

**Al-Quds University**

**Faculty of Health Professions**

**Department of Clinical Laboratory Sciences**

**General Biology Laboratory**

**Manual**

**Fifth Edition  
2014/2015**



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## Preface

The lab technicians of the Department of Clinical Laboratory Sciences of this manual are pleased to present the fourth edition of manual of General biology laboratory. This manual was first published in 2007 by Dr. Akram Karouby; the head of the Department of Clinical Laboratory Sciences at Al-Quds university- Jerusalem – Palestine.

The major goal of the authors continues to be to provide the junior students in the Faculty of Health Professions the basics of biology techniques in a user-friendly and interesting manual. A second major ongoing goal is to reflect the most significant advances in biology that are important to the biomedical field. However, a third major goal of this edition was to achieve a substantial reduction in size, as feedback indicated that many readers prefer shorter texts.



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*I believe God controls the universe. I don't believe biology works in an uncontrolled fashion.*

*Richard Mourdock*

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*We have to pay close attention to what we see, and be ready to work with the unexpected according to the basic principles of systems biology and medicine.*

*Mark E. Hyman*

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## Overview

### Student Responsibilities

1. Students should carefully review the attached sheets.
2. Students are expected to participate in all laboratory activities and complete all lab assignments on time.
3. Students are expected to be prepared in advance when they arrive to lab. being prepared includes the following: having already read text materials (handouts) assigned for that day's activities, bringing required work materials (e.g., lab notebook, handouts, writing supplies, lab coat, etc.).

### Attendance

Attendance is obligatory. If you miss more than one laboratory session, you will get a zero grade for the Lab., and therefore, your grade in the general biology course will be affected.

### Evaluation and Grading

1. Attendance constitute 10% of the total lab. grade.
2. The Lab. reports constitute 20% of the total lab. grade.
3. Quizzes constitute 10% of the total lab. grade.
4. The mid-term lab. exam is 20% of total lab. grade.
5. The final lab. exam constitutes 40% of the total lab. grade.



## Safety Rules

### General Lab Safety Guidelines

Students should be familiar with safety procedures, follow instructions carefully and take appropriate precautions at all times to insure the safety of every student in the lab especially when hazardous conditions occur or hazardous materials are being used, and they have to familiarize themselves with the emergency and fire procedures reactions.

### General definitions

**Hazardous substance** is defined as a material or substance that poses a level of threat to life, health, property or environment. This includes both *chemical* and *biological* agents.

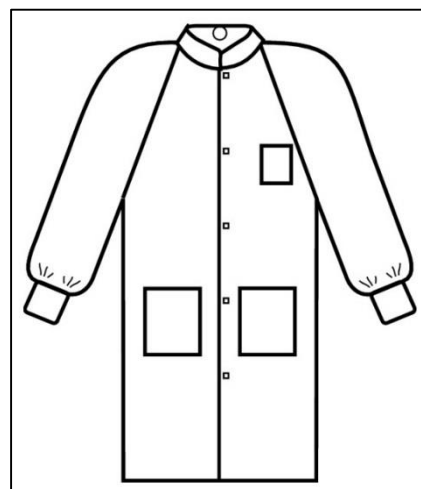
**Chemical hazard** is defined as any chemical potentially harmful to humans, animals or plants.

**Biohazard** is defined as any organism that is capable of replication and causing disease in humans, animals or plants.

**Decontamination** is the removal or neutralization of toxic agents or the use of physical or chemical means to remove, inactivate, or destroy living organisms.

### Personal safety rules

1. Students should dress appropriately in the lab.  
(White, strong cloth, long to knees, long sleeves, tight wrist, pockets, etc.)
2. Wear suitable gloves, masks and goggles as needed.
3. Closed non-absorbent shoes must be worn (no slippers, sandals or open shoes).
4. Long hair must be tied.
5. Do not eat, drink, smoke, chew gum, apply cosmetics or remove/insert contact lenses while in the laboratory.
6. Lessen your movement in the lab as much as you can.
7. Avoid sudden movement and turning around.
8. Playing around is prohibited during the laboratory session.
9. Anyone injured in the lab, should inform the instructor immediately and take immediate action to reduce the risk of further injury.



### Lab. environment

1. The lab environment should be considered extremely dangerous, poisonous and infectious.
2. Do not take any personal stuffs to the lab (i.e. coats, bags, books, food, etc.)
3. All materials in the lab should be considered infectious and dangerous; so they must be handled carefully.

4. All samples and specimens with biological origin (e.g., saliva, urine and blood) or organisms, living or dead, are infectious, even the source of it is the student himself, his relatives or friends, etc. should be treated with care and respect.
5. Students should be familiar with the evacuation plans.
6. Chemicals may be poisonous, corrosive, or flammable; no chemicals, even chemicals known to be safe, should be tasted, ingested, inhaled, or touched unless specifically directed to do so by your instructor.
7. Human organic must be disposed appropriately to eliminate any possibility for contamination and the spread of disease.
8. The safe use of specific equipment and tools (e.g., microscopes, slides, scalpels, and pipettes) will be demonstrated by the instructor during the laboratory sessions.
9. Students should remove and dispose of all trash, clean up any spills, return materials where they belong, as instructed and disinfect the bench area.
10. After completing laboratory activities and cleaning up, students should wash their hands to avoid contamination and hazardous chemicals.

## **Instructions**

1. Do not do anything that you don't know without asking.
2. Label all your samples clearly; the most dangerous substance is the one that has no label.
3. Dispose all contaminated material in autoclave bags.
4. Do not take cultures out from lab area for any reason.
5. Immediately report all accidents and spills to the instructor.
6. It is important to know as much about a chemical as possible.
7. Do not use chipped or cracked glass wares.
8. Use hazardous chemicals under a fume hood.

## **Emergency Procedures for a Laboratory Fire**

1. Do not use a fire extinguisher unless you are trained to use it.
2. In case of a small fire at your desk, smother with a book or piece of cloth; Not your hands.
3. If your clothes are on fire, such as a sleeve, run water over it. If the fire has spread beyond a small portion of clothing or appears that it will, use the fire blanket.
4. If a large fire occurs such as in a waste basket, smother the fire with the fire blanket.
5. If the fire is larger, do not attempt to put out the fire. Leave the laboratory immediately.

## **Electrical Equipment**

1. Do not spray or splash water to electrical equipment which is plugged in.
2. If equipment crackles, snaps or begins to smoke, call the instructor immediately.
3. In case of any worn or broken electrical cords, notify the instructor.

## General signs

**Carcinogen**



**Toxic**



**Corrosives**



**Irritants**



**Explosive**



**Flammable  
(Combustible liquid)**



**Oxidizer**



**Compressed gas**



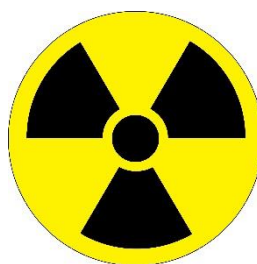
**Water- reactive**



**Biohazard**



**Radioactive**





## Lab. No. 1

# Testing for Biologically Important Macromolecules I (Carbohydrates and Lipids)

### Objectives

- Identifying monosaccharides from polysaccharides.
- Description of varied results of the Benedict's test.
- Testing for the presence of lipids in a solution.
- Identifying amino acids from polypeptides.
- Testing for the presence of free amino acids in a solution.
- Testing for the presence of ascorbic acid in a solution, and comparing between two solutions according to the ascorbic acid composition.

### Overview

All living organisms are composed of various types of organic macromolecules, such as carbohydrates, proteins, lipids, vitamins and nucleic acids. These substances, constitutes the food we eat, provide us with the energy and cellular building blocks necessary for life. Several of these molecules can be detected by simple chemical tests. In today's laboratory, detection of those mentioned macromolecules would be tested.

### Carbohydrates

Sugars are small carbohydrate molecules used as a source of energy by all organisms. There are many types of carbohydrate molecules all of which are made up of various arrangements of carbon, hydrogen, and oxygen atoms. The simplest carbohydrates are **monosaccharides**, or simple sugars, such as glucose and fructose. **Disaccharides** consist of two *covalently* linked monosaccharides. Examples of disaccharides include lactose (the milk sugar; composed of glucose-galactose), maltose (the barley sugar; composed of two glucose molecules), and sucrose (the common table sugar; composed of glucose-fructose) (Figure 1-1). Organisms store simple sugars in the form of **Polysaccharides**, which are more complicated molecules, are composed of multiples of monosaccharides covalently linked together in branched forms to form a large molecule –, for example, glycogen is the storage form of glucose in animals and starch in plants, and these polysaccharides are broken down as needed for energy (Figure 1-2). Other polysaccharides serve a structural function like cellulose – the main component in plant cell walls, and chitin in fungi and exo-skeleton in arthropods.

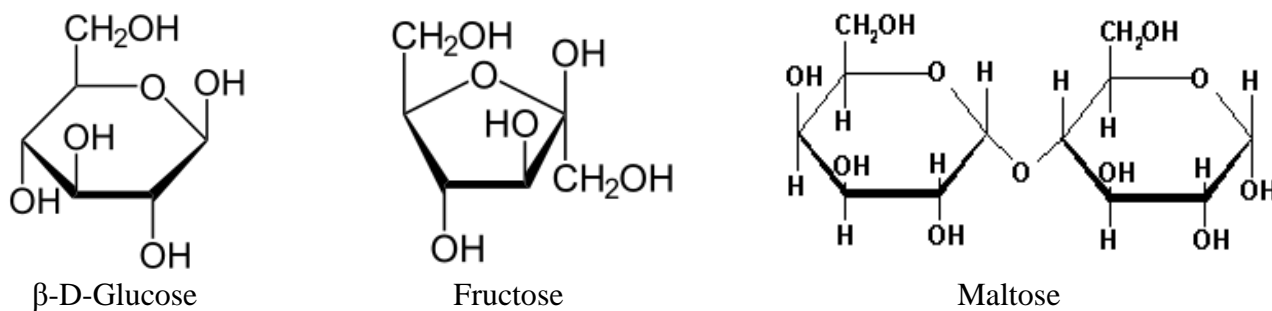


Figure 1-1: An illustration shows the structure of cyclic form of the  $\beta$ -D-Glucose, Fructose and Maltose.

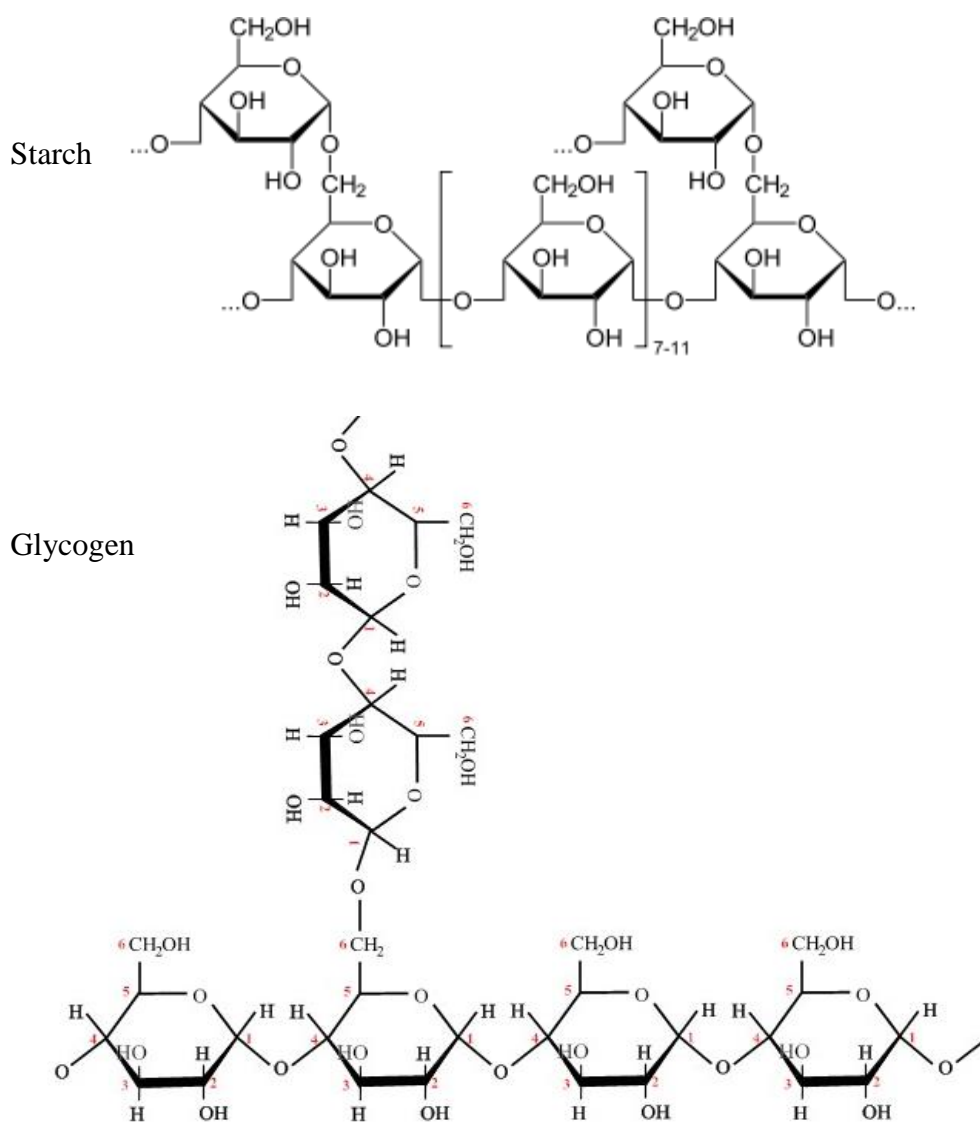


Figure 1-2: An illustration shows the structure of the starch and glycogen molecules.



## Lipids

Lipids are hydrophobic molecules; they do not mix with water, so they dissolve well in organic solvents (i.e. chloroform, Ether, benzene ...). Lipids of animal origin are solid at room temperature while lipids of plant origin are liquid at room temperature; oils. Some classes of lipids include *triacylglycerols*, *steroids*: e.g. cholesterol, *phospholipids*, *Glycolipids* (lipids with carbohydrates attached) and others, all of which contain primarily carbon, hydrogen, and oxygen (Figure 1-3).

Lipids have many functions including energy storage, structural components of cell membranes, signaling molecules and many others.

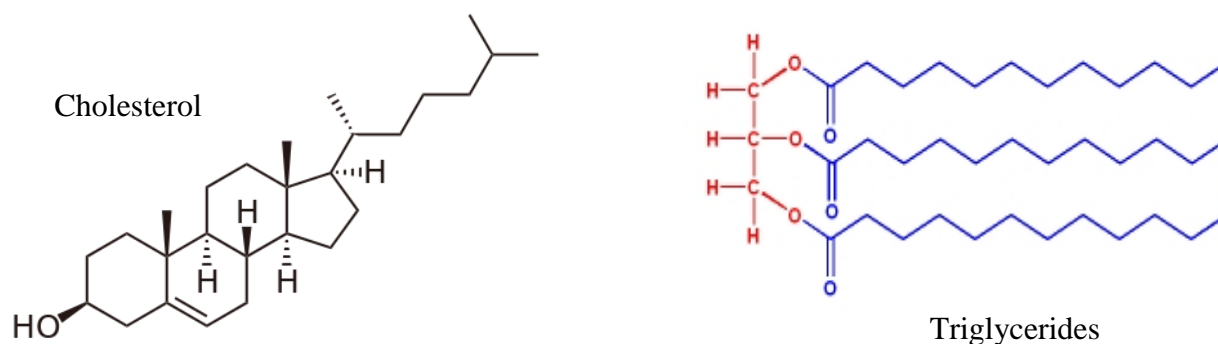


Figure 1-3: An illustration shows the structure of the triglycerides and cholesterol.

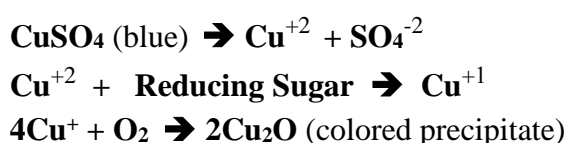
## Experiments

Here are some lab experiments that have to be performed during the lab time, follow the steps and instructions below, and then record your results carefully. After that, you have to write a report and to answer the assigned questions, which have to be handed on time the lab session in the week after.

### Experiment No. 1: Testing for Reducing Sugars

#### Principle of the test

Some sugars can be detected using the *Benedict's Test*. The Benedict's solution contains Cupric ions ( $\text{Cu}^{++}$ ). When heated, the cupric ions react with reducing sugars to give the insoluble, red Copper (I) Oxide ( $\text{Cu}_2\text{O}$ ). The solution changes in a range of green to reddish brown colors as more Cuprous ions are formed. All monosaccharides and some disaccharides (with free *aldehyde* or *ketone* groups) are reducing sugars and react with Benedict's reagent.



#### Procedure

*The test is performed by mixing equal volumes of the Benedict's solution and the solution in question then heating in a boiling water bath for 3 minutes.*

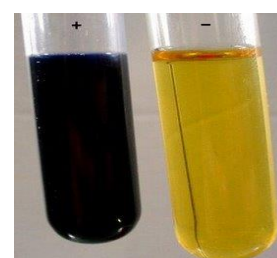
***Benedict's solution + Reducing Sugar-containing solution + Heat → Colored precipitate***

1. Label four clean Pyrex test tubes 1 to 4.
2. In tube #1 add 1.5 ml of glucose solution (10 %) and 1.5 ml of Benedict's solution (what do you call this tube?).
3. In tube #2 add 1.5 ml of distilled water (D.W) and 1.5 ml of Benedict's solution (what do you call this tube?).
4. In tube #3 add 1.5 ml of sucrose solution (10 %) and 1 ml of D.W.
5. In tube #4 add about 1.5 ml of sucrose solution (10 %) and 7 drops of concentrated HCl.
6. Place all tubes in a boiling water bath.
7. After 3 minutes, remove the tubes.
8. Observe and record your results.
9. Add 1 ml of Benedict's solution into tubes #3 and tube #4, boil for 3 minutes.
10. Observe and record your results.

### Experiment No. 2: Testing for Starch

#### Principle of the test

*Lugol's iodine* is used to detect the presence of starch in a solution. Starch interacts with iodine to produce a bluish-black color complex (a yellowish-brown color indicates the absence of starch). Lugol's ( $\text{I}_2\text{-KI}$ ) solution stains starch due to its interaction with the coil structure of the polysaccharides.



**Procedure**

*The test is performed by adding 3 drops of Lugol's solution to the solution to be tested for the presence of starch the solution then observing the presence of the bluish-black color formation.*

**Lugol's iodine (yellowish-brown) + starch → bluish-black color complex**

1. Label three clean plastic test tubes 1 to 3.
2. In tube # 1 add 1 ml of starch solution (5 %) and 3 drops of Lugol's solution (what do you call this tube?).
3. In tube # 2 add 1 ml of distilled water and 3 drops of Lugol's solution (what do you call this tube?).
4. In tube # 3 add 1 ml of glucose solution and 3 drops of Lugol's solution.
5. Observe and record your results.

**Experiment No. 3: Testing for Lipids****Principle of the test**

The aim of the exercise is to distinguish between hydrophilic and hydrophobic substances using the *Sudan III* test. Sudan III is a lipid soluble dye; when added to a mixture of lipids and water, the dye will move into the lipid layer coloring it red.

**Procedure**

*The test is performed by adding some of the Sudan III powder into the solution in question and observing the coloration of the solution.*

1. Label three clean plastic test tubes 1 to 3.
2. In tube #1 add 1.5 ml distilled water.
3. In tube #2 add 1.5 ml of oil.
4. In tube #3 add 1.5 ml D.W and 1 ml of oil.
5. Transfer some of the Sudan III powder into each of the three tubes using a wooden stick.
6. Observe and record your results.

**Lab Exercise**

Obtain an unknown from you lab instructor (record its name in your notebook) and test it for the presence of reducing sugars, starch and its solubility in lipids.

**Questions**

1. In the first experiment, what is the purpose of adding HCl? What is the name of the reaction (braking disaccharides down)? What is the name of the reaction that has the opposite direction (composition)?
2. In the first experiment, what is meant by reducing sugar? Do you think that Cooper has been oxidized or reduced?
3. In the first experiment, a solution gave a light orange color with Benedict's test, but when boiled with HCl it gave a dark red color. So, what do you think about the solution, what does it composed of ?

**Lab. No. 2**

## Testing for Biologically Important Macromolecules II

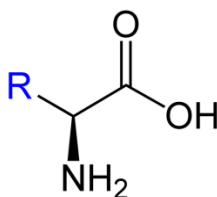
### (Proteins, Amino acids and Vitamins)

In this lab session we will continue what we had started in the previous session.

### Proteins

A *protein* is a polymer that is constructed of *amino acid* monomers which linked together through an amide linkage (*peptide* bond). Proteins perform most of the tasks the body needs to function. All proteins are constructed from a common set of 20 kinds of amino acids. Some functions of proteins include structural involvement (i.e. Collagen in tendons مرايط and keratin in hair), hormonal (e.g. Insulin), transport (e.g. hemoglobin and albumin), biological catalysts; i.e. enzymes (e.g. pepsin), antibodies and many others.

Figure 2-1: General Structure of Amino acids



### Vitamins

Organic compounds required by an organism as a vital nutrient in limited amounts and can't be synthesized by the organism and must be obtained from an external source are called **vitamins**. Vitamins function as coenzymes, or as a part of a coenzyme. Table 5.1 describes some information about vitamins.

Table 2-1: List of vitamins by generic and common names with other information.

Generic name	Common name	Deficiency disease	Food source
Vitamin A	Retinol	Night-blindness,	Carrots
Vitamin B <sub>1</sub>	Thiamine	Beriberi	Vegetables, liver
Vitamin B <sub>2</sub>	Riboflavin	Ariboflavinosis	Dairy products
Vitamin B <sub>3</sub>	Niacin	Pellagra	Meat, fish, eggs
Vitamin B <sub>5</sub>	Pantothenic acid	Paresthesia	Meat, avocados
Vitamin B <sub>6</sub>	Pyridoxine	Anemia, neuropathy	Meat, bananas
Vitamin B <sub>7</sub>	Biotin	Dermatitis, enteritis	Raw egg yolk, liver
Vitamin B <sub>9</sub>	Folic acid	Neuropathy	Leafy vegetables
Vitamin B <sub>12</sub>	Cyanocobalamin	Megaloblastic anemi	Meat
Vitamin C	Ascorbic acid	Scurvy	Citrus fruits
Vitamin D	Cholecalciferol (D <sub>3</sub> )	Rickets	Fish, Mashrooms
Vitamin E	Tocopherols	Mild hemolytic anemia	Fruits and vegetables
Vitamin K	Phylloquinone (K <sub>1</sub> )	Bleeding diathesis	Green vegetables

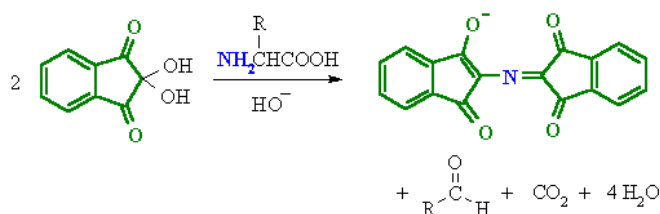
## Experiments

Here are some lab experiments that have to be performed during the lab time, follow the steps and instructions below, and then record your results carefully. After that you have to write a report and to answer the assigned questions which have to be handed on time – the lab session in the week after.

### Experiment No. 1: Testing for Amino Acids

#### Principle of the test

Detection of amino acids is done by mixing the solution in question with Ninhydrin solution, if amino molecules are present the color changes from yellow into purple. **Ninhydrin** is a powerful oxidizing agent that removes the amino groups of amino acids. The reaction liberates  $\text{NH}_3$ ,  $\text{CO}_2$ , and a reduced form of Ninhydrin. The  $\text{NH}_3$  then reacts with Ninhydrin and the reduced Ninhydrin to form a purple color. So, the appearance of the purple color is a positive indicator for the presence of amino acids. Proteins have to be hydrolyzed first to test positive with Ninhydrin.



#### Procedure

*The test is performed by adding 15 drops of Ninhydrin solution to 2 mLs of the solution to be tested, then observing the presence of the purple color formation.*

**Ninhydrin** (yellowish) + **Amino acids** (free) ➔ **purple color complex**

1. Label three clean test tubes 1 to 3.
2. In tube #1 add 2 ml of D.W (what do you call this tube?).
3. In tube #2 add 2 ml of 0.1% glycine solution (what do you call this tube?).
4. In tube #3 add 2 ml of 0.1% egg albumin solution
5. Add 15 drops of Ninhydrin solution to each tube.
6. Place tube #3 in a boiling water bath for 3 minutes, then repeat step (5) for this tube.
7. Observe and record your results.

### Experiment No. 2: Testing for ascorbic acid (vitamin C)

#### Principle of the test

There are two different methods that could be used for the vitamin C testing in a solution; indophenol reagent method and starch-iodine reagent method.

indophenol reagent is a blue color indicator, while starch-iodine reagent is a bluish-black in color, both reagent are bleached in the presence of ascorbic acid.

*Note:* When testing the juices of citrus fruits for vitamin C content, the blue indophenol and starch-iodine reagent may turn pink before turning colorless because of the presence of substances other than vitamin C.

## Procedure

*The test is performed by adding 20 drops of solution to be tested for the presence of ascorbic acid to 1 mL of the starch-iodine reagent, then observing the bleaching of the .*

***Starch-iodine reagent*** (blue) + **Ascorbic acids** (colorless) ➔ **colorless complex**

1. Label four clean test tubes 1 to 4.
2. Put 1 mL of starch-iodine reagent in each tube.
3. In tube #1 add 20 drops of D.W drop wise (what do you call this tube?).
4. In tube #2 add 20 drops of 1% ascorbic acid solution drop wise (what do you call this tube?).
5. In tube #3 add 20 drops of lemon juice drop wise.
6. In tube #4 add 20 drops of orange juice drop wise.
7. Observe and record

## Lab Exercise

Obtain an unknown from you lab instructor (record its name in your notebook) and test it for the presence of free amino acids and ascorbic acid in it.

## Questions

1. What is meant by a negative control? Why do we use it?
2. What is meant by a positive control? Why do we use it?

## Lab. No. 3

# Enzymes

### Objectives

- Identifying enzymes by their reactions
- Utilizing chemical reactions to test for the presence of enzymes
- Studying the effects of different factors on enzymes activity.

All life processes depend on biochemical reactions; food materials are broken down to produce energy, new structures are synthesized and cells divide for growth. However, without assistance, these biochemical reactions occur so slow that an organism will die before the reactions are completed. Therefore, enzymes are used to speed up biochemical reactions and allow them to occur at the physiological temperatures of the living organisms.

Enzymes are called the **organic catalysts** of life; they are organic because they are made up mainly of carbon and hydrogen, they are catalysts because they cause a chemical reaction to occur at a low temperature without actually being used up in the reaction. The material an enzyme works on is called the **substrate** of that enzyme. An enzyme holds its substrates in a pocket called the **active site**, which is formed by the combination of the R groups of the amino acids which form that place, and enables the substrate to be converted into a product. Because the active site of an enzyme has a unique three-dimensional shape, only certain substrates can fit into this active site. As a result, one active site is **specific** for one substrate (Figure 3-1). For example, *glucose oxidase* will oxidize glucose but not galactose. Some enzymes have broad specificity; glucose, mannose, and fructose are phosphorylated by hexokinase. Some enzymes require non protein helpers, organic (**coenzymes**) (i.e. vitamins) or non organic (**cofactors**) (i.e. metals like  $\text{Fe}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Mg}^{++}$ , ...), for their activity.

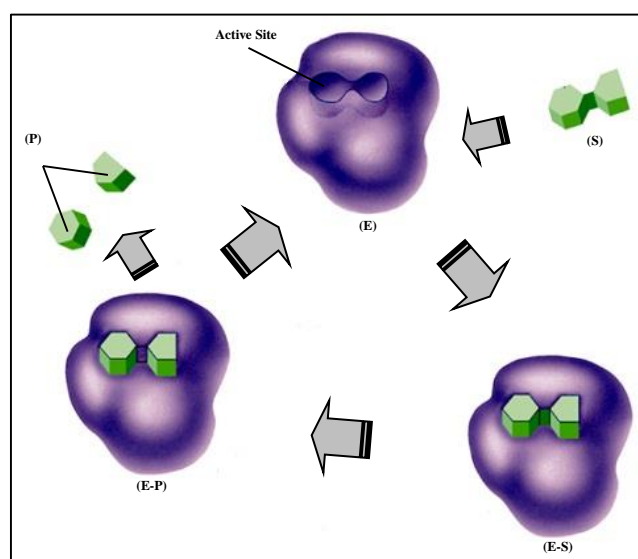


Figure 2-2: A sketched diagram shows the steps of formation of two products from one substrate in an enzyme-catalysed reaction (degradation reaction). (S) Substrate, (P) product, (E) enzyme, (E-S) enzyme-substrate complex, (E-P) enzyme-product complex.

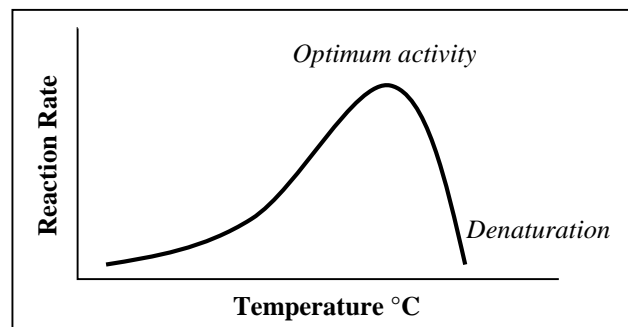
With many biochemical reactions in cells, and therefore many substrates, there are many enzymes each of which catalyzes a different biochemical reaction. There is an estimated of about 1,000 – 4,000 enzymes active in any living cell. Enzymes increase the rate of reactions by lowering the free energy of activation for the substrate conversion to products than the uncatalyzed process.

Because most enzymes are proteins, they must have the correct shape to function efficiently. If the shape of a protein enzyme is distorted, the enzyme will not work as well because the active site will no longer have the same shape and the substrate will not fit into it easily. In extreme cases (e.g. high temperatures, marginal pH conditions) enzymes **denature** (lose their natural shape completely) and will not work at all.

## Effect of Temperature

High temperature generally causes more collisions among molecules, and therefore, increases the likelihood of collision between the substrate and the active site of the enzyme, thus increasing the rate of an enzyme-catalyzed reaction. Above a certain temperature, the activity begins to decline because the enzyme begins to **denature**. The rate of chemical reactions, therefore, increases with temperature but then decreases as enzymes are denatured (Figure 3-2).

Figure 2-3: A graph shows the relationship between temperature and the enzyme activity (indicated by observing the rate of reaction).

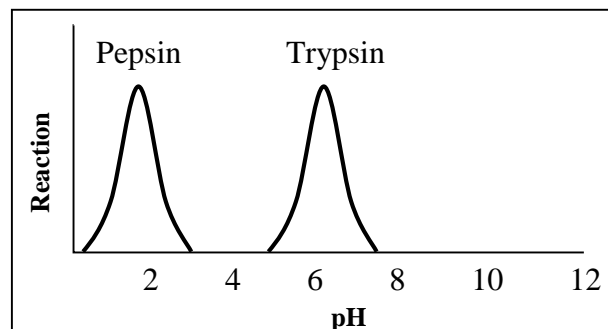


## Effect of pH

Each enzyme has an optimum operating pH. A change in pH can alter the ionization of the R groups of the amino acids. When the charges on the amino acids change, hydrogen bonding within the protein molecule change, and the molecule changes in shape, so, the new shape may not be effective.

The diagram below shows that pepsin functions best in an acidic environment, this makes sense; because pepsin is an enzyme that is normally found in the stomach where the pH is low due to the presence of hydrochloric acid. Trypsin is found in the duodenum, and therefore, its optimal pH is around the neutral to slightly basic range; to match the pH of the duodenum (Figure 3-3).

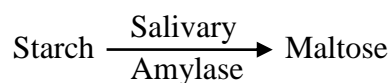
Figure 2-4: A graph compares between the zones of activity for pepsin and trypsin on a pH scale.





## Amylase

Amylase is an enzyme which catalyzes the hydrolysis of starch maltose, glucoses and dextrin. Salivary amylase is produced by the salivary glands and pancreatic amylase is produced by the pancreas.



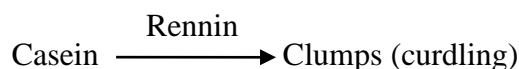
## Pepsin

Pepsin is the principal enzyme in the gastric (stomach) juice. Gastric juice contains HCl which lowers the pH below 2 which is suitable for the gastric proteases like pepsin. Acidity kills microorganisms and denatures proteins. Denaturation makes proteins more susceptible to hydrolysis by proteases. Pepsins are unusual enzymes in that they are acid stable. Gastric protease (pepsin) cleaves peptide bonds formed by amino group of aromatic amino acids (i.e. Phenylalanine and Tyrosine) thus cleaving proteins into peptides. Completion of protein digestion occurs in the small intestine by trypsin.



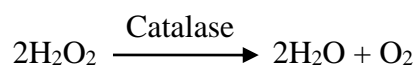
## Rennin

Rennin is a denaturizing enzyme found in the stomach of neonates, where it functions to solidify milk. It causes rapid curdling of milk by causing certain bonds to break in the soluble casein molecules (milk protein) converting it to insoluble casein, thus producing “curdled” milk.



## Catalase

Catalase is an enzyme commonly found just in aerobic living organisms, why? The substrate of catalase is a metabolic by-product called hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Hydrogen peroxide is toxic for cells; since it is a strong oxidizing agent. To prevent the build up of hydrogen peroxide in cells, catalase promotes the breakdown of hydrogen peroxide into water and oxygen, as illustrated by the reaction below.



## Experiments

Today you will examine the activity of four enzymes, amylase, pepsin, rennin, and catalase.

### Experiment No. 1: Effect of substrate concentration and cofactor on the action of the salivary amylase on starch

In this experiment you will test the activity of amylase on a starch solution under different starch concentrations and in the presence and absence of its cofactor.

#### Principle of the test

Amylase is added to a solution of starch in order to be digested into maltoses, glucoses and dextrin. The rate of the reaction increases if the enzyme and substrate mixture are brought to body temperature (37 °C).

The progress of the reaction can be indicated by testing the reaction mixture for either 1) the disappearance of the substrate (starch) or 2) the appearance of product (maltose and glucose). Two simple tests; Lugol's iodine test for starch and Benedict's test for reducing sugars are used for this purpose. A positive Benedict's test is observed as the formation of a brownish-red cuprous oxide precipitate, and a reduction in the bluish-black color formation is noticed for the starch test.

#### Procedure

1. Collect about 2 mL of your own *induced* saliva in a plastic test tube.
2. Dilute the collected saliva 5 times with calcium chloride ( $\text{CaCl}_2$ ; 0.01M) solution. Why?
3. Label six plastic test tubes 1 – 5.
4. In tube # 1 add 2 ml of starch solution (0.1%) and 2 ml of D.W.
5. In tube # 2 add 2 ml of starch solution (0.1%) and 2 ml of saliva in  $\text{CaCl}_2$ .
6. In tube # 3 add 2 ml of starch solution (0.1%) and 2 ml of saliva in D.W.
7. In tube # 4 add 2 ml of starch solution (0.1%) and 2 ml of boiled saliva in  $\text{CaCl}_2$ .
8. In tube # 5 add 2 ml of D.W and 2 ml of saliva in  $\text{CaCl}_2$ .
9. Incubate the tubes on 37 °C for 15 min.
10. Transfer 1 ml from each tube and test for starch using Lugol's solution and another 1 ml from each tube to test for reducing sugars by Benedict's solution (recall both tests from labs. No. 1 and 2).
11. Record your results.

### Experiment No. 2: Effect of Enzyme concentration on the action of catalase on $\text{H}_2\text{O}_2$

In this experiment you will test the activity of catalase on  $\text{H}_2\text{O}_2$  under different enzyme concentrations.

#### Principle of the test

The reaction of catalase is extremely rapid. The action of the enzyme can be demonstrated easily by the evolution of oxygen in the form of gas bubbles when an extract of a tissue containing the enzyme is added to a diluted solution of hydrogen peroxide.

**Procedure**

1. Obtain a suitable tissue containing catalase (i.e. aerobic bacterial culture plate).
2. Transfer one colony of the bacterial culture onto a clean glass slide.
3. Add 1 drop of diluted  $\text{H}_2\text{O}_2$  over the bacterial colonies on the slide and on the cultured plate.
4. Observe and record your results.

**Experiment No. 3: Effect of pH on digestion of protein by pepsin**

In this experiment, you will test the activity of pepsin under different pH conditions.

**Principle of the test**

To test for the activity of pepsin, a suspension of the enzyme is mixed with a protein solution (albumin) in an optimum pH. Turbidity of albumin solution increases with time by the action of pepsin due to the breakdown of albumin.

**Procedure**

1. Label four tubes 1 – 4.
2. Add 1 ml of albumin (3%) in each tube.
3. In tube # 1 add 1 ml of D.W.
4. In tube # 2 add 1 ml of pepsin solution (0.5%) and 1 ml of D.W.
5. In tube # 3 add 1 ml of pepsin solution (0.5%) and 1 ml of acetate buffer (0.1 M, pH 4.5).
6. In tube # 4 add 1 ml of pepsin solution (0.5%) and 1 ml of borate buffer (0.1 M, pH 9).
7. Incubate at 37 °C for 15 minutes.
8. Record your results.

**Experiment No. 4: Effect of temperature on curdling of milk by rennin**

In this experiment you will test the activity of rennin on casein solution under different temperature conditions.

**Principle of the test**

The activity of Rennin could be tested by mixing an enzyme with milk in a test tube. The presence of a reaction is indicated by production of solid milk.

**Procedure**

1. Label five test tubes 1 – 5.
2. Add 1 ml of fresh milk in each of the five tubes
3. In tube # 1 add 3 drops of D.W.
4. In tubes # 2 to 4 add 3 drops of rennin solution.
5. In tube # 5 add 3 drops of boiled rennin solution.
6. Mix the contents of each tube well and incubate tubes (# 1, 2, and 5) in a water bath at 37 °C for 30 minutes.
7. Incubate tube (# 3) in 4 °C and tube (# 4) in 50 °C for, both for 15 minutes.
8. Remove and examine the contents of the tubes in terms of precipitation.
9. Record your results.



## Lab. No. 4

### Diffusion and Osmosis

#### Objectives

- Differentiate between diffusion and osmosis.
- Identify factors that affect diffusion and osmosis.
- Recognize diffusion and osmosis in biological systems.

All molecules display random thermal motion (kinetic energy), which causes molecules to diffuse from a region of higher concentration to a region of lower concentration. This random movement, known as **Brownian movement** (Figure 4-1), continues until the distribution of molecules becomes homogenous throughout the solution. The constant, random motion of molecules enables diffusion to occur. **Diffusion** is the net movement of the same kind of molecules from an area of higher concentration to an area of lower concentration until equilibrium is achieved and molecules are distributed equally. At this point, molecules still move around, but there is no net movement towards any direction (Figure 4-2). The rate of diffusion is dependent on many factors such as temperature, molecular size, molecular weight, pressure, gradient and the type of the medium.

To express the state of change in solute concentration in the same solution, the term **Gradient** is used. *Molecules tend to diffuse down their concentration gradient* unless they were forced to behave differently. If a drop of ink was added to a beaker of water, it will move from its original place to fill the whole beaker (down its concentration gradient).

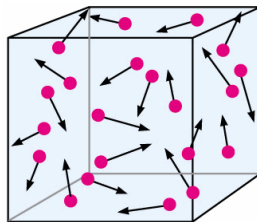


Figure 3-1: A drawing illustrating the Brownian movement.

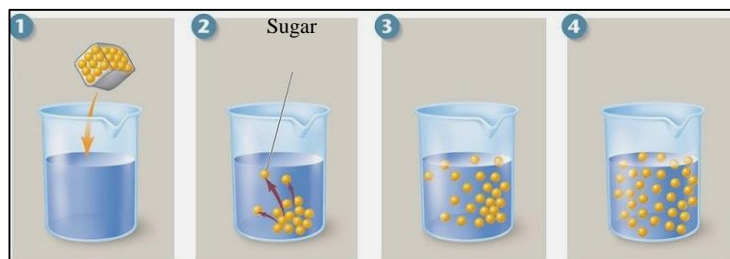


Figure 3-2: (1) A lump of sugar is dropped in a beaker of water. (2, 3) Sugar molecules start to diffuse randomly down their concentration gradient. (4) Eventually, equilibrium is achieved, and the solution is homogenous.

**Tonicity** is a term expresses the relative concentrations between solutions. If two solutions differ in their concentrations, the one with *less solute (and more solvent)* concentrations is said to be **hypotonic** to the one with *more solute (and less solvent)* concentrations, which is said to be **hypertonic** to the former. So, if two solutions differ in their tonicity were separated with a semi-permeable membrane, the *net* movement of the solvent would be *from the hypotonic solution to the hypertonic solution*; because the **hypertonic** solution has less solvent, and therefore, lower solvent potential than the **hypotonic** solution which has more solvent, and therefore, higher solvent potential. On the other hand, **isotonic** solutions, which have *equal solute (and equal solvent)* concentrations exhibit no *net* change in the amount of water in either solution.

The movement of *water* from the hypotonic solution to the hypertonic solution through a selectively permeable membrane is called **osmosis** (Figure 4-3), while the movement of a solute from the hypertonic solution to the hypotonic solution through a selectively permeable membrane is called **dialysis**.

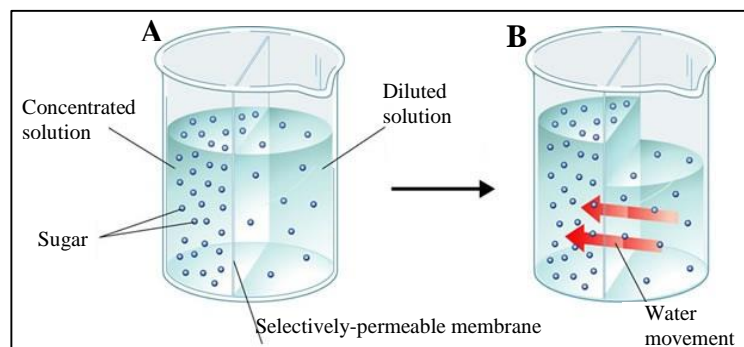


Figure 3-3: Illustrates the process of osmosis. (A) Two solutions differ in their tonicity separated by a selectively permeable membrane. (B) Only water moves across the membrane in order to equilibrate the solutions in both sides of the membrane.

## Osmosis and Living Cells

In biological systems the main solvent is water, in which nutrients dissolve, since they are hydrophilic, and they move down their concentration gradients. The cell membranes of the cells serve as the selectively permeable membranes through which some molecules besides water move freely down their concentration gradients. Larger molecules and those can't pass through the lipid bi-layer need transporter proteins that are dispersed throughout on both sides and transversing the whole membrane thickness, in order to move towards inside and outside the cell according to the cell need (Figure 4-4).

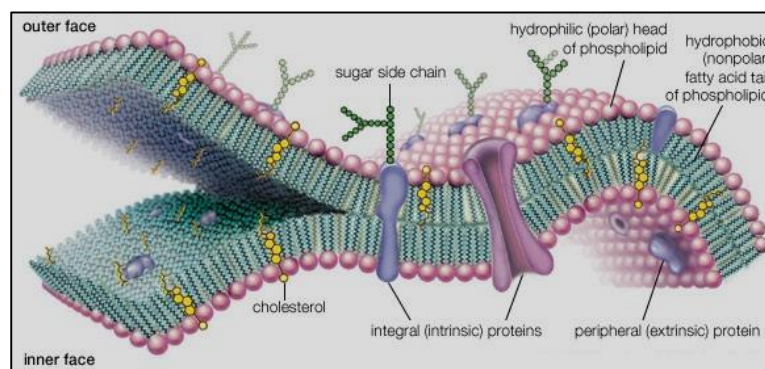


Figure 3-4: A schematic drawing illustrating the eukaryotic cell membrane in the lipid-bi-layer-fluid-mosaic model, showing its accessory protein transporters.

### Osmosis in animal cells

Animal cells are bounded with a cell membrane only, which works as a semi-permeable. So, cells must be in an isotonic medium in order to function well (Figure 4-5.A). Existence in a hypotonic solution results in swelling of the cells which may develop to rupturing of the cells due to the force of the fluid against the inner surface of the cell membrane (Figure 4-5.C). In contrast, putting animal cells in a hypertonic medium causes cells to shrink due to the elimination of water from the cells (Figure 4-5.B).

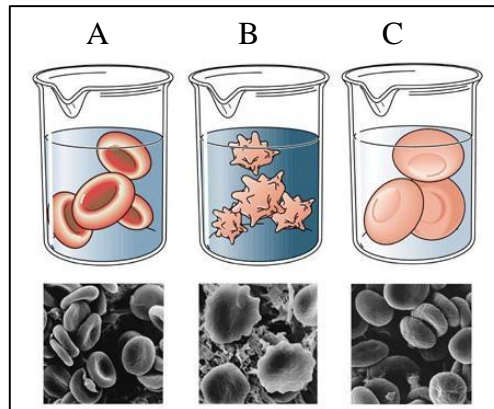


Figure 3-5: This illustration shows the behavior of blood cells in different medium concentrations. In isotonic medium cells be in their native shape because there is no net movement of water from and into the cells (A). In hypertonic solution the net of water movement favors elimination; since water moves towards the outside of the cell in order to equilibrate the high concentration outside the cells (B). In hypotonic solution cells swell, because they gain water which moves from outside towards inside to equilibrate the high concentration inside the cells (C). As water continues to fill in the cells they will burst and cells lyse, so, a turbid blood tube will become very clear if it was filled with distilled water; this is called (*hemolysis*).

### Osmosis in plant cells

Plant cells possess a cell wall superior to the cell membrane. This provides plant cells with strength and rigidity. Plant cells behave as animal cells do, but the cell wall has an impact on this; it prevent cell against rupturing due to overfilling with water, instead plant cells swell if they were put in distilled water. However, the cell wall cannot prevent cells from shrinking if they were put in a hypertonic solution; this is called *plasmolysis* (Figure 4-6).

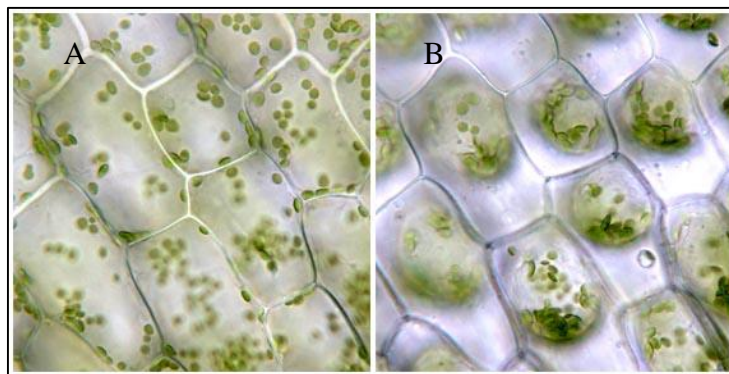


Figure 3-6: This illustration shows the behavior of plant cells in different medium concentrations. (A) Illustrates swelling. And (B) illustrates *plasmolysis*.

## Experiments

Today you will examine the factors that affect diffusion of molecules in a given medium.

### Experiment No. 1: Effect of Solute Concentration on Osmosis

In this experiment you will use cellulose tubing to investigate the relationship between solute concentration and osmosis.

#### Procedure:

1. Obtain three 10-cm tubes of pre-soaked cellulose membrane.
2. Tie a knot in one end of each cellulose tube with short pieces of strings to form 3 sacs.
3. In sac #1 add 1 mL of D.W.
4. In sac # 2 add 1 mL of 20% sucrose.
5. In sac # 3 add 1 mL of 70% sucrose.
6. Remove most of the air from each tube before tying off the other end of it.
7. Weigh each bag separately and record the weight in a table (Initial Mass) (Table.1).
8. Fill three 50 ml beakers, two-thirds full with D.W and label each beaker to indicate the concentration of the solution in the cellulose tube.
9. Immerse each bag in one of the beakers according to its label.
10. After 30 minutes remove the bags from the beakers, carefully wipe off all excess water, and weigh each bag again and record the (Final Mass) (Table.1).
11. Compare the differences in among the three bags.

Table 3-1: Use this table in your experiment.

Tube number	Initial Mass (g)	Final Mass (g)	Net mass (g)

### Experiment No. 2: Effect of Molecular Weight on Diffusion

In this section, you will investigate the effect of the molecular weight on diffusion by observing the movement of colored compounds through an agar gel, which is essentially 98.5% water. Small molecular weight compounds diffuse at a faster rate than large ones.

#### Procedure:

1. Obtain a Petri dish containing agar gel (1.5%).
2. Carefully punch two holes in the agar using a pasture pipette.
3. Fill one hole with a drop of 1% methylene blue solution, and the other hole with 1% potassium permanganate (KMnO<sub>4</sub>) solution. Do not overfill the wells.
4. Incubate the Petri dish at room temperature for 30 min.
5. Examine the Petri dish and measure the diameter in millimeters that each substance has formed in the agar.

**Note:** The molecular weight of (KMnO<sub>4</sub>) is 158 g/mol, and that for methylene blue is 320 g/mol.

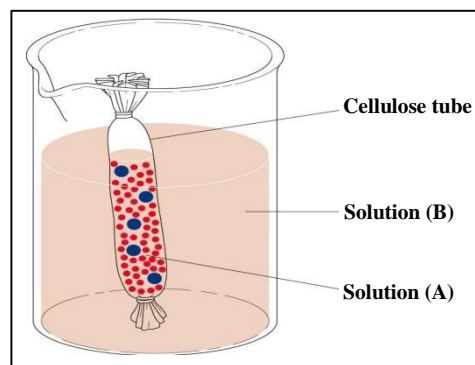


**Experiment No. 3: Effect of Molecular Size on movement through a semi-permeable membrane**

In this experiment diffusion of small molecules through dialysis tubing will be observed. The size of the minute pores in the dialysis tubing determines which substances can pass through the membrane.

**Procedure:**

1. Fill a 50 mL beaker to one third with tap water then add 1 ml of iodine solution into the beaker.
2. Obtain one 10-cm tube of pre-soaked cellulose membrane.
3. Tie a knot in one end of the cellulose tube with a short piece of string to form a sac.
4. Fill the sac with a starch solution (0.1%).
5. Make a knot in the other end of the sac.
6. Hold the sac closed and rinse the outside of it with tap water.
7. Insert the sac into the beaker which you prepared first, with iodine solution.
8. Let it to stand for 20 minutes.
9. Observe and record any changes in coloration and where.

**Experiment No. 4: Effect of Temperature on Diffusion**

In this experiment, you will test the effect of temperature on diffusion.

**Procedure:**

1. Fill a 500 mL beaker, about half full, of tap water and heat it to 50 °C.
2. Fill another beaker about half full of cold water.
3. Keeping both beakers motionless, add one drop of potassium permanganate solution to each beaker.
4. Observe the change in the distribution of the potassium permanganate in each beaker for 1 minute.



**Lab. No. 5****Using the Microscope****Objectives**

- Identify different parts of the student microscope.
- Indicated the function of each part of the microscope.
- Preparing and investigating a wet-mount preparation.
- Practice using the student microscope.

Human eye is a powerful organ that enables us to explore the world around us. However, even a strong sighted person can only detect a spot of just 0.2 mm in diameter. Not until the discovery of optics by the ancient Egyptians and the first lens by the Assyrians 3000 years ago which opened the way to examine small objects and magnify them several times. The invention of the first compound microscope in 1590 was the first step towards the ability to distinguish fine details of small objects, which was a big challenge to humans for many centuries of life. By the mid of the 1600s Antony van Leeuwenhoek was considered the greatest early microscopist who investigated many small objects and wrote many notes about the tiny world.

The Microscope: (μικρός, mikrós, "small" and σκοπεῖν, skopeîn, "to look" or "see") is an instrument for viewing objects that are too small to be seen by the naked or unaided eye.

**Types of the microscopy**

There are many microscope types that are used in many aspects from teaching in schools to the research aspects where very fine sub-cellular parts are examined and tested.

***Optical Microscopes***

Optical microscopes are those which function through the optical theory by using lenses and light in order to magnify the image generated by the passage of a wave through the sample.

Optical microscopes could be simple; where one lens is used for magnification, or compound, where multiple lenses are used for magnification.

According to the field being observed, microscopes could be classified into bright field and dark field, where the image in the former is dark against a bright background, and in the later, the object glows against a dark background.

According to the light source, microscopes could be classified into light microscope; which uses a mirror or a light bulb as a light source, and fluorescent microscope, which uses ultra violet (UV) light source.

### ***Electron Microscopes***

Electron Microscopes are types of microscope that use a particle beam of electrons to illuminate a specimen and create a highly magnified image. Electron microscopes have much greater resolving power than light microscopes that use electromagnetic radiation and can obtain much higher magnifications of up to 2 million times, while the best light microscopes are limited to magnifications of 2000 times.

Most of the microscopic observations in this course will be made with a Bright Field Compound Microscope.

### **Dealing with the microscope**

In order to practice using the microscope you have to know how to handle it, how to use it and how to store it.

1. Always handle the microscope using both hands; one catches the arm of the microscope and the other carries it by the base, making its back facing your body, and the open area out (Figure 5-1.A).
2. Clean all microscope parts using Kim-wipes. To clean the lenses use just the special lint-free lens papers and iso-propanol so they will not be scratched.
3. Always obtain the microscope and store it in a certain situation, the nosepiece is positioned on the scanning lens, and the stage and the condenser are in their lowest position and the mechanical stage is adjusted.
4. Always cover the microscope with the suitable cover to keep it from dusts and store it in its cabinet (Figure 5-1.B).

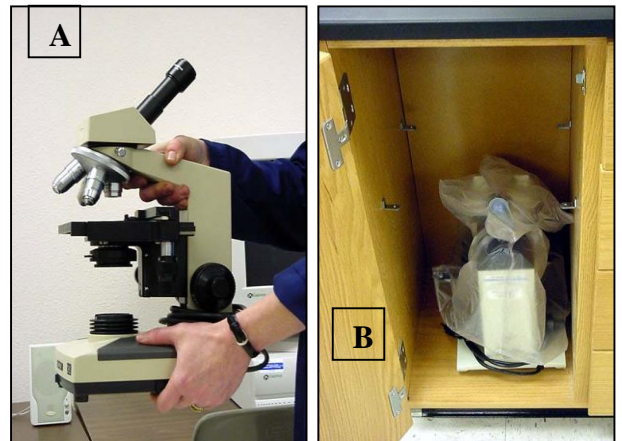


Figure 4-1: Handling (A) and storing (B) the microscope.

### **The Bright field compound microscope**

A microscope consists of a lens system, a controllable light source, a focusing mechanism changes the distance between the objectives and the object under observation.

### **The microscope parts**

The major parts of the compound microscope are shown in (Figure 5-2). Knowing the various parts of the microscope is an essential aspect for the user to master using the microscope.

Here we will focus on the student microscope (bright field compound microscope) and the dissecting microscope – these are the microscope-types that you will use during your studying period.

The bright field compound microscope is composed of three main parts; the head, the nose and the body.

## 1. *The body*

The body of the microscope carries all parts of the microscope and usually is made of metal. It is composed of the base, the back, the arm and the body tube.

**The base** rests on the table, supports the whole body, and contains the electric circuits that control the light\illuminator and the illuminator control knob.

**The back** supports the arm and carries the stage and its accessories and the control knobs; that are used to move the stage and its accessories up and down. *The stage* is the platform that the slide is placed on, in order to be examined. The stage accessories are the *slide holder* or the stage clips that hold the slide to the mechanical stage. *The mechanical stage* is used to adjust the position of the slide. *The mechanical stage control knobs* are used to move the slide and the clips to the four sides. *The sub-stage condenser* is located below the stage and is used to concentrate the light and direct it through the slide. The *condenser control knob* moves the condenser up and down in order to control the light path. *The iris diaphragm* controls the amount of the light that passes through the condenser through the *Iris diaphragm lever*, and it is located below the condenser.

To move the slide up and down in order to position it in the focus of the lens two focusing adjustment knobs are used; the *course adjustment knob* that has a large diameter and moves the stage for longer distance per round than does the *fine adjustment knob* which has smaller diameter and moves the stage for shorter distance per round than does the course adjustment knob. The course adjustment knob is used when dealing with the objectives; 4× and 10×, while the fine adjustment knob is used when dealing with the objectives; 40× and 100×.

**The arm** is the transverse part of the body and carries the head and the nosepiece. It is used to catch the microscope to carry.

**The body tube** is supported by the arm and carries the *head* on its upper side, and the *nosepiece* at its lower side. The *nosepiece* is the revolving part of the microscope that is attached to the body tube of the microscope and carries the *objectives*. The nosepiece can be rotated to choose the suitable lens in order to have the intended magnification. Microscopes carry three to five objectives on their nose-piece differ from each other by their magnification. The *scanning lens* is the shortest one and magnifies objects four times (4×); it is used to scan the smear in order to choose a suitable field – the observed area at a time – to be magnified further. The *low power lens* is a lens that magnifies objects (10×) – some may magnify objects (20×). The *high- dry power lens* can magnify objects (40×). And the *oil immersion lens* which can magnify objects a hundred times (100×).

## 2. *The head*

The head is the upper part of the microscope. It can be rotated as suitable. It carries the ocular lenses or the eyepieces; which is the lens that faces the eye when examining something under the microscope. Some microscopes carry one ocular lens (mononuclear) others carry two (binuclear). The ocular lens magnifies objects ten times (10×). Some ocular lenses may be equipped with a pointer that can be used to point at objects being tested under the microscope.

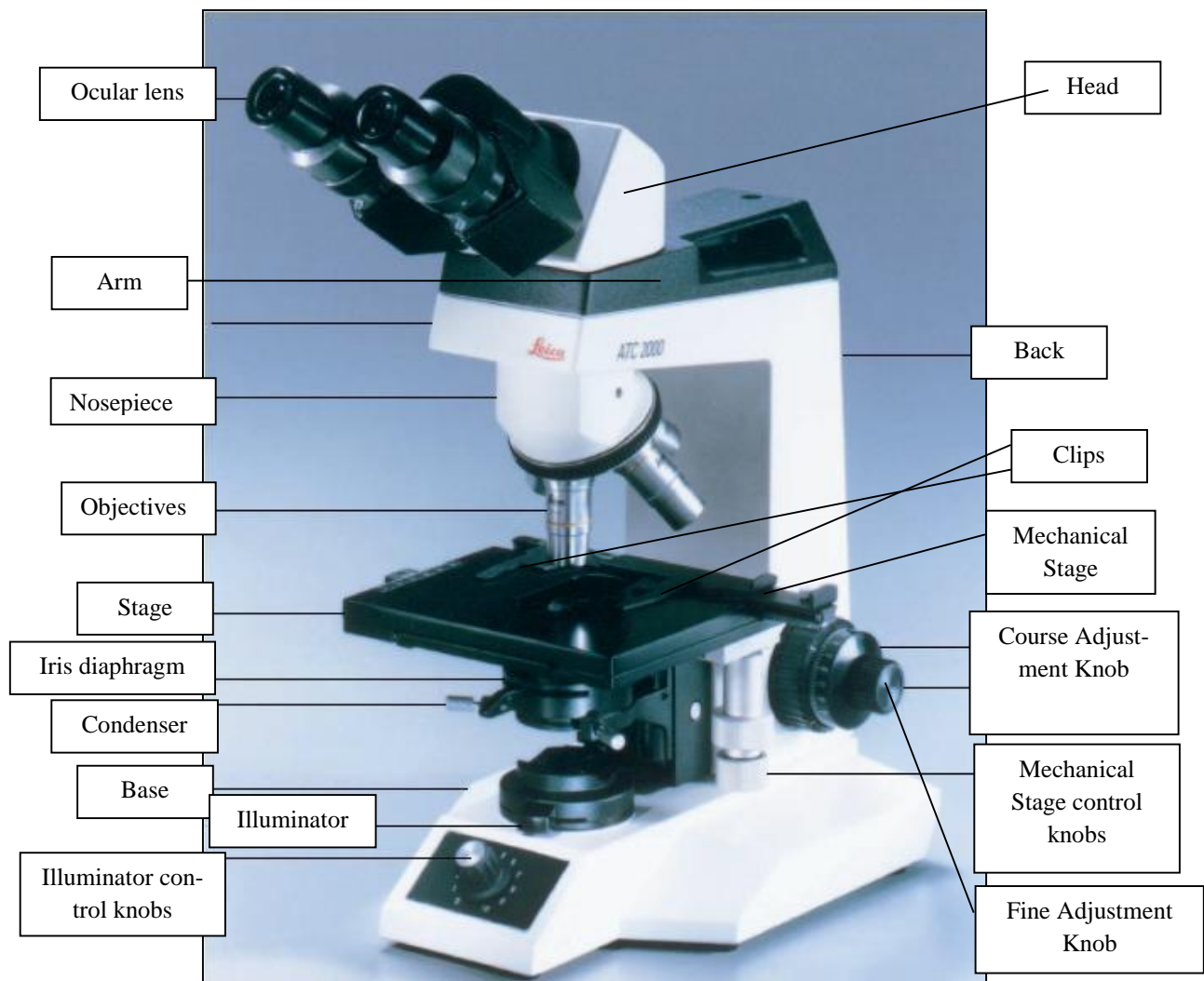


Figure 4-2: The Bright field compound microscope and its parts are indicated.

**Step by step using the microscope using the bright field compound microscope:**

The following steps must be followed carefully in order to get the intended outcome of the microscope.

1. Get the microscope out of the cabinet, put it on the bench, position it as its open area faces your body and remove the cover.
2. The nosepiece must be in the scanning lens, the stage and the condenser are in their lowest position (if not make it as indicated).
3. Prepare your slide.
4. Plug the cable in the electric outlet.
5. Switch the illuminator on using the On/Off button.
6. The light must be dim and the iris diaphragm is almost closed.
7. Place the slide on the stage and fasten it via the clips, and place the examination area over the stage aperture.
8. Look through the ocular lens; you will not see anything but the light.
9. Start elevating the stage by moving the course-adjusting knob towards yourself.
10. Once you observe the image or shades of the image focus the image carefully until you get a clear image.
11. Now scan the smear for the intended area to be examined using the mechanical stage control knobs.
12. Then switch to the 10× lens, raise the condenser a bit and intense the light slightly.
13. Focus the image using the course adjustment knob.
14. Focus the image using fine adjustment knob until you get a clear image, then look for the intended field.
15. If more magnification is needed switch to the 40× and repeat the step 14 (don't use the course adjustment knob at this magnification and above).
16. If the magnification is not enough and you need to focus in more detailed parts then switch to the oil immersion lens.
17. Put the nose piece in a position between the 40× and the 100×, put a drop of immersion oil in the slide and change the lens into the 100×. Here never use the course adjustment knob; just adjust the focus using the fine adjustment knob, you need just a very little distance to focus your image. The 100× lens is very thick and absorbs much light than the others; so, raise the condenser to its highest position and open the iris diaphragm.
18. After you finish examination of your smear switch the light off, move the stage and the condenser to their lowest level, switch the nose piece to the 4x lens and then remove the slide.
19. Clean all microscope parts (except lenses) using kim-wipes. Clean the lenses using just the special lint-free lens papers and iso-propanol; starting with the 4× →→ 100× (don't disorder this pattern).
20. Unplug the cable and wrap it around the microscope's body.
21. Cover the microscope with the dust cover.
22. Carry the microscope firmly and put it in its suitable cabinet facing its back to the outside.

**Notes:**

- As the power of the objectives increases the working distance and the diameter of the field and the light intensity decrease. Therefore, when the image is very clear using the 100× lens, the slide is almost in contact with the objective.
- If you could see a clear image under the 10× and the 40× but not under the 100×; this indicates that the slide is inverted, simply pull the slide invert it upside down and return it on the stage and re examine.
- The image that is seen using the compound microscope is rotated 180°.
- The total magnification is the outcome of the multiplication of the two compound lenses.

***Total magnification = Magnification of the ocular lens × Magnification of the objective***

- The *depth of the field* is the vertical distance within which the structures are in sharp focus. The depth of the field decreases as the magnification increases.
- Most microscopes are *par-focal* that is the image will remain in focus while you change the lenses without the need to refocus it but slight using of the fine adjustment knobs.
- The purpose of using immersion oil is that this type of oil has a refractive index very close to that of the glass, so it will collect the light and prevent its diversion (Figure 5-3).

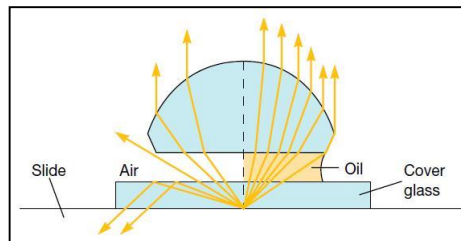


Figure 4-3: The immersion oil prevents the loss of light ray.



## The dissecting microscope

The dissecting microscope which is used to examine larger objects than those examined using the student microscope. The dissecting microscope shares some features with the student microscope and differ in others. The dissecting microscope is composed of a body ocular lens, objective, stage, illuminator and illuminator control knob and a switch On/Off button. Focusing the image is accomplished via two knobs; the zoom magnification knob and focusing knobs. The illuminator could be either in-base or is highlighted from the up (Figure 5-4). Table-1 shows a comparison between the compound bright field microscope and the dissecting microscope.

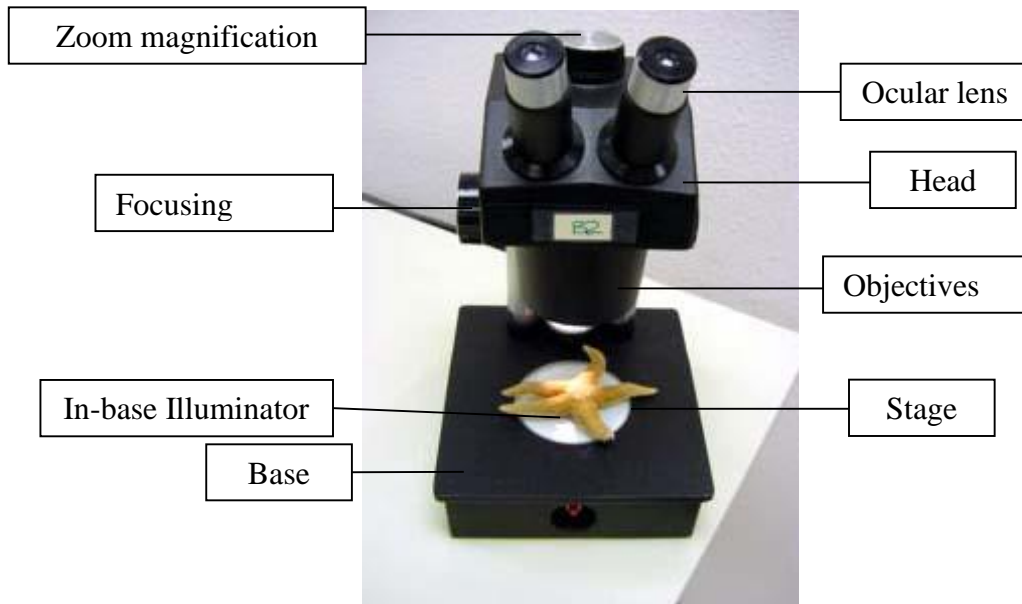


Figure 4-4: The dissecting microscope and its parts.

Table 4-1: A comparison between the compound bright field microscope and the dissecting microscope.

Compound Light	Dissecting microscope
Used to examine materials mounted on microscope slides (usually sectioned & stained)	Used to examine thick materials on microscope slides & whole specimens living or preserved
Generally provides total magnification from 40x to 1000×	Generally provides total magnification from 10x to 45×
Does not provide space for dissection of materials being examined.	Provides space between stage & objectives for dissection

## Experiments

### Experiment: Wet Mount preparation

In this experiment, you will test a typed letter “e” in a wet mount under the microscope and try to draw it.

#### Procedure

1. Get a clean glass slide and a cover slip.
2. With a scissors cut out the letter "e" from a typed paper.
3. Place it on the clean glass slide.
4. Add one drop of tap water over the paper.
5. Hold the cover slip at a 45<sup>0</sup> angle. Gently lower it down.
6. Remove any air bubbles from under the cover slip by gently tapping the cover slip.
7. Dry any excess stain before placing the slide on the microscope stage for viewing
8. Examine the slide under the scanning, low and high power lens.



#### Questions

1. State the procedure that should be used to properly handle a light microscope.
2. Explain why the light microscope is also called the compound microscope.
3. Explain why the specimen must be centred in the field of view on low power before going to high power.
4. Explain why we could not use the 100× with the wet mount.
5. What is the magnification of the following “e” letter under the microscope?



## Lab. No. 6

### Examination of plant and animal cells under the microscope

#### Objectives

- Differentiate between animal and plant cell under the microscope.
- Categorize organisms according to their cellular properties.

Living organisms are so complicated, that many structures must cooperate and coordinate together in order to form the completely competent organism. Atoms integrate together to form molecules which in order form complicated molecules that link together to form very sophisticated polymers. These polymers form many types of organelles each of which have different specialized functions that are important for the cell to persist in life by forming many macro-molecules which are used as the building blocks of the cells, and also, metabolize many molecules in order to extract energy which is the fuel for the cell to progress. Cells, together, form different types of tissues, which collect to form a specific organ. Different organs combine together to form a system that exerts a group of functions which need many other systems to collaborate to constitute a competent organism that can persist and continue in the life.

Cells – which are our subject – are the smallest form of life, and all life forms are made from one or more cells, also, these cells only arise from pre-existing cells. These three pre-mentioned points are known as the “*cell theory*”, which was proposed, by Schleiden and Schwann in 1838.

On the other hand, cells differ according to the level of development; that is, does the cell contain well defined bound organelle structures and nucleus or not? Cells that do not contain a bound chromatin in an envelope (membrane) nor have defined organelle structures were termed to have an old-fashioned nucleus or the *Prokaryotes* (before nucleus), while *Eukaryotes* (true nucleus) are those well developed cells that contained bound and well defined organelles and a nucleus. All prokaryotic organisms are single-celled, such as bacteria and algae.

Also, organisms are classified according to the complexity of their construction and may be composed of one cell only, so they are called *Unicellular* (e.g. bacteria), other organisms are termed as *Multicellular*; where many cell types are specialized and coordinate to execute different functions and constitute a full organism (e.g. mammals, fungi, parasites, arthropods, plants, and many others).

The basic structure of all cells, either prokaryotes or eukaryotes, is almost the same; all cells have an outer covering; called the plasma membrane. The plasma membrane holds the cell components together and selects the passage of different substances into and out of the cell. With a few minor exceptions, plasma membranes are almost the same in prokaryotes and eukaryotes. The interior of both kinds of cells is called the cytoplasm, within which the cellular organelles are embedded in eukaryotes.

In this lab section, you will study only eukaryotic cells (animal cell and plant cell) (Figure 6-1). Review your textbook about the cell before coming to the lab.

Study the structure of these two cells and notice the differences between them.

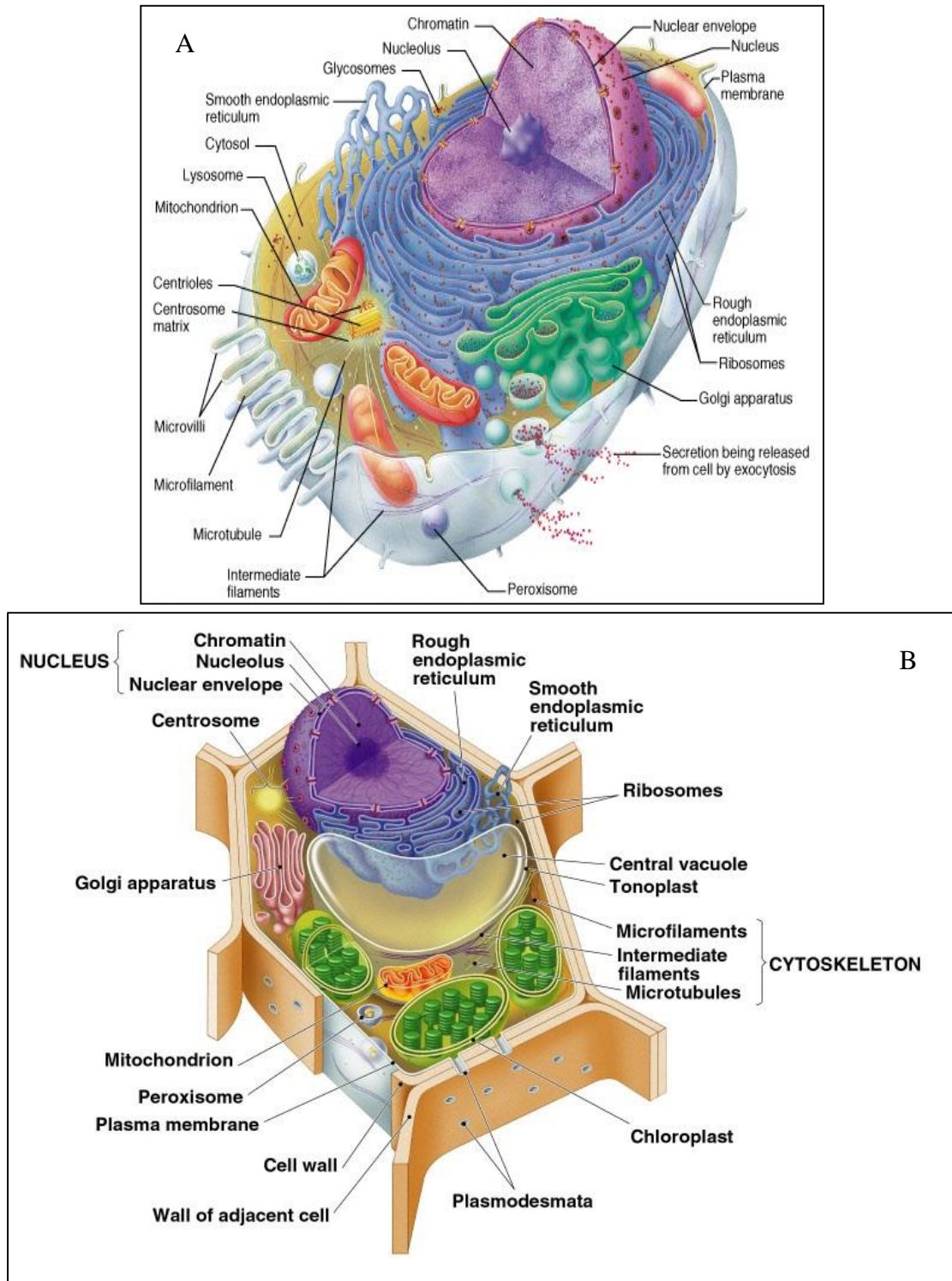


Figure 5-1: Two eukaryotic cells' illustrations; a plant cell (A) and an animal cell (B) and the main organelles are assigned.

## Experiments

### Experiment No. 1: Testing a Wet Mount of onion cells

In this experiment, you will test a wet mount of an onion tissue under the microscope and try to define the tiny structures in the field.

#### Procedure

1. Get a clean glass slide and a cover slip.
2. Peel a delicate transparent thin layer tissue of cells from the inner surface of the skin of the onion as shown in figure 2.
3. Place the thin layer of onion over the clean glass slide.
4. Add one drop of Lugol's Iodine solution over the onion.
5. Hold the cover slip at a 45° angle. Gently lower it onto the drop of stain.
6. Remove any air bubbles from under the cover slip by gently tapping the cover slip.
7. Dry any excess stain before placing the slide on the microscope stage for viewing
8. Examine the onion slide under low power of the microscope. Switch to the high power.
9. Find the cell wall, nucleus, and cytoplasm. Draw living onion cells that you see in the field.

### Experiment No. 2: Preparing a wet mount of cheek cells

1. Obtain a clean slide and add a drop of methylene blue solution in the middle of the slide.
2. Gently scrape the inside of your cheek with the broad end of a clean toothpick.
3. Rub the used toothpick in the drop of methylene blue placed on the slide to transfer your cheek cells.
4. Add a cover slip and observe under low and high power of your microscope.
5. Draw a picture of your cheek cells and label the parts you can see.

#### Questions

1. What is the basic shape of the onion cell?
2. Describe the shape of the cheek cell.
3. What cell parts are found in plant cells that are not found in animal cells?
4. What cell components did you observed under the microscope of both cell types?
5. mention three differences between animal and plant cells.
6. What is the magnification of the following plant tissue under the microscope?





**Lab. No. 7**

## Animal Tissues (Introduction to Histology)

**Objectives**

- Identify different animal tissues under the microscope.
- Indicate the structure and function of each animal tissue.

Cells are the smallest form of life (recall the cell theory). Various types of cells (not necessary identical, but originate from the same origin) integrate and coordinate together to carry out specific functions, this union is called **tissue**. Many tissue types collaborate to form a complete organ that is responsible for exerting specialized functions specified by the cell types composing it. Animal tissues can be grouped into four basic types: **epithelial**, **muscular**, **connective** and **nervous** tissues. The study of tissues is known as **histology**, and connecting tissue abnormalities with diseases is called **histopathology**.

**Epithelial tissue**

Epithelial cells cover the body out surface; which is known as skin, so they provide physical protection of the whole body. This tissue lines the major cavities of the body and form the structure of the lungs; including the alveoli or air sacs where gas exchange occurs. It, also, lines most organs, such as the gastro-intestinal (GI) tract (i.e. mouth, esophagus, stomach, small and large intestine), kidneys, and pancreas. They are also found in ducts and glands, like the bile duct and salivary glands. Epithelia can specialize to act as sensory receptors; they form taste buds, line the nose, and in eyes and ears. Female reproductive organs are lined with ciliated epithelial cells. Capillary beds are made up of epithelium. Epithelia are the first type of cells to differentiate in the embryo; this occurs during the eight-cell stage.

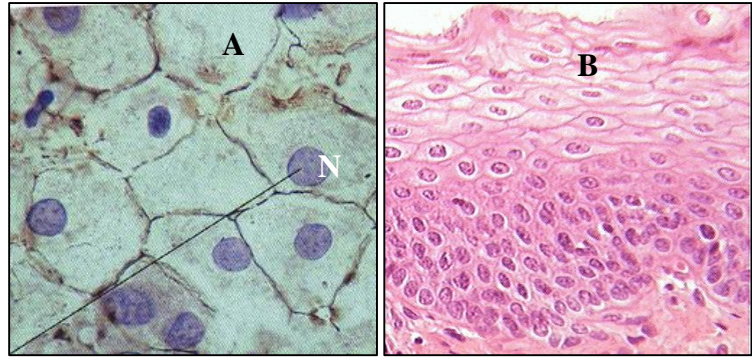
Structurally, **simple** epithelium is the mono-layer epithelium tissue, while **stratified** epithelium is more complex in structure as it is a multi-layer epithelium. Epithelial cells that are amorphous (shapeless) flat in shape are called **squamous** epithelium, and those which are cubic in shape are called **cuboidal** epithelium. Elongated epithelia are known as **columnar** epithelium.

**Simple squamous epithelium** is named because it consists of one layer of flat cells. Squamous epithelia are found lining surfaces utilizing simple passive diffusion such as the alveolar epithelium in the lungs, blood vessels and the major cavities found within the body (Figure 7-1.A).

**Stratified squamous epithelium** is named because it consists of many layers of flat cells. Having many layers makes this tissue ideal for protection. It is found in the epidermis of the skin, the mouth, anus, and vagina (Figure 7-1.B).



Figure 6-1: (A) Simple squamous epithelium tissue of human skin, the indicated part is the nucleus (N). (B) Stratified squamous epithelium from human esophagus, note the multi-layered structure.



**Simple cuboidal epithelium** consists of one layer of cube shaped cells and **stratified cuboidal epithelium** consists of many layers of cube shaped cells. This tissue is suitable for secretion; so, they can be found in exocrine gland of the pancreas as well as in the ducts of the glands. They also constitute the germinal epithelium that covers the female ovary (Figure 7-2).

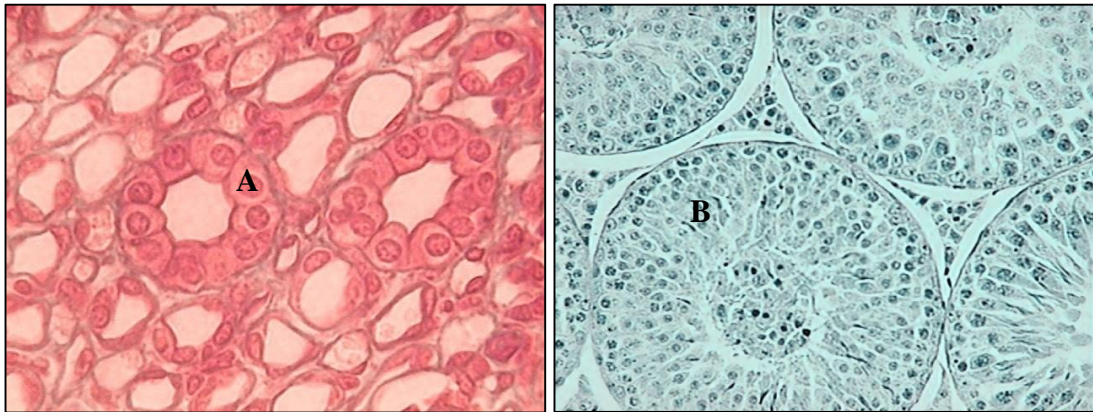


Figure 6-2: (A) Simple cuboidal epithelium. (B) Stratified cuboidal epithelium.

**Simple columnar Epithelium** consists of one layer of elongated epithelial cells and **stratified columnar Epithelium** consists of more than one layer of elongated epithelial cells. This tissue is suitable for absorption and is found in the lining of the respiratory and digestive tract. This tissue could be *ciliated* or *nonciliated*. Cilia are important to move substance in one direction. Some columnar epithelial cells express brush like layer that expands the absorption surface area; like those expressed in the lumen of the small intestine, these appendages are called villi (Figure 7-3).

Some epithelial cells, such as the goblet cells, secrete fluids that are necessary for other processes such as digestion, protection, excretion of waste products, lubrication, reproduction, and the regulation of metabolic processes of the body. As part of its excretory role, certain epithelial cells secrete mucus, which lubricate the body cavities (i.e. peritoneum, pericardium, pleura, and tunica vaginalis) and the passageways that they line. In the trachea, goblet epithelial cells secrete mucous which provides the lubrication to aid ciliated epithelial cells to sweep bacteria and dust away from the lungs. Cilia move mucus, debris, foreign cells up to the throat where they are swallowed to be destroyed in the stomach (Figure 7-3).



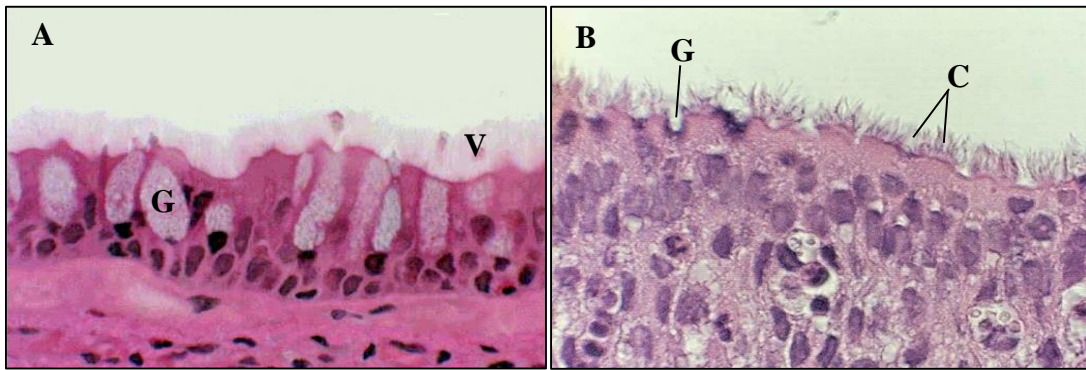


Figure 6-3: (A) Simple columnar epithelium of mouse intestine. (B) Ciliated stratified columnar epithelium. Notice the goblet cells (G), cilia (C) and the villi (V).

## Nervous Tissue

The nervous tissue exists in the central nervous system (CNS, i.e. the brain and the spinal cord) and the peripheral neurons that extend from the CNS and provide sensory information and the reflex commands towards different tissues of the body.

Nerve cells are called neurons. Neurons are composed of the *cell body* that contains the nucleus, the *dendrites* that are short branches extending from the cell body; they receive incoming signals from different organs, and the *axon* (nerve fiber), which is a long, thin extension of the cell body, and carries outgoing electrical signals to their destination (Figure 7-4).

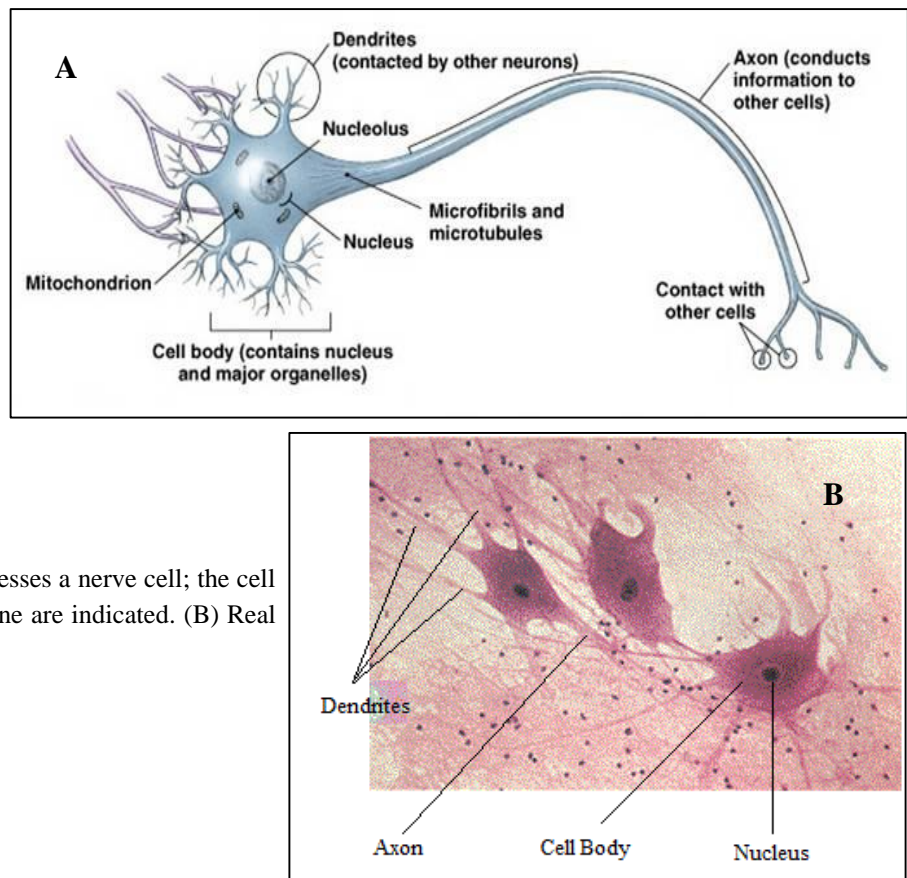


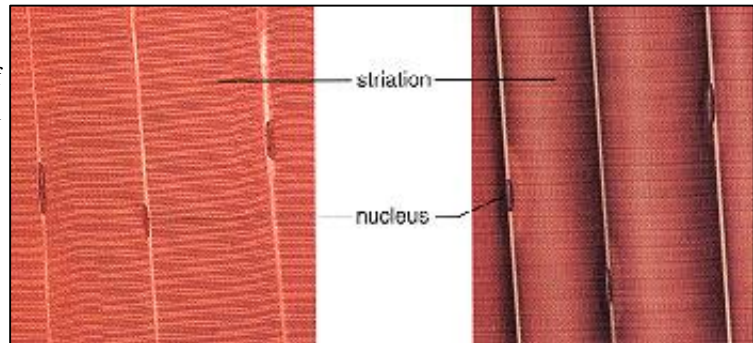
Figure 6-4: (A) A drawing expresses a nerve cell; the cell body, the dendrites and the axone are indicated. (B) Real nerve cells.

## Muscle tissue

Muscle cells are those contractile cells that's main function is contraction and dilation. They can do so because they contain contractile filaments that slide over each other and change the size of the cell. They are classified into **skeletal**, **cardiac** and **smooth** muscles.

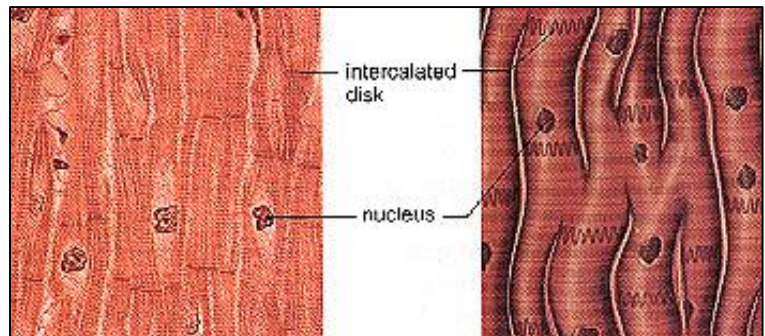
**Skeletal** muscles are striated, voluntary and attached to bones through *tendons*. Their main role is locomotion and provide force for movement. The **muscle fibers** (muscle cells) are long, cylindrical in shape and multinucleated. They lack the ability of cell division. Muscle fibers are composed of *myofibrils* which contain *actin* and *myosin* filaments; that act in contraction (Figure 7-5).

Figure 6-5: A drawing shows characteristics of skeletal muscles, notice the striations, cylindrical shape and the nuclei.



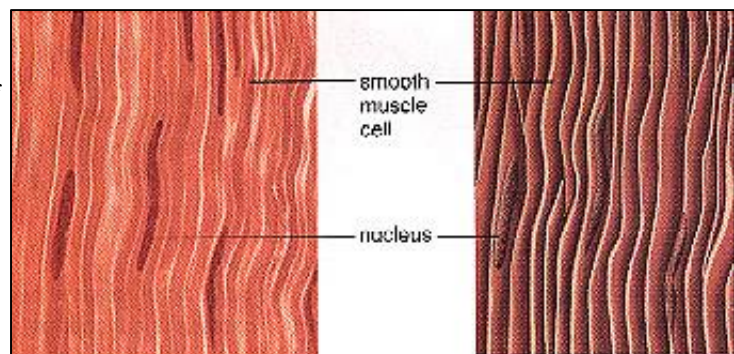
**Cardiac** muscles are unique to heart. The muscle cells are called **cardiocytes**. They are striated, involuntary and mono-nucleated. They form branching networks connected at *intercalated disks* (Figure 7-6).

Figure 6-6: A drawing shows characteristics of cardiac muscles, notice the striations, branching and the intercalated disks.



**Smooth** muscles are small and tapered, nonstriated, involuntary and mono-nucleated. They can divide and regenerate. They are found within the walls of organs and structures such as the GI tract, bronchi, uterus, urethra, bladder, blood vessels, and others (Figure 7-7).

Figure 6-7: A drawing shows characteristics of smooth muscles, notice the absence of striations, the spindle shaped fibers





## Connective Tissue

Connective tissue is a fibrous tissue found throughout the body. It connects body tissues together, provides structural framework for the body, protects various body organs including the brain and the spinal cord and it is the chief energy storing tissue.

Connective tissue makes up a variety of physical structures, including tendons, capsules, ligaments around joints, cartilage, bones, adipose tissue, blood and lymphatic tissue.

Many types of the connective tissue are known, but here, the main types will be discussed; *dense regular* and *irregular* connective tissue, *Loose* connective tissue, *cartilage*, *bone*, *adipose* and *blood*.

**Dense regular and irregular** connective tissue is characterized by a fiber arrangement, which enables this tissue to withstand force in many different directions. Dense irregular connective tissue is found in the coverings of bone (periosteum), cartilage (perichondrium) and nerves (perineurium) and forms the dermis layer of the skin (Figure 7-8).

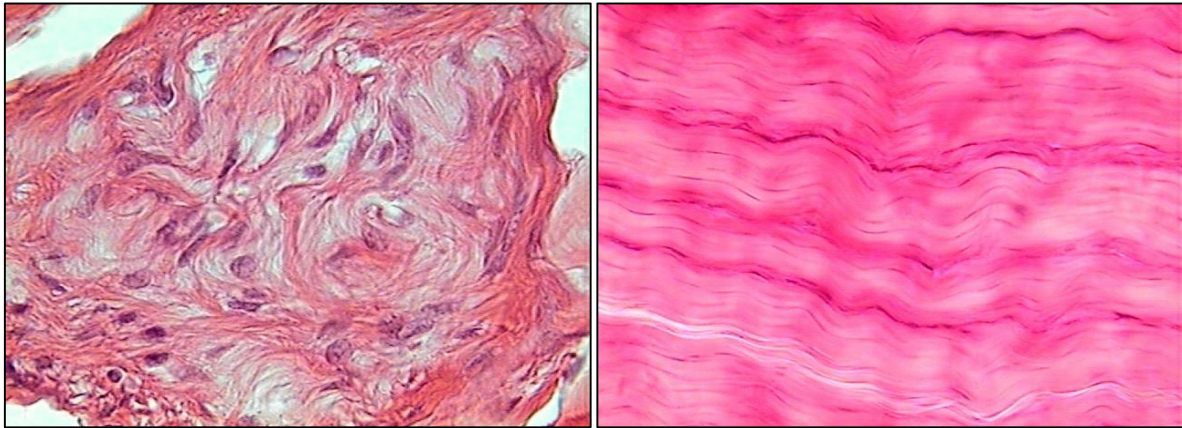


Figure 6-8: (A) Dense irregular connective tissue from human periosteum. (B) Dense regular connective tissue. Note the scarcity of space between cells.

**Loose or areolar** connective tissue is named because the fibers are randomly arranged; there are many spaces between the cells which make this tissue suitable for cushioning and protection. The large spaces between the cells may swell with fluid to cause edema. This tissue forms the hypodermis, surrounds lymph and blood vessels and various organs (Figure 7-9).

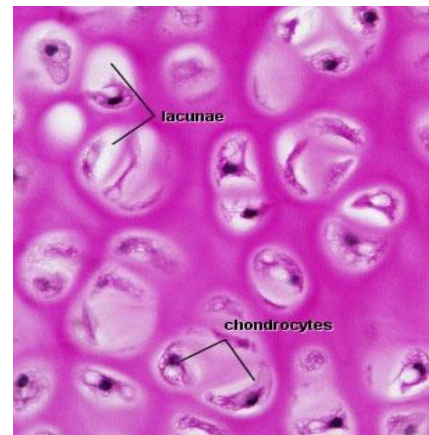


Figure 6-9: Areolar tissue from human dermis.

**Cartilage** tissue contains a gel-like matrix which is stronger than the matrix of the loose connective tissue. Cartilage is avascular, so it doesn't have a blood supply. Cartilage is slow to heal when injured and is often simply removed when torn. Cartilage cells get nutrients and oxygen from the perichondrium which surrounds it. Cartilage consists of cells called *chondrocytes*. Each chondrocyte is enclosed in a space called a *lacuna*. The matrix surrounds each lacuna contains collagen fibers and is made by the immature cartilage cell termed the *chondroblast*. There are three types of cartilage: *hyaline*, *fibrous*, and *elastic*.

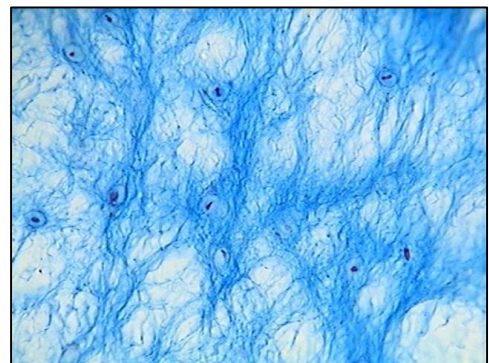
**Hyaline** cartilage is the most abundant cartilage in the body; it is found in the costal cartilage (joins ribs to sternum), articular cartilage (at the ends of bones in a joint), and forms the fetal skeleton (Figure 7-10).

Figure 6-10: Hyaline cartilage, notice the lacuna and the chondrocytes



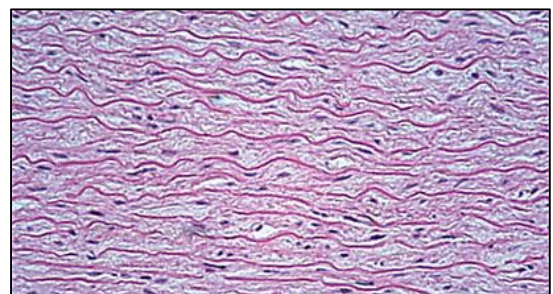
**Fibrous** cartilage is also referred to as fibrocartilage. This cartilage also contains lacunae and chondrocytes but its matrix is shaped in thick bundles or pads. This makes this tissue slightly compressible. It is found in areas that support body weight, such as the intervertebral discs of our back, and the menisci of the knee joints (Figure 7-11).

Figure 6-11: Fibrous cartilage, note the thick bundles or pads that form this type of cartilage.



**Elastic** cartilage has a matrix that contains more elastic fibers than collagenous fibers. This cartilage is found in the external ear (Figure 7-12).

Figure 6-12: Elastic cartilage, note the zigzag shaped fibers.





**Bone** connective tissue contains the hardest and strongest matrix. The matrix mainly contains collagenous fibers. The matrix is mineralized or hardened by the deposition of calcium and phosphate salts. In addition to being stronger and harder, bone differs from cartilage in that bone is vascular and cartilage is not. Also, the *lacunae* of bone are interconnected by little channels called *canaliculi*. The canaliculi form passageways for the capillaries and blood to move through the matrix to get to the *osteocytes* (bone cells) (Figure 7-13).

There are two types of bones: *compact* and *spongy*. Compact bone resembles a tree trunk when viewed under the microscope. Each ring of compact bone is a ring of collagen fibers termed a *lamella*. Between the lamellae are the *osteocytes* (Figure 7-13).

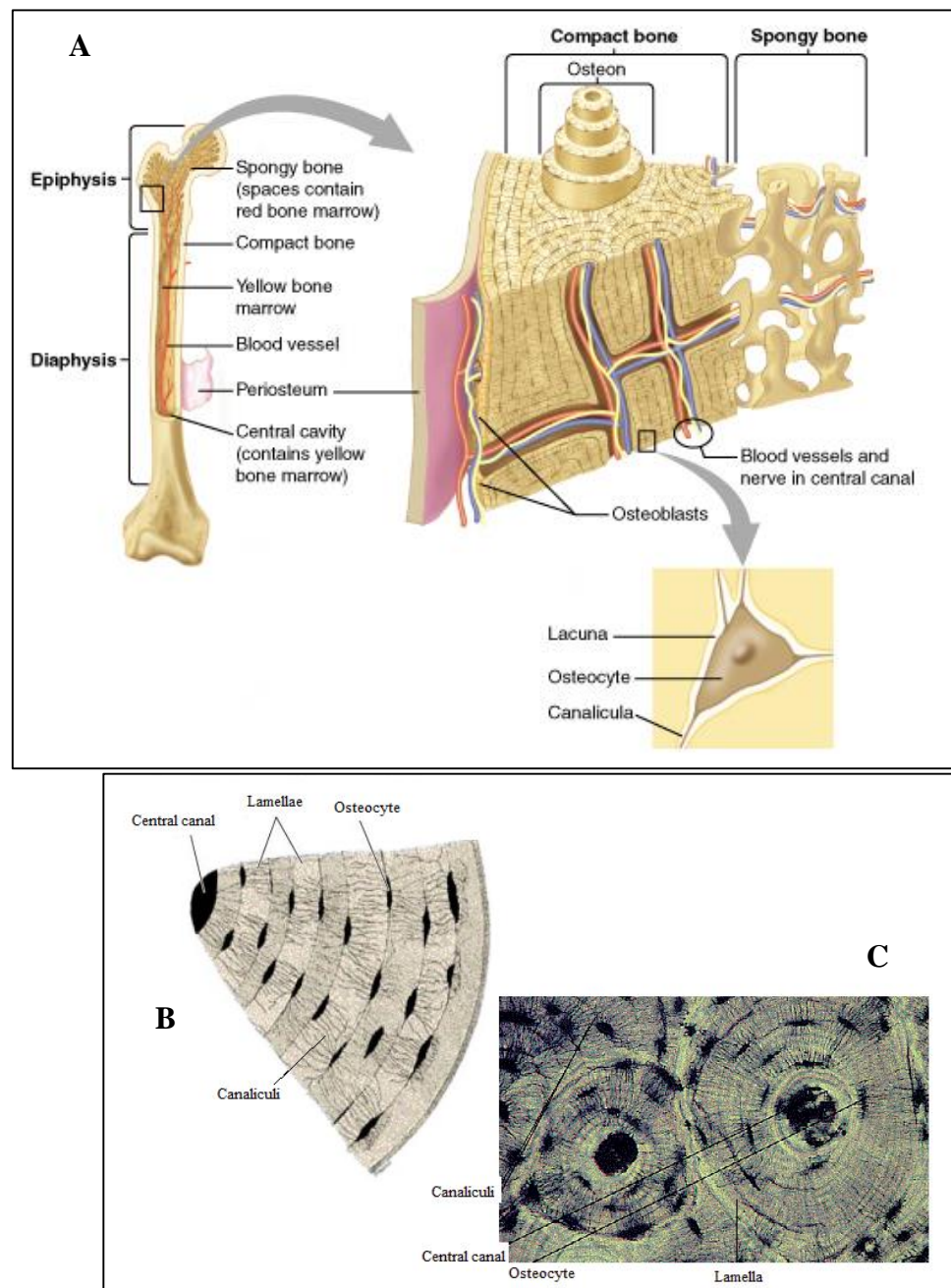


Figure 6-13: (A) Long bone with parts are indicated, compact and spongy bones are shown. (B, C) A transverse section for a compact bone showing the central canal, the lamellae, the osteocytes and the canaliculi.

**Adipose** connective tissue stores fat, it contains *adipocytes* or fat cells (Figure 7-14); when an individual gains weight, the fat cells do not increase in number, but swell to take in and store more fat. Adipocytes multiply few times throughout the life; this occurs during infancy, puberty, and whenever one gains weight rapidly.



Figure 6-14: Adipose tissue; adipocytes are full of lipid storing vacuoles that occupy almost all the cytoplasm and the nuclei are pushed marginally.

**Blood** is also a connective tissue. Blood consists of plasma and formed elements. The plasma is the nonliving component of the blood, and the formed elements are the living portion. The formed elements are red blood cells (RBCs, erythrocytes), white blood cells (WBCs, leukocytes), and platelets (thrombocytes) (Figure 7-15).

Blood has many functions; it transport gases, nutrients and wastes, prevent excessive blood loss, and fight diseases.

More detailed information about blood would be discussed in the next lab. session.

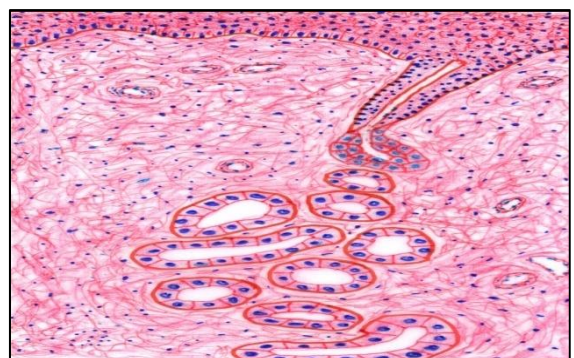
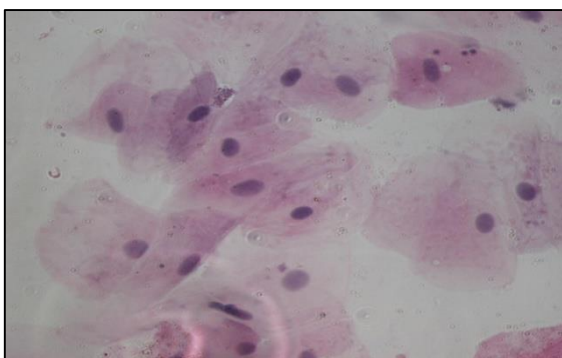
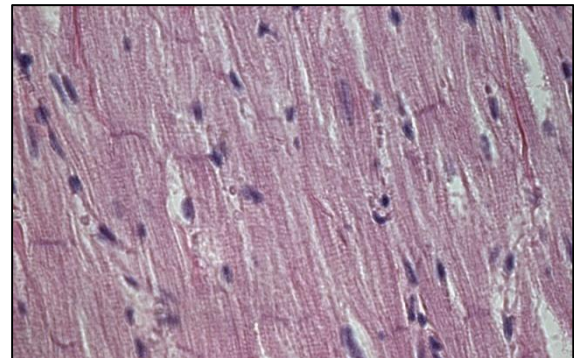
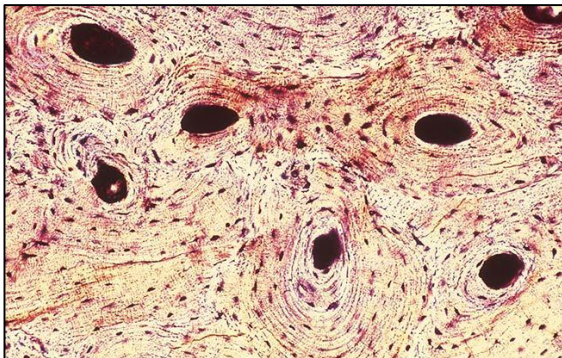
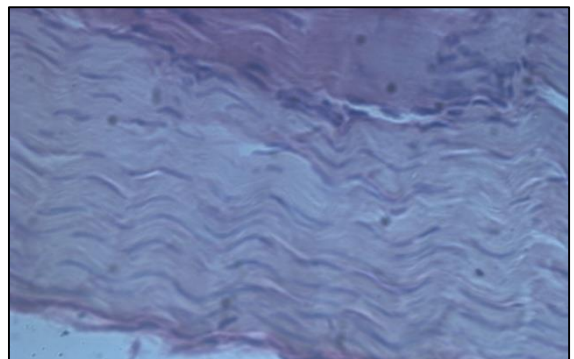
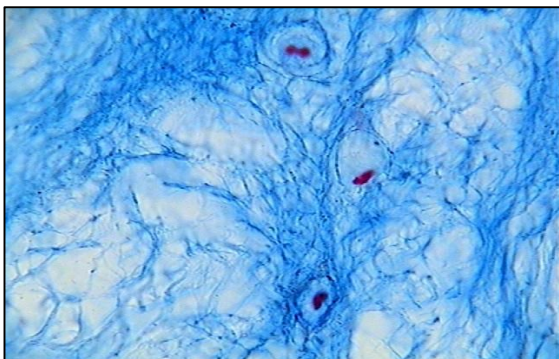
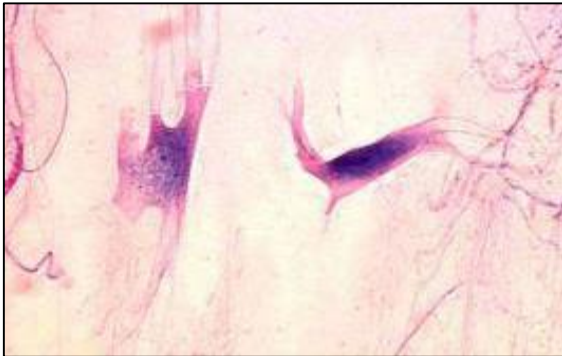


Figure 6-15: A blood film showing RBCs (reddish), WBCs (large, nucleated) and platelets (small dot-like bodies).



## Exercise

Try to identify the following tissues and give an example of an organ they made up of these tissues.







**Lab. No. 8**

## Eukaryotic Cell Division (Mitosis & Meiosis)

**Objectives**

- Identifying different stages of the cell cycle.
- Indicate various events that take place in cell divisions.
- Indicate the stages of different cell divisions under the microscope.

Plenty of vital biochemical processes are needed and must be executed by the cell in order to persist. Cells grow, produce energy, exert specialized function and replicate. All these activities are repeated by cells periodically in a set of events called the cell cycle.

**The Cell Cycle**

In this lab. we will be concerned with the cell division steps; although the cell division is just one phase of the cell cycle, but the other phases are beyond the aspect of this lab session. The division phase is just one phase of the whole cell cycle, which occurs in the (M) phase. Three other main phases make up the cell cycle (Figure 8-1).

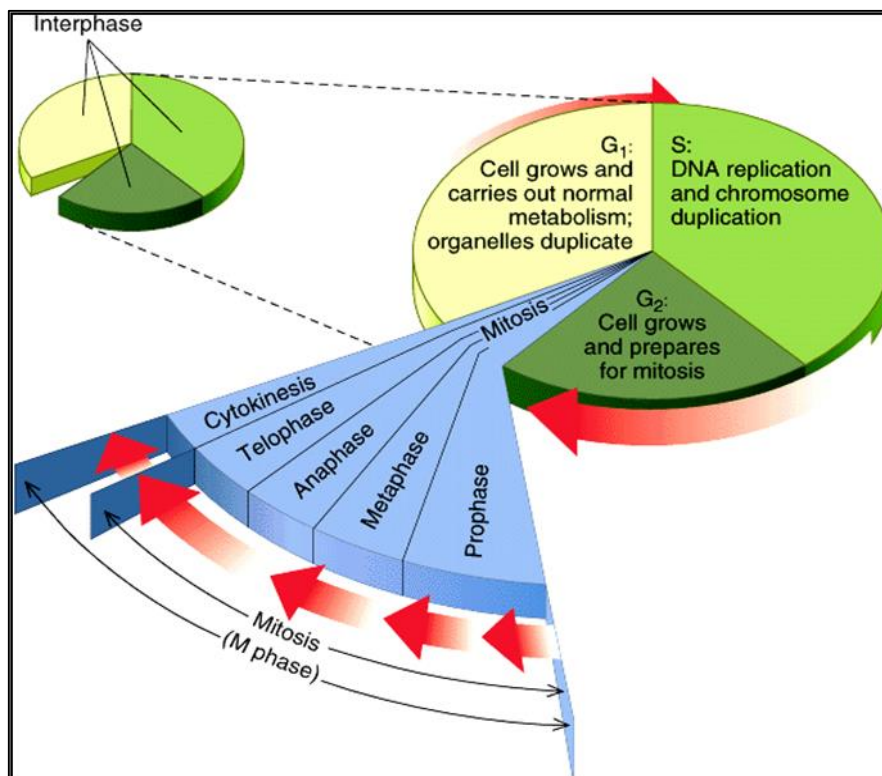


Figure 7-1: A schematic drawing illustrating the cell cycle and showing the four phases that cells go through repeatedly through their life.

The cell cycle begins with the formation of a new cell and ends by the cell division. In the gap 1 (**G1**) phase, vital processes including cell growth and synthesis of structural compounds other than DNA takes place, in the synthesis (**S**) phase DNA replication takes place, in the gap 2 (**G2**) phase synthesis of molecules that are important for cell division occurs, and in the (**M**) phase cell division steps are implemented.

## The Cell Division

A major feature of the cell cycle is the cell division. In eukaryotic cells, two division types are conducted, each of which is exerted in deferent type of cells, and so, result in deferent outcomes. **Mitosis** is the type of division conducted by *somatic* eukaryotic cells and results in two daughter cells which represents the organism's growth. While **meiosis** is executed in *germ* cells, and produces four daughter cells, which are then give the *gametocytes*. Prokaryotes don't possess nuclear envelope; and so, undergo neither mitosis nor meiosis.

### Mitosis

Mitosis is the process by which cells divide and results in the production of two daughter cells from a single mother cell; which are identical to each other and to the original mother cell. Mitosis is divided into four stages: **Prophase**, **Metaphase**, **Anaphase** and **Telophase** (Figure 8-2.A). It is important to realize that the steps are not separated from each other, rather, they are continuous but they are divided into phases in order to simplify the study of the whole process. Cells undergo mitosis in order to restore their volume to surface area ratio. There are many objectives that cells achieve through mitosis including cellular replacement, tissue Repair, development and growth of tumor cells.

**Interphase** is not considered as a mitosis step; rather, it is represented by all steps of the cell cycle except the (M) phase (Figure 8-1). In this phase the cell is metabolically active, the nucleus is clearly visible with one or more nucleoli and contain the diploid number of chromosomes (2N), which is 46 in humans, the chromosomes are long and thin, and the *centrosome*, which is unique to animal cells, is located in the margin of the nucleus.

In the **prophase** the nuclear envelope disappears to prepare the nucleus for division, the chromosomes shorten and thicken and two *chromatids* are clearly visible for each chromosome. The microtubule organizing centre, the *centrosome*, divides into two entities, and each migrate down sides of nuclear envelope, and protein fibers stretch between them forming the mitotic spindle.

The **metaphase** is the final preparatory step for nuclear division; here, the chromosomes line up on the equatorial plate of the cell and the *centromeres* of the chromosomes are attached to the centrioles through the spindle fibers.

In the **anaphase**, the chromatids migrate to the opposite poles of the cell towards the centrioles by sliding of the microtubules.

**Telophase** is the last steps of mitosis; here, the chromatids reach the poles of the cells, the spindle fibers degrade, the nuclear membrane reassembles, the chromosomes elongate and the nucleoli reassemble. Mitosis is considered as the *nuclear division*, while **cytokinesis** is considered as the *cellular*

*division*; it takes place by constriction of the actin fibers as forming a belt around cell in animal cells. Plant cells form a cell plate then cellulose is added. After cytokinesis, the cell reserves the activities of the prophase. By the end of mitosis, the genetic material, that was duplicated in the (S) phase, is separated into the two newly formed daughter cells each of which contains the same amount of the genetic material as their progenitor cell (diploid; 2N).

## Meiosis

Meiosis, on the other hand, takes place in germ cell lines to produce gametocytes; *sperm cells* and *ova* (eggs). Two consecutive cell divisions are performed here; **meiosis I** and **meiosis II** (Figure 8-2.B). In meiosis, the names of the phases of mitosis are used since general features of those phases are shared, but with some critical exceptions especially between mitotic meta- and telophases and meiotic meta- and telophases; I and II (Figure 8-2.B).

Duplication of chromosomes and Distribution of duplicated chromosomes into two daughter cells are two important conditions that are guaranteed by meiosis to insure half of chromosomes are distributed into daughter cells.

In the meiotic **prophase I** the nuclear envelope disappears to prepare the nucleus for division, the chromosomes shorten and thicken and two *sister chromatids* are clearly visible for each chromosome. Chromosomal *tetrads* are formed here; where homologous chromosomes are paired and *crossing over* starts through the *chiasmata* points. The microtubule organizing center, the *centrosome*, divides into two entities, and each migrate down sides of nuclear envelope, and protein fibers stretch between them forming the mitotic spindle. At this phase, cells contain as double as the amount of the nuclear material (2N) as a resting somatic cell.

The **metaphase I** is the final preparatory step for nuclear division; here, the *tetrads* line up on the equatorial plate of the cell and the *centromeres* of the chromosomes are attached to the centrioles through the spindle fibers.

In the **anaphase I** each pair of the *tetrads* migrates to the opposite poles of the cell towards the centrioles by sliding of the microtubules, and the sister chromatids are still connected through the centromeres.

**Telophase I** and **cytokinesis I** are the last steps of meiosis I; here, the homologous chromosomes reach the poles of the cells and the spindle fibers degrade. By the end of meiosis I each newly formed cell contain (1N) chromosomes but each chromosome is composed of two sister chromatids; so, each cell restores the original amount of the nuclear material.

In the **prophase II** the centrosome, divides again and each migrate down sides of nuclear envelope and protein fibers stretch between them forming the mitotic spindle.

The **metaphase II** the chromosomes (1N) line up on the equatorial plate of the cell and the centromeres of the chromosomes are attached to the centrioles through the spindle fibers.

In the **anaphase II** the chromatids migrate to the opposite poles of the cell towards the centrioles by sliding of the microtubules.

**Telophase II** and **cytokinesis II** is the last step of meiosis II. Here, the chromatids reach the poles of the cells, the spindle fibers degrade, the nuclear membrane reassembles, the chromosomes elongate, the nucleoli reassemble and the two cells are separated.

By the end of meiosis four daughter cells each of which contains half amount of the genetic material of their progenitor cell (haploid; 1N).

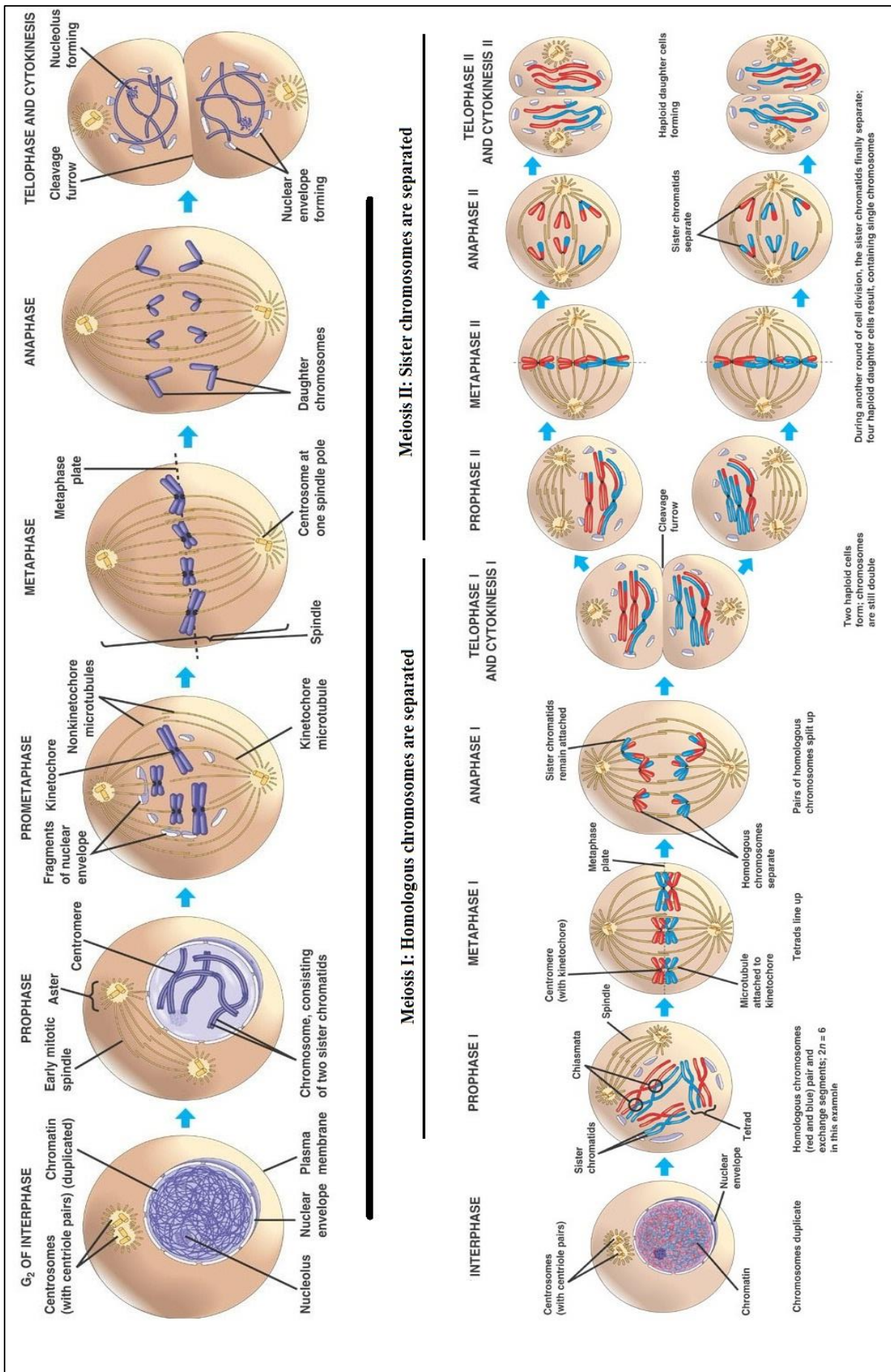


Figure 7-2: A schematic drawing illustrating the main steps of cell division. (A) mitosis, and (B) meiosis.



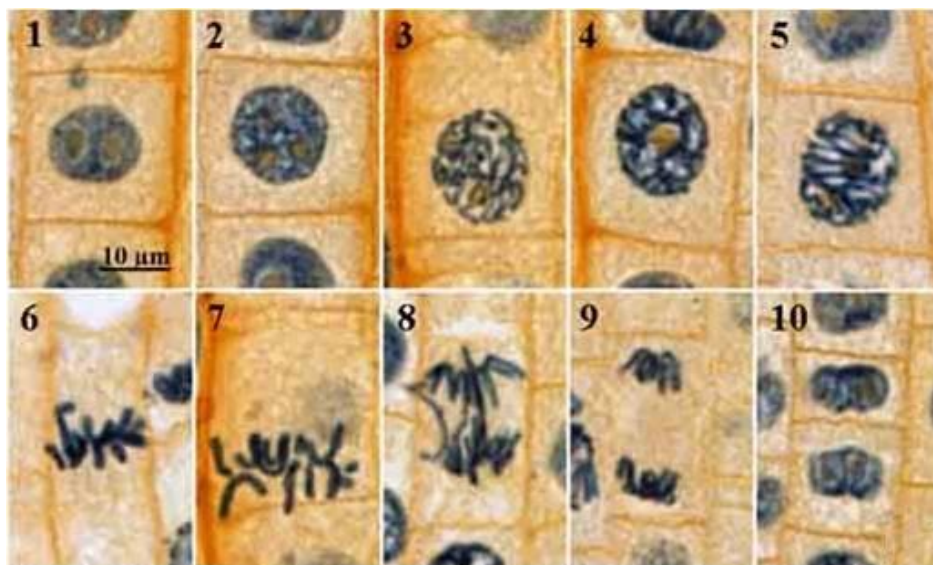
## Experiments

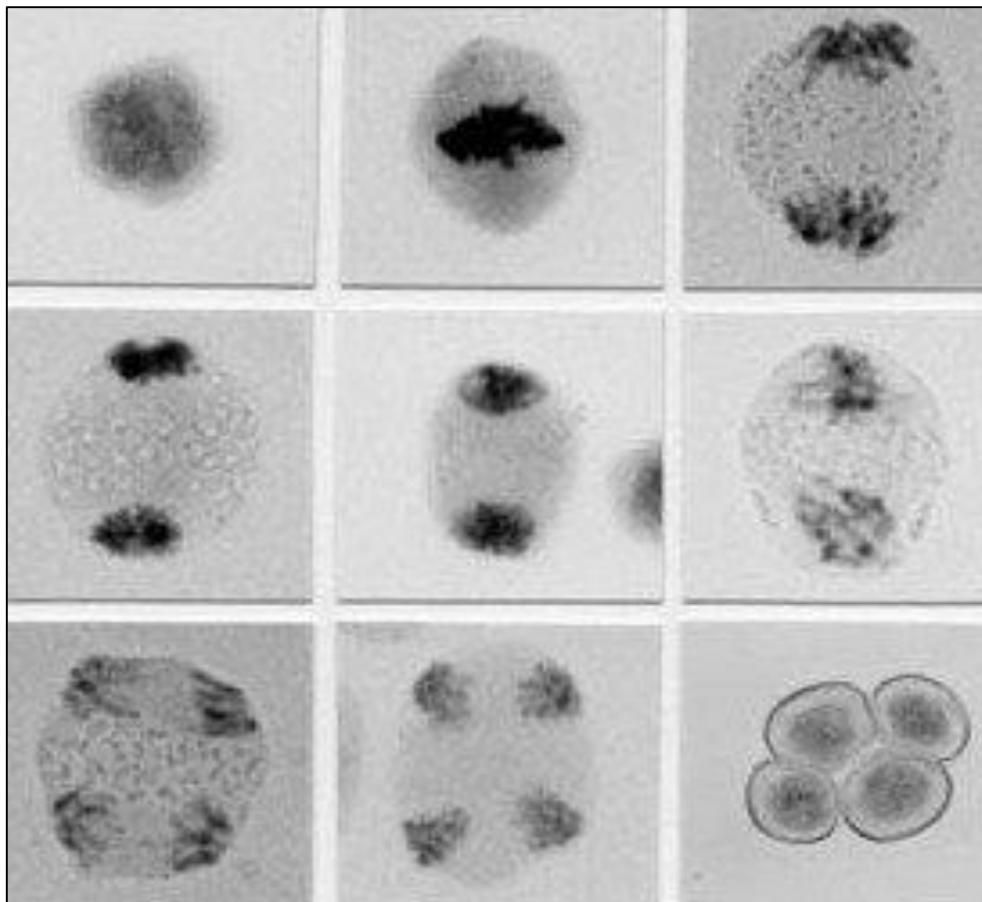
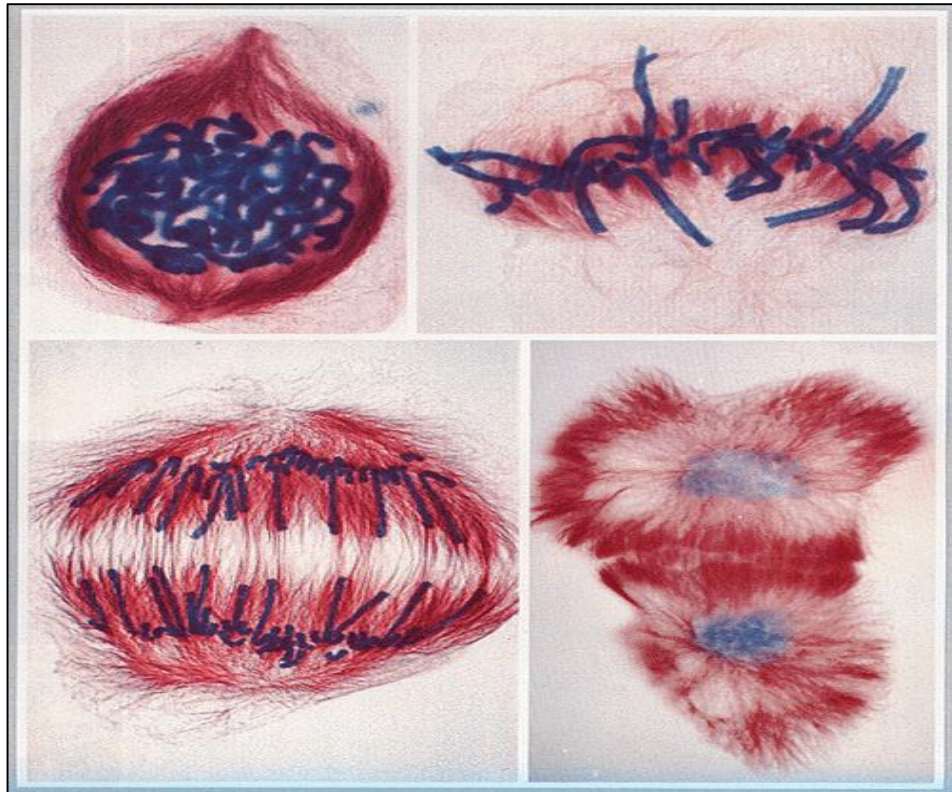
Today you will prepare a slide for observing all stages of mitosis under the microscope. Actively dividing cells will be used; which are onion root tip cells.

### Procedure:

1. Select a large onion bulb with undamaged disc-like stem.
2. Place the bulb on a beaker of suitable size filled with tap water so that the base of the bulb is completely immersed in water. (Change the water in the jar twice daily).
3. Obtain two small cups of HCl (18 %) and Carnoy from your instructor.
4. Using forceps transfer a small piece of an onion root tip into the HCl cup.
5. Leave it for 4 min.
6. Transfer the root tip into the carnoy's cup.
7. Leave it for 4 min.
8. Place it on a slide.
9. Cut off 1 mm piece of the root tip, and discard the rest.
10. Cover the tip with few drops of oricin, or toluidin blue, for 2 min.
11. Blot the stain using a type of tissue. Be careful, not to touch the tip itself.
12. Cover the tip with one drop of water.
13. Gently, lower the cover slip over the tip.
14. Use a Kim-wipes, press over the cover slip in order to spread the cells into one layer. **Don't twist the cover slip.**
15. Place the slide over a steam bath or warm it over a hot plate until the stain at the cover slip edges begins to show signs of drying off.
16. Observe the cells under the microscope.
17. You can make your preparation permanent by freezing the slide on dry ice before removing the cover slip, then, transfer the slide into 70% alcohol. After that dehydrate the slide, and then mount in mounting medium.

**Exercise:** Try to find under the microscope the following structures and identify each one.









## Lab. No. 9

### Introduction to Blood and Cardiovascular System

#### Objectives

- Identifying various blood cells under the microscope.
- Performing ABO and Rh blood grouping (slide technique).
- Indicate blood donation-reception compatibility.
- Practicing measuring blood pressure using the sphygmomanometer.

The cardiovascular system is one of the important systems in the body, it is also known as the circulatory system, it is composed of the heart; that pumps blood to all parts of the body, the vascular system; which serves as a network of pipes which blood travels through, and the blood; which is the chief component of this system and it will be the concern of this lab. session.

#### Blood Cells

Blood is composed of two parts; **plasma** (the fluid part) and formed elements (the **cellular** part). Plasma carries nutrients for various types of body tissues, and the waste products produced by them to be excreted outside. The formed elements include the Red Blood Cells, White Blood Cells and the Platelets. Each of these expresses many vital functions for the body

##### **Red Blood Cells (RBCs, Erythrocytes) ( $5.0 - 6.0 \times 10^6$ cells / $\mu\text{L}$ )**

They are not true cells since they lack nucleus. They are biconcave disc-shaped cells containing hemoglobin; which is the main protein in these cells that binds an iron complex (*Heme*) which has the ability to bind gas molecules (mainly  $\text{O}_2$ ). Hemoglobin is a tetramer protein (composed of four subunits). The name of these cells (red) came from the color of the heme, which is reddish, and gives the blood its characteristic color (Figure 9-1).

##### **White Blood Cells (WBCs, Leukocytes) ( $5.0 - 11.0 \times 10^3$ cells / $\mu\text{L}$ )**

They are the only true cellular elements that circulate in blood. They are heterogeneous in shape, colorless and appear in many sizes. Their main function is fighting against pathogens, so some types have the capability of *phagocytosis* (engulfment). WBCs are subdivided into two main categories; *granulated* and *agranulated*. Granulated WBCs include **Neutrophils**, **Eosinophils** and **Basophils**, the granules are the lysosomes that are distributed in the cytoplasm. Agranulated WBCs include **Monocytes** and **Lymphocytes** (Figure 9-1).

**Neutrophils** are the most abundant WBCs in adults (about 60%). They contain lobed nucleus (usually 3 lobes, but may contain 2, 4 and 5). Their main function is to fight against bacteria; so, their number rises in bacterial infections (Figure 9-1.N).

The presence of **Eosinophils** in blood is very low. Their nuclei are usually bilobed, their cytoplasm contain numerous acidophilic (eosinophilic) granules which stain deep red. They rise in number in parasitic infections (Figure 9-1.E).

**Basophils** are the least in number among the rest of the WBCs. They are usually bilobed, their cytoplasm contain numerous basophilic (azourophilic) granules which stain deep blue. They rise in number in allergic reactions (Figure 9-1.B).

**Lymphocytes** are the smallest in size and the second abundant WBCs in adults (about 30%). They contain no granules. They secrete antibodies and toxins to fight against pathogens. Their number rises mainly in viral infections (Figure 9-1.L).

**Monocytes** are the largest among the WBCs. They possess a convoluted nucleus. They are the body scavengers (of bacteria, dead cells and others), so their main function is phagocytosis.

**Platelets (Thrombocytes) ( $150 - 400 \times 10^3$  cells /  $\mu\text{L}$ )**

They are subcellular elements that's main function is the plug formation during *Hemostasis* (blood coagulation).

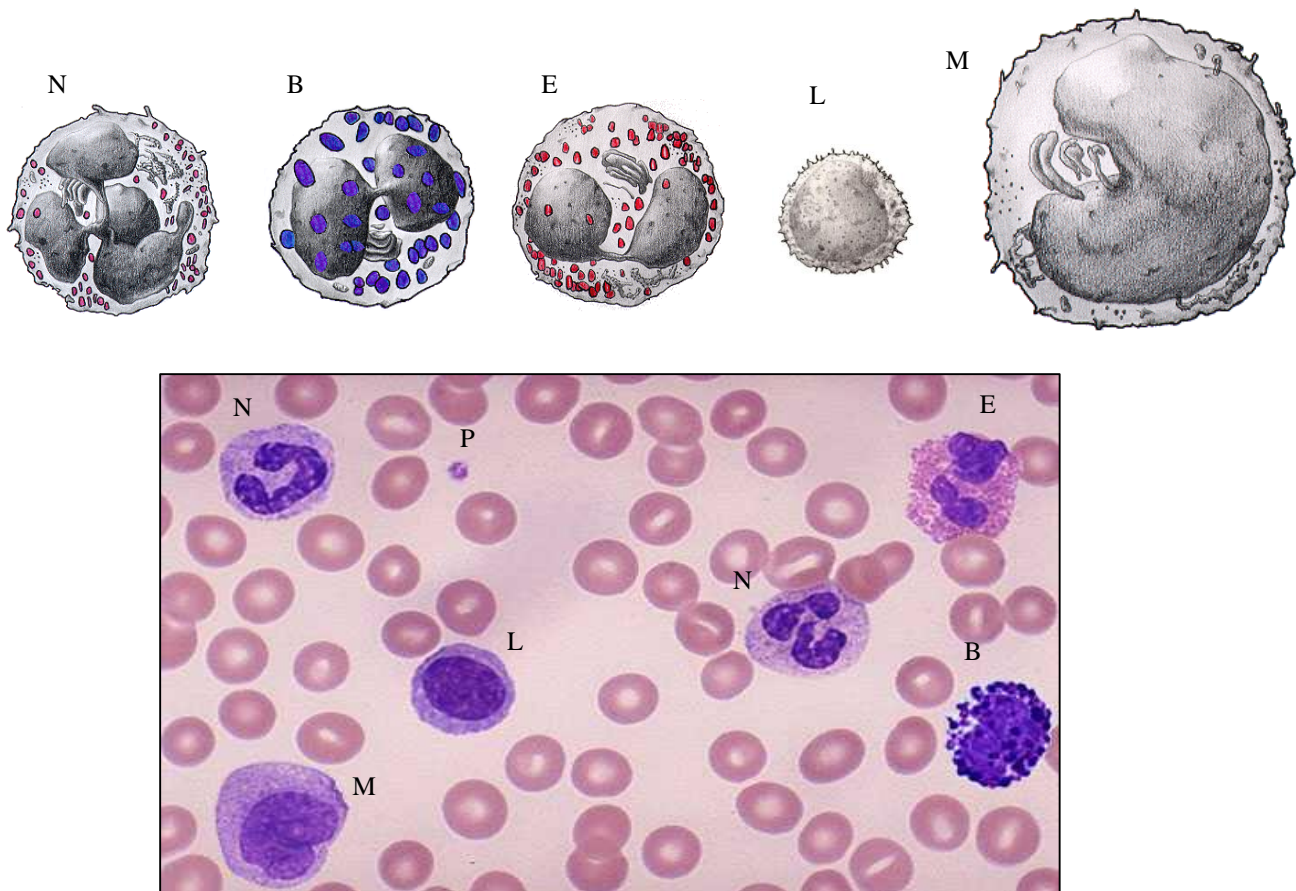


Figure 8-1: Various blood elements are shown; the upper part is drawings of various WBCs. The lower part is a blood smear (film) stained with Wright's stain and magnified 1,000X. (N) Neutrophil, (B) Basophil, (E) Eosinophil, (L) Lymphocyte, (M) Monocyte, (P) Platelet, and the RBCs are distributed in the lower part.

## Blood Groups

Red blood cells contain many surface proteins that are expressed on their cell membrane. These surface proteins have many biological functions for the cell, but many of them differ in structure among individuals; so, when a patient needs blood transfusion his blood cells' surface proteins may differ from those of the donor, so the donor's cellular surface proteins will act as foreign **antigens** (Ag), and the recipient (patient) will produce (or already have) **antibodies** (Ab) against these antigens; so, the patient cells will fight against the new incoming cells and many reactions will take place that may result in the death of the recipient. So, accurate identification of the recipient and donor cells is very important to prevent these complications.

There are many (more than 50) blood group systems that were defined on the RBCs but here just two of them will be focused on; the **ABO** and the **Rh** systems.

### ABO system

The **ABO** system contains two members or antigens (Ags), the (A) Ag and the (B) Ag. Individuals that express the (A) Ag on their RBCs are termed (*Group A*), and those expressing the (B) Ag on their RBCs are termed (*Group B*), and those expressing both (A and B) Ags on their RBCs are termed (*Group AB*) while those who do not express any of the two Ags are termed (*Group O*).

So, Group A individuals contain *anti-B*, and they can be transfused by Group A or Group O blood but not Group B blood, and Group B individuals contain *anti-A*, and they can be transfused by Group B or Group O blood but not Group A blood. On the other hand, Group AB individuals contain no Abs against any of the ABO system proteins so they can be transfused by either Group A or Group B or Group AB or Group O blood. While the Group O patients possess anti bodies against both of the ABO group members (i.e. contain anti-A and anti-B) so, Group O blood is the only suitable blood group to be taken by this patient (Table 8-1).

Table 8-1: Various blood groups of the ABO system and their properties.

Blood Group	Ag	Ab	Take	Give	Comments
<b>A</b>	A	B	A, O	A, AB	***
<b>B</b>	B	A	B, O	B, AB	***
<b>AB</b>	A, B	No	A, B, AB, O	AB	Universal Acceptor
<b>O</b>	***	A, B	O	A, B, AB, O	Universal Donor

Ag: Antigen, Ab: Antibody.

## Rh system

The **Rh** system is the second blood group system in importance. The main Ag in this system is the D- Ag. The individual that possess Rh (D) Ag on its RBCs is termed Rh (D) positive, while who lacks this Ag is termed Rh (D) negative. So, an Rh (D) positive individual can be transfused either by Rh (D) positive or negative blood, but an Rh (D) negative person can be transfused by only Rh (D) negative blood.

The importance of this system appears in another case, where the pregnant mother is Rh (D) Ag negative, while her fetus is Rh (D) positive (obtained it from his father). During the delivery of the *first* Rh (D) positive fetus some of his blood will inter the circulation of the mother influencing her to produce Abs against the Rh (D) Ag. this fetus will be delivered safely and will survive. The problem appears with the following fetuses that are Rh (D) positive; here while the fetus is still developing in the uterus, the maternal anti- Rh (D) will cross the placenta and attack the fetus's RBCs leading to a disease known as the Hemolytic Disease of the New Born (HDNB) which may lead to death of the fetus. Note that all the fetuses that are Rh (D) negative will not be affected by this Rh (D) system change between the mother and the father (Figure 9-2).

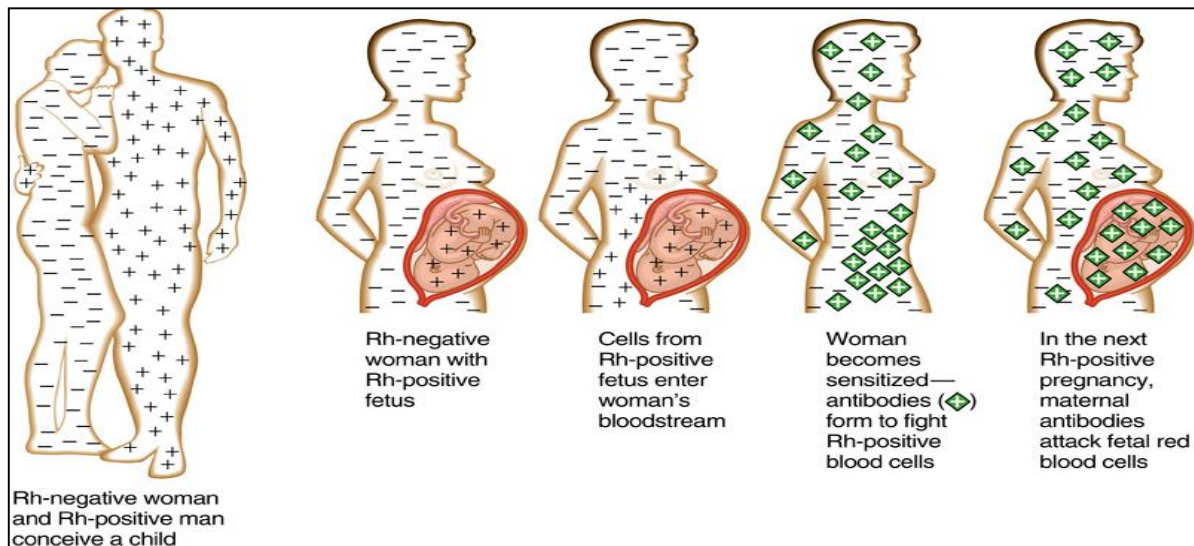


Figure 8-2: The role of the Rh incompatibility between the mother and the father in the developing HDNB.

## Heart beat and blood pressure

During the heart beat, the atria contract first, followed by the contraction of the ventricles. Therefore, two heart sounds will be heard when applying the stethoscope on the chest; the (*Lub*), which is low and dull and lasts longer than the second one, represents the **systolic** pressure which occurs in the contraction state of the heart. The second sound (*Dab*), which is higher and shorter, represents the **diastolic** pressure, which occurs in the relaxing state of the heart. The normal heart beat rate of a resting young adult person is between 60 and 80 beats per minute. The blood pressure is the force of the blood against the walls of blood vessels. It is expressed as the systolic pressure over the diastolic pressure. The normal blood pressure of a resting young adult person is 120/80 mmHg.

## Experiments

### Experiment No 1: Blood Grouping

#### Procedures

1. Obtain two clean slides
2. Put the Ab vials (anti-A, anti-B and anti-D) at room temperature for 3 minutes.
3. Divide one slide into two portions labelled (A) & (B), and the other slide labelled (D).
4. Add one drop of the anti sera on the corresponding portion.
5. Add one drop of the blood specimen to be tested on each of the anti sera (use the lancet to collect blood).
6. Mix using deferent wooden stick for each anti-sera.
7. Rock the slide for 2 minutes.
8. Look for agglutination in each portion.
9. Record your results

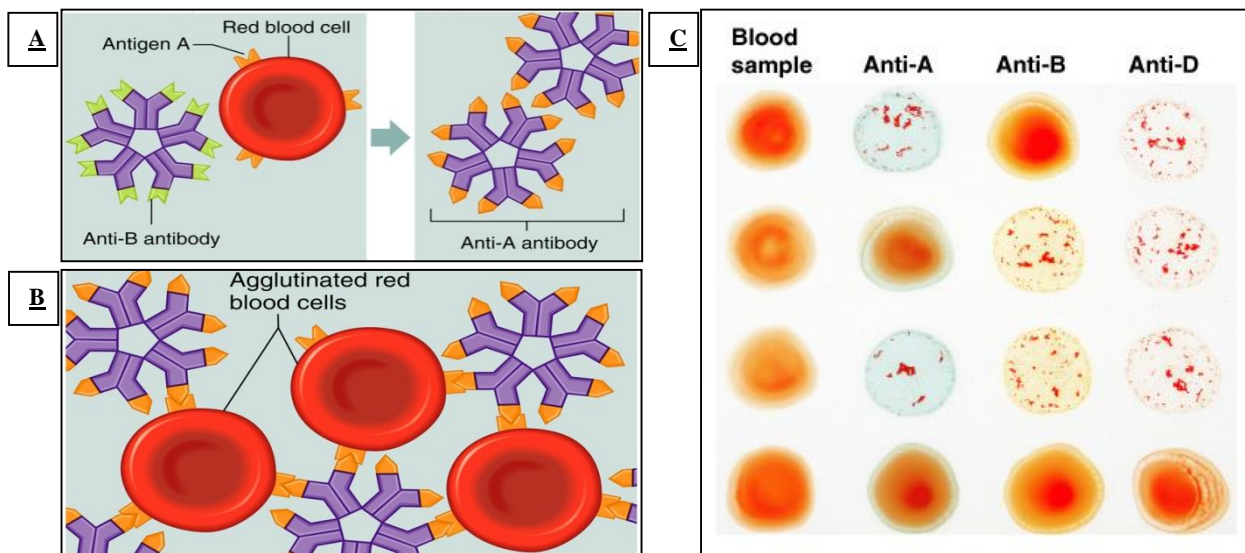


Figure 8-3: (A) & (B) illustrates the process of agglutination. (C) illustrates the possible results you may obtain in the lab.

### Experiment No 2: Measuring Blood Pressure

In this experiment, you need a sphygmomanometer and a stethoscope (Figure 9-4).

#### Procedures

1. Position the patient in a sitting lying position.
2. Put the patient's arm at the heart level and expose.
3. Indicate the *brachial* artery.
4. Deflate the cuff and position it about 2.5 Cm above the brachial artery palpation site.
5. Place the diaphragm over brachial artery palpation site.
6. Tighten the pressure valve.
7. Inflate the cuff to the pressure of 150 mm Hg.
8. Release the valve to allow the mercury to fall in a rate of 2.3 mm Hg per sec.
9. Note the production of a muffled sound and read the pressure here (systolic pressure).



10. Continue following the sound of the heart beats.
11. Read the point at which the beats stop to be heard and record the pressure here (diastolic pressure).
12. Release the valve and deflate the cuff rapidly.
13. If you want to repeat the procedure, wait for about one minute.
14. Restore the sphygmomanometer properly.

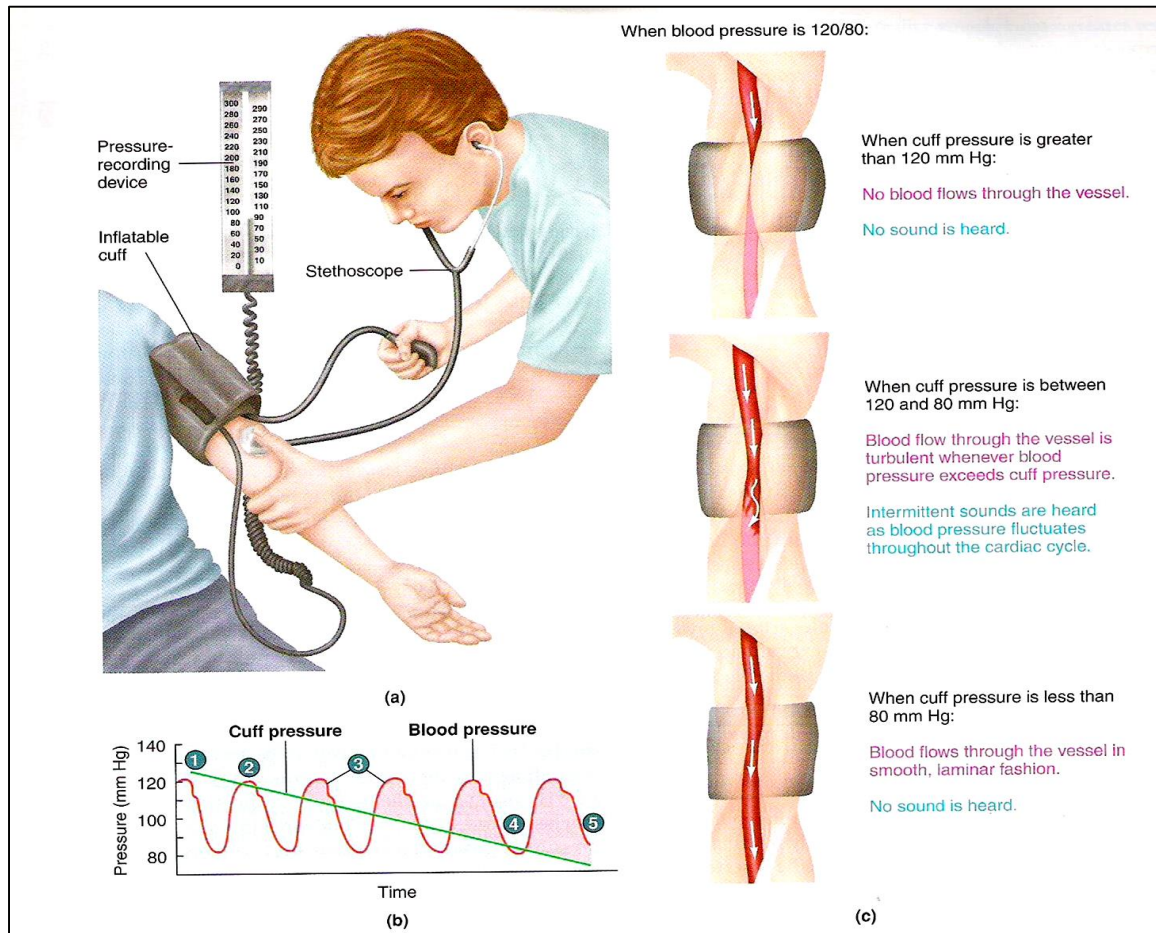


Figure 8-4: (a) Use of a sphygmomanometer in determining blood pressure. (b) Pattern of sounds in relation to the cuff pressure compared with the blood pressure. (c) Blood flow through the brachial artery in relation to cuff pressure and sounds.

**Lab. No. 10****Introduction to Microbiology****Objectives**

- Describe various microbial categories.
- Identify various types of bacterial culture.
- Identify various microorganisms under the microscopically and macroscopically.
- Indicate the mode of classification in each microorganism.
- Indicate the mode of replication in each microorganism.
- Indicate the mode of locomotion in various microorganisms.

Living organisms that are too small to be seen with the naked eye and are visible only through a microscope are called **microorganisms**. Therefore, the science that studies these organisms is called **microbiology**. Van Leeuwenhoek (1674) was the first to observe such organisms through a compound microscope constructed by him. Microorganisms include *bacteria*, *fungi*, *Algae*, *protists* and *viruses*. Scientists estimate that only one percent of the species of microorganisms that exist have been studied so far.

Many microorganisms are pathogenic to humans; many types of bacteria cause infections and diseases like tuberculosis and bubonic plague. Some protists cause diseases like malaria and sleeping sickness, some yeast also cause infections and viruses are responsible for causing many diseases such as *Rabies*, *flue*, *hepatitis* and the *acquired immunodeficiency syndrome* (AIDS). On the other hand, most of the microorganisms play a significant role to keep the planet running; indeed, they carry out about 90% of the biochemical reactions conducted on the earth, without these microorganisms life on the earth would be destroyed; many foods, beverages and medicines are made with the help of bacteria and yeast, some bacteria fix atmospheric nitrogen in soil making it available for plants to grow. Certain fungi grow symbiotically with plant roots increasing their ability to obtain food and moisture from the soil, other fungi are used in our food. Microorganisms that live in our intestines help us to get nutrients from food. Finally, microorganisms have also gained importance as tools in the scientific world.

## Bacteria

The main prokaryotes among all microorganisms are the bacteria; they are unicellular and reproduce asexually by *binary fission*. The discipline of biology that is interested in the study of bacteria is known as *bacteriology*.

Most bacteria are classified according to their shape, i.e. rod-shaped are called *bacilli*, spherical-shaped are called *cocci* and the spiral form are called *spirilli* (Figure 10-1).

Bacteria are also classified according to their behavior in specific tests. **Gram staining** is one of the major tests that are useful in classifying bacteria; those stain bluish purple are termed *Gram positive*, while those stain red are termed *Gram negative*.

Bacteria can be grown in the lab using mixtures of nutrients called **culture medium**. There are many types and classes of culture media that are used for many purposes. Liquid media mixtures are called **broths** while those supplemented with *agar* are solid. Microorganisms can grow in **colonies** (i.e. clusters of organisms growing on a solid medium) (Figure 10-2).

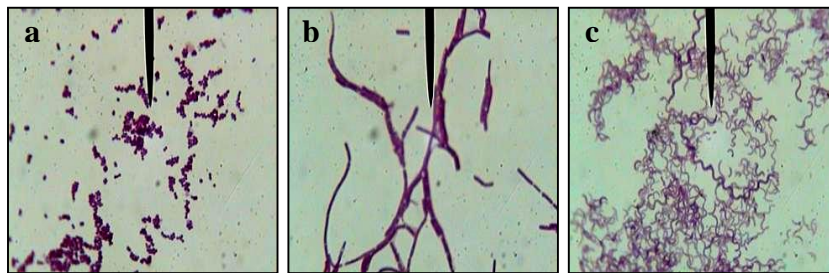


Figure 9-1: Some bacterial forms under the 100 ×. (a) Gram Positive Cocci. (b) Gram Negative Bacilli. (c) mixed Gram Positive Spirilli and Gram Negative Spirilli.



Figure 9-2: Culture plates show bacterial (*Staphylococcus aureus*) colonies growth.



## Fungi

Fungi, is a large group of eukaryotic microorganisms that include yeast and molds (or moulds). The Fungi kingdom separates from others by possessing cell walls that contain *chitin* (unlike the cell walls of plants, which contain cellulose). Molds reproduce mainly by spores (conidia). The discipline of biology that is interested the study of fungi is known as *Mycology* (from the Greek *μύκης*, mukēs, "fungus").

Most molds grow as *hyphae*, which are cylindrical, thread-like structures; hyphae grow at their tips by a process called *branching*, hyphae combine together to form *mycelium*. Hyphae can be either *septate* (partitioned) or *coenocytic* (not partitioned), and when mycelia grow on a plate and become visible they are called colony (Figure 10-3).

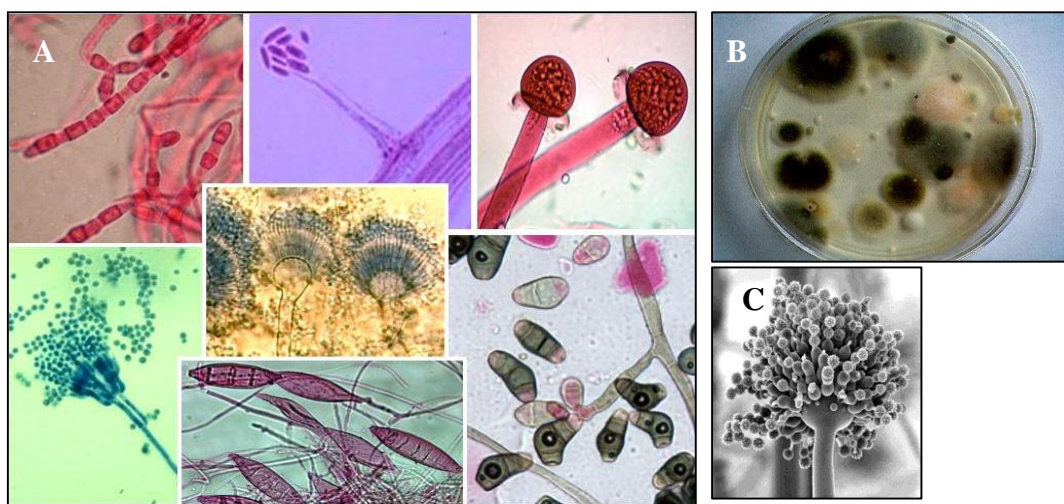


Figure 9-3: Different forms of molds. (A) Different types of molds stained and observed under the 40 × lens. (B) Different types of molds grew on culture media (C) A mold under an electron microscope.

Yeasts are unicellular, oval or spherical fungi, which increase in number asexually by a process termed **budding** (Figure 10-4). Yeast could be differentiated from bacteria by noticing the size under the microscope through a wet mount preparation, and by observing budding of yeast.

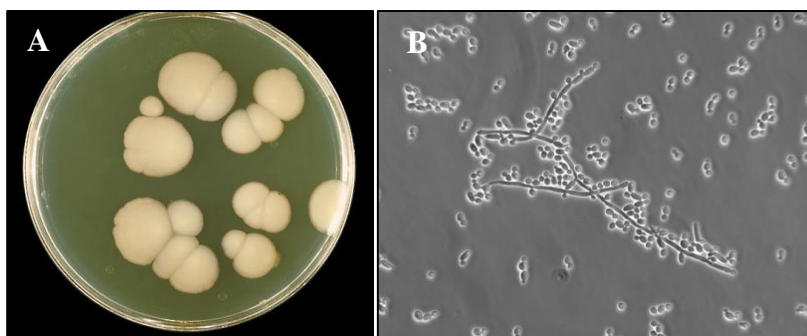


Figure 9-4: Yeast. (A) Yeast colonies grown on a solid culture medim. (B) Yeast cells showing budding under the 40 ×.

In general, fungi are beneficial to humans. They are involved in the decay of dead plants and animals (resulting in the recycling of nutrients in nature), the manufacturing of various industrial and food products, the production of many common *antibiotics*, and may be useful as food. Some fungi, however, damage wood and fabrics, spoil foods, and cause a variety of plant and animal diseases, including human infections.

## Protista

Protista (or protists) are group of eukaryotic microorganisms that exist as unicellular, or multicellular, without specialized tissues. They are classified according to the means of locomotion they adopt. **Pseudopodiates** move via cellular extensions called *pseudopodes* (e.g. *Amoebes*). **Ciliates** use short hair-like structures attached to their cell membrane called *cilia* (e.g. *Paramecium*). **Flagellates** use long whip-like structures called *flagella* (e.g. *Trypanosomes*) (Figure 10-5).

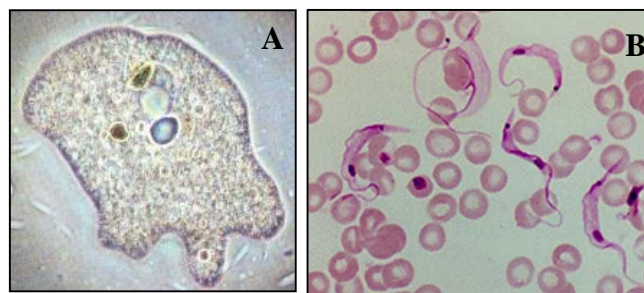


Figure 9-5: Protists. (A) *Amoeba*. (B) *Trypanosoma cruzi* in a blood film under 40 ×.

Protozoans can be free-living or parasitic; some protozoans (i.e. *Euglena*) have chloroplasts like plants and can make their own food. Some protozoans are very dangerous and can cause diseases in humans (i.e. *Amoebes* cause dysentery, *Plasmodium* cause malaria, *Ascaris* and *Taenia* cause gastrointestinal tract infestations).

## Algae

Algae are organisms that include both prokaryotic and eukaryotic members. They range in size from the microscopic to giant algae that reach (60 m) in length. They are photosynthetic, like plants, and “simple” because they lack the many distinct organs found in land plants. Algae provide much of Earth’s oxygen, serve as the food base for almost all aquatic life, and provide foods and industrial product (e.g. agar). They are usually classified according to their colour; blue-green algae (e.g. *Cyanophyta*), Green algae (e.g. *Chlorophyta*), Golden, Brown and Red (Figure 10-6).

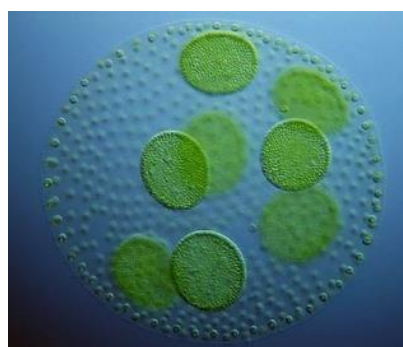


Figure 9-6: Green algae showing chloroplasts.

## Viruses

Although viruses are not considered living organisms, they are classified as microorganisms. Viruses are much smaller than common microbes. They are made of a nucleic acid molecule covered with a protein shell called a *capsid*. Viruses cannot reproduce by their own outside the host cell. Viruses can infest (attack) prokaryotic and eukaryotic cells, and often causing diseases in those organisms (Figure 10-7).

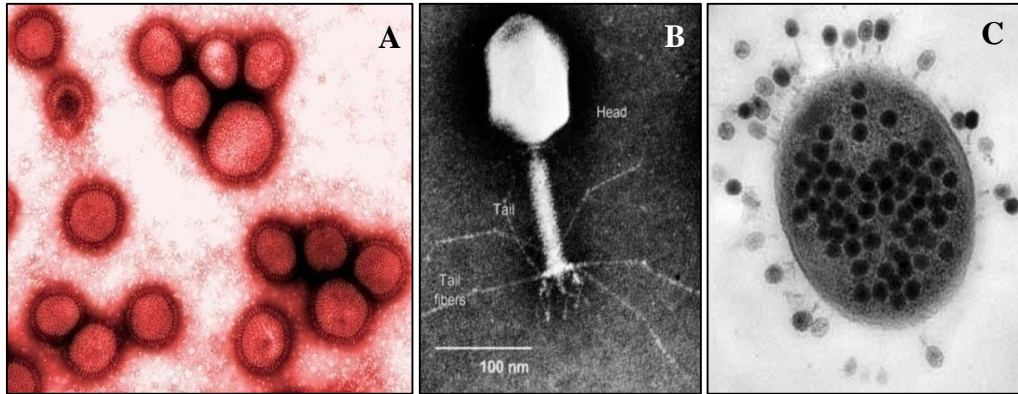


Figure 9-7: Viruses. (A) *Influenza* virus. (B) A bacteriophage. (C) Many bacteriophages attacking a bacterial cell. All the photos are taken under the electron microscope.

## Experiments

### Experiment No 1: Culture media overview

Different culture media types will be shown to you investigate them and notice the differences between them.

#### Procedure

- Investigate the culture media containers that are used in the microbiology lab; lose capped test tube, screw capped test tube, 9 cm glass Petri dish, 9 cm plastic Petri dish, 4.5 cm plastic Petri dish, 9 cm plastic septated Petri dish.
- Investigate the culture media forms that are used in the microbiology lab to grow microorganisms; broth tubes, solid ager tubes, solid agar plates.

### Experiment No 2: Bacterial examination

#### Procedure

- Investigate different types of bacterial growth on culture media.
- Investigate different types and forms of bacteria stained by Gram's method under the oil immersion lens and wet mount.

### Experiment No 3: Yeast examination

#### Procedure

- Investigate yeast growth on a culture media.
- Investigate yeast smear stained using Gram's method under the oil immersion lens, and a wet mount under the 40 x.

### Experiment No 4: Mould examination

#### Procedure

- Investigate various mould cultures on different types of culture media
- Investigate different mould slide preparations under the 40 x.

### Experiment No 5: Protists examination

#### Procedure

- Investigate different pathological parasites macroscopically (*Ascaris lumbricoides* and *Taenia saginata*).
- Investigate different pathological parasites microscopically (*Entamoeba histolytica*, *Trypanosoma cruzi* and *Enterobius vermicularis*)

## Appendices



## Appendix A

### Instructions for Writing a Scientific Lab Report

Lab reports should have *four sections*: **Introduction**, **Materials and methods**, **Results**, and **Discussion**. Each of these sections is described below.

#### Introduction

- This section should be labelled with the word "Introduction" as is done above. There are no colons after the word Introduction. There is only one Introduction in the lab report
- In this section, discuss the problem or topic. Why is it important? What are you trying to find out? State any hypotheses that are being tested. Provide the reader with enough information that they will be able to read and understand the rest of the lab report.
- One of the labs is about tests for carbohydrates, lipids, and proteins. In this section, you might discuss what they are, why they are important, and why you might want to test for them.
- The objective of the experiment is stated at the end of the introduction with one or two sentences.

#### Materials and methods

- This section is labelled "Materials and methods" as shown above. There are no colons after the word Methods.
- In this section, you should give enough detail so that if a reader who did not know anything about the lab wanted to repeat your procedure, they could. *Use only whole paragraphs with complete sentences*. An outline or list is not acceptable (no itemization).
- The section should be written in *past tenses*. Describe exactly what you did in the lab. If the procedure that you followed was different than that given in the lab manual, write what you did, not what the lab manual says to do.
- Avoid using the words I, he, she, or we. This section should be written in a *passive voice* and there should not be references to other people. For example, the following is incorrect: "After we added the solution to the test tube, we heated it until it began to boil". A better alternative is: "After the solution was added to the test tube, it was heated until it began to boil."

#### Results

- Label this section "Results" as shown above. There are no colons after the word "Results".
- There is only one results section in the lab report so put all of your results in this section.
- Put all of the results, statistical analyses, graphs, and tables in this section.
- Do not discuss procedures or give explanation of results in this section, just the results of your experiments.
- Data can often be best summarized using *tables* and *graphs*.
- Use paragraphs with complete sentences in this section. If you use tables or graphs, you must still use sentences and paragraphs to describe general trends and summarize the tables and graphs.

## Instructions for creating graphs and tables

- Be sure that the dependent variable is on the X-axis of your graph and the independent variable is on the Y-axis.
- Identify tables as Table 1, Table 2, etc. Graphs, drawings, and photographs should be identified as Figure 1, Figure 2, etc. Graphs and tables must be captioned (titled). A caption is a sentence or several sentences that provide enough information to understand the table or figure without reading the rest of the paper. See the examples below of a table and a graph.
- Locate graphs and tables near where they are discussed, or at the end of the paper.
- Any symbols or abbreviation are used must be explained under the table or the figure.

Table 1. Survival of young corn plants grown indoors at two different temperatures.

Temperature	Number of seeds planted	Number of plants alive after 4 weeks
20 degrees	100	87
35 degrees	100	53

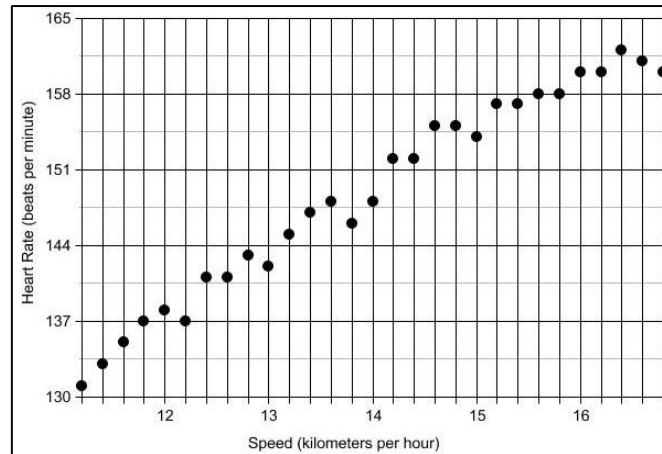
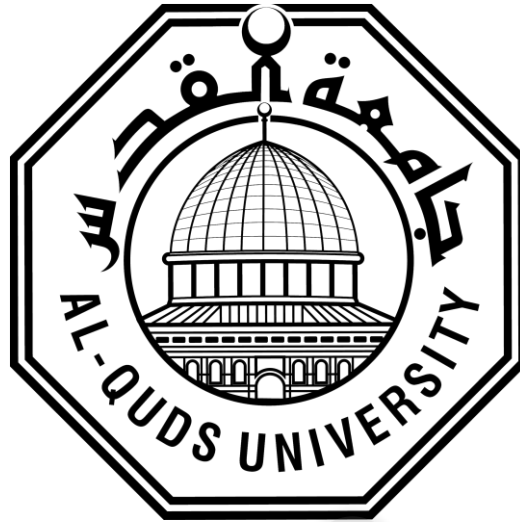


Figure 1. Heart rate of a runner while running at different speeds on a treadmill. Speed was increased every 40 seconds.

## Discussion

- This section should be labelled "Discussion" as shown above. There are no colons after the word Discussion.
- There is only one discussion section in the lab report, so put the discussion for all parts of the lab here.
- In this section, you must discuss whether you accept or reject your hypothesis or hypotheses and explain why. If you reject a hypothesis, state an alternative.
- Explain why your results came out the way they did. If your results did not come out as expected, explain why and what should have happened. Explain what your results mean. How do they fit into the biology of the system that you are studying? Your explanation should be sufficient so that an average intelligent reader without an understanding in biology would be able to understand.





**Al-Quds University**

*Faculty of Health Professions*

**General Biology Lab**

**Session #**

**Title**

Instructor: AAAAAAAAAAAAAAAAAA

Student: AAAAAAAAAAAAAA

No.: 000000000000

## Introduction

Testing of various biologically important macromolecules i.e. carbohydrates, amino acids and proteins could be carried out in the lab qualitatively using various reagents; Benedict's reagent can reveal the presence of reducing sugars, while iodine solution can complex with starch. Ninhydrin reagent – on the other hand – can react with free amino acids. Therefore, this exercise provides us with various procedures to test the previously mentioned molecules, and pipetting and dropping techniques superior to emphasizing on all of the safety rules and labeling techniques that were taught in the previous sessions.

Also this lab. session reviews the technique of using the student microscope and investigating a prepared slide microscopically.

## Materials and Methods

To test for the presence of reducing sugars in the provided unknown solution (number X), 2 mL of the unknown solution were mixed with 1 mL of Benedict's reagent and the whole mixture was incubated in boiling water for three minutes.

According to free amino acid testing, 1 mL of the unknown solution (number X) was mixed with few drops of ninhydrin solution, then, the whole mix was put in a boiling water bath for three minutes.

To test for the presence of starch in the unknown solution (number X) few drops of the Lugol's solution were added over 1 mL of the unknown solution.

Testing a blood smear (film) was accomplished under the scanning, low and high power lens.

## Results

Positive results were obtained for the three tested analytes; orange color was noticed in the reducing sugar test, purple color for the amino acid testing tube and a bluish black complex was formed in the starch investigation tube (Table 1).

Table 1. Illustrates the results of testing various macromolecules in the unknown number X.

<i><b>Test</b></i>	<i><b>Result</b></i>	<i><b>Interpretation</b></i>
Reducing sugar	Orange	+ve
Amino acid	Purple	+ve
Starch	Bluish black	+ve

+ve : positive

Figure 1 shows a blood film that was tested using a student microscope showing many blood cell types under several magnifications.

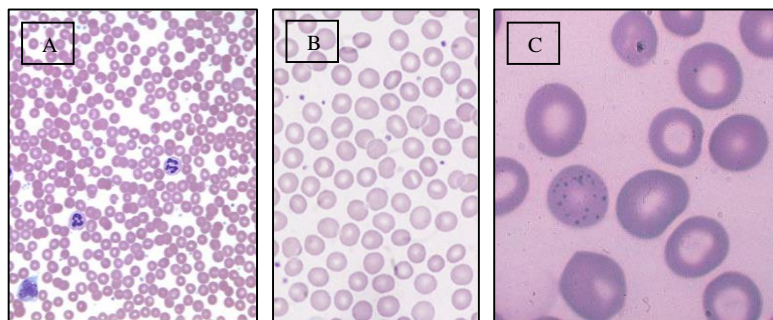


Figure 1. Shows three photos of a single blood smear stained with Wright's stain and tested under various magnifications using a bright field compound microscope; (A) is a field being observed under the 4× lens showing very small blood cells, (B) is a field being observed under the 10× lens, (C) is a field being observed under the 40× lens revealing very small details in blood cells.

## Discussion

The formation of Copper Oxide ( $\text{Cu}_2\text{O}$ ) was represented by the production of the green to brownish precipitate after the addition of the Copper Sulfate ( $\text{CuSO}_4$ ) – in the Benedict's solution – which indicates the reduction of the  $\text{Cu}^{++}$  to  $\text{Cu}^+$  by a reducing sugar in the unknown solution, while the blue color indicates the lacking of any reducing sugar in the tested solution. Negative results with disaccharides or polysaccharides could be converted into positive ones by acidifying the condition of the reaction (i.e. addition of  $\text{HCl}$ ; that lowers the pH) in order to hydrolyse the bonds and forming free monosaccharides (i.e. aldose; glucose) which can reduce the copper in the Benedict's solution.

The formation of the purple color after the addition of ninhydrin solution to a solution containing free amino acids is due to the reaction between two molecules of ninhydrin with one amino acid molecule; this reaction requires the presence of ammonia or a primary amine in the test solution in order for the reaction to take place and the purple color to form. So, if a polypeptide was one of the constituents of the solution with no free amino acids, denaturation of the polypeptide is needed in order for the color to develop, otherwise the reaction will not take place and the result would be negative and the color will remain unchanged (light yellow).

The combination between the polysaccharide – starch – and the Lugol's Iodine solution occurs due to the coil and complex structure of the starch; so hydrolyzing this form into free monomers (glucose) results in a failure of formation of the intended complex and consequently the color of the solution will remain unchanged.

Testing a smear under a microscope produces an inverted image and the field of view being smaller as the magnification gets higher, and as the field is being observed under the oil immersion lens fine details could be observed that couldn't be obtained with lower magnifications.



## Appendix B

### Reagents preparation

#### **Benedict's reagent**

(*Benedict's solution*) is a copper sulfate solution prepared as follows: dissolve 173 g sodium citrate and 100 g anhydrous sodium carbonate in 750 ml distilled water. Stir, filter, and to the filtrate slowly add a solution of 17.3 g copper sulfate pent hydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in 100 ml distilled water. Dilute to a total volume of one liter (1.0 L) with distilled water.

#### **Lugol's Solution**

(*Iodine-Potassium Iodide*,  $\text{I}_2\text{-KI}$ ): Dissolve 200 g potassium iodide (KI) in 800 ml distilled water. Add 100 g iodine ( $\text{I}_2$ ) and stir until solution is complete. Potassium iodide makes the iodine water soluble through the formation of the  $\text{I}^{-3}$  ions.

#### **Ninhydrin Reagent**

Is prepared by dissolving 3.5 g of the Ninhydrin powder in a 100 mL acetone/n-butanol solution.

#### **Indophenol Reagent**

- Stock solution: dissolve 100 mg of 2,6 dichloro-indophenol salt in 100 mL of distilled water.
- Prepare a working solution by diluting the stock solution at a 1:10 ration with distilled water.

#### **Starch-Iodine Reagent**

- Add 2 g of cornstarch or potato starch in 200 mL of cold, distilled water. Bring the mixture to a full boil in a glass beaker.
- To 1 liter of water, add 8 mL of the starch solution and 1 mL of tincture of iodine.
- Note: The color of the starch indicator should be a royal blue. Just before doing the lab check the indicator and dilute the concentration so that a workable number of drops of fresh orange juice (5 to 25) turn the indicator pink.

*The End*