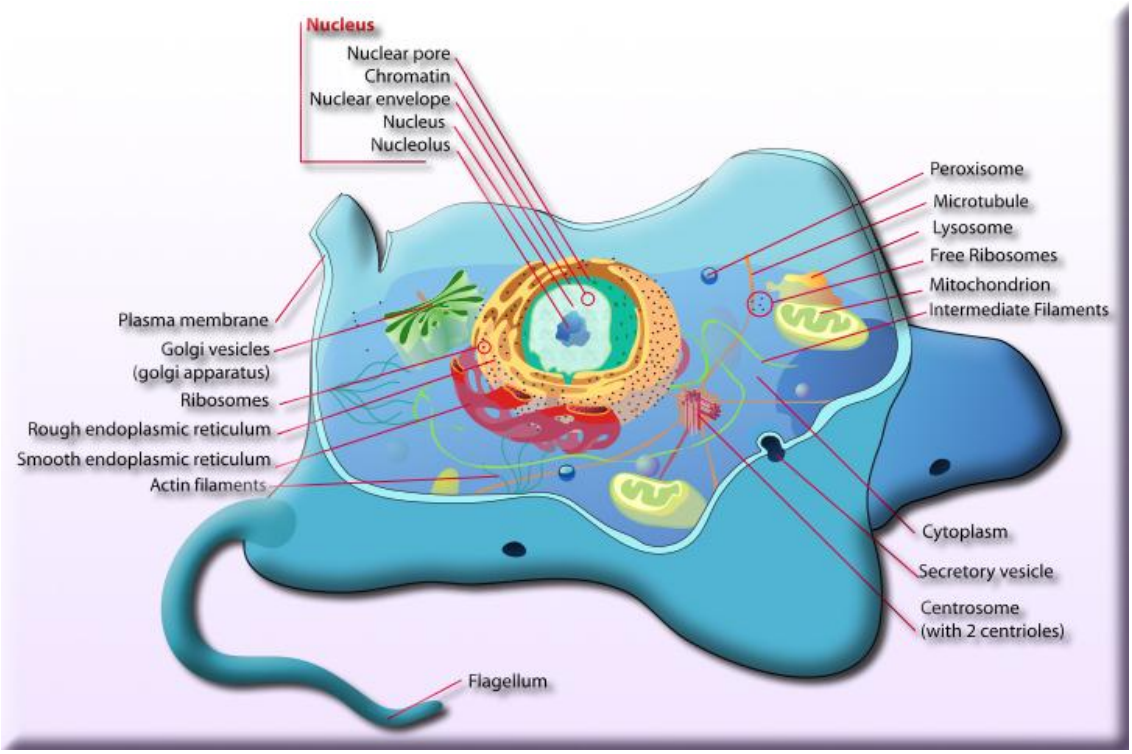
Practical Cell Biology Clinical Pharmacy Level 1

By Staff members of Biochemistry Department



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Microscopes:

Types, Parts and Specification

Types of Microscopes

Various types of microscopes are available for use in the cell biology laboratory. The microscopes have varied applications and modifications that contribute to their usefulness.

1-The light microscope.

The common light microscope used in the laboratory is called a **compound microscope** because it contains two types of lenses that function to magnify an object. The lens closest to the eye is called the **ocular**, while the lens closest to the object is called the **objective.** Most microscopes have on their base an apparatus called a **condenser**, which condenses light rays to a strong beam. A **diaphragm** located on the condenser controls the amount of light coming through it. Both coarse and fine adjustments are found on the light microscope.

To magnify an object, light is projected through an opening in the stage, where it hits the object and then enters the objective. An image is created, and this image becomes an object for the ocular lens, which remagnifies the image. Thus, the **total magnification** possible with the microscope is the magnification achieved by the objective multiplied by the magnification achieved by the ocular lens.

A compound light microscope often contains four **objective lenses:** the scanning lens (4X), the low-power lens (10X), the high-power lens (40 X), and the oil-immersion lens (100 X). With an ocular lens that magnifies 10 times, the total magnifications possible will be 40 X with the scanning lens, 100 X with the low-power lens, 400 X with the high-power lens, and 1000 X with the oil-immersion lens. Most microscopes are **parfocal.** This term means that the microscope remains in focus when one switches from one objective to the next objective.

The ability to see clearly two items as separate objects under the microscope is called the **resolution** of the microscope. The resolution is determined in part by the wavelength of the light used for observing. Visible light has a wavelength of about 550 nm, while ultraviolet light has a wavelength of about 400 nm or less. The resolution of a microscope increases as the wavelength decreases, so ultraviolet light allows one to detect objects not seen with visible light. The **resolving power**

of a lens refers to the size of the smallest object that can be seen with that lens. The resolving power is based on the wavelength of the light used and the numerical aperture of the lens. The **numerical aperture (NA)** refers to the widest cone of light that can enter the lens; the NA is engraved on the side of the objective lens.

If the user is to see objects clearly, sufficient light must enter the objective lens. With modern microscopes, entry to the objective is not a problem for scanning, low-power, and high-power lenses. However, the oil-immersion lens is exceedingly narrow, and most light misses it. Therefore, the object is seen poorly and without resolution. To increase the resolution with the oil-immersion lens, a drop of **immersion oil** is placed between the lens and the glass slide. Immersion oil has the same light-bending ability (index of refraction) as the glass slide, so it keeps light in a straight line as it passes through the glass slide to the oil and on to the glass of the objective, the oil-immersion lens. With the increased amount of light entering the objective, the resolution of the object increases, and one can observe objects as small as bacteria. Resolution is important in other types of microscopy as well.

Compound microscope Parts and Specifications

Before purchasing or using a microscope, it is important to know the functions of each part.

Eyepiece Lens: the lens at the top that you look through. They are usually 10X or 15X power.

Tube: Connects the eyepiece to the objective lenses

Arm: Supports the tube and connects it to the base

Base: The bottom of the microscope, used for support

Illuminator: A steady light source (110 volts) used in place of a mirror. If your microscope has a mirror, it is used to reflect light from an external light source up through the bottom of the stage.

Figure1: Compounds Microscope's Parts

Stage: The flat platform where you place your slides. Stage clips hold the slides in place. If your microscope has a mechanical stage, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.

Revolving Nosepiece or Turret: This is the part that holds two or more objective lenses and can be rotated to easily change power.

Figure 2: objective lens, typical numerical apertures

Objective Lenses:

Usually you will find 3 or 4 objective lenses on a microscope. They almost always consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X , 400X and 1000X.

Condenser Lens:

The purpose of the condenser lens is to focus the light onto the specimen.

condenser (right) and its respective diaphragm (left)

Diaphragm or Iris:

Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide.

2-Electron microscopy.

The electron microscope is a type of microscope that uses **a beam of electrons** to create an image of the specimen. It is capable of **much higher magnifications** and has a **greater resolving power** than a light microscope, allowing it to see **much smaller objects in finer detail.** They are large, expensive pieces of equipment, generally standing alone in a small, specially designed room and requiring trained personnel to operate them.

Figure 3: Electron microscope

a)Transmission electron microscope (TEM)
b) scanning electron microscope (SEM)

Scanning vs. Transmission Electron Microscope

The difference lies in their cost, effect on human health, resolution and the information they can obtain from a specimen.

The resolution of a scanning electron microscope is lower than that of a transmission electron microscope. While a transmission electron microscope can view the images of objects to atomic level (which is less than 1nm), a scanning electron microscope can only be used to view images that require tens of nm at most.

A scanning electron microscope only scans a specimen. This limits the amount of information you can get from the specimen – it can only show the morphology of the specimen. Conversely, a transmission electron microscope can help you see a lot of characteristics of the specimen, such as the stress of the specimen, its crystallization, and morphology.

When preparing samples to be viewed under these microscopes, each requires different levels of effort. A scanning electron microscope, for instance, can sometimes view specimens directly without preparation. A transmission electron microscope, on the other hand, requires time in order to appropriately thin a specimen.

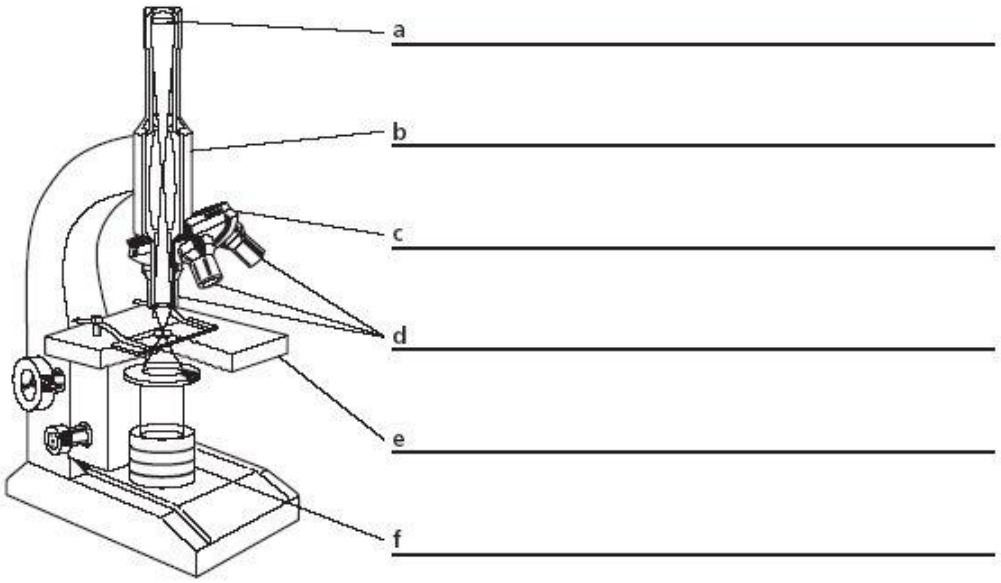
In addition, a transmission electron microscope costs more than a scanning electron microscope. It is also more detrimental to human health since it has higher energy electron beams.

Comparison between Scanning electron microscope and Transmission electron microscope

Scanning electron microscope (SEM)	**Transmission electron microscope (TEM)**
Lower resolution	Higher resolution
Three-dimensional views of microorganisms and other objects, because the whole organism is used.	Flat (2D) images, due to the use of ultra-thin slices of the microorganism or viruses.
Simple to prepare specimens	Specimen preparation requires thinning which is tiring and time consuming
Cheap	Expensive
Relatively safe to use	Relatively detrimental to human health.

Quiz Activity

STRUCTURES AND FUNCTIONS Label each part of the figure in the spaces provided.



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Practical Cell Biology- Section 2

Units, Amounts, Concentrations

&

Automatic Pipettes

Units, Amounts and Concentrations

Units and abbreviations

The common units, and their respective abbreviations, used in biomedical sciences are based on the International System of Measurements (SI units): grams (g), meters (m) etc. Often it is necessary to deal with large quantities e.g. 1500 g or, more commonly, very small quantities, e.g. 0.0000015 g. In such cases, such numbers are either expressed by use of powers of 10 (positive or negative) or by use of the appropriate prefix.

Thus: 1500 g $= 1.5 \times 10^3$ g $=$ 1.5 kg (kilograms)

and 0.0000015 g $= 1.5 \times 10^{-6}$ g $=$ 1.5 µg (micrograms)

Laboratory scientists most commonly use the prefix notation, particularly those that differ by a factor of 1000 in magnitude, so you should try to become familiar with them. Use of the prefixes can greatly simplify calculations, especially mental ones.

Note also that biomedical scientists normally express volumes and concentrations in terms of litres rather than in cubic measurements:

e.g. 1 litre, rather than 1 dm3
1 millilitre (1 ml) rather than 1 cm3

A concentration of 1 milligram per litre is also still commonly expressed in the form 1 mg/l rather than 1 mg.l-1 or 1 mg.dm-3.

The commonly used prefixes are:

Prefix	Name	Which modifies an amount by	Examples
m	milli	1/1000th, i.e. by 10^{-3}	mmol, mg, ml
µ	micro	1/1000 000th, i.e. by 10^{-6}	µmol, µg, µl
n	nano	by 10^{-9}	nmol, ng
p	pico	by 10^{-12}	pmol, pg
f	femto	by 10^{-15}	fmol, fg
k	kilo	by 1000 times, i.e. by10^{3}	kg
M	mega	by 10^{6}	MPa

The prefixes centi (10-2) and deci (10-1) are only commonly used in specific cases e.g. cm

Concentrations

The determination of the concentration of a substance in a biological fluid is central to many areas of medical and dental practice (e.g. electrolytes in serum or glucose in urine). It is important, therefore, that concentrations are expressed in clear unambiguous terms. There are several ways of doing this. The simplest is to express the concentration as the weight or mass of the substance per unit volume:

e.g. 10 g/l or 20 mg/ml or 2 µg/ml

Another way is to express the concentration of a solution or mixture in terms of per cent (%). This is a somewhat outdated method but you may still come across it, particularly if you read the older medical literature, so you should know what it means. Note that there are different types of % concentration:

% (v/v) (volume by volume)
% (w/v) (weight by volume)
% (w/w) (weight by weight)

- A 1% (v/v) concentration is obtained by diluting 1 volume of a substance into 100 volumes (total) of solution, e.g. 1 ml ethanol diluted with water to a final volume of 100 ml gives a 1% (v/v) ethanol solution.

- A 1% (w/v) concentration is obtained by dissolving 1 g of substance in a final volume of 100 ml solution, e.g. 1 g glucose dissolved in water to a final volume of 100 ml solution gives a 1% (w/v) glucose solution.

- A 1% (w/w) concentration is obtained by mixing 1 g of substance with something else in a total weight of 100 g, e.g. 1% (w/w) salt in sand.

Another old method of expressing concentration that you may still see (just look at the side of a tube of toothpaste) is "parts per million (ppm)". One ppm is one part of anything in one million parts of total material, e.g. 1 g of compound X in a million g total, or 1 litre of Y in a million litres total.

Moles and molarity

By far the most important unit defining an amount of a biological substance is the mole, with the corresponding concentration being the molarity. As far as possible, the concentrations of specific compounds in serum, urine etc, are now expressed as molarities in clinical laboratories.

One mole of a substance is the molecular weight of that substance expressed in grams. Thus, the molecular weight of glucose is 180, so:

1 mole of glucose = 180 g
1 mmol glucose = 180 mg
1 µmol glucose = 180 µg
100 µmol glucose = 18,000 µg = 18 mg

Note the abbreviations: 1 mmol = 1 millimole; 2 mmol = 2 millimoles; 5 µmol = 5 micromoles.

Concentrations in molarities are given by expressing the number of moles of the substance present in a defined volume of solution:

A 1 molar (1 M) solution contains 1 mole per litre (1 mol/l)
a 1 millimolar (1 mM) solution contains 1 millimole per litre (1 mmol/l)

Note: **mol** and **moles** mean the same thing (an amount) and **moles/litre** (long winded but correct) is a concentration and can be expressed as **mol/l**, or **mol.l^{-1}**, or (best and simplest of all) — **M**. **Ensure you know the distinction between concentrations and amounts.**

So, if you dissolve 0.5 **mol** of a compound in one litre of solvent the concentration of the compound is 0.5 **mol/l**, or 0.5 **M.** 100ml of the solution contains 0.05 **mol.**

Prefix notation

The value of the prefix notation can now be seen as it allows rapid mental calculations to be performed (after much practice!). The following concentrations are all the same:

0.5 M, 0.5 mol/l, 0.5 mmol/ml, 0.5 µmol/µl, 0.5 pmol/pl

You see that by scaling both the units (amount and volume) in the concentration up or down by a factor of 1000, the value of the concentration remains the same. If you only scale one of the terms (amount or volume), then you can express the same concentration in yet more ways:

0.5 mol/l = 0.5 mmol/ml = 500 µmol/ml = 500,000 pmol/µl

Now, let's say you wanted to determine the amount of cholesterol in the blood of a newborn infant. You know it will be around 5 mM. The detection limit of the method you are going to use is about 20 nmol and you can only take 20 µl of blood. Will this be enough?

5 mM cholesterol contains 5 mmol/l, or 5 µmol/ml, or 5 nmol/µl.

It is easy to see now that 20µl contains 100 nmol of cholesterol - **ENOUGH**.

Example

- *How many µmol are dissolved in 2 l of a 20 mM solution?*

  20 mM = 20 mmol/l, so 2 l contain 40 mmol. 40 mmol = **40,000 µmol**.

- *The molecular weight of NaCl is 58. How many mg are in 50 µmol of NaCl?*

  1 mol of NaCl is 58 g, so 1 µmol is 58 µg, so 50 µmol is 2,900 µg = **2.9 mg**.

- *What is the molarity of a 1% (w/v) solution of glucose? (molecular weight = 180)*

1% (w/v) contains 1 g in 100 ml and, therefore, 10 g in 1 litre.
A 1 M solution of glucose contains 180 g/l, so 10 g/l represents a molarity of 10/180 = **0.056 M** (or **56 mM**).

Dilutions

This is another source of great confusion. Most experiments require you to make dilutions of reagents, either before use or as a consequence of the actual assay. When you are asked to make a ten-fold dilution of a reagent, the objective is to produce a solution that has a reagent concentration one-tenth of the original. It follows that the molecules of reagent that occupied a volume of "x" before must now occupy a volume of "10x". This is obtained by adding to one volume of reagent to nine volumes of diluent (sometimes referred to as a "1 plus 9" dilution or, more commonly, a "1 in 10" dilution).

When a solution of known concentration is diluted, it is obvious that the concentration will fall. Less obvious is the amount by which it falls! Take a typical example:

A solution of a compound is maintained as a stock solution at 5 mM. What is the final concentration of the compound in which 0.15 ml of stock solution is mixed with 0.4 ml of buffer and 0.2 ml of water?

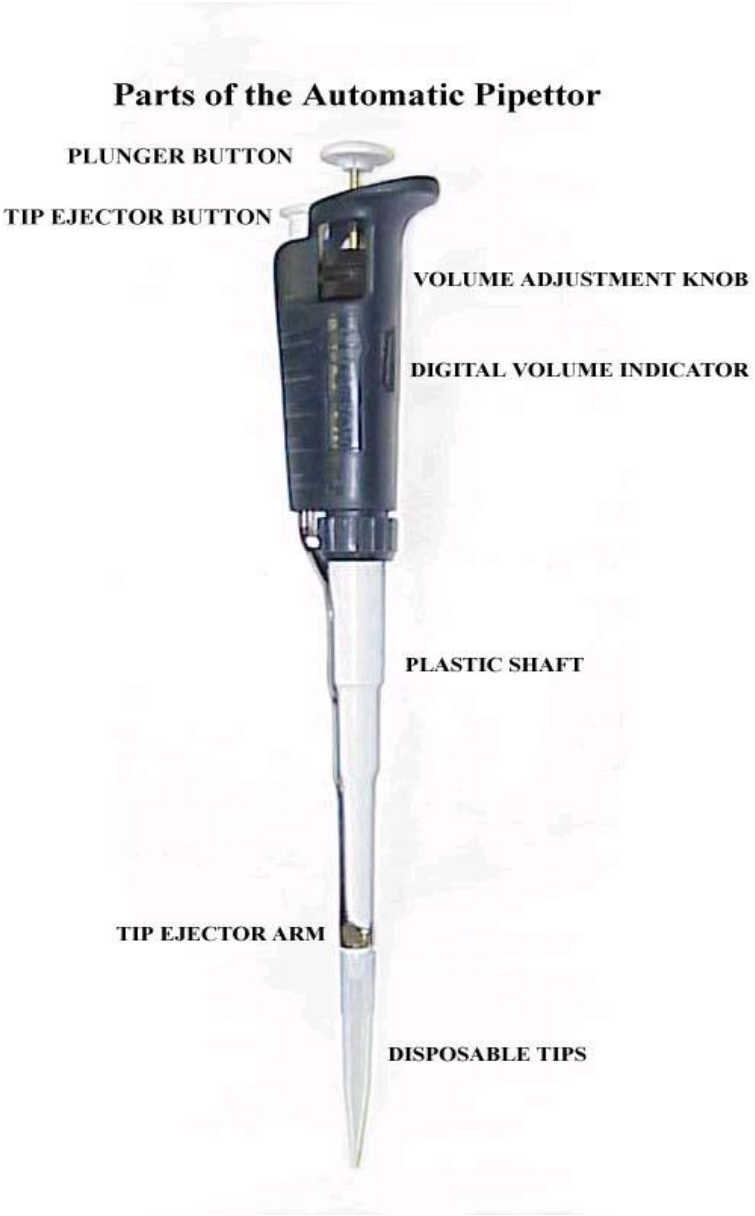
The formula for a dilution is v1 x c1 = v2 x c2 (where c1 and c2 are the concentrations before and after dilution, and v1 and v2 are the volumes before and after dilution). Therefore, c2 = v1/v2 x c1, which works out to be c2 = 0.15 / 0.75 x 5 mM , i.e. **1 mM**

Using Automatic Pipettes

Introduction

- Automatic pipettes are used to accurately transfer small liquid volumes.
- Glass pipettes are not highly accurate for volumes less than 1 milliliter (1 ml), but the automatic pipettes are both accurate and precise.
- These are continuously adjustable digital pipettes.
- Each pipette can be set to transfer any volume within its own volume range.

Parts of Automatic Pipette



Operating Automatic Pipettes

1. Set the volume
2. Attach disposable tip
3. Depress the plunger to the first stop
4. Immerse tip in sample
5. Draw up the sample
6. Pause
7. Withdraw the tip
8. Dispense the sample
9. Withdraw the pipette
10. Release plunger
11. Discard the tip

Pippetting Guidelines and Precautions

For optimal reproducibility, use the following pipetting procedures:

(1) Consistent SPEED and SMOOTHNESS when you press and release the PLUNGER
(2) Consistent pressure on the PLUNGER at the FIRST STOP
(3) Consistent and sufficient IMMERSION DEPTH
(4) Nearly VERTICAL POSITIONING of pipette
(5) AVOID ALL AIR BUBBLES: Since the plastic pipette shaft can be damaged if liquids are drawn beyond the tip into the shaft
(6) NEVER lay the pipette on its SIDE nor INVERT the pipette if liquid is in the tip

Quiz Activity

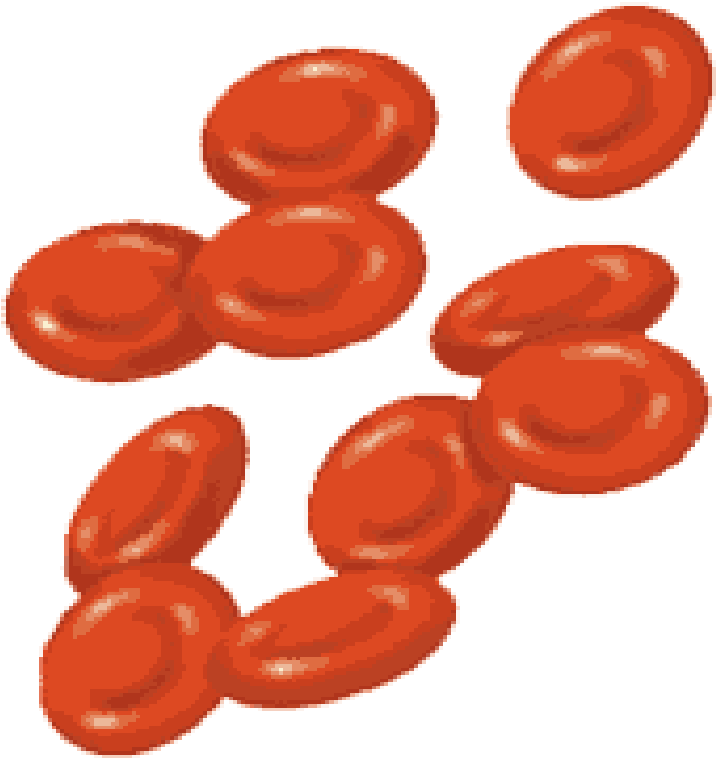
1. *What is the molarity of a 1% (w/v) solution of glucose? (molecular weight = 180)?*

2. *If The molecular weight of NaCl is 58, then how many mg are in 50 µmol of NaCl?*

Solutions:

1. 1% (w/v) contains 1 g in 100 ml and, therefore, 10 g in 1 litre. A 1 M solution of glucose contains 180 g/l, so 10 g/l represents a molarity of 10/180 = **0.056 M** (or **56 mM**).

2. 1 mol of NaCl is 58 g, so 1 µmol is 58 µg, so 50 µmol is 2,900 µg = **2.9 mg**.



Clinical pharmacy - Level 1

Practical Cell Biology- Section 3

Types of blood cells (Red blood cells)

Types of blood cells:

1- Red blood cells.
2-White blood cells.
3-Platelets.

Red blood cells (erythrocytes)

Shape:

- Red blood cells or erythrocytes are small, circular, biconcave (thinner in the center than around the edge) discs that have no nuclei.
- The shape of the red blood cells can change markedly as the cells pass through the narrow capillaries.

Red Blood Cell Count:

- In adult females ⟶ 4.5 -5.0 millions / mm3 (average 4.8).
- In adult males ⟶ 5.0 - 5.5 millions /mm3 (average 5.3).

Red Blood Cell Structure:

a) Red Cell Membrane:

Composition:

The outer surface of the membrane is composed of a lipid bi-layer which is attached firmly to the sub-membranous cytoskeleton. Proteins traverse the lipid bi-layer and are responsible for:

a) The antigenic property of the red cells e.g: blood grouping.
b) Act as ion channels
c) Act as attachment sites for the sub-membranous cytoskeleton.

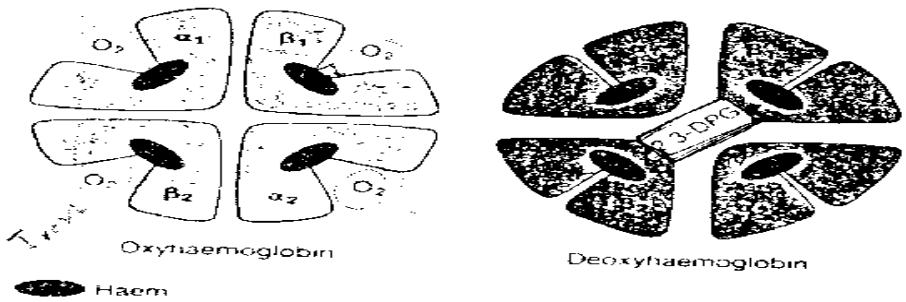
b) Membrane cytoskeleton:

It is a protein network under the cell membrane. It is attached to the trans-membrane proteins. It gives the red cell its shape and its ability of deformation while passing through the narrow blood capillaries.

c) Contents of the red blood cells:

1) Haemoglobin:

It is the oxygen carrying red pigment found in the red blood cells. It is present in the cells in a maximum concentration (33 %}. Each molecule of hemoglobin consists of four subunits, each containing one polypeptide chain (globin) and one iron-protoporphyrin complex (haem).



2) Carbonic Anhydrase Enzyme:

The red cells contain a large amount of this enzyme which catalyses the reaction between carbon dioxide and water increasing the rate of this reversible reaction several thousand folds. This helps CO2 carriage and transport from the tissues to the lungs in the form of bicarbonate ions.

3) Cytoplasmic Enzymes:

These are needed for metabolic reactions inside the red blood cells e.g glucose 6 phosphate dehydrogenase (G6PD) and pryruvate kinase.

Functions of the red blood cells:

1. Oxygen carriage: (combines with the haem)
2. Carbon dioxide carriage:(carbonic anhydrase and globin part)
3. Acid-base buffer: (Hemoglobin)

4. Maintaining the blood pressure: (RBCs and plasma proteins) The red cells together with the plasma proteins are responsible for the blood viscosity which is necessary for maintaining the blood pressure at normal level.

Anemia

Defin: Anemia is a medical condition in which the **red blood cell** count or **hemoglobin** is less than normal.

Symptoms of anemia: fatigue, weakness

Physical signs of anemia: pale skin.

Cause of signs and symptoms of anemia: Anemia leads to decrease blood ability to transport oxygen to the tissues. The decreased oxygen supply to the tissues explains most of the signs and symptoms of anemia.

Types of anemia:

1) haemorrhagic anemia:

Types:

A) Acute blood loss anemia: due to sudden massive loss of blood.

B) chronic blood loss anemia: due to repeated small loss of blood.

2)Haemolytic anemia:

It results from increased rate of haemolysis (destruction of red blood cells).

3)Dyshemopoietic anemia: due to deficiency of one or more of the factors required for the haemoglobin syenthesis. **It includes:** Iron deficiency anemia.

Experiment : Colourimetric Determination of the Blood Haemoglobin

Princible:

Hemoglobin + Potassium Ferricyanide ⟶ methemoglobin + KCN ⟶ cyanomethemoglobin (orange color) its absorbance measured by colorimetry.

Conditions:

1-Reagent: Drabkin´s reagent (k.ferricyanide+KCN)

2-Temp: Room Temperature

3-Incubation Time: 5 minutes

4-Filter: 550 n.m

5-Blank: Drabkin´s reagent

Procedure:

1) Add 20 µl blood to 5 ml Drabkin´s reagent in clean dry Wasserman tube.
2) Incubate at Room Temperature for 5 min.
3) Measure A against blank.

Calculations:

- Hb concentration=A_{Sample} X 36.8 g/dL
- Hb%= measured value/normal value X 100 %

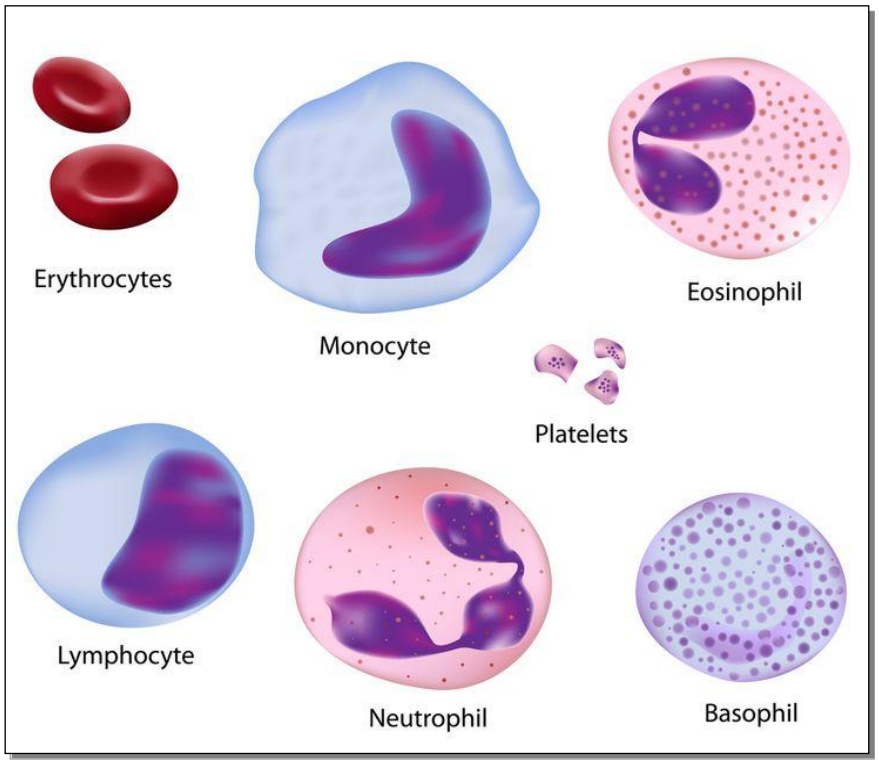
Results:

- Normal levels: female: 14±2 g/dL male: 16±2 g/dL
- If Hb% is less than 70% ⟶ Anemia

Quiz Activity

Complete the following statements:

- Proteins traversing the lipid bi-layer in red blood cells are responsible for....., ... and .....
- Drabkin´s reagent consists of ..... and .....
- Normal Red Blood Cell Count in adult males is .....
- Carbonic Anhydrase Enzyme helps.....carriage and transport from the tissues to the lungs in the form of.....
- The oxygen carrying red pigment found in the red blood cells is present in the cells in a maximum concentration of.....



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Practical Cell Biology- Section 4
White Blood Cells

White Blood Cells

White Blood Cells (WBCs) or Leukocytes

Although leukocytes are far less numerous than red blood cells, they are important to body defense against disease. On average, there are 4000 to 11.000 WBCs/mm3 , and they account for less than 1 percent of total blood volume.

White blood cells are the only complete cells in blood; that is; they contain nuclei and the usual organelles. Leukocytes form a protective, movable army that helps defend the body against damage by bacteria, viruses, parasites, and tumor cells. As such they have some very special characteristics.

Red blood cells are confined to the bloodstream and functions in the blood. White blood cells, by contrast, are able to slip into and out of the blood vessels (by process called diapedesis). The circulatory system is simply their means of transportation to areas of the body where their services are needed for inflammatory or immune responses. In addition, WBCs can locate areas of tissue damage and infection in the body by responding to certain chemicals that diffuse from the damaged cells. This capability is called positive chemotaxis. Once they have "caught the scent," the WBCs move through the tissue spaces by ameboid motion (forming of cytoplasmic extensions that help move along). By following the diffusion gradient, they pinpoint areas of tissue damage and rally round in large numbers to destroy microorganisms or dead cells. Whenever WBCs mobilize for action, the body speeds up their production, and as many as twice the normal number of WBCs may appear in the blood within a few hours.

Types of WBCs:

WBCs are classified into two major groups, depending on whether or not they contain visible granules in their cytoplasm.

A-Granulocytes are granule-containing WBCs.

Granulocytes are a category of white blood cells characterized by the presence of granules in their cytoplasm. These granules are little sacs containing enzymes that digest microorganisms. Granulocytes are part of the innate immune system.

They are also called polymorphonuclear leukocytes because of the varying shapes of the nucleus. The granulocytes include the neutrophils, eosinophils and basophils.

1. Neutrophils: type of white blood cell that is characterized histologically by its ability to be stained by neutral dyes have a multilobed nucleus (3-5 lobes).

2. Eosinophils: type of white blood cell (leukocyte) that is characterized histologically by its ability to be stained **by acidic dyes (e.g., eosin)** and have a bilobed nucleus.

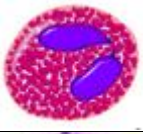
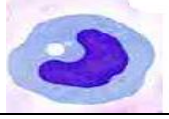
3. Basophils: type of white blood cell (leukocyte) that is characterized histologically by its ability to be stained by basic dye. It is the rarest of the WBCs, they have a 2 or 3 lobed nucleus. The lobes are usually not as well defined as in neutrophilic granulocytes and the nucleus may appear S-shaped.

B- Agranulocytes lack visible cytoplasmic granules.

Leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific granules, which are lysosomes. The agranulocytes include lymphocytes and monocytes.

1. Lymphocytes have **deeply staining, eccentric nucleus.**
2. Monocytes have **kidney shaped** nucleus. When they migrate into the tissues, they change into macrophages.

The normal percentages of the different types of white blood cells are approximately the following:

W.B.C	Mean Range	Nucleus	Diagram
Neutrophil	**60 %**	**Multilobed**	
Eosinophil	**2 %**	**Bi-lobed**	
Basophil	**0.5 %**	**Tri-lobed**	
Lymphocyte	**30 %**	**Deeply staining, eccentric**	
Monocyte	**4 %**	**kidney shaped**	

Procedure:

A. Preparation of a Blood film and staining it with Leishman's stain:

1. Clean thoroughly three microscopic slides, two to be covered with the blood film and one to be used as a spreader.
2. Clean the finger with spirit, allow it to dry and then prick (puncture) it with a sterile lancet to obtain a drop of blood.
3. Touch one end of the two slides to the drop of the blood, only a small quantity is required. Lay the slide on a flat surface.
4. Place the edge of the spreader on the surface of the slide just in front of the drop of blood, and at an angle of 45º.
5. Draw the spreader back until it makes contact with the drop. The blood will now run along the full width of the spreader at the line of junction.
6. When this has happened, push the spreader slowly and smoothly to the other end of the slide.

*****The faster the spreader is moved, the thicker the film; a properly made film should be only one cell thick throughout.

7. Allow the film to dry in air. You can fasten this by waving it. (Repeat with the second slide).
8. Carefully drop Leishman's stain on to the blood film until the film is covered. Allow the stain to act for one minute.

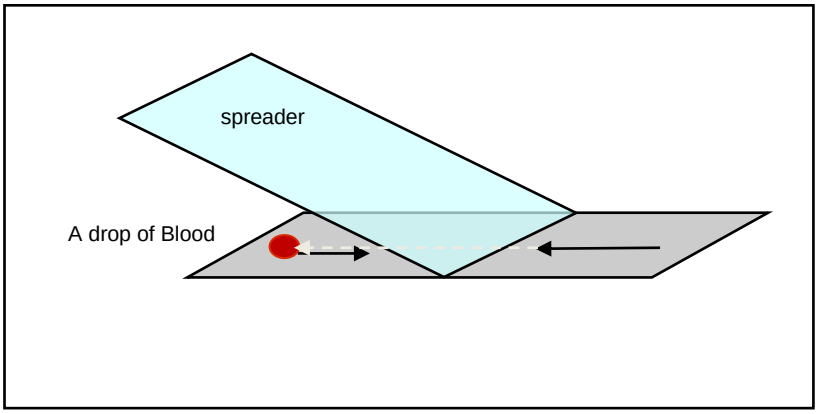
*****Leishman's stain: is a mixture of methylene blue and eosin in methyl alcohol (prepared by mixing 0.6g Leishman's powder into 400 ml methanol, using glass beads or a magnetic stirrer or carefully warming to 37 degree C may help in dissolving).

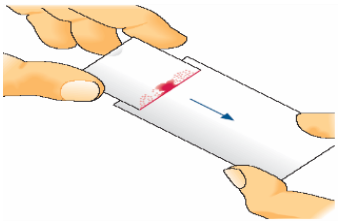
9. Then add as much distilled water to the stain as will stay on the slide without spilling over.

*****This gives a dilution of (1:1 or 1:2). If the dilution of the stain is correct, the fluid will be covered by a thin greenish scum.

10. The diluted stain should be allowed to act for 7-10 min., then wash it off with water. Make sure that none of the greenish scum settles on the surface of the blood film.
11. Continue washing until the film has a salmon pink color.

12. Shake off excess water and allow it to dry in air without heat.





B. Examination of the Blood smear:

Fix the stained slide on the mechanical stage of the microscope. Add a drop of cedar oil to the slide. Examine the blood film using oil immersion lens and write your notes.

Clinical pharmacy - Level 1

Practical Cell Biology- Section 5
Blood Grouping

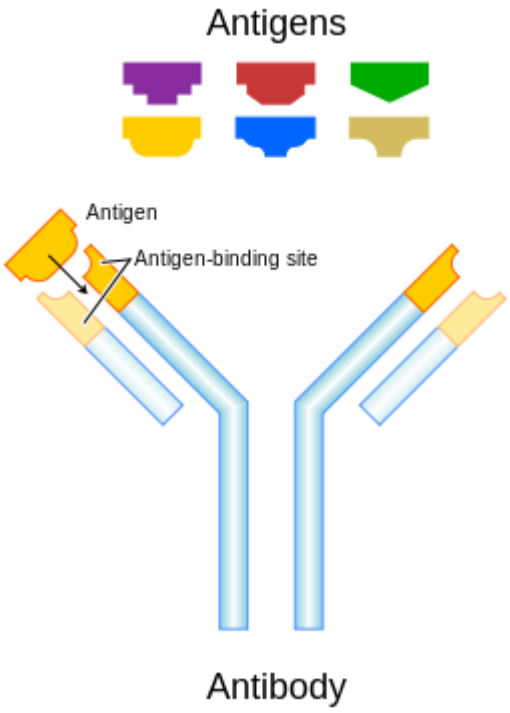
Blood Grouping

Introduction:

Antigen:

An antigen is a substance that evokes the production of one or more antibodies. Each antibody binds to a specific antigen by way of an interaction similar to the fit between a lock and a key. The substance may be from the external environment or formed within the body. The immune system will try to destroy or neutralize any antigen that is recognized as a foreign and potentially harmful invader. The term originally came from antibody generator.

Antibody:



An antibody, also known as an immunoglobulin (Ig), is a large Y-shaped protein that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The antibody recognizes a unique part of the foreign target, called an antigen. Each tip of the "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision.

Basis of Classification:

The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies. The antigens are located on the surface of the red blood cells and the antibodies are in the blood plasma. Individuals have different types and combinations of these molecules.

Significance of the Blood Grouping:

For a blood transfusion to be successful blood groups must be compatible between the donor blood and the patient blood. If they are not, the red blood cells from the donated blood will clump or agglutinate. The agglutinated red cells can clog blood vessels and stop the circulation of the blood to various parts of the body. The agglutinated red blood cells also crack and its contents leak out in the body. The red blood cells contain hemoglobin which becomes toxic when outside the cell.

What are the different blood groups?

There are more than 20 genetically determined blood group systems known today, but the AB0 and Rh systems are the most important ones used for blood transfusions.

(A) AB0 blood grouping system:

According to the AB0 blood group system there are four different kinds of blood groups:

1-Blood group A:

If you belong to the blood group A, you have A antigens on the surface of your red blood cells and B antibodies in your blood plasma.

2-Blood group B:

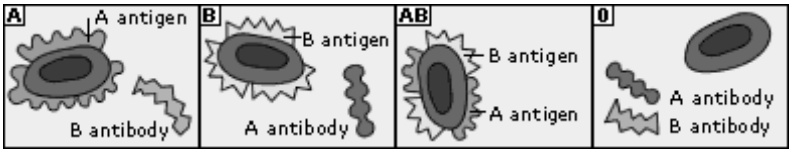
If you belong to the blood group B, you have B antigens on the surface of your red blood cells and A antibodies in your blood plasma.

3-Blood group AB:

If you belong to the blood group AB, you have both A and B antigens on the surface of your red blood cells and no A or B antibodies at all in your blood plasma.

4-Blood group 0:

If you belong to the blood group 0 (null), you have neither A or B antigens on the surface of your red blood cells but you have both A and B antibodies in your blood plasma.



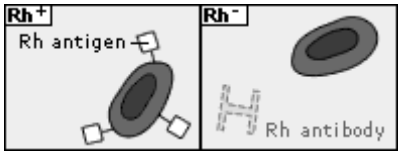
(B) Rh(Rhesus) factor blood grouping system:

1-Rh+:

Many people also have a so called Rh factor on the red blood cell surface. This is also an antigen and those who have it are called Rh+.

2- Rh-:

Those who haven't Rh factor on the red blood cell surface are called Rh-.



According to above blood grouping systems, you can belong to either of following **8 blood groups**:

A Rh+	B Rh+	AB Rh+	0 Rh+
A Rh-	B Rh-	AB Rh-	0 Rh-

Blood transfusions – who can receive blood from whom?

The transfusion will work if a person who is going to receive blood has a blood group that doesn't have any antibodies against the donor blood's antigens. But if a person who is going to receive blood has antibodies matching the donor blood's antigens, the red blood cells in the donated blood will clump.

Blood Group	Antigens	Antibodies	Can give blood to	Can receive blood from
AB	A and B	None	AB	AB, A, B, 0
A	A	B	A and AB	A and 0
B	B	A	B and AB	B and 0
0	None	A and B	AB, A, B, 0	0

A person with Rh- blood does not have Rh antibodies naturally in the blood plasma (as one can have A or B antibodies, for instance). But a person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood

from a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies. A person with Rh+ blood can receive blood from a person with Rh- blood without any problems.

Experiment: Blood typing: How do you find out to which blood group someone belongs?

1.You mix the blood with three different reagents including either of the three different antibodies, A, B or Rh antibodies(anti A/antiB/antiD).

2.Then you take a look at what has happened. In which mixtures has agglutination occurred? The agglutination indicates that the blood has reacted with a certain antibody and therefore is not compatible with blood containing that kind of antibody. If the blood does not agglutinate, it indicates that the blood does not have the antigens binding the special antibody in the reagent.

3.If you know which antigens are in the person's blood, it's easy to figure out which blood group he or she belongs to.

Antibodies with Agglutination	Blood Group
Anti-A	A^-
Anti-B	B^-
Anti-A & Anti-B	AB^-
Anti-A & Anti-D	A^+
Anti-B & Anti-D	B^+
Anti-A & Anti-B & Anti-D	AB^+
Anti-D	O^+
None	O^-

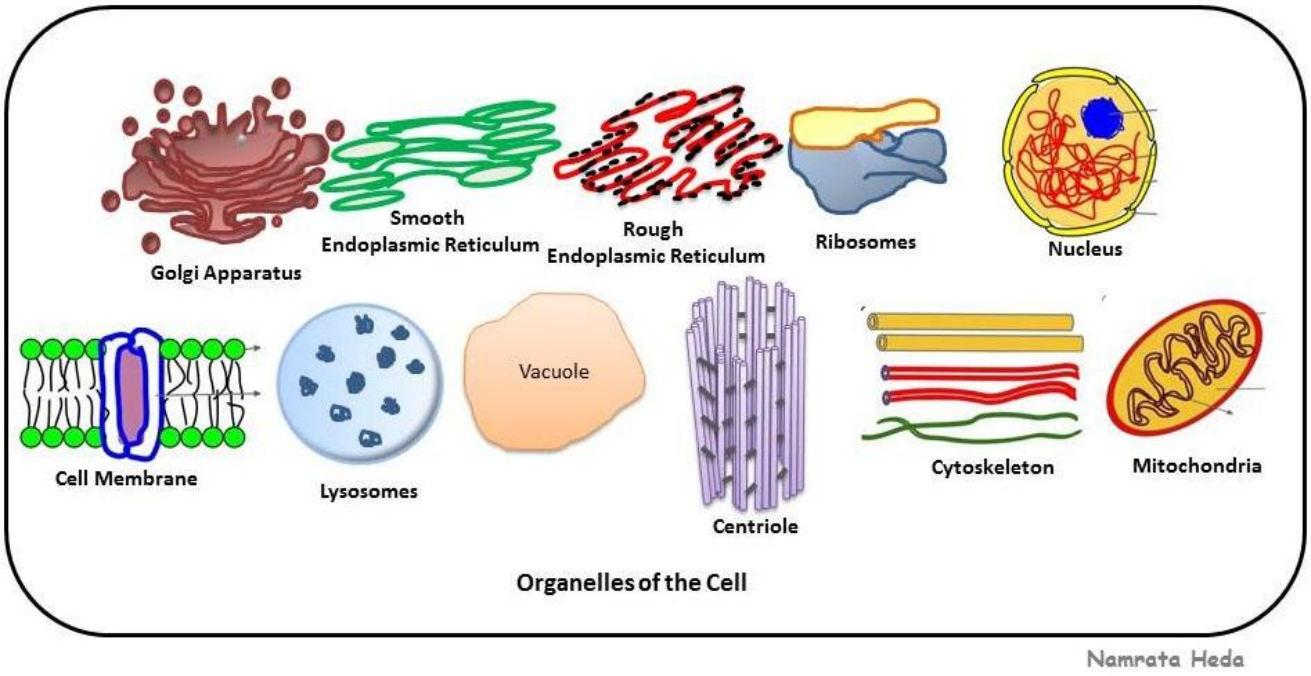
Quiz Activity

- Mention the blood group(s) that could be donated to the following blood groups safely:

Blood Group of Acceptor	Possible Blood Group(s) of Donor
AB^+	.....
A^-	.....
B^+	.....
0^-	.....

Clinical pharmacy - Level 1

Practical Cell Biology- Section 6
Sub-Cellular Fractionation & Sub-cellular Fraction Identification



Sub Cellular Fractionation & Subcellular Fraction Identification

Definition of Cell Fractionation:

Cell fractionation is the separation of homogeneous sets, usually organelles, from a heterogeneous population of cells.

Steps of Cell Fractionation:

1. Disruption (homogenization) of cells and liberation of organelles.
2. Filtration.
3. Purification of cell components.
4. Identification of different organelles and fractions.

1-Homogenization:

Several methods could be embloyed for homogenization of cells and liberation of organelles:

I. Physical Disruption:

Tissue is typically homogenized in an isotonic buffer solution using a variety of mechanisms. A homogenizer is often used as it is relatively gentle. Other procedures include grinding, chopping, pressure changes, osmotic shock, freeze-thawing, and ultra-sound homogenization. The solution is homogenized in an isotonic solution to stop osmotic damage, with a pH buffer to regulate pH, and at an ice-cold temperature to prevent enzyme damage. The organelles are kept either cold, isotonic or buffered.

Figure 4 : Tissue glass homogenizer

II. Detergent Methods:

Detergent-based cell lysis is an alternative to physical disruption of cell membranes, although it is sometimes used in conjunction with homogenization and mechanical grinding. Detergents disrupt the lipid barrier surrounding cells. The ideal detergent for cell lysis depends on cell type. Animal cells, bacteria and yeast all have differing requirements for optimal lysis due to the presence or absence of a cell wall. Because of the dense and complex nature of animal tissues, they require both detergent and mechanical lysis to effectively lyse cells.

III. Enzymatic Method:

The use of enzymatic methods to remove cell walls is well-established for preparing cells for disruption, or for preparation of protoplasts (cells without cell walls) for other uses such as introducing cloned DNA or sub-cellular organelle isolation. The enzymes are originally isolated from biological sources (e.g. lysozyme from hen egg white). The enzymes commonly used include lysozyme, zymolase, cellulose, glycanases, and proteases.

Disadvantages of Enzymatic Method:

- Not always reproducible.
- Not usually applicable to large scale.
- The enzyme must be removed (or inactivated) to allow cell growth or permit isolation of the desired material.

2- Filtration:

This step may not be necessary depending on the source of the cells. Animal tissue however is likely to yield connective tissue which must be removed.

Commonly, filtration is achieved either by pouring through gauze or with a suction filter.

3- Purification:

Achieved by differential centrifugation. The sequential increase in gravitational force resulting in the sequential separation of organelles according to their density. Since different fragments of a cell have different sizes and densities, each fragment will settle into a pellet with different minimum centrifugal forces. Thus, separation of the sample into different layers can be done by first centrifuging the original homogenate under weak forces, removing the pellet, then exposing the subsequent supernatants to sequentially greater centrifugal fields. Each time a portion of different density is precipitated to the bottom of the container and extracted, and repeated application produces a rank of layers which includes different parts of the original sample.

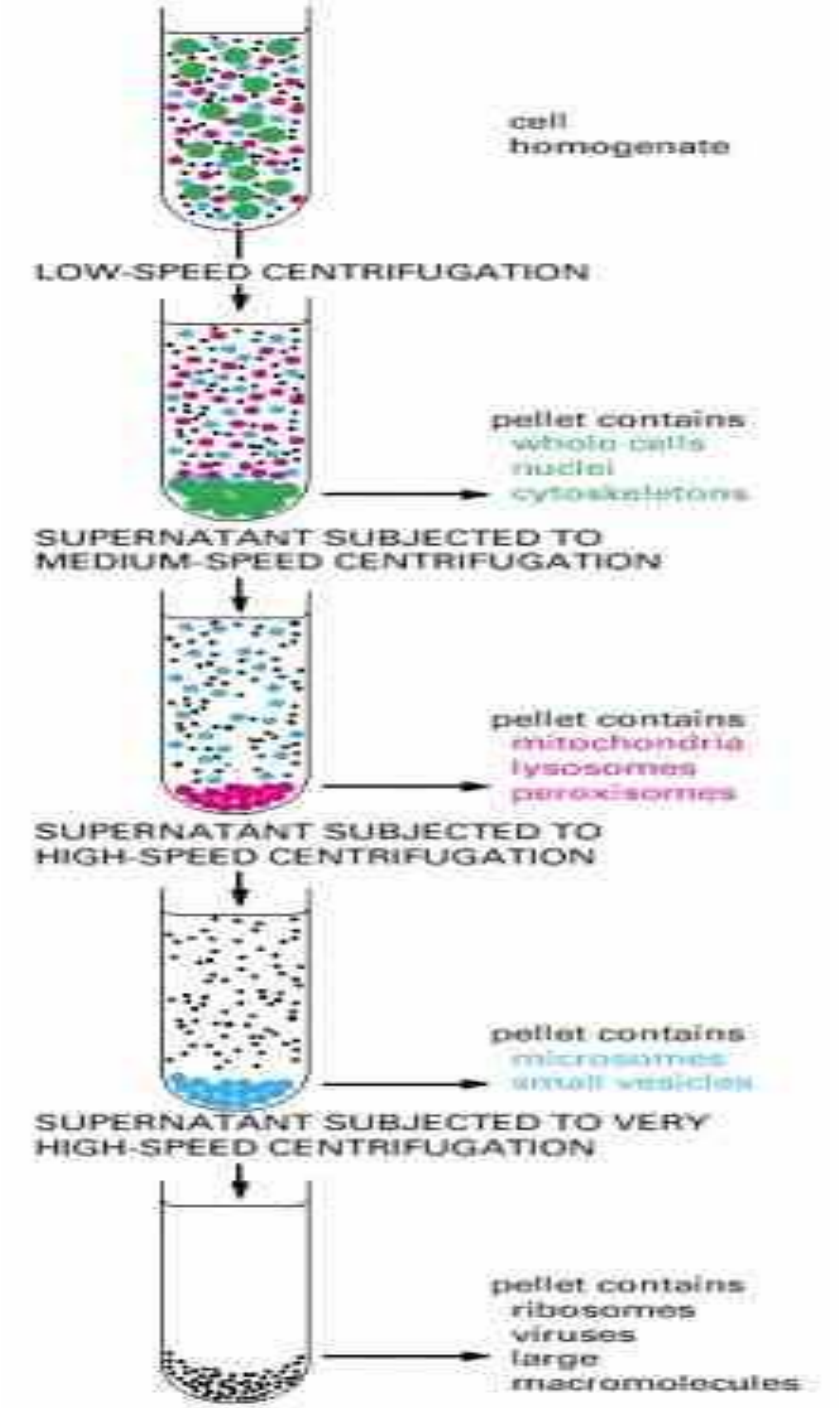


Figure 5 : Differential Centrifugation

4-Fraction Identification:

The constituents of different fractions could be identified by:

1. Morphological examination by using electron microscope.
2. DNA/protein ration or RNA/protein ratio.
3. Enzymatic analysis depending on the presence of marker enzymes.

- **Marker enzymes:**

They depend on the principle that usually (but not always) one particular enzyme is associated with one morphological constituent of the cell.

Examples of marker enzymes:

1. Glucose 6-phosphate for the liver endoplasmic reticulum.
2. Succinate hydrogenase for the mitochondria.
3. Galactosyltransferase for Golgi membranes.

Experiment: Cytosolic/Mitochondrial Fraction Identification

Principle:

The likely steady-state NAD/NADH ratio in the cytoplasm is of the order of 1000 and in the mitochondria less than 10. Therefore, the NAD/NADH ratio could be used for differentiation between mitochondrial and cytosolic cellular fractions.

The lactate dehydrogenase (LDH) equilibrium in the cytosolic compartment is described by:

$$H^+ + \text{Pyruvate} + \text{NADH} \leftrightarrow \text{Lactate} + NAD^+$$

This emphasizes the fact that the equilibrium of the LDH lies to the right, even more so in acidic conditions. The redox ratio of the $[NADH]_c/[NAD]_c$ couple in the cytosol can be calculated from:

$$\frac{[\text{Lactate}]}{[\text{Pyruvate}]} \times K_{LDH} = \frac{[NADH]_c}{[NAD]_c}$$

where K_{LDH} is the equilibrium constant for the LDH reaction, having a value of 1.11×10^{-4} (Krebs, 1973).

1- Determination of Lactic acid concentration:

Principle:

Lactic acid is commonly estimated by the method of Barker and Summerson. After oxidation in strong sulphuric acid solution to acetaldehyde the latter is coupled with p-hydroxydiphenyl in the presence of cupric ions to yield a violet compound that can be measured by colorimetry.

Procedure:

1- In a wasserman tube add 10 µL of the cellular fraction to 1000 µL of Lactic acid reagent and incubate for 10 minutes.

2-Measure A_{sample} and then determine lactic acid concentration using the equation:

Lactic acid Concentration = $(A_{sample}/A_{standard}) \times conc_{standard}$ (100 mg/dl)

2- Determination of Pyruvic acid concentration:

Princible:

Pyruvic acid forms red colored dinitrophenyl hydrazone derivative with dinitrophenylhydrazine that can be measured by colorimetry.

Procedure:

1- Add 10 µL of the cellular fraction to 1000 µL of Pyruvic acid reagent and incubate for 10 minutes.

2-Measure A_{sample} and then determine pyruvic acid concentration using the equation: Pyruvic acid Concentration = $(A_{sample}/A_{standard}) \times conc_{standard}$ (2 mg/dl)

3- Calculation of NAD/NADH ratio:

$$\frac{[\text{Lactate}]}{[\text{Pyruvate}]} \times K_{LDH} = \frac{[\text{NADH}]_c}{[\text{NAD}]_c}$$

where K_{LDH} is the equilibrium constant for the LDH reaction, having a value of 1.11×10^{-4} (Krebs, 1973). The lactate/pyruvate (L/P) ratio both in the intracellular

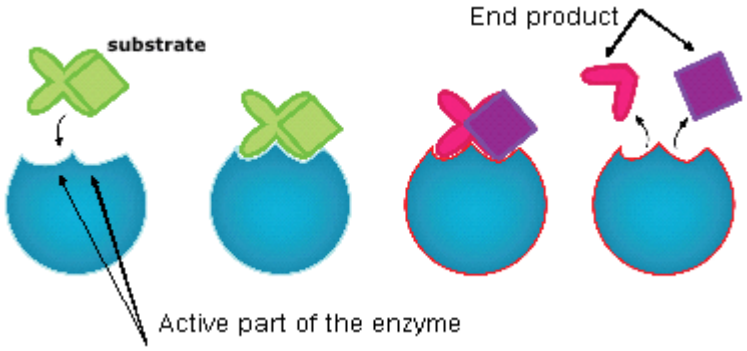
Quiz Activity

Complete the following statements:

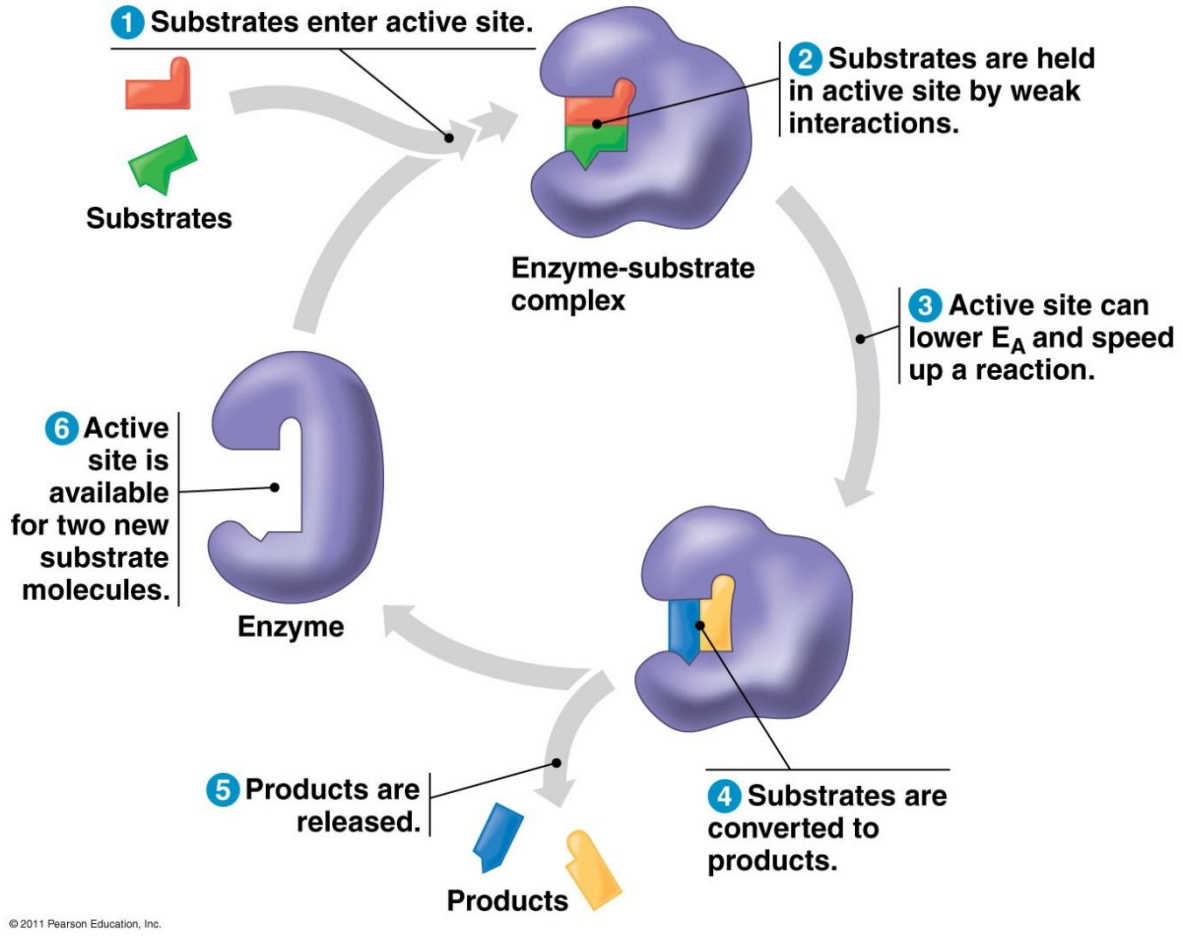
- Detergents are employed for cell homogenization by disrupting the.....surrounding cells.
- The use of.....to remove cell walls is well-established for preparing cells for disruption.
- During differential centrifugation the final fraction obtained using the highest centrifugation speed contains.....
- Succinate hydrogenase is used as marker enzyme for subcellular fractions containing.....
- NAD/NADH ratio in the cytoplasm is of the order of .....

Clinical pharmacy - Level 1

Practical Cell Biology- Section 7
Enzymes



Enzymes



Definition:

Enzymes are "specific" protein catalysts that increase the rate of biological reactions without being changed themselves.

Composition of enzymes

All enzymes are protein in nature. They are divided chemically into:

1- Simple protein enzymes: consisting only of proteins, e.g. pepsin, maltase, etc..

2- Conjugated protein enzymes (Holoenzymes): consisting of a protein part and a non-protein part. The protein part of the enzyme is heat labile and known as apoenzyme. The non-protein part can be:

A) Coenzymes (Cosubstrate):

They are non-protein derived from vitamins .They are loosely attached to the protein part.

B) Prosthetic Group:

It is an inorganic metal which is firmly attached to the protein part of the enzyme.

Forms of enzymes:

i) Zymogens (Proenzymes or Preenzymes):

Enzymes secreted in inactive form especially proteolytic enzymes to prevent destruction of the proteins of the cells that synthesize them. EX:

PH changes: Pepsinogen ⟶ pepsin.

ii) Zymase:

An enzyme that is secreted ready for action, such as salivary amylase.

iii) Isoenzymes or isozymes:

Enzymes that catalyze the same reaction but differ in their structure, properties and activity. Ex: creatine kinase (CK) occurs as three isoenzymes. Each isoenzyme is a dimer composed of two polypeptides, called B and M subunits. CK1=BB, CK2=MB and CK3=MM.

Enzyme Specificity:

There are 4 types of specificity:

Absolute specificity: Here the enzyme acts on one substrate only e.g. arginase acts only on arginine, and histidase on histidine.

Group specificity: In this type, the linkage and another part of the substrate must be of the right type. Examples:

Trypsin hydrolyzes peptide bond whose COOH group belongs to of one of the basic amino acids only.

Relative specificity: The enzyme acts on substances which are "similar" in structure, and posses the same type of bonds e.g. lipase which hyhdrolyzes ester bonds of "lipids"only.

Stereo specificity: Includes.

Optical specificity:

Enzymes of glycolysis act on D-sugars only.

Amino acid oxidase acts on L-amino acids only.

Cis-trans specificity: e.g. fumarase enzyme acts on fumaric acid but not maleic acid.

Location within the cell:

Enzymes are distributed among many organelles within the cell. Some are founds in:

a- The cell-membrane (particulate)

b- Others are floating in the cytosol (soluble)

c- Some are found in organelles as the mitochondria or in the nucleus.

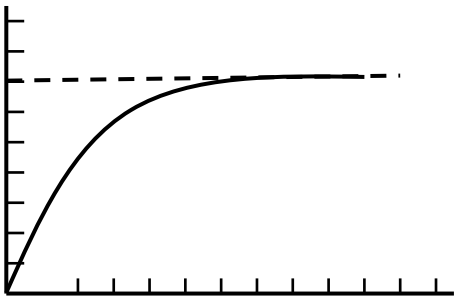
Factors Affecting Enzyme Activity:

1- Nature of the Enzyme (e.g.Isoenzymes).

2- Substrate Concentration:

The rate of an enzyme-catalyzed reaction increases proportionally with the substrate concentration; until a maximal velocity (Vmax) is reached.

The leveling of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme

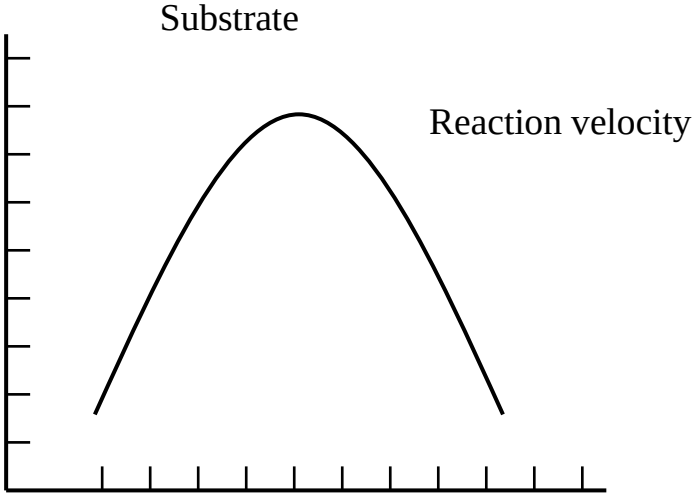


3- Temperature:

HCL

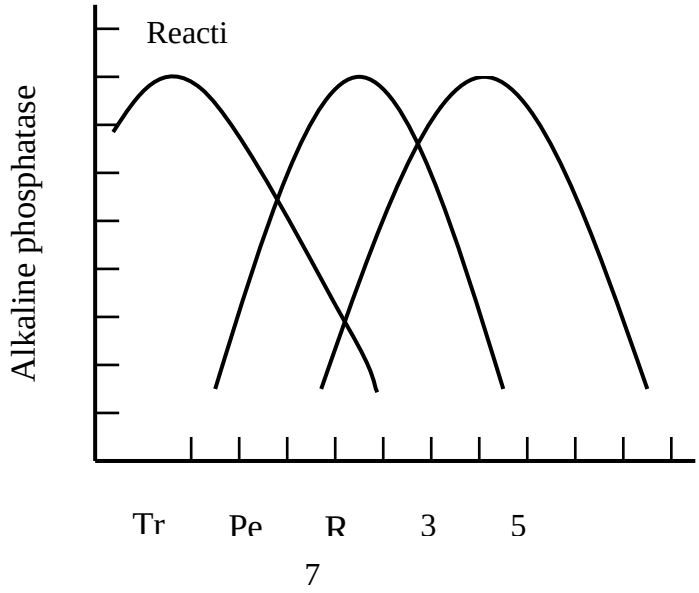
The reaction velocity increases with temperature until a peak velocity is reached. This increase is due to the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.

Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme.



4- pH:

pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the [H+] at which the enzyme functions in the body. For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment.



5- Enzyme Concentration:

The velocity of the reaction is directly proportional to the concentration of the enzyme. Further increase in the enzyme concentration will not increase the velocity of the reaction (within limit).

6- Concentration of Reaction Products:

When the products of the reaction are removed as fast as they are formed, the reaction would be 100% complete.

7- Effect of Time:

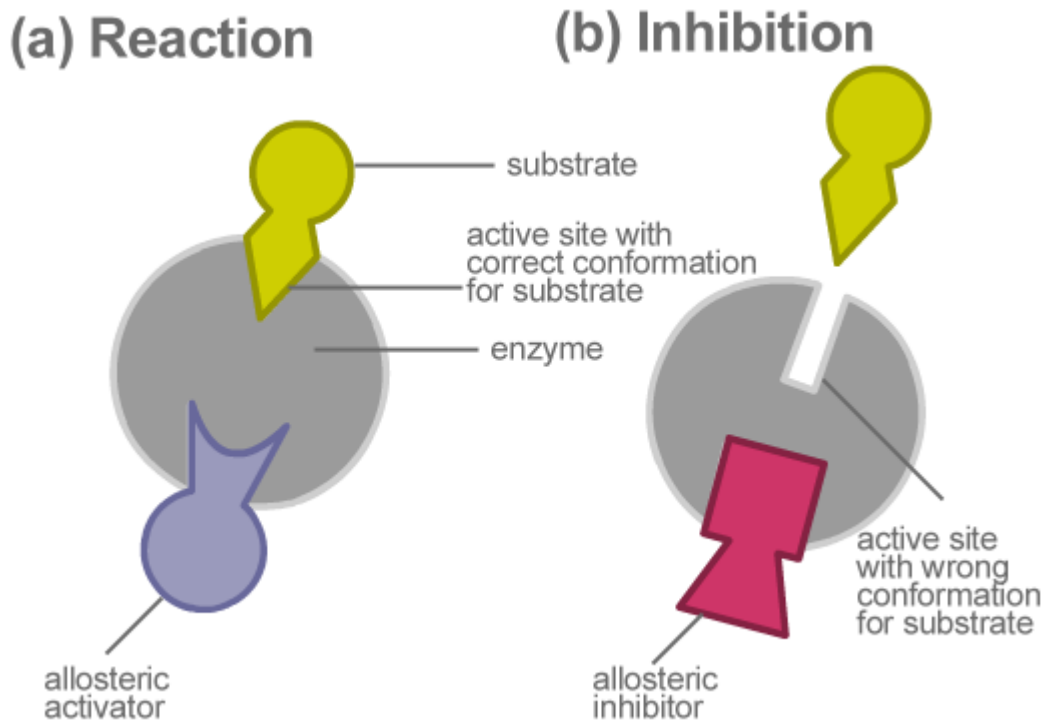
As the reaction proceeds, the velocity of the reaction decreases due to decreased concentration of substrate, and increased concentration of products, which tend to reverse.

8- Activators:

They are inorganic ions which increase the activity of enzyme. Chloride ions activate amylase, magnesium ions activate kinases and calcium ions activate thrombokinase.

9- Inhibitors:

The activity of enzymes is inversely proportional to its inhibitor concentration.



Enzymes in clinical diagnosis

Some enzymes work intracellularly and have no physiologic use in plasma. Their level is very small in healthy individuals. The presence of elevated enzyme activity in plasma may indicate tissue damage that is accompanied by increased release of intracellular enzymes. Some enzymes show relatively high activity in only one or a few tissues. The increase in plasma levels of these enzymes reflects damage to corresponding tissue. Here some of these enzymes are listed:

Transaminases: glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) mainly present in liver. GOT also present in heart. Increased GPT level alone or in association with GOT indicates liver disease especially hepatitis virus, while the increase in GOT alone indicates myocardial infarction.

Diagnosis of myocardial infarction: In addition to GOT, it depends on CK2 (MB) isoenzyme in 4 to 8 hours following onset of chest pain and reaches a peak activity at approximately 24 hours.

Quiz Activity

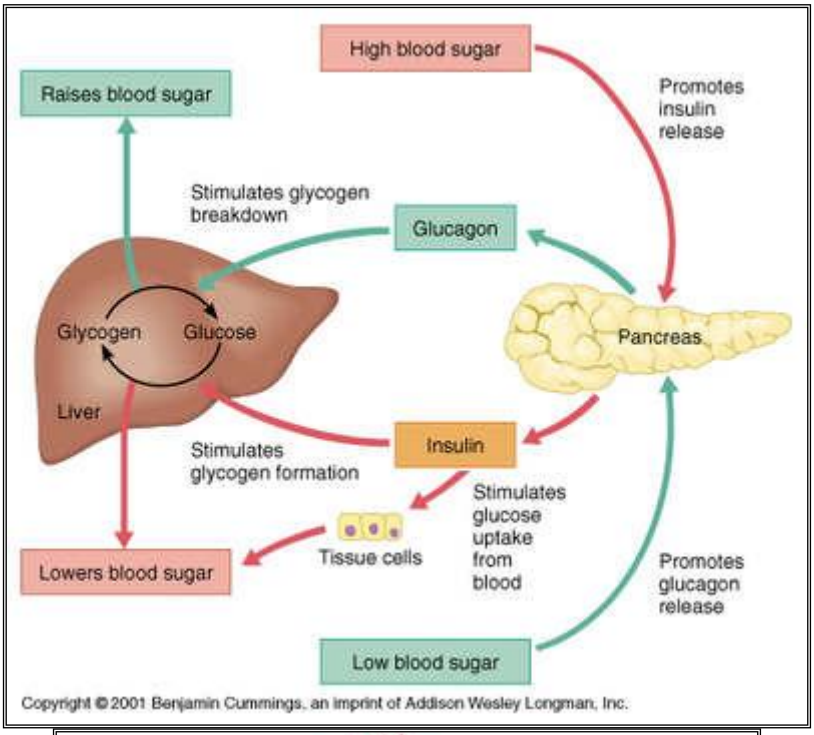
Complete the following statements:

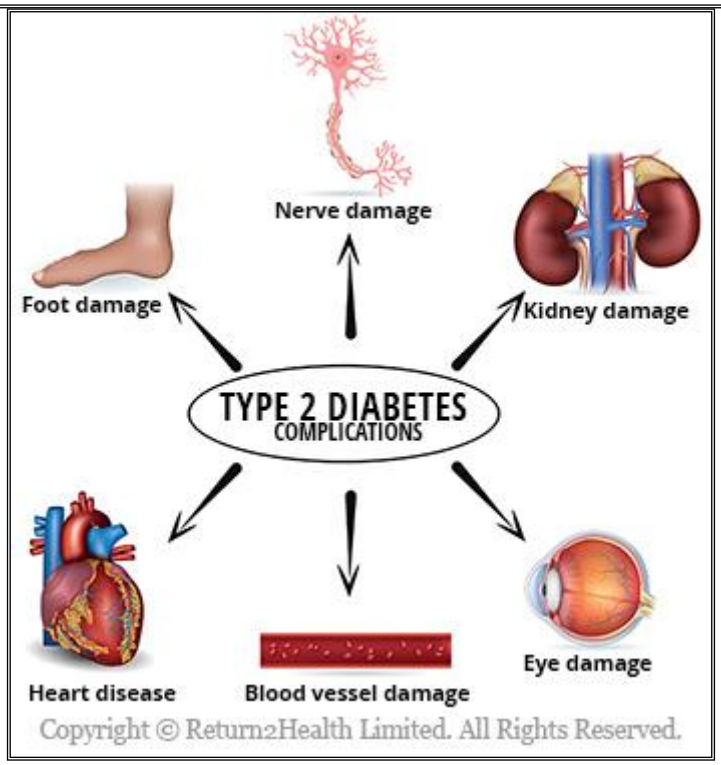
- Coenzymes are derived from ....., while prosthetic group is .....
- ..... are enzymes that catalyze the same reaction but differ in their structure, properties and activity.
- Trypsin hydrolyzes peptide bond whose COOH group belongs to of one of the ..... amino acids only.

Clinical pharmacy - Level 1

Practical Cell Biology- Section 8

Glucose detection test





serum glucose level

Normal levels:

-Fasting: 70-110 mg/dl (Mmole/l*18=mg/dl ? calculate)

-postprandial: 80-140mg/dl

1-Diabetes mellitus:

Causes:

1-Insufficent insulin.

2-Increase in anti-insulin hormones: adrenaline, thyroxine and growth hormone.

3-Excessive treatment with glucocorticoids.

4-High intake of CHO in diet.

Characters:

1-Hyperglycaemia: increased blood glucose level than normal (Fasting: 70-110 mg/dl, postprandial: 80-140mg/dl).

2-Glucosuria: appearance of glucose in urine because its level in blood exceeded the renal threshold of glucose (max capacity of the renal tubules to reabsorb glucose) {180 mg/dl}

3-Polyuria: due to increased blood glucose level which acts as osmotic diuretic.

4-Polydypsia: thirst sensation due to dehydration

5-Loss of body weight.

2-Renal Diabetes:

Causes:

Congenital disease in which patients suffer from low renal threshold of glucose. e.g.: renal threshold becomes 120mg/dl instead of 180mg/dl so if the blood glucose level becomes 140 mg/dl, 120 mg will be reabsorbed and 20mg will be excreted in urine.

Characters:

1-Normal blood glucose level. 2-Glucosuria.

Experiment: Differential Diagnosis of Diabetes

Principle:

glucose $+O_2$+water $\xrightarrow{\text{glucose oxidase}}$ gluconic acid + H_2O_2

H_2O_2 + 4-aminophenazone + phenol $\xrightarrow{\text{peroxidase}}$ Quinoneimine + water

(Rose Red colour)

Glucose oxidase enzyme is specific for glucose so will act on glucose substrate only. This principle could be employed for both qualitative detection of glucose in urine and quantitative estimation of glucose in serum which allows differential diagnosis.

Procedure:

A. Qualitative Detection of Glucose in Urine:

1. Add 100 µL of urine sample to 500 µL of glucose reagent in a Wassermann tube.
2. Incubate for 10 minutes at room temperature.
3. Observe the produced color, If urine sample contains glucose a rose red color would be obtained.

B. Quantitative Estimation of Glucose in Serum:

1. In a wasserman tube add 10 µL of serum to 1000 µL of glucose reagent and incubate for 10 minutes.
2. Measure A sample and then determine glucose concentration using the equation:

Glucose Concentration in sample = $(A_{samp}/A_{stand}) \times Conc_{stand}$ mg/dl

C. According to the results you will be able to differentially detect diabetes -if present- as follows:

Condition	Blood Glucose	Urine Glucose
Normal	Normal	Absent
Diabetes Mellitus	Increased	Present
Renal Diabetes	Normal	Present

Quiz Activity

Complete the following statements:

- **The renal threshold of glucose is defined as .....**
- **Diabetes mellitus maybe caused by ....., ..., ..., or .....**

Good Luck