MARMARA ÜNİVERSİTESİ YAYIN NO : 529
ATATÜRK EĞİTİM FAKÜLTESİ YAYIN NO : 15

EXPERIMENTS IN BIOLOGY

LABORATORY MANUAL

Prof. Dr. Sema S. ERGEZEN

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MARMARA ÜNİVERSİTESİ 1883

EXERCISE: 1

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THE MICROSCOPE

The naked (unaided) human eye cannot see objects smaller than 100 μm (0,1 mm). The microscope is an instrument designed to allow such objects to be seen with eye; that is, it magnifies. The microscope is one of the principal tools of the biologists for the study of living things which are too small to be seen by the unaided eye.

The microscope produces an image (a picture) of an object that becomes magnified or enlarged as it is transmitted through two or more optical lenses. In the light microscope, a beam of visible light produces an image that is magnified by glass optical lenses (Figure 1).

PARTS OF MICROSCOPE:

A compound microscope has four types of elements (Figure 2).

I. Supporting elements: These are the metal parts of the microscope which support the magnifying elements

The foot: It is the base of the microscope that supports the microscope.

The arm: It is the part that supports the upper part of the microscope.

Body tube: It is a narrow long tube which supports the magnifying elements (or optical systems: ocular and objectives).

Stage: A metal plate upon which objects to be examined are placed. The stage is usually stationary (non-moving), but on some microscopes it may move up and down. Flexible "stage clips" or a movable mechanical stage hold the specimen in position. Note the stage "aparture" (hole) in the center of the stage.

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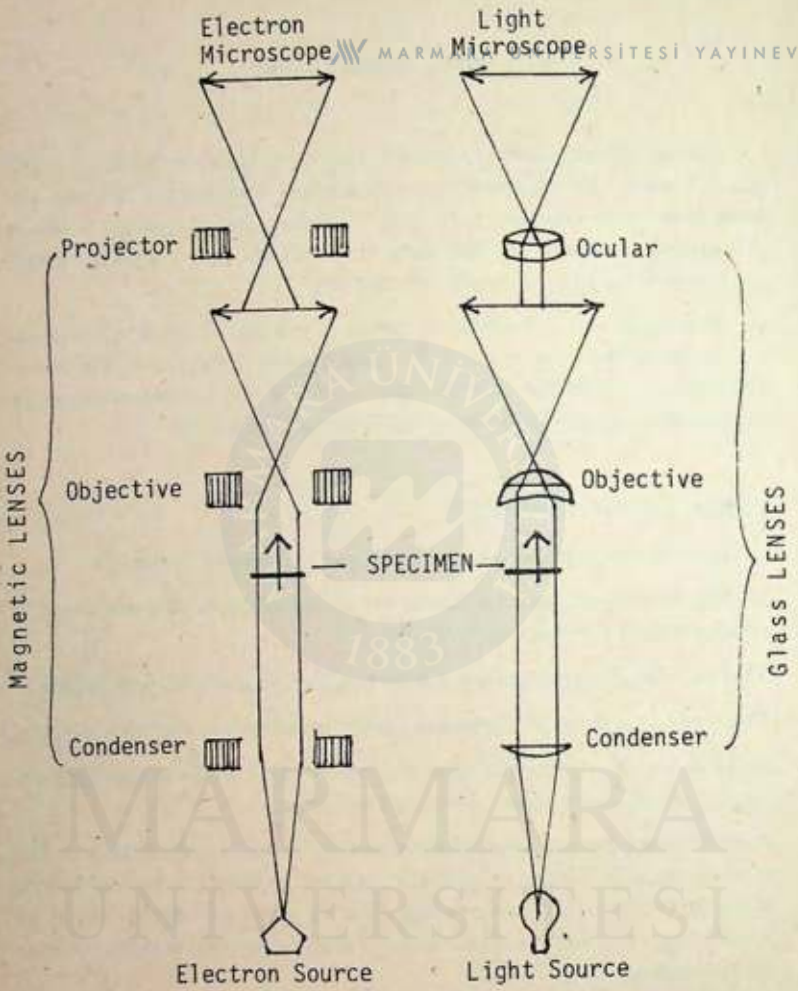


Figure 1. Diagrammatic comparison of the optical systems of the light and electron microscopes.

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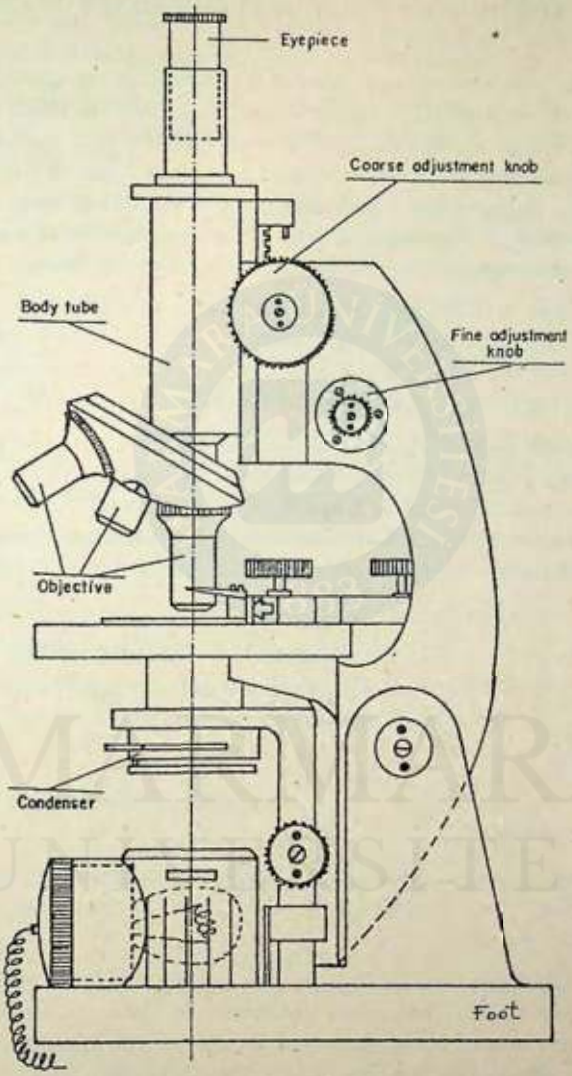


Figure 2. A compound monocular microscope

II. Magnifying elements (optical systems): These are the magnifying lenses of the microscope. The light microscope has three sets of lenses.

The ocular lens system (eye pieces): It is located at the upper end of the body tube and usually removable (but you should not remove it or you should not take it into your hand). Observations will be through the external ocular lens of the ocular. A marking may be found on the ocular which shows the magnifying power of the ocular (Table 1.1). Your microscope ocular has 10 X magnifying power. It means that ocular yields an image ten times larger in dimension than the object being viewed.

The objective lens system: It consist of two or four cylindrical objectives, with different length. These objectives are screwed into a revolving **nose piece** at the bottom of the body tube. The markings, which show the magnifying power, are also found on the objectives (Table 1.1)

The total magnification of the specimen achieved by the compound system is the product of the magnification of the separate lenses. Thus, the total magnification of a 10 X ocular (which magnifies 10 times) and a 10 X objective is, 100 X (10 X 10 = 100 times). Table 1.1 shows total magnification of various objectives with 10X ocular.

Table 1.1

Objective	Ocular	Magnification
4X	10X	40X
10X	10X	100X
40X	10X	400X
100X (oil immersion)	10X	100X

Condenser: A system of lenses, or a single lens is located beneath the stage opening. The function of the condenser is to throw a wide cone of light on the specimen.

III. The light source: The light source usually is a low-voltage bulb which is placed on the foot, under the condenser. For some microscopes, day light may be used for a light source. In this case, the surface of a mirror used to concentrate the light from environment onto the microscope field.

A narrow beam of light, necessary for all observations, is projected upwards through the diaphragm, the stage aparture, an objective lens and ocular lens. When the beam of light passes through the specimen on the stage, the resulting image is also projected upward.

An **iris-diaphragm** located below the stage, may be opened or closed by a small handle, regulates the amount of light that passes through a specimen.

IV. Adjustment knobs (Focus knobs): In order to get a sharp and clear image of the specimen being viewed, the objective lens should be put at a specified distance from the object, that is image of the observed specimen is brought into **focus.** This distance is called as **working distance;** it is a property of the lens system and is constant for any particular objective. The working distance decreases considerably when you move the objectives from low to high power.

The distance between the various objective lenses and the specimen being examined is controlled by the manipulation of the focus knobs. Every compound light microscope has two focusing knobs:

Coarse focus knob: It serves to move either the stage or the body tube "long distance with few turns".

Fine focus knob: It serves to move either the stage or the body tube (relatively short distances with many turns).

The two focus knobs may be positioned sperately on the microscope or they may both be contained in a dual control knob.

USING THE MICROSCOPE

Follow the directions explicitely. If you do not understand a certain procedure or if you encounter a problem, always ask the laboratory instructor for help.

1. Microscope should be placed on a firm, flat base, its arm toward you. Open the iris diaphragm of the condenser as far as it will go. Turn on light source.

2. Revolving nose-piece should be rotated to the low power (10X) or lowest objective before and after use of microscope. Be sure the low power objective clicks into position.

3. Place the specimen (a prepared slide or a dry/wet mount of a letter), on the stage and center it over the stage aperture. Clamp the slide in position with the stage clips.

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4. By rotating the coarse focus knob slowly, reduce the working distance between the objective and stage (lowel the objective or raise the stage) as far as possible, (but the objective should not touch to specimen!).

All these must be done while looking at the microscope from side.

5. Next look into the ocular (eye piece) for observation. Your microscope will probably be **monocular** (one eye piece); hence, only one eye will be involved in observing. There will be a tendency to "squint" with eye not used. This may cause eye strain or headaches, therefore,learn to work at the microscope with both eyes open. Use your left eye to observe with the microscope if you are right handed, the right eye if you are left-handed. This frees the proper hand for recording your observations. Eyeglasses need not be worn during observation. Observation of any specimen is difficult unless proper light is reflected through the lens. Illumination of the field can be achieved by adjusting the mirror and diaphragm opening. Locate the specimen in the field of view (the circular view seen in the eye piece) and move it to the center. With coarse focus knob, increase the working distance until the image of specimen is visible. Then, using the fine focus knob, focus until the sharpest possible image is attained.

6. Next, without moving the slide and without touching the coarse adjustment knob, watch from the ocular and turn the nose piece, switch to high power, (40X) objective. Use only the fine adjustment knob to get the specimen sharply in focus.

Precautions:

The microscope is a delicate and expensive instrument, thus, it must be used very carefully. For the proper use of the microscope, the following precausions should be kept in mind.

1. Your microscope will be placed on the desk in front of you. Please, do not carry it somewhere else! If it is necessary to move it, you should always carry it by holding it upright and supporting the base with

your free hand. A microscope should always be carried in this way with both hands.

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2. Never touch the lenses of the microscope with your hands . For cleaning the lenses in the ocular and objectives use only special lens páper.

3. Always locate the object to be studied with the low-power objective and then turn to the high-power objective if a more detailed study is desired, and always use fine adjustment with the high power objectives. Leave the objective with the lowest magnifying power in position when microscope is not in use!

4. Observation with an oil inmersion objective, if available on your microscope, requires special instruction. Do not use this objective unless otherwise directed.

5. Always use a coversilip on wet mount preparations. Do not allow liquids (stains) to get on the microscope or objectives, if they do, report immediately to the laboratory instructor.

Practice with microscope:

For this exercise use the small papers with the letter "k" written on them. Put the letter "k" on a slide and place it on the stage. The letter should be in a reading position in the center of the opening in the stage (stage aperture). Using the coarse adjustment knob, turn the low power (10X) objective down as far as it will go; do not forget to watch from the side of the microscope. Look into the ocular and focus slowly upward. As you turn the coarse adjustment knob, the objective moves upward, when it reaches to the correct distance from the specimen, the letter will be in focus.

Is the letter in the same upright position when you view it through microscope? How does it appear?

Move the paper a slight distance to the left while looking into the microscope, which way does the image appear to move? Follow the direction of movement of image when you move the paper to the other direction. These phenomena are called inversions, because of the microscope optics, the object and its movements are inverted.

When you want to observe in more detail, you must switch to a higher power objective. Center the detail you want to examine, right in the middle of the lower power field. Then rotate the nose piece until the next higher power objective clicks into place. Use the fine focus knob to make object clear. It is usually unnecessary to raise the body tube before changing the objectives. **Do make certain,** however that, the nose of the high power objective does not contact the specimen.

How does changing from low to high power affect the brightness of the field of view? How did changing to high power affect the size of the field of view? Is it larger or smaller than the field of view seen with the low power objective in position?

Microscopic measurement:

The eye piece of a microscope may be equipped with a measuring device (ocular micrometer). Such device provide a relatively accurate estimation of size (length and width) of an object. If your microscope lacks such a device, it is possible to estimate the actual size of microscopic objects by comparing the size of object with the diameter of the field view (the luminous circular area that is seen through the eye piece of the microscope). This measurement is based on the fact that the field of view for a given microscope will have a constant diameter for each combination of lenses in use.

The first step is to measure the diameter of the field view by using the scanning or low-power lenses by placing a plastic millimeter ruler on the stage. Estimate the diameter of the field and record this distance in **microns** (1000 microns (μ) = 1 mm).

The high-power field is smaller in diameter than the low power field and the millimeter divisions are too large to be used satisfactorily for making an accurate estimate of the field diameter.

To estimate the size of an object under the microscope, line up one edge of the object against the edge of the field of view (Figure 3).

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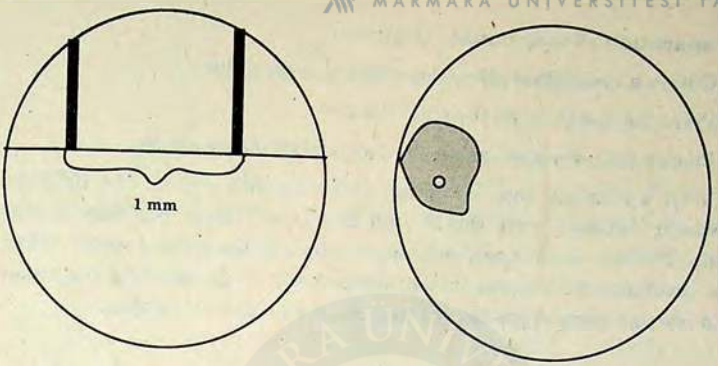


Figure 3. Measuring the field (low power) and a specimen (high power)

Knowing the diameter of your microscope field, the size of any object can be roughly determined by estimating the fraction of this diameter occupied by the object. Multiplying this fraction by the field diameter in microns will give the approximate size of the object in microns. For example, the diameter of field view of low power objective is 1.5 mm = 1500 µm. If the diameter of observed object is about 1/3 of that field. You can calculate its diameter as 1/3 of that field. You can calculate its diameter as, 1/3 x 1500 = 500 µm.

Specimen preparation:

Specimens to be observed with the compound light microscope are always placed in a **mounting medium** on a **glass slide** and covered with the **coverslip**. Specimen must always be either thin enough or few enough in number to allow light to pass through. Preparations can be either permanent or temporary.

Specimens that have been chemically fixed are mounted in a permanent mounting medium so that they may be re-examined at any time in the future.

The **wet mount** is a temporary preparation in which the specimen (often living) is placed in a liquid mounting medium (usually water).

Preparation of wet mount: (Figure 4)

1. Obtain a clean glass microscope slide and coverslip.

2. Place the specimen on the slide (center).

3. Place a drop of water (using eye dropper) on the specimen.

4. Wait a moment, then cover the specimen with a coverslip. Hold the coverslip between your thumb and forefinger. Lower one edge to the slide. Tilt it at a 45° angle and move it toward the drop of water. When the coverslip contacts the water, slowly lower it. Do not drop the coverslip from an upright position as this produces many air bubbles.

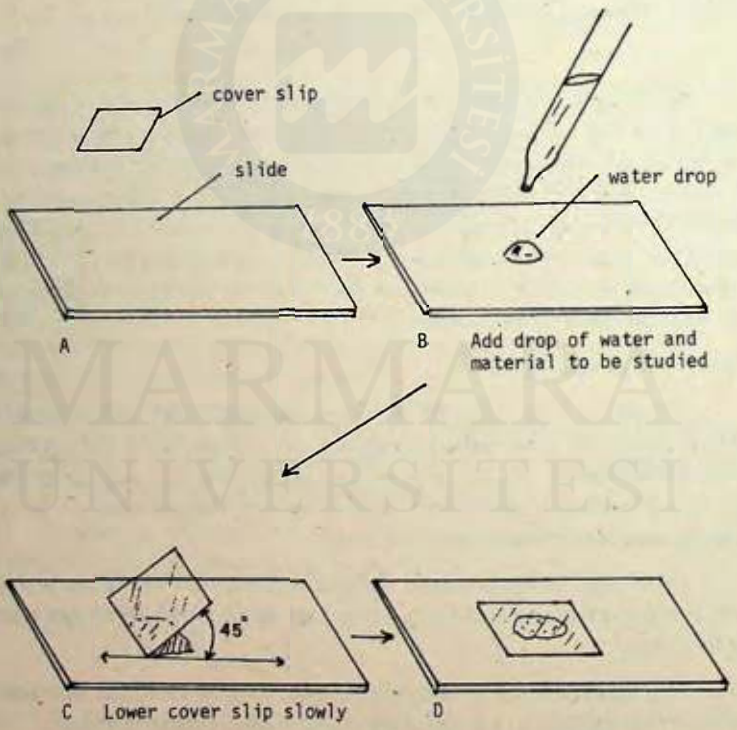


Figure 4. Preparation of wet mount

EXERCISE: 2

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CELL STRUCTURE AND FUNCTION

All organisms are composed of cells. Some organisms like a bacterium, a yeast, a protozoan consist of a single cell (unicellular). More complex animals and plants are composed of many cells (multicellular).

Cells of different organisms show great diversity in structure and function. A bacterium, a yeast, a protozoan, a plant and an animal cells all have different structural arrangements which reflect the specific functions they are involved in. Although cells show great diversity in form and function, all cells constructed according to a fundamental design and share certain common features.

In this exercise, you will examine structure of some eucaryotic (plant and animal) cells with the light microscope (compound microscope). To see a particular structure it may be necessary to stain the cell. The choice of stain for the cell is important because certain stains are specific to certain structures; for example **"acetocarmine"** stains the nucleus and its contents, **"iodine"** stains starch grains and so on.

PLANT CELLS

Onion Epidermal Cells

Cut onion bulb into quarters. Remove one of the fleshy "scale" leaves.Using a forcepts remove small piece of the transparent, paper thin epidermis from the inner face of the leaf and place it evenly in a drop of water (or iodine solution) on a slide. Gently lower a cover slip to prevent trapping air bubbles (Figure 4). Examine this wet mount with the low-power objective (10X). Next examine the cells under high power.

The "lines" forming the border between the individual cells are nonliving cell walls composed of cellulose. The cell wall immediately surrounds the cell membrane (plasma membrane) which encloses the cytoplasm. The central part of many plant cells (difficult to see in living cells) is occupied by a fluid - filled vacuole. The nucleus as a dense body in the translucent cytoplasm located centrally or compressed and pushed against the cell wall. The onion is a plant organ but it does not contain chloroplasts. Study the cells and try to answer following questions.

What is the three dimensional shape of the cells? How many layers of cells make up this tissue? (Study by using fine adjustment).

Elodea Cells

Obtain an Elodea leaf from tip of the plant. This is important because the tip region of the plant has the most metabolically active cells. Use forceps to pluck one of those new leaves, and place it in a drop of water on a slide and put a cover slip. Examine the preparation with the low-power objective (10X). Observe a group of cells near the center of the leaf. Carefully switch to high power. Note the chloroplasts small structures with green pigment in the cytoplasm. As you examine the chloroplasts you should see them moving in the cell. Since chloroplasts cannot move by themselves, this movement is resulted from the "cytoplasmic streaming".

ANIMAL CELLS

Human Epidermal Cells Cheek Epithelial Cells

Animal cells differ from plant cells in a few funamental ways. Animal cells lack a cell wall and they also lack chloroplasts. Animal cells usually have several small vacuoles rather than one large central vacuole. Animal cells may have cilia, flagella and contractile vacuoles.

The cheek epithelial cells are covering cells on the inner surface of mouth. They are easily rubbed off. Gently scrape the inside of your cheek with the broad end of a tootpick. Put a drop of water on a clean slide and then stir the scrapings into water drop and spread on slide. Add a cover slip and examine with the low power of your microscope. It will probably be necessary to close the diaphram to cut down the light and provide some contrast. Locate a single cell and examine it under high power (40X). Locate the cell membrane, nucleus and cytoplasm of cheek cells.

Since it is difficult to observe the structure of living cells, they are frequently stained with dyes to bring out cellular detail. Make a second temporary wet mount of cheek cells, but this time put the cells into a drop of **methylene blue** stain or add a drop of stain to the edge of the cover slip of first preparation, withdraw the fluid from the opposite side of the cover slip by a piece of absorptive paper. The stain thus flows in, replacing the fluid removed by paper.

Examine prepared slide with low power of your microscope. Locate a single cell/cells, center them for viewing under the high power objective. Switch to high power. The nucleus should be clearly visible. Note the general shape of the cells. Think of ways that the shape is related to their function.

What do these epidermal cells have in common with the Onion and Elodea cells? In what ways are they different?

Sketch the cell, putting in as many structures as you have been able to observe. Label nucleus, nuclear membrane (border), chromatin granules, nucleolus, granular cytoplasm, cell membrane (border).

Human Red Blood Cells

Place a drop of saline solution (to which some sodium citrate has been added to prevent coagulation) on a clean slide. Then, wash the ball of one of your fourth fingers with a piece of cotton soaked in 70 % alcohol, and puncture the skin with a sterile lancet, as demonstrated by your instructor. As the first drop of blood oozes out the wound, touch it immediately (before the blood had a chance to coagulate) to the drop of citrated saline solution. Place a cover glass over the diluted blood on the slide. Examine this wet mount of human blood under low and high power.

How do cells differ in appearance from human cheek and Elodea cells? What are the approximate width and diameter of these cells in microns?

With a Pasteur pipet, apply a small drop of a solution of **methylene blue** in normal saline solution to he edge of the cover glass, upon which some of the dye should diffuse into the suspension of cells.

Do there appear to be nuclei in these cells? Are all the cells a like? Can yo distinguish any other sorts of cells?

CELL DIVERSITY

The cells of multicellular organisms are differentiated, that is, they are specialized to carry out certain specific activities. For example red blood cell transport gases (0_2, CO_2), kidney cells excrete waste products, and nerve cells conduct stimuli. These differentiated cells are organized into tissues and organs. Similar organization is present in plants.

Study prepared sections of various organs from animal and plants such as liver, lungs, stomach, leaf, root ect.

Can you distinguish group of similar cells in the organ? How does the arrangement of the tissues allow for successful accomplishment of the primary function of the organ?

UNICELLULARITY (SINGLE CELLED LIFE)

Unicellularity is a condition in which the whole organism consists of a single cell. The cell in this condition is capable of living as an independent unit and displays all the activities of "life" in multicellular organisms.

Prepare wet mounts of the free-living cells provided by your instructor. These could be cultures of Amoeba, Paramecium, Euglena or mixed culture as a pond water. When you identified an unicellular organism compare its structure with the stained slides of same organisms (you will be provided in the laboratory) or the other cells you have studied in this laboratory session.

Yeast Cells

Yeast cells are also free-living organisms that have largely plant-like characteristics, although they do not contain chloroplasts. A suspension of yeast cells will be available. Using a Pasteur pipet, place a drop of this suspension on a clean microscope slide, cover the preparation with a cover glass, and examine it with the high power objective lens.

What is the general shape of the cells? Do they have a cell wall around the plasma membrane?

THE INTERRELATIONSHIP OF CELLS

in the previous exercises you have seen the organized community of cells in tissues and organs of multicellular organisms, and freeliving cells as self sufficient (unicellular) organisms. Some other organisms, however, consist of loosely organized cells. They are most primitive multicellular forms or intermediate between unicellular and multicellular organisms.

The fresh forms of **Volvox**, **Spirogyra** and **Hydra** or their fixed and stained preparations will be available in the laboratory. Study each of these organism with low power objective.

Can you differentiate individual cells in each of these organisms? Which one of these organisms has most extensive division of labor among its cells? Why?

EXERCISE: 3

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CHEMICAL ORGANIZATION OF CELLS

All organisms consist of cells and the cell is the simplest independent structure that possesses all the essential properties of life. In order to understand life, it is essential to know the molecular basis of cellular activities. All cellular activities, including the formation of every structure in a cell depend on the interactions of particular set of molecules

The three major kinds of components of the cells are **water, inorganic ions** and **organic molecules.**

Organic molecules are responsible for most of the chemical complexity of cells. All organic molecules consist primarily of carbon, hydrogen, oxygen and nitrogen. The principal organic molecules in cells are very large molecules (macromolecules). These are polymers, such as, **proteins, nucleic acids** and **carbohydrates,** which are made up of a few kinds of small molecules known as **monomers. Lipid** molecules, are the other major constituents of cell with an intermediate size between macromolecules and the other small organic molecules in the cell. The remaining organic constituents of cell are the monomers of these macromolecules and lipids, intermediates that form during synthesis of monomers or digestion of sugars, carbondioxide, water, vitamins and helper molecules for certain enzymes (coenzymes).

The chemical composition of cells can be investigated in two ways:

1. To grind up the tissue (animal or plant), or extract the juices from it and perform chemical analyses on this material.
2. To obtain thin slices from the tissue and stain with a reagent specific to a certain chemical constituent and examine under the microscope.

You will be using both of these methods in this laboratory exercise.

CARBOHYDRATES

Carbohydrates are the sugars, starches and related molecules. Their general formula is$(CH_2O)_n$. "n" Represents number of $(CH_2O)_n$ units that make up carbohydrates. They are divided into three classes.

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1. Monosaccharides (Simple sugars)

These are the monomers or subunits of more complex carbohydrate polymers. A monosaccharide contains carbon, hydrogen and oxygen in 1 : 2 : 1 ratio and represented as $(CH_2O)_n$. Monosaccharides contain 3 to 7 carbon atoms in an unbranched chain (or ring) and a carbonyl (C=0) group. They are classified by the number of carbon atoms they contain as **triose** (3C), tetrose (4C), pentose (5C), hexose (6C) and heptose (7C).

The most important monosaccharide in cells is **glucose**, a hexose sugar with a formula $C_6H_{12}O_6$. Glucose occupies a central position in energy metabolism and is also the molecule from which other sugars are derived.

2. Disaccharides

Two monosaccharides may bond (Glycosidic bond) together to form a disaccharide, such as, **sucrose, maltose and lactose,** by a condensation reaction, Figure 5.

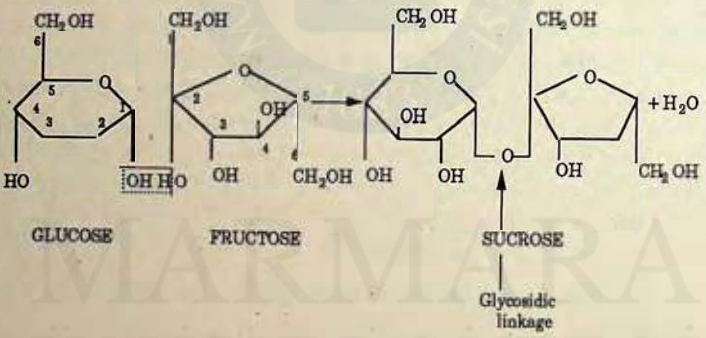


Figure 5. Condensation of monosaccharides

3. Polysaccharides

By a series of condensation reactions, many monosaccharides can be joined to form a polysaccharide. Such as, glucose molecules can be joined together in several different ways to yield long chains (polymers). The most common polysaccharides of glucose are **glycogen** which is made and stored in animal cells; **starch** which is made and stored in plant cells; and **cellulose.**

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Test for reducing sugars (Benedict's Test)

This test is based on the reducing property of sugars. A carbohydrate with a free or potentially free aldehyde ($-C\begin{matrix}O\\H\end{matrix}$) group, is a reducing sugar. Because, this kind of sugar, in a solution of sufficiently high pH (alkaline), can reduce a weak oxidizing agent to an monovalent oxide. Benedict's solution (reagent) is a mixture of sodium bicarbonate, sodium citrate and copper (II) sulfate Copper (II) ion-(Cu^{++}) is the oxidizing agent. In this test, after heating with a reducing sugar (such as, glucose or fructose), divalent copper ions (Cu^{++}) of copper sulfate ($CuSO_4$) is reduced to monovalent copper ions (Cu^{+}) of cuprous oxide (Cu_2O), which forms a red precipitate. The color of the precipitate will range from green to reddish brown depending on the quantity of the reducing sugar present. In the reaction, aldehyde group of sugar is oxidized to a carboxylic acid group.

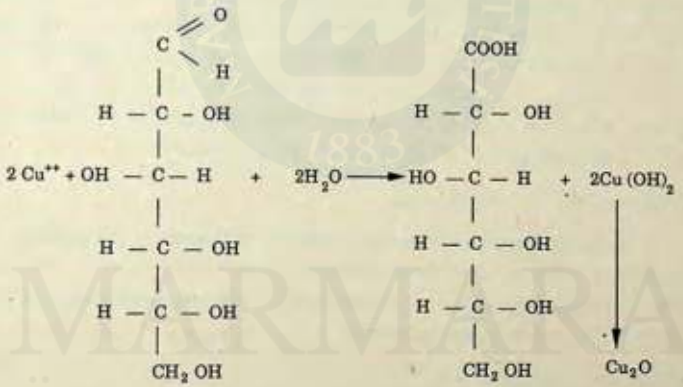


Figure 6. Reaction for Benedict's Test

Procedure:

a) Place in a test tube 2 ml of a 1 % glucose solution and 5 ml of Benedict's solution shake and boil vigorously for 2 minutes (avoid boiling over), and allow to cool. A precipitate is formed, observe the color and texture of the precipitate formed. Repeat the same test with solutions of

1. Distilled water (control)

2. sucrose

3. starch

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b) Cut a cube of apple (1 cm^3) grind it up in a mortar with a small quantity of water. Transfer the material to a test tube and add water to make total volume of approximately quarter of a test tube. add an equal volume of Benedict's reagent and heat to boiling. Record your results and conclusions.

The color and density of the precipitate gives a rough indication of the amount of sugar present. A green precipitate means relatively little sugar, a brown or red precipitate means more sugar is present.

Cytochemical test for reducing sugars

The reducing sugar in apple tissue is **fructose.** To investigate its distribution in the apple tissue, cut a thin section from apple and mount it on a slide in a few drops of Benedict's solution. Examine the cells under the microscope. Now heat the slide gently over a flame until the tissue turns brown. Add water if the tissue looks like drying up. Allow the slide cool and then reexamine the cells. What changes have occured in them? Is sugar present in all the cells or only certain ones?

Hydrolysis of Carbohydrates

A dissaccharide can be **hydrolysed,** that is split by the addition of water, into its two monosaccharide units. A mild acid is used in the process to break the glycosidic bond. Similarly, a polysaccharide can also be hydrolysed its monosaccharide units.

Hydrolysis of Starch

Procedure:

Prepare some starch suspension using a small amount of starch and distilled water. Place 5 ml of starch suspension in a test tube and add 1 ml of 10 % hydrochloric acid. Place the tube in a boiling water bath for 5-10 minutes. Remove, cool and neutralize the solution by the addition of 1 ml of 10 % sodium hydroxide. Test the solution with Benedict's reagent for the presence of reducing sugars. What sugar has been formed by the reaction of acid on starch?

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Test for starch: Starch - iodine test

Procedure:

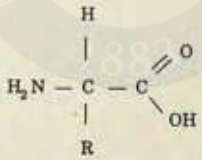
Add a few drops of iodine solution to a test tube containing 5 ml of water. Note the typical iodine color (yellowish - light brown). Add a few drops of starch solution (suspension) and observe the color change.

Test the potato, apple and banana scrapings or egg white for the peresence of starch. Record your results.

PROTEINS

Proteins are the principal organic components of cells. They may perform variety of functions in the cell. Most of them are enzymes, the catalysts of the biological reactions. Some of them are the basic structural components of cell.

Proteins are composed of chains of **amino acids** (monomers), with a general formula



The various amino acids differ in their side chains or R groups. There are twenty different amino acids which make up the structure of most proteins. The amino acids in a protein are joined by **peptide bonds.** When amino acids joined by a condensation reaction, the amino group of one forms bond with carboxyl group of another (Figure 7).

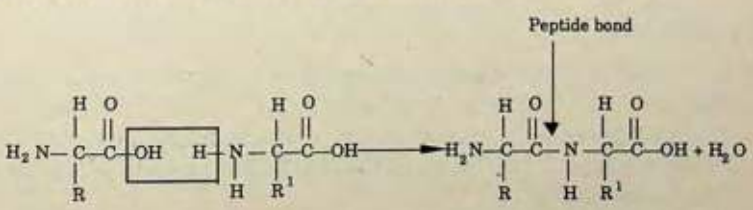


Figure 7. Condensation of Amino acids

To form such – C(=O) – N(H) – bond, one molecule of water is removed.

The structures resulting from the formation of peptide bonds are called **peptides** or **polypeptides.** The peptide bond is stable, but it may be hydrolysed by the catalysis of acid, base or various proteolytic enzymes.

Test for presence of proteins: Biuret reaction

When proteins containing two or more peptide bonds are treated with dilute **alkaline copper sulfate** solution a characteristic **purple** color develops (biuret reaction). The reaction is based on the formation of a purple-colored complex between the copper atom and four nitrogen atoms of peptides. Two of the four nitrogen atoms in the complex come from each of two adjacent peptide chains (Figure 8).

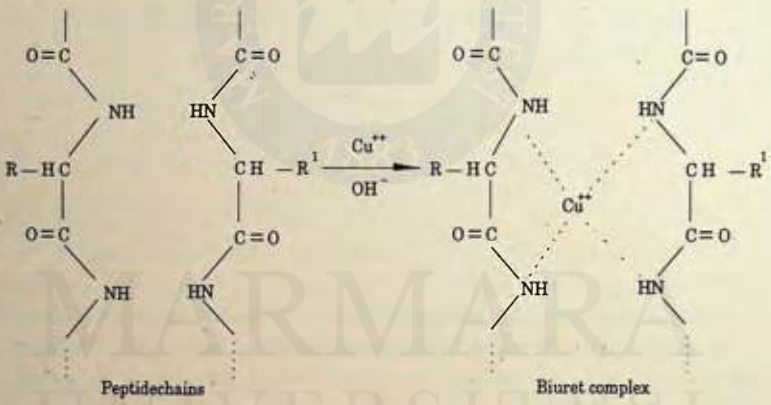


Figure 8. Biuret reaction

Procedure:

Place 1-2 ml of egg albumin (egg white) in a test tube, add an equal volume of 10 % NaOH and then add 5-10 drops of 1 % $CuSO_4$ solution. Mix well. Describe the color of solution. In a second test tube place 1-2 ml of water, add an equal volume of 10 % NaOH and then add 5-10 drops of 1 % $CuSO_4$ solution.

How do you explain the color differences of these two tubes?

LIPIDS

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Lipids are chemically heterogenous group of biological substances that are soluble in organic solvents such as acetone, benzene,ether and they are insoluble in water. The three biologically most important lipids are: **triglycerides,** serve as energy storage compounds; **phospoglycerides,** are important components of cellular membranes; and **steroids,** some of them are important hormones.

Triglycerides consist of a glycerol molecule with three fatty acids linked to it by **ester bonds** (Figure 9).

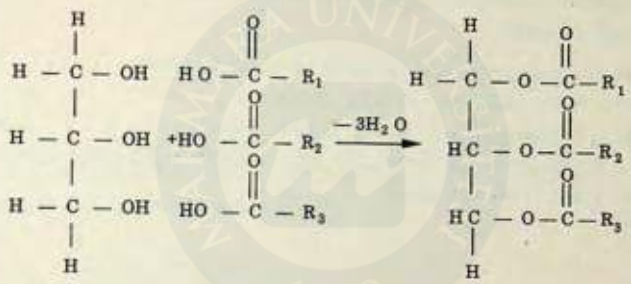
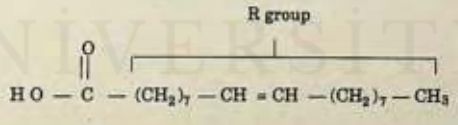


Figure 9. Formation of a Triglyceride

Fatty acid is a long hydrocarbonchain with a carboxyl (COOH) group at one and a methyl group (CH_3) at the other end.



Oleic acid

Triglycerides containing saturated fatty acids are usualy solid or semisolid in room temperature, called as **fats**; such as animal fat and butter. In plants, most triglycerides are liquid (vegetable oil), because they contain, predominantly, unsaturated fatty acids.

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Test for Lipids

Sudan IV, a dye, dissolves in fatty materials and turns a reddish - orange colour.

Procedure:

Add a few Sudan IV (or Sudan III) grains to some water in a test tube, Shake gently. Add a small amount of Salad oil, mix again, and observe the results.

a) Test whole milk, egg yolk, egg white, nuts in a similar way for the presence of lipids and record your results. Let each test stand 5 minutes before reading.

b) Adding appropriate amounts of water to a saturated solution of Sudan IV in absolute alcohol, bring the solution to a 70 % alcoholic solution. Stain sections of fatty nuts (wallnut, peanut or bean) in this solution in a watch glass, for 5 minutes. Then wash rapidly in absolute alcohol and mount in 25 % glycerol.

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EXERCISE: 4

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ACIDS, BASES, PH AND BUFFERS

Acid-base chemistry plays an important role in living systems. Since most of the cellular activities proceed within a narrow pH range, many homeostatic mechanisms in cell operate to maintain appropriate pH values.

The solvent inside the cells and in all exlracellular fluids is water. Water molecules have little tendency to dissociate into its hydrogen (H^+) and hydroxide (OH^-) ions. The dissociation of water is reversable.

$$H_2O \rightleftharpoons H^+ + OH^-$$

Since water splits into one hydrogen and one hydroxide ions the concentrations of hydrogen and hydroxide ions in pure water are exactly equal. At 25°C this concentration is;

$$[H^+] = [OH^-] = 10^{-7} \text{ Molar,}$$

that is, the tendency of water molecules to form $[H^+]$ or $[OH^-]$ ions concentrationy is 0.000 000 1 (10^{-7}) mole per liter. In other words, in pure water, only one molecule in ten million (1 in 10^7) will undergo dissociation.

When certain substances are dissolved in water, it is possible that they release hydrogen ions $[H^+]$, and $[H^+]$ ion concentration will be higher than $[OH^-]$ concentration. Those solutions which increase the $[H^+]$ are called **acids,** the substances which increase the $[OH^-]$ are called **bases.** Water is a very weak acid, since it dissociates only slightly. On the other hand, hydrochloric acid, Hcl, dissociates very readily and almost completely in water to form H^- ions and cl^- ions. Therefore Hcl is a strong acid.

Salts are compounds in which a metal ion, such as Na^+ and K^+, has replaced the H^+ group of an acid:

$$Na^+ + CH_3COOH \longrightarrow CH_3COONa + H^+$$

acetic acid — sodium acetate salt

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When an acid undergoes ionization to produce an H^+ ion, it also produces a negatively charged ion, which is called the **conjugate base.** The conjugate base of water is OH^- and the conjugate base of acetic acid is the acetate ion, CH_3COOH^-. When a conjugate base, A^-, is added to water, it tends to attract H^+ ions from water molecules and therefore produces OH^- ions

$$A^- + H_2O \rightleftharpoons AH + OH^-$$

Thus, a **base** can be thought of either as a molecule that liberates OH^- ions or as one that picks up H^+ ions from water.

The concentration of hydrogen ions in a solution is expressed as its pH. The pH scale ranges from 0 to 14 and has a mid point of 7. The pH of a solution is the negative logarithm to the base 10 of the hydorogen ion concentration, or

$$pH = -\log [H^+] = \log \frac{1}{[H^+]}$$

For example; if the H^+ concentration of a solution is 10^{-4},

$$pH = -\log_{10} 10^{-4} = -(-4) = 4$$

In pure water at 25 C, $[H^+] = 10^{-7}M = (0.000\ 000\ 1\ M)$

$$[H^+] = 10^{-7}M, \text{ so, } pH = -\log 10^{-7} = 7.0$$

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On pH scale from 0 to 14, 7.0 is considered **neutral.** Values of pH above 7.0 represent solutions in which the $[H^+]$ concentration is lower than the $[OH^-]$ concentration; these solutions are said to be **basic** or **alkaline**. In **acidic** solutions, which contain more $[H^+]$ than $[OH^-]$ ions, the pH will be lower than 7.0

Exercise :

In this experiment the pH of unknown solutions will be determined. You will have seven unknown solutions labeled A through G on the work table

1- Using a pair of forceps, dip a piece of pH paper into solution A.

2- Compare the color of the pH paper with the information on the paper dispenser chart and record the pH as

Solution	pH
A	4.0

3- Repeat same procedure with solutions B,C, D, E, F, G and make a complete table.

4- Mix 2 ml of 0.5 m Hcl with 2 ml of 0.5 m NaOH in a test tube. Measure pH of the resulting solution. What kind of reaction will occur?

pH Meter

Of course, the use of an electronic pH metering is a better method of determining pH. The pH metering machine can be thought of as a battery that can develop different electrical potentials depending on the hydrogen ion concentration of the solution in which it is placed. The solution serves as a source of the electrical potentials beacuse of its ionic concentration. Even though these electrical potentials are relatively weak, the extreme sensitivity of this machine is able to detect them and display them on a pH scale.

Your instructor will demonstrate the use of pH meter and provide you with seven solutions to be tested. Compare the results with those you foun previously?

BUFFERS

In living systems, many homeostatic mechanisms operate to maintain appropriate pH values. For example, the pH of human blood is about 7.4 and must be maintained within very narrow limits. Even a slight shifts in the $[H^+]$ concentration (pH) in the cytoplasm of a cell may have marked effects on the functioning of proteins, including enzymes. Therefore, the biological systems have **buffers** to protect cells against significant pH changes.

A **buffer** is a substance or combination of substances that resists changes in pH when acids or bases are added. The buffer either accepts or donates hydrogen ions. A buffer consists of a weak acid and its conjugate base, or a weak base and its conjugate acid. For example, sodium bicarbonate in solution exists as Na^+ and HCO_3^- (bicarbonate) ion. One would expect hydrochloric acid Hcl, when added to a $NaHCO_3$ solution, to increase the acidity of the solution. But H^+ ions from Hcl react instead with HCO_3^- to form the weak acid, Carbonic acid, H_2CO_3. The free H^+ ions contributed by the Hcl are tied up, because the resulting weak acid does not dissociate to any great extent and therefore does not appreciably change the pH. Hence, the bicarbonate ions functions as a buffer.

Thus, living systems possess a variety of buffering systems that tend to hold H^+ ion concentrations within narrow limits.

A buffer system is a solution to which it is possible to add either hydrogen ions or hydroxide ions without affecting the pH of the solution. Buffer system usually consist of a weak base and its salt with a strong acid, or a weak acid and its salt with a strong base. Let us consider a buffer system containing **Monosodium dihydrogen phosphate** (NaH_2PO_4) and **Disodium hydrogen phosphate** (Na_2HPO_4). Two salts of phosphoric acid which are very frequently present in biological systems. NaH_2PO_4 dissociate to give $H_2PO_4^-$ ions and can act in the same way as a weak acid by acting as a H^+ ion donor to remove additional OH^- ion as water. The other salt, Na_2HPO_4 completely dissociates to

give HPO_4^- ions which possess a strong affinity for hydrogen ions which remove from the system. The presence of these two salts gives a buffer system, **Phosphate buffer** system.

$$NaH_2PO_4 \rightleftharpoons Na^+ + H_2PO_4^-$$

$$Na_2HPO_4 \rightleftharpoons Na^+ + HPO_4^-$$

Exercise :

1- Mix 0.05 M KH_2PO_4 and 0.05 M $K_2H\ PO_4$ in the following proportions in a test tube or beaker.

Tube number	1	2	3	4	5	6	7	8	9	10
K_2HPO_4 (ml)	0	0.1	0.5	1	2	3	4	4.5	4.9	5
KH_2PO_4 (ml)	5	4.9	4.5	4	3	2	1	0.5	0.1	0

2- Determine the pH of the solution in each tube.

What is the relationship between the proportions of salt solutions and pH of their mixture in each tube?

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EXERCISE: 5

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ENZYMES

Every living organism carries out a large number of chemical reactions. It is essential for the life of the organism that these reactions should be carried out at an extremely rapid rate and at a safe temperature. All organisms utilize **enzymes** to speed up the rate of chemical reactions without increasing the temperature.

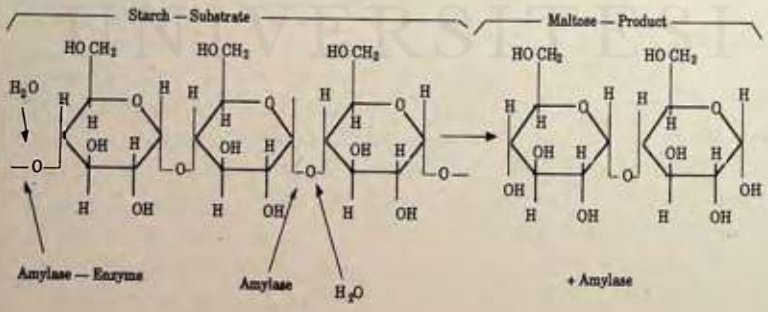
Enzymes are protein catalysts that direct the biochemical reactions. Enzymes do not start a reaction; they speed up the reaction that is already under way, by reducing the need for large amounts of **activation energy.**

Each biochemical reaction in a cell requires a **specific enzyme** to allow the reaction to proceed at the proper rate. Since there are thousands of reactions necessary in the life of the cell, there are thousands of different enzymes available in cells to carry out the reactions.

In order for an enzyme to work in a reaction, it must fit with a **substrate.** Each type of enzyme has a specific physical shape that fits the physical shape of its substrate. Thus, each enzyme has a specific affinity for the substrate of the chemical reaction it catalyzes.

A substrate is a substance that is changed in a reaction and converted into a **product** of the reaction. A general equation for an enzymatic reaction is:

Substrate + Enzyme → End product + Enzyme



Exercises:

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Hydrolysis of Starch by Human salivary Amylase

Human saliva contains en enzyme called salivary amylase, which catalyzes the digestion of starch. The final product of the reaction is a sugar, maltose, (disaccharide).

The activity of salivary amylase can be followed by the iodine test. Iodine yields a deep, blue-black color with starch. As the starch is digested, repeated tests with iodine will give results that vary from the initial blue-black color of the undigested starch to a reddish color for intermediate products in digestion. When the digestion of starch by salivay amylase is complete, no change in the color of iodine will occur.

Collect about a fourth of a test tube of saliva. The flow of saliva can be stimulated by chewing a gum. Add an equal amount of distilles water. Mix the saliva and water thoroughly by shaking the test tube. Then filter the mixture through a double layer of cheese cloth into a smaller beaker. Add 5 ml of starch solution to each of six test tubes numbered 1 to 6. Follow the procedure for each tube. Write your observation of the reaction in each tube on table 5-1 at page 30.

The presence of the sugar maltose can be shown by heating the sample solution for several minutes (in a boiling water bath) with an equal amount of Benedict's solution. If a Bunsen burner is used to heat the contents of the tubes do not allow the solutions to boil, the content of the tube may spurt out of the tabe. As an added precaution, do not aim the open end of the tube toward anyone. If maltose is present the solution will become colored (green, red, orange, or brown) depending on the amount of sugar. Green indicates a small amount of sugar; brown, a large amount.

Effects of temperature on the activity of salivary amylase.

Collect the saliva and prepare your stock enzyme solution as described in exercise 1. You may use the same stock or dilute whatever amounts is collected with an equal volume of distilled water.

Pour 5 ml of starch solution in to 5 test tubes set aside. Take one tube each time and do the following steps.

Immerse the starch solution tube 1 into a beaker filled with icy water. When the temperature of the starch solution in the tube has reached to 5 C (verify this by placing a thermometer in the starch solution), add 5 drops (about 2 ml) of enzyme solution on to it. Mix the tube thoroughly, immediately upon adding the enzyme. Note the time as "0" and with a pasteur pipet immediately remove a few drops of starch solution and place it on a white plate (a seramic tile). Test it for the presence of starch, using iodine. (Recall that starch plus iodine gives deep blue-black color. At one-minute intervals following your "0" reading, remove a sample from the tube and test for the presence of starch. In the first stage of digestion, starch is broken down to dextrins. These give a red color with iodine. When dextrin is digested to the sugar maltose, no color change will occur in the iodine test. The length of time it takes to reach this stage will be considered the length of time for complete digestion to occur.

Repeat same procedure for each of the five tubes for 15 C, 30 C, 45 C and 70 C. Bring the starch solutions in tubes, to these temperatures as immersing them in large beaker with warm (boiling) water.

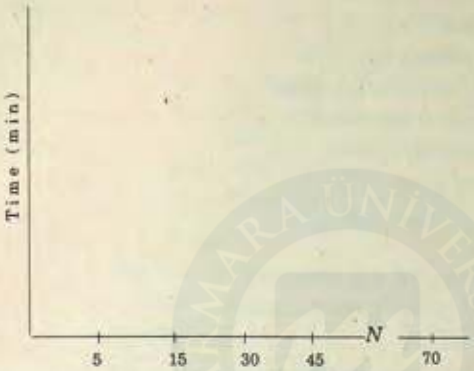
Work in pairs, one of you can read the time interval changes while the other can repeat the second temperature test. Record your observations in a table, indicating the color change observed with each sample.

Table for the effect of temperature on the activity of salivary amylase

TIME	TEMPERATURE				
	5	15	30	45	70
"O"					

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Graph the results by plotting time against temperature. Note the shape of the curve, analyze the data, and interpret the effect of temperature on the activity of human salivary amylase.



Effect of pH on the activity of salivary amylase

You will be supplied by 0.2 M Phosphate buffers having pH of 5.3, 6.2, 7.15, 9.05 (or any other solution having pH of 3, 5, 7 and 9).

Fill the test tubes with 3 ml of these solutions and label them. Now add 1 ml of concentrated starch solution (or equal amount of diluted starch solution) to each tube. Shake each tube thoroughly.

Add 1 ml (5 drops) of your enzyme solution. Shake each tube thoroughly.

With a pasteur pipet immediately remove a few drops of solution from each tube and test it for the presence of starch, using iodine, as you have done in the previous exercise.

At one minute intervals following your "0" reading, remove a sample from each tube and test for the presence of starch.

Record your results in the table as, putting a (+) sign if the starch test is positive, and a (-) sign if the test is negative. Plot your data pH versus time.

What appears to be the **optimum** pH for the activity of salivary amylase?

Table 5-1: Digestion of Starch by Salivary Amylase

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Tube no.	Procedure	Observations
1	5 ml of starch and three drops iodine.	
2	5 ml of starch an 5 ml Benedict's solution. Heat for several minutes in a boiling water bath.	
3	5 ml of starch and 1 ml of your stock enzyme solution. Set aside for 10 minutes and then add three drops of iodine.	
4	5 ml of starch and 1 ml of stock enzyme solution. Set aside for 10 minutes and then add 5 ml of Benedict's solution. Heat for several minutes in a boiling water bath.	
5	5 ml of starch and 1 ml of enzyme solution which has been boiled vigorously for several minutes in a boiling water bath. Set the tube aside for 10 minutes and then add three drops of iodine .	
6	5 ml of starch and 1 ml of boiled enzyme solution. Set the tube aside for 10 minutes and then add 5 ml of Benedict's solution. Heat for several minutes in a boiling water bath.	

Note: 5 % Iodine used in the experiments.

EXERCISE: 6

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MEMBRANE TRANSPORT

The distinction between the interior of a cell and the medium which surrounds it, is maintained by a membrane. Continued existence of the cell is dependent on the ability of this membrane to permit passage of some substances and prevent that of others. Thus, biological membranes are highly selective permeability barriers. Penetration of molecules and ions through the cell membrane is regulated by specific transport systems such as, simple diffusion, osmosis, active transport, endocytosis and exocytosis. Transport processes have several important roles:

1. They regulate cell volume and maintain the intracellular pH and ionic composition within a narrow range to provide a favorable environment for enzyme activity.

2. They extract and concentrate metabolic fuels and building blocks from the environment and extrude toxic substances.

3. They generate ionic gradients that are essential for the excitability of nerve and muscle.

In this laboratory you will observe first simple diffusion processes within red blood cells and also in onion skin cells. Secondly you will exercise on **selective permeability (Active transport)** of yeast cell membrane.

Exercise:

Simple diffusion:

1. Animal cells - Erythrocytes

Like any other cell, red blood cells (erythrocytes) are enclosed in a semipermeable membrane. If red blood is placed in a **hypotonic** sulution (a solution whose osmotic pressure or concentration is less than that of the cell, is said to be **hypotonic)**, water enters by **osmosis** (net movement of water from a less concentrated solution to a more concentrated solution) and erythrocytes swell. In extreme cases, when the external solution is markedly hypotonic to the cell's contents, the erythrocytes bursts and its hemoglobin is released **(haemolysis).**

Place a drop of blood on each of four slides, labelled A-D. To A add a drop of distilled water; to B, C and D add a drop of 0.75 %, 1.0 % and 3.0 % salt solution (Nacl). Observe red blood cells under high power for some period of time and interpret your observations. (Blood may be provided from your own thumb under supervision of your instructor. Sterilize the outside of the thumb with 70% ethanol. Move the arm vigorously and squeeze the base of the thumb. Take sterile lancet and make one sharp jab into the flesh near the base of the nail).

2. Plant Cells

Strip off two small pieces of epidermis from the inner surface of an onion scale. Mount one piece in distilled water and other in a strong solution of sucrose (1.0 M is satisfactory).

Compare the two slides at intervals and interpret your observations. Look for plasmolysis and turgor. When plant tissues are immersed into a **hypertonic** solution, shrinkage of the cytoplasm away from the cell wall takes place and **plasmolysis** occurs.

In water or hypotonic solution, unlike the red blood cells the plant cells will not "burst" or "lyse", because of the elasticity and high tensile strength of the cell wall, which prevents over expansion and bursting of the cytoplasm by excessive water uptake so they become quite turgid.

Active transport

In the previous experiments you have seen that in many respects the cells are subject to different laws of diffusion because of their differently permeable membranes. However in some cases the living cell does control the passage of different substances across its plasma membrane. When the direction and the rate of passage of a substance is not predicted by the simple laws of diffusion, for example, when substances are carried against a concentration gradient, the plasma membrane is said to participate actively in the transport process. Active transport involves the **expenditure of energy** (metabolic energy) by the cell obtained through some chemical reactions as opposed to the thermal energy involved in the simple diffusion process. This is because in active transport the substances which are carried (rather transported) are being moved against a concentration gradient than the one occuring in the simple diffusion process, therefore, this transport can be viewed as an **uphill** transport therefore expenditure of energy by the cell is required.

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This demonstration is one of the easiest and most successful in showing the presence of active transport.

Weight out 0.1 g of beaker's yeast, place in a 125 ml Erlenmeyer flask, then add 25 ml of 0.75 % sodium carbonate solution. Mix well by swirling the flask, to suspend the cells, then add 25 ml of 0.02 % neutral, red solution again mix them a few times by swirling.

1. Filter a few millilitres of the solution through a filter paper into a test tube.

What color changes did occur in the suspension before and after the addition of the dye? What color is the filtrate and what is the color of the cells remaining on the filter paper, how does this compare with the initial color of the yeast?

2. Divide the remaining of the yeast in 3 equal portions in three test tubes.

A. Add a few milliliters of 0.01 N NaOH to the first tube note the color.

B. To the second test tube add a few milliliters of 0.01 N ammonium hydroxide what color change if any, did you observe?

C. Heat to boiling the contents of the third tube what color change, if any, did you observe by this treatment.

Try to interprete the results of parts 1, 2A, 2B, 2C. From which of the above observations can you conclude that active transport has occured?

3. Control: Put a few milliliters of NaOH, NH_4OH and Na_2CO_3 into Separate three tubes. Add one milliter of Neutral red to each tube. Observe the color changes.

In none of the above experiments we had any idea about the site of accumulation of the dye molecules. Therefore observe microscopically the suspension/s which you think gives enough evidence for active transport. Try to localize any organelle or part of the cell where the dye seems to have accumulated.

EXERCISE: 7

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PHOTOSYNTHESIS

All organisms require a constant supply of usable energy to maintain themselves, to grow and to reproduce. The immediate energy source for most organisms is organic food molecules. **Heterotroph** organisms can obtain energy or energy intermediates from the stepwise chemical break down of these molecules.

The sun is the ultimate energy source on earth. However, only certain organisms, **autotrophs** can trap the energy of sunlight directly and convert it into potential energy; this energy-trapping process is called **photosynthesis.**

Procaryotic blue-green algae, certain photosynthetic bacteria, eucaryotic plants use different light trapping pigments as **chlorophyll a** (blue-green light), **chlorophyll b** (yellow-green light), **xanthophyll** (yellow light), and **carotene** (orange light). Among these pigments chlorophyll is most common one. Chlorophyll and other pigments are found within the membranes of **thylakoid** of **chloroplasts** of eucaryotes or within the inner extensions of plasma membrane of procaryotes.

Photosynthesis is the conversion (fixation) of carbon dioxide and water into organic compound carbohydrate. The energy that drives the process comes from light-activated chlorophyll.

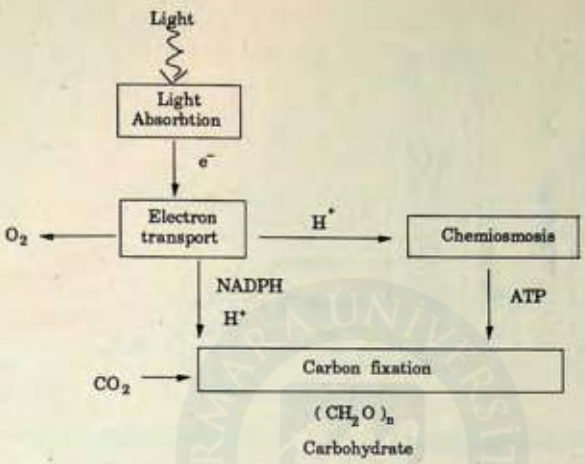
A simplified equation for photosynthesis is:

$$12H_2O + 6CO_2 \xrightarrow[\text{chlophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2 + 6H_2O$$

inorganic raw materials — food — waste product of this reaction

Photosythesis proceeds by a large number of chemical reactions that can be divided into two phases; **light dependent** reactions (or light reactions) and **light independent** reactions (or dark reactions). These reactions can be summarized as follows:

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Chlorophyll trapped light energy is used to split H_2O, which yields protons H^+, 1/2 0_2 and electrons. Electrons are transferred by series of carriers, to $NADP^+$, forming the reduced NADPH molecule. H^+accumulates in thylakoid space and proton gradient forms across the thylakoid membrane which used to synthesize ATP from ADP+P_i.

In dark reactions (not directly dependent on light) CO_2 is fixed and converted to glucose and these reactions fueled by ATP and NADPH produced by the light reactions.

Exercise:

1- Effect of light intensity on the rate of photosyhthesis.

Light plays a key role in the process of photosynthesis. In this experiment the efect of light intensity on the rate of photosynthesis will be investigated by measuring the evolution of oxygen from an equatic plant at different levels of illumination. Since oxygen is a by-product of photosynthesis, the rate of oxygen evolution be taken as a direct measure of the rate of photosynthesis. Oxygen released from subemerged equatic plants is readily observed because it escapes as a stream of bubbles.

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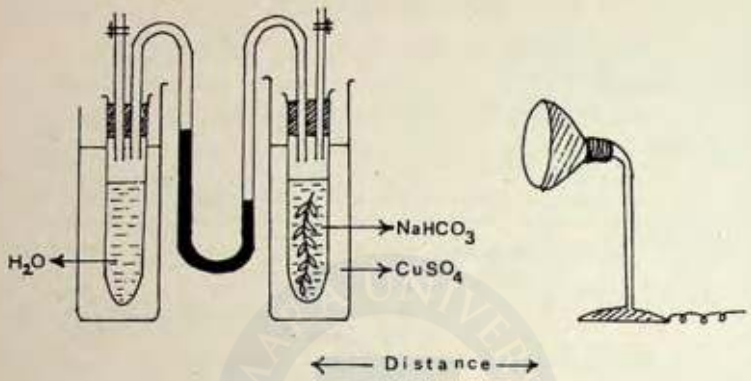


Figure 10. Set - up for Photosynthesis

1. Pour sodium bicarbonate solution into one of the test tubes until 3/4 of it fills, and fill the other test tube with water to the same level.

2. Put a shoot of **Elodea** plant which should have a groving tip, into the test tube which contains sodium bicarbonate.

3. Place the tube containing elodea in a large beaker which contains 1 % $CuSO_4$ (which will absorb the infrared rays emitted by the light source). Infrared rays do not contribute to photosynthesis directly, but they heat the beaker and lead to erroneous results.

4. Allow 5-10 minutes, for the temperature of the tube containing the plant material to come to the temperature of the $CuSO_4$ beaker.

5. Arrange the light source to a appropriate distance from the apparatus.

6. Air tied the tubes and measure the rate of gas evolution as length mm/minute, by taking readings 2-5 minutes intervals (the difference in the arms of monometer).

7. During the course of experiment, you will investigate the effect of various light intensity. This can be done by varying the distance of light source from the plant. Use the following light intensities:

a. No light.

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b. Place the lamp at increasing distances from the plant (10 cm, 15 cm, 30 cm, 50 cm, 100 cm). The intensity of light falling on a given object from a constant source is inversely proportional to the square of distance between them.

Intensity = $1/d^2$

d = distance between the lamp and plant. Work out the light intensity as $1/d^2$ or more conveniently $100/d^2$ for each distance used in your experiment.

Plot your results on graph, putting light intensity on the horizontal axis and length of bubble in mm/min on the vertical axis. What relationship between the gas production and light intensity is demonstrated by your results?

2- The effect of temperature on the rate of photosynthesis

Use the same apparatus. This time the light intensity should be kept constant. Follow the steps 1-7 as described in exercise 1.

In each experiment, the rate of photosynthesis should be measured at 20°C (room temperature) and 30°C or two other temperatures 10°C apart.

In step 3, you can use the larger outer beaker as a waterbath. ($CuSO_4$ beaker). Before you immerse the shoot into the $NaHCO_3$ solution adjust the temperature of water in outer beaker. Allow 5-10 minutes for the inner beaker to come to the temperature of water bath. During the experiment, you can add small amounts of hot water to the outer beaker to requlate the temperature.

The experiment will be performed twice, once with a low light intensity once with a high light intensity.

In general, increasing the temperature of a chemical reaction produces in increased reaction velocity and for a temperature rise of 10°C, the velocity is doubled. This is called the temperature coefficient, Q10.

$$Q_{10} = \frac{\text{The rate of reaction at temp of (T+10)°C}}{\text{The rate of reaction at temp of T°C}}$$

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Calculate the Q_{10} for the high intensity light experiment and for the low intensity light experiment.

3- The effect of various concentrations of carbondioxide on the rate of photosynthesis

The apparatus should be used as in the previous experiments. The intensity of light and temperature should be kept constant. At each experiment, at step 3, use a different concentration of sodium bicarbonate. Repeat the experiment using 0.05, 0.1, 0.3, 0.5, 1,2,4 percent bicarbonate solutions. Make sure the temperature of the water in the outer beaker is the same at each experiment.

Plot a graph of the concentration of CO_2 (horizontal axis) against the rate of photosynthesis (vertical axis).

In conclusion, what do experiments A and C suggest about the nature of photosynthetic reaction? Explain your results briefly.

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EXERCISE: 8

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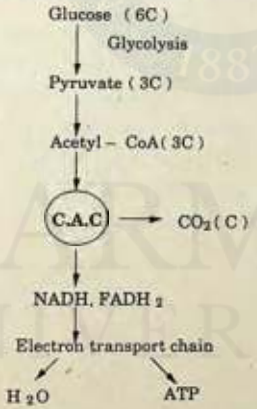
ANAEROBIC FERMENTATION

All organisms can make energy intermediates by breaking down organic food molecules. Most break down their food **by aerobic respiration**, a process that uses molecular oxygen in the oxidation of high-energy food molecules; respiration produces the low-energy molecules carbon dioxide and water as by-products.

One of the principle pathways of cellular respiration involves the breakdown of the common nutrient **glucose.** The over all reaction for the metabolism of glucose can be summarized as:

$$C_6H_{12}0_6 + 6CO_2 + 6H_20 \rightarrow 6C0_2 + 12H_20 + \text{Energy}$$

The oxidation of glucose to carbon dioxide and water occurs in a sequence of reactions that can be grouped into four phases: glycolysis, formation of Acetyl-CoA, the citric acid cycle, the electron transport system and chemiosmotic phosphorylation.



Many cells (organisms) can obtain energy in the absence of oxygen by the **anaerobic process of fermentation.** The most common type of fermentation uses the pathway of glycolysis, making **pyruvate** from **glucose.** Organisms that live under anaerobic conditions, such as yeast and certain bacteria, convert pyruvate to **ethyl alcohol or lactate.**

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Glucose (6C) → Pyruvate (3C) → Ethyl alcohol (2C) + CO_2 (C)

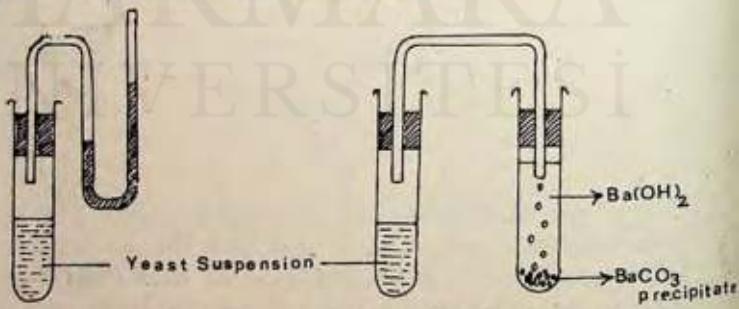
Fermentation in yeast cells:

In yeasts, pyruvate is first broken down to acetaldehyde and carbon dioxide. In the second step acetaldehyde is converted to **ethyl alcohol.** By this process of **alcoholic fermentation,** carried out in the absence of oxygen, yeast can convert fruit (grape) juices into wine by converting their glucose into ethy alcohol. Since yeasts do not tolerate to alcohol, they die in the culture and fermantation ceases when the ethyl alcohol concentration reaches about 12 percent.

Exercise

1. A set up for measuring CO_2 evolution from yeast fermentation, solution of 10 % glucose and the yeast suspension will be prepared for you before the laboratory session. Please listen to the instructions of your laboratory instructor carefully before you start your experiment.

Fill the test tube of the apparatus with glucose solution and yeast suspension, in equal amounts. The tube should be 2/3 full. Connect the manometer (U shape portion of graduated end will be half full with a dye (or ink) tightly). Observe the rate of gas evolution at room temperature. the dye will move up in the graduated arm of the manometer owing to the production of CO_2. The rate of gas evolution is measured as the movement of the dye in mm per minute, Figure 11.



Set-up for measuring CO_2 evolution

Set-up for measuring CO_2 production by precipitation of

Temperature effect on fermentation

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Repeat the experiment, placing the yeast-glucose suspension (tube) in a warm water bath. You can use a large beaker filled with hot water as a water bath. Take the temperature of water with a thermometer. adjust the temperature of water to about 40°C. You can regulate the temperature by adding small amounts of hot or cold water into the beaker.

Do you observe any change in the rate of CO_2 evolution? If so explain. Fermentation rates should be presented and analyzed graphically (plot CO_2 production versus time).

2. In the first part of the test, you have observed the gas evolution as a result of fermentation. In this part of the test, you will verify that the gas liberated is CO_2.

Put the yeast glucose solution in a test tube. Connect the tube by means of plastic tubing to another tube containing Barium hydroxide ($Ba(OH)_2$) solution will turn milky due to $BaCO_3$ precipitation.

$$CO_2 + Ba(OH)_2 \rightarrow BaCO_3 + H_2O$$

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EXERCISE: 9

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CELLULAR REPRODUCTION

The life span of every individual organism is finite. All organisms grow old and die or they are eliminated by predators, by extreme fluctuations in environmental condition. But, all organisms have the ability to reproduce themselves. Reproduction assures the continuation of each species.

Every kind reproduction involves the detachment of a viable portion of the organism. The detached fragment, which can be as simple as a single **cell,** always contain at least one set of the organism's genes.

In most organisms, life begins as a single cell, the **zygote.** The growth and development of one cell to the higher multicellular complexity is due to the activity of the original cell (zygote) and its progeny of cells in reproducing themselves. Cells reproduce by the process of cell division. In a large majority of eucaryotic organisms cell division involves two distinct processes that often, but not always, occur together: division of the **nucleus** (karyokinesis) and division of the **cytoplasm** (cytokinesis). The process by which the nucleus divides to produce two new nuclei with the same number of chromosomes as the parenthal nucleus, is called **mitosis**. Thus, mitosis serves to maintain a constant number of chromosomes in **somatic cells** (body cells).

In sexual reproduction of eucaryotic organisms, two gametes (the egg and sperm) unite to form the first cell (zygote) of the new individual. The zygote produced by their union would have double the normal number of chromosomes, and thus the number of chromosomes would double in each generation. However this does not happen; the chromosome number normally remains constant within a species from generation to generation. therefore, a different kind of cell division must occur in the production of gametes, whereby the **diploid** number of chromosomes present in all other cells of the body is reduced to the **haploid** number found in gametes. This special process of reproduction division is called **meiosis**.

MITOSIS

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Mitosis is a process by which the nucleus divided into two nuclei, each of which contains identical genetic material. Usually the cell divides also. Although mitosis is a continuous process, it is convenient to speak of distinct phases: **prophase, metaphase, anaphase and telophase** (Figure 12). When we look at a plant or animal cell that has been killed and stained while in the process of mitosis, we are looking at the stopped action.

Prophase is the stage when the cell is preparing itself for division. During prophase, individual **chromosomes** become visible because they undergo a coiling process that shortens their length while increasing their diameter. As this occurs, the chromosomes become visible as paired structures, **chromatids** joined at the **centromere.** Meanwhile, the spindle apparatus forms, the nuclear membrane breaks down, and nucleoli disappear.

Metaphase: At this stage, the rod-shaped chromosomes (pair of chromatids) are lined up in a row across the middle of the cell

Anaphase is the stage during which, the equal distribution of genetic material is accomplished. Each centromere divides and the identical chromatids move to opposite sides (poles) of the cell. Each chromatid is now called a **daughter chromosome.**

Telophase: During this phase, the spindle apparatus breaks down. The chromosomes form two groups and the individual chromosomes go back into an uncoiled condition. A nuclear membrane forms around each group and these are now called **daughter nuclei.** If cell division is complete, it is at this point that **cytokinesis** will occur by the formation of a **cell plate** (cell furrow in an animal cell). Nucleoli are also reformed.

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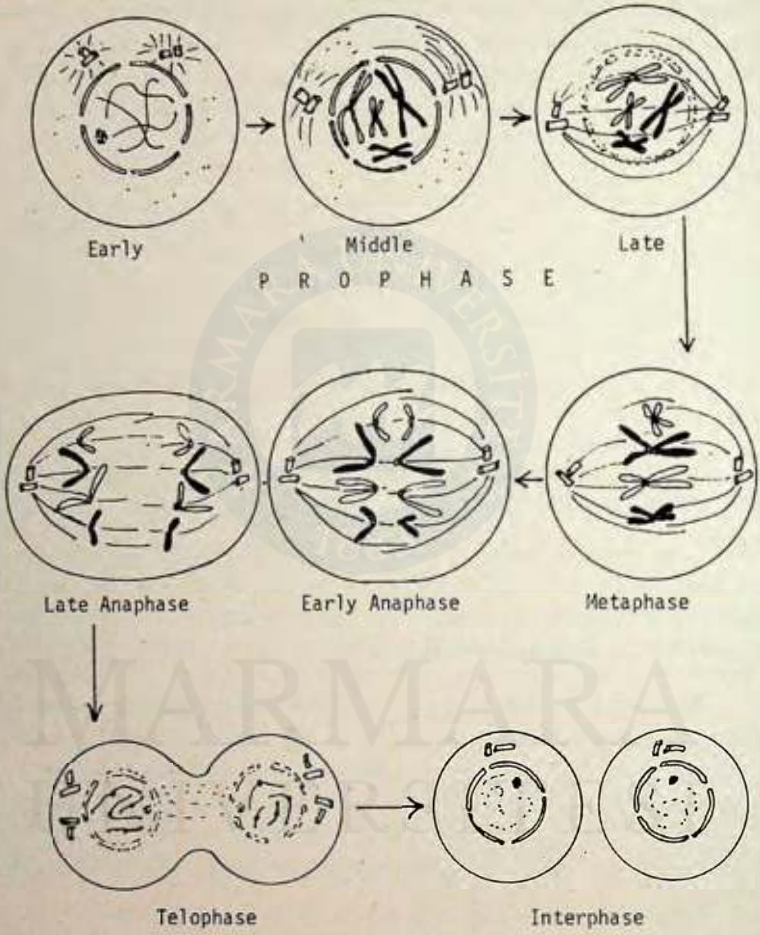


Figure 12. Mitosis in an animal cells.

Exercise:

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1- Squash preparations of **Onion** root tip.

One week in advance of this experiment, place onions in small containers with water (or seeds of broad bean were germinated).

Root tips contain many actively dividing cells. Remove an onion from the container and cut a tip 5 mm long from a root. Place it on a microscope slide. Add a few drop of **acetocarmin** (preferably iron-acetocarmin) stain. Tear apart the root tip into smaller pieces using dissecting needles. Don't let the stain dry out on the slide, add some more stain as it starts to evaporate. Carefully put a cover glass over the pieces in the stain and place a small piece of paper towel over the cover glass. Gently mash the cover glass with the thumb, being careful not to break it. Heat the slide slightly by passing it over alcohol flames a few times. Be careful not to let the stain solution boil. Scan the preparations, first at low power to find cells showing chromosomes. Turn to high power (40X), for details.

Try to find cells representing, interphase, prophase, metaphase, anaphase and telophase. What do the chromosomes look like? Can you count the chromosomes? Draw pictures of one of the good representative of each phase. What proportions of the cells seems to undergo divisions?

2- Prepared slides of onion root tip.

Study prepared slides of an onion root tip (longitudinal section) with scanning or low power. Locate the tip or cone-shaped portion of the section. Locate the cells have a boxlike appearance with large round nucleus (interphase cells) or cells at prophase, metaphase, anaphase and telophase. Study these cells with high power.

Answer the following questions for each cell you observe: How are the chromosomes arranged? What happens to the nuclear membrane? Do you see spindle fibers? How can you determine the poles of the cell? Are the chromosomes visible? Describe the appearance of the cell.

MEIOSIS

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Meiosis is the process of cell division that reduces the **diploid** (2N) number of chromosomes in the parent cell to a **haploid** (1N) number in the daughter cells; hence, it is called a process. In all animals that are multicellular, meiosis takes place at the time gametes are produced. Meiosis consists of two cell divisions. During the first division of meiosis, reduction from the diploid number of chromosomes to the haploid number of chromosomes has been accomplished. Each of the two cells produced as a result of meiosis I goes through a second division. This second division results in the formation of four cells, which mature into gametes. Each cell has the haploid number of chromosomes and each chromosome is composed of a single chromatid (Figure 13).

Exercise:

To understand the mechanism involved the production of gametes, you will make model chromosomes using two different color toy-clay. Make a pair of, two different colors (red and blue) metacentric chromosomes with the chromatids 20 cm long and 1 cm thick. Make another pair of chromosomes with the chromatids 10 cm long and 1 cm thick.

Compare your four chromosomes with Figure 14, to make sure that you start with a proper information. These chromosomes will represent the genetic make up of a gonad cell 2N = 2 at **prophase** stage.

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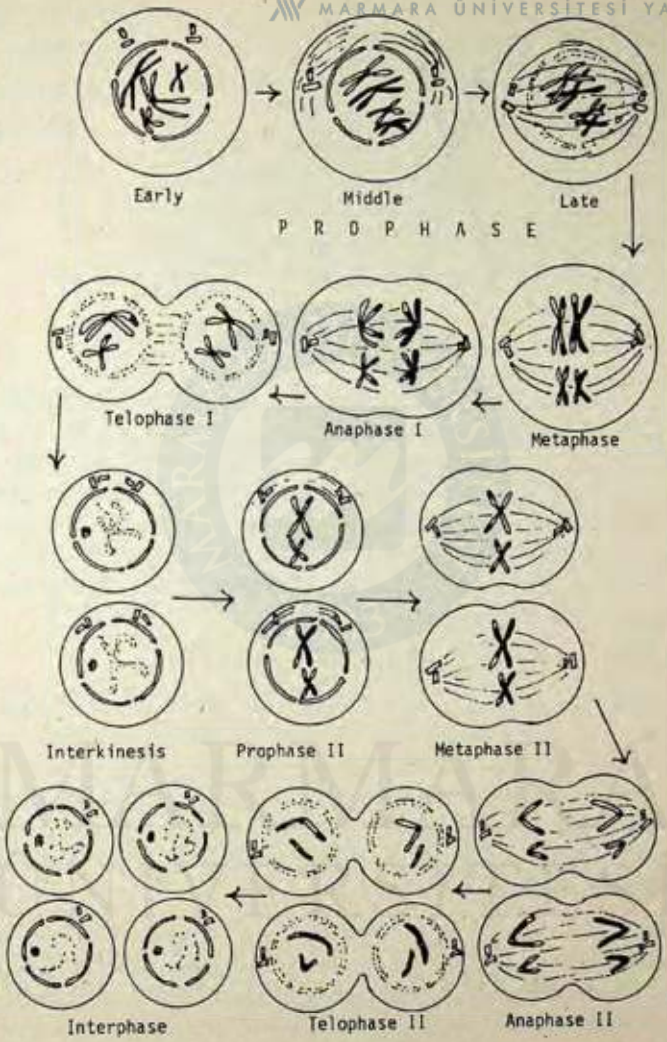


Figure 13. Meiosis in an animal cell.

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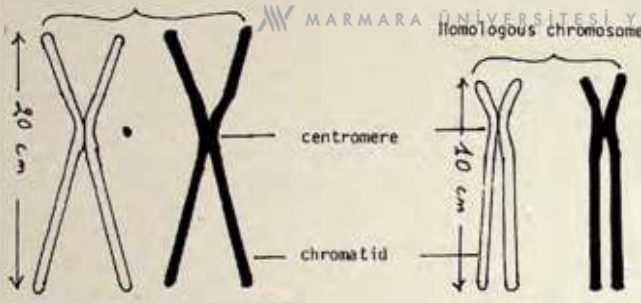


Figure 14. Metacentric and Homologous pairs of chromosomes

The chromosomes of each are in same size, have centromers in the same position and have genes for the same characteristics (alleles), at equivalent points along their length. Different color (red and blue) chromosomes in a pair represents the contributed chromosomes of female and male parents. These pair of chromosomes are **homologous** chromosomes with the chromatids. The DNA of the chromosomes duplicate during **interphase**, before they enter to prophase of meiosis, so each chromosome is composed of two strands.

Prophase I (Synapsis and Crossing-over):

During the prophase stage of meiosis the nucleus prepares itself for division. The chromosomes become visible, the nuclear membrane begins to disintegrate, the nucleolus fades, and the spindle apparatus develops. Homologous chromosomes become associated with each other, they are paired, interwined point for point along their entire length, that is they are in **synapsis.** Put your two pairs of homologous chromosomes near each other to simulate their synapsis.

While in the synapsed condition, equivalent pieces of homologous chromatids will be broken off and exchanged. This process, called **crossing-over**, can occur several times along the length of homologous chromosomes. Simulate this process in your model by detaching, exchanging, and reattaching equivalent parts. As a result of the processes of crossing-over, a new combination of genes has been formed.

If you don't understand the process of crossing-over, ask your instructor's help before going on.

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Metaphase I (Alignment of pairs):

The chromosome pairs line up at the equatorial plane. move your chromosomal models to show this arrangement.

Anaphase I:

During this state, the separation of homologous chromosomes occurs. This separation is called **segregation.** Segregate the metacentric pair of chromosomes by moving them on the drawn spindle fibers. Each-color chromosomes of a tetrad move to a opposite pole, together with their chromatids.



Figure 15. Chromosome movement at Anaphase.

How do the chromosomes at the end of anaphase I differ from the chromosomes as they would appear at the end of the anaphase stage of mitosis?

Telophase I (Cytokinesis):

During this stage cytokinesis occurs and two daughter cells form. Each of these daughter cells has one each of the homologous pairs of chromosomes. Each is haploid, and each has a complete set of genetic information.

Separate the two sets of your model chromosomes by a distance to remind you that they are in different cells.

Prophase II:

The cell prepares for a second division. What characteristics would you expect in any prophase stage?

Metaphase II:

During metaphase II the chromosomes in one cell are lined up on equator. They are not homologous pairs; each chromosome, however, is made up of two chromatids.

Move your chromosome models to the center of the one-half sheet of paper to represent this activity in both daughter cells.

Anaphase II:

During anaphase II the centromere slit and the chromatids migrate toward opposite poles.

Separate your chromatids (cut into two from the centromere region), and move the chromosomes toward opposite poles. Do this in each of the two daughter cels. In what ways does anaphase I differ from anaphase II?

Telophase II:

During telophase II cytokinesis occurs in each daughter cell. This results in the formation of four cells. What other processes would you expect to occur in telophase?

Prepared slides of **grasshopper** testis:

In this part of experiment stages of meiosis in testis of grasshopper will be examined from permanently prepared slides.

In animals, meiosis always takes place in the gonads. A convenient place where meiotic figures can be seen is the testis of the grasshopper testicular tissue sections. Look for the meiotic figures to be found in the cells within the seminiferous tubules. The products of the first meiotic division are called primary **spermatocytes,** and the products of the second meiotic divisions are called secondary spermatocytes,or **spermatids.** The spermatids then develop tails to become **spermatozoa,** which are usually found in the lumen of a tubule.

EXERCISE: 10

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MENDELIAN GENETICS

Sexual reproduction is characterized by the uniting of two haploid (n) nuclei of gametes which bring together genetic material from two parents of a diploid organism. The result, a **zygote,** diploid cell (2N), then develops into a new individual.

The offspring will not be identical to either parents but instead will be a different individual. The various offspring may have traits of one parent, both parents, or neither parent. This depends upon the genes that the offspring receive from the parents.

To understand this universal phenomenon we must investigate how hereditary material pass from parents to offspring, how this material express itsef in new combinations, and whether principles can be formulated that apply to the complex events.

The study of heredity (genetics) is dependent upon information about the appearance of traits within lines of organisms of direct descent. The patterns of occurrance through successive generations enables us to develop knowledge about the nature of the genetic material. Thus, to study genetics of an organism in the laboratory we must choose one with a short life cycle to follow the pattern of inheritance for several generations.

In order to determine the probable appearance of an offspring we must first know something about the parents. What are their **phenotypes?** What genes do they posses, **(genotype)**? What possible genes can be found in the germ cells of each parent, and the possible ways these germ cells may combine during fertilization?

The chromosome theory of heredity

Genes are located on chromosomes at discrete loci (locus-singular) and pairs of chromosomes may contain alternate forms of the same gene loci (allelic genes).

The chromosomes are in pairs in a diploid cell. They duplicate at every cell division to provide identical sets for the daughter cells, as do

the alleles. And, during production of germ cells by meiotic division, only one of each pair is retained in gamete (sperm or ovule), so that, upon fertilization, fusion of the male and female nuclei results in formation of **zygote,** in which a full set of chromosomes are presented. Therefore in zygote every pair of chromosome has each member contributed by each parent, which is exactly what alleles are expected to do.

Meiosis, therefore, is the process by which the alleles are separated **(segregated)** and fertilization, is the process by which the alleles are restored to "doubleness", **(recombination).**

We can simulate the effects of segregation and independent assortment by using models The prime objective will be to see the magnitude of the possible genetic variation resulting from segragation and recombination.

Exercise:

1- Segregation and recombination at a single locus.

Working in pairs you will use two containers to serve as the sex organs for two parents, each with colored toothpicks in them to serve as the gametes. The segregating gene which is found at one locus on the chromosomes is reflected by the color of the gametes (or toothpicks).

a. The gametes (single toothpicks) will first be constituted from pure breeding parents. First produce from each contrasting container, 12 gametes. Then mate these. You should now have..... number of zygotes and offspring, each with like/unlike (cross out one) chromosomal make-ups.

b. Now simulate the sex organs of two of these offspring with the right number and color of chromosomes to make 12 (twelve) diploid cells per individual. Randomize the chromosomes and then draw them "**meiotically**" (one at a time) but simultaneously from each container. Mate them two at a time to form the zytoges of the next generation. Record the number of zygotes (pairs of toothpicks) in their respective different classes (e.g., all red, 1 red - 1 yellow, all yellow, etc.)

Were the chromosomes bearing allelic genes blended or mixed or modified in the course of their journey from diploidy to haploidy and then to diploidy again? Explain.

How many genetically different types of diploid progeny are possible in the 1st generation and in the 2nd generation?

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2- Segregation and independent assortment of contrasting alleles at two independent loci.

This part of the exercise will demonstrate the importance of probability in inharitance.

In the fruit fly, **Drosophila melanogaster,** a recessive mutation on chromosome number 2 resulting in a mutant called "dumpy" **(d)** causes shortened wings to develop. Thus a fly of genotype **dd** has dumpy wings whereas one of genotype **DD** or **Dd** has normal wings.

A mutation resulting in a mutant called **"ebony" (e)** on chromosome number 3 causes dark body color. An ebony fly has the genotype **ee** where as one of genotype **EE** or **Ee** would have normal body color.

a. Work in pairs. Each pair of students obtains a cup labeled "2" as well as one labeled "3" and the toothpicks contained therein. These represent the following:

Cup "2" Green toothpick = chromosome number 2 carrying gene d

Cup "3" Blue toothpick = chromosome number 2 carrying gene D

Cup "2" Red toothpick = chromosome number 3 carrying gene E

Cup "3" Yellow toothpick = chromosome number 3 carrying gene e

Both cups together represent a population of individuals whose genotype is DdEe.

b. Mix the toothpicks within the cups and draw **one from each cup,** sight unseen. (This is comparable to what pappens in meiosis when **one of each pair** of chromosomes randomly goes to each daughter cell.) Record the genetic composition of the gamete you have drawn (DE, De, dE, or de) on Table 1 under the column labeled **"first gamete"**. Return the toothpicks to their appropriate cups and repeat the procedure. List the results of your second drawing under the column marked **"second gamete"**. Repeat the procedure until you have filled both columns.

c. Next complete the third column in Table 1 by writing the genotype derived by combining the two gametes in fertilization (a.g., De plus de .... Ddee).

d. Tabulate your findings by listing the 16 offspring in Table 2. Fill in the phenotype column. (Phenotype = external appearance of an indivudual irrespective of genotype).

e. List in Table 3 the total obtained of each, of the 4 possible phenotypes. Do you get the ratio which would be expected due to random independent assortment? If not, can you explain why?

f. On the board combine your results with those of others in the class. Your instructor will calculate a ratio using this large sample. How does this ratio compare with the expected ratio? Why?

g. We will now extend the idea of the **Independent Assortment** of loci to the more general case. Assume two contrasting alleles for each pair of the entire set of chromosomes in an organism. How many different gametic and zygotic (diploid progeny) combinations would be present in an organism with 5 pairs of chromosomes? (gametic) (zygotic)

10 pairs : (gametic) (zygotic)

23 pairs : (gametic) (zygotic)

(It will be useful to express your answer as exponents, and derive them from a general rule or formula).

Table 1 :

Matings	First Gamete	Second Gamete	Genotype of offspring
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

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Table 2 : Tabulation of offspring

Genotype	Number Obtained	Phenotype
DDEE		
DDEe		
DdEE		
DdEe		
DDee		
ddEE		
ddEe		
ddee		

Table 3:

Phenotype	Exp. Number	Class Obtained
normal wings; normal color		
normal wings; ebony body		
dumpy wings; normal color		
dumpy wings; ebony body		

3- Drosophila melanogaster: Wild and mutant types

Drosophila, the fruit fly, is an ideal animal for experimental genetics, because it has many properties that make it suitable for genetic experiments. For example; it can be cultured easily in the laboratory in a bottle, on an artificial diet. It takes only 10 days to produce a new generation of adult flies once parent flies have been mated. This allows us to see the results of experimental crosses relatively short period of time. They can be raised in large numbers. A single female lays between 80 and 200 eggs, so it does not take long to produce a large population. This is also important because the frequent mutants with altered phenotypes can be detected in the population. Its wild type and mutants can be easily observed with a dissecting microscope.

Drosophila is a member of the insect order **Diptera** and has a characteristic life cycle which contains four stages: the egg, the larva, the pupa and adult fly as you see in the Figure 16.

Exercise: Examination of wild type Drosophila.

Each group of students will be given a culture of wild type Drosophila. The culture contain all stages; adult, pupa and larvae. The wild type organism in this laboratory exercise is a strain of **Drosophila melanogaster,** known as fruit fly. Handling and anesthetization of flies

1. Obtain a stock bottle of wild type (normal) flies.

2. Saturate the cotton pad of an etherizer with ether.

3. Gently tap the stock bottle so that the flies fall to the bottom of the bottle.

4. Remove the plugs from the stock bottle and the etherizer.

Quickly invert the stock bottle over the etherizer.

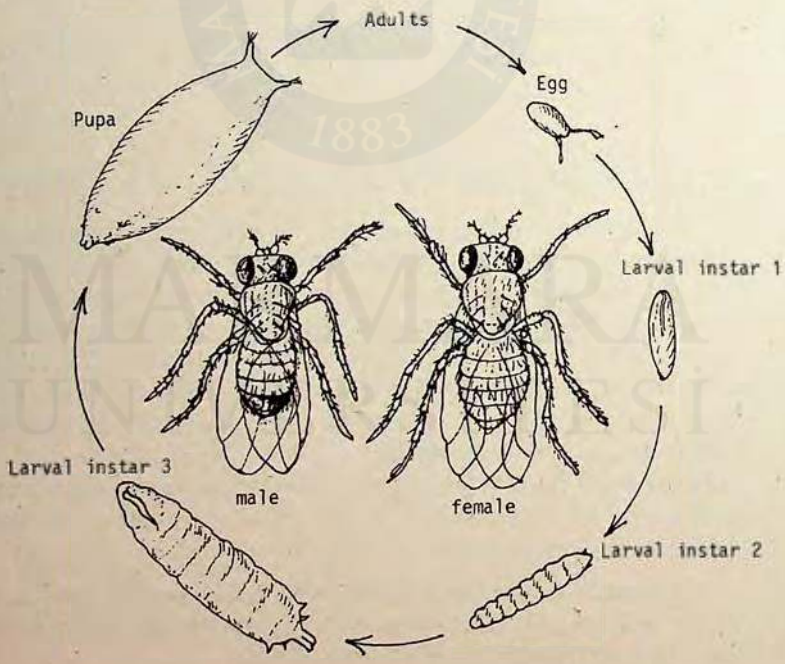


Figure 16. Life cycle of Drosophila

5. Tap the jars a number of times until the flies have dropped into the etherizer.

6. Replace the plugs.

7. Allow the flies to remain in the etherizer only as long as it takes to anesthetize them. Overexposure will result in serious damage to the tissues and death.

8. Dump the flies onto a white card, and observe them with a dissecting microscope. Move the flies around with a brush. Note the red eyes, the straight wings, and the gray body. You will see back bands on the abdomen (the posterior portion of the body). Some flies have a blunt abdomen with three broad bands; there are the male flies. Those that have a pointed abdomen with five narrow bands are the female flies.

Exercise:

Identification of mutant characters

Drosophila has many easily distinguishable mutants. The mutant traits in Drosophila, with obvious morphological changes, allow us to examine and study the transmission of characteristics from parents to offspring by direct observation.

Your laboratory instructor will make available to the class number of mutant Drosophila cultures or etherized specimens. Observe the given mutants under dissecting microscope and record your observations.

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EXERCISE: 11

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THE DIVERSITY OF LIFE

We share the world with enormous variety of living things. From whatever point of view biology is approached, whether that of pure research, medicine, agriculture, ecology or economics, classification is necessary to bring menagable order, out of this great diversity of life.

Biological classification goes back to time of Aristotle (300 B.C.). The system of classification in use today is the "***binomial system***" established by Carolus Linnaeus in 1758.

The theoretical basis of the Linnaeus's system was different from the one we use today. He attempted to divide organisms into groups which had certain morphological features in common. However modern taxonomists classify organisms according to their phylogenetic relationships (evolutionary history). In many cases it leads to the same result as Linnaeus's morphological classification. Because, morphological characters are produced as a result of evolution and organism that share morphological features often are phylogenetically related. The modern taxonomists also use the information provided by the detailed molecular studies on genetic material and proteins; computer assisted statistical analysis of physical measurements of body parts; behavioral studies and etc., for the grouping of organisms.

TAXONOMY AND THE CATEGORIES OF CLASSIFACATION IN THE BINOMIAL (TWO-NAME) SYSTEM

Taxonomy is the classification of organisms. The classification system consists of a hierarchy of categories (taxa; singular taxon).

In the binomial classification system each finer level category restricts the set of properties that specific organisms posesses. Each broader level category includes more properties and a larger set of related organisms.

The broadest category of all is the ***kingdom.*** The broadest category within the kingdom is tehe ***phylum*** (or division). Each phylum (or division) contains a group of related ***classes*** , each class contains a group of

related ***families;*** and each family contains a group of related ***genera*** (singular genus). The final unit is the ***species.*** Species is the fundamental unit of classification which is defined as, the population of individuals similar in their structural and functional characteristics that in nature breed only with each other and share common ancestry, or in other words, share a common gene pool.

In the binomial system, every species is given Latin names. The first word designates the genus to which the species belongs; a genus contains one species or a group of similar species. The second word indicates species itself. For example, the binomial for house fly is ***Musca domestica.***

genus : Musca

species : domestica

THE CLASSIFICATION OF ORGANISMS

There are several classification systems proposed and in use. All of them proposed on the basis of the most recent thoughts on evolutionary relationships of organisms. The classification scheme presented in this text is the system proposed by Robert Whittaker in 1969.

According to this system all organisms classified under five kingdoms as MONERA, FUNGI, PROTISTA, PLANTAE and ANIMALIA.

Although the kingdom is the most inclusive category, a bigger category than the kingdom, **the domain**, is used to hold the "anucleate" and "nucleate" organisms of different kingdoms. The kingdom Monera alone is classified under domain **Procaryota** and four other kingdoms together are classified under domain **Eucaryota**.

DOMAIN PROCARYOTA

Anucleate organisms (organism that lack of true nucleus)

KINGDOM MONERA

Prokaryotic organisms. All have procaryotic cellular organization. Nutrition absorbtive, chemosynthetic or photosynthetic with anaerobic or aerobic metabolism. Reproduction asexual, nonmotile or motile by bacterial flagella. Solitary, unicellular flamentous or colonial.

PHYLUM CYANOBACTERIA: Blue-green algae.

Photosynthetic. Chlorophyll a and accesory pigment on photosynthetic lamella (specialization of membranes). Internal gas bubbles for floating near the surface of water. Gelatinous outer sheath with various pigments. Flamentous or clustered colonies. Some species fix nitrogen.

Oscillatoria, Nostoc.

PHYLUM BACTERIA

Variety of metabolic adaptations to various kind of environmental conditions (habitats).

Class Archeobacteria: Ancient bacteria.

Methanogens, Holophiles, Thermophiles.

Class Eubacteria: True bacteria.

A diverse group. All have rigid cell walls. Some move by flagella. Reproduce by binary fission. Some are ecologically important. Divided into subclasses by their shape, such as, Bacilli, Cocci, Sprilla.

Escherichia coli, Clostridium botulinum
Staphylococcus aureus.

Class Myxobacteria	: Slime bacteria
Class Spirochetes	: Some are important pathogens
Class Actinomycetes	: Mold like bacteria
Class Mycoplasmata	: Bacteria without cell wall
Class Rickettsiae	: Obligate intracellular parasite,
Class Chlamydiae	: Obligate intracellular parasite.

DOMAIN EUCARYOTA

Nucleate organisms (organisms that have true nucleus and membraneous organelles).

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KINGDOM PROTISTA

Primarily unicellular (one-celled), some may form colonies which are composed of several similar cells more or less tightly associated with one-another. They are found in many different habitats. All types of nutrition except chemosynthetic autotrophy. Life cycles may include both sexually and asexually reproducing phases. Some are parasitic. Locomotion is by cilia, flagella or ameboid movement. The evolution of the protists is obscure. They are thought to be the descendants of common ancestor.

SUBKINGDOM PROTOPHYTA

Algal protists, mainly autotroph.

PHLYLUM EUGLENOPHYTA: Euglenoids.

No cell wall; mostly fresh water forms; 1-3 flagella, chlorophylls a and b and caretenoids.

Euglena

PHYLUM CHRYSOPHYTA: Diatoms, golden-brown and yellow-green algae.

Cellulose and pectin walls, containing silica in diatoms; marine or fresh water forms, Diatoms lack flagella, algal forms have one or more flagella; chlorophyll a and c, carotenoid pigments.

Navicula, Chaetoceros

PHYLUM PYRROPHYTA: Dinoflagellates.

Pectin and celllulose cell walls; mostly marine forms, usually two flagella; brown red and yellow (Chlorophyll a and c, carotenoids).

PHYLUM CYPTOPHYTA: Cryptomonads.

SUBKINGDOM PROTOZOA

Animal like protists. Mainly heterotrophs.

PHYLUM MASTIGOPHORA: Zooflagellates.

Without chloroplast. Locomotion by flagella; no cell wall, fresh water or parasitic forms.

Trypanosoma

PHYLUM SARCODINA: Amoebas, foraminiferans, heliozoans and radiolarians.

No cell wall but some secrete shells containing silica, calcium carbonate etc., movement or feeding associated with pseudopods. Marine or fresh water forms.

Amoeba, Entoameba

PHYLUM SPOROZOA: Sprozoans.

Parasitic sprozoans that reproduce by spores. They lack special locomotory organelles. Complex life cycles.

Plasmodium (causes Malaria)

PHYLUM CILIATA: Ciliates.

Locomotion is by cilia. They have two types of nuclei (macronucleus and micronucleus). Reproduction asexual process (conjugation). Fresh water, marine and parasitic forms.

Paramecium, Stantor

KINGDOM FUNGI

All eukaryotes; mainly multicellular organisms. They do not posses photosynthetic pigment. Cells often form a mycelium. All heterotrophs with saprobic (absorbing food from dead organic matter) or parasitic. Many multinucleate with nuclei dispersed in a walled and often a septate mycelial syncytium. Most are non-motile; live embedded in a medium or food supply. Fungi may reproduce vegetatively but mainly reproduce by means of spores which may be produced sexually or asexually. Reproductive cycles include both sexual and asexual processes.

PHYLUM EUMYCOPHYTA: True fungi.

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All classses have cell walls and hyphae and reproduce both sexually and asexually by means of spores. Generally terrestrial. All classes contain saprobic and parasitic forms.

Class Oomycetes: Water molds and their terrestrial relatives. Flagellated spores. Some are unicellular; others consist of branched mycelia with coenocytic hyphae. Cellulose cell walls. Important parasites exist in this group.

Phytophtora infestans - late blight of potatoes,

Plasmopara riticola - downy mildew of grapes,

Saprolegnia , mold on dead insects, fish, frogs etc. in water

Class Zygomycetes

Sexual reproduction by zygospores. Hyphae are coenocytic and cell walls consist mainly of chitin. Heterothallic (two mating types).

Rhizopus nigricans - the black bread mold

Class Ascomycetes: Sac fungi.

Sexual reproduction by ascospores formed in asci. Asexual reproduction by conidia (spores) which pinch off from connidiophores.

Saccharomycetes cerevisia - **Yeast** (unicellular) - bread and wine yeast

Aspergillus - common food mold

Penicillium

Class Basidiomycetes: Club fungi - Mushrooms, rusts and smuts

Sexual reproduction by basidiospores born by basidia. Septate hyphae; heterothallic and dikaryotic stage in life history.

Agaricus compestis - Eadible mushroom

Amanita - Poisonous mushroom

Puccinia graminis - wheat rust

Ustilago - smuts on corn or wheat

PHYLUM MYXOMYCOPHYTA: Slime molds.

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Organisms with life cycles that include separate cells, aggregations of cells, and spore-producing stages.

Class Myxomycetes: Plasmodial slime molds.

Class Acrasiomycetes: Cellular slime molds.

KINGDOM PLANTAE

Plants. Eukaryotic, multicellular and photosynthetic (autotrophs) organisms with stiff cell walls containing cellulose. Cells frequently contain vacuoles. Main photosynthetic pigments chlorophyll a and b, accesory pigments carotenoids. Sessil (non motile) with a few exception. Growth continuous throughout life. Differentiated tissues and organs. Both sexual and asexual reproduction with alternation of gametophyte and sporophyte generations. Plants range in complexity from flaments, colonies and sheet of the simplest green algae to the most advanced, the flowering plants which with their roots, stems, leaves and characteristic flowers, are the most recent group of plants to have evolved on earth.

The groups of **seed plants** emerged as the vascular plants evolved. The seed is a structure that enabled the embryo to survive under advese conditions on land.

SUBKINGDOM THALLOPHYTA

They show little or no tissue differentiation, entire plant as thallus. The reproductive structures are often unicellular and lack a protective wall or jacket of non-reproductive cells. Zygotes do not develop into embryos within the female reproductive organs where they are produced.

The three groups of multicellular algae are believed to have arisen from three different groups of unicellular ancestors.

PHYLUM CHLOROPHYTA - Green algae.

Members have chlorophylls a and b, carotenoids; store starch. unicellular to multicellular but not very large or elaborate. Mostly fresh water forms, many of them marine forms, some terrestrial forms exist. Some are motile and flagettated. Some form colonies. Asexual reproduc-

tion by fragmentation of colony, various types of sexual reproduction (isogamy, anisogamy and oogamy).

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Chlamydomonas, Spirogyra, Volvox,ulva

PHYLUM PHAEOPHYTA - Brown algae.

Contain chlorophylls a and b, fucoxanthin. Store **laminarin** (a carbohydrate). All multicellular some are very large and elaborate. Almost all are marine forms (giant kelps). Zoospores with two lateral flagella.

Fucus

PHYLUM RHODOPHYTA: Red algae.

Contain chlorophyll a, phycobilins and carotenoids. Store modified starch. Multicellular, usually marine, Some fresh water forms. No flagellated cells in life history.

Porphra (used as food in Japan)

SUBKINGDOM EMBRYOPHYTA (EMBRYONTA)

Embryophyta have evolved numerous adaptations for the terrestrial life. The reproductive structures are multiellular and protected by jacket of sterile (non-reproductive) cells. The early stages of embryonic development take place in the female reproductive organ. The **cuticle**, a waxy outer protective layer covering the soft, watery tissue underneath. Plant body is differentiated into different functional parts and tissues. Higher groups have distinct roots, stems and leaves. Chlorophyll a, b and corotenoids are the principal pigments. They store starch. The plants of embryophyta are biochemically similar to the chlorophyta. They are thought to have arisen from flamentous green algae.

PHYLUM BRYOPHYTA: Non-vascular plants.

They are lack of conducting tissues. Usually terrestrial, with a marked alternation of **gametophyte and sorophyte** generations. The gametophyte is the prominent generation (sexual generation) on which the sporophyte is dependent. They need moist environment for swimming of sperm.

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Class Musci: Mosses.

The gametophyte plant (haploid) has an errect stem and its leaves are arranged in a spiral. In some species the sexes separate, in others both male organ (antheridia) and female organ (archegonium) develop on the same plants Diploid sporophyte is leafless structure, grows on the gametophyte tissue. Spores in sporangium are produced by meiosis (Figure 17).

Sphagnum

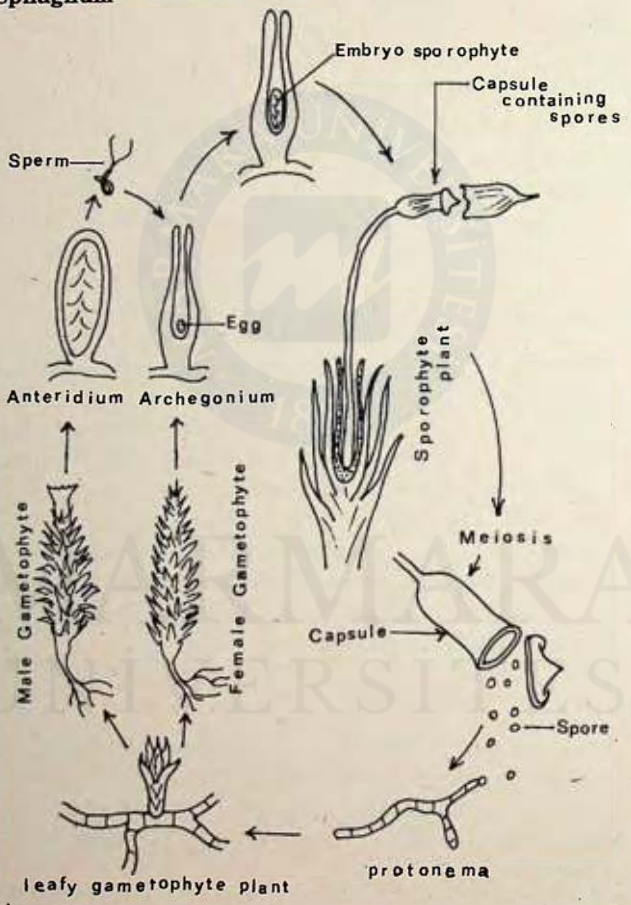


Figure. 17 Life cycle of a Moss

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Class Hepaticae: Liver worths.

Simpler than mosses with a similar life cycle.

PHYLUM TRACHEOPHYTA: Vascular plants.

They have a protective layer of sterile jacket cells around the reproductive organs; multicellular embryos retired within the **archegonia;** cuticles on the aerial parts; vascular tissue is present in sporophytes. Most modern forms have roots, stems and leaves in sporophyte generation. Usually more than few centimeters high. Sporophyte is independent, self sufficient and dominant generation.Gametophyte is independent (lower vascular plants) or dependent on sporophyte (gymnosperms and angiosperms). Wide range of habitats in terrestrial environment.

Class Psılopsida:

The earliest vascular plants, from which other vascular plants probably evolved. Have many fossil forms. Simple plants with a central strand of vascular tissues and dichotomous branching.

Living genus: **Psilotum**

Class Lycopsida: Club mosses.

They have true roots and leaves. Certain leaves become specialized for reproduction, they bear sporangium on their surface (club shape sporophylls).

Lycopodium, Selaginella

Class Sphenopsida - Horse tails

Equisetum

Class Pteropsida: Ferns.

They have evolved from the Psilopsida and have many fossil forms. They are fairly advanced plants with a very well developed vascular systems. Most have underground stem (rhizome), roots and large many veined leaves (fronds). Gametophytes are usually photosynthetic, but need a moist environment to survive. Sperms are flagellated and must swim. (Figure 18).

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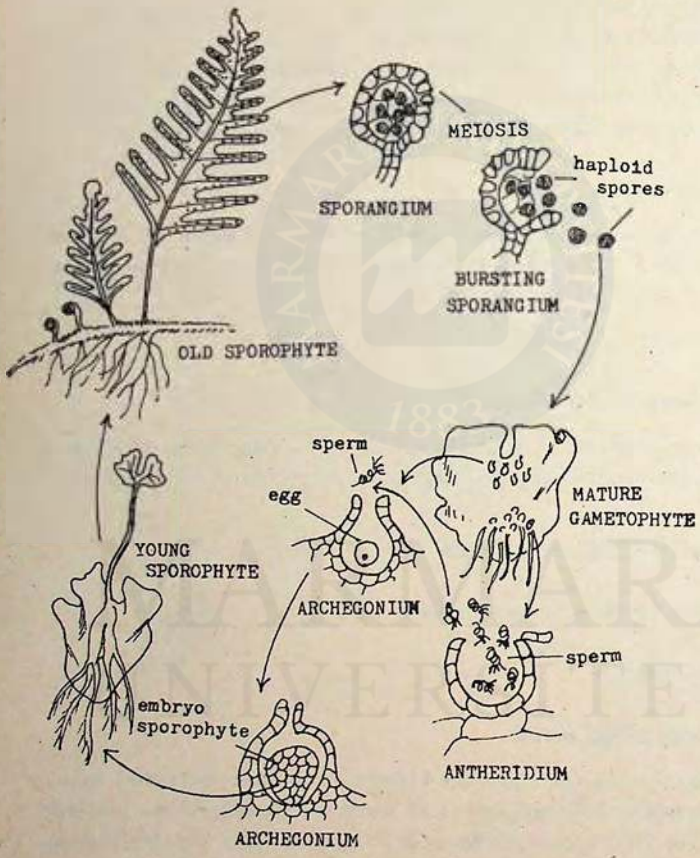


Figure 18. Fern life cycle.

SUB-PHYLUM SPERMATOPHYTA: (Spermopsida) - Seed plants.

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Separate male and female spores (heterospory). Large megaspores give rise to megagametophtes. Megagametophyte consists of cells among which an egg is produced. **Megagametophyte** (megaspore) is retained within the megasporangium (ovule) (Figures 19, 20).

Male spores (microspores - pollen grains) produced give rise to male gametophyte (pollen grain and pollen tube). Fertilization con occur after pollination (delivery of male gamete to the egg by pollen tube) in the **megagametophyte.** The zygote develops into an embryo **sporophyte** with the rudiments of leaves, stems and roots, while still within the nutritive tissue of gametophyte. The protective seed coat derived from the previous sporophyte. The seed thus consists of structures belonging to three generations. The seed formation is advantageous for the protection of embryo and the dispersal of seed plants.

Super Class GYMNOSPERMAE: Gymnosperms.

No true flowers or ovules are present. Seeds are usually naked on the surface of cone scales. Fruit like structures homologous to cones or fruit like cones are sometimes present. Endosperm like tissue derived from gametophte is haploid (Figure 19). Gymnosperms appear to have evolved from ancient group of plants called the "Pteridospermae" which resembled ferns but had xyleme tissue like gymnosperms.

Class Cycaddae: Cycads.

Palm like plants with flagellated sperm, found in tropical and subtropical regions. Considered the most primitive of modern seed plants.

Class Gınkgoae: Ginkgo tress.

Ginkobiloba

Class Conıferae: Conifers (cone bearers).

Common evergreen tress and shrubs with needle-shaped leaves.

Pines, firs, cedars, hemlocks, junipers, spruces and sypresses.

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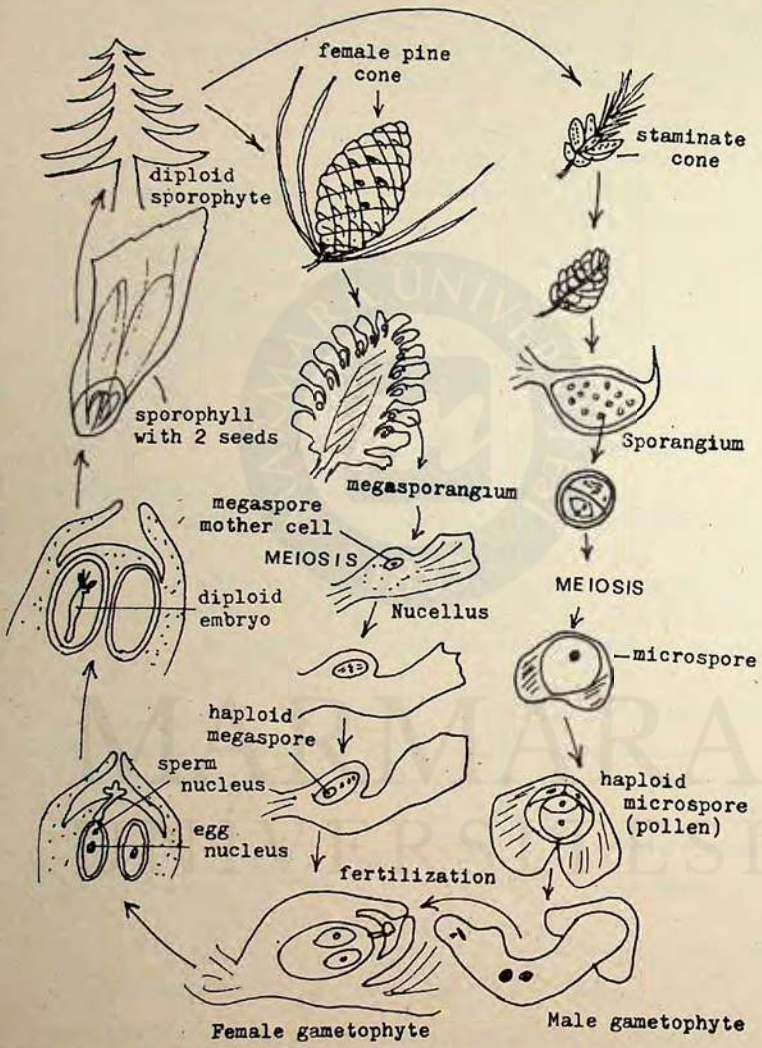


Figure 19. Fymnosperm life cycle.

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Super Class Angiospermae: Angiosperms.

The flowering plants with seed is enclosed in ovary which becomes (sometimes along with other flower parts) the fruit (Figure 20). Stored food in seed nourishes the embryo until it can begin its own photosynthesis. They have large amount xyleme elements. They are adapted to various seasonal conditions and various environments.

Class Dicotyledonae: Dicotyledons.

Embryo has two seed leaves (cotyledons), leaves generally have network of veins; stems contain ring of vascular bundles; secondary growth occurs. Flower parts in multiples of five, four or sometimes two.

Most flowering plants. About 175.000 species, including familiar trees, flowers plants and vegetables.

Class Monocotyledonae: Monocotyledons.

Embryo has one seed leaf (cotyledon) and usually a large endosperm. Leaves generally have parallel veins; stem contain scattered vascular bundles. Secondary growth usually do not occur (except palm tress). Flower parts in multiples of three.

Includes the **bamboos**, **grasses**, **lillies**, **orchids** and **palms**.

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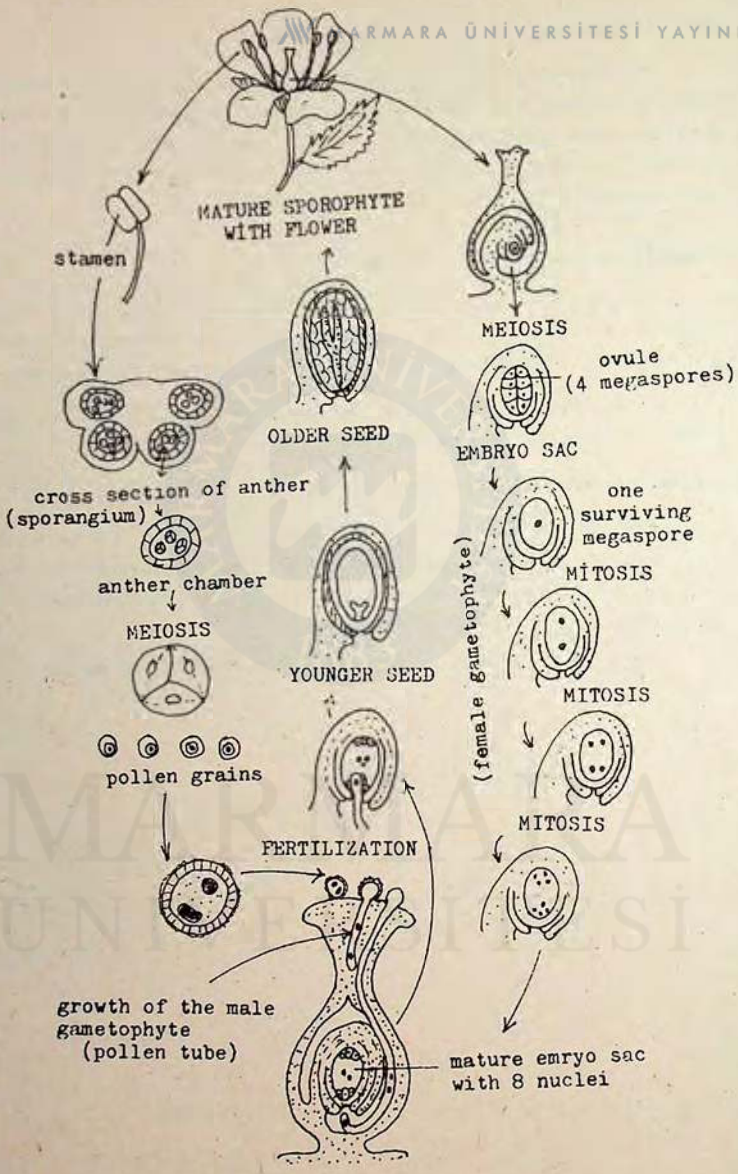


Figure 20. Angiosperm life cycle.

KINGDOM ANIMALIA

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Animals. Multicellular, eucaryotic organisms. In all, except simplest animals, cells are organized to form tissues and tissues are organized to fom organs. They are **heterotrophic.** Most are capable of locomotion, some are sessil. Most of them have well developed sensory and nervous systems and respond to external stimuli with appropriate behavior. Most animals reproduce sexually, with large nonmotile eggs and small flagellated sperm. They live in all types of environments.

The approaches used to determine phylogenetic relationship of different species to each other are:

1. BODY SYMMETRY:

A. Radial : Radially symmetrical animals have a central axis and are radially symmetrical around this axis. They can be cut into two identical pieces by a lengthwise cut (but not a cross-wise cut) through the center in any direction.

B. Bilateral : Bilateral symmetry is a condition in which a section along the median segittal plane (and no other) will divide the animal into right and left halves which are mirror images of one another. Bilaterally symmetrical animals have **dorsal** and **ventral** surfaces and anterior-posterior ends.

2. DEVELOPMENTAL PATTERNS

Tne developmental patterns of embryos and larvae provide important information about phylogenetic relationships. Bilaterally symmetrical metazoan animals have been divided into two groups **Deuterostomia** and **Protostomia.** Each have characteristic mode of development including a basic difference in the ultimate destiny of **blastopore.** These two groups reflect two main lines of evolution.

Deuterostoma: During embryonic development, **blastopore** develop into the **anus,** new mouth (second mouth) forms later in development elsewhere. Cleavage pettern of deuterostome egg is **Radial** and **Indeterminate** type.

Protostomia: During embryonic development **blastopore** opening develops into **mouth.** Clevage pettern of **mosaic** egg of protostomes, is **spiral** and **determinate** type.

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In the simplest emetazoons the body is a **double-walled sac** with a single digestive cevity. They do not have body cavity, so these animals are acoelomates (Cnidarians and Platyhelminthes). The higher animals have **a tube - within - a - tube** body plan. An outer tube, body wall and an inner tube, the digestive tract. Between the two tubes is a second cavity, the body cavity. If the body cavity develops between the mosoderm and endoderm, it is called a **pseudo-coelom (Nematods).** If it forms within the mesoderm and is completely lined by mesoderm, it is called as **coelom** (true coelom). Figure 21 shows the body cavity in different group of animals.

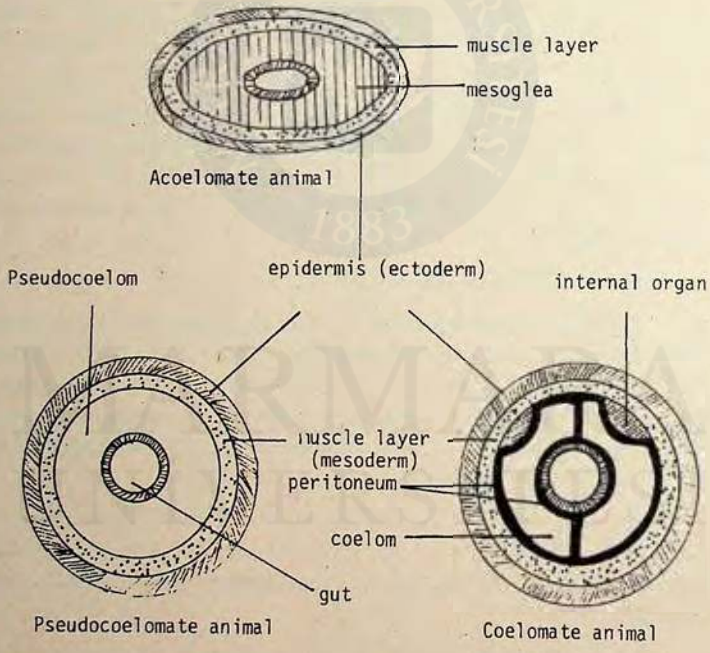


Figure 21. Cross sections of animal body illustrating three basic animal body plans.

EVOLUTIONARY RELATIONSHIPS OF ANIMALS

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Figure 22 shows the evolutionary relationships of animals, which is based on the developmental characteristics.

It is generally agreed that the flagellate protists are the ancestors of multicellular organisms (plants and animals) and animal like protist is the ancestor of animals. It is probable that multicellular animals and sponges evolved different directions (independently) therefore sponges put in a **Subkingdom Parazoa,** while all other multicellular animals are classified as, Eumetazoa. But essentially they are all multicellular Metazoans.

Sponges arose from flagellate colonies of the collar flagellates. Although there are several theories about the evolution of multicellular animals (metazoans) it is generally accepted that they arose from the protists.

We certainly know that the coelenterates and the flat worms branched off from the main line of metazoan evolution at a very early time. After the evolution of a circulatory system, an anus and a body cavity the metazoans split into two branches (super phyla). Annelids and Echinoderms. Within these phyla, all the other phyla arose (Figure 22).

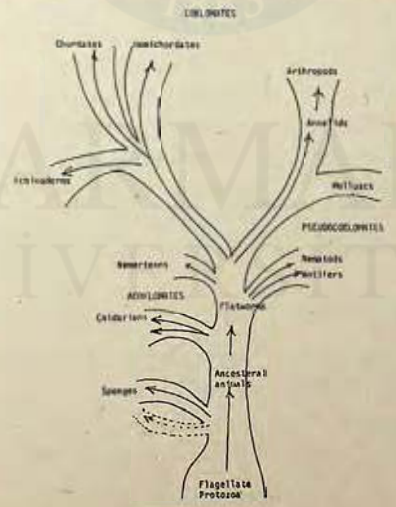


Figure 22. Phylogenetic tree, illustrating evolutionary relationships of animals.

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SUBKINGDOM PARAZOA

PHYLUM PORIFERA: Sponges.

They have specialized cells (cell diferentiation) but not much tissue differentiation. No anterior end, mouth or digestive cavity is present. Often an indefinite form is organized around water canals which are connected to exterior by pores. Body cavity lined by flagellated colar cells for creating water current, these cells resemble Protozoan collar flagellates. Calcerous or silicerous skeleton or horny spicules are present sessile. Solitary or form colonies. Asexual reproducion by budding; sexual reproduction with externally swimming sperm and internal egg. Larva is motile.

Class Calcarea: Calcerous sponges

They have $CaCO_3$ skeleton.

Class Hexactinellida: Glass sponges

They have siliceous spicules.

SUBKINGDOM EUMETAZOA

All animals other than Sponges. They have the tissue and organ system level organization. Most have mouth and digestive cavity.

PHYLUM CNIDARIA: (Coelenterates).

Radially symmetrical, aquatic, free swimming or sessil animals. They have inner and outer tissue layers with differentiated cells. Mouth surrounded by tentacles bearing **nematocysts** (cnidoblasts - sting cells). They have gastrovascular cavity, no anus. Solitary or colonial. They have **polyp** or **medusa** forms. Sexual reproduction (medusa is the sexual stage) or asexual reproduction by budding off. They have **planula** larva.

Class Hydrozoa: Hydra like animals.

Most have polyps and medusain life history. Polyp stage often is colonial. Marine and fresh water forms.

Hydra, Obelia.

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Class Scyphozoa: Jelly fishes.

They are all marine forms and have free swimming medusa.

Class Anthozoa: Sea anemones and corals.

Medusa stage is completely absent in this group. They are sessil, often cloonial and all marine forms.

PHYLUM PLATYHELMINTHES: Flatworms.

They are most primitive bilaterally symmetrical animals. They are protostomes, acoelomates. They have three well developed tissue layers (ectoderm, mosederm and endoderm) with organs.Gut has mouth but no anus. Flattened body. Sexual reproduction. They are hermaphroditic but cross fertilization occur.Asexual reproduction by budding.

Class Turbelleria: Planarians.

Free living marine, fresh water and terrestrial forms. They have cephalization in nervous system. Body surface is ciliated.

Planaria.

Class Trematoda: Flukes.

They are mostly parasitic (external and internal parasides of vertebrates) with complicated life cycles with or without intermediate host. They have suckers attack to host. Mostly hermophoroditic.

Fasciola hepatica

Class Cestoda: Tape worms.

Endoparasites of vertebrates. They have no head or digestive system. Life history with one or more intermediate host.

Taenia solium

PHYLUM NEMATODA: Round worms.

They are pseudocoelomates (fluid flilled body cavity) and protostomes. They have bilateral symmetry, complete digestive tract (gut with mouth and anus). body surface covered with cuticle. Free living or parasitic. They are found in fresh water and damp soil habitats.

Ascaris lumbricus - human parasite

Trichinella spiralis - human parasite

PHYLUM ANNELIDA: Segmented worms.

Coelomate (true coelom), protostoms. They have bilateral symmetry and segmentation (metamerism) of body. Cephalization, developed segmented ventral ganglia (ventral chain of ganglia) and brain. They have closed circulatory system, completely differentiated gut with mouth and anus. Excretory organ is **protonephridium** or **meta nephridium.** Respiration is via body surface. Some have appandages (parapodia). They have spiral cleavage and **Trocophora** larva. Hermaphroditic, sexual reproduction.

Class Polychaeta:

Mostly marine forms. They have well developed head and segmented body with parapodia. Sexual reproduction with separate sexes. Asexual reproduction by buding

Nereis

Class Oligochaeta: Earth worms.

Terrestrial and fresh water forms. Parapodia is absent. Head is reduced. Hermaphroditic with cross fertilization.

Lumbricus - Earth-worm

Class Hirudinea - Leeches.

They have segmented body. They are ectoparasites.

Hurido medicinalis

PHYLUM MOLLUSCA

They have protostome characteristics. They are bilaterally symmetrical. Non-segmented body (soft body) is covered by a mantle, which secrete a shell. Head and muscular foot usually present. They have a reduced coelom. Gas excretion by nephridia (opens into pericardial coelom). Heart is a circulatory organ. They have a basic nervous system composed of a ring of nervous tissue around the esophagus and several pairs of ganglia. Sexual reproduction with separate sexes. They are related to the annelids.

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Class Amphineura - Chitons.

They are orally flattened and have tough mantle and shell of eight skeletal plates. Single foot used for locomotion. Head is reduced, have radula (specilized feeding apparatus). Marine forms.

Chiton

Class Bivalvia - Pelecypods

They have two flattened shells or valves. These are usually symmetrical. Head is reduced. They have paired gills. Marine or fresh water forms and most of them filter feeders. Muscular blade - like foot is used for burrowing.

Pecten (Scallop), **Mytilus** (mussel)

Ostrea (Oyster), **Mya** (Clam)

Class Gastropoda - (Belly footed) - Snails and slugs

Asymmetrical body with a coiled shell. Shell is reduced or absent in some. Large foot for locomotion. Radula is present. Marine, freshwater or terrestrial forms. They have large head with sense organs (eyes and tentacles). They have all types of feeding. respiration by mantle cevity. They have six pairs of ganglia, in some fused to form brain.

Lymnea (Pond snail), **Helix** (Escargot) **Limax** (Slug)

Class Cephalopoda - head footed - Squids, Octopods

Head is surrounded by grasping 8-10 tentacles with suckers. Shell is external, internal or absent. Mouth with or without radula. Locomotion by jet propulsion using siphon made from mantle. They have large eyes. Marine animals, all carnivorous and very active. They have well-developed head with advanced sensory structures and brain. They have closed circulatory system with capillary beds at tissues.

Octopus, Sepia, Loligo (Squid)

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Bilaterally symmetrical, protostome animals. They are segmented but some segments fused to form body regions (heteronomous segmentation). Appendages are jointed , body and appandages covered with a hard chitinous exoskeleton. Body cavity is a hemocoel (reduced coelom). Open circulatory system with dorsal heart, haemolymph (blood) flows in hemocoel; respiration through body surfaces, gills or trachea. Segmented ganglionic nervous system, similar to annelids, compound eyes in some. Growth is by molting. Marine, fresh water and terrestrial forms.

Class Arachnida - Spider, scorpions, ticks and mites.

Their body has one or two main parts, 6 pairs of appendages. The first pair, the chelicera ends in pincers, the second pair - pedipalps are used as jaws and the last four pairs are used for walking. Mostly terrestrial.

Class Crustacea - Lobsters, Crabs, Barnacles, Copepods

Body two or three parts. Antennae, chewing mouth parts, three or more pairs of legs. $CaCO_3$ added to skeleton. Mostly marine forms.

Class Insecta

Their body has three parts (head, thorax and abdomen). They have antennae, mouth parts modified for chewing, sucking or lapping. Usually have two pairs of wings and three pairs of legs. Respiration by trachea, excretion by malphigian tubules. Mostly terrestrial, some are fresh water forms. Direct postembryonic development (Hemimetabolous) or indirect postembryonic development (Holometabolous), metamorphosis. They include many important orders as

Coleoptera - True bugs, **Hemiptera** - Stink bugs,

Diptera - Flies, **Hymenoptera** - Bees,

Lepidoptera - Butterflies,

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PHYLUM ECHINODERMATA

Deuterostomes with radial cleavage pettern. Adults with **pentaradial** symmetry (adaptation to sedentary life style). They have calcerous skeleton, complete digestive tube and coelom (enterecoelomfluid filled cavity), vascular system (ring canal and radial canals) with tube feet. Respiration through body-wall, nervous system scattered around body. Separate sexes with specialized gonads. Larva is bilaterally symmetrical, metamorphoses to become radially symmetrical. Mostly bottom dwelling, marine forms.

Class Crinoidea - Sea Lillies

Class Asteroidea - Sea Stars (or Star fishes)

Class Echinoidea - Sea Urchins (or Sand dolars)

Class Holothuridea - Sea Cuccumbers

PHYLUM CHORDATA , Chordates

Deuterostomes with bilateral symmetry. All have notochord at some stage of life. Hollow dorsal tubular nerve cord, the brain develops from a swelling of anterior end of the tube. Pharyngeal gill slits are present at some stage in the life history. Segmentation at least in part of the body in most groups. Post anal tail at some stage in the life history. Ventral heart, endoskeleton.

SUBPHYLUM UROCHORDATA - Tunicates

Marine animals with sac like body which is covered by cellulose tunic. Filter feeding. Larvae are free swimming and have notochord in tail and metamorphoses into adult form.

Class Ascıdıacea - Sea squirts

SUBPHYLUM CEPHALOCHORDATA - Lancelets

Marine animals with a segmented, elongated fish like body. Burrow in sand as its anterior end protruding. At anterior bears tentacles with chemical and tactile (touch) sensory cells, which help to feed. V shaped muscles for movement.

Amphioxus

SUBPHYLUM VERTEBRATA - Animals with vertebrae.

Animals with a notochord, pharyngeal gill slits and a dorsal hollow nerve cord. In higher vertebrates the notochord is present in the embryo only and in later stages of life head and trunk supported by a series of skeletal (bone or cartilage) structures called **vertebrae,** surrounding or replacing the notochord in the adult. They have closed circulatory system with ventral heart, well developed brain protected with specialized skeletal box (skull), eyes and other well developed sense organs. They have developed liver and kidney and two pairs of limbs. Reproduction is bisexual. They are highly diverse and abundant and have important ecological roles.

Class Agnatha - Jawless fishes

Skeleton cartilaginous, notochord persist throughout life, lack of true Jaws, lack of paired appandages. Marine and freshwater forms.

Lamprey, Hagfishes

Class Chondrichthyes - Cartilaginous fishes

Cartilaginous skeleton, jaws present, notochord replaced by vertebrae in the adult. Gill openings separate. Paired pectoral and pelvic fins. Tail fin usually asymmetrical and modified to form claspers in male

Sharks

Class Osteichthyes - Bony fishes

Bony skeleton and jaws. Gill openings all covered by a single bony operculum. Paired pectoral and pelvic fins, tail fin usually symmetrical. Many have swim bladder. Marine and freshwater forms.

Herring, Salmon, Eels, Seahorse, Floander and most other familiar fishes.

Class Amphibia - Frogs, Toads, salamanders

Poikilothermic Tetrapods (four legged animals). They lay eggs without an amnion or shell. Larva has gills for respiration.Adult respiration via lung and skin. They have three - chambered heart with mixed blood. Scale absent on skin, moist and slimmy for respiration.

Order Urodeles - Salamenders and Newts

Order Anurans - Frogs and Toads

ClassReptillia-Lizards,Snakes,Crocodiles

Poikilothermic tetrapods with lungs. Amniotic eggs without complete septum between ventricles. Blood is mixed.

Class Aves - Birds

Homeothermic vertebrates with feather. Bipedal tetrapods. Anterior limbs modified as wings for flight. They have more than one mode of locomotion.

Sparrows, Penguins, Ostriches

Class Mammalia

Homeothermic tetrapods with young nourished by milk from mammary glands of females. Most of them viviparous and covered with hair.

Subclass Monotremata

Primitive egg laying mammals with reptilian features (egg laying and have cloaca).

Echidna, duck-billed Platypus

Subclass Marsupialia

Young born alive but undeveloped state. Development is completed in a mother's pouch (marsupium) on the abdomen. Mammary glands open into marsupium.

Kangaroos

Subclass Eutheria - Placental mammals

Young develops within the uterus of the mother.

Humans, Bats, Whales, Rodents, Dogs, Elaphants, Horses, Cattles, Cats ect.

EXERCISE: 12

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FORM AND FUNCTION IN ANIMALS

You have seen the adaptive changes in external morphology of variety of animals when you have studied their classification. Your instructor will introduce the comparative anatomy and function (physiology) of organ systems in the class. In this laboratory session you will be familiar with the internal organ systems of vertebrates as dissecting a lower vertebrate animal, the frog.

Exercise:

FROG ANATOMY

A living animal is much more interesting oorganism to study than a dead or preserved one. It is possible to show many types of physiological process with living animals. For this reason you will use an anesthesized frog in this exercise.

The frog is the most commonly used animal in introductory biology laboratory because it has all the characteristics of vertebrate, it can be obtain and anesthesized easily. Frogs can be anesthesized with ether, uratane or by pitting. Your laboratory instructor will provide you with the anesthesized frog in dissecting tray and with the dissecting equipment.

External Anatomy

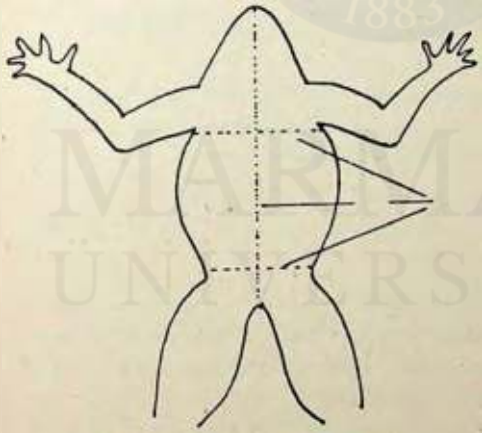
The frog's basic body consists of the head, trunk, the pectoral girdle (the front legs and associated structures) and the pelvic girdle (hind legs and associated structures). Identify these structures of your frog. also identify the **dorsal** (back), **ventral** (belly), **anterior** (head end), and **posterior** (tail end) of your frog. Locate these structures: the anus on the dorsoposterior surface between thehind legs; the tympanum, a circular area found posterior and lateral to the eyes; the external nares, small pit-like openings on the anterior of the head.

Studies of Circulation

1- You can observe the capillaries either in the frog's tonque or in the webbing between the toes. Place the anesthetized frog on its belly on a frog board and stretch the tonque or toes over the opening in the edge

of the frog board or on a piece of plastic foam. Secure the tohgue or toes in place with pins and observe with low power through the compound microscope. You might be able to see three different kinds of blood vessels with the blood flowing through them: arteries, capillaries, and veins. The arteries will branch into smaller and smaller blood vessels until eventually the blood vessels are about the same diameter as the red blood cells. These smallest blood vessels are capillaries. these capillaries will combine into larger and larger blood vessels called veins. The red blood cells are oval in outline and will flow from the arteries through the capillaries to the veins. Usually the blood flowing in the arteries will flow in pulses and the blood in the veins will flow at a continuous pace. determine the rate of blood flow in these three blood vessels for comparison.

2- After you have studied the circulation in the tongue or toes of the frog, open the body cavity of the frog, and study the action of the-heart. Place the frog on his back in a dissecting tray and use a pair of scissors to cut through the skin from the cloaca to the point of the chin. Make cuts to the side at the level of the shoulder and hip so that the skin can be peeled back on either side as indicated in figure below.



lines for cuting
the skin and body wall

Notice that are a large number of blood vessels inside the skin. The skin is the major organ of gas exchange (oxygen + Co_2). The whitish-

colored thin layer remaining over the belly of the animal is the belly musculature. Make a similar pettern of cuts through this muscle, but cut to either the right or left of the midline to perevent cutting the ventral abdominal blood vessel. When you get to the region of the arms, you will find it necessary to cut through the bones of the sholder girdle. Immediately under these bones is the heart. It still should be beating. This portion is the ventricle. There is a smaller chamber at the anterior end of the heart that is called the atrium. Locate and distinguish the atrium and ventricle of the heart. Determine the heart rate in beats/minute, and record. After a few minutes, place some cool Ringer's solution on the heart (10°C) and record the heart rate. Repeat a few minutes later with warm Ringer's solution (30°C). What effect does temperature have on heart rate? Why?

1. Warm _____ beats/min.
2. Room temperature _____ beats/min.
3. Cold _____ beats/min.

Internal Anatomy

After your experiments on the heart, locate the following structures:

1- The **liver,** a large reddish-brown organ located posterior to the heart, stores food and releases it as needed. The liver is important in the digestion of fats and helps blood coagulation in wounds.

2- The **gallbladder;** lit the lobes of the liver until you locate a bluish,green sac. This sac stores bile, which contains salts that aid in fat digestion.

3- The two **lungs** are located on either side of the heart and should be seen protruding from under the liver. These are thin sacs, usually speckled with black. Some exchange of gases between the atmosphere and the blood takes place in the lungs.

4- The **stomach, small intestine** and **large intestine** take up most of the abdominal cavity. The huge J-shaped structure is the stomach. This can be seen at the frog's left side and just below the liver Diges-

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tion starts in the stomach of the frog rather than in the mouth, as in humans. The long, slender middle portion, extending from the stomach, is the small intestine. This is called the small intestine because of its small diameter, even though it is 5 - 8 cm long.

Digestion is completed here. The small intestine empties into an abruptly expanded portion at the base of the abdominal cavity, which is the large intestine. Undigested food is held in the large intestine until the animal defecates.

5- The **spleen** is a small reddish structure seen near the small intestine. It is sometimes dificult to locate. The spleen stores and cleans the blood.

6- The two **kidneys,** located on opposite sides of the backbone, are about 2 cm long and reddish in color. There may be a thin membrane separating the kidneys from the rest of the abdominal cavity; if so, remove it. The kidneys serve the important job of removing cell wastes from the blood.

7- The yellowish stripe on the surface of the kidney is the **adrenal gland.** This complicated organ furnished the hormone adrenalin that helps the organism respond to emergencies.

8- The presence of **testes** is the easiest way to verify that your frog is a male. These are the two white, bean-shaped structures located at the anterior end of the kidneys. As in humans, they produce the male sex cells. If you have a female frog with mature eggs, you will see the enlarged saclike **ovaries** that contain the eggs. You may want to remove these to see the remaining parts.

9- On the right and left sides you should see each of two small coiled tubes. These **oviducts** conduct eggs from the ovary to the outside. In males they are present but are much smaller and nonfunctional.

10- **Fat bodies** are irregularly shaped, yellowish-white masses of stored food. They may be found in the lower part of the abdominal cavity.

11- The white threads that run from the backbone are **nerves.** Pinch some of them and see what hapens.

12- The **urinary bladder** is attached to the large intestine very near the point where the legs join the body. It is very thin and is easily damaged during dissection. Its function is to hold the urine.

13- While you are studying internal anatomy, notice and locate the major, specific arteries and veins entering into and leaving from heart, lungs, liver, kidney and gonads. Your laboratory instructor will demonstrate the dissection of frog. You must be careful not to cut the major arteries. If blood remains in the circulatory system you will be able to locate all major arteries and veins, as they are shown in Figures 23 and 24.

14- When you finish to study internal anatomy, you may cut the head of frog from the neck region and open the cartilageous skull to study parts of the central nervous system (Figure 25).

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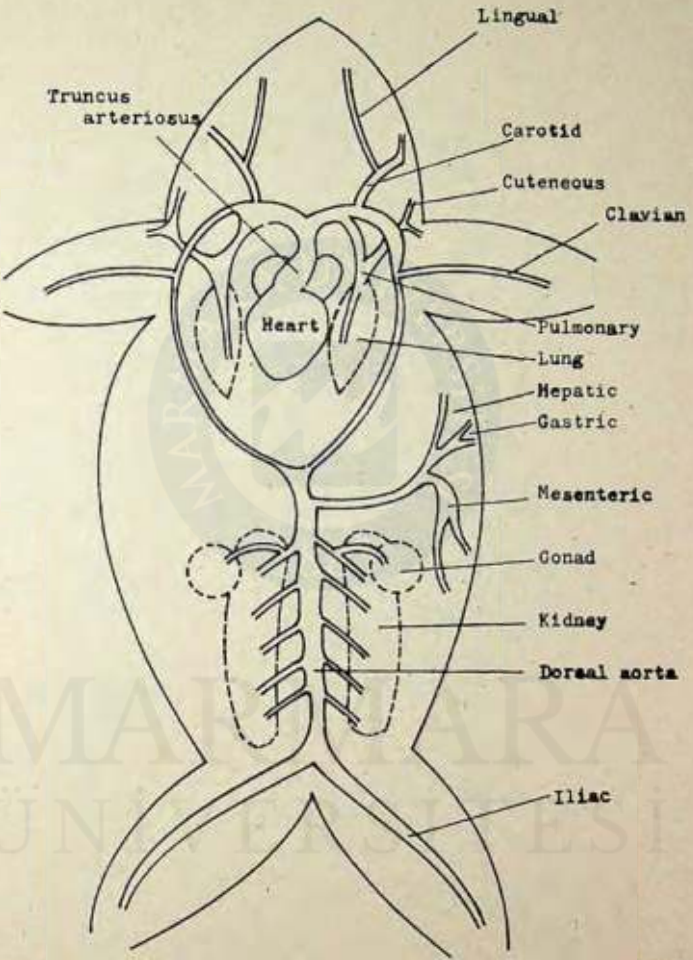


Figure 23. Arterial system of a frog.

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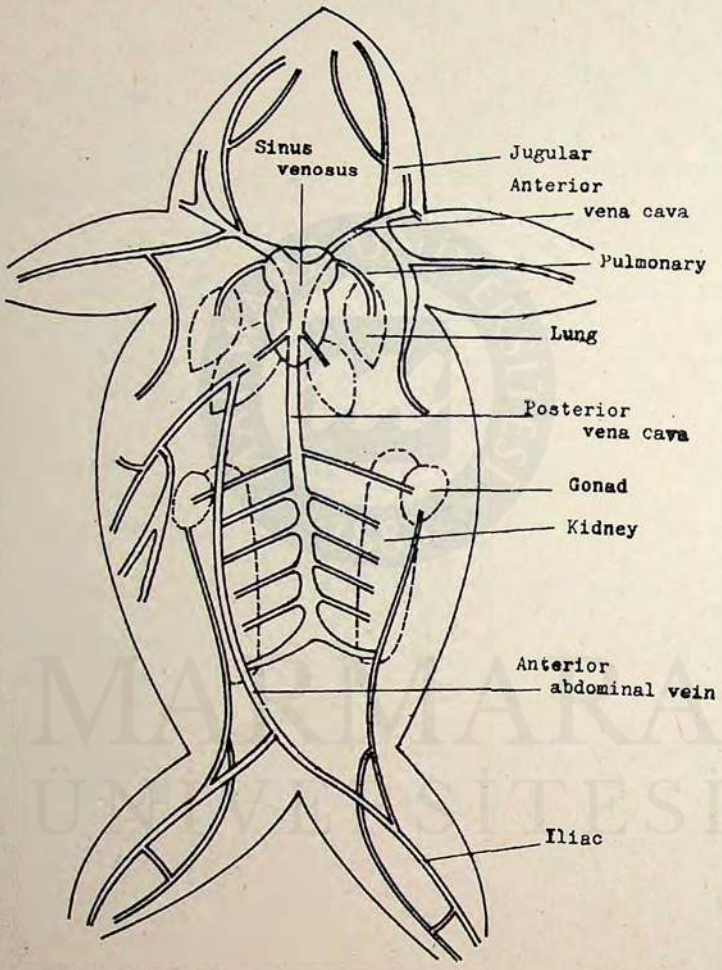


Figure 24. Venous system of a frog.

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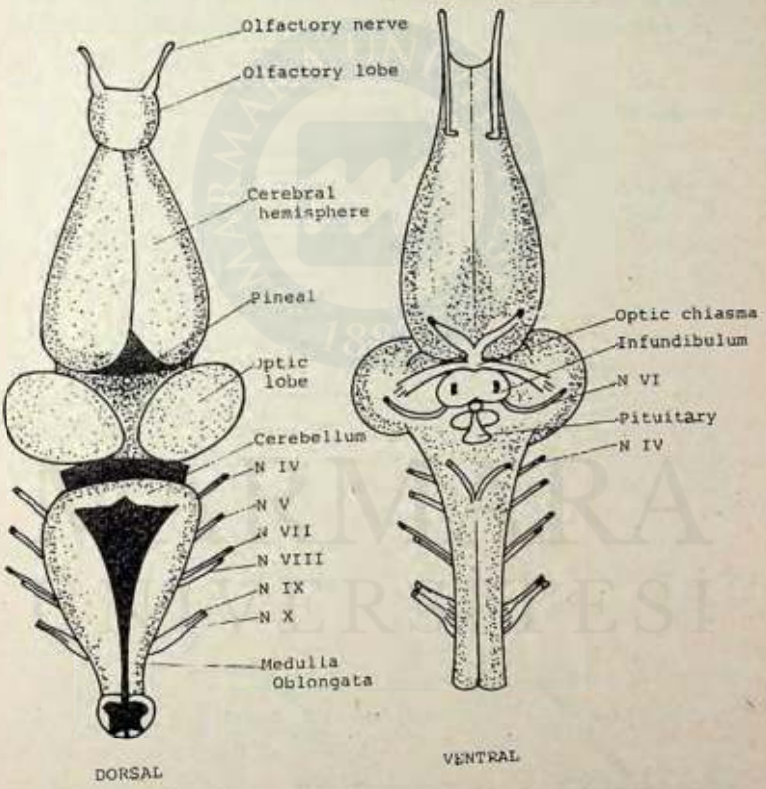


Figure 25. Brain of a frog.

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CELLULAR COMPONENTS OF THE BLOOD

The cellular components of the blood are red blood cells (**erythrocytes**), white blood cells (**leukocytes**), and platelets (**thrombocytes**). Of these, the red blood cells are the principal vehicle of O_2 and CO_2 transportation. The white blood cells, of which five different types are commonly distinguished, are active mainly in combating bacterial infections. The platelets release substances at the site of injury, involved in the blood clotting.

Method Of Making A Blood Smear

Obtain two clean microscope slides. Wash the ball of one of your fourth fingers with a piece of cotton soaked in 70 % alcohol, and puncture the skin with a sterile lancet, as demonstrated by your instructor. As the first drop of blood oozes out of the wound, place a drop of blood near one end of first slide and immediately (before the blood has had a chance to coagulate) touch an end edge of the second slide to the middle of the second slide and draw it at an angle towards the drop of blood (Figure 17). As the second slide contacts the drop of blood, it will spread out in the acute angle formed by the two slides. Then push the second slide forward along the surface of the second slide, whereupon the blood will become spread out in a thin film, or smear. Set the smear aside to dry, and wipe your finger free of blood with a piece of dry, sterile cotton.

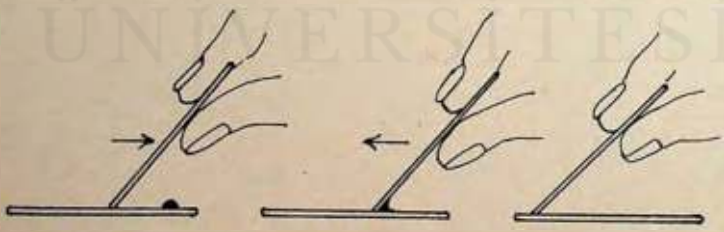


Figure 26. Method of making blood smear.

While holding the smear slide over a staining tray or a sink, place a few drops of Wright's stain on it (appendix I); wait for about 2 minutes and then place an equal number of drops of distilled water on the stain. Mix the stain and the water by tipping the slide from one side to the other; let it stand for 4 minutes and then spill off the excess fluid. Wash the slide with a few drops of additional distilled water, spill it off, and allow the slide to dry. Then examine it without a cover glass, using high power, or oil immersion lens of your microscope. The majority of the cells on the slide will consist largely of red blood cells (erythrocytes), which should appear pinkish. The leucocytes will appear in far fewer numbers than erythrocytes. How do they differ in appearance from the erythrocytes?

Red Blood Cell (Erythrocyte) Counts

The formed elements of the blood include the red blood cells, the white blood cells, and the platelets. Any change in the normal number of these formed elements is important to the clinician, because it indicates that certain physiological changes are occurring within the body. Thus, in many cases, it is important to make a count of the RBC. The normal values are:

	RBC	WBC
Male	5,000,000 / mm^3	5,000 - 10,000 / mm^3
Female	4,500,000 / mm^3	5,000 - 10,000 / mm^3

Procedure:

For all routine blood work, blood is usually taken from the fingertips. The fingertip must be perfectly dry to prevent the blood from flowing off. Discard the first drop. You are now ready to prepare the blood for cell counting. Materials needed to make counts are counting chamber, diluting pipettes, and microscope.

Red cell count is made in the central primary square. Always find the central primary square under low power and then switch to high. The central primary square is divided into 25 squares (tertiary squares), and each tertiary square is divided into 16 squares called **quaternary** squares. The red cells are usually counted in the five groups of 16 small squares in the center and four corners of the central square millimeter.

To avoid counting a corpuscle twice, those on a line are counted only when they are on the top and left lines, or on the bottom and right lines (Figure 27).

Red cell diluting fluid is 2 1/2 percent solution of sodium citrate.

Filling Pipettes

Use a clean and perfectly dry pipette; be sure it is the correct pipette. Obtain a fresh large drop of blood. Remove cork from diluting fluid bottle. Grasp pipette near point and place on drop of blood and draw up. Keep graduations on pipette facing you; pass into diluting fluid in horizontal position and draw up; place pipette in vertical position and rotate it so that air bubbles don't form. Remove pipette at a horizontal position; plug one end with finger and remove rubber tube; plug other end with thumb and shake. Shake pipette for 2 min. Set up hemocytometer; place cover slip on surface; using thumb as control, discard first two drops of blood. Place tip below cover slip at incline of charging surface. If a good charge is received, count 1/2 min before going into counting of cells.

RBC Counts

Draw blood to 0.5 mark, diluting fluid 101 mark. Shake for 2 min. Discard first two drops. Charge chamber. Allow cells to settle for 1/2 min before examination. Check distribution of cells under lower power. Transfer to high dry power - 40X (diaphragm always closed in red and white cell counting). Make contact with slip. Look in eyepiece and focus up. Go to every corner of tertiary square. Add four zeros to total number = total number of cells per cubic millimeter of blood (same as multiplying total count in five tertiary squares by 10,000).

Human Blood Groups

The surface of human red blood cells (erytrocyte) contain a wide variety of **antigens** (glycoprotein) that vary from individual to individual. Each surface antigen may exist in several different forms, and each form is produced by a different allele of a specific gene (multiple allelism). Thus there are several different **blood group systems.** The best known among these systems are the ABO and Rh systems. The antigenic deter-

minants of each of these systems are specified genetically by alleles of a single gene.

If we consider ABO system, we see that all people in the human population fall into one of four blood groups: A, B, AB and O. Each blood group is characterized by a specific glycoprotein antigen on the surface of the red blood cells. Thus group A people have antigen A on their RBCs; group B people have antigen B on their RBCs; group AB people have both antigen A and B; and 0 people have neighter antigen A nor antigen B.

Human blood plasma (serum) also contains some proteins (antibodies). For example, group A people have an antibody (anti-B) in their blood plasma that specifically combines with the red blood cell antigen of group B people. This causes clumping or **agglutination** of the red blood cells. Antibodies (agglutinins), for each type of blood designated as Anti-A and Anti-B. Thus, if blood from one individual is mixed with blood or plasma containing corresponding antibodies, the red blood cells clump together, or agglutinate, due to the adhesion of their antigens. Obviously, a person's blood cannot contain the antibodies corresponding to his own blood cells, but his/her plasma normally contains antibodies for all other antigens in the series. When blood transfusions are to be given, then, it is always best to obtain a donor of same blood type as the patient

Summary of blood proteins in human ABO blood groups

Blood type	Antigen on Red blood cell membranes	Antibody in blood plasma
A	A	Anti - B
B	B	Anti - A
AB	A and B	Neither
0	Neither	Anti-A & Anti-B

The Rh blood groups differ from the ABO groups in that the ABO antibodies are always present in the blood, whereas Rh antibodies are produced only when foreign Rh antigens enter the body. Rh positive people have an Rh antigen on their blood cells. People without Rh antigens are Rh-negative. Normal human blood does not develop the anti-body

(anti-Rh) until the individual is exposed to it. If an Rh-negative person is accidentally given a transfusion of Rh-positive blood, anti-Rh antibodies will develop in few months period in the Rh-negative person. Any Rh-positive blood that subsequently enters this person's circulation will agglutinate.

Blood Typing

An individual's blood group can be determined by mixing a sample of his blood with a series of sera each containing a reagent corresponding to a specific type of antibody. The reagents contained in the sera are anti-A (corresponding to type A antibodies), anti-B (corresponding to type B antibodies), and anti-D (corresponding to Rhesus antibodies, also called anti-Rh).

1. Each student will be provided by a white plastic plate which is divided into three sections with a wax pencil and each section labeled as A, B, D.

2. Obtain a drop of blood by pricking your thumb or finger with a sterile lancet.

3. Place a drop of blood into each section on the plate. place a drop of sera anti-A, anti-b and anti-D respectively onto blood drops. Mix each of the mixture with a clean toothpick or similar object.

4. After about half a minute, observe each drop on your slide.

If agglutination occurs the blood samples in the test panel will form red streaks and clumps which were resulted from the agglutination of red blood cells. If no agglutination occurs, the erytrocytes will be scattered uniformly throughout the liquid. The results can be interpreted as follows:

Agglutination in the anti-A panel means you belong to group A.

Agglutination in the anti-B panel means you belong to group B.

Agglutination in both anti-A and anti-B panels means you belong to group AB.

Agglutination in neither anti-A nor anti-B panels means you belong to group 0.

Agglutination in the anti-D panel means you are Rh-positive.

No agglutination in the anti-D panel means you are Rh-negative.

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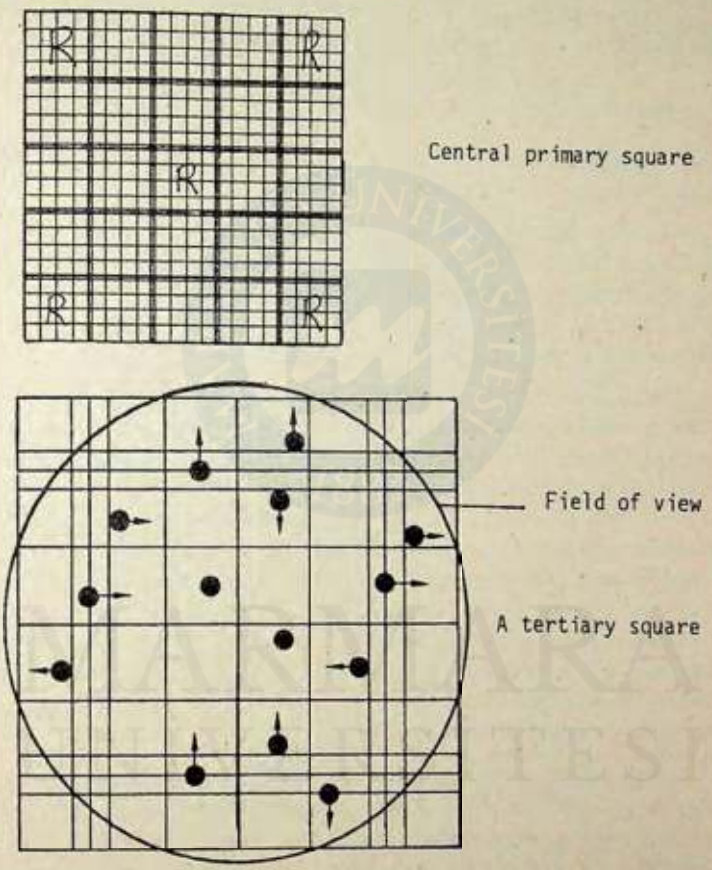


Figure 27. A blood cell counting area in a counting chamber (Hemocytometer)

EXERCISE: 14

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OXYGEN CONSUMPTION IN RELATION TO AMBIENT TEMPERATURE

Energy is released by cell respiration (tissue or internal respiration) which generally involves the oxidation of sugar:

$$C_6H_{12}0_6 + 60_2 \xrightarrow{\text{Enzymes}} 6H_20 + 6C0_2 + \text{Energy} + \text{Heat}$$

All the biochemical reactions occuring within cells are in some way related to this fundamental process.

The quantity of 0_2 consumed or CO_2 produced by a respiring animal is a measure of the sum total of all the biochemical processes (metabolism) taking place in the animal. Thus by carefully measuring the rate of 0_2 usage or CO_2 production, the role of metabolic activity in an organism can be determined.

Most animals, and plants as well, have such poor insulation that their internally generated heat is lost to the environment before their own body temperature rises; similarly, if the environmental temperature rises, they have no mechanisms to keep their body temperature at a lower level. Such organisms are called **Ectothermic** (cold-blooded or **Poikilotherms**) because their body temperature is controled principally by external factors. A few groups, notably birds and mammals, regulate their body temperature against environmental fluctuations. They conserve their metabolically generated heat when ambient temperature is low and they lose heat by evaporative mechanisms such as sweating and parting when ambient temperature rises. These animals are called **Endothermic** (warm-blooded or **homeotherms**).

When the environment is cold, an endotherm often has to generate extra heat as well as conserve heat better by such means as ruffing its feathers or raising its fur. Extra heat is the consequence of an increased metabolic rate and this will be reflected in increased oxygen consumption. An ectoderm, in contrast, will lower its metabolic rate and oxygen consumption in a cold environment. Why?

This laboratory offers you the opportunity to study the metabolic consequences of altered ambient temperature in an endoderm (a mouse)

and an ectoderm (a frog). We will provide you with a simple device for measuring oxygen consumption, a water bath at room temperature and another containing ice water, and animals.

The Apparatus: Several different methods may be used to measure respiratory rate (respirometry). Most methods involve either chemical, instrumental, or volumetric measurements of respiratory gases. In this laboratory session you will measure the volume of oxygen consumed by using a simple device called **Manometer** (Respirometer)

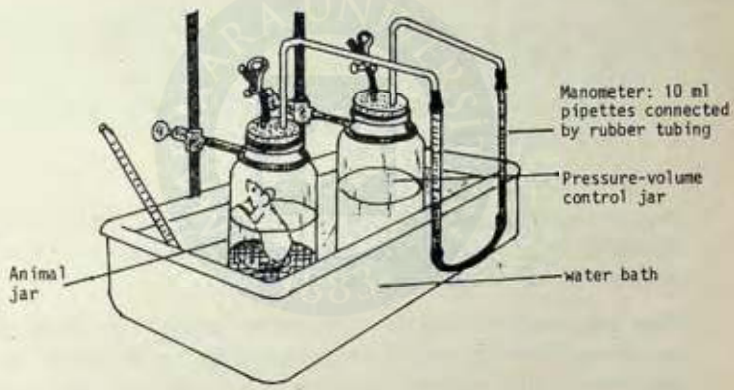


Figure 28. A respirometer for measuring oxygen consumption in small animals.

The respirometeres you will use, operate on a very simple principle **Volume change.** As an organism respires, it utilizes oxygen and releases carbon dioxide. If the carbon dioxide is eliminated from the container in which an animal is placed, then the diminishing supply, of oxygen can be measured as the movement of stained fluid in a manometer connected to the otherwise tighty sealed animal chamber. Note that the carbon dioxide absorbent, soda lime, is caustic and must be kept out of reach of the animal. It is separated by a wire screen in the animal jar.

Exercise:

During this laboratory period, you and your laboratory partner will work together on different animals. Watch out for

a. leaks in the system;

b. incomplete equilibration of the chamber temperature;

c. excessive activity of the animal.

In working out the exact protocol, remember that;

a. time has to be allowed for the animal to consume a measurable amount of 0_2;

b. time has to be allowed for the chamber air to reach a temperature other than room temperature, and

c. time has to be allowed for the animal to be affected by the altered temperature.

The protocol can be made as following:

1. **Room temperature:** Adjust the temperature of the water bath to that of the room air and maintain it within ± 1°C during the test period by addition of cool or warm water.

2. **High temperature:** Place the animal (in its container) in a warm water bath at about 35-40°C for a minimum of 30 min. Adjust the temperature of the water bath by addition of hot water and maintain it within ± 1°C during the test period.

3. **Low temperature:** Place the animal (in its container) in an ice filled bucket or in a cold water container (5-10°C) for about 30 min.

After an animal and the water bath have been brought to the correct temperature, place the animal in the respirometer and allows the system to come to equilibrium for 5 min with the vent tubes open. Then close the vents with pinch clamps. Record the initial height ou the stain level (in mm) in the arm of monometer after an exactly measured interval of 1-10 min, again record the height of the stain level in the manometer. Now release the vent clamps, and allow the stain columns to equilize. Make three such measurements of oxygen consumption for each animal, and calculate the average value.

Metabolic rate can be calculated as following:

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1. Measurements of oxygen consumption are made simply by measuring the difference (in mm) in the stain levels in the two arms of the manometer. This will give the volume of oxygen consumed (in ml) during the test period.

2. Divide the volume of oxygen consumed by the time of test period, and convert to ml of oxygen consumed per hr. For example:

$$\frac{3 \text{ ml } O_2}{5 \text{ min}} \text{ X } 60 \text{ min} = 36 \text{ ml } O_2/\text{hr}.$$

3. Determine the average O_2 consumed value.

4. Divide average consumption rate (ml O_2/hr) by the weight of your animal in Kg to obtain the "Metabolic Rate" in ml O_2/Kg body weight/hr.

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EXERCISE: 15

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ANIMAL REPRODUCTION AND DEVELOMENT

Animal organisms reproduce in two different ways, **sexually** and **asexually.** Asexual reproduction is common among unicellular and simple colonial animals. In many lower animal groups, both sexual and asexual reproduction occur but, sexual reproduction is the only known reproductive mechanism among vertebrates.

Many variations in sexual reproduction of animals exist, but all involve the **fusion of the gametes** (sex cells): The egg, female gamete and the sperm, male gamete. The gametes are produced in specific sex organs called **gonads** (ovary and testis).

The sexual reproduction process is initiated by the maturation and release of gametes. As both the egg and sperm mature, they must complete the process of chromosome reduction from 2N (diploid) to N (haploid) through a meiotic divisions. During the maturation process most of the egg cells also accumulate great amount of yolk material into their cytoplasm and are furnished with new organelles. Thus a mature egg is larger than a mature sperm.

The fusion of gametes is celled **fertilization,** and the fertilized cell produced is called the **zygote.** The zygote contains the mixture of genetic programs of two parents. The new organism then arises by mitotic divisions of the zygote.

EMBRYONIC DEVELOPMENT

Development is a continuous process, it starts when a sperm fertilizes an egg, and ends with death. Between the beginning and the end of this process a sequence of progressive changes occur, ranging from early embryonic development to changes in sexual maturity and aging.

In this laboratory session you will have an opportunity to compare early embryonic development of different vertebrate and invertebrate species.

There are cosiderable variations in the details of development among animals but in most cases the early stages are quite consistent

and basic pattern is similar. The early development in animals involve three kinds of activities:

a. Thes increase in the number of cells and subsequent growth.

b. The differentiation of cells.

c. The morphogenetic activity.

The common sequential changes in all developmental patterns can be outlined as follows:

1- **Fertilization** involves the fusion of nuclei and other events associated with the union of egg and sperm cells (gametes) and the zygote (fertilized egg) forms.

2- **Cleavage** is the series of mitotic divisions undergone by the zygote. No growth occurs during early cleavage. The pettern of cleavage varies in different species of organims depending on the amount and distribution of yolk (food material) contained in the egg.

In egg with little yolk cleavage is total and completely divide the egg successively, 2, 4, 8, 16 etc. separate cells. **Isolecithal** eggs, such as those of echinoderms and mamals, have this type cleavage. So, also do the **moderately telolecithal** eggs of frogs. In the extremely **telolecithal** eggs, such as those of birds where there is only a tiny disc of cytoplasm on top of a large yolk, cleavage does not divide the entire ovum into separate cells. Cleavage here involve only the thin disc on top of the egg (Figure 29).

3- **Blastulation** is the formation of a mass of cells that, in most organisms, become arranged into a thin-walled sphere. The central cavity of the blastula is called blastocoel.

4- **Gastrulation** is characterized by morphogenetic movements that lead to the formation of the three distinct germ layers of cells, the **ectoderm, mesoderm** and **endoderm** from which the organs of the embryos are later formed.

5- **Morphogenesis** is the development of characteristic internal and external forms.

This exercise will give you an opportunity to study and compare the patterns of changes in Sea urchin, frog, chick and mammalian development using live or preserved material.

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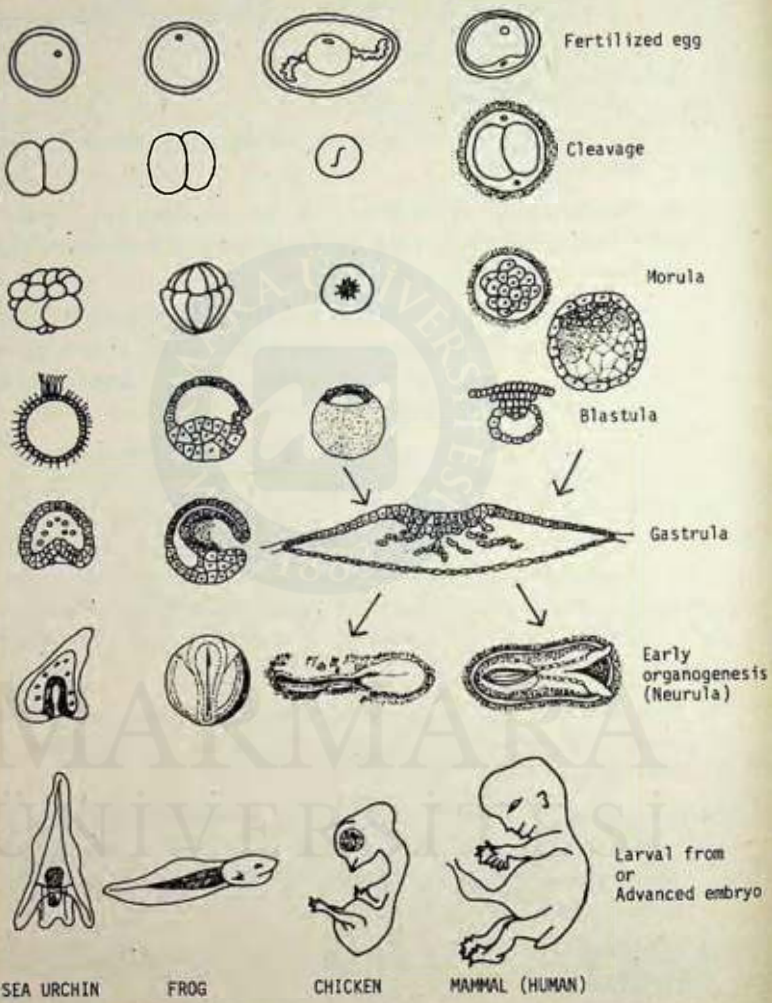


Figure 29. Variousdevelopmental patterns

REPRODUCTION AND DEVELOPMENT IN AQUATIC ENVIRONMENT

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A. SEA URCHIN (ECHINODERM) DEVELOPMENT

Study with living material: The laboratory instructor will demonstrate how gametes are obtained from live female and male sea urchins (Appendix 3).

Exercise:

1- With clean pipettes transfer egg and sperm suspensions into the separate depression slides and cover (or make wet mounts on regular slide, but support the coverslip with vaseline at the corners). Observe with low power objective.

2- While the eggs are beneah the microscope lenses add carefully a small amount of sperm suspension to the eggs on the slide, and examine immediately under high power objective. Note the time to follow the cleavage stages. It is not possible to view sperm penetration but the appearance of the fertilization membrane is a recognizable sign of fertilization.

The cleavages are very easily observed in sea urchin embryos. At 25 °C, in an hour the first cleavage begins and you will be able to follow two cell stage of the embryo.

You may need special instructions and controlled conditions to follow the continuing cultivation of sea urchin development. This is not usually possible in the laboratory which does not have supply of fresh sea water and temperature controlled pool for the cultivation. Therefore you will follow the further developmental changes of sea urchin from the preserved materials (prepared slides).

Stages of Sea urchin (or Sea star) development in preserved material

You are provided by slides of stained specimen illustrating echinoderm development. Observe the slides, compare with the photographs and drawings suplied in the laboratory. Record your observations including drawings of each stage (Figure 30).

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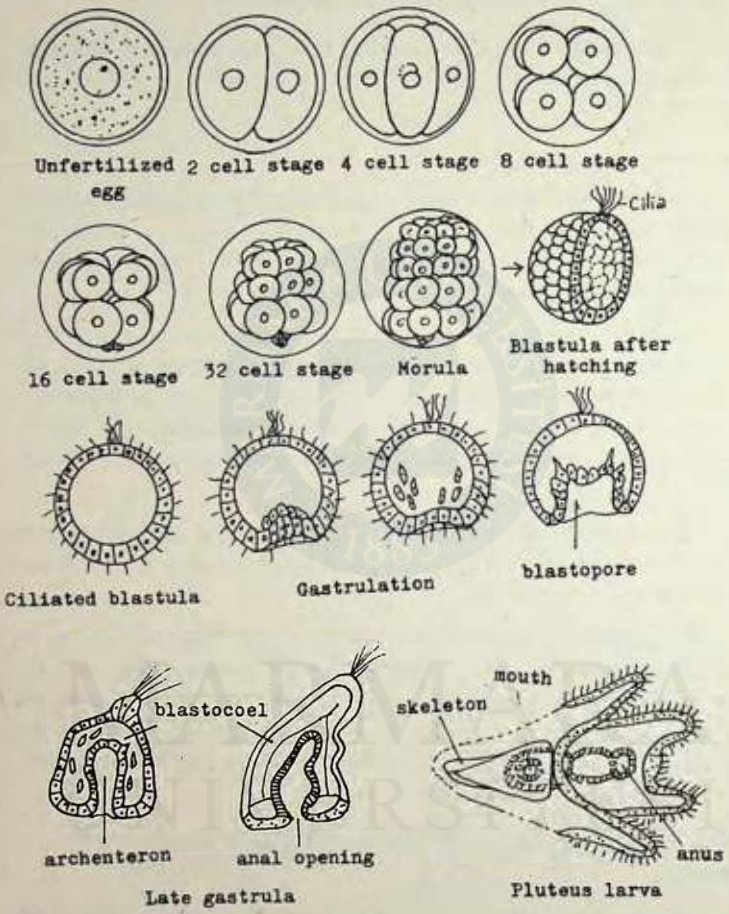


Figure 30. Sea urchin development

Unfertilized egg has a large nucleus and nucleolus. It contains small amount of yolk distrubuted evenly in the cytoplasm (isolecital equal yolk). Estimate the size of the egg. Are all eggs the same size? Why?

Fertilized egg (Zygote) contains smaller or unconspicious nucleus. The fertilization membrane may not be evident in preserved material. Differentiate it from the unfertilized egg.

Cleavage stages: Examine the slides of 2, 4, 8, 16, 32 and 64 cell stages. Is there any difference in size of these embryos compare to zygote? Are all these cells produced in these embryos of the same size? What has happened to the individual cell size?

As cleavage continues, the embryonic cells become arranged in sphere (hollow sphere), the **blastula.** The central cavity is called **blastocoel.** How does the size of the blastula compare to that of the zygote, to that of the early cleavage stages? What is the arrangement of cells? Do you detect any differences among the cells in size, in shape?

Cilia are present in the living blastula, and within 12-24 hours after fertilization the blastula freely swims.

Gastrulation in the sea urchin embryo comes about by an infolding or invagination of one side of the blastula. The cells of this region gradually work inward and upward until they lie in contact with the inner side of the cells of the opposite side of the blastula. Thus, two primary cell layers, an outer **ectoderm** and an inner **endoderm** are produced. The third primary layer **mesoderm** is formed by out-pocketings of the endoderm. Does the invagination occur just anywhere on the blastula surface or specific region of cells involved? Do the invaginating cells are different from the other cells of the embryo? How do they differ? In size? In shape?

The gastrula stage is reached within a day or two after ferilization.

Larval stages: The gastrula does not become an adult directly. Within a day or two the gastrula changes into a free swimming, feeding larva, **pluteus larva.** The gastrula - to - larva transition involves the development of digestive system with the appearance of a mouth opening and formation of skeletal elements. In several weeks to several months the larva grows and finally changes morphologically into a small sea urchin (or sea star). This process of conversion of form is called **metamorphosis.**

B. AMPHIBIAN DEVELOPMENT

In this exercise you will study the living embryo of frog which will be complemented by the preserved embryonic material. During this study, you will obtain fertilized eggs with artificial fertilization to follow the stages of embryonic development. You also will be provided by preserved, stained embryo sections on silides.

Induced ovulation and fertilization of frog

The mature frogs possessing fully developed ovarian eggs will have been experimentally treated to induce **ovulation** by your laboratory instructor (Appendix 4). The eggs will be stripped from these females at the beginning of the laboratory period.

Active sperms can be obtained by dissecting and mincing the testes of a wintering male frog in the frog ringer (or Holtfreter's solution or fresh pond water) until a milky suspension is obtained. This should be done about 1/2 hour before the fertilization and stripping the eggs so that active sperm will be available at the time the eggs are stripped from the female.

The unfertilized eggs: Obtain some unfertilized eggs from the culture dish, cutting them away from the rest of the egg mass with forcepts and scissors. Put them into a small petri dish and cover with pond water. Observe with dissecting microscope. Notice that the unferilized egg is a large cell with an outer surface half black (the pigmented hemisphere) and half yellow (the yolk-rich hemisphere). The nucleus is usually located near the pole of pigmented hemisphere.

Examine the cross sections of a preserved egg. Is the cytoplasm homogenous? Is there any difference in the location of particles with the different size (yolk particles)?

Fertilization of eggs:

To fertilize the eggs, drop the motile sperm suspension on to unfertilized eggs inserted into pond water in the petri dish or stripp the eggs from frog directly on to sperm suspention.

When egg is fertilized, it shrinks slightly, and a fertilization membrane appears. The eggs are then free to rotate within their membranes, and shift so that the animal pole is uppermost. If fertilization occur the

fertilized egg will rotate in ten to fifteen minutes after insemination (fertilization). The fertilized egg will have the pigmented hemisphere up and the yolky hemispere (more dense) under.

Cleavage Stages: (Figure 31).

At room temperature the initial cleavage occurs within 2 hours after fertilization. Study different cleavage sages of both live and preserved embryos and compare with sea urchin cleavage stages (Figure 30).

Does the first clevage completely divide the zygote into two equal cells? How does this compare to echinoderm cleavage? Observe the later cleavage stages and answer the following questions. What is the location of the cells undergoing more rapid cleavage? Where are the cells dividing more slowly? On what basis do you decide whether the cells of a region are dividing more rapidly than those of another area. What is the reason for the difference in the rate of divisions?

The Blastula: The presence of the blastocoel (internal cavity) identifies the blastula phase of development.

At what point in the sequence of cleavage stages did you first detect the presence of this cavity? How does this internal cavity compare to that of echinoderm?

The Gastrula: It is characterized by a fold or lip of cells that develops on the surface of the embryo. Surface cells develop inward turn and the progression of such a fold leads to cell layering and the formation of a new interior cavity (**gastrocoel**).

Because of the large amount of yolk, gastrulation cannot occur as it does in the sea urchin embryo, but the end result is the same as in the sea urchin the three germ layers are formed (**endoderm, ectoderm** and **mesoderm**).

Examine the whole embryos and the sectioned preparations at early and late gastrula stages.

Where does a new internal cavity (gastrocoel) form. How does it compare to that of echinoderm? What happens to the blastocoel? Does cell multiplication continue during gastrulation? Can you observe the different kinds of cells in the late gastrula? Are the different kinds of cells mixed in a random distribution. Or does each group or layer of cells generally consist of the same kind of cells?

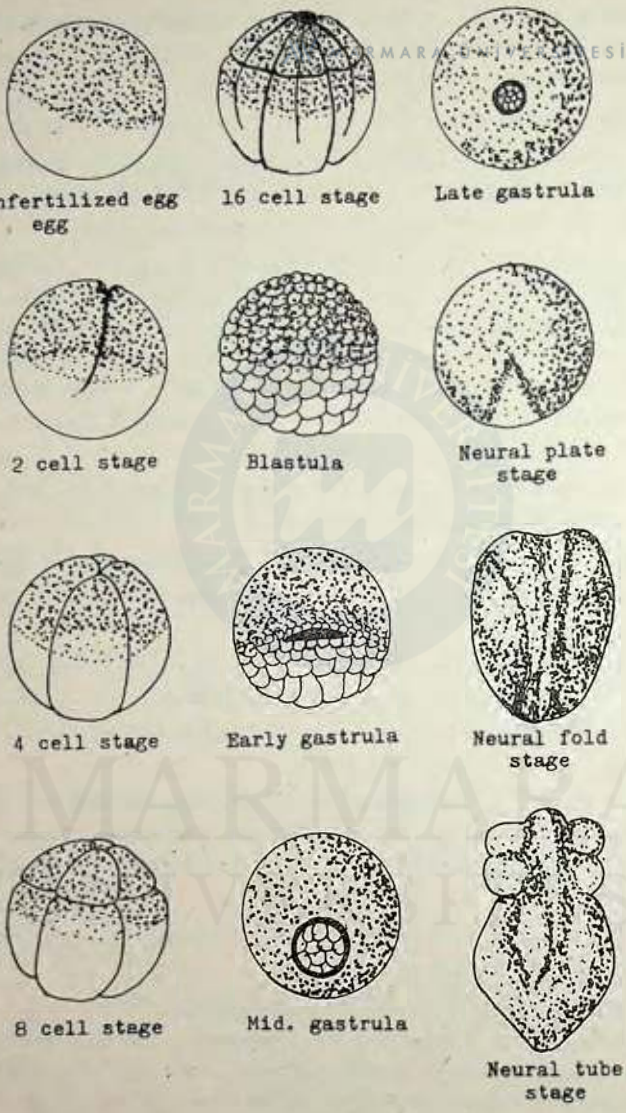


Figure 31. Frog (amphibian) development

Neurulation: After very late gastrulation, the embryo undergoes a general external reshaping which accompanies formation of neural tube. At this stage the cell differentiation and morphogenetic activity are evident.

2-3 days after the neural tube formation most of the other tissue-forming processes occur in the embryo and embryo develops into tadpole.

ANIMAL REPRODUCTION AND DEVELOPMENT IN THE TERRESTRIAL ENVIRONMENT

C. BIRD (CHICK) DEVELOPMENT

The egg cell released from the hen's ovary consists of the yellow yolk mass plus a small area of yolk free cytoplasm called **germinal disc,** which is located on the surface of the yolk. Because of the large amount of yolk, cleavage occurs only in this disc, and the embryo grows by absorbing raw materials for its metabolism from the yolk.

Since eggs are held in the terminal, portion of the oviduct for variable periods before they are laid, the stage of development at the time of laying varies with each egg. However, cleavage, blastula formation, and gastrulation have usually been accomplished by the time the egg is laid.

Because of the high concentration of yolk in the ovum, cleavage is partial, occuring only in the germinal disc. Gastrulation differs from that in the sea urchin or frog. Blastula is a flat plate of cells rather than a hollow ball (Fig. 32).

The yolk of the chick egg remains extaembryonic, but becomes enclosed within a membrane, the yolk sac, which grows out from the body of the embryo.

In the laborator, you will be provided by live and stained slides of whole mount 33, 48, 72 and 96 hours embryos. Observe these under dissecting microscopes. Notice the development of the following structures: Neural groove and neural folds - future brain region. Somites - blocks of mesoderm cells - future skeleton and striated muscles.

At 33 hour embryo notice the formation of the heart: Is there any beating or circulation? Has blood formed?

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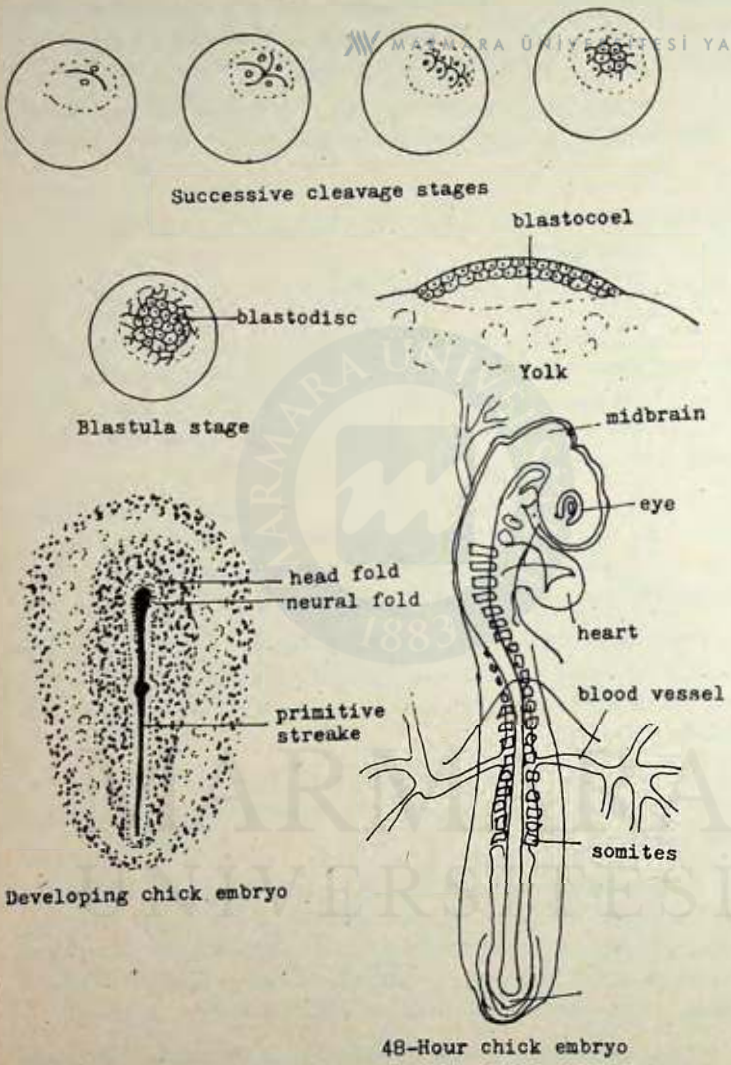


Figure 32. Chick (bird) development

EXERCISE: 16

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PLANT REPRODUCTION AND DEVELOPMENT

Plants exhibit three patterns of reproduction, **vegetative, sporulative** and **gametic**. Sporulative and gametic reproduction are major components of the life cycle, but the degree of vegetative reproduction may vary greatly among the various plant groups. All **mosses and vascular plants** possesses the following reroductive characteristics:

1- Distinct sprophyte and gametophyte generations
2- Multicellular sporangia that produce spores by meiosis
3- Multicellular sex organs
4- Dissimilar male and female gametes
5- A distinct multicellular embryonic stage for the sporophyte.

All plants that reproduce sexually follow a life cycle as seen below

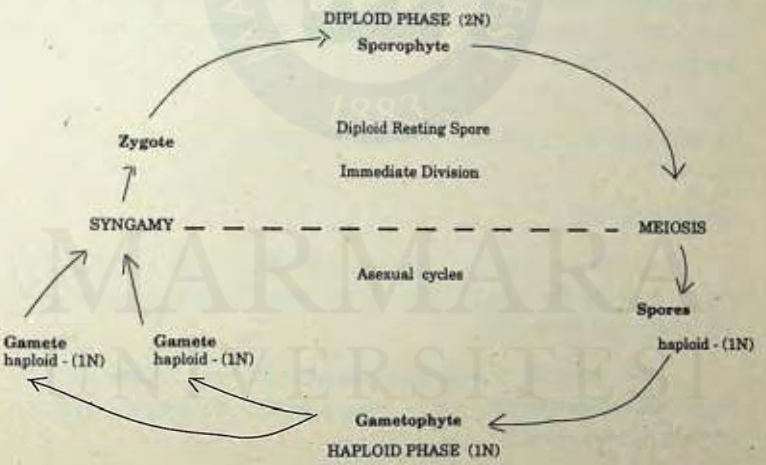


Figure 33. Life Cycle of Sexually reproducing Plants

In all plant life cycles, the haploid **gametophyte** phase always alternates with the diploid **sporophyte** phase. This gametophyte - sporophyte pattern is called an **alternation of generations.**

REPRODUCTION AND DEVELOPMENT OF MOSS (NON-VASCULAR PLANT)

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The distribution of mosses is limited to moist places because they require water for sexual reproduction. Male organs, **anteridia** and female organs **archegonia** occur, in season, at the tips of the gametophyte plants.

You will be provided by the fresh moss plants and its prepared slides in the laboratory. Examine both materials.

There will be a number of club-shaped antheridia and vase-shaped archegonia with a long neck and an egg containing base. Flagellated sperms, released from the antheridia, swim through the dew or in drops of water from the male to the female plant. Fertilization occurs in the base of the archegonium. The zygote is the new diploid sporophyte generation and begins to develop on the gametophyte plant (Figure 17). The sporophyte is a capsule - tipped stalk and meiosis occurs in the capsule, forming many haploid spores. Note that, the moss gametophyte generation is the dominant generation and the sporophyte is dependent upon the gametophyte.

THE REPRODUCTION AND DEVELOPMENT OF VASCULAR PLANTS

SEED PLANTS:

Cone bearing plants (Gymnosperms) (Figure 19) and flowering plants (Angiosperms) (Figure 20) exhibit several important reproductive characteristics. These are:

1- The sporophyte generation produces not one, but two tppes of spores; **microsporangia** produce **microspores** and **mega-sporangia** produce **megaspores.**

2- Microspores develop into **male gametophyte** (pollen grains). Megaspores develop into **female gametophytes.**

3- Pollen grains are transferred from microsporangia to megasporangia or associated structures by wind or insects. This process is called **pollination.**

4- The pollen grain forms a pollen tube which carries the sperm nuclei to the egg within the female gametophyte.

5- The **embryo sporophyte, female gametophyte and associated tissues** compose **the seed.**

Examination of the fresh speciments:

A pine or other conifer branch with cones attached, a simple typical flower (e.g. gladiolus, lilly) and the prepared slides of male and female gametophytes. Answer the following questions for each representative plant:

Which generation is haploid? Which generation is diploid? Which generation produces spores or gametes? Are gametes formed by mitosis or meiosis? Are spores formed by mitosis or meiosis?

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EXERCISE: 17

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POPULATION GENETICS

Artificial populations can be established with known genotypes in specific frequencies. By following these populations through several sexual generations, it is possible to relate any changes which occur in the genetic make-up of the populations to the variable or variables introduced into the experiment. A more feasible means of studying such change in the laboratory is to establish models of populations, and after making certain assumptions based on observations of natural populations, deduce the effect of a particular environmental or genetic factor in such theoretical systems. In this exercise you will experiment with a model system to illustrate how this can be useful in understanding the genetics of populations and their evolution.

There are a series of containers in the laboratory, each having two kinds of beans, white and black. Each container is labeled with a letter; there are different proportions of white and black beans in each one. Imagine that each represents a population of **Dosophila** which consists of **wild type alleles** for **grey bodies** (white beans) and **ebony alleles** for **black bodies** (black beans). Also assume that each bean is a gamete and that each population broods randomly. The question to be asked is: In a random breeding population, what will be the frequencies of the wild type allele and the ebony allele in the next generation?

1. Each pair of students should obtain one of the containers of beans. Stir the beans and extract two beans at a time to represent a mating. In order to asure randomness, do not look into the container while you make your selection. Record the type of mating on the available chart, such as wild X wild, wild X ebony, ebony X ebony. Indicate the proportions and kinds of alleles each cross (individual fly) would produce, for example, in a wild X wild cross, the resultant fly would form only wild type alleles for body color. Wild type + is dominant to ebony **e**; thus, in a wild X abony cross, the resultant fly would proportionately form 2 wild + alleles and 2 ebony **e** alleles via meiosis.

Repeat the selection process 25 times, stirring the beans before youselect two. When you have completed the sampling, tally your results to determine the total numbers of wild-type and ebony alleles that would

result from these matings. Since your sample of matings is a relatively small one, add your results to the tally from your population made in other lab sections. (In the next lab period, obtain from the lab instructor the results from all of the lab sections.) At this time, ask the lab instructor to tell you the frequencies of the + and e alleles in the original population.

MATTING NO.	TYPE OF MATING	ALLELES EXPECTED

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2. Using the same model of a population, assume that there is some problem in the mating process with the ebony gametes so that, on the average, every third ebony gamete fails to mate successfully. In your sampling, discard every thir ebony gamete that is drawn and raplace it with another gamete. If the next gamete you draw is an ebony one, it represents the first of a new series of three ebony gametes and is counted. proceed as you did before and record the results on the chart; also, add your results to the tally for all lab sections.

What effect does the selective advantage of the wild type alleles have on the composition of the population of the next generation? Is it possible to predict the effect of the wild type allele after a number of generations?

MATTING NO.	TYPE OF MATING	ALLELES EXPECTED

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3. The capacity of a particular genotype to survive and to reproduce, that is, to contribute its genes to the next generation, is called the **adaptive value** of the genotype. In any given environment, the most successful genotype is assigned an adaptive value of **1**. In our first experiment, we asumed that the matings with the wild type + allele (gamete) as well as the ebony **e** allele are equally successful in mating. If **q** represents the frequency of the **e** allele and **p** the frequency of the + allele, then in a random mating population, the following matings are possible:

PARENTAL GENERATION	FREQUENCY
$p + q = 1$	$p \times p$ ---- 4 p
	$p \times q$ ---- 2 p, 2 q
	$q \times p$ ---- 2 q, 2 p
	$q \times q$ ---- 4 q

The alleles from the matings are indicated on the right. If we tally these, we find that the frequencies of alleles in the new generation are identical to those of the parental generation, that is, composed of **p + q**. If we substituted an arbitrary value for example **0.6**, the value of **q** is **0.4** since **p + q = 1**. Substituting in the matings then yield.

0.6 X 0.6	0.36	(the frequency of this mating of p X p is 0.36 and gives only p alleles)
0.6 X 0.4	0.24	(gives 1/2 p or 0.12 and 1/2 q or 0.12)
0.4 X 0.6	0.24	
0.4 X 0.4	0.16	(gives only q)

The total **p** in the new generation will be **0.36 + 0.12 + 0.12 = 0.6;** the total **q** in the new generation will be **0.12 + 0.12 + 0.16 = 0.4.** Note that the frequency of the two alleles remains the same from generation to generation provided that there is (a) no change in the adaptive value, (b) no mutation ofalleles, (c) no new alleles introduced into the population by migration, and (d) a random breeding population.

In the second experiment, one out of three gametes carrying the e allele is incapable of mating and thus does not contribute its gene to the gene pool of the next generation. The adaptive value of the e genotype must be less than that of the + genotype, that is an adaptive value of less than **1**. The degree of disadvantage of a genotype is measured by **s**, the selection coefficient. In other words, the adaptive value of such a genotype is then **1 - s.** If **s = 0**, then there is no disadvantage. If **s = 1**, then the genotype is **lethal**. In our particular experiment, **s = 1/3** since one gamete out of three could not mate. The adaptive value of the **e** genotype must then be **1 - 1/3 = 2/3** (relative to the adaptive value of **+**).

The contribution of a particular genotype to the next generation depends on both its frequency and on its adaptive value; in other words, the frequency of **e** in the next generation (q_1) is equal to $(1\text{-}s)q_0$ where q_0 represents the frequency of the original generation. The frequency of **p** must then be **1 - (1-s)q.** In our particular example, $(1\text{-}s)q_0$ is equal to **(2/3) (0.4)**, thus $q_1 = 0.266$ and $p_1 = 1 - 0.266$ or **0.734**. The frequency of **q** in the following generation (q_2) will be $(1\text{-}s)q_1$ which in our example is **(2/3 (0.266)** or **0.177**. Thus we can arrive at a general formula for deriving the frequency of **q** after **n** generations, namely $q_n = (1\text{-}s)^n q_0$. In our example, $(2/3)^2$. **(0.4)** should give the value of q_2 or **0.177**. Now, if you compute the frequency of p_5 (the value of **q** after five generations), you will find that there is a continuing decrease in the frequency of **q**, and that this gene tends to be reduced in the population. So, selection plays a major role in determining the gene frequencies of populations; the environment acts as the selective force operating on the variability in the population to determine the levels at which it will be maintained, or to what extent it will be transmitted to the subsequent generations. This affects the hereditary constitution of populations, or in other words, results in evolution.

Calculate the frequencies of alleles in the new generation with data from your first and second experiment.

	1st Exp.	2nd Exp.
Frequency of **p**		
q		

In the second experiment, what is the adaptive value of the + allele? _____ of the e allele? _____

Using your data from the second experiment, what will be the frequencies of the + and e alleles after 3 generations? After 10 generations?

How do your actual results in the mating sample compare with the theoretical expectation on the basis of the formula given above?

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EXERCISE: 18

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DROSOPHILA BEHAVIOR - A RESPONSE (PHOTOTAXIS) TO AN ENVIRONMENTAL STIMULUS (LIGHT)

To be successful in nature, that is in order to survive and reproduce, organisms must be able to cope with their external environment and adapt to it. Adaptations can be morphological (structural), physiological (functional) or behavioral.

Organisms adapt themselves to changing external environment by making useful responses to changing environmental stimuli. Thus they keep their internal conditions as nearly unchanged as possible and react in ways which favor their survival.

Behavior is a biological process like any physiological process (digestion, circulation), it has genetic basis and like any genetic trait, it evolves. Behavioral traits, as with all other adaptations, are products of natural selection, which has acted through time to produce a population that tends toward the maximum possible fitness in its particular habitat.

The response of an organism to light as a stimulus, called **phototaxis,** may also influence the population structure and evolution of a species. Phototoxis is a complex behavioral response in **Drosophila**. Possessing complex sensory, nervous, and locomotor mechanisms, animals are capable of moving their body (fly) toward light (stimulus).

Exercise:

In the present exercise phototaxis response of different strains of Drosophila will be studied.

The phototaxis response will be measured by distribution of flies after they have selected one of two alternatives (dark or light) at a choice point in a Y tube. One arm of the Y tube is darkened by aluminium foil cover preventing penetration of light. The other arm is illuminated by an incandescent light bulb.

Introduce 10 males and 10 females into the Y tube. Count the number of flies in the dark or light arms of the Y tube after a certain period (15 minutes). Repeat the same procedure with another strain.

Is the reaction of flies to light random or do the flies prefer light or dark? Is there any difference between Drosophila strains and between the sexes with respect to light preference? How can you explain the differences in the light preferences between the strains? Is there any difference in mobility of flies in different strains? Are the differences due to genetical or environmental effects? Are these differences between individuals within the same strain in phototactic response?

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APPENDICES

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APPENDIX 1:

Solutions and Stains

Iodine solution: Iodine dissolves easily in water only when 1 % to 2 % KI is present.

Methylene blue: Dissolve 20 mg of Methylene blue in distilled water and dilute to 200 ml with more distilled water.

Aceto Orcein or Aceto Carmin stain: 1-2 % Acetic,Orcein in N Hcl solution.

Frog's Ringer solution:	Nacl	6.50 g
	Kcl	0.14 g
	$NaHCO_3$	0.20 g
	$NaH_2PO_4.H_20$	0.011 g
	$Cacl_2$	0.12 g
	$Cacl_2$	0.12 g
in	H_20 (DW)	1 lt

Dissolve each salt, separately in 200 ml, wait and mix, so calcium phosphate will not precipitate.

Holtfreter's stock solution:	Nacl	3.5 g
	Kcl	0.05 g
	$Cacl_2$	0.1 g
	$NaHCO_3$	0.02 g
in	H_20 (DW)	1 lt

(Use 10 % Holfreter's solution for pitiutary extract, fertilization and embryonic development.)

Benedict's reagent: Dissolve 173 g sodium citrate and 100 g $NaCO_3$ in 800 ml of warm water. If the resulting solution is not clear, filter it. Add water to make a total volume 850 ml. Dissolve 17.3 g of $CuSO_4$ in 100 ml of water and pour it into carbonate-citrate solution as stirring. Dilute the solution to 1 lt with distilled water.

Wright's stain: Dissolve 0,5 gm of Sodium bicarbonate in 100 ml of distilled water and add 1,0 gm of Methylene blue. Heat the solution, and keep it at the 100 °C for 1 hour. After cooling, transfer the solution to a flask of about 1 lt capacity and add 500 ml of 0.1 % solution of Eosin Y while shaking the flask.

Filter, collect the precipitale on the filter paper and dry it. The staining solution is made by dissolving 0.3 gm of the dried precipitale in 100 ml of pure Methyl alcohol. Alow to stand at room temperature 1 or 2 days.

Filter, and 200 ml of Methyl alcohol to reach 80 ml. 80 Use for staining.

APPENDIX 2:

Computing of RBC count factor of 10.000

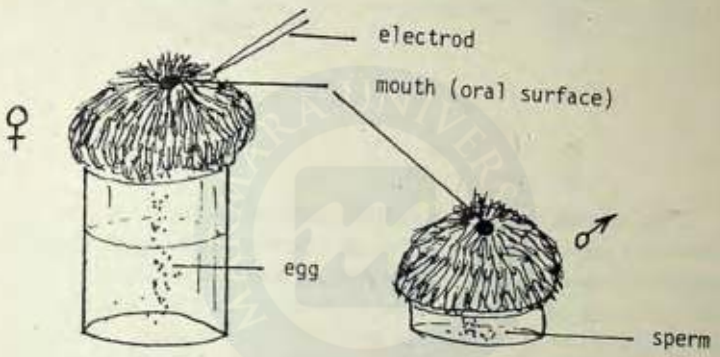
Red cell count is made in the four corner and center tertiary squares (R). Difference in count should not exceed 15. The diagram in Figure 18 indicates which square the particles are to be included in counting. Total the counts in the five tertiary squares (R). A central primary square has an area of 1 mm^3 (contains 25 primary squares). The count was made in 5/25 or 1/5 of the area, or 1/5 mm^2. The depth is 1/50 mm^3 = (1/5 mm^2 area x 1/10 mm depth). The standard count is for 1 mm^3 (to convert 1/50 mm^3 to standard, multiply by 50, that is 50 x 1/50 = 1 mm^3). The dilution factor is 200 or 100/0.5 = 200. To compare with standard count, multiply total count in the tertiary squares by, 10.000 (50 multiplied by 200).

APPENDIX 3:

Obtaining of gametes from sea urchin males and females

The sexes are not externally distinguishable, but the animals can be sexed and gametes obtained in several ways. One easy method in-

volves passing a 10-volt alternating current through the animal. The other method is to inject 2.0 ml of 0.5 M Kcl into body cavity through soft membrane surrounding mouth (oral surface). both methods bring about immediate shedding of gametes. Eggs are collected into a beaker containing sea water, and sperms collected into a petri dish as shown in the figure below.



Dilutions of egg and sperm solutions can be prepared before artificial insemination.

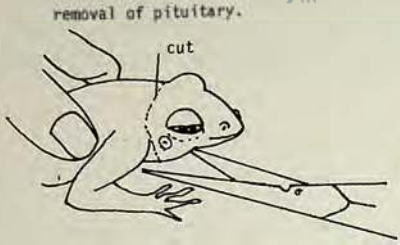
APPENDIX 4:

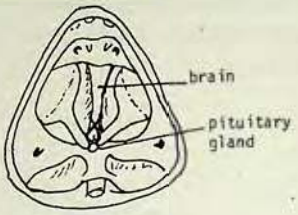
Induction of ovulation in Frog females

Frogs brought into the laboratory during winter or early spring months can be artificially induced to release eggs by injecting them with frog pituitary or pituitary extracts. This procedure stimulates the natural hormonal changes that occur in spring.

Pituitary glands can be obtained from mature females or males. pituitary glands of the females are about twice as potent as those of the males, and the required number of glands for injection varies with the season. The technique as shown in the figure below, involves the removal of pituitary.

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Ventral of skull

The pinkish, oval pituitary gland is attached to the brain at the anteroventral region of the brain. Cut the head from neck region. Hold the skull with a piece of tissue paper (it is slimy and can be easily slipped out of your fingers). Cut the skin at the top of the head. Make a shallow cut on the dorsal line of cartilageous skull with a razor blade. by holding with the tissue paper from two sides, open the skull, expose the brain.cut the optic nerves from 2 sides, invert the brain and expose the pituitary gland.

The pituitary is removed and sucked into a 18-gauge needle. Syringe and needle should be full with Frog Ringer's before the gland drawn into the barrel. The gland should stay in the needle (not in the Syringe).

Select the female to be injected and holding her in your left hand, insert the needle into the body cavity, and quickly inject the pituitary gland with a minumum amount of ringer solution. You may repeat the procedure for several pituitaries or collect the pituitaries inject at ones.

The eggs released into oviduct and remain there for several days, but they can be forsed out through the cloaca by gentle pressure on the frog's abdomen.

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