جامعة بيرزيت
BIRZEIT UNIVERSITY
Department of Biology
and Biochemistry

BIOLOGY 111 LAB MANUAL

Seventh Edition

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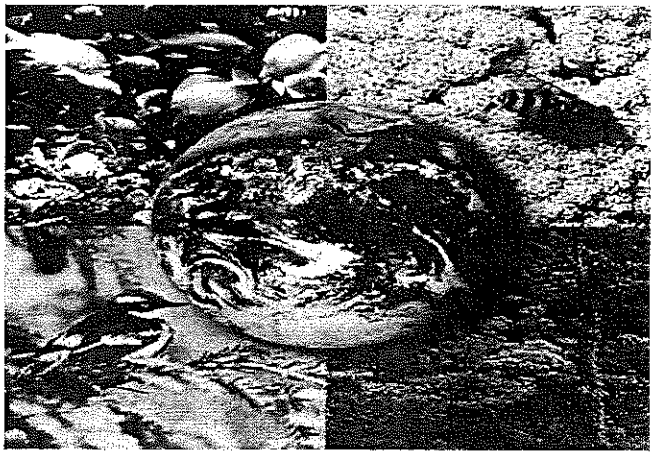

Prepared by: *Dr. Ahed Abdulkhaliq*
and Dr. Sabrin H. Albeituni

Reviewed by: *Sanade O. Barakat*

Edited by: *Dr. Johnny Stiban*

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Foreword

About the Authors

Dr. Ahed Abdulkhaliq is a Ph.D. graduate of Ohio State University, USA, specializing in quantitative animal genetics. His Ph.D. research focused on the estimation of genetic parameters for animal reproductive traits. He joined the department of biology and biochemistry at Birzeit University as assistant professor in 1979. He was awarded the Fulbright scholarship to join Oregon State University, USA as a postdoctoral researcher for one year. During his visit he worked on a major gene (the callipyge) which proved to be responsible for shifting the metabolism from fat to red muscle deposition. After that, he was promoted to associate professor. He joined Madrid University for his sabbatical year. His research focused on increasing milk production through cross breeding. He served as a chairperson of the department of Biology and Biochemistry for two terms. He has written the first few editions of this lab manual.

Dr. Sabrin Albeituni is a B.Sc. graduate of our department in 2008. She served as a teaching assistant in the department for 2 years after her graduation. During her work as TA, she developed new experiments and had written an excellent manual, which we have been using since 2010. She was awarded Fulbright scholarship to study in the USA, where she obtained her Ph.D. in Microbiology and Immunology at the University of Louisville. She is currently doing post-doctoral work at St. Jude Children's Research Hospital where she is studying the regulation of T cell responses by myeloid cells during inflammation. Dr. Albeituni was an integral figure in the success of this course and the department.

Sanade Barakat graduated from our department in 2012 with a B.Sc. degree in Biology. She is the longest serving teaching assistant in the department. Having published her first research in the genotoxicity assessment of treated wastewater under the direction of Prof. Khalid Swaileh, she is currently active in a research allied with β-casein variants in local cows under the supervision of Dr. Ahed Abdulkhaliq. At present, she is a graduate student in the department's Master's Program in Environmental Biology (MPEB). Her dedication to helping teachers and students is unparalleled, as throughout her five years she has drove innovation and creativity in the process of teaching in our labs, which drew great interest in new students entering Biology. She reviewed this new version of the manual thoroughly and had a great impact in suggesting new and improved experiments.

Dr. Johnny Stiban is a former chemistry student at Birzeit University. He received his B.Sc. degree in biochemistry from the University of Maryland College Park, USA. He obtained his Ph.D. degree under the tutelage of Prof. Marco Colombini at Maryland where he helped discover ceramide channels in mitochondria. He joined the department in 2009 and continued working on his research in areas of apoptosis, ceramide biochemistry and mitochondrial DNA metabolism in collaboration with Prof. Laurie Kaguni at Michigan State University. He edited the fifth edition of the manual when he worked closely with Sabrin to finalize the product. As a chairperson of the department he embarked on updating the sixth edition of the manual with new and improved experiments.

Activity One

The Microscope

INTRODUCTION

Microscope (*micro:* small, *scope:* to see) is an instrument used to magnify and resolve the image of objects that are too small to be seen by the naked eye. It is considered one of the most important tools in biology, for being essential in exploring and studying the structures of different biological systems, such as, cells, microorganisms, etc. In general, a microscope consists of a system of lenses that focuses the illumination on the specimen under study and then magnifies its image that will be seen by the viewer.

The **magnification** of an image is defined as the *ratio* of the size of image to the real size of object:

$$\boldsymbol{Magnification = \frac{image\ size}{object\ size}}$$

When using a compound microscope, the total magnification can be calculated as follows:

Total Magnification = magnification of the eyepiece x magnification of the objective

In addition to enlarging the image of an object, it is important to obtain a clear image where its details can be resolved. **Resolution** is the shortest distance between two objects that can be distinguished as separate. Resolution is a function of three parameters: the **wavelength** of the light, the **angular aperture** of a lens (Figure 1.1), and the **refractive index** of the medium surrounding the specimen. The resolution is quantitatively described in the following equation:

$$r = \frac{0.61\lambda}{n \sin \alpha}$$

where r is the resolution, λ is wavelength of the light, n is the refractive index of the medium between the specimen and the objective lens, and α is the angular aperture of the objective (Figure 1.1).

Note that (n sin α) is the **numerical aperture** of the objective lens (NA), and it is specific for each lens, therefore the previous equation can be simplified as follows: $r = \frac{0.61\lambda}{NA}$

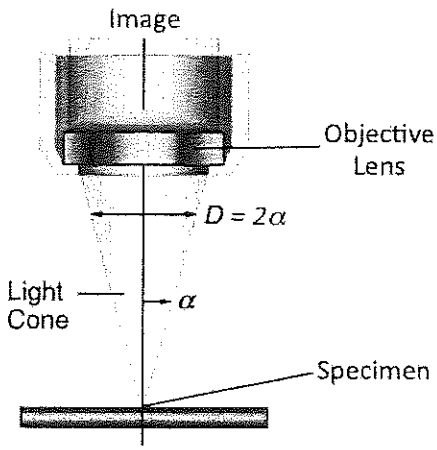


Figure 1.1: The angular aperture of the objective lens. It is half the angle of the light cone projecting from the specimen to the objective lens. (Adapted from: http://philschatz.com/physics-book/contents/m42491.html)

The resolution of the microscope is limited by the wavelength of the light being used. For example, the resolution value that can be obtained theoretically using a light microscope, with a lens with NA 0.94 and a source of light with λ = 450 nm is approximately 300 nm. Techniques to improve the resolution of light microscope were developed, such as using an oil immersion lens, to obtain a resolution of approximately 200 nm. This means that, the viewer can distinguish between two points in the specimen as being separate if the distance between the points is 200 nm or more.

There are two main types of microscopes based on the source of illumination used: **Light Microscope** and **Electron Microscope**.

I- Light Microscope:

As its name refers, uses light (photons) as a source of illumination. There are different types of light microscope for different purposes. Examples of light microscopes include the compound microscope and the dissecting microscope. Compound microscopes can be used in different forms of microscopy such as: bright-field microscopy, dark-field microscopy, ultraviolet microscopy, phase contrast microscopy, differential interference contrast microscopy, fluorescent microscopy, etc. In this laboratory, you will be introduced to two forms of light microscope, the compound microscope and the dissecting microscope.

A) Compound Microscope: It consists of the following parts (Figure 1.2):

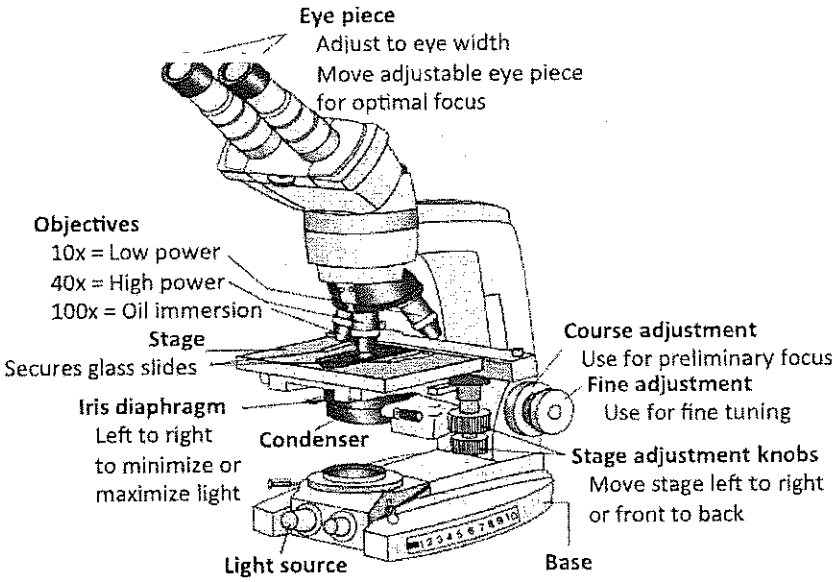


Figure 1.2: The main parts in a compound microscope (Davis, 2007).

1- Base: it is also called the supporting stand. It rests on the bench and is generally horseshoe-shaped.

2- Illuminator: it is the source of light. Follow the path of light (Figure 1.3) to understand how the image is magnified by a compound microscope.

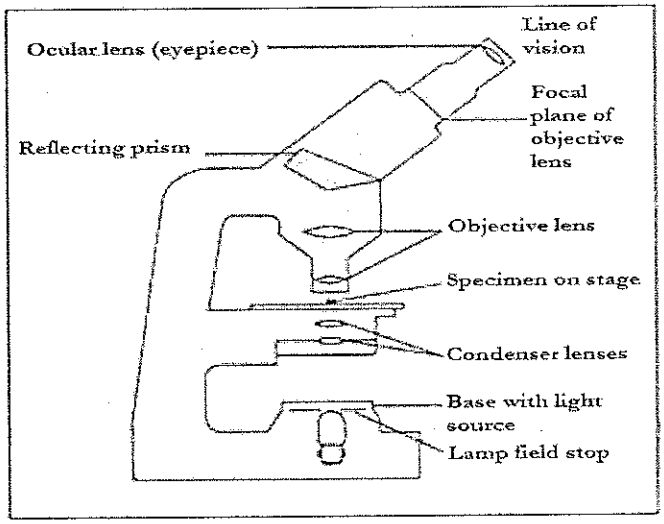


Figure 1.3: The path of light in the compound microscope.
(From www.ncbi.nlm.nih.gov/bookshelf/picrender.fcgi)

3- Iris diaphragm: is located below the condenser, and it consists of a circular structure with an aperture that can be adjusted to control the amount of light that reaches the condenser; therefore, the student can change the field brightness and contrast. This structure resembles the iris in the human eye with an aperture (the pupil).

4- Condenser: consists of one or a system of lenses that focuses light onto the specimen located on the stage. The specimen image is then projected onto the objective lens.

5- Arm: for use when carrying the instrument.

6- Stage: carried on the arm and serves as a support for the microscopic slide being examined. The specimen mounted on the slide must be centered over the opening in the center of the stage. Sometimes two clips are used to hold the slide in place. The slide can be moved in the *xy* planes with a knob attached to the stage. The distance between the stage and the objectives can be adjusted using the coarse and fine adjustment knobs.

7- Course adjustment: is a large knob located at both sides of the microscope. It is used to move the stage upwards and downwards quickly, bringing the image of the specimen approximately into focus. This adjustment should **ONLY** be used in conjunction with the scanning objective lens, to prevent the slide from hitting the objectives.

8- Fine adjustment: is a small knob located at both sides of the microscope. It is used for accurate focusing, allowing the details of the specimen to be seen. It can be used with all the objectives, especially when changing from one objective lens to another.

9- Objective lenses: are lenses attached to the nosepiece. There are four objective lenses with different magnification powers: **scanning lens (4x)**, **low power lens (10x)**, **high power lens (40x)**, and **oil immersion lens (100x)**. When using the oil-immersion lens, a drop of cedar wood oil is added on the specimen increasing the magnification and resolution of the microscope. In this lab, the oil immersion lens will not be used. It is important to note that the objectives are subsequently used starting from the scanning lens (4x), then the low power lens (10x), and finally the high power lens (40x).

10- Eyepiece or ocular lens: it is a lens inserted at the top of the body tube, and is used to further magnify the image obtained from the objective. Its magnification is written on it (usually **10x**). It can be replaced with an eyepiece of lower or higher power. An eyepiece micrometer and a stage micrometer can be used, as a ruler to allow the measurement of specimen dimensions. It is important to adjust the **interpupillary distance** (the distance between the center of the eyepieces) to see one only one image.

11- Body tube: it is a tube to connect the eyepiece with the revolving nosepiece.

12- Revolving nosepiece: is the structure to which the objective lenses are attached. It rotates to bring the desired objective into position.

General Instructions when using a compound microscope

1- Lift the microscope by placing one hand under the base and the other on the arm.

2- Place the microscope at a comfortable distance from the edge of the bench *(Do not slide the microscope from place to place; you must pick it up as indicated).* Plug in the light and turn it on. You can control the light intensity according to your sight; usually it is about two-thirds of the maximum.

3- Make sure that the low power objective is in place. You will feel a click when the objective is in position.

4- Clean the microscope lenses with lens paper.

5- While looking through the ocular lens, open BOTH eyes. Make sure that the interpupillary distance is suitable and properly adjusted, so that you see a single circular field of vision.

6- Place the slide on the stage, with the specimen over the stage hole. Use the clips to hold the slide in place.

7- To obtain a clear image, first start with scanning objective lens (4x). Use the coarse adjustment to obtain an approximately clear image, and then use the fine adjustment to sharply focus the image.

8- Once the image is in focus, you can change the objective to low power (10x) by rotating the nosepiece, until the objective clicks in position. The image usually gets out of focus, to obtain a clear image ONLY use the fine adjustment. The same procedure is applied when using high power objective (40x).

9- Adjust the condenser to its position, according to the objective lens used.

10- When you have finished, turn down the lamp. Unplug the microscope. With the coarse adjustment, move the stage downwards to the maximum. Place the scanning objective lens (4x) in position. Remove the slide from the stage. Clean the eyepiece and objective lenses with lens paper. Cover the microscope with an appropriate cap. *Place the microscope in the assigned cabinet with the ocular lens positioned towards the inside of the cabinet.*

B) Dissecting Microscope (Figure 1.4): it is a type of stereomicroscope used to examine an object at low magnification (usually less than 100x). It consists of two lens systems, one for each eye. Allowing the image to be seen in its three dimensional form. There is an available working space between the objectives and the stage, making it widely used in the dissection and identification of insects, small animals, microsurgery, botanical study, and

for the examination of different specimens in fresh state without the need of previous preparation.

Most of the instructions of how to use a compound microscope, apply to the dissecting microscope. However, the student has to take in consideration some differences:

1- The specimen to be examined does not need further preparation; the specimen is only placed on the stage in a Petri dish or on a white paper.

2- Turn the focus adjustment knob to focus on to the specimen.

3- Turn the Magnification knob to magnify the image of the specimen.

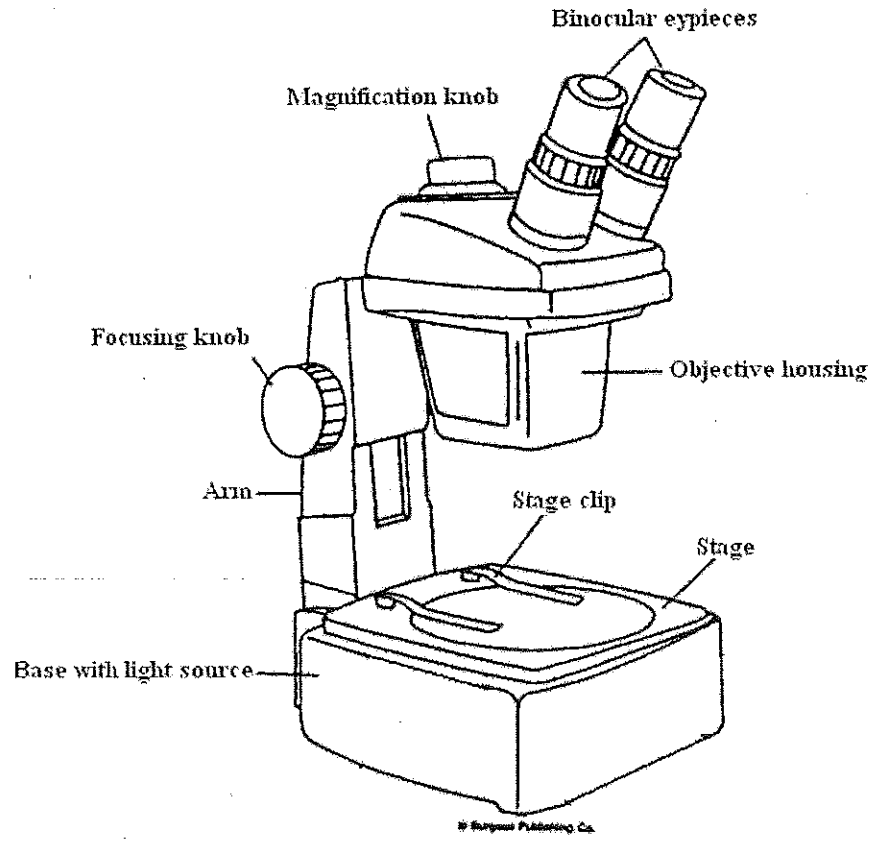


Figure 1.4: Parts of a dissecting microscope
(from http://uwstudentweb.uwyo.edu/e/ehotchki/MICRO.pdf)

II- Electron Microscope: a beam of electrons produced by a tungsten filament is used instead of photons to produce images with better resolution (0.2 nm), since the wavelength of electron beams have very short wavelengths. A system of electromagnetic lenses is used to focus the electron beam on the specimen, and then the image is formed on a fluorescent screen or a photographic film. However, the specimen needs further and a more complex

preparation before it can be seen by an electron microscope. There are two main types of electron microscope: TEM (transmission electron microscope) and SEM (scanning electron microscope).

A- **TEM**: the beam of electrons passes through the specimen and a two dimensional image is obtained.

B- SEM: the beam of electrons is deflected from the surface of the specimen allowing the formation of three-dimensional images.

MATERIALS

1-	Light microscope	**2-**	Scalp hair
3-	Microscopic slides and cover slips	**4-**	Letter "e" microscopic slides
5-	Plastic Pasteur pipettes	**6-**	Etherized fruit flies (*Drosophila melanogaster*)

PROCEDURE

- **Wet mount (temporary mount) preparation:**

1- Pull out a hair from your scalp. Cut the hair leaving about 2 cm of the part containing the bulb.

2- Place a drop of water in the center of a microscopic slide.

3- Place a glass cover slip on the specimen by holding the cover slip at a 45° angle to the drop of water with one edge of the cover slip on the slide and then slowly lowering the cover slip over the drop forcing trapped air out of the preparation.

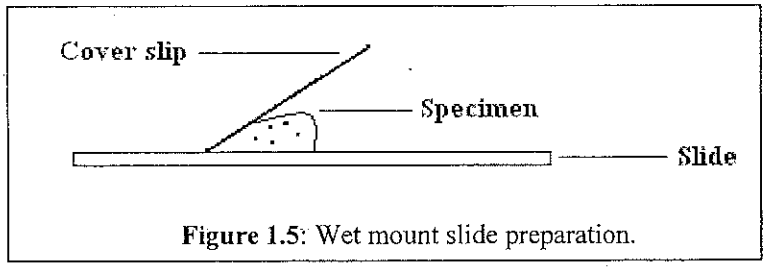


Figure 1.5: Wet mount slide preparation.

4- Examine the specimen using a compound microscope, and follow the general instructions of how to use a compound microscope as mentioned above.

- **Permanent slide:**

1- Obtain a letter "e" permanent slide.

2- Examine the specimen using a compound microscope, and record your observations.

- **General part of the fruit fly (*Drosophila melanogaster*):**

1- Using a dissecting microscope, study the external anatomy of etherized fruit flies (Figure 1.6).

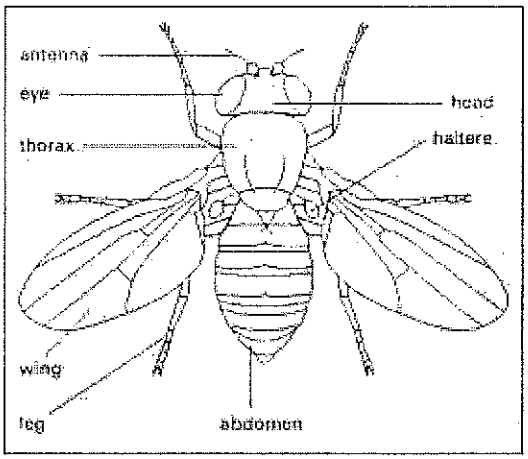


Figure 1.6: The external anatomy of *Drosophila melanogaster* – Dorsal view (Alberts et al, 2002).

- **Extra activity:**

As biologists, our inner curiosity expands to a wide range of questions, generating further enthusiasm to learn and observe more. Obtain ***anything*** from nature or home you would like to observe its details under a microscope.

Activity Two

The Diversity of Life

INTRODUCTION

Living organisms are classified on the basis of the evolutionary relationships among them. *The study of biological diversity in an evolutionary basis is called* **biological systematics**. One of the major objectives of systematics is the naming and classification of species, in what is known as **taxonomy**. Organisms are classified in a hierarchical mode into different taxonomic levels as follows: **species, genus, family, order, class, phylum, kingdom,** and **domain**. A **binomial** system is used to scientifically name the organism; the first part of the binomial refers to the **genus** (plural: *genera*) and the second part refers to the **species**. The whole binomial is *italicized* and the first letter of the genus is capitalized. For example, the scientific name of humans is written as: *Homo sapiens* and that of the fruit fly is *Drosophila melanogaster*. In taxonomy, related species are placed in the same genera; related genera are placed in the same family and so on.

All organisms on Earth can be classified into one of the following domains: **Eubacteria, Archaea** and **Eukarya**. Organisms in the Eukarya domain can be grouped into the following kingdoms: **Protista, Plantae, Fungi** and **Animalia**.

In this laboratory, you will be briefly introduced to the general characteristics of each domain.

I- Domain Eubacteria:

This domain consists of prokaryotic unicellular organisms. The cells of most Eubacteria can be classified based on their shape: **coccus** (spherical shape), **bacillus** (rod shaped), or **spirillus** (helical shape).

Organisms in this domain can be classified according to *their modes of nutrition*:

1- **Photoautotrophs**: photosynthetic organisms that use light energy to make organic compounds from carbon dioxide (e.g. *Cyanobacteria*).
2- **Chemoautotrophs**: these organisms synthesize organic compounds from carbon dioxide but use a chemical source of energy instead, such as hydrogen sulfide (H_2S), ammonia (NH_3) and ferrous ions (Fe^{2+}).
3- **Photoheterotrophs**: these organisms use light as a source of energy but obtain their carbon from organic compounds.
4- **Chemoheterotrophs**: in these organisms, organic compounds are the source of energy and carbon.

II- Domain Archaea:

Species of the domain Archaea are considered **extremophiles**, i.e. they "love" to live in extreme environments. Archaea can be classified into three groups as follows:

1- **Methanogens**: obligate anaerobes (they cannot survive under normal atmospheric oxygen) that produce methane as a metabolic by product. They live in the guts of humans and ruminants as well as swamps and ponds.
2- **Halophiles**: live in highly concentrated salt water such as the Dead Sea (around 31.5%).
3- **Thermoacidophiles**: grow in hot sulfur springs and volcanic vents at temperatures 65– 80 °C and pH of 1 – 2.

III- Domain Eukarya

As humans, we belong to this domain of life. All cells that have nuclei and internal organelles are eukaryotic. Eukaryotes can be uni- or multi-cellular organisms, meaning that they are composed of one or many cells, respectively. Eukarya are divided into the following groups:

1- **Kingdom Protista:**

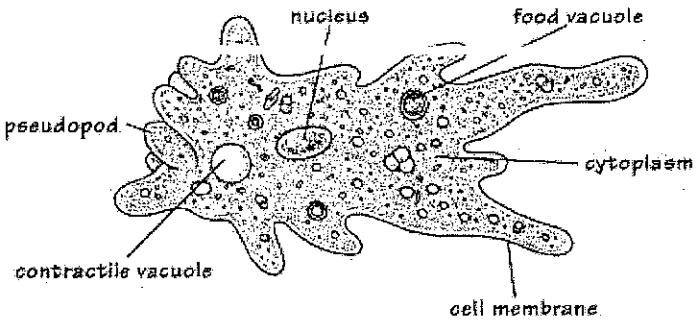
Protists are highly diverse; most of them are unicellular and microscopic such as *Amoeba,* while others can be multicellular and macroscopic. For this reason, scientists have classified protists into about 20 kingdoms, but for simplicity the term "protist" can be used as an informal term to refer to all eukaryotes that are not plants, fungi or animals.

According to the mode of nutrition, protists can be classified into three groups:

1. **Animal-like protists** (protozoa): these are ingestive protists, such as *Amoeba, Plasmodium,* and *Paramecium.*
2. **Fungus-like protists:** these are absorptive protists.
3. **Plant-like protists:** these are photosynthetic protists such as *Euglena.*

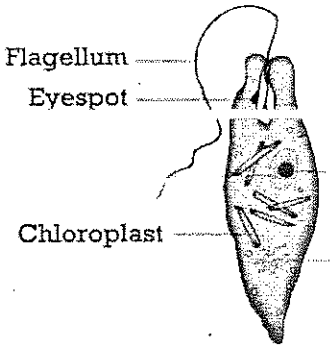
In this laboratory you will be introduced to *Amoeba, Paramecium* and *Euglena.*

a) *Amoeba*



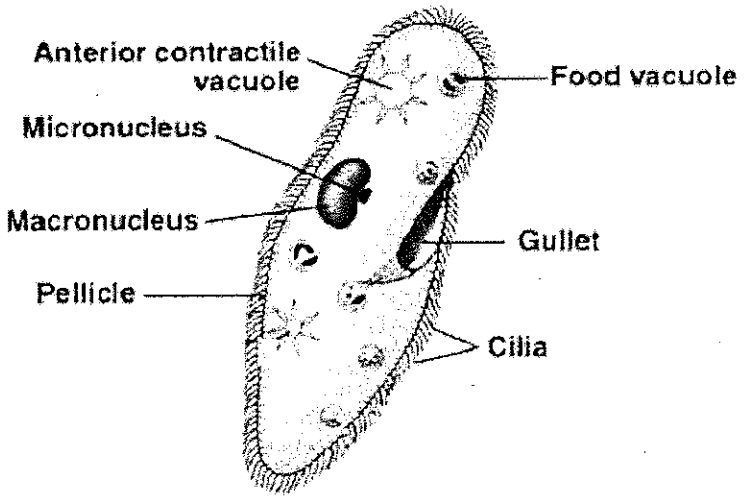
It is a unicellular organism that uses pseudopodia to move and feed.

c) *Euglena*



It is an elongated, ovoid cell with a long flagellum emerging from its end. *Euglena* lacks a wall and has chlorophylls *a* and *b*. Stigma (eyespot) is located near the anterior (front) end of the cell, and functions as a light detector.

b) *Paramecium*



This protozoan uses many cilia for movement. It has a special oral groove (Gullet) into which food particles are pulled in by beating cilia, and has an anal pore through which wastes are expelled. *Paramecium* has one or more micronuclei and one macronucleus.

Figure 2.1: The structure of a) *Amoeba* b) *Paramecium* c) *Euglena*

2- Kingdom Fungi:

Fungi are eukaryotic organisms that lack chlorophyll and reproduce by means of spores. Fungi are heterotrophs and acquire their nutrients by absorption. Fungi are primarily filamentous and multicellular. The fungal body usually consists of microscopic that branch and spread over the surface of the food supply. Each filament is known as a hypha; a mass of hyphae is called a mycelium. Examples of fungi are: bread molds, yeast, mushrooms, etc. ***Rhizopus nigricans*** (Figure 2.2) is the common black bread mold. The mycelium includes three types of hyphae: horizontal hyphae that form a network on the surface of bread; root-like hyphae (called rhizoids) that penetrate into the bread; and upright hyphae that hold sporangia on their ends.

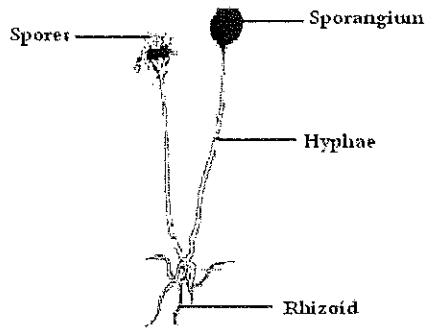


Figure 2.2: Structure of *Rhizopus nigricans*

3- Kingdom Plantae:

They are photosynthetic eukaryotes. Plants have cells with rigid cell walls mainly composed of cellulose. In the following are some examples of plants:

a) ***Spirogyra*** (Figure 2.3): They are green filamentous algae. Each filament is divided into cells joined end to end. Each cell contains one or more long, green, spiral chloroplasts. The nucleus is found near the center of the cell.

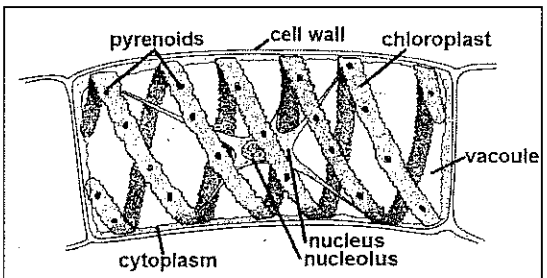


Figure 2.3 Structure of *Spirogyra*

b) Mosses and liverworts are small plants that grow in moist places on land.

c) Vascular plants: The vascular plants show a greatest internal specialization into tissues and organs (roots, stems, leaves and reproductive organs). Examples of vascular plants are the angiosperms (flowering plants).

4- Kingdom Animalia:

Animals are heterotrophs, and intake their food by ingestion. Their cells lack cell walls. The common phyla of this kingdom are briefly described as in the following:

a) Phylum Porifera: sessile (not moving) organisms with porous bodies (e.g. sponges).

b) Phylum Cnidaria (*Coelenterates*): primitive aquatic animals whose body is saclike body composed of two distinct tissue layers. There is a digestive cavity, but it has only one opening, which serves as both mouth and anus. Tentacles are often present around the opening to capture food (e.g. jellyfish, sea anemones, corals and *Hydra*).

In this laboratory, you will be introduced to the general characteristics of *Hydra*.

- ***Hydra*** (Figure 2.4):

The body is cylindrical with a crown of tentacles on one end. The other end allows the organism to stick on surfaces by the mucus secreted from the foot or **basal disc**. The **mouth** lies in the middle of the oral cone, around which the **tentacles** are arranged. The gastrovascular cavity extends into the tentacles and has one inlet, the mouth, which at the same time acts as an exit (the anus). The body wall is formed of an outer ectoderm and an inner endoderm. These organisms are **carnivorous**. The tentacles can poison and hold prey and pull the prey toward the mouth, which opens wide to receive it. Once the food is inside the gastrovascular cavity, digestive enzymes are secreted into the cavity, and extracellular digestion begins. Indigestible remains of the food are expelled from the gastrovascular cavity via the mouth. Hydra can reproduce asexually by **budding**. The buds, not always present, are of different sizes; they are usually connected to the body of the parent. In addition, *Hydra* can reproduce sexually.

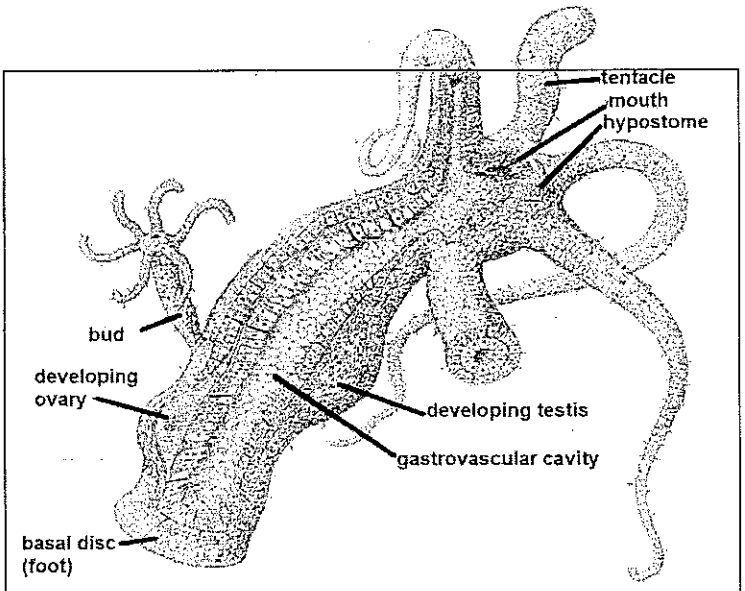


Figure 2.4 Internal and external anatomy of *Hydra*

c) Phylum Platyhelminthes (Flatworms):

Flatworms include tapeworms and Planarians. In this laboratory, you will be introduced to *Planaria*.

- ***Planaria*** (Figure 2.5):

Planarians are non-parasitic flatworms. Their body is small and elongated. The anterior (front) end develops two lateral lobes and is broader than the posterior (back) end which is pointed. A pair of rounded black eyes lies on the surface near the anterior end. The mouth opening is located closer to the posterior end.

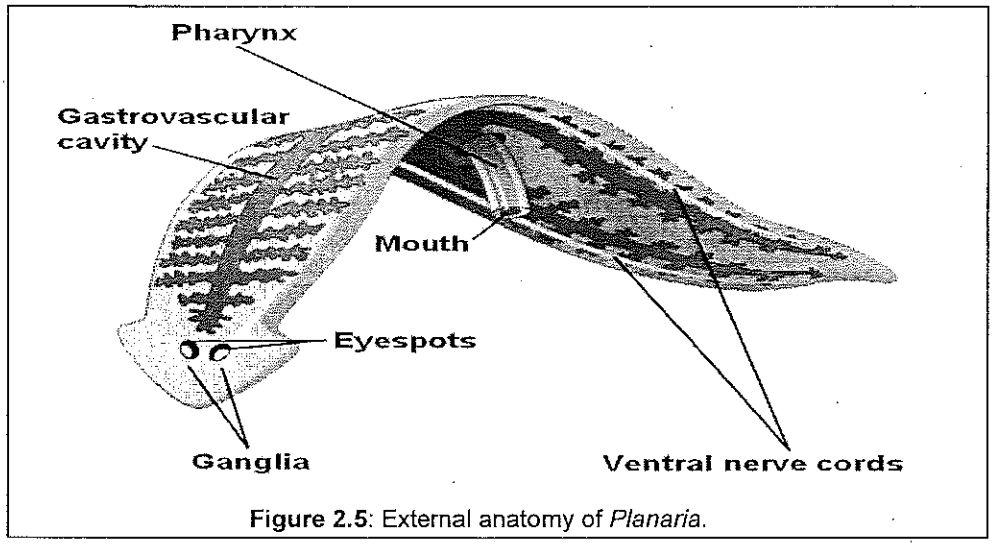


Figure 2.5: External anatomy of *Planaria*.

d) Phylum Nematoda (round worms): they are worms with cylindrical and non-segmented bodies. It includes free-living worms and parasitic worms such as *Ascaris lumbricoides*.

e) Phylum Mollusca: Fairly complex animals, most of which have shells such as snails and clams, others do not have obvious shells such as octopuses and squids.

d) Phylum Annelida (segmented worms): Their body is divided into a series of units or segments (externally and internally). Most annelids are aquatic, but some occur on land in moist places such as earthworms.

e) Phylum Arthropoda: All arthropods have jointed legs and a hard outer skeleton. Arthropods include spiders, scorpions, centipedes, millipedes, crustaceans and insects.

f) Phylum Echinodermata: They are strictly marine organisms. Their symmetry is radial and they are fairly advanced animals (e.g. sea stars, sea urchins, brittle stars, sea cucumbers).

g) Phylum Chordata: it includes a major subphylum vertebrata, which includes fish, amphibians, reptiles, birds, and mammals.

PROCEDURE

- **Prepared slides:**

Examine the following prepared slides using a light microscope. *Draw and label.*

1- *Amoeba* 2- *Paramecium* 3- *Euglena* 4- Mold types

5- *Spirogyra* 6- *Hydra* 7- *Planaria*

- **Bread mold:**

1- Prepare a wet mount of bread mold and examine it under the compound microscope. *Draw and label.*

- **Fresh water microorganisms:**

1- Prepare a wet mount of a drop of water obtained from the pond and examine it under the compound microscope. *Draw and label* and compare what you see with Figure 2.6.

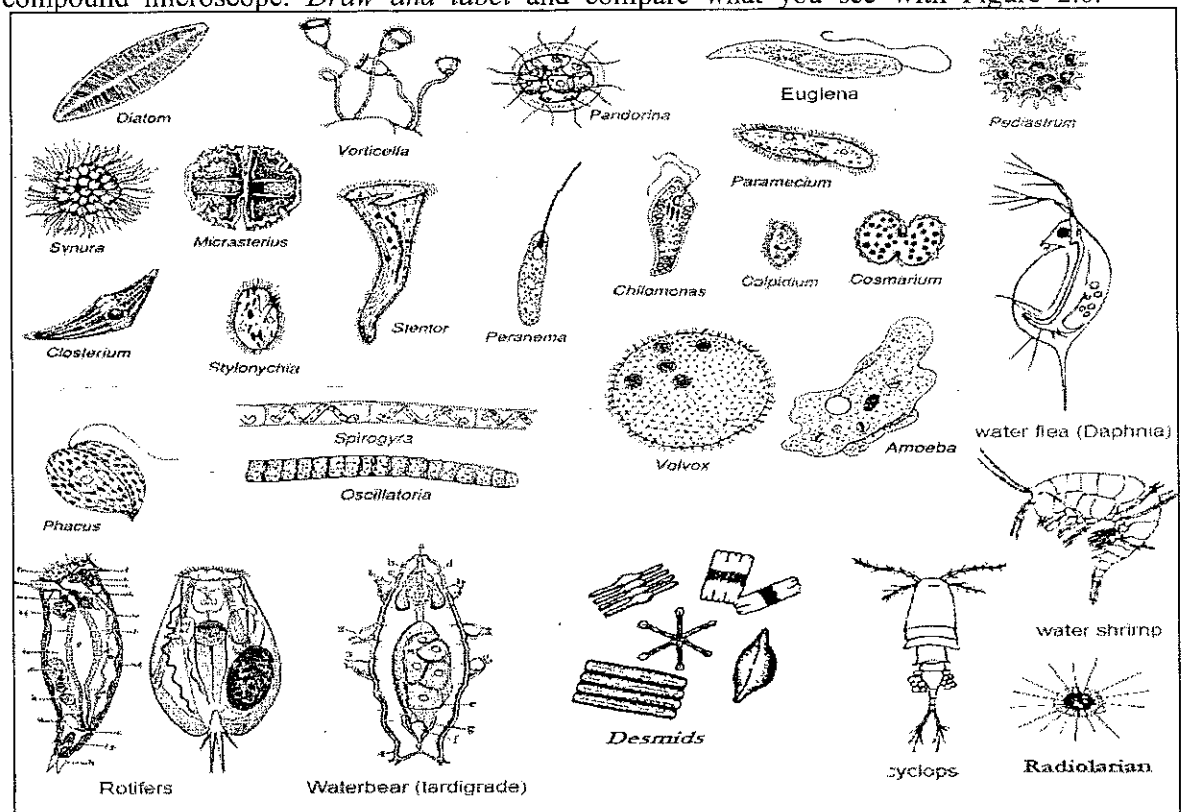


Figure 2.6: Identification guide for common microorganisms in pond and freshwater

Activity Three

Carbohydrates

INTRODUCTION

Carbohydrates are organic compounds often with the general formula $C_x(H_2O)_n$, where x and n could be equal or different integers. Carbohydrates sometimes contain other elements such as phosphorus, nitrogen or sulfur. Although the term "carbohydrates" refers to "hydrates of carbon", this fact was disproved since the arrangement of atoms in these compounds have little to do with water. According to the number of molecules they contain, carbohydrates can be classified into: monosaccharides, disaccharides and polysaccharides.

Monosaccharides (*mono*: one, *saccharide*: sugar) are the simplest form of carbohydrates, and are considered the monomers (building-blocks) of the more complex carbohydrates. Monosaccharides can be classified based on their carbonyl carbon (>C=O) and their number of carbons. According to the location of the carbonyl functional group, monosaccharides can be either **aldoses** (terminal carbonyl group; aldehyde) or **ketoses** (internal carbonyl group; ketone). According to the number of carbons they contain, monosaccharides can be classified as *trioses* (three carbons sugar), *tetroses* (four carbons sugar), *pentoses* (five carbons sugar), *hexoses*, *heptoses*. Both modes of classification are used to describe sugars. For example, a five-carbon aldose such as ribose is an *aldopentose*, and a six-carbon ketose like fructose is a *ketohexose*. Examples of monosaccharides are shown in Figure 3.1. An aldose contains a terminal carbonyl carbon (aldehyde) and thus it can perform organic reactions specific for aldehydes. An aldehyde can perform as a reducing agent in acidic or basic media to reduce other species such as copper ions while the aldehyde itself becomes oxidized. Similarly, aldoses can be considered "reducing sugars" because of this property (Figures 3.2 and 3.4).

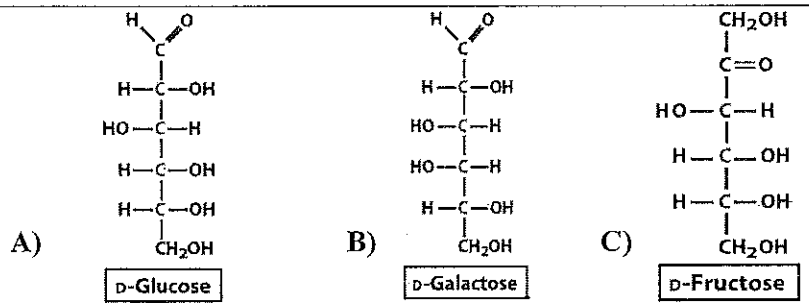


Figure 3.1: The chemical structure of (A) glucose an aldose, (B) galactose an aldose, (C) fructose a ketose (Nelson and Cox, 2008).

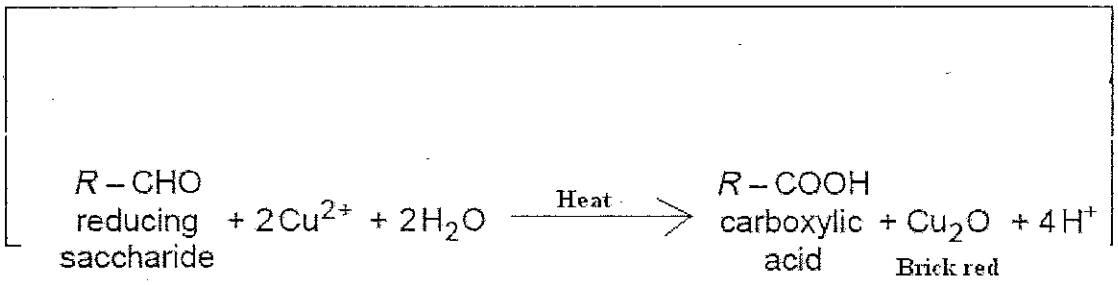


Figure 3.2: The reduction of copper (II) ions to Cu_2O by a reducing sugar in an acidic medium (Schreck and Loffredo, 1994).

When two monosaccharides are joined together by a **dehydration reaction**, a **disaccharide** is formed. The two monosacharides are joined by a covalent bond called **glycosidic linkage**. The common examples of disaccharides are maltose (glucose-glucose), sucrose (glucose-fructose), and lactose (glucose-galactose).

Polysaccharides are polymers with hundreds to thousands of monosaccharides joined by a glycosidic bond. Some common examples of polysaccharides that are polymers of glucose monomers are starch, glycogen and cellulose. **Starch**, is a helical storage polysaccharide found in plants. There are two forms of starch: amylose (simple and unbranched), and amylopectin (complex and branched). Another storage polysaccharide is **glycogen**, a helical and highly branched polysaccharide found in animals. On the other hand, **cellulose** is a straight and never branched structural polysaccharide found in the cell wall of plant cells. Cellulose molecules are arranged in parallel chains stabilized by hydrogen bonds.

In this experiment, different qualitative tests will be performed to identify carbohydrates. The following flowchart (Figure 3.3) summarizes the qualitative tests used to identify different carbohydrates.

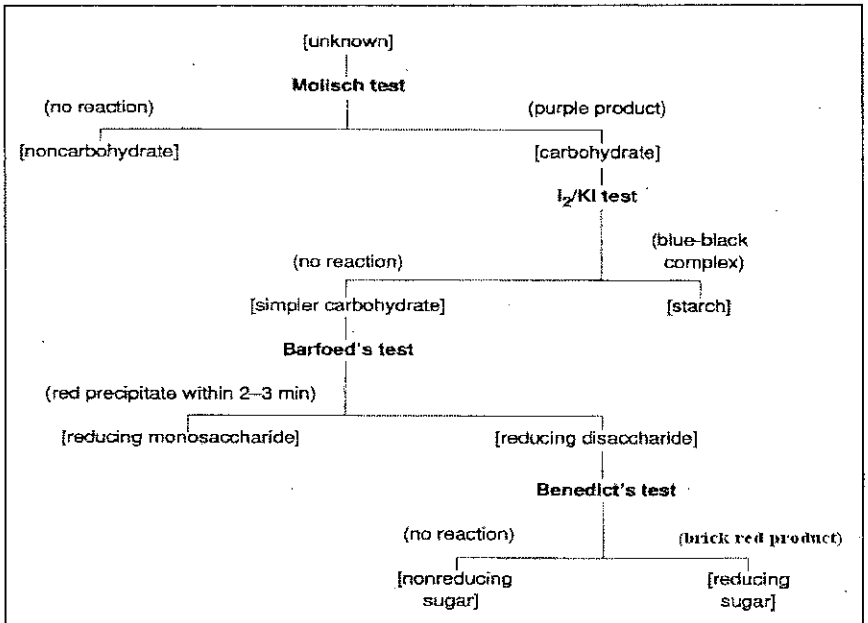


Figure 3.3: Flowchart for the identification of an unknown carbohydrate. (Schreck and Loffredo, 1994)

1- **Molisch test**: It is a sensitive test for the identification of carbohydrates or carbohydrate moieties in other macromolecules. Nucleic acids and glycoproteins give a positive result by this test, since both contain carbohydrate portions. A concentrated sulfuric acid (H_2SO_4) solution dehydrates the carbohydrate to form an aldehyde that condenses with α-naphthol in the Molisch reagent to produce a **purple compound**.

2- **I_2/KI test:** Many polysaccharides can be identified by their reaction with IKI reagent [Iodine (I_2) dissolved in potassium iodide (KI)]. IKI reagent interacts with the helices of amylose and glycogen, and the chains of cellulose forming colored complexes. A **deep blue-black** color is obtained with **starch**; a **red-brown color** is given by **glycogen**; and a **violet-brown** to **red-brown** color with **cellulose**. The colors fade away by heating and return by cooling.

3- **Barfoed's test:** It is test that determines if the reducing sugar in question is a reducing monosaccharides or a reducing disaccharides. The Barfoed's reagent consists of copper (II) ions in an **acidic medium**. Copper (II) ions are reduced by the sugars to insoluble brick red cuprous oxide Cu_2O (Figure 3.2). The brick red precipitate is formed in 2-3 minutes, if the sugar is a reducing monosaccharide. Note that, if heated for a long time the glycosidic linkage of the disaccharide molecule can be broken down with heat and acid, giving a false positive result (however this requires more than 3 minutes and therefore can be easily detected).

4- **Benedict's test**: It is used to identify reducing sugars regardless if they are monosaccharides or disaccharides. Therefore, in order to further distinguish between a reducing and a non-reducing disaccharide, Benedict's test is performed. All reducing sugars have a free aldehyde group[1]. However, ketoses can also be reducing sugars due to the conversion of ketoses to aldoses by **tautomerization**. Benedict's reagent differs from Barfoed's reagent in that it consists of mainly copper (II) ions in a strongly **alkaline** solution. The reaction is described in the following equation (Figure 3.4).

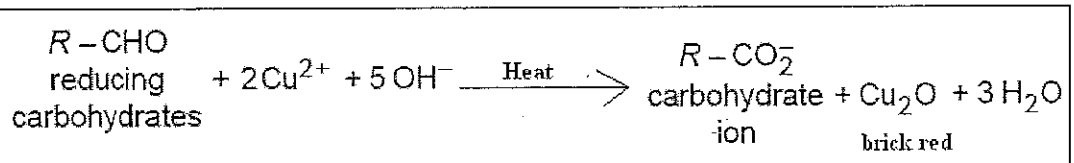


Figure 3.4: The reduction of copper (II) ions to Cu_2O by a reducing sugar in an alkaline medium (Schreck and Loffredo, 1994).

MATERIALS

[1] Aldoses in solution have cyclic hemiacetal groups. These sugars are reducing sugars since the hemiacetal is readily interconvertible with the aldehyde functional group.

1-	Molisch reagent[2]	**2-**	Barfoed's reagent[3]	**3-**	Benedict's reagent
3-	Glucose (0.02%)	**4-**	Glucose (0.4%)	**5-**	Glucose (0.2%)
6-	Glucose (2%)	**7-**	Fructose (2%)	**8-**	Lactose (2%)
9-	Sucrose (2%)	**10-**	Maltose (2%)	**11-**	Glycogen (2%)
12-	Starch (2%)	**13-**	Concentrated H_2SO_4	**14-**	Test tubes
15-	Concentrated NaOH	**16-**	IKI	**17-**	Plastic Pasteur pipettes
18-	Tissue paper				

PROCEDURE

- **Molisch Test:**

1- Into four clean and labeled test tubes, add 2 ml of the following substances: (A) 2% glucose, (B) 2% sucrose, (C) 2% starch, (D) water (control).

2- To each tube, add two drops of the Molisch reagent and mix well.

3- Add 2 ml of a concentrated sulfuric acid solution (H_2SO_4) into four *OTHER* test tubes.

4- One at a time, hold the test tubes containing the concentrated sulfuric acid solution at an angle of 30°, and then ***slowly pour*** the solutions prepared in step #2 into them (BE VERY CAREFUL HANDLING ACIDS!!). *Do not mix. Two layers will form.* Record your results.

A positive result is the formation of a purple ring between the two layers.

- **IKI testing:**

1- Add one drop of IKI on a piece of tissue paper and a piece of absorbent cotton. Record the color.

2- Add one drop of IKI to each of the three test tubes containing 2 ml of the following substances and record the colors:

(A) 2% starch, (B) 2% glycogen, (C) 2% glucose, (D) 2% maltose, (E) water (control)

[2] Molisch reagent consists of 5 ml of 10% α-naphthol in 100 ml of 95% ethanol.

[3] Barfoed's reagent preparation: dissolve 70g of copper acetate monohydrate in 9ml glacial acetic acid, and complete the final volume of one liter with water.

- **Barfoed's Test:**

1- Into seven clean and labeled test tubes, add 1 ml of the following substances:

(A) 2% glucose, (B) 2% fructose, (C) 2% sucrose, (D) 2% lactose, (E) 2% maltose, (F) 2% starch, (G) water (control)

2- Add 3 ml of Barfoed's reagent to each tube.

3- Transfer the tubes to a boiling water bath for 2 minutes. Record your results immediately.

- **Benedict's Test:**

1- Add 1 ml of each of the following solutions into eight clean and labeled test tubes:

(A) 2% glucose, (B) 2% fructose, (C) 2% sucrose, (D) 2% lactose, (E) 2% maltose, (F) 2% starch, (G) water (control).

2- Add 3 ml of Benedict's reagent to each test tube. Shake and transfer the tubes to a boiling water bath for 2 minutes. Record your results immediately.

- **Determination of the performance of Benedict's reagent with different concentrations of sugar solutions:**

1- To four separate test tubes, add 2 ml of the following glucose solutions: (A) 0.4% glucose, (B) 0.2% glucose, (C) 0.02% glucose, (D) 0% glucose (water only).

2- Add 2 ml of Benedict's reagent to each test tube. Shake well and transfer the tubes to a boiling water bath for 2 minutes. Record your results (take a picture if you like).

- **Hydrolysis of disaccharides:**

1- To a test tube, add ten drops of concentrated HCl and 1 ml of 2% sucrose. To another tube add 10 drops of water and 1 ml of 2% sucrose.

2- Transfer both test tubes to a boiling water bath for 2 minutes. Cool the tube. Then add 3 ml of Barfoed's reagent to each.

3- Transfer the test tubes to a boiling water bath for 2 minutes. Record your results (compare both tubes).

- **Unknown:**

Your instructor or TA will provide you with an unknown solution and given information to determine the nature of your unknown.

Activity Four

Lipids, Proteins and Vitamins

INTRODUCTION

I. Lipids

Lipids are a diverse class of biological molecules that play a variety of different roles in cells. Lipids are important for energy storage; they are considered indispensible structural molecules in all cellular membranes; and moreover they have been recently found to be powerful signaling molecules. Although lipids differ widely in their chemical structures, they share a solubility property: All lipids have little or no water affinity (i.e., they are all **hydrophobic** molecules). However, some are **amphipathic**, having a hydrophobic and a hydrophilic (polar) portion in their structure.

According to their chemical structures, lipids can be generally classified into: fatty acids, triacylglycerols, phospholipids, glycolipids, sphingolipids, steroids and terpenes. In this section, you will be introduced to fatty acids and triacylglycerols.

Fatty acids are amphipathic molecules, with a polar carboxylic "head" attached to a hydrophobic, long and unbranched hydrocarbon "tail" (Figure 4.1). If the fatty acid tail has no double bonds, it is called a **saturated fatty acid** since all its carbons are saturated with hydrogen atoms. On the other hand, if the hydrocarbon chain has one or more double bonds it is referred to as an **unsaturated fatty acid**.

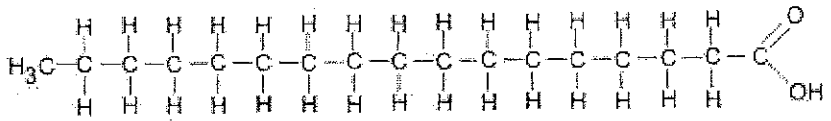


Figure 4.1: The chemical structure of palmitate; a saturated fatty acid.

When three fatty acids link to a glycerol molecule by **ester bonds** a triacylglycerol (or triglyceride) is formed (Figure 4.2).

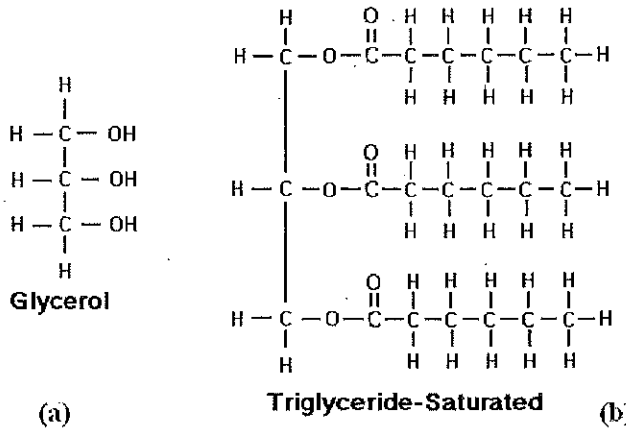


Figure 4.2: The chemical structures of (a) glycerol and (b) a saturated triglyceride.

In this laboratory Sudan III dye and emulsion test will be performed for the identification of lipids. **Sudan III dye** is a hydrophobic dark red dye that turns to **red-orange** when added to a hydrophobic substance. In addition to lipids, a positive result is obtained with other hydrophobic substances.

In addition, the **emulsion test** can be used for the identification of lipids. Since lipids are hydrophobic, a white cloudy emulsion is formed when a lipid dissolved in ethanol is slowly added to water.

II- Proteins:

Proteins are the macromolecules that actually do work in the cell. The name "protein" is derived from the Greek work *proteios*, which means "the first place". They can be enzymes, hormones, signal receptors, structural proteins, storage proteins, regulatory proteins, transport proteins or defensive proteins. Proteins are polymers of **amino acids** formed by **dehydration reactions** forming peptide bonds between amino acid residues (Figure 4.3). All amino acids have a carboxyl group, an amino group, a hydrogen atom and an R group; all attached to a carbon atom. The amino acids differ in the R group they contain.

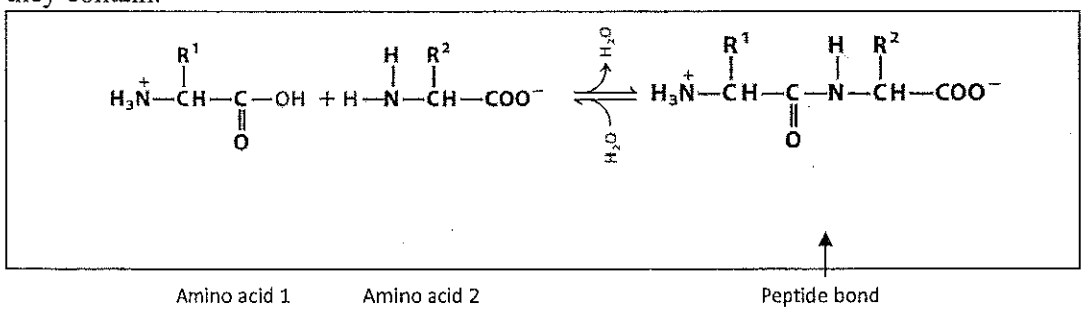


Figure 4.3: The formation of a peptide bond from two amino acids by a dehydration reaction. (Nelson and Cox, 2008).

In biochemistry labs, **Biuret reagent** is used to detect peptide bonds. In this test, the amide group (N–H group) in the peptide bond forms a complex with the copper (II) ions in the Biuret reagent, changing its color from blue to **violet**. The higher the number of peptide bonds the more intensive is the violet color.

Another test for the identification of proteins, is by disrupting the three dimensional structure of the protein in a process called **denaturation**. Denaturated proteins lose their solubility allowing the protein to precipitate. Heat, a strong acid or base, a concentrated salt solution and many organic solvents such as 95% ethanol can precipitate proteins.

III. Vitamins:

Vitamins are organic molecules that are required in the diet in relatively small amounts because the organism cannot synthesize them. They are important for the body as they have diverse physiological functions. Some vitamins function as coenzymes and enzyme cofactors, while others play important roles in blood clotting, bone formation, connective tissue production, etc. Vitamins can be classified as: **water-soluble vitamins** (vitamins C and B complex), and **lipid-soluble vitamins** (vitamins A, D, E and K).

In this laboratory, the identification of vitamin C (ascorbic acid) will be performed. Due its antioxidant properties, vitamin C can be detected by two methods:

1- The reduction of DCPIP: The blue indicator, DCPIP (2,6-dichloro-phenolindophenol) is reduced by ascorbic acid to a colorless compound (Figure 4.4):

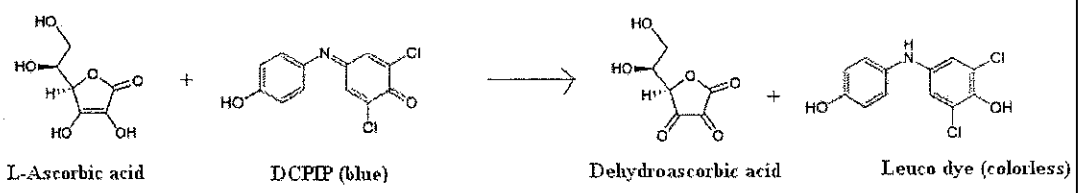


Figure 4.4:The reduction of the blue DCPIP to a colorless compound by ascorbic acid.

2- The reduction of iodine to iodide: The iodine dissolved in a potassium iodide solution (IKI) is used to identify polysaccharides. When ascorbic acid is added to a blue-black starch IKI complex, iodine is reduced to iodide ions which no longer can form a colored complex with starch, making the blue-black color disappear.

MATERIALS

1-	Corn oil	**2-**	Distilled water	**3-**	Sudan III dye
4-	Ether	**5-**	Flour extract	**6-**	Milk extract
7-	Butter extract	**8-**	Nuts extract	**9-**	Egg white
10-	Liver extract	**11-**	10% $CuSO_4$	**12-**	1% $CuSO_4$
13-	Saturated KCl	**14-**	15% TCA	**15-**	95% ethanol
16-	Glycine	**17-**	2% starch	**18-**	Concentrated NaOH
19-	DCPIP	**20-**	IKI	**21-**	Xylene
22-	Filter paper	**23-**	Test tubes	**24-**	Plastic Pasteur pipettes
25-	Lemon juice	**26-**	Orange juice	**27-**	Ascorbic acid

PROCEDURE

- **Lipid identification by Sudan III test:**

1- Add 2 ml water to a test tube and then add 2 ml of corn oil. Allow layers to be formed.

2- Slowly add 2 drops of Sudan III dye to the test tube. Record your results.

3- To another test tube, repeat the same experiment but add xylene (a hydrophobic substance) instead of corn oil. Record your results.

- **Comparison of lipid content in different lipid sources:**

1- Using a *pencil*, draw five small circles on a filter paper and label each circle with the following extracts: flour (F), butter (B), milk (M) and nuts (N). The fifth circle label it with (E) for ether, which is the solvent, used to extract lipids from the other sources.

2- With a dropper, add a small drop of each of the mentioned substances to the appropriate circle spot on the filter paper. Make sure when you take out the extract to use the upper layer of the solution and not to mix the components. Also, make sure your drop does not extend very much. Use a blow dryer to completely dry the filter paper.

3- Add 3 drops of Sudan III solution on each drop and let it soak for 3 minutes. Use forceps to remove the paper from the stain.

4- Rinse the paper with water for to remove excess stain.

5- Record the color intensity in each spot as: no color (-), faint orange (+), definite orange (++).

- **Identification of lipids by emulsion test:**

1- Add 4 ml of 70% ethanol to a test tube containing 1 ml of corn oil. Shake vigorously.

2- Slowly, decant the solution to a test tube containing 2 ml of water. Observe the formation of a white emulsion.

- **Identification of proteins by precipitation reaction:**

a) By heat:

1- To a test tube add 3 ml of egg white, and to another tube add 3 ml of liver extract.

2- Transfer the test tubes to a boiling water bath for 3 minutes. Record your results.

b) By chemicals:

1- Label five test tubes as (1, 2, 3, 4 and 5).

2- Add 1 ml of egg white to each of the labeled test tubes.

3- To test tube #1, add 1 ml of distilled water (control).

4- To test tube #2, add 1 ml of 95% ethanol.

5- To test tube #3, add few drops of 10% $CuSO_4$.

6- To test tube #4, add few drops of saturated KCl.

7- To test tube #5, add few drops of 15% trichloroacetic acid (TCA). Record your results.

- **Identification of proteins and polypeptides by Biuret Test:**

1- To one test tube, add 1 ml of egg white, and to another test tube add 1 ml of glycine (an amino acid).

2- To each test tube, add 2 ml of concentrated sodium hydroxide (NaOH). Mix well.

3- To each test tube, add 6 drops of 1% copper sulfate ($CuSO_4$). Mix and record your results.

- **Reduction of DCPIP dye by ascorbic acid (vitamin C):**

1- To four different test tubes, add 1 drop of DCPIP.

2- To each test tube, *drop wise* add the following substances: lemon juice, orange juice, ascorbic acid and distilled water (control).

3- Record the number of drops that are needed to make the blue color disappear for each substance. *Which substance contains more vitamin C?*

- **Reduction of iodine by ascorbic acid:**

1- Add one drop of IKI to a test tube containing 1 ml of 2% starch.

2- To the test tube, add the ascorbic solution drop wise until the blue color fades away.

- **Unknown:**

Your instructor or TA will provide you with an unknown solution and some information to determine the nature of your unknown; is it a protein, lipid or vitamin C?

Activity Five

Scientific Investigation:
What Makes Fruits Go Brown

INTRODUCTION

It is a well-known fact that some fruits and vegetables go brown when they ripen. This browning has implications in commercial food processing and important for food growers and sellers, since fruits and vegetables with brown spots lose their appeal by consumers. Hence studying this phenomenon is important by biologists.

In order to study phenomena, biologists first make observations and do preliminary background inquires about the subject, and then they construct testable hypotheses about the topic in question. The hypotheses are then tested by experiments to see whether or not these hypotheses explain the phenomena. The experiments are then repeated to confirm the results. If the experimental results do not support the hypotheses, then these hypotheses need to be revised. After the hypothesis is supported by experimentation, a conclusion (or conclusions) can be made to explain the original observation.

In this lab session, students will evaluate the process of fruit browning by first observing the process and considering different hypotheses that might explain the reaction. Further, students will test each hypothesis through experimentation. Eventually conclusions about fruit browning will be made in light of all testing. For the sake of scientific inquiry and investigation, the authors will not elaborate more on fruit browning in this introduction and therefore it will be the students who will discover the causes of the phenomenon by experimentation, reasoning and accessing available knowledge.

MATERIALS[4]

1-	Scalpels or knives	**2-**	Mortar and pestle	**3-**	Tongs or forceps
4-	Apples, bananas or potatoes	**5-**	1% Phenol	**6-**	Kettle (to boil water)
7-	2% HCl	**8-**	Toothpicks	**9-**	Thermometers

[4] This activity was adapted almost in its entirety from Nuffield Foundation http://www.nuffieldfoundation.org/print/3207), accessed 31-August-2016.

10-	Ascorbic acid solution at different concentrations (5%, 2.5%, 1.5%, 1%, 0.5%)	**11-**	1% Catechol solution in a dark dropper box	**12-**	2% Citric acid
13-	2% Sodium hydrogensulfite	**14-**	2% NaCl	**15-**	2% Sucrose

PROCEDURE

- **Initial Observation:**

1- Cut an apple into eight equal pieces. Take one piece and crush it in the mortar and pestle. Put the crushed sample on a tile next to an uncrushed piece. Compare the rate at which both samples go brown. You can use a stopwatch.

2- When the uncrushed piece becomes brown cut a small piece from it using a knife. In addition, break a small piece from it using your hands. Note the color of the freshly exposed surfaces.

3- Note which piece browns more quickly – the cut or broken surface?

- **Investigation 1** – Is the reaction an effect of microorganisms acting on the tissue?

1- Take two apple slices, soak one in a 1% phenol solution phenol (to kill microorganisms) for 1 minute. Soak the other piece in pure water as a control.

2- Use tongs to remove the slices, shake off excess liquids.

3- Wash one surface of each slice with 4 drops of catechol.

4- Observe the rate of browning of each slice and answer the main question of this section (i.e. is the hypothesis validated?)

- **Investigation 2** – Do enzymes control the reaction?

1- Take four apple slices and treat as described below:

- Immerse in a water bath at 100 °C for 1 minute.
- Immerse in a water bath at 60 °C for 1 minute.
- Immerse in a water bath at 40 °C for 1 minute.
- Immerse in water at room temperature for 1 minute.

2- Remove the slices using tongs, and shake off excess liquid. Wash the surfaces with 4 drops of catechol.

3- Compare the rates at which the four samples go brown. Answer the main question of this section (i.e. is the hypothesis validated?)

- **Investigation 3** – Does the reaction require air or some part of the air?

1- Wash the upper surfaces of two apple slices with 4 drops of catechol and place each in a flasks, one kept open (normal atmospheric air) and the other filled with CO_2 gas[5].

2- Note the rate at which each slice goes brown. Answer the main question of this section (i.e. is the hypothesis validated?)

- **Investigation 4** – How does ascorbic acid affect the browning reaction?

1- Take six apple slices and soak each slice in one of the following solutions for 2 minutes:

- 5% ascorbic acid
- 2.5% ascorbic acid
- 1.5% ascorbic acid
- 1% ascorbic acid
- 0.5% ascorbic acid
- distilled water

2- Remove the slices, shake off excess solution, and wash the upper surfaces with 4 drops of catechol.

3- Arrange the tubes in sequence to show which goes brown first and which last. Answer the main question of this section.

[5] CO_2 can be generated by adding 50 ml of 0.1 HCl to marble chips ($CaCO_3$) in a 250 ml conical flask. CO_2 is can be then collected in the tube to use in the experiment and kept covered. Alternatively, CO_2 can be pumped directly from a gas tank.

- **Investigation 5** – How do other chemicals affect the browning reaction?

1- Take seven apple slices and soak one in each of the following solutions for 2 minutes:

- 2% hydrochloric acid
- 2% citric acid
- 2% sodium hydrogen sulfate(IV)
- 2% sodium chloride
- 2% sucrose
- boiling water
- cold distilled water

2- Remove the slices, shake off excess solution, and wash the upper surfaces with 4 drops of catechol.

3- Note how soon each slice goes brown. Answer the main question of this section.

Activity Six

The Cell

INTRODUCTION

The cell is the "building block" of all living organisms. The general principles of cells are summarized in the **cell theory** as follows:

1- *All living organisms are composed of cells.*
2- *The cell is the unit of structure, physiology and organization of living organisms.*
3- *All cells come from pre-existing cells.*
4- *Cells carry their genetic material and pass it to daughter cells through cell division.*

There are two types of cells depending on whether the genetic material is enclosed by a membrane-bound nucleus or not. If the DNA is stored in a nucleus it is referred to as **eukaryotic** (*eu-* means "true"; *karyon* means "nucleus"). On the other hand, **prokaryotic** cells (*pro-* means "before") do not have a cell nucleus. There are many different types of eukaryotic cells, such as animal cells and plant cells (Figure 6.1).

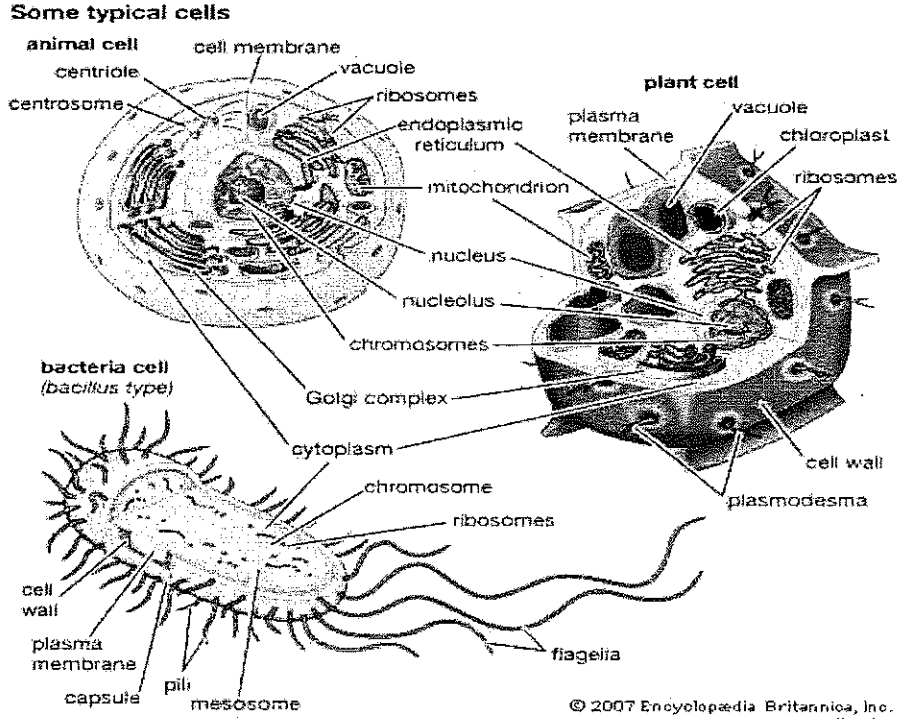


Figure 6.1: The main parts of different cell types: animal, plant and bacterial cells. (From: Encyclopedia Britannica, 2007)

Cells are composed of many components. A summary of the main components of cells is briefly described here:

1- Cell Wall: It is a hard layer that surrounds some types of cells, giving the cell support, strength and protection. It is found in some Archea, Eubacteria, Plantae and Fungi. In these organisms, the cell wall content differs in its structure and chemical composition. For example, in bacteria, the cell wall is composed of peptidoglycan. In fungi, the cell wall is composed of chitin, while in plants it is mainly composed of cellulose, hemicellulose, pectin and lignin.

2- Plasma membrane: It is a selectively permeable membrane that separates the intracellular components from the extracellular environment. It is effectively the boundary of the cell. It is composed of a phospholipid bilayer with proteins embedded through it (integral proteins) or attached to its surface (peripheral proteins).

3- Cytoplasm: It is the entire region between the nucleus and the plasma membrane. It consists of the cytosol (the fluid medium inside cells), organelles and cytoskeleton.

4- Nucleus: It is the center of cellular activities. It encloses the chromatin (DNA and proteins) and nucleoli which is the site of ribosome synthesis. It is enclosed by a double membrane, the *nuclear envelope*, which contains pores allowing the interchange between nucleus and the cytoplasm.

5- Plastids: These are cytoplasmic organelles found in most plant cells. There are three main types of plastids:

(a) Chromoplasts (colored plastids): They contain pigments such as orange carotene and yellow xanthophylls that give color to flowers, ripened fruits and autumn leaves.

(b) Chloroplasts (green plastids): They are the site of photosynthesis. They contain the green pigment, chlorophyll, and other pigments such as carotenoids. They are surrounded by two membranes. In addition, there is a third membrane system found inside the chloroplasts called the *thylakoid*. The thylakoid membranes (which are arranged in structures called *grana*) are suspended in the aqueous matrix of the chloroplast (called the *stroma*). Carbon assimilation reactions occur in the stroma while light-harvesting reactions occur on and in the thylakoid membranes. Chloroplasts contain their own DNA that is different from nuclear DNA.

(c) Leucoplasts (*leuco-* means "white"): These are a variety of organelles used to store materials such as *starch* (stored in ***amyloplasts***), *oils* (stored in ***elaioplasts***) and *proteins* (stored in ***proteinoplasts***).

6- Mitochondria: Mitochondria are the "powerhouses" of eukaryotic cells. They are the site where cellular respiration takes place. In cellular respiration, energy is extracted from food in the form of ATP. Mitochondria produce the most ATP molecules via electron transport chain and oxidative phosphorylation. Mitochondria are elongated organelles that are surrounded by two membranes, termed *mitochondrial outer membrane* (*MOM*) and *mitochondrial inner membrane* (*MIM*). The inner membrane is infolded forming finger-like projections called ***cristae***, where the electron transport chain takes place. Inside the MIM is the matrix where the enzymes of the citric acid cycle (Krebs cycle) are located. Like chloroplasts, mitochondria contain their own circular DNA molecules in the matrix. In addition to DNA ribosomes and DNA enzymes that are responsible for synthesizing proteins in the organelle are present in mitochondria.

7- **Endoplasmic reticulum (ER)**: It is a network of membranes distributed inside the cytoplasm starting from the nuclear envelope. Under the electron microscope this membrane can look *rough* or *smooth* depending on the presence of ribosomes on its surface. Hence there are two types of ER, **rough** and **smooth endoplasmic reticulum (RER** and **SER)**. RER, due to the presence of ribosomes, is the site of protein synthesis while SER has functions in lipid synthesis, calcium storage and detoxification among others.

8- Ribosomes: They composed of RNA and protein molecules in complexes that are large enough to be detected by electron microscopy. Their role is to produce proteins from the mRNA template. They can be either attached to the RER or free in the cytosol.

9- Golgi apparatus: It is made up of flattened unconnected sacs called *cisternae*. It is the sorting place in the cell. Materials coming from the ER arrive at the Golgi where they are sorted, sometimes modified, packaged and sent to their correct destination.

10- Vacuoles and vesicles: Vesicles are produced from ER and Golgi to be used as transport vehicles for materials coming in and out of the Golgi. *Transport vesicles* carry materials from the ER to the Golgi and *secretory vesicles* carry cargo from Golgi to the plasma membrane. Vacuoles are larger than vesicles, and they have other roles. There are many different types of vacuoles depending on their functions: *Food vacuoles* form by phagocytosis and allow bulk food to be stored and digested. *Contractile vacuoles* expel water outside the cell of freshwater organisms. The *central vacuole* found in plant cells takes most of the volume of the cell and maintains turgor pressure.

11- Centrosome: It is an organelle associated with the nuclear envelope of animal cells, and is essential in the formation of mitotic spindle in cell division. In each centrosome, there are two central bodies called centrioles, which are absent in plant cells.

12- Lysosome: It is a small compartment surrounded by a single membrane. It contains hydrolytic enzymes that functions in breaking down of biological macromolecules. Hence

it is the site of cellular digestion. Vesicles from the Golgi or from the plasma membrane can come to the lysosome.

13- Peroxisome: It is a single membrane organelle that contains enzymes that make and breakdown the toxic hydrogen peroxide (H_2O_2). It is a cellular detoxification center.

MATERIALS

1-	Piece of cork	**2-**	Yogurt	**3-**	Tomato
4-	Light compound microscope	**5-**	IKI	**6-**	Potato
7-	Ethanol 70%	**8-**	Toothpicks	**9-**	Microscope slides
10-	Microscope cover slips	**11-**	Cotton	**12-**	Lancets
13-	Plastic Pasteur pipettes	**14-**	Razor blades		

PROCEDURE

- **Cork:**

1- In one hand, hold a piece of cork and start taking very thin sections with the aid of a sharp razor blade. You may need to take more than one section to succeed in getting a thin one. *Be extremely careful handling blades; they are* ***very*** *sharp.*

2- Make a wet mount of the thin section.

3- Examine and draw what you see.

- **Prokaryotes:**

– Eubacteria (*Lactobacillus*):

1- With a dropper, place one drop of diluted yogurt on your slide.

2- Place the coverslip on the drop of yogurt.

3- Examine and draw what you see under the compound microscope. The rod-shaped organisms that you see are cells of yogurt bacterium *Lactobacillus*.

– Eukaryotes:

(a) Tomato cells:

1- Make a wet mount of the epidermis of tomato.

2- To obtain a thin layer, you may scrape some of the sub epidermal cells of tomato.

3- Examine the slide under a compound microscope. Draw and label.

Further observation: Distinguish between the inner and outer epidermis of tomato cells.

(b) Potato cells:

1- Make a wet mount by scratching the fleshy part of potato.

2- Draw and label the potato cells.

3- While looking through the eyepiece, put a drop of IKI solution on the edge of the cover slip and absorb the water by a piece of paper from the other edge.

4- Observe the gradual coloration of the starch granules with a deep blue-black color.

5- Examine the slide under a compound microscope. Draw and label.

(c) Animal cells:

i. Mouth cheek squamous epithelium:

1- To 10 ml of saline solution (0.09% NaCl) add 1-2 drops of methylene blue.

2- With a toothpick, gently scrape the inner lining of your mouth.

3- Place what you collect in a drop of the prepared solution on a slide.

4- Continue preparing the wet mount.

5- Examine the slide under a compound microscope. Draw and label.

ii. Human blood:

1- Clean the tip of your hand finger with a piece of cotton soaked with 70% ethanol.

2- With a sterile lancet puncture the tip of your cleaned finger.

3- Place a drop of saline solution on a slide and add a drop of blood and clean your punctured finger again.

4- Continue preparing the wet mount and examine the slide under a compound microscope. *Draw and label.*

Activity Seven

Biological Membranes: Diffusion and Osmosis

INTRODUCTION

Membranes are formed from lipid molecules arranged in a bilayer with the hydrophobic part of the lipid shielded from the aqueous environment. Different models to describe the boundary of cells have been suggested throughout history, but the currently accepted model for membrane structure is the *fluid mosaic model.* According to this model, which was proposed by Singer and Nicolson, the plasma membrane consists of proteins embedded in or attached to the surface of a fluid lipid bilayer (Figure 7.1).

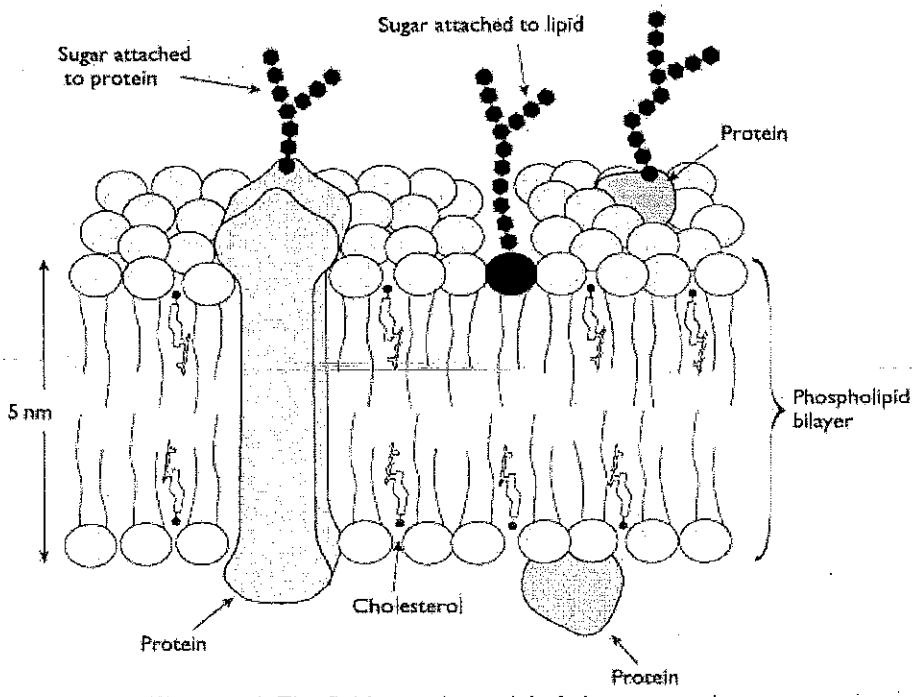


Figure 7.1: The fluid mosaic model of plasma membrane.
(From: http://www.biochemistry.org/Portals/0/Education/Docs/BASC08_full.pdf).

As a dynamic barrier to most molecules, the plasma membrane regulates movement of solutes across its hydrophobic part into and out of cells since it is selectively permeable to solutes. Solutes can cross the plasma membrane by simple diffusion, facilitated diffusion

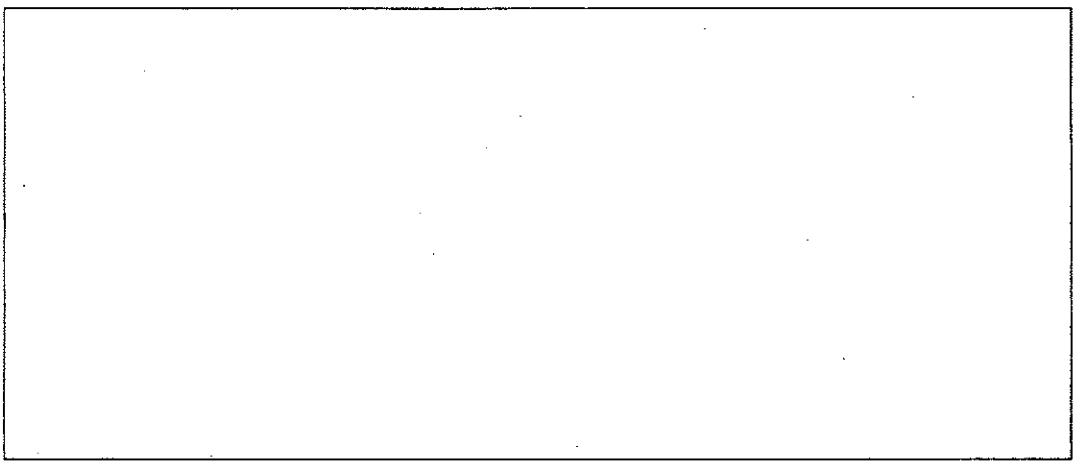
or active transport depending on their sizes, polarity, charge, the presence of specialized proteins, etc.

Simple diffusion is *the process by which molecules move towards equilibrium by crossing the membrane down their concentration gradient (from the side of higher solute concentration to the side of lower concentration).* This process depends on the properties of the solute. For instance, solutes that are charged cannot pass by simple diffusion due to the presence of the hydrophobic interior of the membrane, whereas solutes with reduced polarity (such as propanol and O_2) can easily cross the membrane. The smaller the size of the solute the quicker it is to pass through. This process does not require proteins and is only dependent on the concentration gradient between both sides of the membrane.

Not only can solutes move by simple diffusion but so can water molecules. **Osmosis** is the diffusion of water molecules across a selectively permeable membrane is. In osmosis, water molecules move from a region of higher water potential (low solute concentration) to a region of lower water potential (higher solute concentration) through a selectively permeable membrane.

When a cell is immersed in a solution, the *tonicity* of the solution can be classified by comparing its concentration to the concentration inside the cell as follows:

1- **Hypotonic solution**: is a solution of lower solute concentration than that inside the cell. Therefore, water molecules tend to move *inside the cell* causing it to *swell and then burst* (animal cell) or become *turgid* (plant cell).
2- **Isotonic solution**: the solute concentration of the solution is the same as that inside the cell. Thus the rate of water entering the cell is equal to the rate of water exiting the cell resulting in *no net gain or loss of water by the cell.*
3- **Hypertonic solution**: is a solution of higher solute concentration than that inside the cell. Therefore, water molecules move *outside the cell*, causing it to *shrink.* As the plant cell is surrounded by a cell wall, the cell membrane is partially detached from the cell wall in a process called ***plasmolysis*** (Figure 7.2).



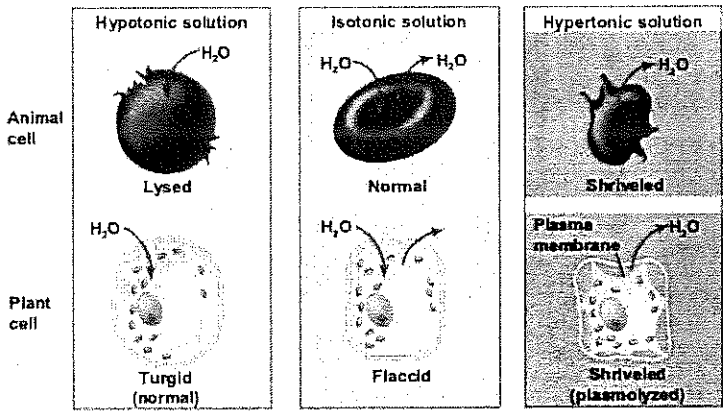


Figure 7.2: Response of the plasma membrane in animal and plant cells in hypotonic, isotonic and hypertonic solutions (Campbell et al, 2009).

The rate of diffusion (or osmosis) across membranes is determined by many important factors such as cell size and shape. Specialized cells, such as epithelial cells lining the small intestine are elongated with villi. Their main function is the transport of nutrients, hence the excess surface area by these protrusions allows for faster diffusion. The **surface area-to-volume ratio** plays an important part in controlling diffusion.

MATERIALS

1-	Methylene blue	2-	Petri dish of 1% agar	3-	1M $AgNO_3$
4-	1M KBr	5-	1M $K_3Fe(CN)_6$	6-	$KMnO_4$ dye
7-	0.1% NaCl	8-	0.9% NaCl	9-	10% NaCl
10-	1M NaCl	11-	IKI	12-	Starch 10%
13-	70% ethanol	14-	Onion	15-	Light compound microscope
16-	Watch glass	17-	Beakers	18-	Dialysis bag
19-	Lancets	20-	1% Phenolphthalein	21-	2% agar containing NaOH and phenolphthalein
22-	0.1 M HCl	23-	0.1 M NaOH	24-	Dull knife

PROCEDURE

- **Diffusion in a liquid:**

1- Fill a 50 ml beaker with water and do not move it for 10 minutes.

2- Gently add a drop of methylene blue to the surface of water. Do not allow the drop of dye to splash.

3- Observe the spreading of the dye through the solution. Notice the beaker at the end of the lab session.

Alternative: Place a tea bag slowly to the beaker

Extra Activity: *Experiment on what will happen when the temperature of the water inside the beaker changes or when the color of the dye is changed.*

- **Diffusion in a solid:**

1- Take a Petri dish of 1.5% agar that already was prepared to have 4 holes according to Figure 7.3

2- With a dropper add one drop of the following solutions to the corresponding holes: (A) 1M $AgNO_3$, (B) 1M $K_3Fe(CN)_6$, (C) 1M KBr, (D) 1M NaCl. Be careful not overfill the holes and try to fill them all to about the same level.

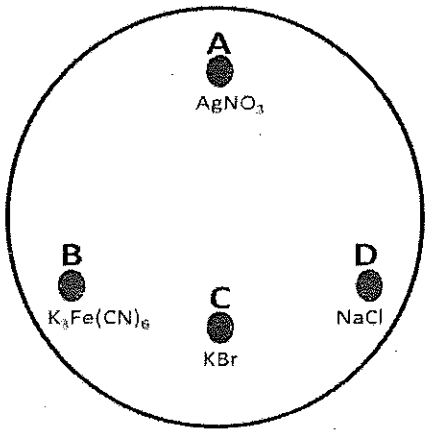


Figure 7.3: Agar Petri dish with four different chemicals.

3- To another Petri dish, add one drop of the following solutions: (A) Potassium permanganate ($KMnO_4$), (B) methylene blue, (C) diluted potassium permanganate solution, (D) diluted methylene blue solution.

4- Place the Petri dishes on the bench, until you can see colored bands forming where the solutions meet solution A. Ag^{+} will react with negative ions (Cl^{-}, Br^{-}, $Fe(CN)_6^{-3}$) from other solutions to form a precipitate. Also, observe the halo formed due to the diffusion of potassium permanganate and methylene blue in the agar.

5- In the first Petri dish, measure the distance between each hole and the colored band, record your measurements.

6- Rank the relative rates of diffusion of Cl^{-}, Br^{-}, $Fe(CN)_6^{-3}$ ions as slow, faster, fastest.

7- Measure the diameter of the halo formed due to the diffusion of potassium permanganate and methylene blue. Also, observe the effect of dilution on the rate of diffusion in both dyes. Record your results.

- **Diffusion in an artificial cell:**

1- Fill a dialysis bag with 10% starch solution. Make sure you tie up both ends well and that the bag is rinsed thoroughly with water.

2- Immerse the dialysis bag in a beaker containing a diluted iodine solution.

3- Record your results after two hours.

- **Surface area and cell size:**

1- In two clean test tubes, add three drops of phenolphthalein

2- Add three drop of 0.1 M HCl to one test tube, swirl to mix the solutions, and observe the color. Record your observation.

3- To the other test tube with phenolphthalein, add 3 drops of 0.1 M NaOH. Record your observation.

4- Answer the following questions: *What color is the dye in the base? What color is the dye in the acid?*

5- Prepare your model cells by cutting 3 blocks of agar of different sizes using a dull knife.

6- Calculate the *surface area* of each of your three cells.

7- Calculate the total volume of each of your cells.

8- Put each model cell into a beaker of 0.1 M HCl. Observe what happens next. Determine which model cell allows the fastest diffusion of the solution and which one allows the slowest. How do you explain the difference?

- **Osmosis in animal cells (red blood cells):**

1- To four test tubes add 4 ml of the following: (A) distilled water, (B) 0.1% NaCl, (C) Saline (0.9% NaCl), (D) 10% NaCl

2- Clean the tip of your finger with a piece of cotton soaked in 70% ethanol. With a sterile lancet puncture the tip of your cleaned finger.

3- Add 1-2 drops of blood to each of the solutions in step 1. Clean your finger.

4- Take a drop from each solution mixed with blood and make a wet mount.

5- Examine the slides under the compound microscope. Draw your observations.

- **Osmosis in plant cells (onion cells):**

1- To four watch glasses add 4 ml of the following: (A) distilled water, (B) 0.1% NaCl, (C) Saline (0.9% NaCl), (D) 10% NaCl.

2- Cut small and uniform sections of onion and add them to the referred salt solutions for 5 minutes.

3- Pull the epidermis of the onion section soaked in the previous salt solutions and make a wet mount.

4- Examine the prepared slides under the compound microscope. Draw your observations.

5- While examining the wet mount of the onion epidermis in 10% NaCl, put few drops of distilled water on the edge of the cover slip and absorb the water by a piece of paper from the other edge.

6- Draw and explain your observations.

- **Unknown:**

Prepare a slide of an onion tissue soaked in an unknown solution and determine the possible percentage of the solution's concentration. Draw and indicate the state of the cell and the term given for the tonicity of the solution.

Activity Eight

Cellular Activities

INTRODUCTION

Cells are constantly doing chemistry. The set of all chemical reactions performed by cells in order to stay living is called **metabolism**. Metabolism can be categorized into **catabolism** (breaking down of molecules to extract energy) and **anabolism** (using chemical or light energy to make molecules). In this lab we will study photosynthesis (an anabolic process) and fermentation as an example of a catabolic pathway.

I- Photosynthesis

Photosynthesis is a process by which organic compounds are synthesized from CO_2, and water in the presence of light. Hence, most plants are strictly *photoautotrophs*. Chloroplasts are the sites of photosynthesis. This pathway involves two main processes:

1- **Light reactions**: light energy is captured by the chlorophyll pigments and is converted to chemical energy (ATP) by **photophosphorylation**. A water molecule splits into O_2 and electrons and protons (H^+), which are eventually transferred through a series of protein complexes to the coenzyme $NADP^+$ forming NADPH. Energy of electron transport is stored in a proton gradient that is used to generate ATP. *The products of light reactions provide the energy and reducing power for the carbon assimilation reactions.*

2- **Calvin cycle (Carbon assimilation reactions)**: the carbon atom from carbon dioxide (CO_2) is incorporated into organic molecules that are essential to provide energy for plant cells and for the whole biosphere for that matter.

The equation in Figure 8.1 summarizes the inputs and outputs of photosynthesis:

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

Figure 8.1: General equation of photosynthesis

In this experiment, you will study the effect of different factors such as light and temperature on the rate of photosynthesis. The rate of photosynthesis can be assessed by measuring the volume of O_2 produced during the reaction and trapped in an inverted tube. The more oxygen produced the higher the rate of photosynthesis.

II- Fermentation in Yeast:

Fermentation is a metabolic process that converts sugar to acids, gases or alcohol, extracting energy from it. Fermentation does not supply ATP directly, but its action complements glycolysis by the regeneration of NAD^+ that helps glycolysis continue with the production of 2 ATP molecules per glucose. Fermentation occurs in bacteria and yeast cells as well as in oxygen-depleted muscle cells.

Yeasts are unicellular fungi. These organisms obtain energy from different carbohydrates by fermentation. In this experiment, you will measure the volume of CO_2 produced during the fermentation of different carbohydrates by yeast cells under anaerobic conditions (Figure 8.2):

$$C_6H_{12}O_6 \xrightarrow[fermentation]{glycolysis} 2CO_2 + 2C_2H_5OH + 2ATP + heat$$

Glucose *Ethanol*

Figure 8.2: General equation of fermentation.

MATERIALS

1-	*Geranium* leaves	**2-**	Sodium Bicarbonate ($NaHCO_3$)
3-	Funnels	**4-**	Graduated test tubes
5-	Beakers	**6-**	Sucrose (10%)
7-	Galactose (10%)	**8-**	Molasses (10%)
9-	Glucose (10%)	**10-**	Fructose (10%)
11-	Dry yeast and 6 fermentation tubes	**12-**	Ice bucket
13-	Lamps (40W, 150W)		

PROCEDURE

- **Effect of light on the rate of photosynthesis:**

1- Put 500 ml of Sodium Bicarbonate ($NaHCO_3$) in a 1000 ml beaker
2- Add 5 green leaves to the beaker
3- Place an inverted funnel on top of the leaves. Make sure that no air bubbles are formed and left on the leaves by rotating the funnel in a circular motion or by inserting a rod through the opening of the funnel and stir out the bubbles.
4- Cover the end of the funnel with a graduated test tube filled with sodium bicarbonate.
5- Place the beaker under a source of light.
6- As photosynthesis proceeds oxygen gas is released and collected in the inverted graduated test tube. Measure at regular time intervals how much oxygen is released (as ml in the graduated tube).

The experiment is carried out under different environmental conditions:

a. At room light in ice
b. At room light in 50°C water bath
c. At room light & room temperature ----- "Control"
d. At room temperature in the dark
e. At room temperature under a 40 Watt light bulb
f. At room temperature under a 150 Watt light bulb
g. With water rather than sodium bicarbonate in the beaker.

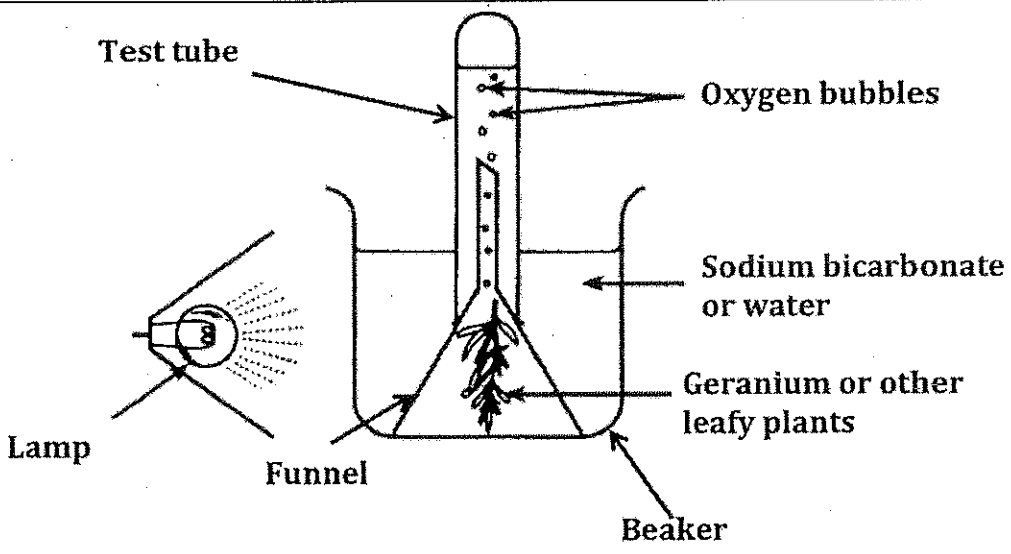


Figure 8.3: A simple apparatus to measure the volume of O_2 released with time due to photosynthesis by plant leaves.

- **Fermentation in Yeast:**

1- Available are six fermentation tubes and six test solutions (for the whole lab section):

(A) 10% sucrose

(B) 10% glucose

(C) 10% galactose

(D) 10% molasses

(E) 10% fructose

(F) water (control)

2- Each group takes one solution and one fermentation tube.

3- In a beaker, measure 0.5 g of dry yeast and add 20 ml of a test solution on it. Mix well and leave at room temperature for 5 minutes.

4- After room temperature incubation, stir the solution gently and pour it into one fermentation tube slowly. Make sure to tip the fermentation tube so the tail of the tube completely fills with the solution and that no air bubbles evolve. *Make sure to label your fermentation tube!*

5- Your initial reading should be zero at zero time point. Place the tube in the 37°C incubator and start the timer.

6- When the timer reads 10 minutes, remove the tube from the incubator and measure the distance from the tip of the tail to the level of the solution. This is your reading (volume of CO_2 in ml) at time 10 minutes.

7- Return the tube to the incubator for 10 more minutes and repeat the procedure. Take readings every 10 minutes for a period of 60 minutes.

8- After the 60 minutes has passed, collect the readings from all other groups and discuss your findings.

Data collection sheets:

Photosynthesis

Table 1: Volume of O_2 (in ml) collected in the graduated test tubes under different environmental conditions for 40 minutes.

Time (min)	Room light/Room temp (control)	Dark / Room temp	40 W Light/Room temp	150 W Light/Room temp	On Ice/Room light	50° C / Room light	Water
0							
5							
10							
15							
20							
25							
30							
35							
40							

Fermentation in yeast

Table 2: Volume of CO_2 (in ml) collected in the graduated centrifuge tubes for 60 minutes.

Time (min)	10% Sucrose	10% Fructose	10% Glucose	10% Galactose	10% Molasses	Water
0						
10						
20						
30						
40						
50						
60						

Activity Nine

Cell Division – Mitosis

INTRODUCTION

Cells are produced from pre-existing cells by cell division. In this process, cells divide to produce daughter cells. The division of the nucleus is referred to as **mitosis**, which is followed by **cytokinesis**, the division of the cytoplasm. Mitosis occurs in somatic cells, producing genetically identical daughter cells (diploid), that is, each cell has virtually the same DNA sequences. In a unicellular organism, mitosis is responsible for the production of a new entire organism. While in a multicellular organism, mitosis functions in the organism growth, and the replacement and renewal of cells.

The cell accomplishes the equal distribution of its DNA set to the daughter cells through different stages known as the **cell cycle** (Figure 9.1). Mitosis is only one stage in the cell cycle, and the rest of the cycle is referred to as **interphase**. Interphase is divided to three subphases: **G_1 phase** (first growth/gap), **S phase** (DNA synthesis) and the **G_2 phase** (second growth/gap). In the G_1 phase, organelles and proteins are synthesized preparing the cell for the next stage, the S phase in which the DNA is replicated. The DNA is in the form of greatly extended fibers that form a tangled mass called **chromatin**. The fibers are too small to be seen in the microscope, so the nucleus looks homogenous and it is bounded by the double nuclear membrane and contains nucleoli, which may not be visible in every cell. After the replication of DNA, the cell resume its growth in the G_2 phase and ensures that the DNA was properly replicated.

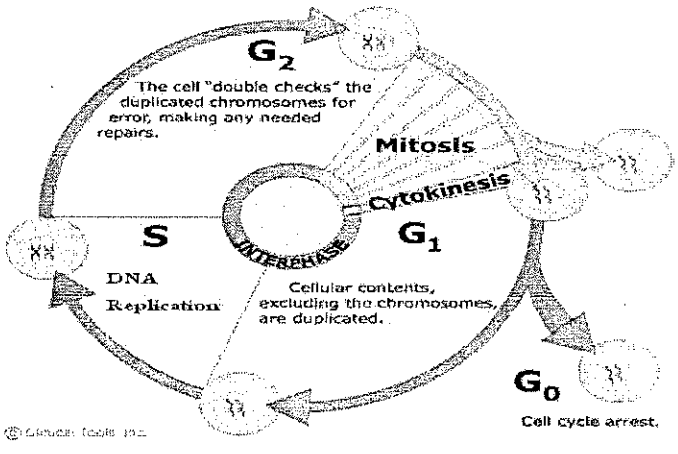


Figure 9.1: A Eukaryotic cell cycle (Becker et al, 2009).

The mitosis phase is also divided into different continuous subphases (Figure 9.2): Prophase, prometaphase, metaphase, anaphase and telophase.

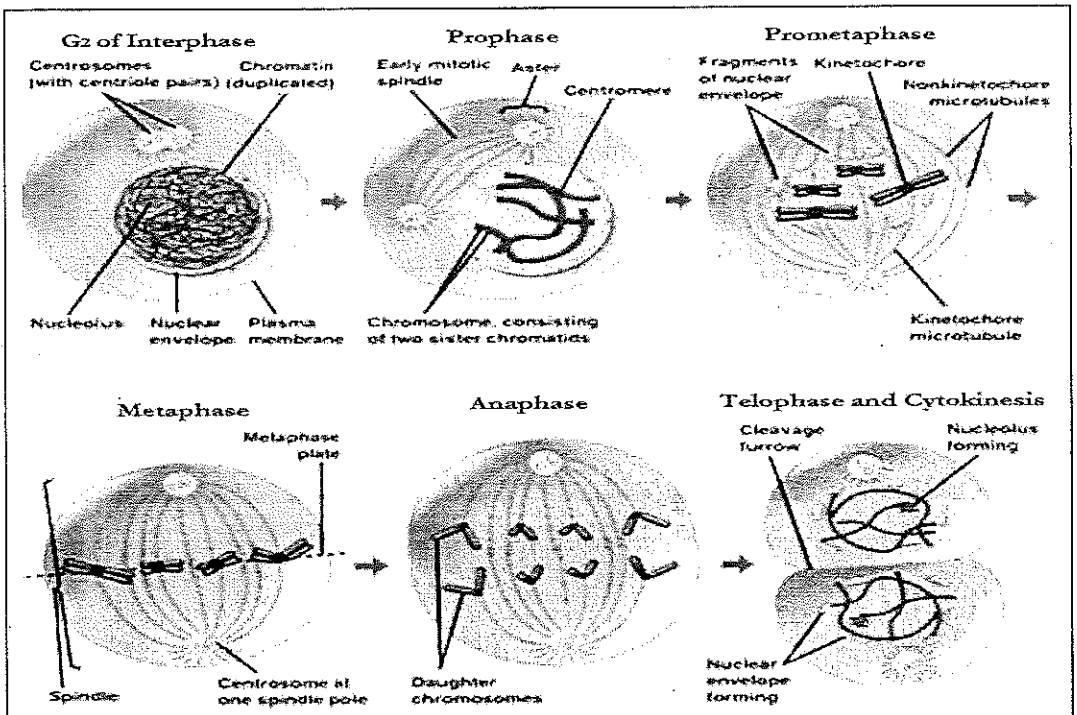


Figure 9.2: The G_2 phase of interphase and the distinct phases of mitosis in an animal cell. (Campbell and Reece, 2009).

1. Prophase: Chromosomes become visible under the light microscope, due to the coiling of the chromatin. Each duplicated chromosome appears as two sister chromatids joined together at the **centromere**. The nucleoli disappear. The two centrosomes[6] become surrounded by radiating microtubles called asters and spindles. While the centrosomes move apart to the opposite poles of the cell as the mitotic spindles push them apart.

2. Prometaphase: The nuclear envelope fragments, allowing the microtubules of the mitotic spindle to attach to center of each chromatid in a specialized protein structure called **kinetochore**. This attachment causes the chromatids to begin to move to the opposite poles of the cell.

3. Metaphase: The spindle is completely formed, and the maximally condensed chromosomes are aligned at the **metaphase plate** in the cell equatorial plane due to the equal pull of forces on the kinetochores from both directions.

[6] In the S phase the cell's centrosome is divided into two.

4. Anaphase: This phase begins when the centromeres of each chromosome separate as certain severing proteins are activated to cut the attachment of both chromatids. The kinetochore microtubules shorten causing the movement of the sister chromatids (now referred to as chromosomes) apart from each other to opposite poles of the cell. The cell elongates due to the elongation of the non-kinetochore microtubules.

5. Telophase and **cytokinesis**: The nuclear membranes and nucleoli in both daughter cells reappear. In the case of plant cells, a **cell plate** forms a boundary between the two daughter cells before cytokinesis. While in animal cells, there is only a formation of a constriction midway between the already divided nuclei. This constriction is called the **cleavage furrow**, as if a belt were being drawn tightly around it. This furrow deepens and finally penetrates through the whole cytoplasm, dividing it completely into halves and bringing into existence two daughter cells (*cytokinesis*).

Cell division also takes place in reproductive cells, however daughter cells that have half the set of DNA (haploid) of the original cell, are produced in a process called **meiosis**, which is a specialized form of mitosis. Meiosis, takes place in gonads (ovaries and testes) leading to the formation of gametes (eggs and sperms). In sexual reproduction, gametes fuse together forming a **zygote.**

In this laboratory, you will be introduced to mitosis in onion cells of a root tip and in the buds of yeast (*Saccharomyces cerevisiae*).

MATERIALS

1-	Prepared slides of onion root tip	2-	Fixed onion root tips
3-	Carnoy fluid	4-	6M HCl
5-	Toluidine blue dye	6-	Fermented yeast solution
7-	Watch glass	8-	Microscope slides
9-	Cover slips	10-	Light compound microscope

PROCEDURE

- **Mitosis in cells of onion root tips:**

1- Obtain a prepared slide of an onion root tip and determine the stage of the cell cycle in 100 cells, and record your results in a table.

2- Calculate the percentage of cells in each stage, and the time that is spent in each stage.

NOTE: By determining the percentage of cells in each stage of mitosis and in interphase, you can calculate the amount of time spent in each stage. For example if 20 cells out of 100 were found to be in prophase, the percentage of cells in prophase is 20/100 x 100 = 20%. Assuming that the cell cycle of an onion root tip cell takes about 16 hours, you can determine the amount of time spent by the cell in prophase by multiplying the percentage by the length of the cell cycle. So, if you can assume that the onion cell spends 20% of the time in prophase, it spends 0.20 x 16 hr or 1.6 hr (1 hr and 36 min) in that stage.

- **Preparing mitotic squashes:**

1- *(For technicians and TAs only):* Suspend a mature onion bulb with its bottom third in water. Let it develop roots in the dark, otherwise, crooked roots will develop. When roots are about 1 to 2 cm long (in 1 to 2 days), they should be cut and transferred to Carnoy fluid (no chloroform) for killing and fixing the cells. After 4 hours in Carnoy fluid, the root tips can be used. In no case should the root tips be left in Carnoy fluid for more than 48 hours. If it is necessary to keep the roots for more than 48 hours, transfer them to the holding solution. They can be kept for three to four months before use.

2- Obtain two small cups. Label one "HCl" and pour enough 18% HCl into it to cover the bottom. Likewise, label the other "Carnoy" and pour enough Carnoy fluid in it to cover the bottom. Use forceps to transfer an onion tip into the HCl cup, allow the root to remain for 4 minutes.

3- Transfer the root into the Carnoy fluid and allow it to remain for 4 minutes.

4- *(Students' procedure starts here):* Place the root on a slide. With a razor blade or any other sharp instrument, cut off 1 to 2 cm of the root tip and discard all but the tip.

5- Cover the root tip with 3 drops of Toluidine blue for 2 minutes. After 2 minutes, blot away the stain; be careful not to touch the root tip. When the stain has been blotted away, cover the root tip with 2 drops of water and blot again. Then add one drop of water.

6- Gently lower a coverslip over the root tip. Cover the slide with a paper towel or other absorbent, and with your thumb, firmly press (squash) on the coverslip; do not twist the coverslip. This pressure will spread the cells into a single layer. Observe your preparation

under the microscope. If the cells are not sufficiently separated to permit viewing of each, squash the preparation again.

7- Collect the class data for each group, and calculate the mean and standard deviation for each group. You must make a table in your notebook for the class data.

8- Compare the number of cells from each group in each of the phases.

- **Asexual reproduction in Yeast (budding):**

1- Make a wet mount of a drop of an actively dividing yeast culture, and observe the cells in the microscope under high power. Note the cells that are producing smaller buds or short chains of buds. Draw what you see.

Activity Ten

Genetics

INTRODUCTION

Genetics is the *scientific study of the transmission of traits from parents to offspring*, or simply the *scientific study of heredity and heredity variation*. The heredity "factor" or "units" are called **genes**. A gene is a sequence of nucleotides in the DNA that code for the amino acid sequence of the polypeptide chains, or for different RNA types such as transfer RNAs, ribosomal RNAs, and micro RNAs.

Each gene has a distinct place in the chromosome referred to as **locus** (plural: loci). Therefore, in a diploid organism two copies of the gene are present. Each copy is referred to as an **allele**. If an organism has two identical alleles for a certain gene it is considered **homozygous** for that gene. Otherwise, an organism with two different alleles for a certain gene is referred to as **heterozygous** for that gene. In a heterozygous organism, usually one of the alleles is **dominant** and the other is **recessive**.

Dominant alleles are responsible for the determination of the appearance of a certain trait, while the traits determined by the recessive alleles cannot be noticed in the organism when a dominant allele is present. Therefore, the genetic makeup of an organism, or its **genotype** can differ from the organism's **phenotype**, or its observable trait.

Our analysis in this laboratory will be confined to certain well-defined traits, which *were believed* to be inherited in a relatively simple fashion (*fact check, they are NOT!*) The methods will be those of observation, and an elementary statistical analysis.

According to Hardy-Weinberg principle, results of a monohybrid cross are nothing other than the expansion of the simple binomial $(p + q)^2 = (1)^2$. If we let the letter **p** stand for **the frequency of the dominant allele** of a pair (e.g. T), and **q** stands for **the frequency of the recessive allele** (e.g. t), it is obvious that the expression $p^2 + 2pq + q^2$ expresses the results of the F2 of a monohybrid cross, with the familiar 3 : 1 ratio, or TT + 2 Tt + tt.

It is then possible to define a population by the frequency with which various alleles appear in it. All people who show the recessive phenotype must be genotypically homozygous for the recessive allele; however, those who are phenotypically dominant may be either homozygous or heterozygous.

When considering complete dominance, the frequency of the recessive allele, q, can be calculated according to Hardy-Weinberg principle by the following equation:

$$q = \sqrt{f(aa)}$$

And from the equation $p+q = 1$; $p = 1 - q$.

For example if 36 per cent of the class are non-tasters, the frequency of non-tasters is 0.36. The square root of this is 0.60. Thus we can say that although only 36 percent of the population shows the recessive trait, that 60 percent of all the genes in that population are recessive alleles.

Since this is true, simple subtraction gives us the figure for dominant allele $p = 0.40$. Substitution in our equation,

$$p^2 + 2pq + q^2 = 1$$

with $p = 0.40$ and $q = 0.60$ we find the following $0.16 + 0.48 + 0.36 = 1$. Now we can make the following statements:

1. In any population with 36 percent of the members showing the recessive trait:
   a) 16 percent are homozygous dominant.
   b) 48 percent are heterozygous.
2. To apply the Hardy-Weinberg principles the following assumptions have been made:
   a) There is complete random mating among the members of the population studied.
   b) There is a very low rate of mutation from one allele to the other.
   c) All genotypes are equally viable and equally likely to make - no selective value.

MATERIALS

1-	PTC papers	2-	Thiourea papers
3-	Sodium benzoate papers	4-	Fruit flies (*Drosophila melanogaster*)
5-	Light dissecting microscope	6-	Corn color genetics display

PROCEDURE

- **Human characteristics:**

NOTE: In this exercise, you will *ASSUME* that each character is determined by **one gene**, and that each gene has **two alleles**. This is because not many human traits are controlled by a single gene with two alleles and these examples are *ONLY* for the sake of understanding basic genetics.

1- Study the following traits, and specify if you are dominant or recessive for the described traits.

a) The ability to taste different chemicals:

Certain people have the ability to taste one or all of the following chemicals: phenylthiocarbamide (PTC), thiourea, and sodium benzoate, while others do not have this ability. Do you taste anything?

The ability to taste such compounds is inherited as a dominant trait. It is important to note that the sensitivity to such compounds can be affected by having a dry mouth, or eating and drinking before tasting such compounds thus other genes or environmental factors can influence the ability to taste.

b) Free and Attached Ear Lobes:

Observe whether the fleshy part of the lower ear lobe is free or attached to the skin of the head (Figure 10.1). Do you have free or attached ear lobe? Free ear lobes are said to be inherited as a dominant trait, attached ear lobes are recessive traits. This characteristic though has continuous variation thus making it an invalid example.

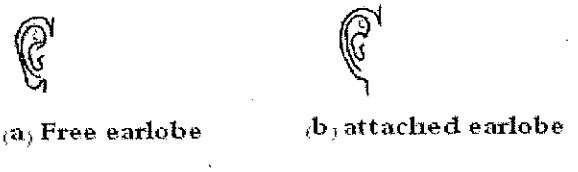


Figure 10.1: A picture showing and individual with a free earlobe (a), and an attached earlobe (b).

c) Hairline (Widow's peak)

The presence of a widow's peak (a V-shape) at the hairline is inherited as a dominant trait. Recessive individuals will have a straight hairline (Figure 10.2). This trait, nonetheless, was not proven scientifically to be influenced by genetics.

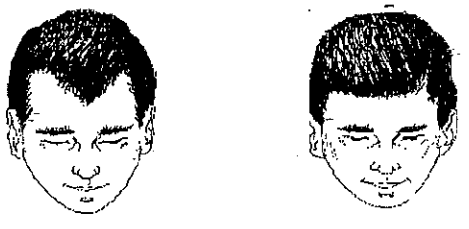


Figure 10.2: Comparison between widow's peak (left) and straight hairline (right).

d) Tongue rolling

Those who can roll their tongue into a tube are considered to have at least one of the alleles thus being dominant. Some people can't therefore they are said to have the recessive trait. This trait may be learned and hence not considered controlled by a gene.

LACK OF ABILTY TO ROLL TONGUE ABILITY TO ROLL TONGUE

Figure 10.3: Comparison between a person who is able to roll their tongue (right) and one who lacks the ability (left).

2- Record the traits mentioned above for your classmates.

3- For each character, determine which trait is recessive and which is dominant.

4- In the classroom population, calculate the phenotypic ratio and the frequency of the recessive and dominant alleles.

- **Corn color**

Your instructor or TA will provide you with a display showing the inheritance of the characteristic, corn color, in two different traits yellow and purple.

1- Count the corn kernels, purple and yellow, of the parents and offspring and determine which trait is dominant and which is recessive.

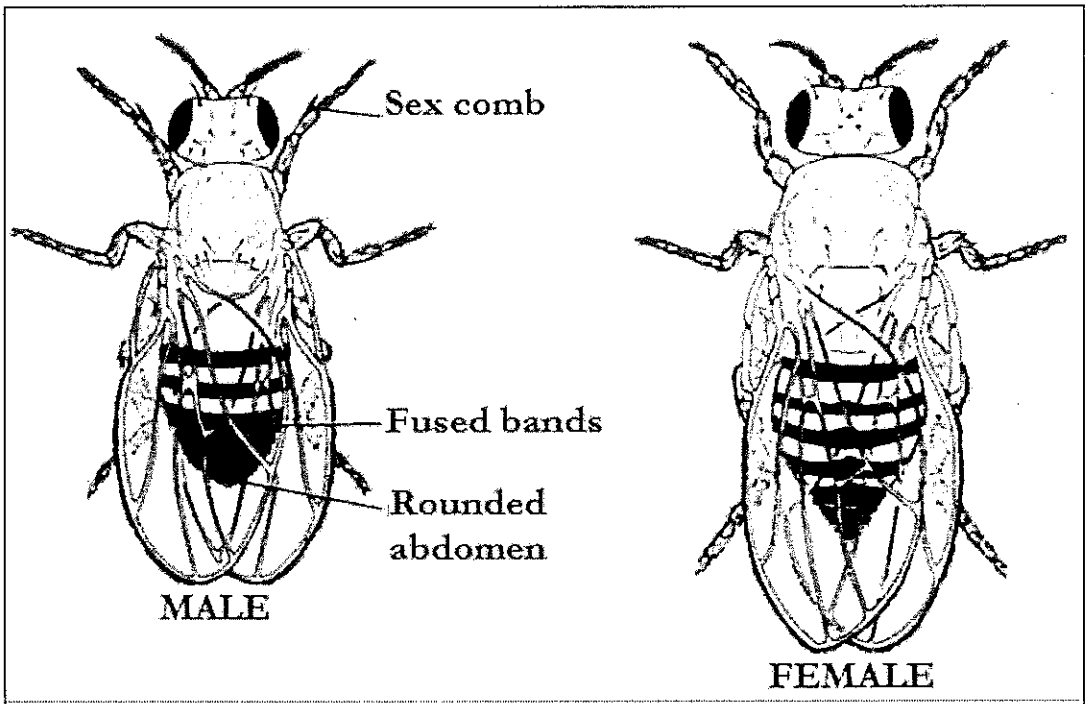
2- Calculate the phenotypic ratio and the frequency of the recessive and dominant alleles.

- **Going outside class borders:** *Go around campus and sample many students with regards to these traits. Record your observations and discuss them in class.*

- **Distinguishing between male and female fruit flies (*Drosophila melanogaster*):**

1- Obtain etherized fruit flies and examine under a dissecting microscope.

2- Distinguish between male and female fruit flies (Figure 10.4).



(a) Male fruit fly (b) Female fruit fly

Figure 10.4: Male and female fruit flies. (a) A male fruit fly is smaller in size, with a dark abdomen and a sex comb in the first leg. (b) A female fruit fly is larger, with a striped abdomen, and present no sex combs (from http://alpha.sci.ege.edu.tr/~genetik/alpagut/dr.pdf).

Activity Eleven (optional)

Anatomy of Ascaris and Earthworm

INTRODUCTION

I. Nematoda – *Ascaris*:

Ascaris is an invertebrate parasite of phylum Nematoda. Humans can serve as hosts of *Ascaris lumbricoides*. Adult worms are found in the intestine of the host. In this section, you will be introduced to the external and internal anatomy of *Ascaris lumbricoides.*

a) External Anatomy (Figure 11.1):

The body form is cylindrical, long (length varies according to species and sex) and tapering at both ends. *Ascaris* is dioecious (i.e., has separate sexes). The female is larger and has a straight posterior end, while the male is more slender and has its posterior end sharply curled ventrally.

The *Ascaris* body is covered by a cuticle layer. Four longitudinal streaks run the entire length of the body: two thin, white dorsal and ventral lines, and two broader and darker lateral lines (some of the internal organs show through the transparency of the body wall along the latter two streaks). These four lines mark the internal division of the muscles in the body wall into four quadrants. The mouth, lies at the anterior end of the body and is guarded by three finely **papillated lips**, one dorsal and two ventrolateral. The minute **excretory pore** lies on the ventral side, two millimeters behind the mouth. The **genital aperture** in the female is situated on the ventral side some distance from the anterior end (at about one third the length of the body in *Ascaris lumbricoides*). In the male, the genital duct joins the hindgut and both open by a common **cloaca** a short distance from the posterior end. A pair of minute **copulatory spicules** project from the latter aperture. The female has a separate slit-like anus.

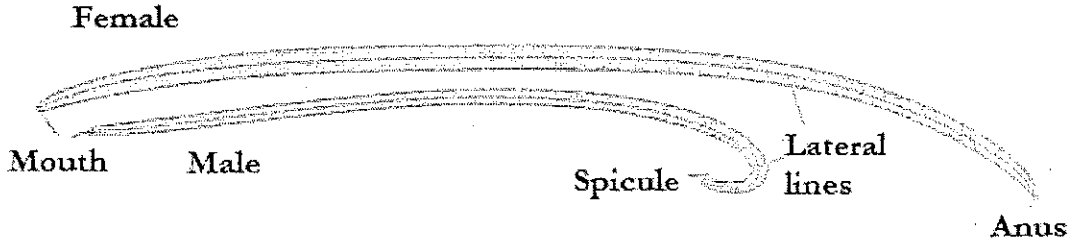


Figure 11.1: The external anatomy of a female *Ascaris* (upper) and a male *Ascaris* (lower).

b) Internal Anatomy (Figure 11.2):

1. **Digestive system:** The **alimentary canal** is a ribbon-like structure that runs along the body and consists of three parts: a short **stomodaeum**, a **long midgut** and a short posterior **proctodaeum**. The first and the third are ectodermal in origin; while the middle one is of endodermal origin. The stomodaeum is differentiated into a short buccal cavity followed by a short muscular esophageal swelling (often called pharynx). While the proctodaeum consists of the anal region.
2. **Excretory system:** There are two **excretory canals**, each extending along a lateral line.

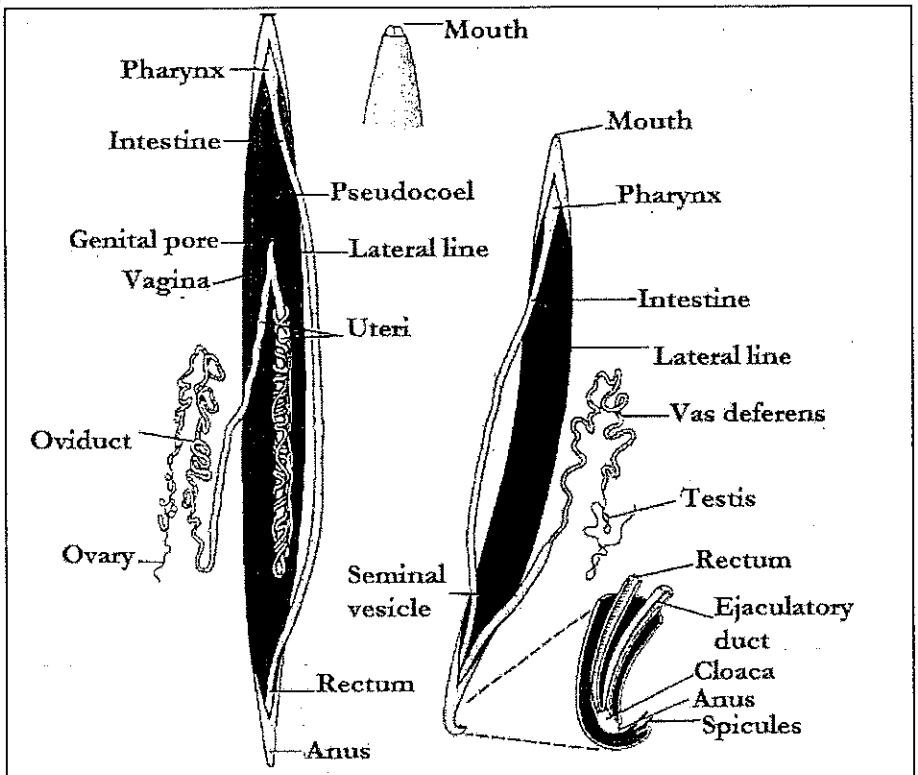


Figure 11.2: The internal anatomy of: (A) a female *Ascaris*, (B) a male *Ascaris*.

3. Reproductive systems: The **male system** consists of a single thread-like, much coiled **testis**, which lies in the anterior half of the body and passes out into a coiled **vas deferens**, which dilates into a wider but straight **vesicular seminalis**. This opens with a short muscular ejaculatory duct into the hindgut, and to the outside by a **cloaca**. Note also the presence of two **copulatory spicules** lying in two small pouches dorsal to the ejaculatory duct.

The **female system** consists of two thread-like structures called **ovaries**, one anterior and the other posterior. Each leads into a coiled and somewhat less opaque

oviduct. This leads into two wide straight uteri. In the case of *A. vitulorum*, the oviduct dilates into a spherical chamber, the receptaculum seminis, before leading into the uterus. The two uteri unite together and form the single **vagina**, which tapers anteriorly and opens by the genital aperture. The reproductive system can better be traced from the genital aperture to the gonads. Note that the coils of the gonads and their ducts embrace the midgut.

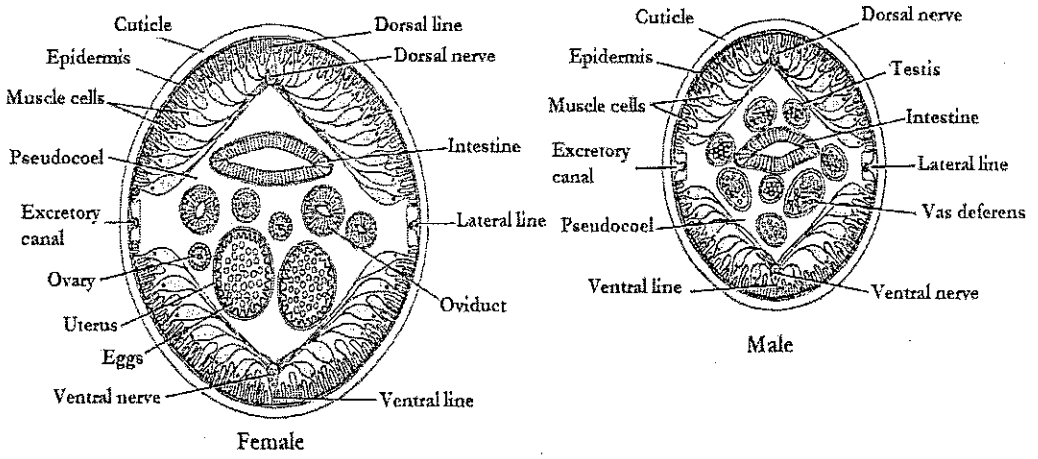


Figure 11.3: Cross section of left Female *Ascaris* and right male *Ascaris*,

II. Annelida – *Earthworm*:

Bilaterally symmetrical worm characterized by a ringlike series of similar (metameric) segments containing paired setae, nephridia, ventral ganglia, and other duplicated structures; a complete digestive tract, large coelom, thin non-chitinous cuticle, closed circulatory system. Annelida are widely distributed, mostly marine worms. They can also be found in fresh water and soil. There are about 15,000 known species of Annelida. In this section, you will be introduced to the external and internal anatomy of Earthworm.

a) External anatomy:

The earthworm body is segmented. Around the mouth, there is a lip-like structure, the **prostomium**. Note the presence of a sac-like and light pigmented structure called **clitellum**. The clitellum plays an important role in the sexual reproduction of earthworms. Although earthworms are hermaphrodites (monoecious), they cross-fertilize. The clitellum secretes a mucous cocoon that stores the eggs and sperms, and then slips off the worm to the soil, where the embryos develop.

b) Internal Anatomy:

1. **Digestive system:** The segments where each organ is located is referred by using roman numerical system. The digestive system is a continuous, highly specialized, food-handling tube and consists of the mouth and buccal cavity (I-II), pharynx (II-V), esophagus (VI-XIV), crop (XV-XVI), gizzard (XVII-XVIII), intestine, and anus. The animal can be visualized as a tube within a tube, that is, an intestine within the outer body wall. The intestine is enclosed by the inner and the outer walls of the coelom. Specialization of the digestive tract in earthworm is an adaptation for processing large quantities of soil and detritus. The ingested soil is passed through the **mouth and buccal cavity**, then to the well-developed **muscular pharynx**, and the **esophagus** (a passageway leading to the storage and digestion area). In segments X and XI, the esophagus may have three paired glandular swellings (calciferous glands) for calcium and iron regulation. Immediately posterior is the thin-walled **crop** for storage, and a thick, muscular, grinding **gizzard** followed by the long **intestine** for food digestion and absorption. Specialized cells along the outer intestinal wall appear to function for glycogen synthesis and breakdown of proteins to ammonia and urea. The **typhlosole**, a fold extending into the lumen of the intestine, greatly increases the intestinal surface area. The alimentary canal ends at the **anus**, through which soil and unused food material is ejected. Piled up fecal castings are often seen on lawns. These casting represent a substantial amount of tunneling and turnover of soil, an important contribution to aeration and the loosening of millions of tons of surface soil.
2. **Circulatory System:** The earthworm has a closed **circulatory system.** There are three major vessels: one dorsal that functions as the main heart "pumping" blood by peristalsis, and two ventral vessels. The dorsal and ventral vessels are connected by five pairs of vessels, the **pseudohearts** that loop around the digestive tract near the worm's anterior end.

PROCEDURE

- **Dissection of *Ascaris lumbricoides*:**

1- Before dissection, study the external anatomy of a female and a male *Ascaris lumbricoides*.

2- Lay the worm in the dissecting dish with its dorsal side upwards.

3- Pin it down to the dish at its two ends, pour water to cover the specimen.

4- Make a longitudinal incision along the mid-dorsal line using a pin to avoid cutting the internal organs. Since the body may be twisted, care is needed to maintain the proper position of the cut throughout the length of the worm.

5- Pin the sides of the body wall, so that you can identify the inner organs. The digestive and reproductive systems are easier to be identified.

- **Cross section of a female and a male *Ascaris lumbricoides* (Figure 11.3):**

1- Examine the following prepared slides under the compound microscope: a) Cross section of a male *Ascaris*, b) Cross section of a female *Ascaris*.

- **Internal anatomy of Earthworm (Figure 11.4):**

1- Make a shallow dorsal incision a few segments posterior to the clitellum.

2- Cautiously make a continuous shallow cut just to the right of the median dorsal wall, continuing to the anterior end of the worm. Do not cut deeper than the thickness of the outer body wall or the scissors will injure organs lying below the thin epidermis.

3- Carefully separate the flaps by inserting a scalpel under the cut edges. Keep the blade as close as possible to the body wall.

4- Cut away adhering tissues and septal walls, moving toward the anterior until the full length is cleared along each side.

5- Pin back the exposed flaps, and place the pins firmly, pointed away from the specimen. For orientation, observe the general layout of **pharynx, esophagus, hearts, and dorsal blood vessel, genitalia (mostly seminal vesicles), crop, and gizzard, and crosswalls** that mark the internal segmentation. By placing pins every five segments to serve as markers, it will be unnecessary to make repeated counts of segments.
6- Keep your dissection clean by using an eyedropper to wash away debris.

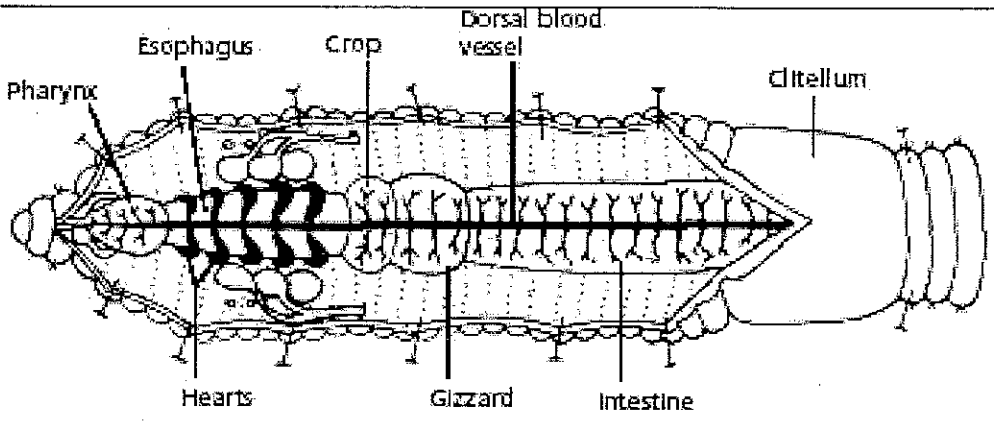


Figure 11.4: The internal anatomy of Earthworm.

- **Cross section of Earthworm (Figure 11.5):**

1- Examine a prepared slide of the cross section of earthworm.

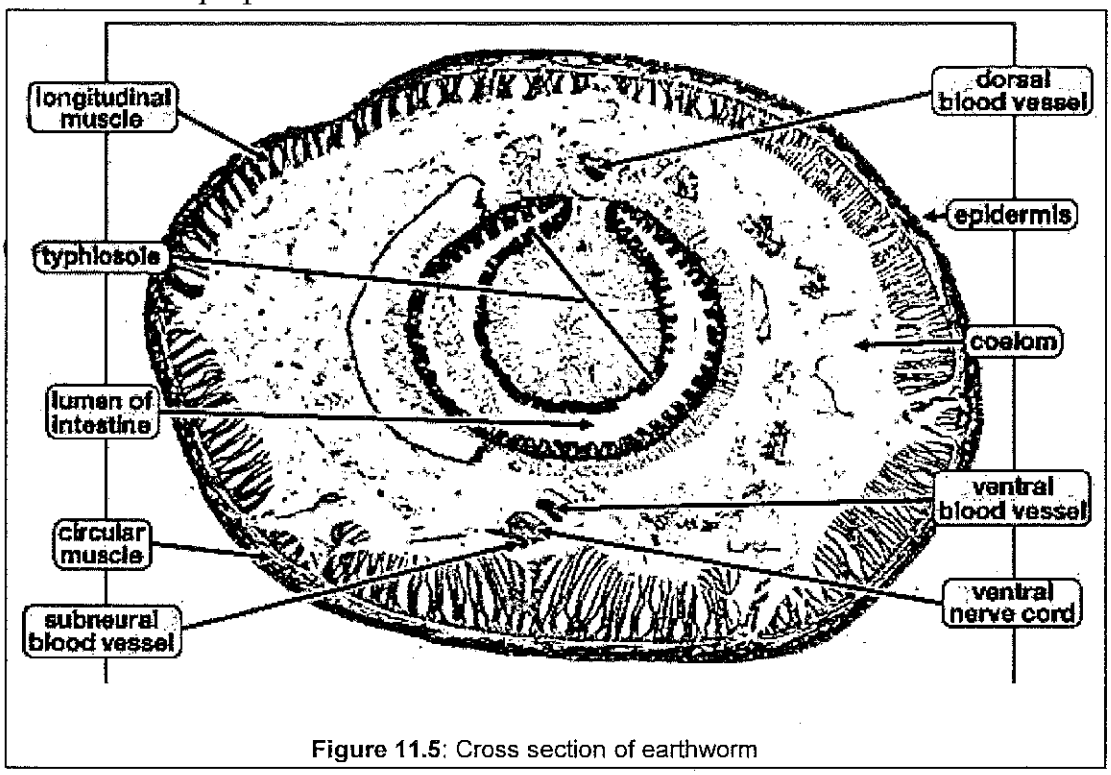


Figure 11.5: Cross section of earthworm

Activity Twelve

Anatomy of the Rat

INTRODUCTION

Mammals are endothermic and maintain a high body temperature. They have **hair** and **sweat glands**, which are important in thermoregulation. Their **mammary glands** produce milk, which is usually released through the nipples to nourish the young after birth. The young are generally retained within the body of the female during their development (**viviparous**), so that they are kept warm. They are, nourished through the blood vessels of the placenta. This organ allows the fetal and maternal circulation exchange nutrients and wastes during development.

a) External Anatomy of the Rat (*Rattus norvegicus*)

Rodents are characterized by long, continually growing incisor teeth that must be worn down as rapidly as they grow. This wearing process removes the dentine surface on the inner face more than it does on the harder outer enamel.

The **vibrissae** (whiskers) project laterally from the snout. These tactile hairs are considerably wider than the animal. In a fraction of a second the rat can tell whether the hole it is diving into, is large enough to accommodate its body. Almost any information discernible by touch reaches the rat through his vibrissae.

Notice the mouth, its anteroventral position, cleft upper lip, and the elongated lower lip. Examine the external nares, the eyes, and eyelids.

In the anterior corner of the eye is a vestigial skin fold, the **plica semilunaris**, apparently homologous to the extra eyelid or nictitating membrane of birds and reptiles.

The **pinna**, or cartilaginous concha of the outer ear, directs sound waves into the ear opening (external auditory meatus) and assists in the determination of the direction or location of the sound source.

Find the anus below the base of the tail. In the **male**, the scrotum, which contains the testes, will be seen near the anus. During the season of reproductive activity, the testes descend from the abdominal cavity into the scrotum, which becomes quite conspicuous. At other times, or in immature males, the scrotum is reduced and the testes are withdrawn into the body cavity. This external location, when testes are spermatogenically active, is

related to the poor tolerance of spermatozoa for normal body temperature. Male gametes are not formed at 37°C or higher, and the scrotal sac is a mammalian adaptation providing a lower temperature for them. Just anterior to the scrotum is the opening of the male urogenital system terminating in the penis, which is usually withdrawn into a skin covering, the prepuce. In the **female** there are three openings: 1) anus 2) vaginal opening in front of the anus, and 3) urinary opening on the tip of a palliate structure in front of the vagina.

The foot digits except the thumb, or pollex, of the forefoot end in claws. Pads, or plantar tubervules, protect the bottom of the feet and provide a gripping surface for walking or climbing, a useful combination with claws. Notice the occasional bristles between the epidermal scales of the tail.

PROCEDURE

- **Internal anatomy of the Rat (Figure 12.1):**

1- Lay the animal on its back and make a medioventral abdominal incision, from pelvis to diaphragm.

2- Cut laterally through the body wall near pelvis and diaphragm to form flaps that can be pinned back to expose the viscera. Remove abdominal fat deposits but do not disturb the alimentary canal.

3- Examine the mouth and the muscular tongue attached to the floor of the mouth cavity. Anterior to this attachment is a more loosely connected portion of the tongue, held by a vertical frenular fold. The roof of the mouth is formed by the anterior hard palate and posterior soft palate. Above the latter is the nasopharynx. Into this large cavity the posterior nares pass, as do auditory tubes that enter the nasopharynx through longitudinal openings near the junction of the dorsal and lateral walls of the pharynx and the posterior border of the soft palate.

4- Locate the **pharynx** that connects with the **esophagus** posterior to the **glottis**, the valve like action of which keeps food from entering the trachea (windpipe). This latter structure, ventrally located in the neck (feel it from the outside), contains the **larynx** (voice box). The glottis is covered by the epiglottis, which is raised when air passes into or out of the larynx but is bent backward over the glottis and trachea while food is being swallowed. Anyone who has choked on food knows the function of the epiglottis.

5- Locate the curved **stomach** that consists of two portions: the thin-walled, large **cardiac sac**, anterior; and the opaque, thicker walled **pyloric sac**, posterior. The esophagus empties into the cardiac sac.

6- Locate the intestine. The food passes into the pyloric sac and then into the anterior part of the small intestine (duodenum) via a muscular fibrous ring, the pyloric sphincter. Find this valve as an externally visible groove.

7- Trace the coiled **small intestine**, supported by **mesenteries** containing numerous blood vessels. Do not damage these structures; simply follow them. The **duodenum, jejunum,** and **ileum** (successive divisions of the small intestine) are inseparable externally. The ileum empties into a saclike **cecum**, which in man is reduced to the vestigial vermiform appendix. The cecum may function as a storage organ for the bulk of ingested food, for bacterial fermentation, or as a special absorptive structure. Digestion of cellulose and other plant materials is particularly difficult, and herbivores characteristically possess a long intestine and large cecum whereas carnivores do not.

8- Trace out the **large intestine**, which starts at the ileocecal junction, to the ascending colon. Bulbous objects inside the terminal portion or descending colon are fecal pellets. Waste passes through the colon, to a short **rectum** (near and within the pelvis), and finally outside via the anus, guarded by an anal sphincter muscle.

9- Observe the large, multilobed brown **liver**. This organ (largest of all the organs) produces, among numerous other substances, bile, which enters the duodenum through the bile duct found in the hepatoduodenal mesentery. **The rat lacks a bile storage organ or gall bladder**. Find the point near the pyloric sphincter where the bile duct joins the duodenum.

10- The **pancreas**, lighter in color than the liver, is a rather diffuse organ in the first (duodenal) loop of the small intestine. Its large dorsal lobe can be seen near the pyloric sac. Pancreatic juice with a number of digestive enzymes flows through many small pancreatic ducts that enter the bile duct, and passes into the gut through the latter tube. (Look near the dorsal surface of the pancreas for the pancreatic ducts). Trace a food bolus from mouth to anus, reviewing all major structures passed along the way.

11- Find the dark, red, spherical **spleen**, posterior to the greater (outside) curve of the stomach. This organ is functionally a part of the circulatory system and a major phagocytic and white-blood-cell-forming center.

12- Locate the bladder.

13- Locate the right and left **kidneys**, with the **adrenal glands**.

14- Examine **the reproductive system of a male rat**. Locate the penis, testes, epididymis, vas deferens, seminal vesicles, and prostate (Figure 11.2.a).

15- Examine **the reproductive system of a female rat**. Locate the ovaries, the uterus and the fallopian tube (Figure 12.2.b).

16- Locate the **diaphragm, heart** and **lungs**.

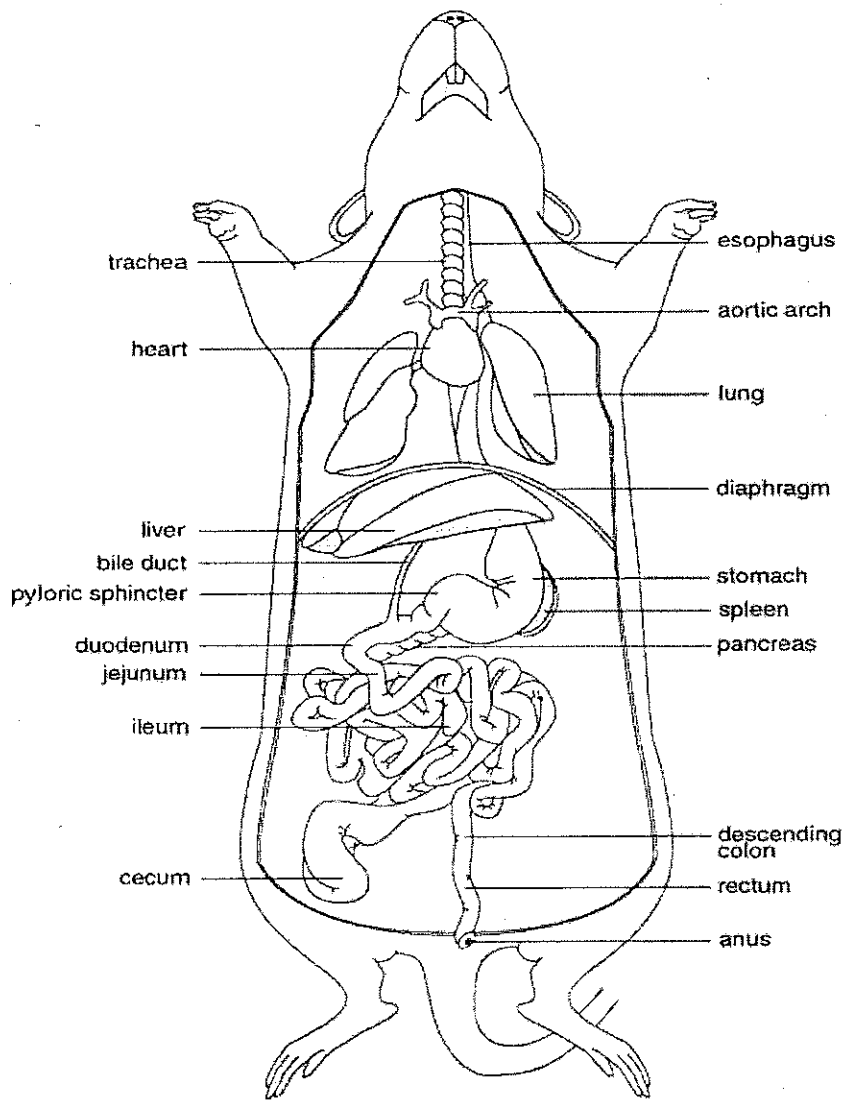


Figure 12.1: The internal anatomy of the Rat (the excretory and reproductive systems are not shown (from http://media.wiley.com/CurrentProtocols/PH/ph0701/ph0701-fig-0001-1-full.jpg).

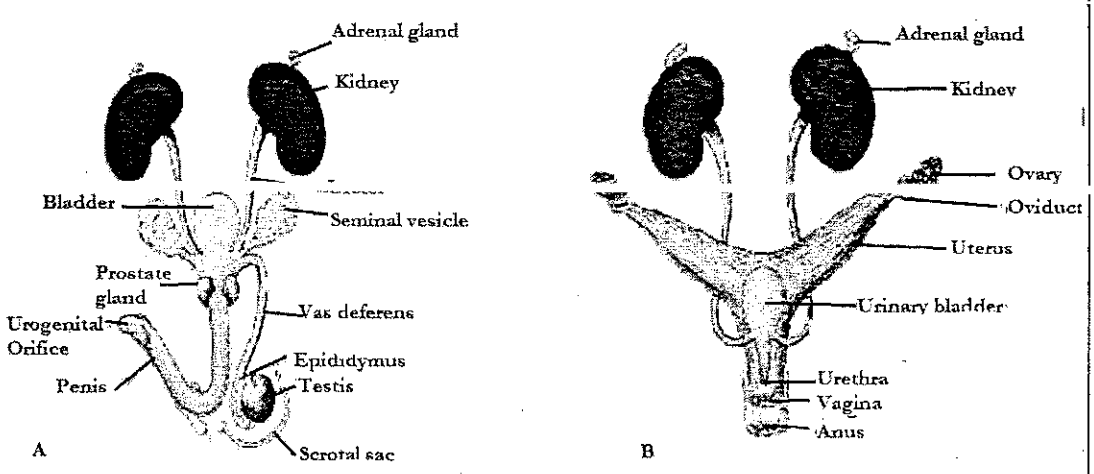


Figure 12.2: The reproductive system and excretory systems of: **a)** a male rat, **b)** female rat (from http://jrsowash.wikispaces.com/file/view/rat.student.pdf).

Activity Thirteen (optional)

Cellular Organization – Animal Tissues

INTRODUCTION

Some animals are unicellular. Other animals are composed of aggregations of cells living together in a semi - independent state. As we go up the scale of animals, we find that they have gradually developed groups of cells which cooperate with each other to perform specific functions. These similar cells which work together are called **tissues**. Certain tissues, in turn, cooperate with each other in higher organisms to form **organs**. There are four main types of tissue in animals: **Epithelial tissue, connective tissue, muscular tissue** and **nervous tissue**.

1. Epithelial Tissue (Figure 13.1):

This tissue forms a membrane covering the external surfaces and lining the internal parts of the body. It is devoid of blood vessels and is nourished by the fluid which passes to the cells by way of the intercellular substance. The general functions of epithelial tissues are protection, excretion, secretion, absorption, and the reception of stimuli.

According to the shape of the constituent cells, this tissue is classified as:

a) Squamous epithelium

b) Cuboidal epithelium

c) Columnar epithelium

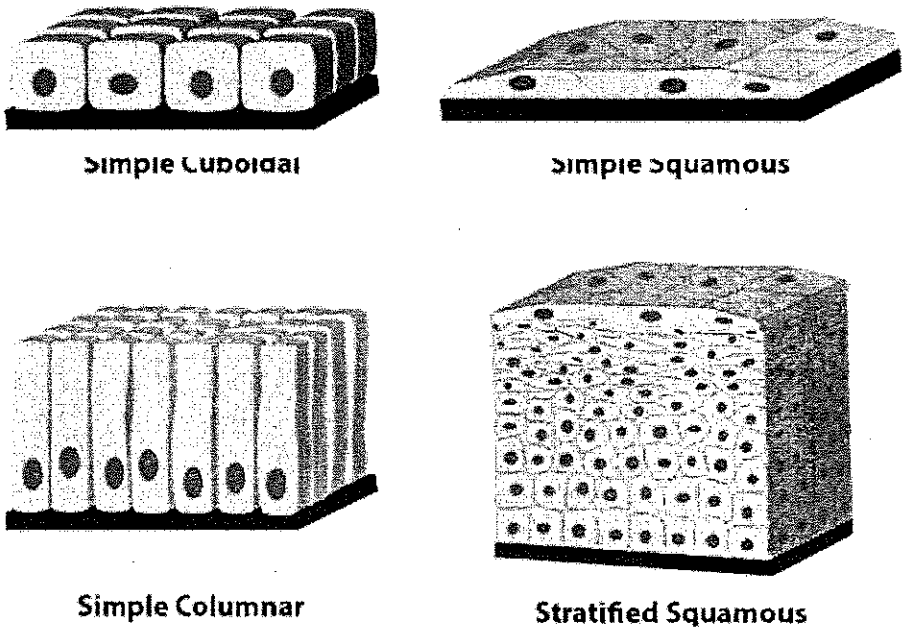


Figure 13.1: Simple and stratified epithelium.

If this tissue consists of a single layer of cells, it is described as **simple epithelium**. Otherwise, it is called **stratified epithelium.**

Squamous **epithelium** is so called because the cells on the free surface are flattened, and fitted together to form a mosaic. Simple squamous epithelium is found lining the alveoli of the lung, and in the membranous labyrinth of the inner ear. These tissues are derived from the ectoderm and endoderm. The serosa of the intestine is another example.

A similar tissue in structure is found lining the heart and the blood and lymph vessels, and forming the capillary networks. This tissue is derived from the mesoderm and is called endothelium. Mesothelium is the name given to this tissue where it lines the serous cavities.

Stratified squamous epithelium consists of several layers of cells. It covers the body forming the epidermis and is found wherever the ectoderm folds in from the outside, i.e., mouth, nose and anus.

The cells of the **cuboidal epithelium** have equal heights and widths. In a cross section they have a square outline. Simple cuboidal epithelium is found in salivary glands and kidney tubules. The sperm ducts of earthworms and some other animals are lined with ciliated cuboidal epithelium.

The cells of simple columnar epithelium have a cylindrical shape and are set upright on the surface which they cover. It is found lining the stomach, small and large intestines and digestive glands.

In some locations, the free surface of columnar epithelium is provided with threadlike processes which carry on ciliary motion. This tissue is found in the respiratory tract from the nose to the end of the bronchial tubes, in the uterine tubes and the upper part of the uterus in the female, and in the efferent ducts of the testes in the male. Numerous mucous - secreting goblet cells are found in ciliated epithelium.

2. Connective Tissues:

These tissues differ in appearance, but are alike in that the number of cells is relatively few and the intercellular material is abundant. They serve to connect and support the other tissues of the body, and with few exceptions they are highly vascular. The intercellular **matrix** determines the physical characteristics of the tissue. The connective tissues may be classified to:

a) Connective tissue proper:

1. Adipose (fat) (Figure 13.2)
2. Areolar (Figure 13.3)
3. Elastic
4. Fibrous
5. Reticular

Note: The proper connective tissue can be also classified into two main groups:

a) **Loose connective tissue** e.g. Areolar, Adipose

b) **Dense connective tissue** e.g. Fibrous - tendons, ligaments.

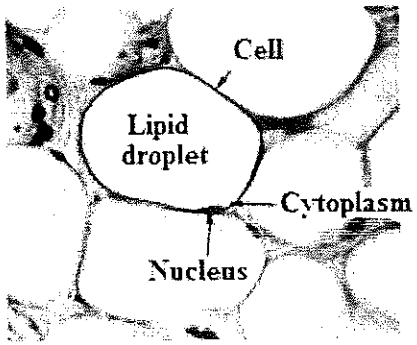


Figure 13.2 A micrograph of adipose cells

Figure 13.2: Areolar connective tissue.

b) Liquid or vascular tissue (Blood) (Figure 13.3): The cells of this tissue are in a liquid intercellular substance. The blood appears opaque and homogenous with the naked eye. But, on microscopic examination, it is seen to consist of cells, or corpuscles, in an intercellular liquid, the plasma. The volume of cells and plasma is approximately equal.

The blood cells are: Erythrocytes (red blood cells), leukocytes (white blood cells) and platelets. Erythrocytes or red blood cells in human beings are biconcave disks about 7.7 μm in diameter. The stroma of these contains hemoglobin which has a nonprotein part named hematin and a protein part named globin. Leukocytes are less in numbers and larger. Blood platelets or thrombocytes are disk - shaped bodies, always smaller than red or white blood cells.

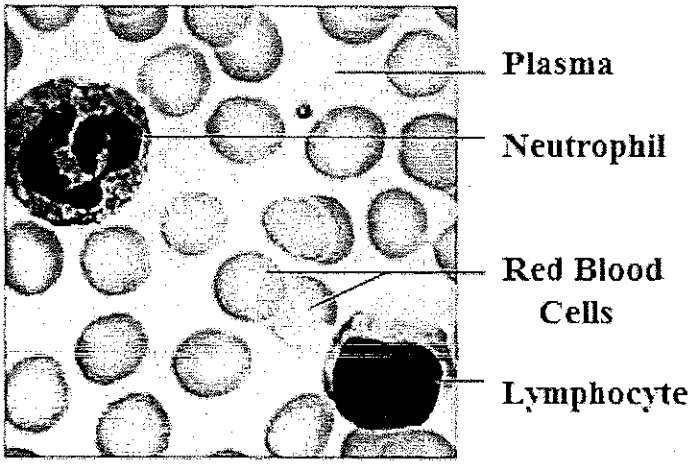


Figure 13.4: Different types of human blood cells.

c) Cartilage: The well-known substance called gristle is firm, tough and flexible. When a section is examined microscopically, it is seen to consist of groups of cells in a mass of intercellular substance called the matrix. As to the texture of the intercellular substance, three principal varieties can be distinguished:

1. **Hyaline or true cartilage** (Figure 13.5)
2. **Fibrocartilage**
3. **Elastic cartilage**

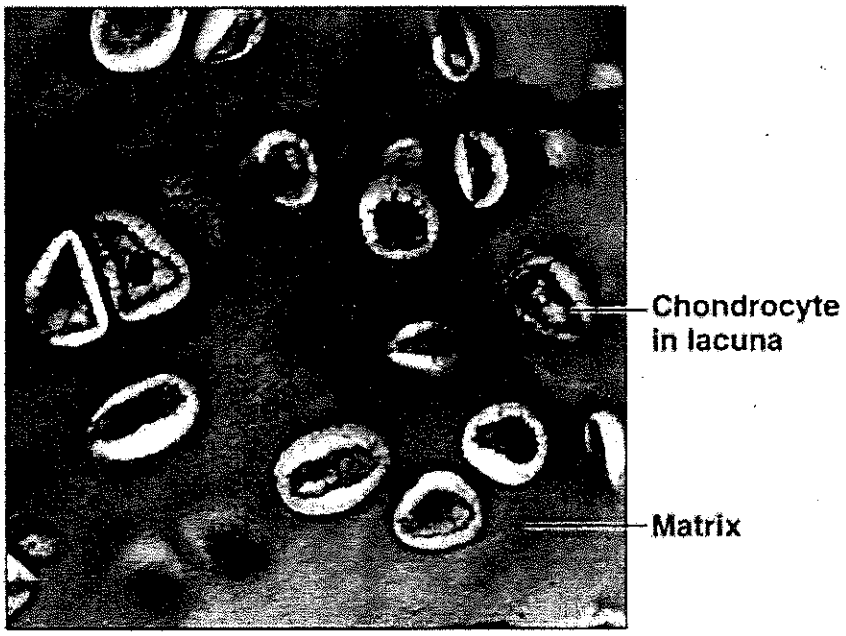


Figure 13.5: A photomicrograph of Hyaline cartilage

In the hyaline cartilage, cells are lying in fluid spaces called **lacunae** that are embedded in a homogenous intercellular substance. This substance is made up of chondrin which contains collagenous fibers forming a felt like mass. This tissue enters into the formation of the external ears, nose, larynx, trachea, bronchi and bronchial tubes.

In the fibrocartilage, the intercellular substance is pervaded with bundles of white fibers, between which are scattered cartilage cells. It serves as a strong, flexible connecting material between bones and is found wherever great strength combined with a certain amount of rigidity is required. It is found joining bone together.

In elastic cartilage, the intercellular substance is pervaded with elastic fibers which form a network. In the meshes of the network the cartilage cells are found. This tissue is found in the epiglottis, cartilage of the larynx, auditory tube, and external ear, it strengthens and maintains the shape of these organs, yet it allows a certain amount of change in shape.

d) Bone: The intercellular substance in this tissue is rendered hard by being impregnated with mineral salts, chiefly calcium phosphate and calcium carbonate. The inorganic matter constitute about two-thirds of the bone weight. Bone freed from inorganic matter by soaking in dilute acid is called decalcified.

By examination, a bone section will appear in some parts consisting of slender fibers and lamellae, whereas in others it is dense and close in texture, appearing like ivory. There are two forms of bony tissue:

1. **Spongy**
2. **Dense or compact** (Figure 13.6).

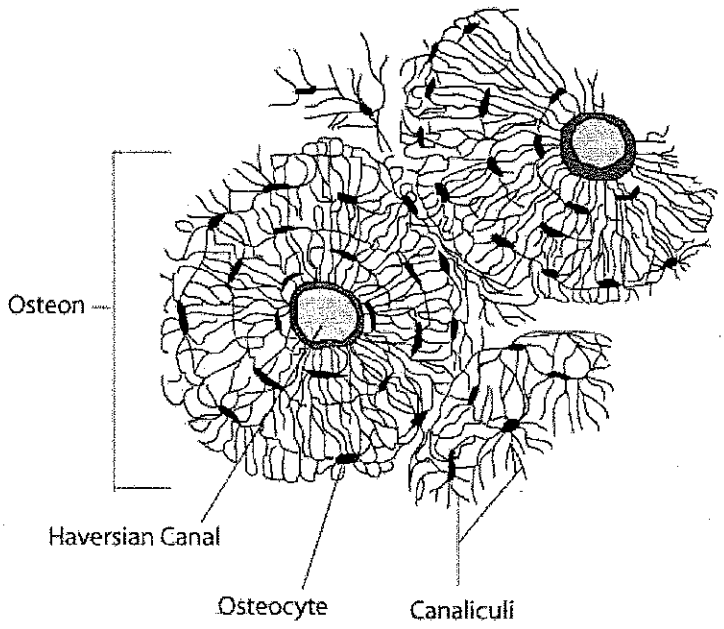


Figure 13.6: The histological structure of compact bone

The compact tissue has fewer spaces and is always found on the exterior of a bone, whereas the spongy has large cavities and more slender intervening bony partitions and is found in the interior of a bone. The shafts of long bones are made up entirely of compact

tissue, except that they are hollowed out to form central canal, the medullary canal, which is lined by a vascular tissue called the medullary membrane. All bones are covered by a membrane called periosteum. If this membrane is stripped from a fresh bone, many bleeding points representing Volksmann's canals through which the blood vessels enter and leave the bone are seen. These blood vessels proceed from the periosteum to join the system of **Haversian canals**. Around the Haversian canals the **lamellae** are deposited. Between them, arranged in circles, are found the lacunae which contain the bone cells. Radiating from one lacuna to another and toward the center are the **canaliculi**.

3. Muscular Tissue (Figure 13.7):

Although the property of contractility is possessed by all living protoplasm, it is particularly well developed in muscular tissue. In such tissue the direction of contraction is along the axis of muscle cells. Muscular contraction is responsible in vertebrates, not only for locomotion, but also for movements of the various internal organs, for the beat of the heart, for the propulsion of blood and lymph through vessels, for the passage of food through the digestive tract, and for the passage of glandular secretions and excretory products through ducts.

- Three main types of muscular tissues are recognized:

A. Striated or skeletal muscle.

B. Nonstriated or smooth muscle.

C. Cardiac muscle.

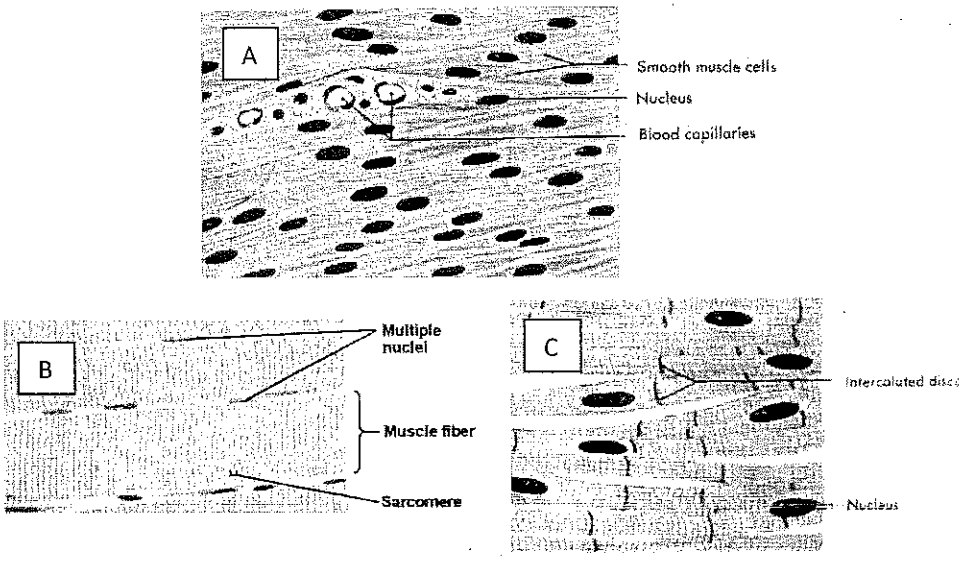


Figure 13.7: Different types of muscles in animals. A. skeletal muscle. B. smooth muscle. C. Cardiac muscle.

A. Striated Muscle:

They are voluntary muscles. The voluntary muscles are so called because they act under conscious control, and called striated because of the bandings or cross- striations which characterize the microscopic structure of such muscle fibers. The muscle cell contains many nuclei. The striated muscles are often referred to as skeletal muscles because they are attached to the boned of the skeleton.

The skeletal muscle fiber or cell is made up of numerous longitudinal fibrils which may be aggregated into small bundles. The **sarcoplasm** (or muscle protoplasm) surrounds these fibrils. The membrane of the fiber or cell enclosing the fibrils and sarcoplasm is called the sarcolemma.

The striated muscle tissue is made up of numbers of **fasciculi** (bundles of muscle fibers), bound together by connective tissue, and varying greatly in number and in size. Each fasciculus is surrounded by connective tissue (perimysium) and all the fasciculi forming the muscle are encased in a tough connective tissue sheath (epimysium).

B. Nonstriated (Smooth) Muscle:

Nonstriated or smooth muscle consists of delicate spindle - shaped cells, each with one central oval nucleus and homogenous fibers. The cells are arranged in layers, or sheets, held by fibrous connective tissue. Such muscle is found in the internal organs of the vertebrate body, as in the walls of the digestive tract, blood vessels, respiratory passages, urinary and genital organs. That is the reason for calling it visceral muscle, sometimes. It is not under control of the will, it is called sometimes, involuntary muscle.

Study a prepared slide of nonstriated muscle and note the shape of the fibers and the location of the nucleus.

Draw your observations and label.

C. Cardiac Muscle:

The heart muscle of vertebrates is called cardiac muscle. It has delicate cross striations, and the fibers are branched to form an interconnecting network. Cardiac muscle is striated, yet involuntary. Throughout the life of an individual, its only rest period is between successive contractions of the heart.

4. Nervous Tissue (Figure 13.8):

The structural units of nervous tissue are **neurons**, or nerve cells, together with their processes. The part of the cell in which the nucleus lies is called the cell body. The processes of a neuron are of two kinds:

a) Dendrites, usually numerous, which are short processes showing a high degree of branching close to the cells body.

b) Axon, which is a long process with branches, the terminal arborization, at its end. Collateral branches may be given off by the axon along its course, but these are frequently lacking. The number of cytoplasmic processes extending from a nerve - cell body varies and forms the basis for classifying different types of neurons.

The axon with its enveloping sheath, or sheaths, is spoken of as a nerve fiber. Those nerve fibers located outside the brain and spinal cord are covered by a thin, continuous encapsulating membrane, the neurolenma (sheath of Schwann). This is composed of sheath cells, which are present in series beginning at the brain or spinal cord, or at a ganglion, and ending almost at the terminal arborization. Each sheath cell, with its cytoplasm and flattened nucleus, surrounds a section of the axon.

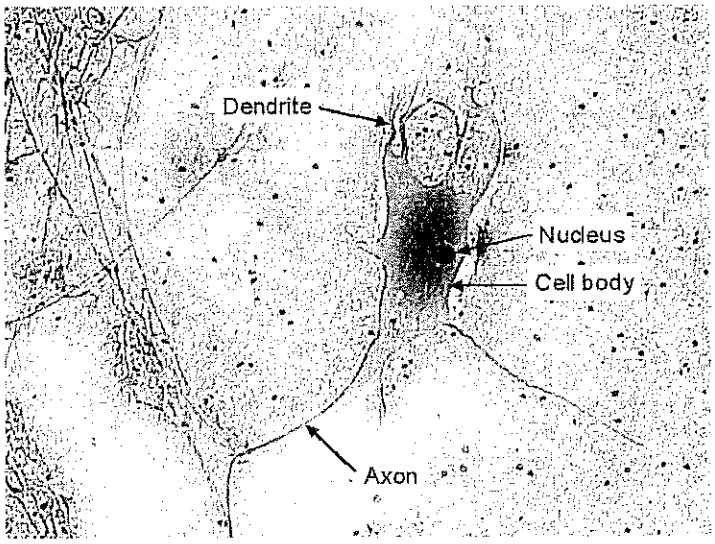


Figure 13.8: A photomicrograph of a neuron

Nerve fibers are said to be either medullated or nonmedullated, depending upon whether or not they are surrounded by a sheath of glistening-white material called myelin, which lies between the axon and neurolemma. Unlike the neurolemma, which is continuous, the myelin sheath is interrupted at rather regular intervals by circular constrictions, the nodes of Ranvier.

Sections between adjacent bodies are called internodes. A single sheath cell covers an internode. It is believed that the myelin sheath may serve as an insulating substance, much in the manner of the coating on an electric wire preventing loss of energy of the nerve impulse during its passage along the fiber. Nonmedullated fibers lack myelin sheath, and

the neurolemma surrounds the axon directly. Lacking a myelin sheath, they appear grey in cooler in contrast to the glistening - white appearance of medullated fibers.

A nerve, or nerve trunk, is made up of numerous nerve fibers outside the central nervous system, bound together in parallel bundle by connective tissue. Most peripheral nerves are composed of medullated fibers. Nonmedullated fibers are found chiefly as components of nerves making up the sympathetic nervous system. Nerve fibers located in the brain and spinal cord are medullated but lack a neurolemma. Instead they are surrounded by neuroglia cells.

In addition to nerve cells of various types, other cells are present in the central nervous system **glial cells.** These are interspersed among the nervous elements, providing support, protection, nourishment and have many other functions.

Appendix A

Data Representation

In quantitative experiments, the representation of data is essential in demonstrating the relation between two or more variables. After the collection of data, it is important to extract information from numbers, that is, to interpret the main results revealed by a certain set of data.

There are different methods used in data representation. In this section, you will be introduced to two of these methods which are described as follows:

1. Tables:

During an experiment, tables could be used in recording and representing data. For example, in an experiment conducted to measure the volume of CO_2 produced by yeast in sucrose and galactose solutions at 37°C over one hour, the following table should be designed:

Table A.1: CO_2 volume (ml) produced by Yeast in Sucrose and Galactose solutions at 37 °C over one hour.

Time (minutes)	**CO_2 volume (ml)**	
	Sucrose	Galactose
10	0.2	0.0
20	0.5	0.1
30	0.8	0.1
40	1.0	0.2
50	1.3	0.2
60	1.7	0.3

Notice the main characteristics that describe a well-organized table:

1- Tables should be sequentially numbered, beginning with Table 1.

2- Each table should include a title which includes the dependent and independent variables and allow the reader to interpret the represented data.

3- Cells in the table should be equally spaced giving the table a well-organized look.

4- The units of the different variables should be included in the table or in the title.

2. Graphs:

Graphs are usually used for a visual representation of data. A properly drawn graph should allow the reader to interpret the presented data and understand the main results in a glance.

There are general rules that should be followed when drawing a two dimension graph:

1- To determine the dependent and independent variables:

The independent variable is a set of conditions monitored by the researcher, such as Temperature, pH, sucrose concentration, time, etc. The independent variable is always represented on the x-axis. The parameter measured under a certain set of conditions is the dependent variable, and it is always represented in the y-axis (Figure A.1).

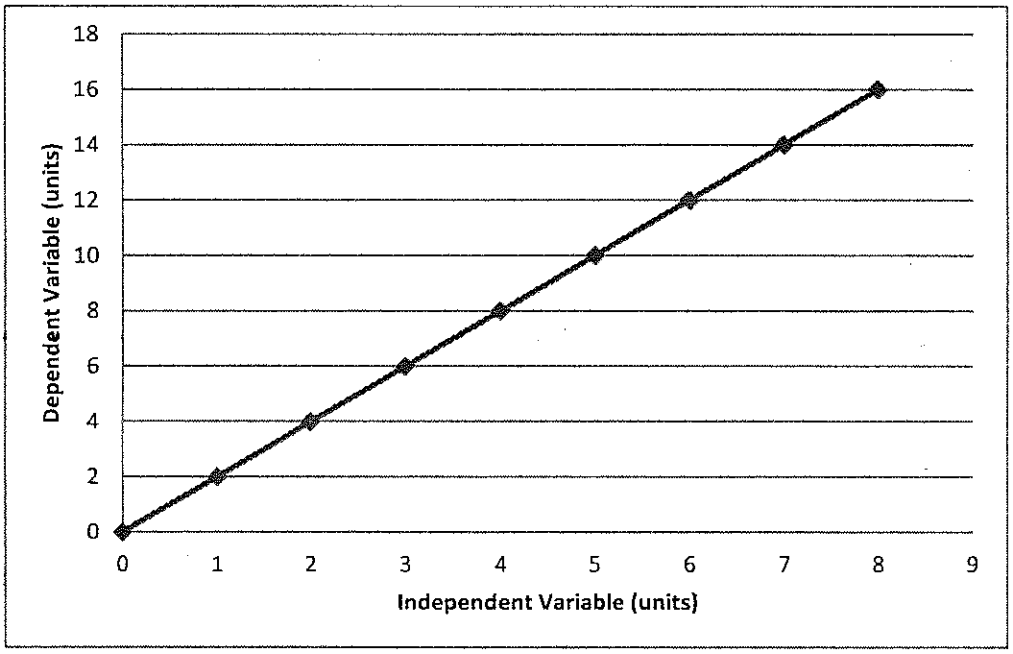


Figure A.1: Sample graph- The independent variable is represented in the x-axis while the dependent variable is in the y-axis.

2- The units of the independent and dependent variables should be demonstrated in the graph.

3- Graphs are referred as figures. In scientific writing, figures are sequentially numbered and are followed by a title. The title includes a brief explanation of the conducted experiment. In a certain experiment, an acceptable title could be: " The effect of nitrogen concentration (mg/l) on the Tomato plant height (cm) at 35°C". On the other hand, a title such as: " Nitrogen concentration against tomato plant height" is not acceptable.

4- The scaling of the x and y-axis:

The scale of both axes should be related to the data range. For example, if the values of a certain set of data range are from 80-100, the axis scale should be from 70-110 (slightly below the initial value and above the final value). In addition, the student should avoid starting the scale from zero, as it is considered highly below the initial value of the above data set (Figure A.2).

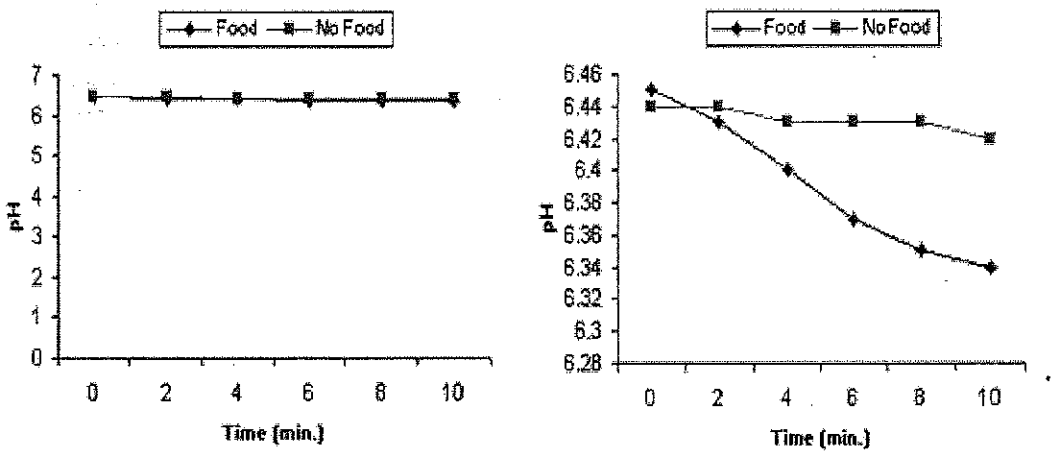


Figure A.2: Effects of axis scale on data presentation.

In the graph on the left, it seems that there is no difference in the pH values between the two treatments. However, when using the appropriate scale it is clear that there is a significant difference between pH values in both treatments.

5- The values on both axes should be numbered at regular intervals.

6- Plotting more than one function in a set of axes: In many biological experiments, the student can be asked to compare between two different treatments and plot the graphs in the same set of axis; for example, in comparing between the activity of a specific enzyme on different substrate concentrations with and without an inhibitor, the following graph can be plotted (Figure A.3):

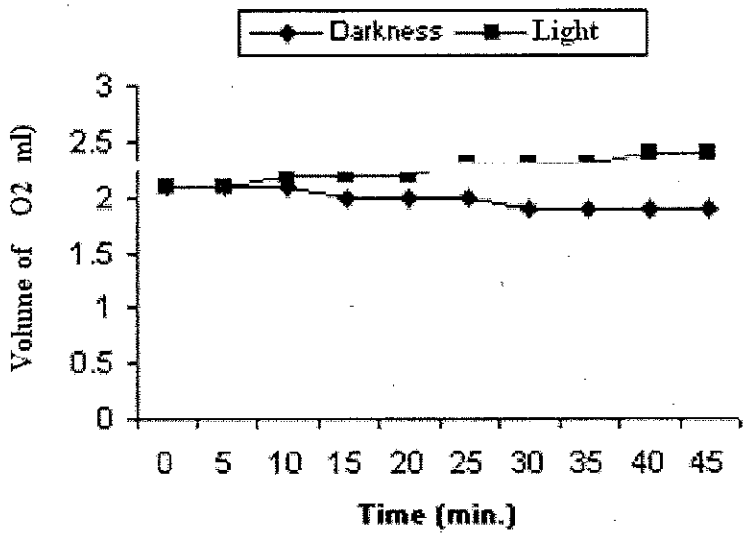


Figure A.3: The change in the volume of O_2 (ml) produced with time by a plant put in darkness and exposed to light.

You can notice from the previous graph, that both functions can be differentiated by using different symbols such as (■, ♦,●,▲, etc). In addition, a key in the right side of the graph is added to explain the meaning of the symbolic representation.

- **Types of Graphs:**

There are different well known types of graph representation. In this section, three types of graphs will be introduced:

1- Plotted curves: used when there is a continuous relationship between the independent and dependent variables (Figure A.4).

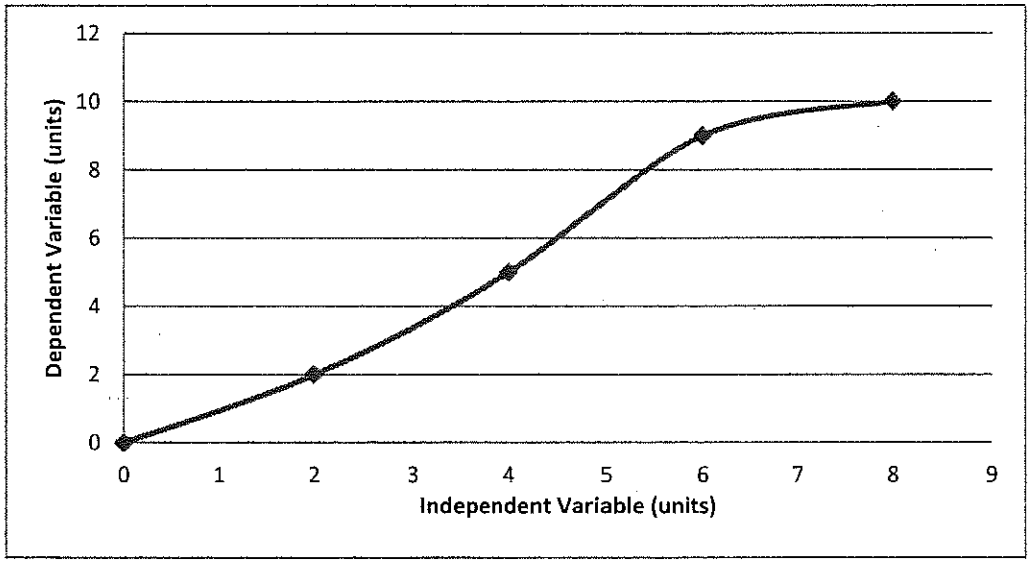
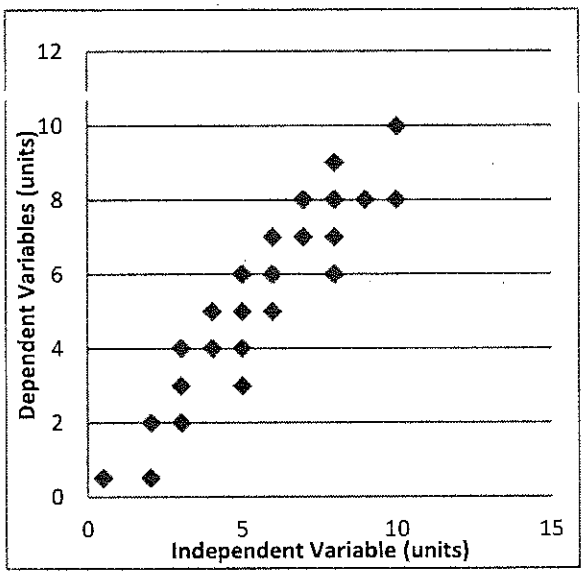


Figure A.4: An example of a plotted curve.

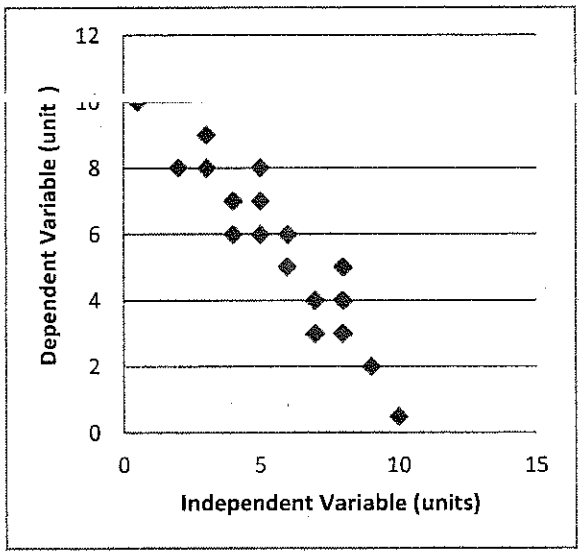
If the slope of the function is constant a line graph can be plotted, as shown in Figure A.1.

2- Scatter diagrams: used to represent the correlation between both the dependent and independent variables. The correlation can be positive, negative or no correlation can be found between both variables (Figure A.5).

A-Positive Correletion



B-Negative Correlation



C- No Correlation

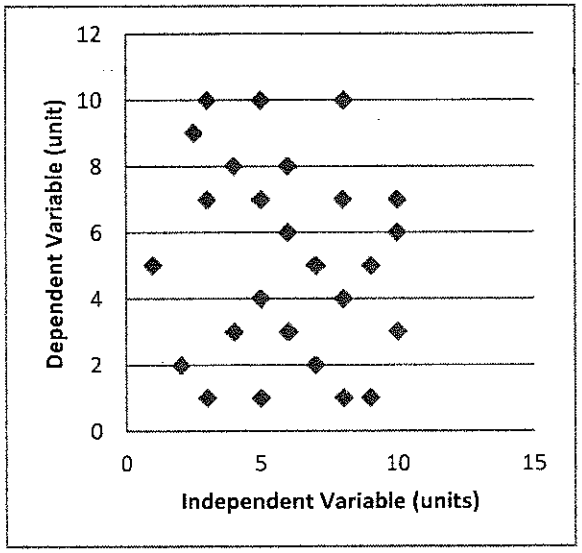


Figure A.5: Shows the different correlations between dependent and independent variables. A- High positive correlation B- High negative correlation C- No correlation.

3- Bar Charts: used to represent the frequency of a distinct variable, also it is usually used for the comparison between two distinct categories, as shown in the following figure:

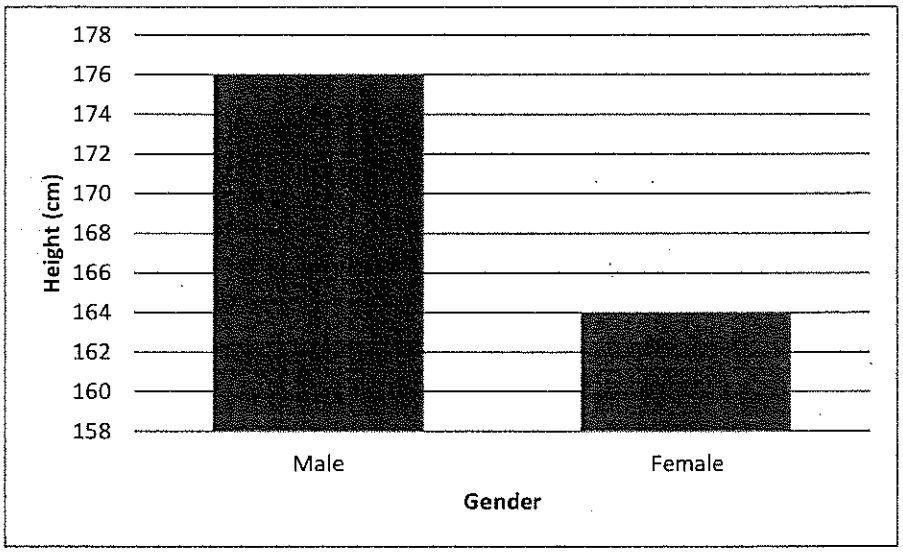


Figure A.6: The average height (cm) of males and females in a general biology class.

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