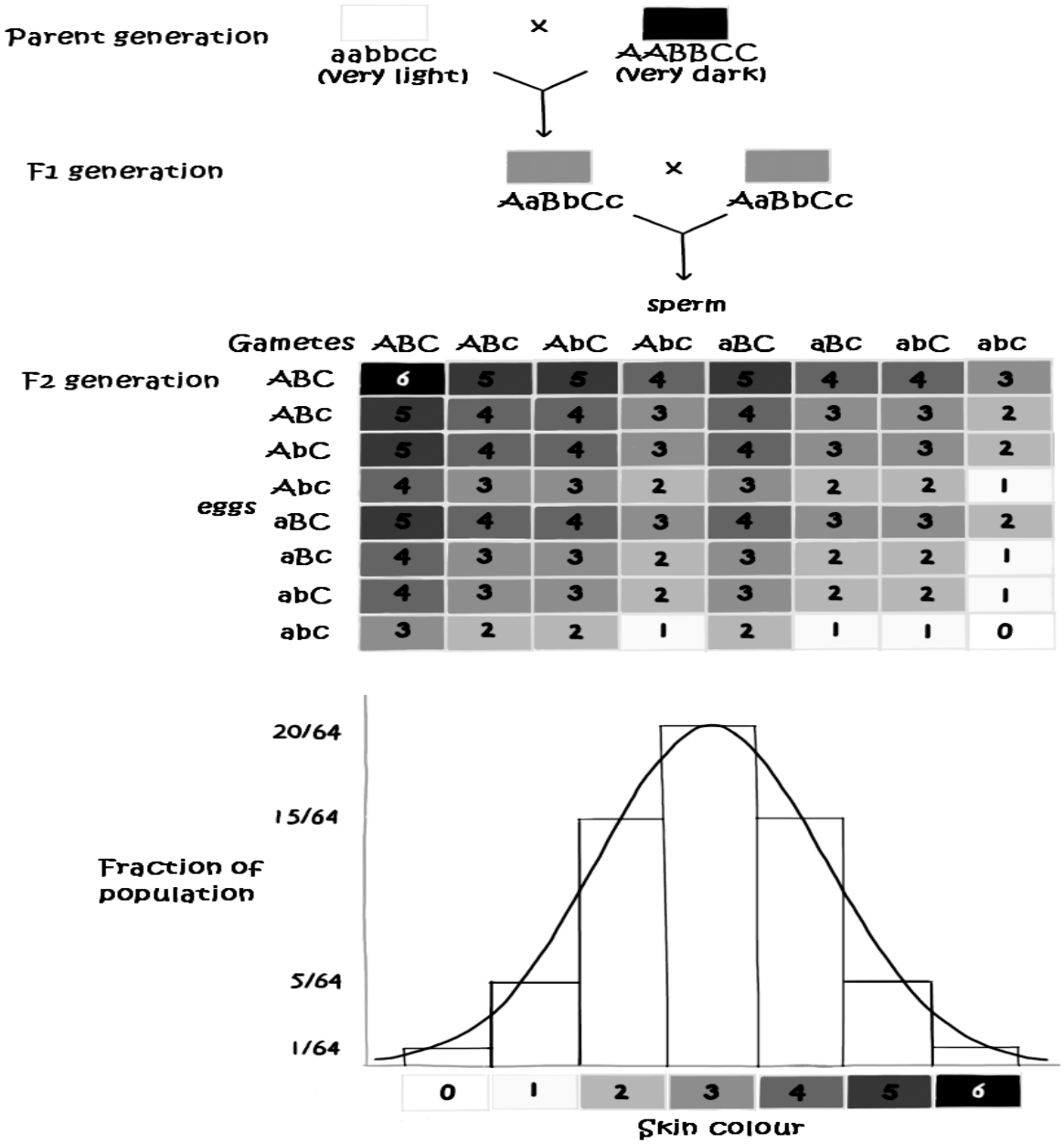
When in dihybrid crosses the epistatic interactions occur between two genes less than four phenotypes appear in F_2. Such epistatic interactions may be of the following types:

1. Dominant epistasis (12:3:1). When the dominant allele (e.g., A) of one gene masked the activity of alleles of another gene (e.g., B) and expressed itself phenotypically, then A gene locus is said to be epistatic to the B gene locus. Because the dominant allele A can express itself only in the presence of either B or b allele, therefore, such type of epistasis is termed as dominant epistasis. The alleles of hypostatic gene B will be able to express themselves phenotypically only when gene locus A may contain two recessive alleles (aa). For example, among dogs the colours of coat depend upon the action of two genes. One gene locus has a dominant epistatic inhibitor allele (I) of coat colour pigment. The allele I prevent the expression of colour allele at another independently assorting hypostatic gene (B or b) and produces white coat colour. The alleles of hypostatic gene locus (BB, Bb or bb) express only when two recessive alleles (ii) occur on the epistatic locus, i.e., iiBB or iiBb produces black and iibb produces brown individuals. When two

such white coat colour dogs are crossed, in F_1 the white, black and brown coat colours appear in 12: 3: 1 ratio.

2. Recessive epistasis. Sometimes the recessive alleles of one gene locus mask the action (phenotypic expression) of alleles of another gene locus. This type of epistasis is called recessive epistasis. For example, in very rare Bombay phenotype, blood of the person of genotype —hh does not react with anti-A, anti-B, or anti-H. The allele h is found to be epistatic to the multiple alleles in the A-B-0 locus.

Polygenic Inheritance.



The phenotypic traits of the different organisms may be of two kinds: qualitative and quantitative. The qualitative traits are the classical Mundelein traits such as form (e.g., round or wrinkled seeds of pea); structure (e.g., horned or hornless condition in catties); pigments and so on. The organisms possessing qualitative traits have distinct (separate) phenotypic classes and are said to exhibit discontinuous variations.

The quantitative traits are economically important measurable phenotypic traits of degree such as height, weight, shape, skin pigmentation, metabolic activity, amount of fruits, seeds, milk, meat produced by plants or animals, etc. in contrast to qualitative traits, the quantitative traits may be modified variously by the environmental conditions and are usually governed by many factors or genes, each contributing such a small amount of phenotype that their individual effects cannot be detected by Mendel methods but by only statistical methods. Such genes which are non-allelic and affect the phenotype of a single quantitative trait are called polygenes or cumulative genes. The inheritance of polygenes is called polygenic inheritance.

The example of quantitative inheritance is inheritance of the kernel colour in Wheat. This problem was studied by Swedish geneticist H.Nilsson-Ehle in 1908. When he crossed a certain red strain to a white strain, he observed that the F1 was all light red and that approximately 1/16 of the F_2 was as extreme as the parents, i.e., 1/16 was white and 1/16 was red. He interpreted these results in terms of two genes, each with a pair of alleles exhibiting cumulative effect.

Each of the contributing alleles ABC adds some red to the phenotype of kernel colour, so that the genotypes of whites contain neither of these alleles and a red *genotype contains only ABC; alleles. In F_2 five phenotypic classes* are obtained; each "dose" of a contributing allele for pigment production increases depth of colour. In case there were two genes involved, there would be obtained 15:1 ratio (15 coloured: 1 white).

Another classical example of polygenic inheritance is the inheritance of skin colour in man. It was found that two pairs of genes (A-a, B-b) cause the difference in skin pigmentation between Negro and Caucasian people. These genes were found to

affect the character in additive fashion. Thus, a true Negro has four dominant genes (AABB) and a white has four recessive genes (aabb). The F1 offspring of mating aabb with AABB are all AaBb and have an intermediate skin colour termed mulatto. A mating of two such mulattos produces a wide variety of skin colour in the offspring, ranging from skins as dark as the original Negro parent to as white as the original white parent.

P ♀AABB X ♂aabb
Negro white
Gametes AB ab
F_1 AaBb – mulattoes
Intermediate skin colour
P ♀AaBb X ♂AaBb
Mulattoes Mulattoes
Gametes AB; Ab; aB; ab AB; Ab; aB; ab
F_2 1-AABB (like negro); 4-AABb, AaBB (darker then mulattoes);
6-AaBb, Aabb, aaBB (like mulattoes);
4-Aabb, aaBb (lighter than mulattoes); 1-aabb (like white).

These results are clearly showing that A and B genes produce about the same amount of darkening of the skin and, therefore, the increase or decrease of A and B genes cause variable phenotypes in F_2 in the ratio of 1 Negro : 4 dark : 6 intermediate : 4 light: 1 white. Other examples of quantitative traits of human beings include height, hair colour and eye colour.

Pleiotropy. A single gene often influences more than one phenotypic trait (major effect) then the others with less evident phenotype (secondary effect). Most genes have their multiple effects and are called pleiotropic genes. The phenomenon of multiple effects (multiple phenotypic expressions) of a single gene is called pleiotropy.

Examples: 1. In Drosophila the recessive gene for vestigial wings causes vestigial wings in homozygous condition. However, careful observations show that other traits as well are affected: the tiny wing-like balancer behind the wings; certain bristles; the structure of the reproductive organs; egg production is lowered; longevity is reduced.

2. In human, the gene for disease phenylketonuria has pleiotropic effect and produces various abnormal phenotypic traits, collectively called syndrome. For

example, the affected individuals secrete excessive quantity of amino acid phenylalanine in their urine, cerebrospinal fluid and blood. They become short stature, mentally deficient, with widely spaced incisors, with pigmented patches on skin, with excessive sweating, and with non-pigmented hairs and eyes.

Practice.

Assignment 1. Draw a table "Possible genotypes of blood groups in the ABO system".

Assignment 2. Solve the problems and explain the kind of gene interactions.

1. A black mouse mates with a brown mouse, and all of the offspring are black. Why are no brown offspring produced?
2. A tall plant with red flowers from a true-breeding line was crossed with a homozygous short plant with white flowers. All the F_1 was tall with pink flowers. If the F_1 are allowed to self-pollinate, what proportion of the F_2 will resemble the F_1 in appearance?
3. F_2 plants segregate 9/16 coloured : 7/16 colorless. If a coloured plant from the F_2 is chosen at random and self-pollinated, what is the probability that there will be no segregation of the two phenotypes among the progeny? The plant's colour is controlled by two non-allelic genes, which are located in the different pairs of chromosomes.
4. Bulb colour in onion is depending upon two pairs of non-allelic, non-linked genes. Dominant gene of one allele is an inhibitor of pigmentation. The kind of pigment produced depends upon another allele. When a pure white strain is crossed to a pure red strain and produces an all white F_1 and F_2 with 12/16 white, 3/16 red and 1/16 yellow. How to explain these results?

Topic 12. Linkage. Inheritance of Sex.

Key concepts:

1. Morgan's experiments. Linkage. Chromosome Theory.
2. Crossing-over and incomplete linkage.

3. Sex determination.

4. Sex-linked inheritance.

Linkage

The hereditary units or genes which determine the characters of an individual are carried in the chromosomes and an individual usually has many genes for the determination of various different characters. As there are more genes than the chromosomes, it can be expected that each chromosome contains more than one gene. The genes for different characters may be either situated in the same chromosome or in different chromosomes. When the genes are situated in different chromosomes, the characters they control appear in the next generation together or apart, depending on the chance alone. They assort independently according to Mendel's law of independent assortment. But if the genes are situated in the same chromosome and are fairly close to each other, they tend to be inherited together. This type of coexistence of two or more genes in the same chromosome is known as linkage. The difference between independent assortment and linkage are shown the following examples:

1. Genes on different chromosomes assort independently giving a 1 : 1 : 1 : 1 test cross ratio is as follows:

P ♀AABB x ♂aabb

Gametes AB ab

F_1 AaBb

Test cross: **P** **♀AaBb x ♂aabb**

Gametes AB, Ab, aB, ab ab

F_2 ½ AaBb : ½ Aabb : ½ aaBb : ½ aabb

2. Linked genes do not assort independently, but tend to stay together in the same combination as they were in the parents:

P ♀AABB x ♂aabb

Gametes AB ab

F_1 AaBb

Test cross: **P** **♀AaBb X ♂aabb**

Gametes AB, ab ab

F_2 ½ AaBb : ½ aabb or 1:1

The hypothesis that linked genes tend to remain in their original combination because of their location in the same chromosome was advanced by T.Morgan.

Morgan stated that the pairs of genes of homozygous parent tend to enter in the same gametes and to remain together, whereas same genes from heterozygous parent tent to enter in the different gametes and remain apart from each other. He further stated that the tendency of linked genes remaining together in original combination is due to their location in the same chromosome. According to him the degree of strength of linkage depends upon the distance between the linked genes in the chromosome.

Chromosome Theory of Linkage.

Morgan formulated the chromosome theory of linkage which is as follows:

1. The genes which show the phenomenon of linkage are situated in the same chromosomes and these linked genes usually remain bounded by the chromosomal material so that they cannot be separated during the process of inheritance.
2. The distance between the linked genes determines the strength of linkage. The closely located genes show strong linkage than the widely located genes which show the weak linkage.
3. The genes are arranged in linear fashion in the chromosomes.

Kinds of Linkage T.Morgan and his co-workers by their investigation on the Drosophila and other organisms have found two types of linkage, viz., complete linkage and incomplete linkage.

1. The complete linkage is the phenomenon in which parental combinations of characters appear together for two or more generations in a continuous and regular fashion. In this type of linkage genes are closely associated and tend to transmit together.

Example. The genes for bent wings and shaven bristles of the fourth chromosome mutant of Drosophila melanogaster exhibit complete linkage.

In most of the organisms crossing-over takes place both in males and females. But in male Drosophila and female silkworm, Bombyx mori crossing-over takes place either very rarely or not at all. This becomes clear from Morgan's experimental results from Drosophila. In 1919 T.H.Morgan mated gray bodied and vestigial winged fruit flies with flies having black bodies and normal wings. F_1

progeny had gray bodies and normal wings, indicating thereby that these characters are dominant. When F1 males were backcrossed (i.e., test crossed) to double recessive females (black vestigial), only two types of progeny (one with gray bodies and vestigial wings, and the other with black bodies and normal wings), instead of four types of phenotypes were obtained:

Parents:	Gray, Vestigial ♀Aabb	x	Black, Long ♂aaBB
Gametes:	Ab		aB
F_1:	All Gray, Long AaBb		
Test cross:	F_1 male Gray, Long ♂ AaBb	x	Female Black, Vestigial ♀ aabb
Gametes:	Ab, aB (only two types of gametes due to complete linkage and lack of crossing over in male Drosophila)		ab
Test cross ratio:	½ Gray, Vestigial Aabb	½ Black, Long aaBb	or 1:1

The linked genes do not always stay together because homologous non-sister chromatids may exchange segments of varying length with one another during meiotic prophase. This sort of exchange of chromosomal segments in between homologous chromosomes is known as crossing over. The linked genes which are widely located in chromosomes and have chances of separation by crossing over are called incompletely linked genes and the phenomenon of their inheritance is called incomplete linkage. When F_1 females of the Morgan's classical cross in Drosophila between gray, vestigial (Aabb) and black, normal or long (aaBB) were test-crossed to double-recessive (aabb) males, all four types of progeny were obtained in following ratio, showing occurrence of crossing-over:

Parents: Gray, Vestigial ♀Aabb x Black, Long ♂aaBB

Gametes: Ab aB

F_1: Gray, Long AaBb

Test cross: F_1 Female Gray, Long ♀AaBb x Male Black, Vestigial ♂aabb

Gametes: Ab, aB = Non-crossovers ab

AB, ab = Recombinants

Test cross ratio: 1.Gray, Vestigial; Aabb = 41,5% } 83% parental combination showing linkage

2.Black, Long; aaBb = 41,5% }

3.Gray, Long; AaBb = 8,5% } 17% recombinants due to crossing over

4.Black, Vestigial; aabb = 8,5% }

All the linked genes of a chromosome form a linkage group. Because, all the genes of a chromosome have their identical genes on the homologous chromosome, therefore linkage groups of a homologous pair of chromosome is considered as one. The number of linkage group of a species, thus, corresponds with haploid chromosome number of that species.

Examples:

Drosophila has 4 pairs of chromosomes and 4 linkage groups.

Man has 23 pairs of chromosomes and 23 linkage groups.

Genetic and Cytological Mapping of Chromosomes

The percentage or frequency of crossing over appears to be closely related to physical distance between genes. It becomes possible to determine the relative distances between the genes in a linkage group and also their order and may give diagrammatic representation of chromosomes showing the genes as points separated by distances proportional to the amount of crossing over. Such a diagrammatic, graphical representation of relative distances between linked genes of a chromosome is called linkage or genetic map.

The distance between genes on the- chromosome cannot be measured in the customary units employed in light microscopy; geneticists use an arbitrary unit to measure the map unit to describe distances between linked genes. A map unit is equal to 1% of crossovers (recombinants); that is it represents the linear distance along the chromosome for which a recombination frequency of 1% is observed.

These distances can also be expressed in morgan units; one morgan unit represents 100% crossing over. Thus, 1% crossing over can also be expressed as 1 centimorgan Examples:

If a F_1 hybrid having the genotype AaBb produces 8% of cross- over gametes AB and ab, then the distance between A and B is estimated to be 16 map units or centimorgan.

If the map distance between the gene loci B and c is 12 centimorgan, then 12% of gametes of genotype BbCc should be crossover types.

Sex determination.

In sexually dimorphic organisms besides morphological and behavioral differences between both sexes, the sexual diversity also occurs at the level of chromosomes. Two types of chromosomes were recognized which are as follows:

Autosome. The chromosomes which have no relation with the sex and contain the genes which determine the somatic characters of the individuals are known as autosomes (A).

Sex chromosomes. The chromosomes which are responsible for the determination of sex are known as sex chromosomes, e.g. X and Y chromosomes. The X and Y sex chromosomes exhibit structural differences. The cytological studies have shown that the X-chromosomes of most organisms are straight, rod-like and comparatively larger than Y chromosomes. The Y chromosome is smaller in size with one end slightly curved or bent to one side in Drosophila; in man no such curvature of Y chromosome occurs. The X-chromosomes have large amount of euchromatin and small amount of heterochromatin. The euchromatin has large amount or DNA material, hence, much genetic information. The Y chromosome contains small amount of heterochromatin. The Y chromosome has little genetic information therefore sometimes it is referred to as genetically inert or inactive.

In organisms following two systems of chromosomal determination of sex have been recognized: 1. heterogametic males; 2. heterogametic females

Heterogametic males. In this type of sex determination female has two X-chromosomes and male has only one X-chromosome. Because male lacks an X-

chromosome, therefore during gametogenesis produces two types of gametes: 50% gametes carry the X-chromosomes and the rest 50% gametes lack in X-chromosome. Such a sex which produces two different types of gametes is called heterogametic sex.

The female produces similar type of gametes is called homogametic sex. The heterogametic males may be of the following two types:

1) XX-XO type. In certain plants and insects the female having two X chromosomes (hence, referred to as XX) and are, thus, homogametic, while the male having only one X chromosome (hence, referred to as XO). The presence of an unpaired X chromosome determines the masculine sex. The male lacking in one X chromosome produces two types of sperms: half with X chromosome and half without X chromosome. The sex of the offspring depends upon the sperm that fertilizes the egg.

2) XX-XY type. In the somatic cells of females (man, mammals, certain insects) all chromosome pairs are identical, they have a pair of X chromosomes because they have no Y chromosome, thus a female is designated as XX. As a result of gametogenesis the male animal produces two different kinds of sperms; one kind will have an X chromosome and the other Y chromosome. But the ova produced in oogenesis are all alike, each has an X chromosome. Drosophila, a fruit fly, has 8 chromosomes, of these three pairs are identical in both sexes, but one pair is not. Human somatic cells have 46 chromosomes, man having 22 pairs of autosomes and one pair of heterosomes, while a woman has 23 pairs of homologous chromosomes. In meiosis the chromosome pairs segregate, so that each egg has 22 autosomes and one X chromosome, while sperms have 22 autosomes and either an X or a Y heterosome, thus there are two kinds of sperms. Sex of the offspring is determined by the kind of sperm that will fertilize an egg. The X and Y sperms are produced in approximately equal numbers, and either kind has an equal chance of fertilizing an egg. If the egg is fertilized by an X chromosome sperm, the zygote will have XX chromosomes and it will develop into a female. If the egg is fertilized by a Y chromosome sperm, the zygote will have XY heterosomes and it will develop into a male. Recently the gene in the Y

chromosome responsible for determining the sex has been identified and named SRY gene.

In some animals the sex is not determined by the sperms. In birds and some insects all the sperms are of one kind only but there are two kinds of eggs, so that males are XX and females are XY, in such cases the type of egg will determine the sex of the offspring.

Heterogametic females. In this type of sex chromosomal determination the male possesses two X-chromosomes, therefore, is homogametic and produces single type of gametes, each carries a single X-chromosome. The female sex either consists of single X-chromosome or one X-chromosome and one Y-chromosome. The female sex is heterogametic and produces two types of eggs, half with an X-chromosome and half without an X-chromosome (with or without a Y-chromosome). To avoid confusion with that of XO-XX and XX-XY types of sex determination mechanisms, instead of the X and Y alphabets, Z and W alphabets are generally used respectively.

ZW-ZZ system of sex determination *occurs* in *certain* insects and vertebrates such as fishes, reptiles and birds. Here the female sex has one Z chromosome and one W chromosome. The male sex has two Z chromosomes. The sex of the offspring depends on the kind of egg, the Z bearing eggs produce males but the W bearing eggs produce females as shown in the following chart:

P	♀2A+ZW	x	♂2A+ZZ
Gametes	(A+Z) (A+W)		(A+Z)
F_1	2A+ZZ	:	2A+ZW
	male		female

Sex-linked inheritance. The sex chromosomes (heterosomes) not only have the genes for sex but they also carry genes for some recessive characters, such characters are said to be sex-linked, and they may be possessed by either sex. The character is sex-linked when its gene is carried in the X chromosome of a male or female. The inheritance of these characters is different from the inheritance of those characters whose genes are on autosomes. The pattern of heredity of sex-linked characters does not follow Mendel's laws because his results require the presence of pairs of similar genes in both sexes. Moreover, if the recessive type of

genes occur in X chromosomes of males, they express themselves phenotypically. Because in this case Y-chromosome contains no gene to overcome the recessive gene of X chromosome. The genes which occur exclusively on the X chromosome (mammals, Drosophila) or on the analogous Z chromosome (in birds and other species) are called X or Z-linked genes. The genes which exclusively occur in Y chromosome are called holandric genes. The inheritance of X or Z-linked and holandric genes is called sex-linked inheritance. In XX-XY type organisms,sex-linked genes can be classified into following three types:

X-linked. The X-linked genes are localized in the non-homologous sections of X-chromosome and have no corresponding allele in Y chromosome. These genes are commonly known as sex-linked genes.

Y-linked. This type of inheritance is performed by those genes which are localized in the non-homologous section of Y-chromosome, and that have no alleles in X-chromosome. The Y-linked genes are commonly known as holandric genes.

XY-linked. This type of inheritance is performed by those genes which are localized in homologous sections of X and Y chromosomes.

Inheritance of X-linked genes.

The X-linked genes exhibit following characteristic patterns of inheritance: The differential region of each chromosome (i.e., X) contains genes that have no counterparts on the other kind of sex chromosome. These genes, whether dominant or recessive, show their effects in the male phenotype. Genes in the different region are called hemizygous in males.

The X-linked recessive genes show the following two more peculiar features: criss-cross pattern of inheritance, i.e., in criss-cross inheritance, a X-linked recessive gene is transmitted from male parent (father) to F_2 male progeny (grandsons) through its F1 heterozygous females (daughters), which are called carries.

In the fruit-fly Drosophila melanogaster the eye-colour is sex-linked and the normal eye-colour is red. Morgan found one fly which had white eyes. This white-eyed male (the white-eyed condition is confined to males) was mated with a pure red-eyed female. It was found that all the flies of F_1 are red-eyed, but their females

have the gene for white eyes, while the males do not because they receive their X chromosome from the mother. When these F_1flies are interbred, the F_2 flies have an average of two red-eyed females, one red-eyed male, and one white-eyed male. In other words, only half of the males, but none of the females, have white eyes like their grandfather. Thus the appearance of the white-eyed character is linked with the male sex, but is inherited through the female.

Sex-linked characters are known in a number or animals, some common sex-linked characters in human beings are haemophilia, colour-blindness, and a kind of night blindness. Haemophilia is a condition in which blood fails to coagulate or coagulates very slowly after an external or internal injury. Some persons with haemophilia can bleed to death even from a small cut. In haemophilia (bleeder's disease) only males show the disease but it is transmitted through females who generally do not show the disease. The cause of haemophilia (as also of colour-blindness) lies in the sperms or eggs having a defective X chromosome which carries the gene for haemophilia. If the father has the defective X chromosome sperm, then all his sons escape the disease because they are conceived by his Y chromosome sperms, but all his daughters will be "carriers" of the disease because they have been conceived by his defective X chromosome sperms. If a carrier female mates with a normal male, then she will produce about half haemophilic and half normal sons, and about half carrier daughters and half unaffected daughters.

	Normal female		Haemolimphic male
P	♀X^HX^H	x	♂X^hY
Gametes	X^H		X^h, Y
F_1	X^HX^h,		X^HY
	Carrier daughter		Normal son

Colour-blindness is a condition in which a person cannot distinguish red colour from green; it occurs in about 8 per cent of males but rarely in females. It is caused due to lack of pigment in the retinal cone cells. Colour-blindness is a recessive character; its gene (as well as its dominant allele) is located in the X chromosomes of gametes. Colour-blindness is transmitted from a colour-blind man through his daughters who have normal colour vision to about half of her sons

only. One kind of night-blindness (inability to see in dim light) is hereditary. It affects only men, but women transmit the disease. The daughter of a night-blind man and his normally sighted wife is likely to pass the defective gene to one of her sons (by a husband whose sight is normal).

Inheritance of Y-linked genes.

Genes in the non-homologous region of the Y-chromosome pass directly from male to male. In man the Y-linked or holandric genes hypertrichosis (excessive development of hairs on pinna of ear) are transmitted directly from father to son. Recently, certain other holandric genes have been reported in humans, e.g. genes for H-Y antigen, spermatogenesis, height (stature) and slower maturation of individual.

Inheritance of XY-linked genes.

The genes which occur in homologous section of X and Y chromosome have inheritance like the autosomal genes. The XY-linked genes are partially or incompletely sex-linked, because sometime the crossing over may occur in the homologous sections of X and Y chromosomes. Certain XY-linked genes of man are two skin diseases (Xeroderma pigmeritosum and Epipermolysis bullosa, Retinitis pigmentosa, etc.).

Practice.

Assignment. Problem solving.

1. 50% of the daughters and 50% of the sons of a married woman are colour-blind. Illustrate the condition of this disease in that woman and her husband by means of a sketch.

2. Could a recessive mutant gene in humans be located on the X-chromosomes if a woman exhibiting the recessive trait and a normal man had a normal son? Explain.

3. In fowl, barring is sex-linked and dominant, the recessive allele producing solid black colour when homozygous. A silky feather is a recessive autosomal allele, as opposed to no silky. If black cocks, heterozygous for silky, are crossed to barred, silky hens, what genotypes and phenotypes will be produced and in what proportions?

4. One form of colour blindness in humans is caused by a sex-linked recessive mutant gene. A woman with normal colour vision and whose father was colour-blind marries a man of normal vision whose father was also colour-blind. What proportion of their offspring will be colour-blind (give your answer separately for males and females).

Topic 13 Practical skills

Solve the problems:

1. In cattle, a recessive gene in homozygous condition causes calves to born "amputated" which die soon after birth. Two normal animals are crossed. What progeny do you expect?
2. Consider three gene pairs each of which affects a different character. These three genes pairs assort independently of each other. Calculate the probability of obtaining an ABC phenotype from a cross of individuals that AaBbCC × AaBbcc.
3. For the following cross involving the comb character in poultry, determine the genotypes of the individuals. A walnut crossed with a single produces offspring ½ of which are walnut, ¼ rose, ¼ pea and ¼ single.
4. In rabbits one enzyme is needed to produce a substance needed for hearing. Another enzyme is needed to produce another substance also required for normal hearing. The genes responsible for the two enzymes are not linked. Individuals homozygous for either one or both of the non-functional recessive alleles are deaf. If cross were made between two double heterozygous, what phenotypic ratio would be expected in the progeny?
5. In Drosophilae genes *a* and *b* are linked with 2,5% recombination. Heterozygous female is crossed with a double homozygous male. What will be the proportions and genotypes of the offspring?
6. In chickens barred plumage is dominant over non-barred and X-linked. The phenotype can be distinguished in newly hatched chick. Commercial chicken breeders in England have used this difference to separate male and female chicks otherwise a difficult ask in order to accomplish this, what must be the genotypes of the parents?

Topic 14. Genetic change and variation.

Key concepts:

1. Variation and its main types.
2. Characteristics of non-inherited variation.
3. Inherited variation. Recombinations.
4. Mutations and their main types.
5. Point mutation.
6. Chromosomal mutations: aneuploidy, euploidy. Aberrations: deletion, inversion, translocation and duplication.
7. Mutation frequency and mutagenic factors.

VARIATION AND ITS TYPES

Variation is an ability of organisms to change their features and properties. It is one of the most important adjective properties which are influenced by the environment. Variations provide the variety of organisms and the unique properties of each and every organism.

There are two types of variations:

- phenotypical variations (modifications)
- genotypic variations (recombinations and mutations)

Modifications are the changes which occur due to environmental influence and do not concern genotype. They are simple and have the same changes in organisms of a certain genotype. For example, regular trainings enhance the growth of muscular volume, lungs capacity, bone firmness, etc. In Chinese Primrose flowers which are red at ordinary temperature are white if the temperature is very high. In a race of rabbits the hair, which is white at ordinary temperature is blackened at certain lower temperature. They occur in a mass way - many individuals respond the influence of the environment by phenotypical changes. They are adjective mechanisms. As a rule, they are reversible. If the factors influence ceases, they disappear, too. Thus, after the end of physical

trainings muscles volume and lung capacity decrease again. If hazardous environmental effects affect an organism for a long time it undergoes certain metabolic changes which result in the irreversible changes. Thus, child's skeleton wrecks during rachitis. Modifications are not inherited - these physical changes do not transmit themselves to next generations.

Modifications are characterized by reaction norm. These are the limits of the features alterations. The norm of reaction is congenital. It is conditioned by two causes: genotype and environmental properties.

The correlation of genotype and environmental properties may vary. Some features depend on genotype only - these are blood groups of ABO system. Their reaction norm is zero. Other signs vary insignificantly. They have a narrow reaction norm. Some other signs vary considerably. For instance, body weight, number of leukocytes or erythrocytes change significantly. They have a wide norm of reaction. Such signs are not qualitative but quantitative. They depend on a great number of genes and are inherited not according to Mendel's laws. Therefore, they are characterized by statistic terms like variation number, variation curve, average arithmetic quantity, and average quadratic deviation and other.

Genotypic variations produce genetic change in the organisms by favoring some gene combinations rejecting others and constantly modifying the gene pool. There are two types of genotypic variations: recombination and mutation.

Recombination-that is new genotypes from already existing genes. Recombination is conditioned by the following mechanisms:

- the production of gene combination containing in the same individual two different alleles of the same gene, or the production of heterozygous individuals(meiosis);
- the random mixing of chromosomes from two parents to produce a new individual (sexual reproduction);
- the mixing of a particular allele with a series of genes not previously associated with it, by an exchange between chromosomal parts during meiosis, called crossing-over, to produce new gene combinations.

MUTATIONS

There are the changes which occur as a result of genetic system alterations - the mutations. They are neither single-vectored, nor simple. One and the same factor may cause the alteration of different signs while various factors may cause the similar changes. Mutations occur suddenly in single individuals. They are not adjective and appear spontaneously. Usually they are irreversible. They are inherited by the following generations.

Dutch botanist Hugo de Vries studied the plant Oenothera lamarckiana in wild form for many years continuously and observed certain spontaneous changes in some of these wild plants. These plants differed considerably in stem height, flower colour and leave's shapes. He observed that these changes were heritable and led to several new varieties. De Vries considered these variations quite significant and for them he coined the term mutation.

De Vries proposed the mutation theory which has the following characteristics:

1. Mutations appear from time to time among the organisms of a naturally breeding species or populations. The organisms with mutations are called mutants.
2. Mutations are heritable and form new species. They do not disappear by crossing.
3. Mutations arise suddenly in one step, i.e., new species arise suddenly in one step and not gradually.
4. Mutations occur in all possible directions and may be advantageous or disadvantageous. Unsuitable mutants are destroyed by natural selection.

Mutations appear full-fledged and, hence, there is no question of incipient stages in the development of an organ. Mutations occur frequently in the nature and have been reported in many organisms, e.g., Drosophila, mice, rats, rabbits, guinea pigs and man. In man, the mutations cause variation in hair colour, eye colour, skin pigmentation and several somatic malformations. Various genetically diseases of human beings such as haemophila, colourblindness, phenylketonuria, etc., form other examples of mutation in human beings.

How does a mutation act? Any change in sequence of nucleotides in the DNA will result in the corresponding change in the nucleotide sequence of mRNA. This may result in alignment of different tRNA molecules on mRNA (during protein synthesis). Thus, the amino acid sequence, and, hence, the structure and properties of the enzyme formed will be changed. This defective enzyme or structural protein may adversely affect the trait controlled by the protein. In consequence, a mutant phenotype makes its expression.

Kinds of mutations.

There exists a lot of controversy about the possible kinds of mutations among geneticists. They have been classified variously according to different criteria as follows: According to their occurrence in somatic and germinal cells following types of mutations have been classified:

A. Somatic mutations. The mutations occurring in non-reproductive body cells are known as somatic mutations. The genetically and evolutionary consequences of somatic mutations are insignificant, since only single cells and their daughter cells are involved. If, however, a somatic mutation occurs early during embryonic life, the mutant cells may constitute a large proportion of body cells and the animal body may be a mosaic for different types of cells. Somatic mutations have been often related with malignant (cancerous) growth. Examples of somatic mutation have been reported in *Oenothera lamarckiana* (Hugo de Vries) and several other cases including man. In man somatic mutation causes several fatal diseases such as *neurofibroma*, *retinoblastoma* and *heterochromia of the iris.*

B. Gametes mutations. The mutations occurring in gamete cells (*e.g.*, sperms and ova) are called gametes mutations. Such mutations are heritable and of immense genetically significance. The gametes mutations only form the raw material for the natural selection.

According to their phenotypic effects kinds of mutations may occur:

- Dominant mutations. The mutations which have dominant phenotypic expression are called dominant mutations. For example, in man the mutation disease aniridia (absence of iris of eyes) occurs due to a dominant mutant gene.

- Recessive mutations. Most types of mutations are recessive in nature and so they are not expressed phenotypically immediately. The phenotypic effect of mutations of a recessive gene is seen only after one or more generations, when the mutant gene is able to recombine with another similar recessive gene.
- Lethal mutations. According to their effects on the phenotype mutations may be classified as lethal, sub vitals and super vitals.

Lethal mutations result in the death of the cells or organisms in which they occur. Sub vital mutations reduce the chances of survival of the organism in which they occur. Super vital mutations, in contrast, cause the improvement of biological fitness under certain conditions.

According to the mode of origin, following two kinds of mutations have been recognized:

1. Spontaneous mutations. They occur suddenly in the nature and their origin is unknown.
2. Induced mutations. The mutations can be induced artificially in the living organisms by exposing them to abnormal environment such as radiation, certain physical conditions (i.e., temperature) and chemicals. The substances or agents which induce artificial mutations are called mutagens or mutagenic agents.

Mutagenic agents are of the following kinds:

✓ Radiations. The radiations which are important in mutagenesis are of two categories: one type is ionizing radiations such as X - rays and gamma rays; alpha and beta rays; electrons, neutrons, protons and other fast moving particles. The second type is non-ionizing radiations such as ultraviolet and visible light.

✓ Temperature as mutagen. It is reported that the rate of mutation is increased due to increase in temperature. For example, an increase of 10°C temperature increases the mutation rate two or three fold. Temperature probably affects both thermal stability of DNA and the rate of reaction of other substances with DNA.

✓ Chemical mutagens. Many chemical compounds which are ordinarily considered to be non-toxic have been found to be mutagenic in contain specific situations. Any chemical substance that affects the chemical environment of chromosomes is likely to influence, at least indirectly, the stability of DNA and its

ability to replicate without error. A chemical mutation can cause mutation only when it enters in the nucleus of the cell.

According to the genetic material, following two types of mutations have been recognized:

- Gene mutations (Point mutations). Any chemical change in a particular gene leading to the appearance of different alleles and affecting the phenotype of the organism is called gene mutation.
- Chromosome mutations. Any change in the number and structure of the chromosome or chromosome sets, leading to ploidy and chromosomal aberrations respectively.

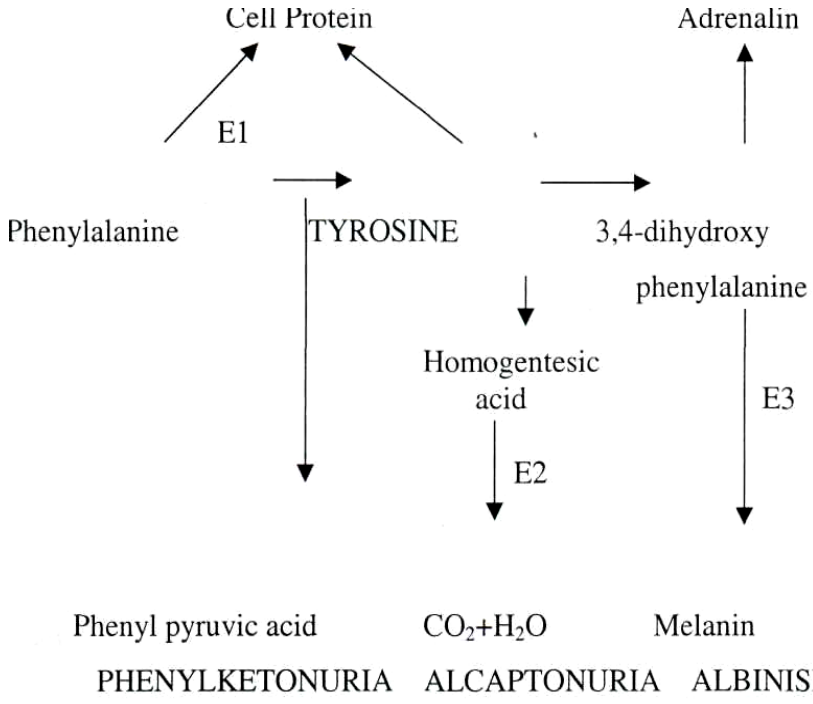
Gene mutations. When heritable alterations occur in a very small segment of DNA molecule, i.e., a single nucleotide or nucleotide pair, then this type of mutations are called "point mutations". The point mutations may occur due to sub nucleotide change in the DNA and RNA.

By now we fully understand that DNA of the organism is responsible for all heritable traits, and that a part of the DNA molecule responsible for coding the synthesis of one enzyme (protein) is termed a 'gene'. If exposure to radiations, for example, brings about a loss in the DNA molecule of one single component of a rung (horizontal bar), say of thymine, when the chromosome (or DNA) undergoes replication, the vacant place may be occupied by any purine or pyrimidine.

Naturally, the changed pattern will code a different sequence of amino acids for the synthesis of proteins, i.e., an altered messenger RNA will be coded and it will lead to the formation of a different enzyme or protein. The effect of altered enzyme will influence the cytoplasm structure of the organism, which would become modified to the extent when mutation might be said to have occurred.

This can be illustrated with the following example, wherein phenylalanine (amino acid) in the liver is partly built into cell-protein, and partly converted into tyrosine-another amino acid with the help of an enzyme, say, E_1. Then, a part of tyrosine is built up into cell protein, a part into 3, 4-dihydroxy phenylalanine, and the rest into homogentesic acid. This acid is normally oxidized with the help of an enzyme, say, E_2, into carbon dioxide and water. Product 3, 4-dihydroxy-phenylalanine is partly

used to synthesize adrenaline hormone, and the rest is converted into a protein melanin by means of an enzyme, say, E_3; melanin is a pigment.



Now, suppose there are genetic changes and the enzymes E_1, E_2, E_3 are altered, let us examine what biochemical effects they produce. If E 1 is altered, most of phenylalanine is not converted to tyrosine, instead, it is converted into phenyl pyruvic acid, which, if in excess, collects in the cerebro-spinal fluid and is believed to cause damage of the brain leading to mental retardation and other symptoms of a disease known as phenylketonuria.

Taking that E_1 was not altered, but E_2 was altered, then homogentesic acid or alcapton will not be oxidized to CO_2 and water. Large quantity of alcapton is passed on to the kidneys which put it out with urine; such urine darkens on exposure to air—a symptom of the disease known as alcaptonuria.

Lastly, supposing E_1 and E_2 remain normal, alteration takes place in E_3, then in the absence of normal E_3, melanin will not be produced and the skin will be without this dark pigment causing albinism.

Mutation, caused by a single gene, often produces such significant effects, which are worth studying. Sickle-cell anemia is a condition known to be the influence of a single mutant gene Si. The disease is common in the American Negroes and is inherited. The disease is caused when a child inherits two mutant

genes, one from each parent. It was first described in 1910 by James B. Herrick. In 1949, L. Pauling showed that it was due to abnormal haemoglobin resulting from a single gene mutation.

In the sickle-cell anemia the normal haemoglobin A in the red blood corpuscle is replaced by abnormal haemoglobin designated as haemoglobin S (HbS). Haemoglobin S is much less soluble than HbA, and it begins to crystallize when oxygen concentration falls in the capillaries of the tissues. This results in the change in the shape of rbc, which change from biconcave disc-shaped to crescent or sickle-shaped. Haemoglobin S has low oxygen-carrying capacity, therefore severe anemia results. Genetically, a person suffering from this disease is homozygous and has two dominant genes, one from each parent. A heterozygous condition produces sickle-cell trait. Sickle-cell trait is not so dangerous. Biochemically, the difference between HbA and HbS is illustrated in a part of their molecules thus: In Normal haemoglobin (HbA) the peptide chain consists of-valine-histidine- leucine— threonine— proline— *glutamic acid*— glutamic asid— lysine. In Abnormal haemoglobin S (HbS) it is the same, except that glutamic acid at eighth position is replaced by valine. This single gene mutation alters the property of haemoglobin and brings a change in the shape of the red blood corpuscles.The substitution of valine for glutamic acid can be interpreted as single base change in the triplet genetic code of messenger RNA; the adenine member of the triplet is replaced by uracil so that the code changes from AUG to UUG.

Changes in the chromosomes - Not only genes, but chromosomes too are subject to change or mutations. Whereas single gene or multiple gene mutations take place at any time of the life cycle, chromosomal mutations generally take place at the time of gamete formation during meiosis. It is at the zygotene stage of meiotic division that sister chromatids of chromosome come together and quite often non-sister chromatids are engaged in an exchange of genetic material.

Chromosome mutations are inherited once they occur and are of the following types:

Changes in chromosomes structure.

Changes in chromosomes number.

Structural changes in chromosome may be of the following types:

(i) Deletion-when a part of the chromosome separates out. Quite frequently such a loss results in death-that is, it is *lethal.* Since loss of a series of genes renders the organism incapable of forming one or more enzymes or other fundamental proteins, this effect is quite understandable.

(ii) Duplication-when a part of the chromatid comes and gets attached to the other non-sister chromatid. The added segment of the chromatid can occupy various positions, sometimes, at the end or sometimes between the ends. This would certainly affect sequence of genes in the duplicated chromatid. As a whole, this class of change is both more frequent and less lethal than deletions. An example of this kind of change is observed in the shape of the eyes of fruit-fly. Normally, the compound eyes of this fly are oval, but duplication of a certain segment of the chromatid results in a narrow form called bar-eye. When such abnormal flies are inbred extensively, an occasional individual is produced in which a second duplication of the same segment occurs. This results in an even narrower eye.

(iii)Inversion-a condition in which instead of a part being lost or added, here a segment is merely turned upside down, so that the sequence of genes is changed. This form of mutation is quite frequently met with. Effects of inversion are sometimes fatal, or create reduction in fertility due to difficulty in the synapses of such abnormal chromosomes.

(iiii)translocations-A condition when two non-homologous, chromatids exchange segments during meiosis. This has serious effects which may be lethal or at least create difficulties at the time of Synapses, thus reduce fertility.

Variations in chromosome number.

Each species has a characteristic number of chromosomes in the nuclei of its gametes and somatic cells. The gamete chromosome number constitutes a basic set of chromosomes called genome. A gamete cell contains single genome and is called haploid. When haploid gametes of both sexes (male and female) unite in the process of fertilization a diploid zygote with two genomes is formed. C

hromosomal aberrations may include whole genomes and entire single

chromosomes. Changes in number of whole chromosomes is called heteroploidy Heteroploidy may involve entire sets of chromosomes (euploidy) or loss or addition of single whole chromosome (aneuploidy) Each may produce phenotypic changes, modifications of phenotypic ratio, or alteration of linkage groups.

Many are of some evolutionary significance.

I. Changes in One or a Few Chromosomes.

Aneuploidy. In aneuploidy one or several chromosomes are lost from or added to the normal set of chromosomes. In most case, aneuploidy is lethal in animals, so in mammals it is detected mainly in aborted fetuses.

In diploid organisms aneuploid variations take four main forms:

Nullisomy (a nullisomic cell) involves a loss of one homologous chromosome pair; that is the cell is 2V-2.

Monosomy (a monosomic cell) involves a loss of a single chromosome; that is, the cellis2N-l.

Trisomy (a trisomic cell) involves a single extra chromosome; that is, the cell has three copies of one chromosome type and two copies of every other chromosome type. A trisomic cell is 2N+1.

Tetrasomy (a tetrasomic cell) involves an extra chromosome pair, resulting in the presence of four copies of one chromosome type and two copies of every other chromosome type. A tetrasomic cell is 2N+2. .

Aneuploidy may involve the loss or the addition of more than one specific chromosome or chromosome pair. For example, a double monosomic it is 2N+2+2. In both of these cases, one explanation is that meiotic nondisjunction involved two different chromosomes in one parent's gamete production.

In mammals aneuploidy of the sex chromosomes is more often found than aneuploidy of the autosomes because of a dosage compensation machanizm by which excess X chromosomes are inactivated.

In humans, autosomal monosomy is only found rarely. Presumably, monosomic embryos do not develop significantly and are lost early in pregnancy. This early lethality, in some cases, may be related to unmasking of recessive lethally.

In contrast, autosomal trisomy accounts for about one-half of chromosomal abnormalities producing fetal deaths. In fact, only a few autosomal trisomies are seen in live births: Down's syndrome,Patau's syndrome,Edward's syndrome.

II. Changes in Complete Sets of Chromosomes.

Monoploidy and polyploidy involve variations from the normal state in the number of complete sets of chromosomes. Since the number of complete sets of chromosomes is involved in each case, monoploid and polyploids are both euploids. Monoploids have a single basic set of chromosomes. Monoploidy is common in plants and rare in animals. Monoploids in some cases are found normally and are produced due to parthenogenesis, as in male (drone) insects such as bees, wasps and ants. In these insects, queen and workers are diploid females. In flowering plants monoploids may also originate spontaneously due to parthenogenesis of egg. Monoploids can be produced by different artificial methods, such as: X-ray treatments, temperature chock, hybridization etc.

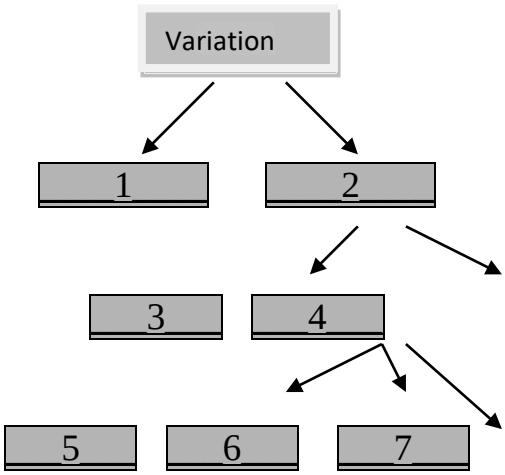
Polyploidy. Any organism with more than two genomes (2n) is called a polyploid. Changes in complete sets of chromosomes can result, for example, when meiotic nondisjunction occurs such that all chromosomes are involved. Fusion of a gamete with two chromosome sets with a normal gamete will produce a polyploid zygote, in this case one with three sets of chromosomes, which is a triploid (3n). Similarly, fusion of two gametes, each with two chromosome sets, will produce a tetraploid (4n) zygote Polyploidy of somatic cells can also occur following mitotic nondisjunction of complete chromosomes sets.

The most important effect of polyploidy is that it reduces the fertility of polyploid plants in variable degrees. The polyploid plants have been found to contain large-sized pollen grains, cells, leaves, stomata, etc. The polyploid plants are more vigorous than diploids. Polyploidy is rare in animals but occur in flatworms, leeches, mice. Polyploidy in humans have been found in liver cells and cancer cells. In them polyploidy is whether complete or a mosaic, it leads to gross abnormalities and death.

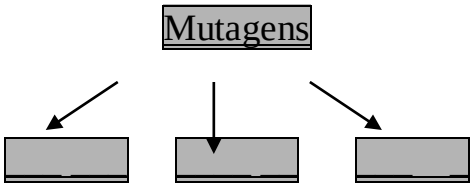
Practice.

Assignment 1. Types of variation.

Complete the following concept map about forms of variation.



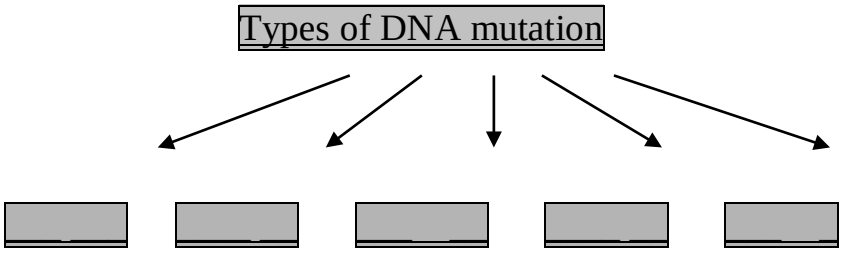
Assignment 2. Make up the diagram "Mutagens".



1. …… …….. ……..

2. …… …….. ……..

Assignment 3. Copy and complete the following diagram to show the types of point mutations.



Assignment 4. Problem solving.

A piece of DNA has the following nucleotides sequence:

A–A–T–T–C–G–C–G–A–T–T–C.

State the change (type of mutation) that has taken place in each of the following variants:

– A–T–T–C–G–C–G–A–T–T–C–C

– A–A–T–T–C–G–A–G–C–T–T–C.

Topic 15. Human genetics. Pedigree analysis. Study of Twins.

Key concepts:

1. Peculiarities of human heredity
2. Methods of the human genetics.
3. Pedigree analysis.
4. Twin method.

Man is not a very favorable subject for studies of inheritance, because:

a) members of the human race are genetically diverse, viz., they are heterozygous for many genes and there are wide variations in their physical, biological and social environments;

b) controlled mating is impossible and non-ethical;

c) has small individual progenies, so unfavorable to the use of certain standard research techniques of the genetics;

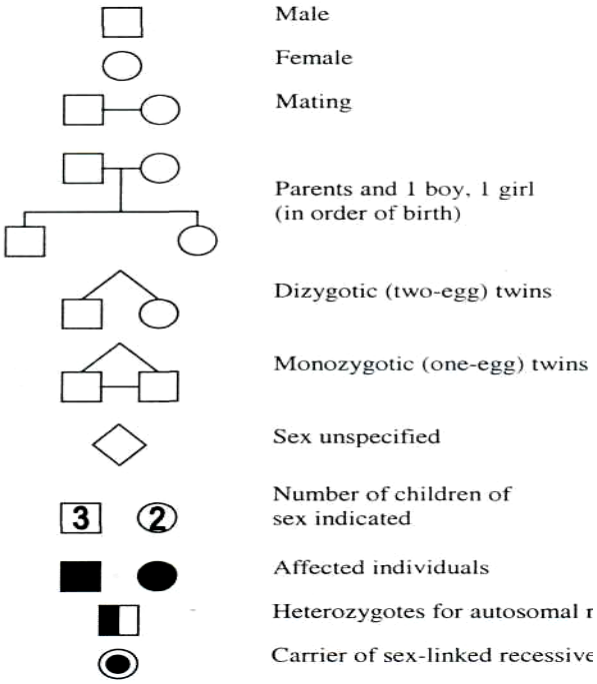
d) has long time between successive generations.

To study the genetics of various human traits special methods are used, such as pedigree analysis, twin's study, populative-statistic, cytogenetic, cell culture, etc.

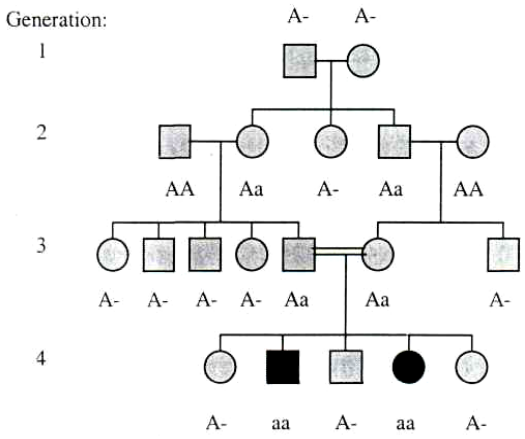
Pedigree analysis.

Since in human beings controlled crosses cannot be made, so human geneticists have to resort to a scrutiny of established mating in the hope that informative mating have been made by chance. The scrutiny of established mating is called pedigree analysis. A member of a family who first comes to the attention of a geneticist is called the propositus. The investigator than traces the history of the character shown to be interesting in the propositus back through the history of family.

Figure presents a hypothetical pedigree to show how the symbols are assigned to the family tree.



A human pedigree, illustrating the use of pedigree symbols.



The trait presented in figure is determined by a recessive allele *a.* Generations are numbered with Arabic numerals, while individuals are numbered with Roman numerals; this makes it easy to refer to particular people in the pedigree. The trait in this pedigree results from homozygosis for the rare allele, brought about by cousins marrying. Since cousins share a fair proportion of their genes, it can be expected that, in their offspring, a number of genes will become homozygous, and in this case one of the genes resulted in an identifiable genetic trait. Gene symbols are included in the pedigree to show the deductive reasoning *possible with such pedigrees; normally, such symbols would not be present and the researcher would have to analyze the pedigrees without that information*. For

example, the following reasoning could take place: The trait appears first in generation IV.

Since neither parent (the two cousins) had the trait, while two children were produced with the trait (IV-2 and IV-4), the simplest hypothesis is that the trait is caused by a recessive allele. Thus, IV-2 and IV-4 would both have the genotype *aa*, and their parents (III-5 and III-6) both must have the genotype Aa. All other individuals who did not have the trait must have at least one A allele; that is, they must be A- (i.e., AA or Aa). Since III-5 and III-6 are both heterozygotes, then at least one of each of their parents must have carried an *a* allele. Further, since the trait appeared after cousins had children, we must assume that the rare a allele is only in the immediate family of III-5 and III-6 so that both II-1 and II-5 are most likely AA in genotype. Therefore, II-2 and II-4 both must be Aa in order to transmit the alleles to III-5 and III-6.

Recessive inheritance. A large number of human traits are known to be caused by recessive genes. For recessive traits to be expressed, the allele must be homozygous (i.e. aa). Many serious abnormalities or diseases result from homozygosis for recessive mutant alleles. Individuals with albinism, for instance, do not produce the melanin pigment that protects the skin from harmful ultraviolet radiation. As a consequence, albinos have considerable skin and eye sensitivity to sunlight. Frequencies of recessive mutant alleles are usually higher then frequencies of dominant mutant alleles because heterozygote's for the recessive mutant allele are not at a significant selective disadvantage. Nonetheless, individuals homozygous for recessive mutant alleles are rare. In the United States approximately 1 in 39,000 of the *white* population and 1 in 28,000 of the African, American population are albinos. Among the Irish, about 1 in 10,000-15,000 are albinos. The following lists some general characteristics of recessive inheritance for a relatively rare trait:

Most affected individual *have* two normal parents, both of whom are heterozygous. The trait appears in the F_1 since a quarter *of progeny* are expected to be homozygous for the recessive allele. If the trait is rare or relatively rare, an individual expressing the trait is likely to mate with a homozygous normal

individual; thus the next generation is represented by heterozygote which does not express the trait. That is, recessive traits often "skip" generations.

Mating between two normal heterozygotes should produce an approximately 3:1 ratio of normal progeny to progeny exhibiting the recessive trait. However, in the analysis of human populations (families), it is difficult to obtain enough numbers to make the data statistically significant, especially if a biochemical test is necessary to confirm the presence of the trait because, in such cases, only the living members of a family can be surveyed. For recessive genes that have less deleterious effects, the allele can reach significant frequencies in the population. An example of such a recessive trait is attached earlobes. There are significant numbers of heterozygotes and homozygous recessives for this trait in the population. As a result, there is a strong possibility of *Aa* x *aa* mating, and half the progeny will have the trait. When both parents are affected, all their progeny will usually exhibit the trait. Dominant inheritance. There are many known dominant human traits.

Dominant mutant alleles are expressed in a heterozygote combination. Because many dominant mutant alleles that give rise to recognizable are rare, it is extremely unusual to find individuals homozygous for the dominant allele. Thus an affected person in a pedigree is likely to be a heterozygote, and most pairings that involve the mutant allele are between a heterozygote and a homozygous recessive (wild type). Most dominant mutant genes that are clinically significant (that is, they cause medical problems) fall into this category. The following are some general characteristics of a dominant trait:

Every affected person in the pedigree must have at least one affected parent.

The trait usually will not skip generations. An affected heterozygous individual will, on average, transmit the mutant gene to half of his or her progeny. Suppose the dominant mutant allele is designated A, and its wild-type allele is *a*. Then most crosses will be *Aa, aa.* From basic Mundelein principles half the progeny will be aa (wild type) and the other half will be *Aa* and show the trait.

Twins. Some of the difficulties in the study of heredity in man can be partially overcome by the study of twins. There are two kinds of twins:

a) fraternal or dizygotic twins who may be of different sexes, who are not more alike in appearance than ordinary "sibs" (brothers and sisters), and who are derived from the ova fertilized each by a distinct spermatozoon;

b) identical or monozygotic twins, who resemble one another very closely indeed, are always both of the same sex, and are both derived from the same zygote, the embryo having at an early stage divided into two parts each of which developed into one of the twins. Two identical twins thus have the same genotype while the genotypes of fraternal twins are not more alike than those of ordinary sibs. It is known that many characters are partly determined by the genotypic constitution and are partly by the environment. Since identical twins are of identical genotype, any phenotypic differences between them must have been caused by differences in the environments in which the individuals have lived. If pair of identical twins is found to differ from one another in some character, they are said to be "discordant" in respect of that character, and the discordance is regarded as due to environmental differences. If they are alike, they are "concordant" and the concordance is regarded as due to their genetic similarity. If a character shows significantly higher concordance between identical twins than it does between non-identical twins, then heredity plays a significant part in the development of that character. If concordance is not higher between identical than between non-identical twins it can be concluded only that there is no evidence for a genetic factor in the development of the character.

It is possible to calculate the coefficient of pair concordance (K) by following formula: K = C / (C+D) x 100%; Kmt - for monozygotic twins; Kdt - for dizygotic twins, where C is the number of concordant twin pairs, D - is the number of disconcordant twins pairs. The coefficient of heredity (H) is calculated by the formula: H = Kmt - Kdt / (100% - Kdt) x 100%; and coefficient of environmental influence -E = 100% - H. If H is nearly 70%, the genotype plays a significant part in the development of that character.

A large number of characters have been studied in this way. In anatomical traits, like height or dimensions of the head, concordance is found to be markedly higher between identical than between non-identical twins, but this is less true of

body weight, in which, of course, environmental differences, for example in food, are naturally expected to be important.

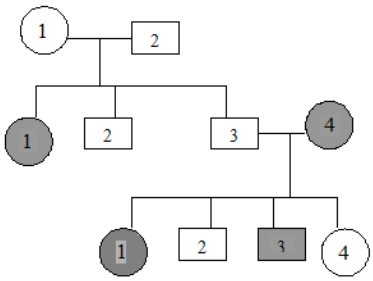
Twins method allows determining the role of heredity and environment in the phenotype formation.

Practice.

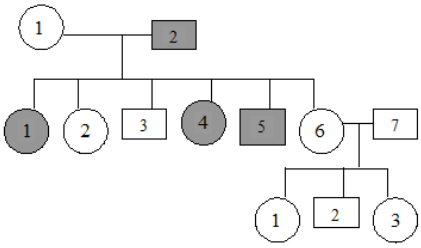
Assignment 1. Pedigree analysis.

Determine the probable inheritance mode for the trait shown in the affected individuals (the shaded symbols):

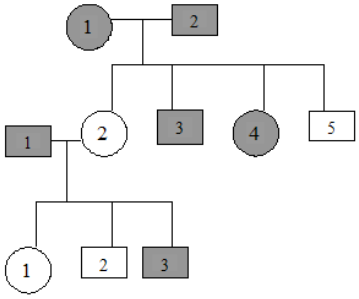
Pedigree A



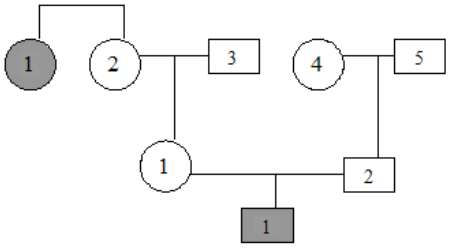
Pedigree B



Pedigree C



Pedigree D



What is the most probable explanation for these pedigrees?

Assignment 2. Determine the value of heredity and environment in the expression of the traits given below using the formulas:

$$H = \frac{Cmz - Cdz}{100 - Cdz} \ (\%)$$

$$E = 100\% - H$$

Where: H – is a coefficient of heredity, E – is a coefficient of environment, C – is a coefficient of concordant twin's pairs in % for:

- mz – monozygotic twins; - dz – dizygotic twins.

Complete the table.

#	*Traits*	*Cmz*	*Cdz*	*H*	*E*
1	Eyes colour	99	28		
2	Epilepsy	67	3		
3	Tuberculosis	67	23		
4	Diabetes mellitus	65	18		
5	Schizophrenia	69	10		
6	Appendicitis	29	16		
7	Hypertensions	25	9		
8	Alcoholism	54	18		
9	Blood group	100	30		
10	Asthma	47	24		

Topic 16. Molecular pathology. DNA analysis.

Key concepts:

1. What is a molecular pathology? Biochemical method.
2. Recessively and dominantly inherited traits.
3. DNA analysis.

Molecular pathology is caused by a mutation of the gene. One and the hereditary disorder may be caused by different can be inherited as an autosomal dominant, or autosomal recessive and as a recessive X-linked trait. Molecular pathology may be:

- congenital malformations of organs and tissue. For example: retinoblastoma, syndactyly, polydactyly, no upper incisors etc. The diagnosis is made up during examination of the phenotype in the clinic;
- defects in metabolism: amino acids, carbohydrates, lipids, minerals and others. The diseases can be manifested at different periods of ontogenesis. Changes the amount of nucleotides or their sequences in the DNA lead to the changes of the DNA code and hence alter the structure of the protein molecules. Proteins are enzymes. Decreasing of enzymes activities leads to accumulation of metabolic

products which causes diseases.

Molecular diseases are diagnosed by using the biochemical methods. On the first stage the screening programs are used (rapid tests). On the second stage is usually use chromatography, mass-spectrometry and other more complex methods for the determination of enzymes, carbohydrates, amino acids, etc. in order to confirm or to disconfirm the previous diagnosis.

About 5000 molecular diseases were found by using biochemical method.

Gene related human disorders are transmitted to the off spring as per Mendel principles.

These are of two types: recessively inherited traits and dominantly inherited traits.

Recessively Inherited Traits. These are caused by recessive autosomal genes in homozygous condition. The following are the common examples of this type of disorders.

Defects in Amino Acid Metabolism.

1. Phenylketonuria (PKU). It is an inborn metabolic disorder in which the homozygous recessive individual lacks the enzyme phenylalanine hydroxylase needed to change phenylalanine (amino acid) to tyrosine (amino acid). Lack of the enzyme is due to the abnormal autosomal recessive gene on chromosome 12. Affected babies are normal at birth but within a few weeks a level of phenylalanine in plasma increases and this impairs the brain development.

2. Alkaptonuria. This metabolic disorder produced due to deficiency of an oxidase enzyme required for breakdown of homogentisic acid (also called alcapton). Lack of the enzyme is due to the absence of the normal form of gene that controls the synthesis of the enzyme. Hence homogentisic acid then accumulates in the tissues and is also excreted in the urine. The most commonly affected tissues are cartilages, capsules of joints, ligaments and tendons. The urine of these patients if allowed to stand for some hours in air turns black due to oxidation of homogentisic acid.

3. Albinism. It is caused by the absence of the enzyme tyrosinase which is essential for the synthesis of the pigment from dihydroxyphnylalanine. The gene for

albinism (a) does not produce the enzyme tyrosinase but its normal allele (A) does. Thus, only homozygous individual (aa) is affected by this disease. Albinos lack dark pigment melanin in the skin, hair and iris, have poor vision.

Defects in Lipid Metabolism.

1. Tay-Sach's Disease (TSD) or Infantile Amourotic Idiocy. Children with Tay-Sach's disease are born normal but develop severe brain and spinal cord damage later in a few months due to an error in fat metabolism. Homozygous children show degeneration of central nervous system due to accumulation of a fatty substance in nerve cells. This is caused by the enzyme-acetyl hexosaminidase which in normal individuals exists in two forms A and B. In TSD, only the A form is present; the B form is not present. The child dies in 3 to 4 years. There is no treatment for this disease and.

2. Gaucher's Disease. In this disorder the breakdown of fatty acid substance is impaired leading to accumulation of lipid materials in body tissues and blood. It is caused by an autosomal recessive gene which inhibits the activity of an enzyme ***glucocerebrosidase,*** Consequently there is accumulation of cerebroside (a sphingolopid). There is enlargement of the spleen and liver and expansion of some of the limb bones.

Nonenzymatic genetic diseases.

1. Cystic fibrosis (CF). It is a recessive disorder of infants, children and yong adults that is due to a recessive autosomal gene present on chromosome 7. It produces a defective ***glycoprotein*** which causes formation of thick mucus in skin, lungs, pancreas, liver and other secretory organs. Sweat of the patient contains very high level of Na^+ and Cl^-. There is failure of chloride ion transport mechanism in cell surface membrane of epithelial cell. Accumulation of airways. Mucus deposition in pancreas blocks secretion of pancreatic juice. Liver may undergo cirrhosis. There is impaired production of bile.

Haemoglobinopathies.

1. Sickle-cell anemia – is an autosomal hereditary disorder in which the erythrocytes become sickle-shaped under oxygen deficiency. The cells can not pass

through narrow capillaries. They have a tendency to clot and degenerate. Blood circulation and oxygen supply are disturbed.

2. Haemophilia – is sex-linked disease due to the presence of a recessive sex-linked gene (h), carried by X-chromosome. The patient does not possess the natural phenomenon of blood clotting due to absence of antihaemophiliac globulin or factor VIII (hemophilia – A) and plasma thromboplastin factor IX (haemophilia – B) essential for it. As a result of continuous bleeding, the patient may die of blood loss.

A female becomes haemophiliac only when both its X-chromosomes carry the gene ($X^h X^h$). Such female generally die before birth because the combination of these two recessive alleles is lethal. If female has only one allele for haemophilia ($X^H X^h$) she is normal. Such female is known as carrie. Transfusion of normal blood checks bleeding because it provides the vital factors for blood clotting.

Dominantly Inherited Traits.

These are caused by dominant autosomal gene. Some of these disorders are:

- Achondroplasy – a form of dwarfism in which long bones do not grow:
- Polydactyly – presence of extra fingers:
- Brachydactyly –abnormal short fingers:

Huntington's disease – a disorder in which muscle and mental deterioration occurs. The mentioned disorders occur due to the mutations of a dominant gene on the autosomes

DNA –analysis is the definition of the sequence of nucleotides in the DNA. It allows determining a cause of the disease. In this method:

- cloned DNA and produce a large number of fragments . They can be used for analysis or for obtaining active functional proteins. Proteins can be used in treating genetic diseases.
- determine the localization of gene mutations using separate DNA fragment. DNA probes (nucleotide sequence is known) . Hybridization was carried out DNA probes of healthy person and the host cell. If the DNA of the host cell is normal,

hybridization is complete (by the principle of complimentarily). If there is a change, it will not hybridize. Then by electrophoresis determine abnormalities in the structure of DNA (each DNA fragment has a definite place in the form of a band at a particular location of the gel).

DNA analysis allows us not only to study the gene defect chromosome, but also monitor the effectiveness of therapy, to establish genetic relatedness, tissue compatibility. This method is indispensable for the development of treatment of a hereditary disease by genetic engineering.

DNA analysis can be used in the diagnosis of hereditary diseases, in investigations of malignant processes, in forensic medicine and for detection of infectious pathogens. Cystic fibrosis may serve as an example of a serious hereditary disease the diagnosis of which improved greatly after introduction of DNA analysis. The diagnosis of this disease is nowadays possible by direct analysis of mutations and indirectly by investigations of the link between the disease and DNA polymorphisms.

What is gene cloning?

1. A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a vector, to produce a recombinant DNA molecule.
2. The vector transports the gene into a host cell which is usually a bacterium, although other types of living cell can be used
3. Within the host cell the vector multiplies producing numerous identical copies not only of itself but also of the gene that it carries
4. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny further vector replication takes place.
5. After a large number of cell divisions, a colony, or clone, of identical host cells is produced.

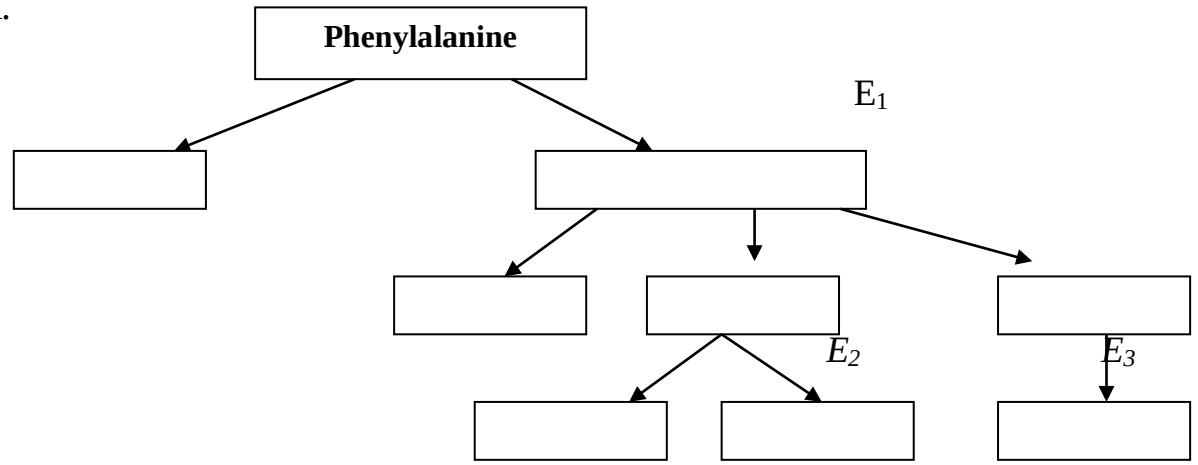
Each cell in the clone contains one or more copies of the recombinant DNA molecule the gene carried by the recombinant molecule is now said to be cloned Other symptoms are mental retardation, decreased pigmentation of hair and skin and ekzema. The heterozygous individuals are normal but carriers.

Practice.

Assignment 1. Metabolism of amino acid phenylalanine.

(A – normal organism, B, C, D – an organism with mutation in gene). Copy and complete the diagram. Fill in empty boxes with the appropriate term.

A.



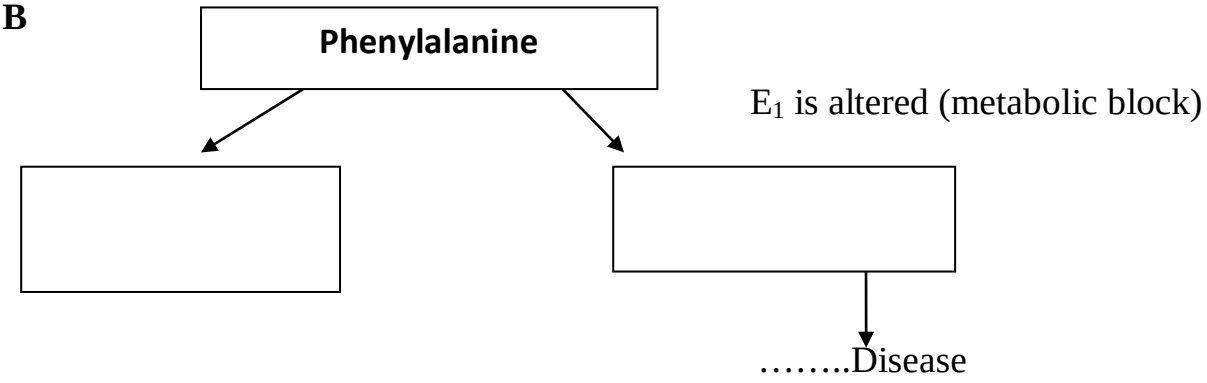
Enzymes:

E_1 – *phenylalanine hydroxylase*;

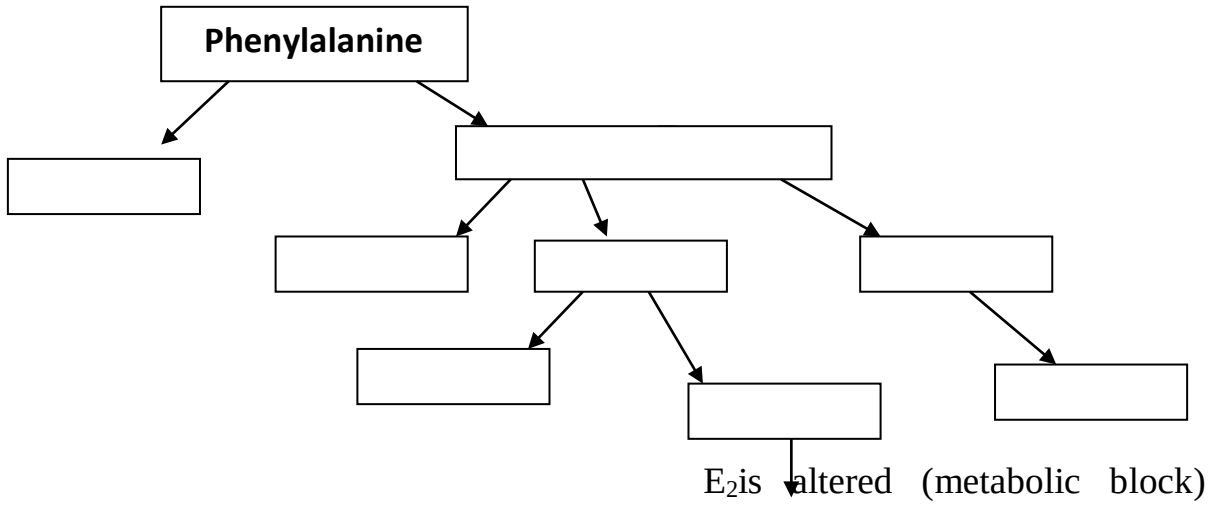
E_2 – *homogentyrosinate*;

E_3 – *homogentisate oxidase*.

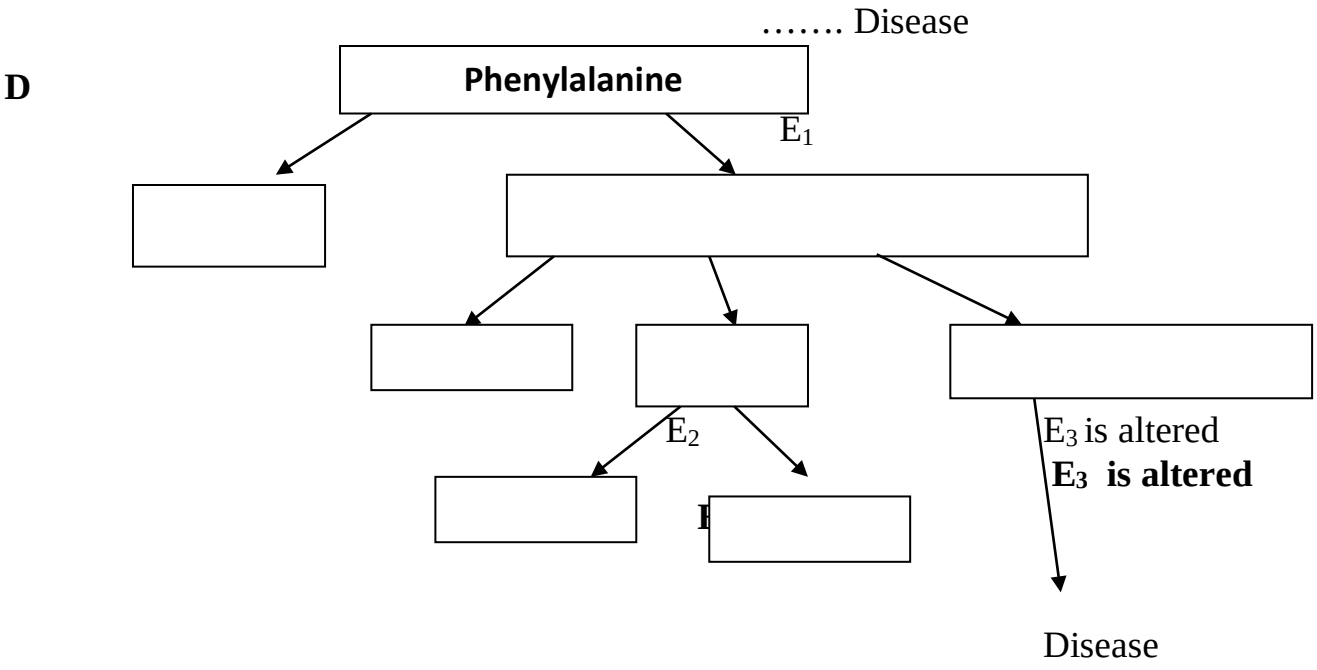
B



C



E 3



Assignment 2. Hereditary disorders in humans caused by gene mutation

Copy and complete the following table, give the main characteristics of disorders and the type of their inheritance.

Disorder	Type of inheritance	Major symptoms

Topic 17 Cytological method.

Key concepts:

1. What is karyotyping method?
2. What types of chromosomes are known?
3. What anomalies are caused by structural changes in autosomes.
4. Anomalies that are caused changes in the chromosome number.
5. Determination of Sex chromatin.
6. Human Sex Anomalies.

Cytological method includes karyotyping human chromosomes and determination of sex chromatin.

For karyotyping of human chromosomes venous blood is taken and blood leukocytes are stimulated to divide (by mitosis) in vitro by the addition of phytohaemagglutinin. Colchicine is added to arrest cell division at metaphase stage. It is further treated with hypotonic saline solution which results in swelling of cells and dispersal and better clarity of chromosomes for counting and morphological study. There after the material is stained (e.g. with Giemsa technique) to demonstrate the banding patterns of chromosomes. Finally, a suitable metaphase spread is photographed through a high power microscope. The individual chromosomes are cut out from the photograph. The chromosomes are then arranged in an orderly fashion in homologous pairs to produce a standard arrangement, the karyotype.

To characterize a chromosome in the karyotype the following parameters are used: shape of chromosome, length of chromosome. The human metaphase chromosomes were first of all classified by a conference of cytogeneticists at Denver in 1960 and are known as the Denver classification. To follow this classification each of the 22 pairs of autosomes has been numbered from 1 to 22 according to their decreasing size. Patau (1960) divided the human chromosomes into the following seven groups designated A to G:

A group: 1 to 3 pairs - metacentric

B group: 4 to 5 pairs - submetacentric

C group: 6 to 12 pairs - submetacentric

D group: 13 to 15 pairs - acrocentric

E group: 16 to 18 pairs - submetacentric (16 is metacentric)

F group: 19 to 20 pairs - metacentric

G group: 21 to 22 pairs - acrocentric

Group A consists of longest metacentric chromosomes. Group G consists of the shortest acrocentric chromosomes. These chromosomes have satellites that correspond to nucleolar organizers. Chromosomes of group D also contain satellites. In males, group G includes a variable Y chromosome which lacks the satellites.

The X-chromosome is the member of group C and can be identified by special banding methods.

In human beings various types of chromosomal variations, both numerical as well as morphological, have been reported. The following structural changes in autosomes which cause different anomalies have been studied:

Deletion — human babies missing a portion of the short arm of chromosome 5 (autosome) have a distinctive cat-like cry; hence, the French name "cri du chat" (cry of the cat) syndrome. They are also mentally retarded, have malformation in the larynx, moon faces, saddle nose, small mandibles and microcephally (small head).

Translocation. In patients with chronic myelocyte leukemia (a kind of cancer) in the bone marrow and in cells derived from it, is present a short chromosome, called the Philadelphia (Ph') chromosome (so named because it was discovered in that city). Detailed cytological study disclose Ph' to be a number 22 chromosome that has lost most of the distal part of its longer arm. The deleted part of autosome 22 is translocated to one of the larger autosomes (most frequently to the distal end of chromosome 9). This translocation is not transmitted to offspring of persons having Philadelphia chromosome. Some mutations involve parts of a chromosome set (aneyploidy) in individuals. Likewise, changes may involve addition of either a single chromosome,

called trisomy (2n+l). In humans the following three syndromes have been studied:

Down's syndrome or Trisomy-21. or mongolian idiocy. It is the most common chromosomal abnormality in live births (1 : 650 births). There are about 50 physical characteristics shown by Down's syndrome infants soon after birth. These include mild or moderate mental retardation; eyes that slant up and out with internal epicantal folds; a tongue that is large, swollen and protruding; small and under developed ears; short stature; stubby fingers and enlarged liver and spleen. Women over 45 years of age are about twenty time more likely to give birth to a child with Down's syndrome than women aged 20. Nondisjunction of chromosome pair 21 during oogenesis is the main cause of occurrence of trisomy-21. This event is found to be affected either by senescence of oocytes, virus infection, radiation

damage, etc. Nondisjunction of chromosome pair 21 during spermatogenesis can also produce child with Down's syndrome, but paternal age does not seem to be associated with its incidence. Lastly in about 2 to 5 percent cases the normal chromosome number is present (2n=46), but the extra chromosome 21 is attached (translocated) to one of the larger autosomes (usually chromosome 14).

Edward's syndrome or Trisomy-18. First described in 1960 by Gohn H.Edwards and his colleagues, trisomy-18 is found to contain an incidence of about 0,3 per 1000 births. It is characterized by multiple malformations, primarily low-set ears; small receding lower jam; flexed and clenched fingers; cardiac malformations; and various deformations of skull, face and feet. Edward's syndrome is related with maternal age (i.e., 35 to 45 year old mothers have more chance of giving birth to trisomy-18 infant).

Patau's syndrome or Trisomy-13. This syndrome was described in 1960 by Klaus Patau and coworkers. Its incidence is about 0, 2 per 1000 births. Individuals with Patau's syndrome appear to be markedly mentally retarded; have sloping forehead, harelip and cleft palate. Polydactyly (both hands and feet) is almost always present; the hands and feet are deformed. Cardiac and various internal defects (of kidney, colon, and small intestine) are common. Death usually occurs within hours or days, but the foetus may abort spontaneously.

Human Sex Anomalies.

Turner's Syndrome (XO Females). A female with 44 autosomes and only with one X chromosome in her body cells exhibits symptoms of Turner's syndrome. Such females are sterile and have short stature, webbed neck, broad shield- shaped chest, low intelligence, poorly developed ovaries.

Poly-X Females (XXX Females). Such females are called super females because they possess an extra X chromosome (44 autosomes + 3X chromosomes). Some females may have 4 or 5X chromosomes besides the normal autosomes. All such poly-X females are mentally retarded and sterile showing abnormal sexual development.

Klinefelter's Syndrome (XXY Males). When an abnormal egg with XX chromosomes is fertilized by a sperm carrying Y-chromosome, a zygote having

three sex chromosomes (XXY-chromosomes) is formed. The resulting young one is an abnormal sterile male showing the following features: small testicles, mental retardation, longer arms, higher pitched voice and sparse body hairs. XYY Males. Presence of an extra Y-chromosome in males results in their unusual height, mental retardation, severe facial acne during adolescence and criminal bent of mind.

Determination of sex chromatin is used for studying human sex anomalies. In 1949 year Bar and Bertram reported a deeply stained chromatin body in the nerve cells of female cat which was absent in the male. This chromatin body is called sex chromatin or Barr body after the name of its discoverer. Such Barr body has also been observed in most of the body cells (e.g., skin, oral epithelium and blood cells) of man. Human females have the Barr body in the nuclei of their body cells in higher proportion than males and are, therefore, referred to as sex chromatin positive. The human males are called sex chromatin negative.

The sex chromatin appears in the interphase nucleus as a small chromocentre, heavily stained with basic dyes. It has a relatively constant position in each tissue and species. It can be attached to the nucleus as in nerve cells of certain species; attached to the nuclear membrane as in cells of epidermis or of the oral mucosa, free in the nucleoplasm, etc.

It has been demonstrated that in homogametic XX female individuals one X-chromosome gets characteristically condensed and inactivated. Such chromatin material is called facultative heterochromatin, since it becomes inactive in certain part of the life cycle and resumes activity before entering the germ line. The phenomenon of inactivation of X-chromosome was confirmed by the observation of the Barr body.

It was demonstrated that whenever the number of X chromosomes was two or more than two, the number of Barr bodies was one less than the number of X-chromosomes.. Thus, in normal female only one active X chromosome is present. Method of sex chromatin determination is used for:

- ✓ diagnostics of sex chromosomes number dependent diseases
- ✓ determination of fetal sex

✓ definition of the chromosomal state of intersexes

Practice:

Assignment 1. Hereditary disorders in humans caused by chromosomal mutations.

Copy and complete the following table, give the main characteristics of disorders.

Disorder	Karyotype	Major symptoms

Topic 18. Population –statistical method. Genetic counseling.

Key concepts:

1. What characteristics of population can be determined with the help of the Hardy Weinberg equilibrium formula?
2. What is the genetic structure of population?
3. What are demes, isolates?
4. What is a real and ideal population?
5. What is the practical application of the Hardy-Weinberg equilibrium formula?
6. Main stages of genetic counseling.

The **Hardy–Weinberg principle** (also known as the **Hardy–Weinberg equilibrium, model, theorem**, or **law**) states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences. These influences include *mate choice*, *mutation*, *selection*, *genetic drift*, *gene flow* and *meiotic drive*. Because one or more of these influences are typically present in real populations, the Hardy–Weinberg principle describes an ideal condition against which the effects of these influences can be analyzed.

In the simplest case of a single locus with two alleles denoted A and a with frequencies $f(A) = p$ and $f(a) = q$, respectively, the expected genotype frequencies are $f(AA) = p^2$ for the AA homozygotes, $f(aa) = q^2$ for the aa homozygotes, and $f(Aa) = 2pq$ for the heterozygotes. The genotype proportions p^2, $2pq$, and q^2 are called the Hardy-Weinberg proportions. [Note that the sum of all genotype frequencies of this case is the binomial expansion of the square of the sum of p and q, and such a sum, as it represents the total of all possibilities, must be equal to 1. Therefore $(p + q)^2 = p^2 + 2pq + q^2 = 1$. The solution of this equation is $q = 1 - p$.]

If union of gametes to produce the next generation is random, it can be shown that the new frequency f' satisfies $f'(A) = f(A)$ and $f'(a) = f(a)$. That is, allele frequencies are constant between generations.

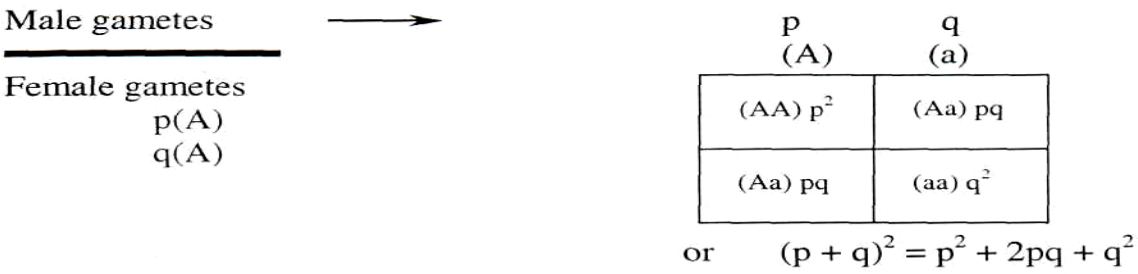
Population-statistic method is used to calculate the results of the mode of inheritance of genes in a given population. Population is a group of a certain species which inhabit a defined territory for a long time and cross freely.

Small populations (up to 1500 individuals) are called isolates. The frequency of incestial marriages is high in isolates (over 90%). Most individuals of an isolate are homozygotes. That's why dominant or recessive genes get accumulated there. Rare genes are often stored in isolates.

Bigger populations (1500 - 4000 individuals) are called demes. The incest rate in demes is lower - up to 80-90%. Demes also have a large number of homozygotes.

A population of a particular species includes many inbreeding groups. If all the gametes produced by a population are considered as a hypothetical mixture of genetic units from which the next generation will arise, we have the concept of a gene or gamete pool. Every male gamete in the gene pool has an equal opportunity of uniting with every female gamete. Zygotic frequencies expected in the next generation from such random gametic unions may be predicted from a knowledge of the gene (allelic) frequencies in the gene pool of the parental population. For example, the expected zygotic frequencies for allele *A* and a of a gene pool can be determined by chanse matig. If p stands for percentage of A alleles in the gene

pool and *q* stands for the percentage of a alleles, the checkerboard of both alleles may predict possible chance combinations of A and *a* gametes as follows:



or $(p + q)^2 = p^2 + 2pq + q^2$

thus, p" is the fraction of the next generation expected to be homozygous (AA), 2pq is the fraction expected to be heterozygous (Aa) and q^2 is the fraction expected to be recessive *(aa).*

When only two alleles are involved, and, therefore, p and q represent the frequencies of all of the alleles concerned p+q=l. Since p+q=l, p=l-q. Now if 1-q is substituted for p, all relations in the formula can be represented in terms of q as follows: $(p+ q)^2 = 1$; $p^2 +2pq +q^2 = 1$

Hardy-Weinberg Law

The formula $(p+ q)^2 = 1$; $p^2 +2pq +q^2 = 1$ is expressing the genotypic expectations of progeny in terms of gametal or allelic frequencies of the parental gene pool and is originally formulated by a British mathematician Hardy and a German physician Weinberg (1908) independently. Both forwarded the idea, called Hardy-Weinberg law equilibrium after their names, that both gene frequencies and genotype frequencies will remain constant from generation to generation in an infinitely large interbreeding population in which mating is at random and no selection, migration or mutation occur. Should a population initially be a in disequilibrium, one generation of random mating is sufficient to bring it into genetic equilibrium and there after the population will remain in equilibrium (unchanged in gametic and zygotic frequencies) as long as Hardy-Weinberg condition persists. Hardy-Weinberg law depends on the following kinds of genetic equilibriums for its full attainment:

- The population is infinitely large and mate at random.
- No selection is operative.

- The population is closed, i.e., no immigration or emigration occurs.
- No mutation is operative in alleles.

Meiosis is normal so that chance is the only factor operative in gametogenesis. The significance of Hardy-Weinberg equilibrium was not immediately appreciated. A rebirth of biometrical genetics was later brought about with the classical papers of R.A.Fisher, beginning in 1918 and those of Sewall Wright, beginning in 1920. Under the leadership of these mathematicians, emphasis was placed on the population rather than on the individual or family group, which had previously occupied the attention of most Mendelian geneticists. In about 1935, T.Dobzhansky and others started to interpret and to popularize the mathematical approach for studies of genetics and evolution. The shifts or changes in gene frequencies can be produced by a reduction in population size, selection, mutation, chance (genetic drift), meiotic drive and migration.

Genetic Counselling

Modern genetic counselling is a communication process between a healthcare professional trained in genetics and an individual or family affected by or at risk for an inherited disorder. The goals of this process include promoting awareness of the medical facts of the condition, understanding the role of heredity in the expression of the condition and its risk of recurrence, discussing the options available for dealing with the disorder, and assisting families in choosing the options that are most appropriate for them. Genetic counseling takes place in medical genetics centers. It includes the following steps:

- Establishing an accurate diagnosis for the affected individual. To determine a diagnosis, the genetics team must gather information. The first step in a consultation involves eliciting a detailed family history with pedigree construction for at least three generations. Other methods may be used: cytological, biochemical, DNA analysis.
- After analysis of the information gained during the consultation, the genetics team may order diagnostic testing for a suspected condition. Counsellors inform families of the benefits, risks and limitations of such testing before testing begins.

Counsellors assist families in understanding the informed consent process. They must ensure that families comprehend the ramifications of test results, whether they are positive or negative. This complete evaluation can establish a diagnosis of an inherited condition, but may also rule out heredity as a cause and greatly reduce a family's risk of recurrence. Calculation of the recurrence risks within a family. Theoretically genetic risk ranges from 0 to 100%. Low: 0-12% -family may have of the child, average: 12-20% - the family can have a child under the supervision of a physician – geneticist, is high: more than 21% - the family is undesirable to have a child.

- The final step in the genetic counselling process is that of follow-up. Counsellors often summarize the important medical and genetic information in a letter to the family so that it can be accessed at any time. These aids in understanding as the material can be studied in a less stressful atmosphere. Inclusion of the results of tests that confirm the diagnosis is helpful for future generations.

Practice:

Solve the problems:

1.The gene determining dark eye colour in humans is dominant and its concentration in the population is 0,8. Determine the percentage of light eyed persons in this population.

2. In humans the concentration of the gene determining Rh negative blood in a certain population is 0,4Determine in this population:

a) percentage of heterozygous carries of this gene

b) percentage of persons with Rh positive blood.

3. The concentration of the blood groups alleles according to the ABO system in a given population is: I^A-0,22: I^B -0,11: I^O – 0,67. Determine the frequency of homozygous and heterozygous people with I^A (A) and : I^B (B) blood groups in this population.

Topic 19. Practical skills

Solve the problems:

1. In the F_2 from a cross of red-flowered plants with white-flowered plants obtained 705 plants with red flowers and 224 with white. How to explain these results?

1. If a mother and her child belong to blood group 0, what blood group could the father not belong to?

2. A man of blood group A and Rh^- marries a woman of blood group IV and Rh^+. What phenotypes and genotypes will have their children?

3. In Drosophilae genes *a* and *b* are linked. Its recombination frequency in females is 12%. A male and female have the same genotypes. What types of gametes and in what proportions will these organisms produce? (Give two variants).

4. In the guppy a dominant allele results in the presence of a black spot on the dorsal fin. Spotted males transmit the trait only to sons and not to daughters, and male and female progeny of such daughters do not show the trait. What way is this trait inherited?

5. Consider three gene pairs each of which affects a different character. These three genes pairs assort independently of each other. Calculate the probability of obtaining an abc phenotype from a cross of individuals that AaBbCc x aaBbcc.

Suggested Reading.

1. K.L. Lazarev. Medical Biology. - Simferopol, 2003, p.

2. T.V. Bihunyak "Medical Biology". - Ternopil, 2010.

3. Lecture material

Topic 20. Module I “Cell - Genetics”

Revise the previous material.