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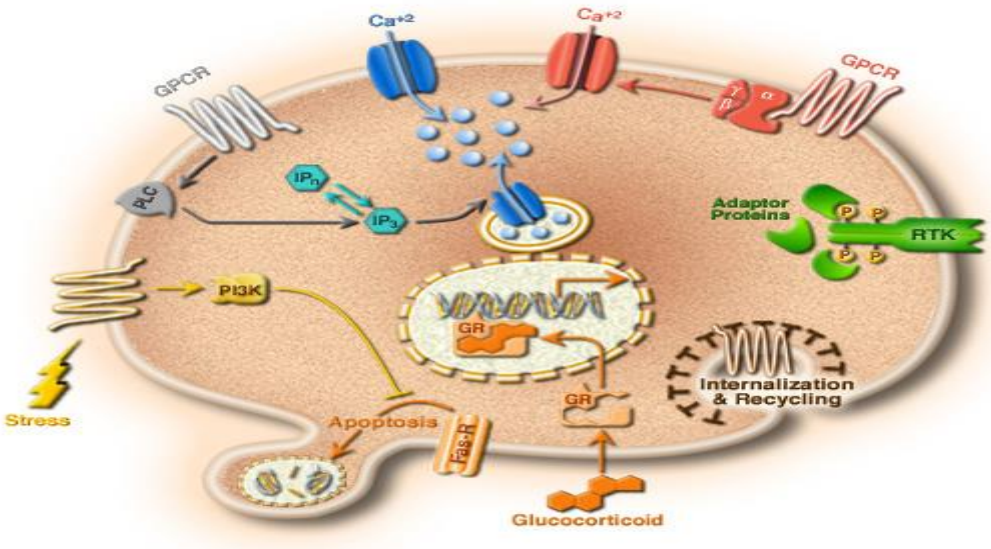
Nature and Life Sciences department

Handout of TD

Subject: Cell biology

Teaching intended for students in the First year of LMD joint trunk

Classes for Natural and Life Sciences



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Introduction

All living things on Earth are made up of cells. The cell is the simplest unit of an organism, and the vast majority of life on Earth. It consists of single-celled microbes. Each cell is like a chemical factory of astonishing complexity, and we have only begun to unravel the mysteries of its inner workings in the past 50 years or so, using modern techniques such as microscopy, biochemistry, and molecular biology. In this the publication, as its title suggests, contains various topics related to the Directed Work course for Cell Biology, which is taught in the first years of the Common Core Biology. The publication contains six directed works. The first directed work includes methods of studying the cell, which in turn is divided into four orientation sessions. The first of these includes light and electron microscopy techniques, while the second session includes Techniques for studying cells and their components or Histochemical and Cytochemical methods While the third includes cell components separation "Cellular fragmentation «The fourth orientation session is about microscopic techniques for studying cells. The second orientation session is titled: Chromatography and its types. The third directed work included the study of animal tissues in one-session and plant tissues in another guidance session. The fourth directed work was the cell wall, its structure, role and how it is formed. The fifth directed work was entitled Protein synthesis in eukaryotes and prokaryotes. Finally, the sixth directed work is the study of the makers. And finally The works conclude This printable contains a series of questions of various styles. In the form of exercises, its aim is to renew the student's mind-set – or the reader in general – and to arouse in him the desire to discuss, recall and review topics. And it is a paragraph These questions are very important to measure the student's level of understanding and comprehension of the content of the topics presented.

I have tried to include in this publication many of the basic and necessary concepts and information, but not all of them that a first-year university student in the common core biology course studying the cell biology course should be familiar with, so that it will be a suitable reference for him. I have also tried to have this reference written in an easy and smooth style and with a clear presentation, far from complexity. I have relied on attaching each directed work with figures and documents to consolidate the concept of what I am explaining. I hope you are my destiny this publication is for the university library and its students... and my utmost hope is that it will be a modest scientific addition that will raise its levels to benefit all people.

D: Bekhouche Naima

TD 01: Methods of studying the cell

The class the Guidance 1: Light and Electron Microscopy Techniques

That our notes on the items in this the world, do not become notes Scientific, unless accompanied by a question scientific, for example let's take This question is one of the important questions that scientists have asked in the past...

Do all living things share a common structural unit basic?

He appears At first glance, the answer to the previous question did not seem to have scientific value, but at the present time it has become clear to us that the answer to this question has great value in the advancement of science and medicine. Man seeks knowledge for the sake of knowledge, whether this knowledge has scientific value at the present time or not. This knowledge may have scientific value in the future. Today we know that the cell is the basic unit of structure in all living organisms. It is worth noting that scientists did not reach this result except after research and experiments that took decades, and after the great progress in the manufacture of microscopes, which enabled us to see many things that cannot be seen with the naked eye. If you look with the naked eye at a human being and a tree Orange, when I found any similarity between them in Composition, but using a microscope you realize that both of them are composed of cells that are very similar in Composition.

Requires viewing cells use examination microscopey for small its sizes (10 to 100µm) alive the it is used to study its structure. Light microscope (photonic) and electron microscope.

1. Optical microscopes (MO)

1.1. Bright field optical microscope

- **Its working principale**

The bright field microscope uses visible light as a light source. It is equipped with three systems of transparent glass lenses. (Shape1):

- Objective lens (the object) it gives an initial magnification and presents a real image.
- Eyepiece (the eye) Provides secondary magnification and allows for the formation of a magnified, imaginary image. The real image presented by the objective lens.
- Condenser (the capacitor) concentrates light on the sample (controlled by the diaphragm).

And so, we are the separator power (separation limitla) as the smallest distance between two adjacent points that we can distinguish with a microscope, as the resolving power of the classical light microscope is estimated at about µm0.2 the magnification can reach up to 2000.

Calculate the magnification power: To calculate the total magnification of the object to be examined under the microscope, follow the following method:

1. Note the magnification power of the eyepiece lens by reading the number written on it, which is usually (10) times (10x).
2. Note the magnification power of the objective lens by reading the number written on it, which varies according to the objective lenses. Let us assume that you used the largest objective lens, whose magnification power is usually (40) times (40x)

The total magnifying power of the object = eyepiece lens × objective lens

10x× 40x= 400x

1.2 Types of light microscopes

There are different types of light microscopes (Table 1), each with its own optical composition. Designed to allow observation of cells under certain conditions.

Table1: The most commonly used light microscopes.

It is used in	Type
Viewing the intracellular structures after staining.	bright field light microscope(MO on clear background)

Note unstained samples and live and moving cells.	Dark field light microscope(MO in black)
Highlighting differences in refractive index and contrast. This means making it easier to see the movement of cells and their structures by increasing the differences in refractive indices. (For light rays, photons) and the contrast of these structures.	Phase contrast microscope(MO in phase contrast)
View sample after labelling with fluorinated materials the fluorescent mark the structures and components of giant molecules.	Fluorescence microscope(MO to fluorescence)
Viewing cells in cell culture media (cellular culture).	inverted light microscope(MO reversed)

2. Electron microscopes (ME)

- **Its working principle**

It is a bit similar to the principle of operation of a light microscope, except that:

- Photons were replaced by electrons.
- Electromagnetic lenses instead of glass.
- The resolving power of the electron microscope is higher than that of the light microscope and is estimated at 0.2nm (That means 1000 times' higher than MO).
- So magnify in ME it can reach 500,000 vs 2000 in MO.

2.1Transmission electron microscope (MET)

In this type, electrons penetrate the sample treated with heavy metals. A clear, magnified image resulting from differential absorption appears on its screen. (The divergent) electrons are attracted to different components of the sample.

This microscope consists of (Figure 2) basically from:

- Source of electrons (distorted metal wiren even glow at a very high temperature in an evacuated tube). Delighted the electrons in this vacuum are moved by applying a different potential from 10 to kV 100.
- Evacuated tubular track.
- Electromagnetic lenses (In rollsdes bobines) allows the path of electrons to be directed.

2.2. Scanning electron microscope (MEB)

Allows the specimen to be viewed in a three-dimensional (semi-3D) for the outer surface. Therefore, it is used in studying the surfaces of large samples after treating them with reflective or electron emitting metallic materials such as platinum silver and gold.

The flow of electrons sweeps the surface of the sample, thus the secondary electrons emitted or reflected from the sample surface are what form the image of the sample on the screen MEB.

3. Comparison between light and electron microscopes

The most important differences are summarized in the following table:

Table 2: The most important differences between the light microscope and the electron microscope

ME	**MO**	
Tungsten wire heated to glow	Electric lamp	**Power source**
Free electronsnn A sample is placed in an evacuated tube to be immersed in the air.	Photons	**Radiation**
Electromagnetic lenses	Glass lenses	**Photosystem**
0.2nm (2Ä)	0.2µm	**Separating power**
300 to 800 Ä	2 to 10µm	**Sample thickness**
500,000 (MET)	40 to 2000	**Zoom**

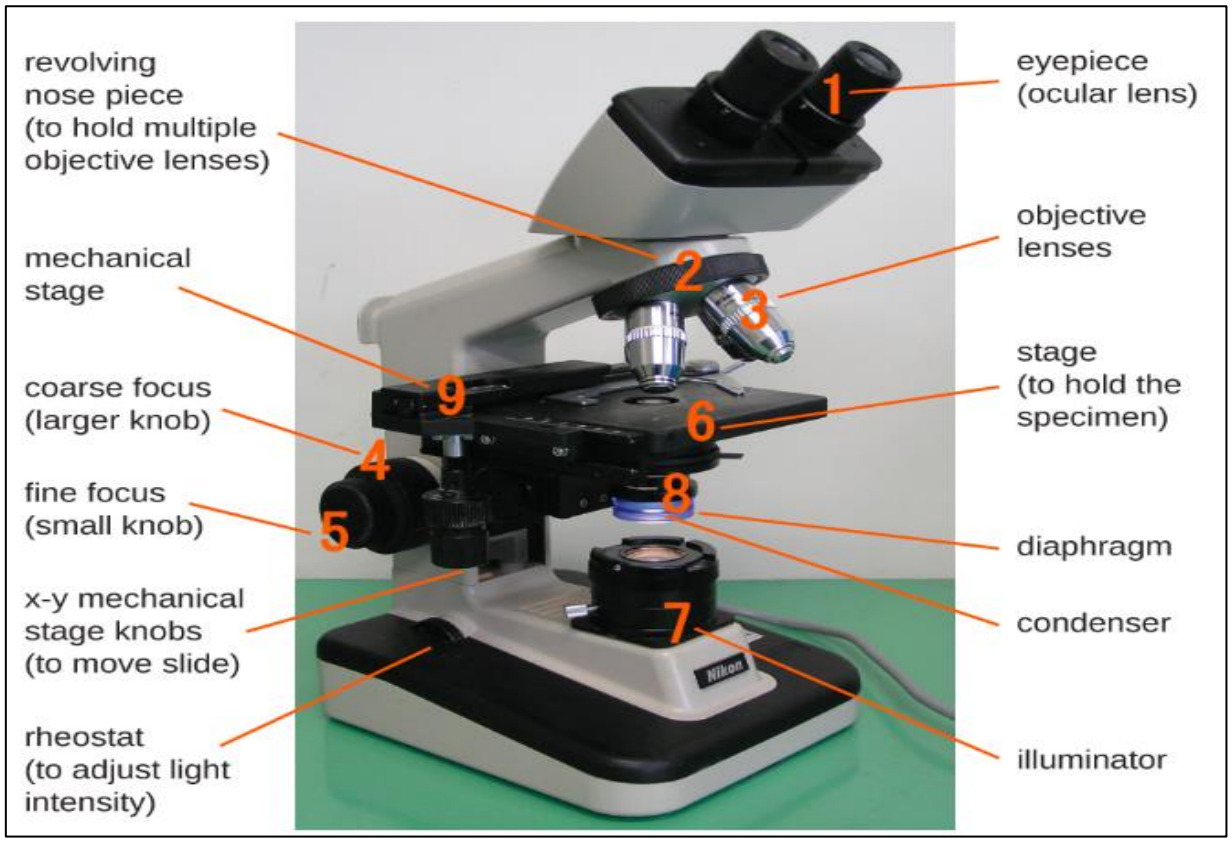


Figure 1: Schematic diagram of a bright field optical microscope (binocular eyepiece) and its components.

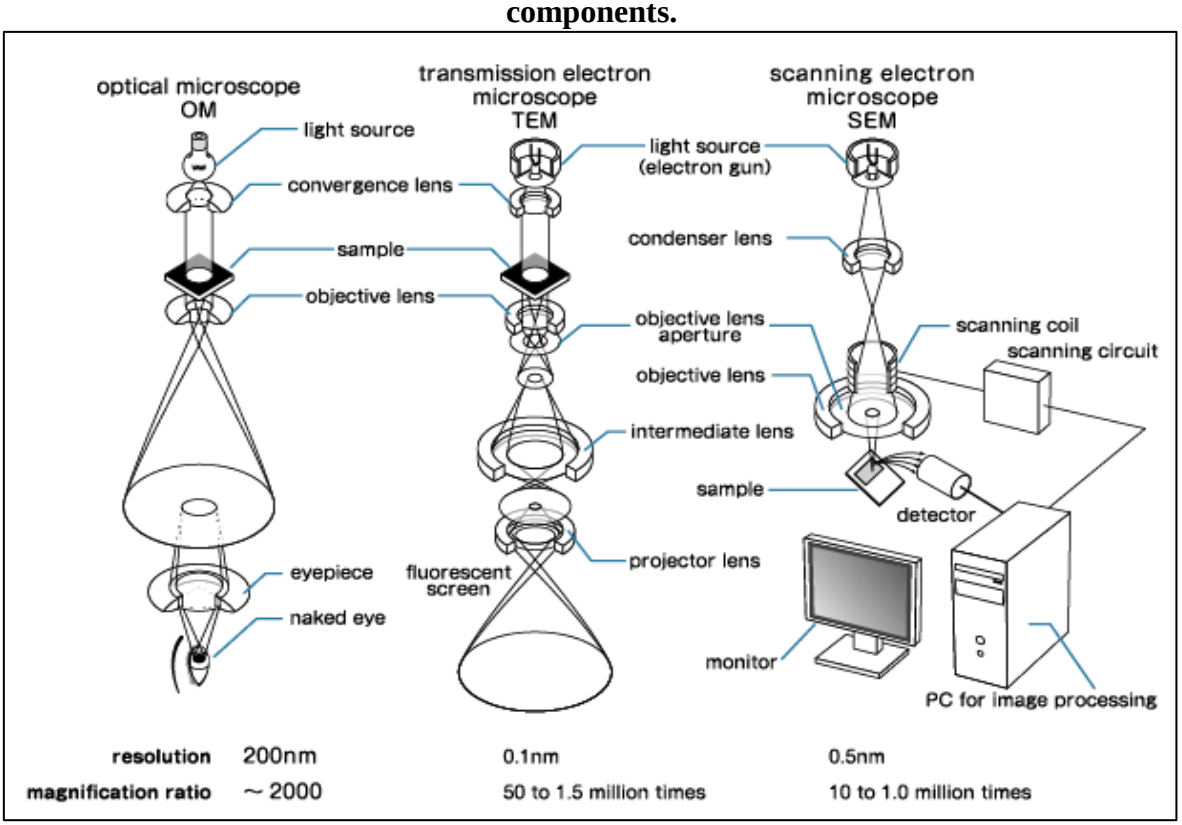


Figure 2: Working principle of optical microscope, transmission electron microscope and scanning electron microscope

Figure 03: Images of transmission electron microscopes (right) and scanning electron microscopes (left).

TD01: Study methodscell

Guidance share2: Techniques for studying cells and their components or Histochemical and Cytochemical methods

Methods that allow determining the nature and location of the chemical components of the cell. Among these methods used are:

1 .Enzymatic Histochemistry technique

The terms Cytochemistry and Histochemistry are used to identify or determine the location of cellular structures in tissue sections by using the enzyme activity in these structures. That is, depending on specific chemical reactions, the mechanism of enzyme action in histochemistry includes the following:

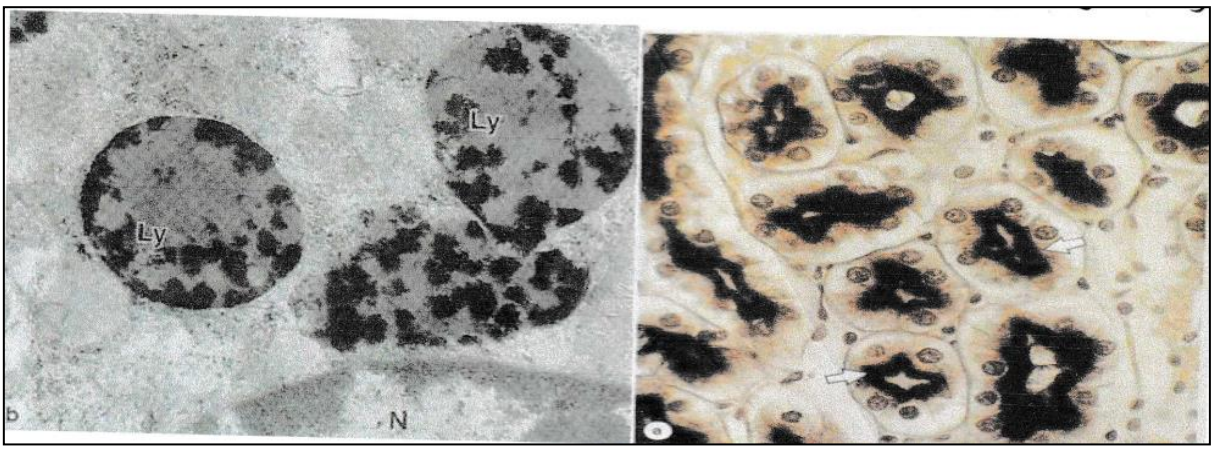
*The tissue sections are immersed in a solution containing a reagent for the enzyme whose location is to be determined.

* Allows the enzyme to react with the reagent.

* The tissue section is then placed in a medium containing a labeled substance.

* The labeled substance reacts with the molecule resulting from the enzyme-reagent reaction.

* The reaction produces an insoluble substance that precipitates over the areas containing the enzyme and can be seen under a light or electron microscope. Examples of enzymes that can be detected by histochemical methods include phosphatase enzymes using lead phosphate or lead sulfate as a substance that reacts with acid phosphatase in the particles. Status (Figure 01).



(a) Photomicrograph of transverse sections of kidneys treated with Gomori's technique for alkaline phosphatase showing strong activity of this enzyme on the apical surfaces of cells in the tubule lumen (arrows).

(b) Transmission electron microscope image of kidney cells showing the localization of acid phosphatase in three lysosomes LY) Near the nucleus (N The black material in these structures represents lead phosphate that precipitates at sites of enzyme activity at 25,000 magnification.

Figure 01 : Enzymatic Histochemistry

2. Technology Histochemistry and Cytochemistry

These techniques are used to detect some special materials in tissues and cells, depending on biochemical reactions that allow the different components of these tissues to be shown in situ. These components may be lipids, sugars, proteins, nucleic acids, or heavy metals. An example of this is the detection of glycogen and protein aglycones in the Schiff's test involves the oxidation of certain polysaccharides with periodic acid, which results in a red color. Brachet which is used to detect nucleic acids by placing the sections in a stain. Contains methyl green which colors DNA and the pyronine who colors ARN.

3.**Immunohistochemistry techniques**

This technique aims to detect the locations of many proteins. It depends on the interaction between the antibody and the antigen. It is done by labeling the antibody with a suitable marker and incubating the tissue section with the labeled antibody so that the labeled antibody binds to the protein X (antigen) and then a microscope is used to examine the section to see where the X protein is located. And its properties. Antibodies learn by molecules that allow them to be located within the cell. These parameters may be : (Markers)

*Fluorescent or rhodamine colored fluorescent.

*Radioactive isotopes.

*Peroxidase or Phosphatase, Alcaline, Enzyme Immunoglobulins

Depending on the information used there are 3 types of immunological techniques.

3-1 Enzyme immunoassay

Known as Enzyme Linked Immuno Sorbent Assay) ELISA in this reaction, the antigen or antibody is identified by an enzyme that allows the conversion of the uncolored reaction substance (substrat) into colored products.

3-2 Radioimmunotherapy.

Also known (Radio-Immuno-Assay) RIA relies on the use of a radioactive marker (Marqueur-Radioactif). This technique is applied less frequently because its use by biologists requires them to obtain accreditation and for The cause of the radioactive waste problem.

3-3 Immunofluorescence technique

Immunofluorescence uses specific antibodies and fluorescent dyes (Fluor-chromes) no Appeared biological molecules in cellular or tissue preparations, the study requires the use of microscope Fluorinated (Mo a fluorescence).

- **Stages**

In the cell. The following steps must be followed : P to detect matter.

- Take protein P from a mouse and injected into another animal (rabbit) to produce antibodies Anti P
- Fluorinated material fixation fluorescence on antibody P.
- After adding fluorescent antibodies to the cell preparation, they attach to the antigen generator P forming a flurescent antigen-antibody Complex.
- Observation under ultraviolet light with a fluorescence microscope allows the position of P thanks to the fluorescence emitted from fluorescent (Figure02).

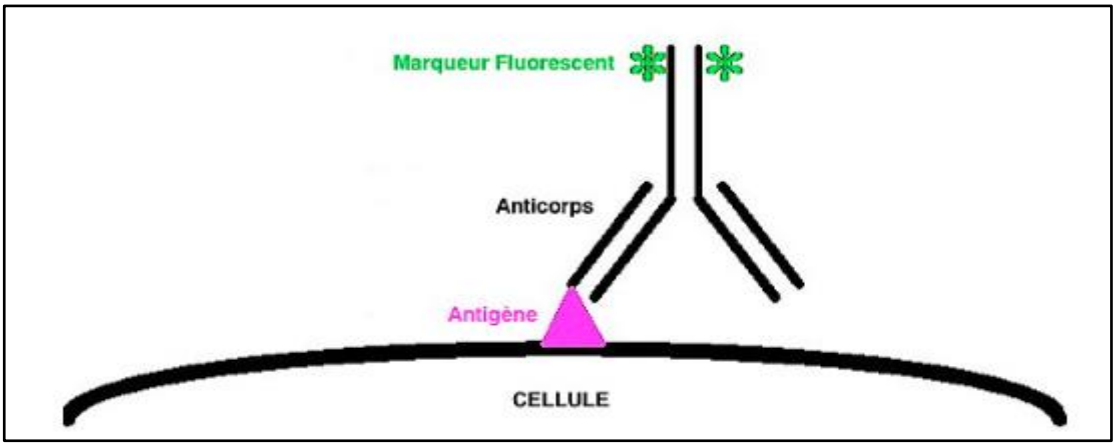


Figure 02 : General principle of immunofluorescence

Immunofluorescence can be direct or indirect.

a. Direct method :

In this way the medium to be detected is directly coupled to the labeled antibody.

b.Indirect method :

The teacher is connected (The marker) with a second antibody (body Secondary antibody) (specific to immunoglobulins of the type producing the primary antibody) (Figure 03).

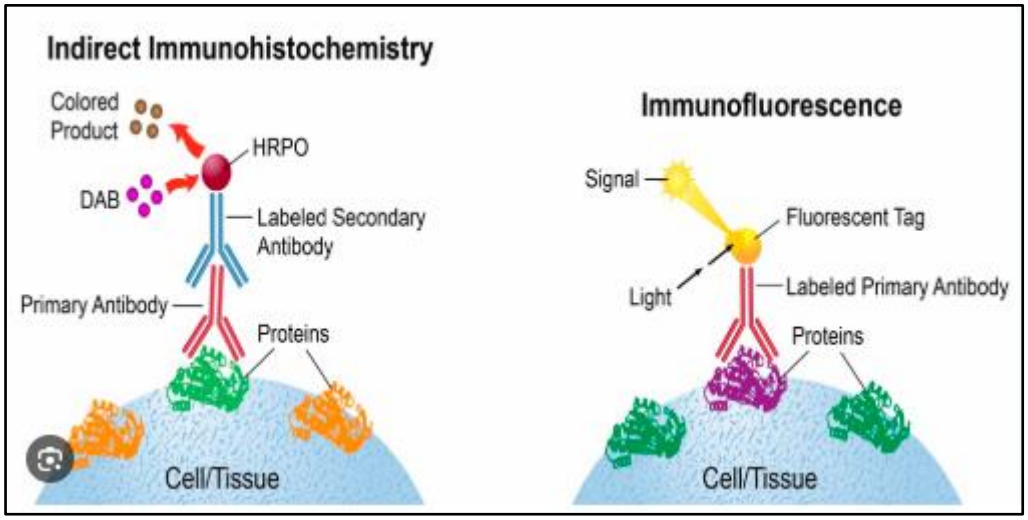


Figure 03 : Principle of direct and indirect immunofluorescence

4- In situ hybridization technique

Hybridization is the bonding of two single strands of nucleic acids.DNA to DNA or RNA to DNA or with ARN to ARN. Which recognize each other if they are complementary chains of nucleotide mechanisms.This technique is used in detection position certain sequences revealed from nucleotides ADN or knowledge of cells containing messenger RNA to the study of gene expression or the location of a gene on a particular chromosome, first you need to disassemble the DNA inside the cell is heated to become single-stranded and then becomes ready for hybridization with a single piece of DNA or RNA called a probe (sondes) complementary to the sequence to be detected or to determine its location.Tag the touch for arefor nucleotides containing a radioactive isotope the solution containing the probe is placed on the sample for a period of time.Certain until the hybridization process occurs and then the location is revealed the probe is placed in the sample by autoradiography such as tritium H_3 or phosphorus (P_{32} P_{33})or sulfur (S_{35}) these are some of the effective radioactive isotopes.(Figure 04).

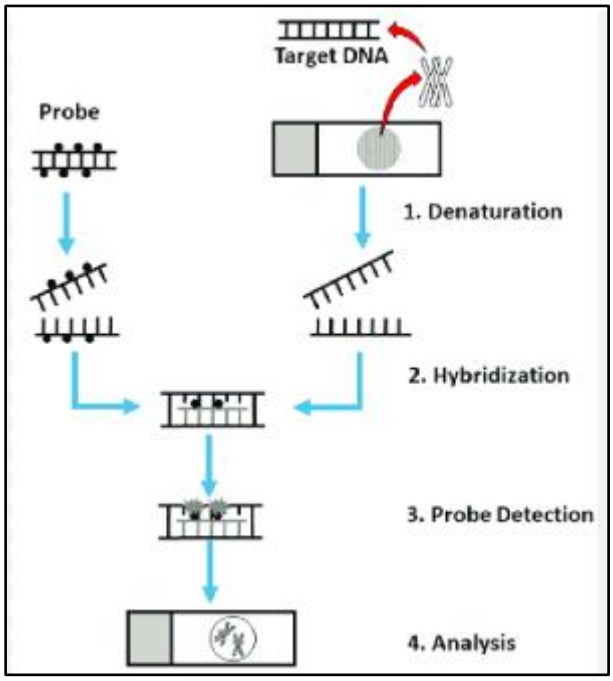


Figure 04 : In situ hybridization technique

5. Flow cytometry technique

This technique is used to study some cells and their granulation, this is like blood cells suspended in plasma, or cells isolated from tissues. The cells are placed in a suspension or liquid, and are quickly passed one by one in front of a laser beam. This technique allows the measurement of the dimensions of the cells or their granularity (the percentage of granules in the cytoplasm). This technique is used to monitor the development of the disease in people with AIDS (Figure 05).

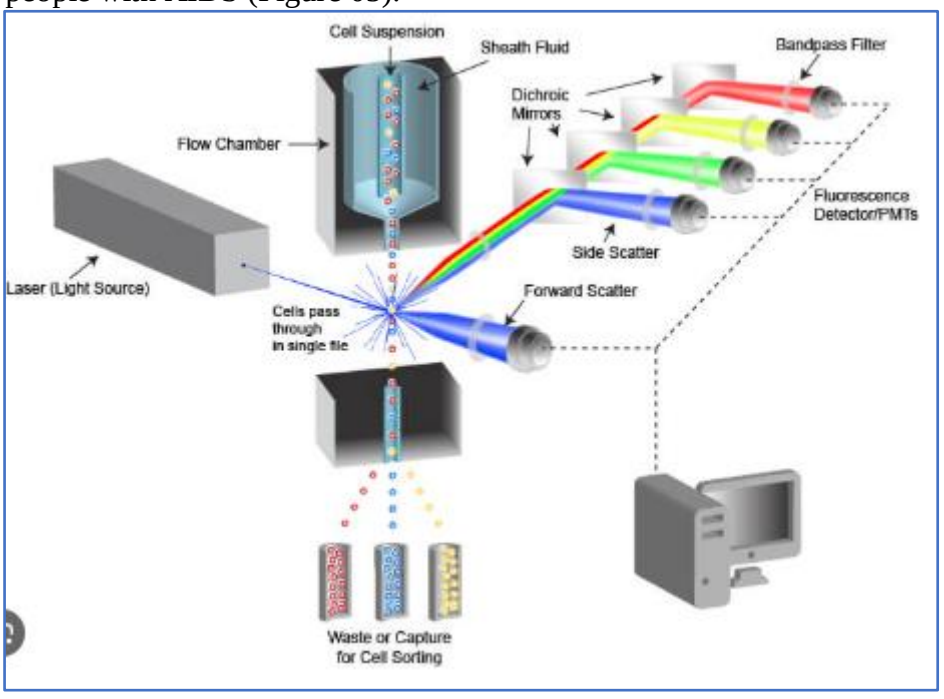


Figure 05 : Flow cytometry technology

6-Technology Tissue autoradiography

Autoradiography is based on the use of a radioactive metabolite (a metabolite containing a radioactive atom) that emits radiation that is detected by photographic emulsions. This technique allows the location of a molecule to be determined and its path or transformations to be followed within the cell.

Stages :

-Injection of a radioactive substance (usually containing H3 or C14 = tritium) in the organic or isolated form can also be Incubate cells in a medium containing this radioactive material.

-The animal is killed and sections are made in the tissue to be studied, or cultured cells are taken, the sample is fixed and the unincorporated radioactive material is removed by washing.

-Cover the sample with a photographic emulsion (a mixture of gelatin and silver crystals of BNAG«Affected by radiation the treated sample is kept for several days or several weeks in the dark during this period.

Represented by:

1- The record (cell) was labeled using radioactive isotopes, which are characterized by emitting specific radiation(α,β,γ) .
2- And Apply a layer of photographic emulsion containing silver compounds such as gelatin bearing silver bromine crystals. AgBr on sensitive plate (Panel Photographic (photographic film).
3- The radioactive elements in the preparation emit radiation that affects the silver bromine, where the silver ions are reduced by the emitted radiation to metallic silver suspended in the gelatin.
4- The areas corresponding to the areas where the radioactive element is present in the report appear as black granular spots in the form (Figure 06).

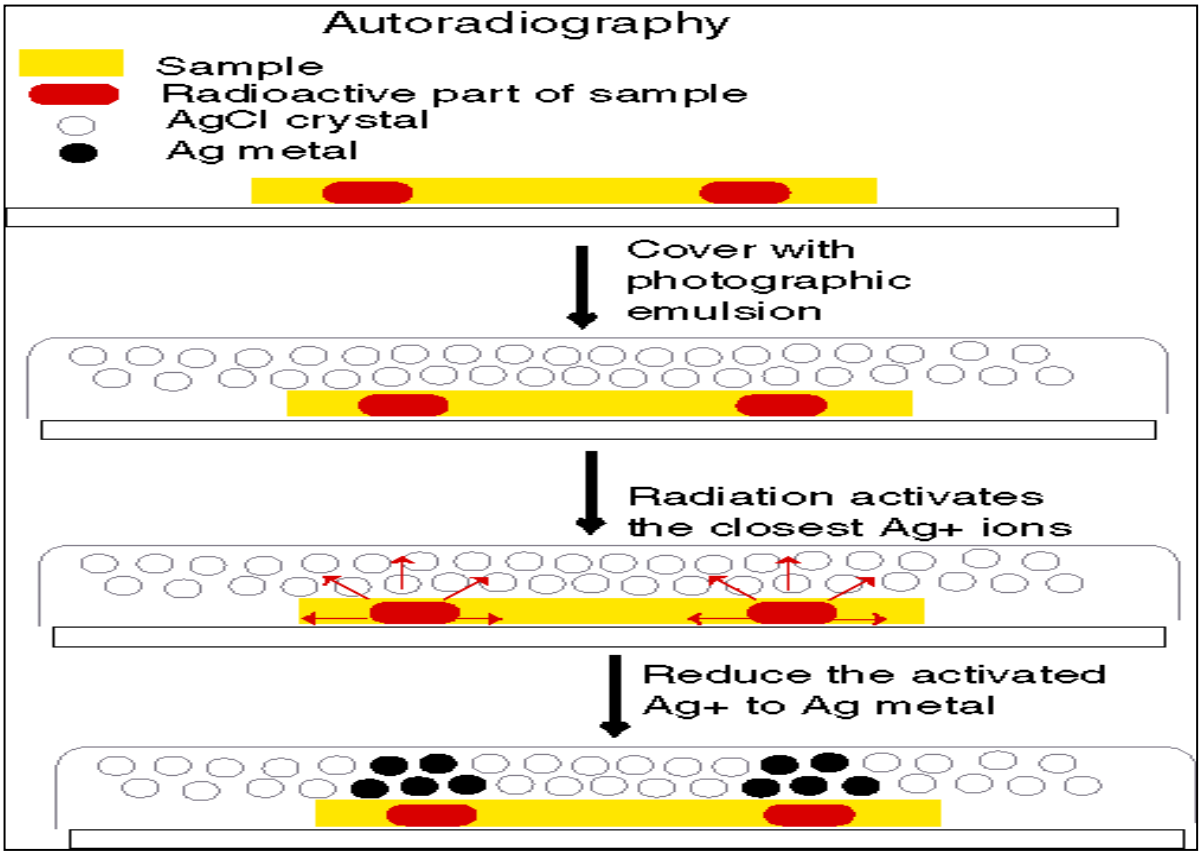


Figure 06: Tissue autoradiography

7-Dialysis:

A process that involves removing salts from their solutions in general. Dialysis is often used to remove salts from proteins after the precipitation process with these salts special tubes are used for this purpose called (Dialysis tubes) are thin tubes resembling sulfur leaves designed to contain holes with diameters measured in angstroms. The diameters of the holes in dialysis tubes vary, which qualifies them to be used to trap proteins depending on their weights. Molecular (diameters) these bags also allow the passage of salt molecules from the solution(Protein-salt) out and replace Solution-water distilled or organized-constantly salt molecules are completely removed from the protein (Figure 07).

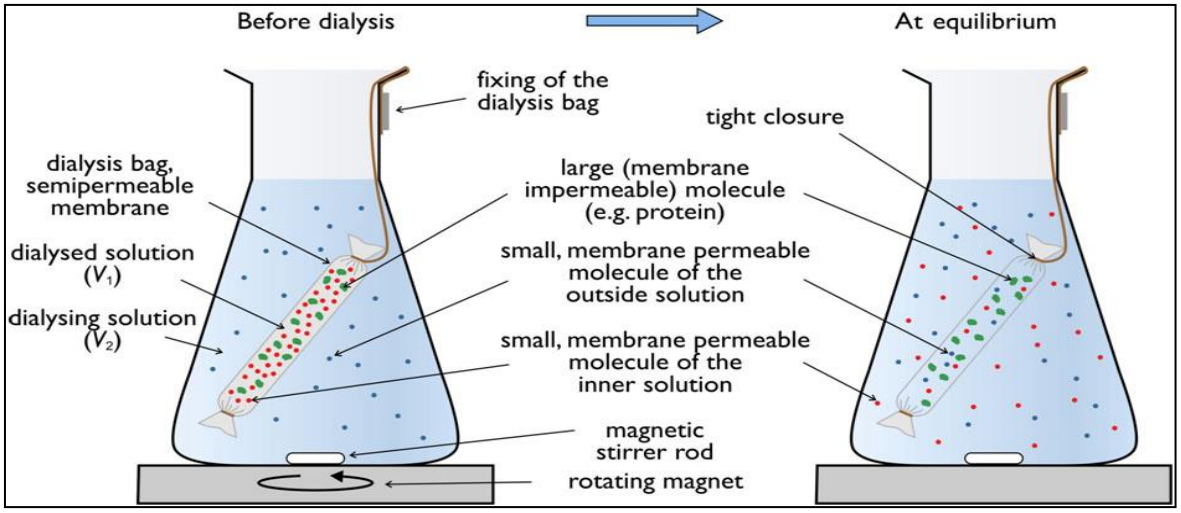


Figure 08: Dialysis

8. Freeze fracture technique

This method is used to study membranes. Biological this method involves rapidly freezing the sample and then cutting it in a vacuum chamber at (-100 degree) the cutting knife cannot cut the model under these conditions, but it works to break the model along weak lines in its natural cohesion, such as the middle part of the plasma membrane, and the broken piece is left in the gas vacuum for a suitable period to allow some water to evaporate from it exposed surface outwards, which causes the soft areas of the model to shrink slightly. The exposed surfaces are shaded with a very thin layer of heavy elements such as carbon and platinum to provide the appropriate contrast. The model is then removed with acids, leaving a copy of the element. Metal copy examined by scanning or transmission electron microscope.

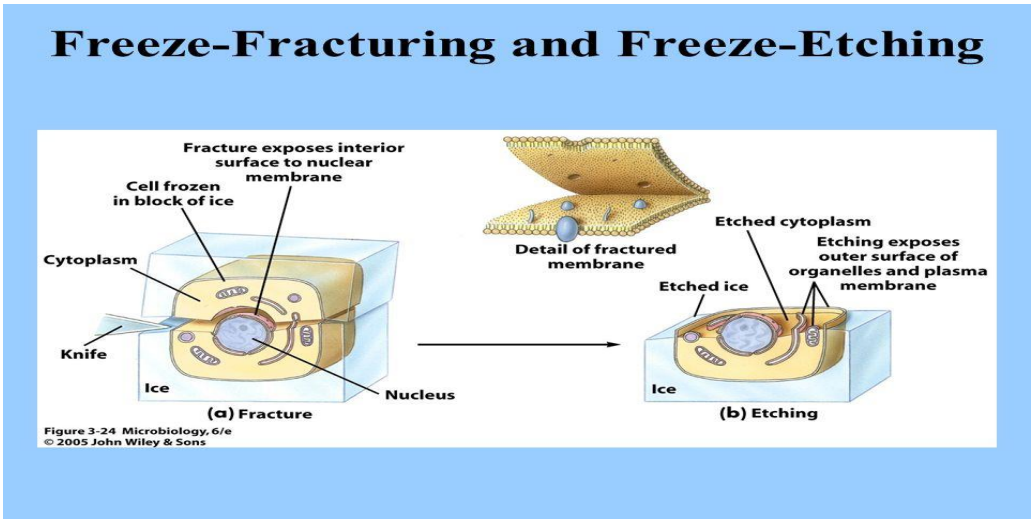


Figure 09: Freeze fracture technique

example: Cells that produce large amounts of proteins or those that produce small amounts in a tissue can be identified using a radioactive amino acid by knowing the number of silver particles formed on top of the cells, which is directly proportional to the density of production Protein.

9. Technology Lectin Texture Chemistry

This technique relies on the use of lectins, which are proteins of animal, plant or bacterial origin that can recognize and bind to sugar compounds from cellular components, especially sugars that enter in the structure of plasma membranes.

10. Methods Study enzyumiya

Enzymes are called Enzymes on compounds of a protein nature thatmediate to speed up chemical reactions, the word enzyme is from the Greek (en: inside, Zyme: yeast) thousands of enzymes have been discovered in biological media and several hundred have been isolated and I studied.Enzymes play a very important role in the vital activity of all living organisms, whether plants, animals or microorganisms. Minute and a single living cell contains about 1000 different enzymes, each of which is specialized in accelerating a specific chemical reaction. Enzymes are divided into two sections:

Simple enzymes are made up of only amino acids, and complex enzymes are made up of a part Protein and an additional organic or inorganic part known as a co-enzyme protein and an additional organic or inorganic part known as a coenzyme co-enzyme some enzymes are in the form of precursors called zymogens.

- **You are aware the effectiveness of enzymes in laboratory diagnosis**

Modern medicinal chemistry research in the diagnosis of many diseases depends on determining the activity of blood serum enzymes, because enzymes are usually concentrated in the cells of the living organism where they are produced. Her work media in the case of disease, certain enzymes leak into the blood serum from the affected cells and tissue. More than forty enzymes have been found in human blood, the activity of which undergoes many changes when infected with certain diseases. Blood enzymes are an accurate detector in diagnosing various pathological conditions affecting the blood, liver, heart, and a pancreas, and in the discovery of some cancerous tumors and others, the most important blood serum enzymes that have entered into practical application in biochemistry laboratories are the following:

-Amylose activity in serum is increased above normal, indicating the early stages of acute pancreatitis and blockage of its ducts.

-Lipase activity is increased in serum in cases of pancreatic disorder (acute pancreatitis).

-Alkaline phosphatase activity in serum is increased in osteomalacia, and hepatitis.

- Acid phosphatase, which is elevated in serum, indicates prostate cancer. Ten say and its inflammation.

- Transaminases that are more active in liver disorders (her agency for) Liver and heart.

TD01: Study Methods Cell

Guidance Lesson 3: Separating Cell Components "Cellular fragmentation"

1. Cellular fragmentation

It is the separation of internal components (such as organelles) from each other for a specific type of cell, and is done in fragmentation Cellular:

- Separating the cells to be studied from other cells present with them in the same tissue.
- The cell membranes of these cells are broken down so that their components (such as organelles) are released and exit them cell.
- Separating the components of a cell from each other (such as separating the nucleus from the rest of the cell) to study this to bite it is easy and accurate. The importance of cell segmentation lies in the following :
  - Study of each of the different types of cells onlimit if found intexture it contains other types of cells.
  - Study the D the different components of this particular type of cell to be studied. This study includes studying the location, function, and components of organelles.

- The study of cellular molecules and the biological processes that occur within the cell.

1.1Cellular fragmentation stages

Stage 1: Separation of different cells:

There may be more than one of the different cells present in a single tissue and separating these cell types from each other will help to obtain a pure sample containing only a specific type of cells to be studied.

Stage 2: Cell membrane disruption and filtration of sap Cellular:

Breaking or destroy the required cells by breaking their cell membranes, which leads to the release of materials and the existing organelles inside these cells to the surrounding external environment.

Stage 3: Separation of cell components:

At this stage, the released organelles are isolated or separated from each other, and the separated organelles and the cellular materials released as a result of this process, such as enzymes, amino acids and sugars, can be studied. The steps of cell fractionation can be summarized as follows:

1.1.1 Separate the required cells by:

-To choose fabric the required one contains the specific type of cells to be studied.

-Separate cells from other cells in fabric using triglyceride enzyme and collage. Responsible for the breakdown or destruction of proteins and helps remove unwanted protein tissues.

-The cells are also separated from blood impurities and other components. fabric by adding chemicals that prevent blood clotting: Chelating Agents such as EDTA (Ethylenediaminetetraacetic Acid) these substances prevent clotting enzymes from binding to Ca++ and Mg++, and therefore if blood is present in the sample to be analyzed, it will not clot and no clots (blood clumps) will form, so that a sample can be obtained in the end naqy cells and healthy.

1.1.2Grinding process: it is the process of breaking down cells, in which:

- Breaking their cell membranes, which leads to the release of the contents of these cells, as the cell organelles become free this process is done by osmotic pressure, which destroys the cell membranes and keeps the internal organelles intact. We obtain a homogeneous cell powder, and this suspension is divided by one of the following treatments:
- **Mechanics**: Crush by piston (Homogenizer (Figure 02).
- **Physically**: by ultrasound or high pressure
- **Chemically**: By detergents (acids or bases) or by enzymes. The grinding medium must meet the chemical requirements and osmosis it has a pH is neutral, and its ionic composition is as close as possible to that of the cytoplasm a 0.25 M sucrose solution is an isotonic medium toward most vesicular organelles.

1.1.3 Nomination:

The resulting solution from the grinding process is subjected to a filtration process to remove blood vessels, connective tissue and large particles.

1.1.4 Purification:

The purification process consists of separating the brown cells are separated into pure fractions by centrifugation (or super centrifugation). There are two types of centrifugation :

- Differential centrifugation or differential centrifugation.
- Centrifugation on density gradient medium "sucrose gradient centrifugation"

1. Expulsion Differential Central

-The homogeneous cell powder is subjected to a series of centrifugations with increasing times and speeds.

-The primary extract is fractionated into a series of sediments (Culots) and fresh liquids (Survivors).

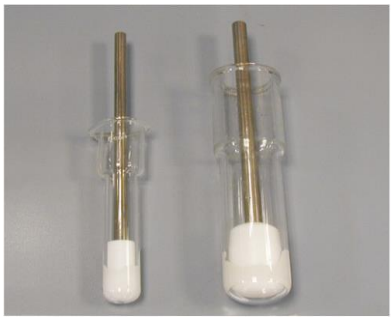
-The sedimentation rate of particles (organelles, macromolecules...) depends on their size, shape and density, so that the larger and more numerous particles in the cell extract form the first sediment (the first cut).

-The sedimentation rate is determined by the sedimentation coefficient expressed in units of Svedberg (S) (Figure 03).

2-Theeviction Central on a density gradient medium.

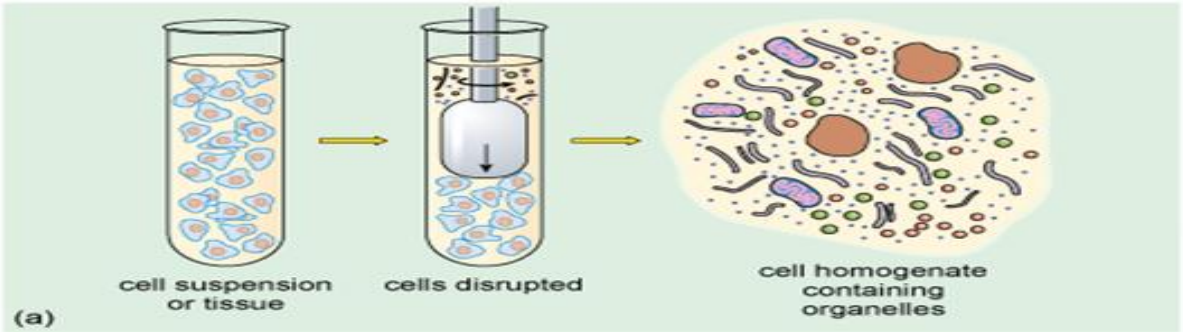
-The supercentrifugal technique allows for a graduated medium density, separates in one go cell organelles and even small biological particles that have similar sedimentation rates very.

-Centrifugation is represented by a gradient medium density in placing a thin layer of cell powder over a sucrose solution whose concentration varies regularly and decreases from bottom to top.
-After centrifugation, each element of the sample settles in a region of the tube whose density is equal to that of the element, thus obtaining different bands (the densest layer is inlower) (Figure 04).



Piston: Crushing or shredding device Homogenizer) which consists of a glass mashing tube (Homogenizer tube) and a pestle which consists of It consists of a metal leg ending in a bulging part made of Teflon (Teflon). The metal leg is connected at the other end to a motor that rotates the mortar handle inside the tube. Or the tissue can be shredded with a regular electric mixer.

(a)



(b)

Figure 02: Piston shapes (a) and piston crushing method grinding Mechanic (b)

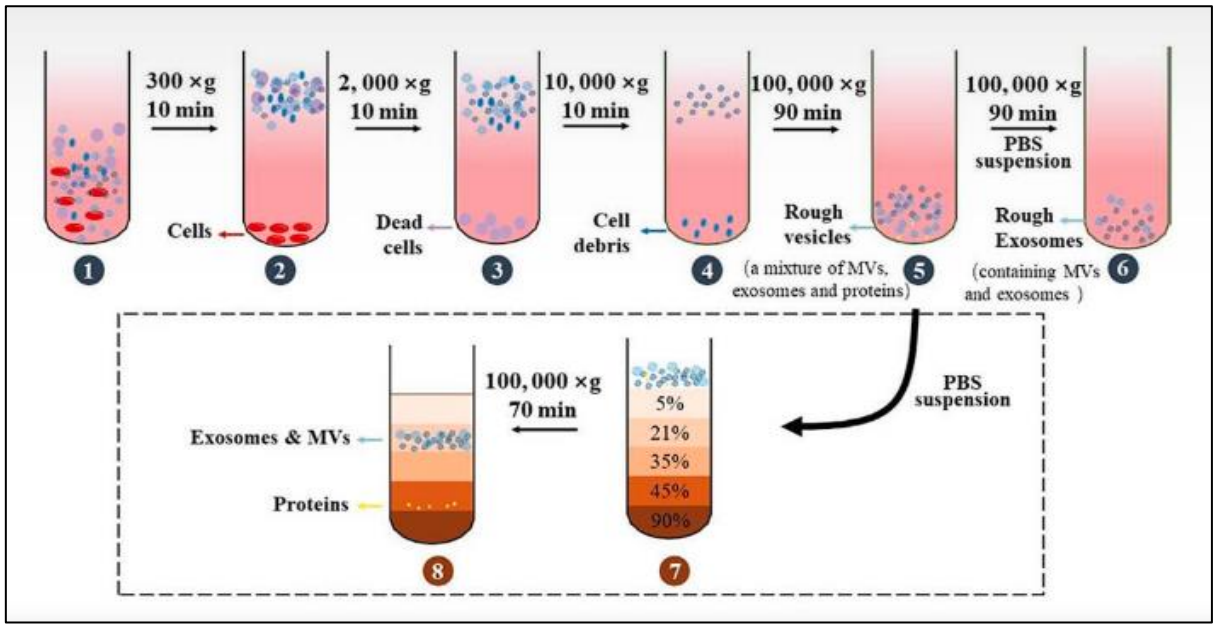
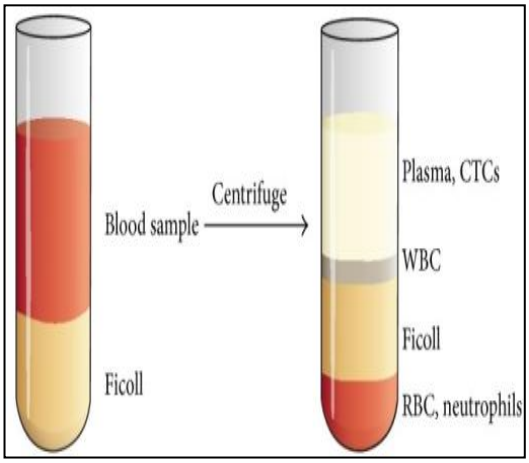


Figure 03 : Cell fractionation by differential centrifugation



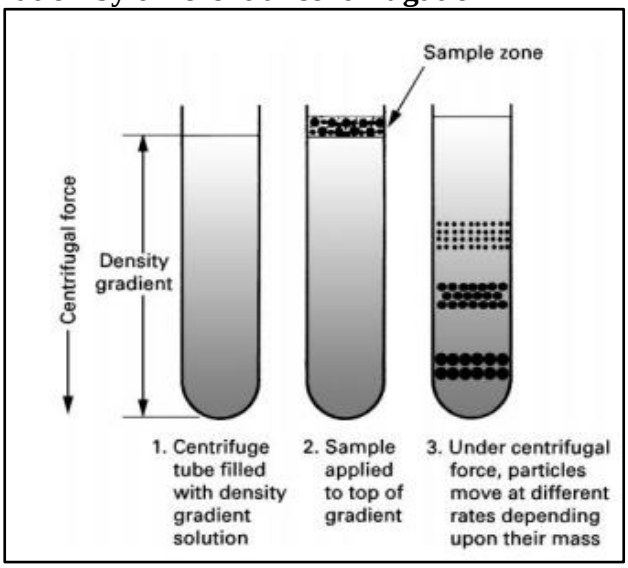


Figure 04: Cell fractionation by ultracentrifugation on density gradient medium

TD 01: Methods of studying the cell

Guidance session 04: Technology Microscopic examination of cells

I mean by technique at Microtechniques (Microscopic Preparations) are the steps by which maybe study of the cellular structures that make up the body of a living organism and that cannot be seen with the naked eye, or parts thereof, or organs of the body, using special devices and equipment for this purpose, and it includes a group of techniques Tissue-Cellular and the choice of the appropriate method depends on the intended goal during the study and there is there are many ways, including the most common ones at the present time:

- Non-segmental preparations you can't make clips in it like: Tissues blood
- Sectional preparations for light and microscopic examinations electronic.

1. The way Segmental: Most common we focus what the following:

1.1Whole mounting:

Where the entire sample is placed on the slide for examination without the need to cut it like liver flukes and lice two types:

a. Download Temporary kidney.

b. Download Permanent kidney.

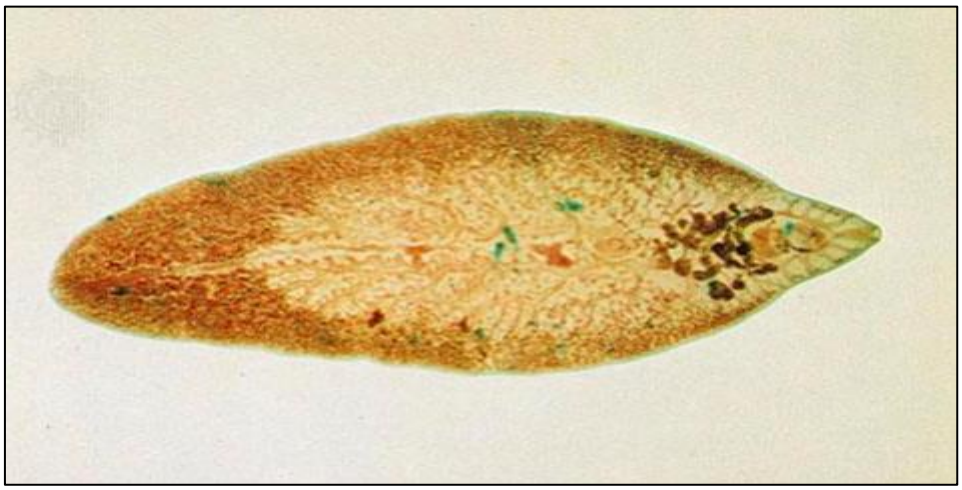
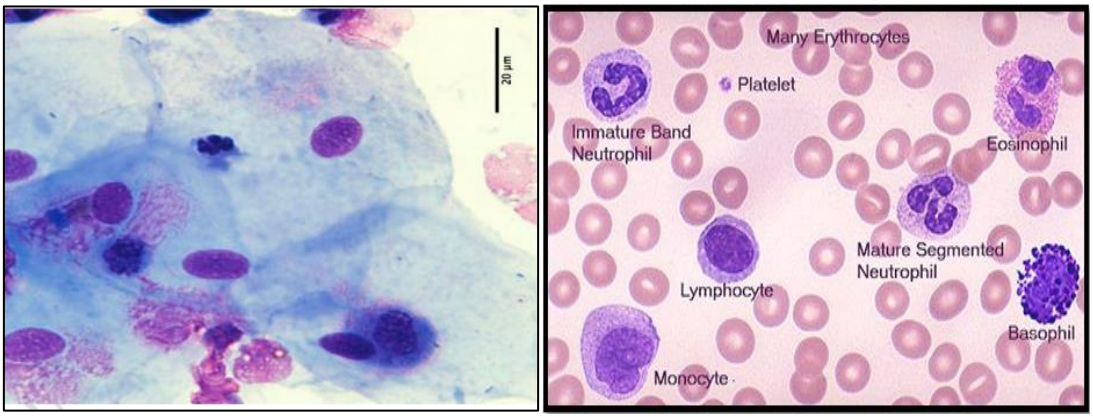


Figure 01: Total download (The worm Hepatic)

1.2 Smearing Method:

This method is used for tissues, which are difficult to cut, especially body fluids such as blood, cerebrospinal fluid, spinal fluid, and coelomic fluid, as well as parts of some tissues such as the marrow. The bone, the smear works by spreading the liquid between two slides or between a slide and its cover to obtain a thin layer. The last step in preparing smears is staining them. Several stains can be used depending on the type of smear. The smear method represents a good diagnostic tool in diagnostic cytology and diseases. Tissue and there are several ways to perform the smear method, and the blood smear is considered blood smear is the most famous.



Oral mucosal swab **blood swab**

Figure 02: Swab method

1.3 Teasing Method:

Used to study parts of a tissue, such as muscle, for example, a small piece of muscle is taken and then, using a dissecting needle, it is disassembled into structural units, such as muscle fibres, which can be penetrated by microscope light.

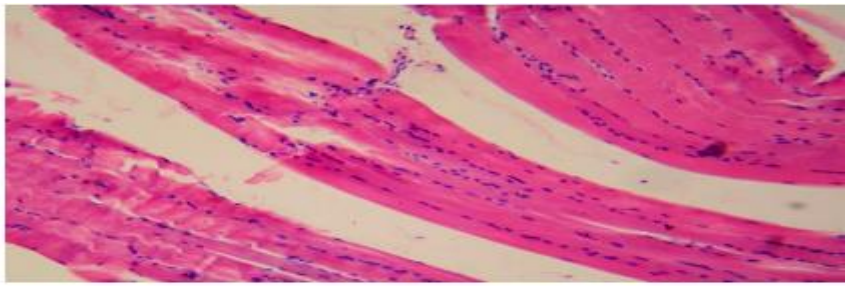


Figure 03: Prose method or publishmuscle tissue

1.4 Squashing Method

Used to crush soft samples and convert them from the tissue state to the cellular state on a glass slide, such as studying the stages of cell division and observing chromosomes.

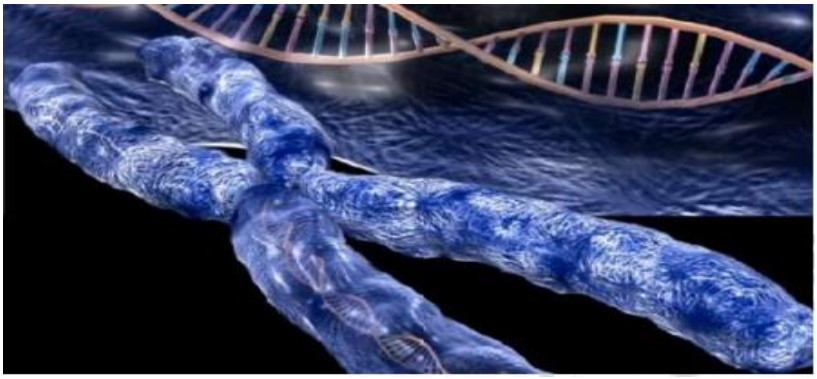
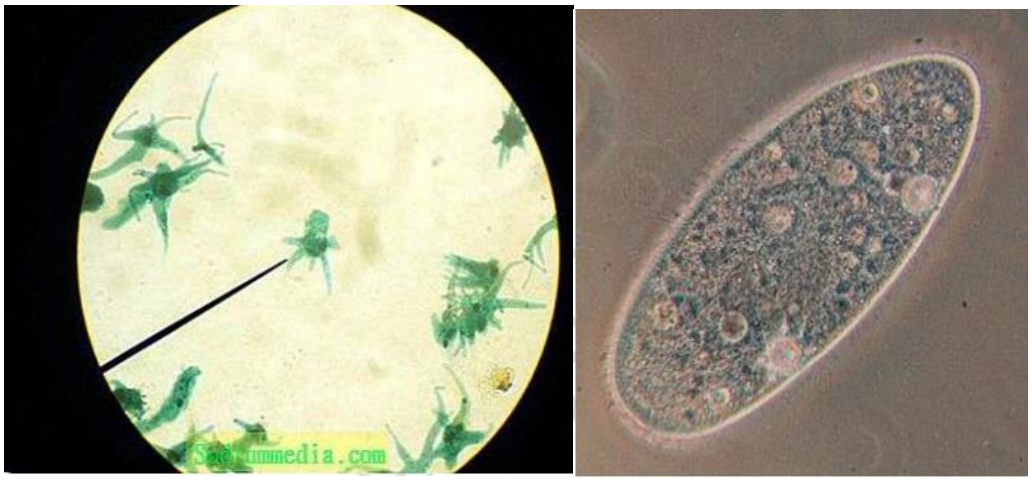


Figure 04: Road Crush or mash «Chromosomes"

1.5 Direct Method:

Used for rapid study of living specimens for a very short time, such as in the examination of oral squamous cells, amoebas, and paramecium.



Amoeba **Paramecium**

Figure 05*:* The method Direct

1.6 Freezing method:

Used for immediate diagnosis in pathology laboratories. Tissue and pathology laboratories for specific disease conditions such as tumors.

In this method, the soft or fixed tissue is frozen and hardened and sections are made from it with an ice microtome or what is called with cryostats features:

1- Road fast and simple, it is often used during surgeries that require rapid diagnosis of cancers.
2- Materia is the chemistry in the fabric does not change due to lack of use the heat.

3- Used in tissue chemistry to study the activity of cellular enzymes, detect alcohol-soluble lipids, xylene and the reaction of antibodies with antigens.

Its disadvantages:

1- No, give a series of clips.
2- Give hick sections due to difficulty of cutting and dyeing.

1.7 Sectioning Method:

And it is the most important thing is to study the samples at their tissue and cellular level, and the purpose is to obtain a very thin tissue section.

There are three main methods used to make Histological sections

1. Technique Paraffin is the method the most famous (The paraffin technique)
2. Technique Silodine is Mostaccuracy (The celloidin technique)
3. Technique Freezing is the fastest (The technique freezing)

2- Foundations theory of preparation for light microscopy

The study of living tissues is more important from a biological point of view, because it allows us to know the normal state. For tissues and the studied cells .However, this encounters many obstacles, which makes researchers resort to studying fixed tissues, as the tissues are fixed, then sections are made, stained and then examined under a microscope, while preserving the tissues as much as possible in a condition close to their natural state.

2.1 Study of living tissues:

Living cells can be observed by placing them between a glass plate and a coverslip, in their natural environment or in a medium close to it such as physiological fluid, in order to evaluate some of their functions such as sperm motility, measuring the frequency of cilia beating, or studying chemo taxis in different cells. In these studies, vital stains are used to evaluate the vitality of the cells, such as tryptan blue, or to show some components such as moderate red, which shows phagocytic vacuoles. Cell culture methods also allow the study of living cells in vitro.in vitro in a liquid medium, or on a support to which it can adhere, such as the polyester plastic cell culture trays commonly used in scientific research laboratories and in medical laboratories for diagnostic purposes.

2.2Study of fixed cells and tissues:

In this procedure, the samples to be studied are killed and then included in a material that allows the preparation of microscopic sections to be stained and prepared for study by light microscopy or electronic. Preparing any chip goes through several stages, starting with preparing for the installation process.Sample, and ends with it being loaded dyed on clean slice. Between these two stages, the preparation goes through several stages.Steps she: Installation, removal water, promoting, impregnation, burial, pieces, download, dyeing, coverage.

3. Preparing tissue sections

Much of our knowledge of the microscopic nature of the body comes from taking small samples and cutting them into very thin slices suitable for microscopic examination. The section must be thin enough to allow enough light to pass through for clear viewing. The tissue sections should not be so thick that the components of the tissue appear to be stacked on top of each other that it is impossible to distinguish their identity. Therefore, sections are usually cut thinner than the diameter of the cells. A section of cellular tissue must be cut through its individual cells sections for light microscopy are paraffin-embedded but there are other methods used for specific purposes.

3.1 Paraffin method for preparing tissue sections

It is a method used to prepare a tissue sample for examination under a microscope. It includes the following stages:

3.1.1 Tissue sample

We get a small sample of the tissue through excision diagnostic or excision surgical or postmortem sampling. When taking a sample from a body, it must be taken immediately after death to avoid damage. The tissue must be cut carefully with a sharp instrument so as not to distort the appearance of the microscopic. For good

fixation, the tissue mass should not exceed one centimeter, and the sample should be immersed in the fixative immediately after taking it.

3.1.2 Fixation

Fixation hardens soft tissue to prevent damage and chemical changes that occur as a result of the activity of proteins in the tissue. Fixation coagulates the protein in the tissue. It may be possible use thermal fixation if it does not cause deformities in the fabric therefore; chemical fixation is an alternative to protein coagulation. Fixatives chemical also by inhibiting cellular enzymes (Yeasts) from digestion cell, it also keeps carbohydrates and fat in tissue cells. Like that, stabilizers contain antiseptics that kill bacteria and germs that transmit diseases in infectious tissues. And became famous some fixatives have the ability to fix specific components in tissues but 4% formaldehyde neutralizer is adequate for most routine work. Bowen's means bouin (mixture of formaldehyde and picric acid), the fixation time and the volume of fixative used vary according to the size of the samples. A volume of fixative equal to 5 times the volume of the sample is recommended.

3.1.3Dehydration

The aim of the process is denial with paraffin is replacing all the water in the tissue mass is covered with paraffin wax so that the tissue can be cut easily later. However, since paraffin does not dissolve in water, the water must be removed first. It is known as the first step in that process by denial, in it, the tissue is passed successively through alcoholic solutions of increasing concentration (50%-100%), leaving the fabric in each solution for a sufficient period of time, until the water is replaced by alcohol. But since paraffin does not dissolve in alcohol, the alcohol is replaced by a solvent that can be dissolved in paraffin. To mix with him this process is known as leaching.

3.1.4 Clearing

Xylol is commonly used to demineralize tissues. The mass is passed through several stages of xylol permeate with alcohol, resulting in the xylol being replaced by all the alcohol and the tissue mass being ready for burial. Or baths xylenes or toluene is considered this step is in preparation for the embedding process because paraffin is hydrophobic. This latter process is known as clarification.

3.1.5 Embedding

The aim of embedding is to make the sample solid, allowing for the production of thin, regular sections.The embedding medium used is paraffin wax, which allows the sample to harden. The piece is immersed in liquid paraffin and kept in an incubator controlled at 56°C. After 4 hours of embedding the liquid paraffin is poured into a small metal mold "**Bars leuckart**», after cooling produces a solid mass of paraffin containing the sample taken.

3.1.6 Sectioning

The excess wax is trimmed off, and the tissue block is then fixed in a cutting device called a microtome it is cut into thin slices of 2 to 5 microns arranged in a regular series in the form of a strip stuck together and can be easily removed later.

3.1.7 Staining and mounting

In order to add dyes to the tissue slice, the paraffin wax must be removed and replaced with water so that the dye appears effectively. For this purpose, the slice is passed through alcohol solutions of decreasing concentration (100%-50%) then xylol until all the paraffin is replaced with water. Or glass slides are placed on a hot plate (at 45-60°C) for 15 minutes to melt the paraffin. Paraffin is removed by passing the slides in toluene baths (Or xylene He has drop or two of dye is added and the process of converting water to wax is repeated again. Finally, the tissue is examined under a microscope.

3.1.8 Structure and observation under a microscope:

The stained sections are fixed between the slide and the coverslip using a synthetic resin called Canada balsam, whose refractive index is close to that of glass, thus obtaining a tissue preparation ready for examination under a light microscope.

3.2 Preparation of sections for electron microscopy

The processing sequence is similar to that shown in the light microscope examination.

3.2.1 Fixation

It is usually done using Glutaraldehyde followed by subsequent fixation with osmic acid (osmium tetroxide) (OsO4)

3.2.2 Dehydration

Samples are passed through increasing concentrations of ethanol and then through propyl oxide.

3.2.3 Inclusion or enrobation

The specimens are embedded in a resinous material that allows the specimen to harden (Araldite) resin by polymerization.

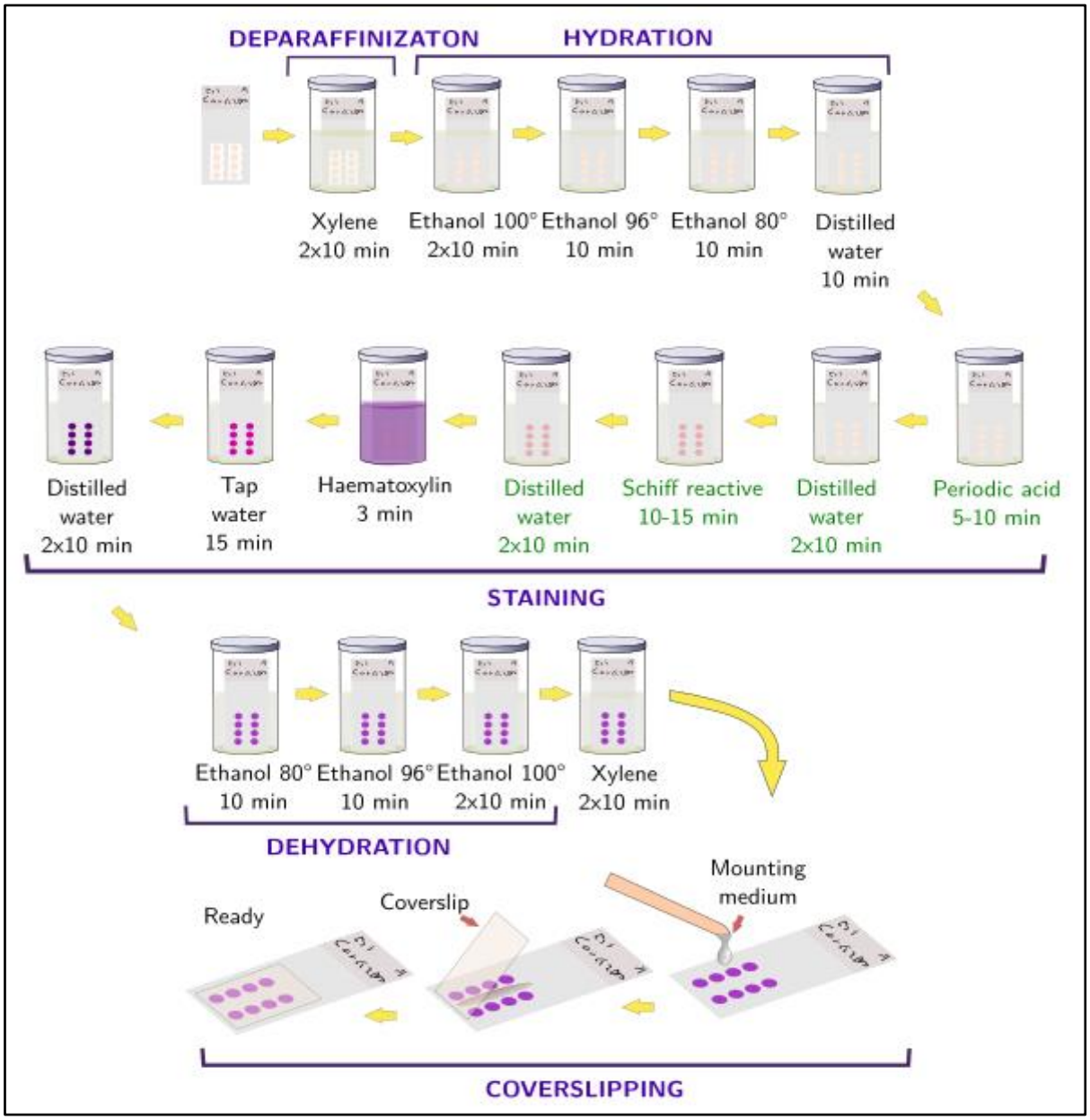
3.2.4 Pieces

Blocks are cut resin containing the sample using what for microscopic section Ultramicrotome equipped with a glass or diamond knife, it allows to obtain ultra-thin sections with a thickness of about 80 nanometers.

3.2.5 Contrast

Cell sections are placed on a copper grid, the grid is immersed in a solution of heavy metals (uranyl acetate and lead citrate) to darken the cell data and increase contrast, the grid is then inserted into the transmission electron microscope (MET) for examination.

Figure06: Manual shredder Dizziness (Rotary Microtome)


DEPARAFFINIZATON
HYDRATION
Xylene 2x10 min
Ethanol 100° 2x10 min
Ethanol 96° 10 min
Ethanol 80° 10 min
Distilled water 10 min
Distilled water 2x10 min
Tap water 15 min
Haematoxylin 3 min
Distilled water 2x10 min
Schiff reactive 10-15 min
Distilled water 2x10 min
Periodic acid 5-10 min
STAINING
Ethanol 80° 10 min
Ethanol 96° 10 min
Ethanol 100° 2x10 min
Xylene 2x10 min
DEHYDRATION
Ready
Coverslip
Mounting medium
COVERSLIPPING

-A-

-B-

Figure 07 : Preparation of tissue sections (A + B)

TD02 : Chromatographic separation

1. Definition Chromatography

Chromatography used to mean the separation of colored substances (the literal meaning of the word is colors), but today the term has become broader than this. narrow concept.Wa it Chromatography process in general is a physical method of analysis and separation using two phases, one of which is the stationary phase.Stationary phase has a relatively large surface area and the other is mobile phase which moves across the stationary phase and contains on the sample to be tested. The widepossibility of testing materials of both phases made it possible to use these methods to separate materials similar in physical and chemical properties. Chromatography is often a dual analytical method that can be used as both separation and quantification methods at the same time. It is easy and fastaddition to that it suitable for separating and estimating small amount of sample.

2. Chromatographic ProcessTechniques

Chromatologic methods include:Gravia now many processes depend on the difference in the distribution of the components of the material to be separated between two phases, one of which remains fixed and is called the stationary phase, which is either solid or liquid fixed on a solid support and is usually placed in a burette column, for example, or spread on a plastic sheet or a piece of paper, and the second is called the mobile phase, which is either liquid or gas and passes through the outer surface of the stationary phase and is sometimes called the carrier because it transports thecomponents of the material through the column, and is sometimes called the outlet because it removes the materials from the column, and the process of moving materials through the column is called discharge the sample is usually placed at the top of the chromato column.Gravia its contents move through the column as a result of the phase passing through. Moving but at different speeds depending on the tendency of the substance to remain in the stationary phase or in the mobile phase in craig's way with slight differences.The distribution that occurs as the mobile phase passes through the stationary phase in the column is very similar to how the explain this classification in the table.

T	Chromatographic methodsA	stationary phase	mobile phase
1	Ion exchangeIon exchange	Solid (resins)	liquid
2	Liquid-liquid chromatography(LLC) and absorption chromatography Liquid Chromatography	liquid	liquid Solid (adsorbent) resins
3	layer chromatographydelicate(TLC)	The paper	
4	Liquid chromatography –solid(LSC)	liquid	Solid Adsorbed
5	Exclusion chromatography (gel filtration)	liquid	solid
6	Chromatographygas-solid(GSC)	Solid/Distinctive	gas
7	Chromatographygas-liquid(GLC)	liquid	gas
8	partial sieve chromatography	Solid/partial sieve	gas

Who was the first to use separation methods ? Chromatography ?

The first use Chromatographic separation methods are attributed to the Russian botanist Tsot, who published in 1906.

Describe Michel's method of chromatographic separation.Use it ?

Description of the separation of chlorophyll and other substances in plant juice using a chromatographic column filled with calcium carbonate. A solution of petroleum ether containing plant substances was passed over this column and it was noted that the substances separated into different color bands.

Why is the color separation method called this ? The name ?

Michel noticed that the materials separated into different color ranges, and for this reason he called this method the color separation method, and this name has continued to this day, despite most uses of chromatophores.Gravia no includes color formation.

Explain the importance of chromatography methods ?

1. Chromatologic ability is considered Gravia on the chapter good, where the contents of a sample containing 10 to 20 substances can be completely separated within minutes.
2. Organic compounds with similar structures can be separated, as can inorganic compounds with similar

properties.Chemical for example, nitrogen 14 can be separated from nitrogen 15 in ammonia.

3. Types of chromatography methods ?

There are several methods used to classify chromatographic methods.Gravia but most of them depend on stationary phase and mobile phase type for example, what It is called liquid-solid chromatography.This means that the mobile phase is the liquid, which is called first, followed by the fixed solid phase, and so on chromatologic methods can also be divided into:Gravia building on the mechanics or how to distribute the materials to be separated between the two phases.

1. **Liquid-solid chromatography**
2. **Liquid – Liquid Chromatography**
3. **Gas-liquid chromatography**
4. **Exchange chromatography**
5. **Planar chromatography**
6. **Sieve chromatography**
7. **Continuous system electrophoresis**

3.1Chromatography Liquid – Solid

A- Chromatography column

Column chromatography is based on adsorption where a solid material (the adsorbent or stationary phase) is placed In a glass tube and placed vertically, the mixture to be separated is placed on the adsorbent at the top of the column, then the solvent (mobile phase) is poured to descend through the column. The components of the mixture differ in their ability to be adsorbed on the column as well as their solubility in the solvent, so they flow through the column at different speeds. The weaker the adsorption of the adsorbent, the faster they will descend first, and vice versa. If the adsorption of the materials is stronger, they will be delayed in reaching the bottom of the column, and thus the separation process is completed. Separation columns come in different shapes and are usually made of glass of different lengths and diameters. Different, the amount of mixture to be separated determines the size of the column used, the more the amount of fixed item the larger the separation, the better and the column should not be more than two-thirds full of the fixed material. The mechanism of distribution of materials between the mobile and stationary phases depends on the strength of the adsorption of the material on the surface of the solid stationary phase, so that the material that is strongly adsorbed is delayed, i.e. remains for a longer period in the column.While the material is a dsorption the least you can do is get out of the column early. Thus, the materials are separated from each other.That's why we can say that chromatography methods gravia in which the stationary phase is a solid that depends on a dsorption sometimes called chromatography gravia a dsorption.

- **Disadvantages :**

- That number of solids that can be used as a stationary phaselimited.
- The distribution coefficient depends on the total concentration, so the separation is incomplete.

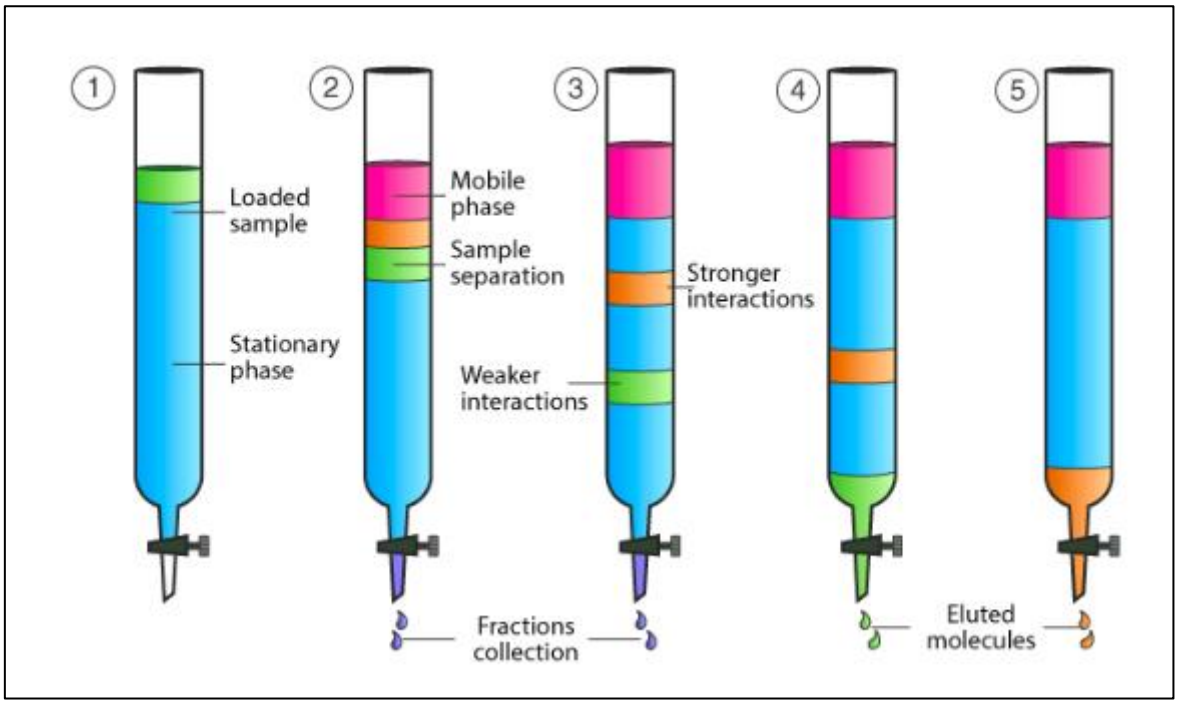


Figure 01 : Chromatography Liquid - Solid (Chromatography column)

3.2 Chromatography Liquid - Liquid

It was completed discover it by Sing and Martin

-Which It consists of ?

The stationary phase consists of a thin layer of a liquid or a mixture of liquids fixed on the surface of a permeable and inert solid.The mobile phase is another liquid.

-Chromato features gravia Liquid - Liquid

-Distribution coefficient no depends on focus therefore, the separation is complete.

-The separation of substances depends on the amount of solubility of the substance in the stationary phase. So that the substance that dissolves more is delayed more.

-And the material that does not dissolved in the stationary phase exits the column early and quickly.

-It is also called chromatography.Gravia partial solubility in which the stationary phase is a liquid

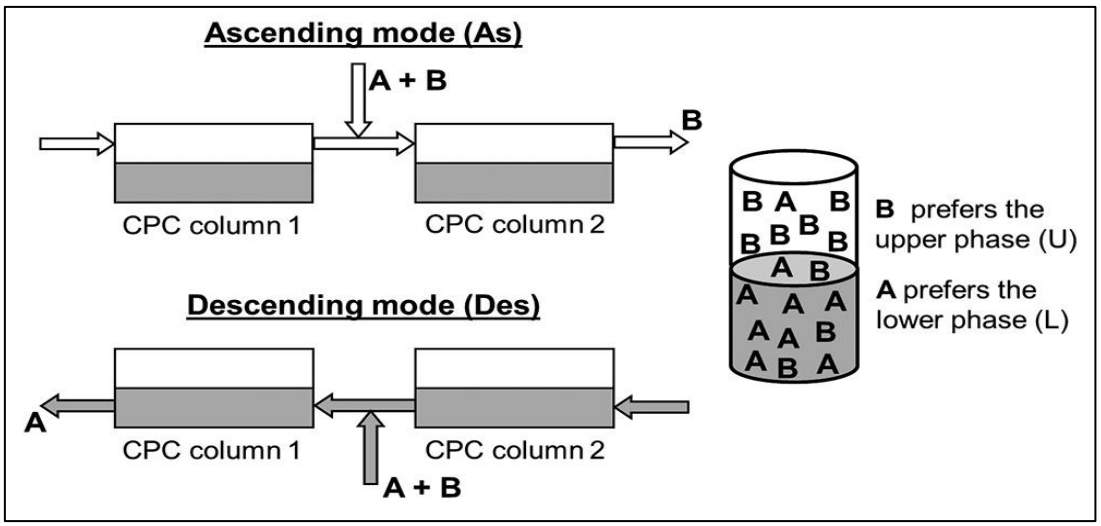


Figure 02 : Chromatography Liquid - Liquid

3.3. Planar chromatography

In planar chromatography methods, chromatographic separations are performed on a surface. Level instead of using a packed column.

A-Chromatography Paper

In this method, the liquid stationary phase, which is the water surrounding the cellulose, is essentially fixed to the paper. The filter and the filter paper itself serve only as a support on which small amounts of different samples are placed. On a line drawn at the beginning the paper so that the samples appear as spots on this line and then the beginning of this paper is dipped into the moving solvent in a closed atmosphere (in a beaker, for example). This is so that the perimeter of the cup becomes saturated with the moving solvent vapor. The solvent moves up the paper by capillary action.Or moves down by action gravity if the solvent was originally placed at the top the paper or moves horizontally by diffusion as the solvent moves, the components of the sample are distributed between the moving solvent and the stationary phase. Therefore, they move at different speeds across the paper depending on that distribution.

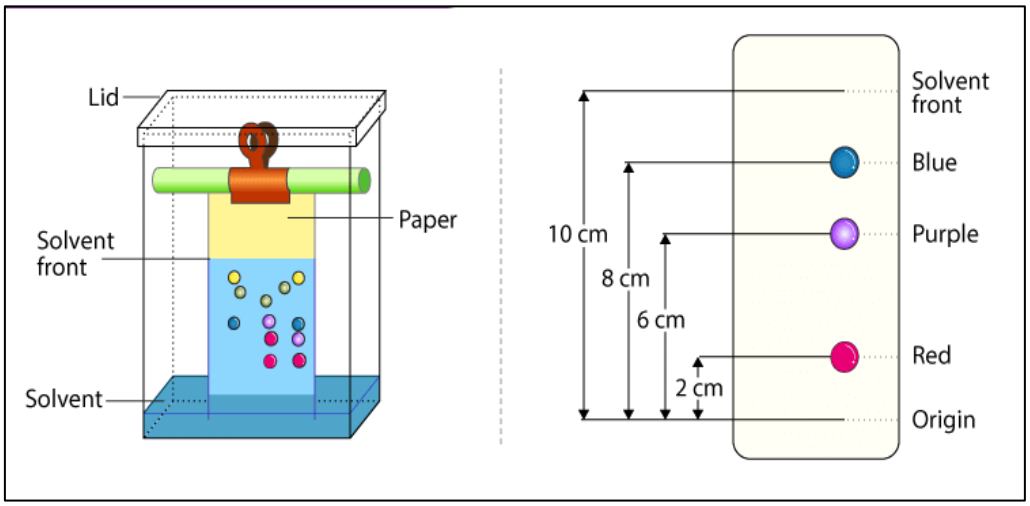


Figure 03 : Chromatography Paper

B-chromatography thin layer

In this method, a glass plate or a small plastic sheet is used, covered with a thin layer. ((0.2-0.3mm from a polar absorbent material such as alumina or silica gel as a stationary phase and can be used any solvent is suitable as a phase.mobile, we conclude from this that the distribution mechanism may depend on adsorption.

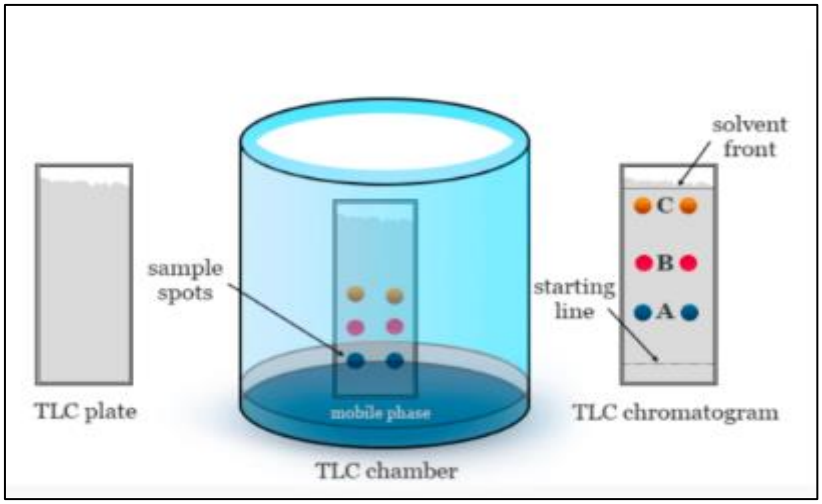


Figure 04 : Chromatography thin layer

3.4 Chromatography Gas-liquid

Like other types of chromatography, it consists of two stages are :

A- The stationary phase is a liquid substance with a high boiling point and is fixed on a solid substance. Known as the support, the support material is packed after being coated with the fixed grade material inside a separation column of a specific diameter and length that can be heated at high temperatures.

B- The mobile phase is an inert gas with a high thermal conductivity, such as nitrogen. Helium is known as the carrier gas.

-Principle of the device

When performing the analysis, a very small amount is injected.(1µml of the sample in the liquid state or in the form of a solution by means of a special syringe (injection needle) in the injection opening or by means of a special valve if the sample is gaseous, where the sample evaporates and then the vapor is pushed by the carrier gas to the spiral-shaped separation column located inside the oven where its components are separated based on the difference in their distribution coefficients between the carrier gas and the stationary phase, and the separated components are detected by an electronic estimator, which responds to each component according to its concentration, and an electrical signal is generated that is recorded on a special recorder in the form of signals.

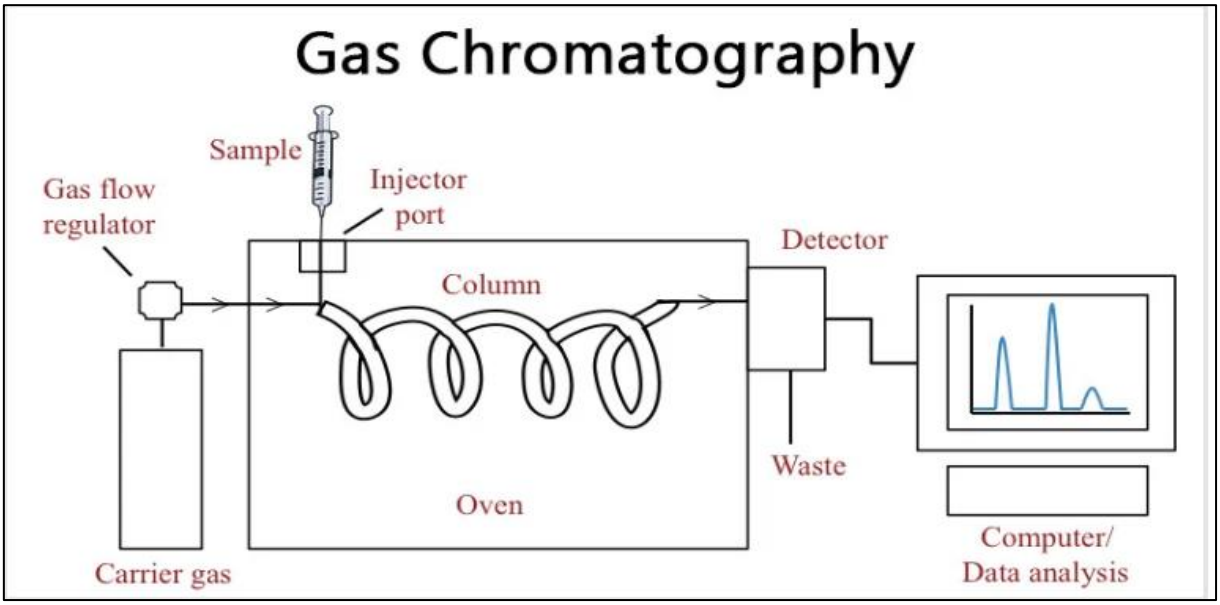


Figure 05 : Chromatography Gas-liquid

3.5 Chromatography Reciprocity

It is considered a type of chromato.Gravia Liquid-Solid where the mobile phase is a liquid and the stationary phase is a solid.

-Mechanic

no it does not depend on adsorption, but rather on ion exchange.

-Use it

Used to separate ions.

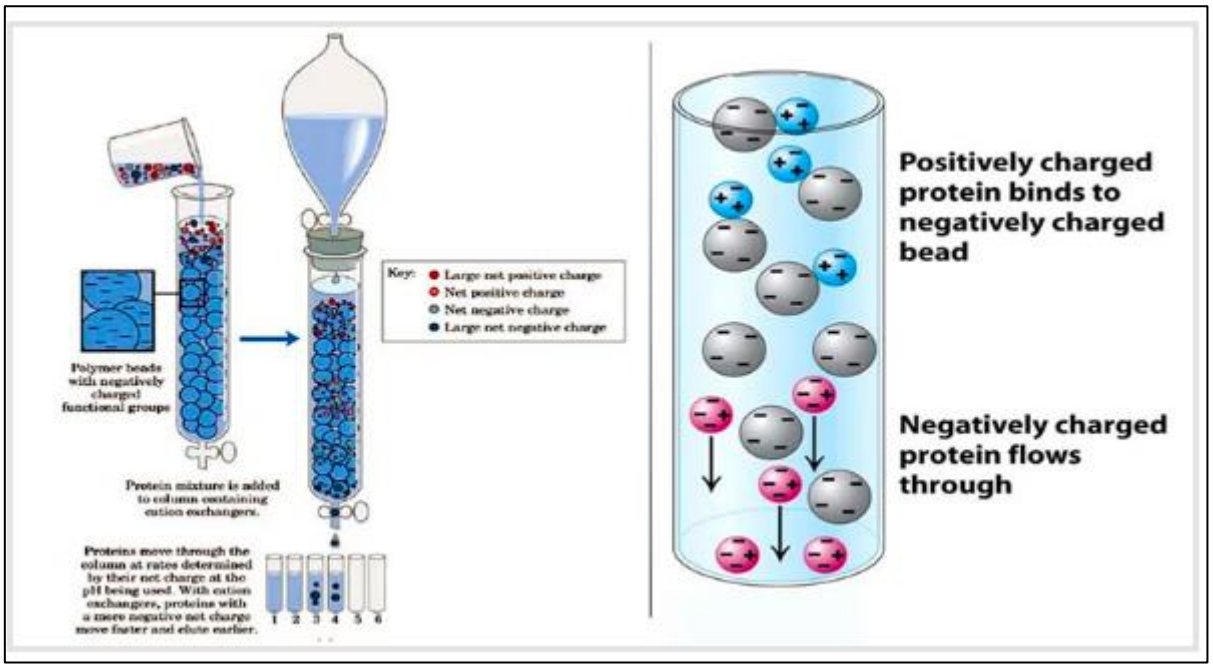


Figure 06 : Chromatography Reciprocity

3.6Chromatography sieve

The column is filled with a gelatinous substance whose very large molecule has a sieve-likestructure, especially after soaking it in water. When passing the materials to be separated based on the size of their particles in a way that is exactly similar to the work of a sieve.

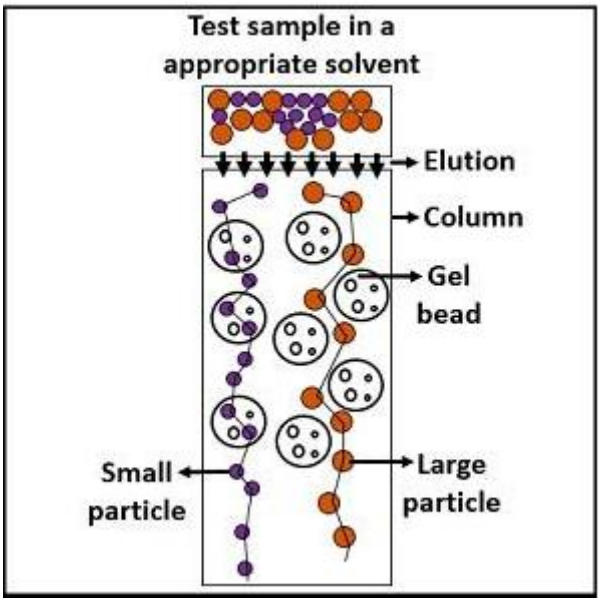


Figure 07 : Chromatography sieve

3.7 System electrophoresis Continuous :

This method is considered to be the development of chromatophores.Gravia Paper where an electric field is applied perpendicular to the direction of solvent flow. The moving one causes the ions to deviate from the solvent flow path at different angles towards the cathode or anode. It depends on both the amount and type of charge of the ion and its size.

-Its disadvantages

It is not sensitive.

-Its uses

It is used in the analysis of proteins and polysaccharides.

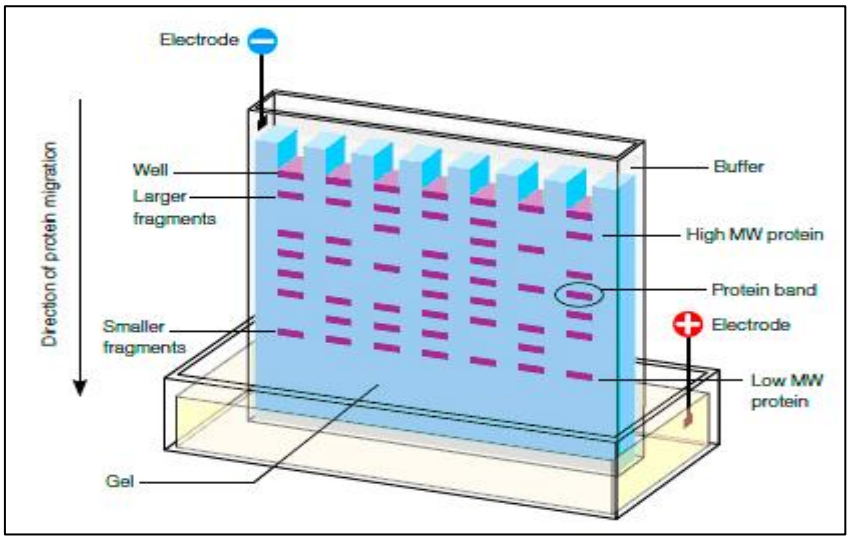


Figure 08 : System electrophoresis Continuous

TD 03 : Cell and tissue culture

Orientation Lesson 01 : Animal Tissue Culture

What does tissue culture mean ?

Tissue culture (or cell culture) means separating cells from any animal or plant tissue and then culturing and growing them in artificial environments suitable for growth .It also includes the cultivation of organs in the laboratory in farms 3D culture , or transplantation of organs inside the living body cell quality the culture may be cells removed from tissue and treated with specific enzymatic or mechanical methods for the purpose of disintegrating them and then culturing them in a suitable environment for growth, or they may be cells derived from a cell line or cell strain the purpose of this process is to keep the transplanted cells alive and able to perform their vital functions.

First : Animal tissue culture

Animal tissue culture is an important field of research. It focuses on the production and reproduction of animal cells in a controlled laboratory environment. Cells are extracted from tissues and prepared in a nutrient medium to stimulate their growth.This technology is used in scientific research and medical applications, including the production of cell cultures and cell cloning.

1. **Key points**

- Animal tissue culture is a sophisticated technique for producing and reproducing animal cells in the laboratory.
- This technique involves extracting cells from animal tissue and preparing them in a suitable environment to stimulate growth and division.
- Primary and secondary cell culture methods are widely used in scientific research and medical applications.
- Animal tissue culture techniques are important in areas such as cell culture production and cell cloning.
- These techniques require a controlled laboratory environment and precise scientific methods to ensure the success of the transplantation and cell growth process.

2. **Introduction to Animal Tissue Culture**

Animal tissue culture is a process of growing cells outside the body. The cells are transferred to a suitable environment in a laboratory. The goal is to keep the cells alive and able to perform their normal functions.

3. **What is animal tissue culture ?**

This technique is used to grow and multiply animal cells and tissues outside the body. The cells are isolated from the living organism. Then they are cultured in a suitable environment with the nutrients and factors necessary for their growth.

4. The importance of animal tissue culture

Animal tissue culture contributes to several fields, including :

- Study of the physiological properties of cells
- Drug and chemical testing
- Production of therapeutic proteins and pharmaceuticals
- Development of cell and tissue therapies
- This technique allows precise experiments to be performed on animal cells. This allows comparison with experiments on whole organisms.

5. Historical Look at Animal Tissue Culture

Animal tissue culture began in the 19th century. In 1885, German physician Wilhelm Rucks isolated a portion of the neural plate of a chicken embryo. He then preserved it in saline, helping to develop modern tissue culture techniques.

In 1907, Ross Granville Harrison cultured frog embryo cells in a nutrient medium of coagulated lymph. This discovery facilitated the development of nerve cells, which was an important step in the field.In 1913, Steinhardt and Limpert used guinea pig corneal tissue to culture smallpox virus. This early research was an important step toward the development of modern animal tissue culture techniques.Historical discoveries and developments in animal tissue culture have been the foundation for what we are today. This field has seen great progress and development.

He should Learn some common terms in animal tissue culture :

1. Primary cell culture

In the world of animal tissue culture, primary cells play an important role. Primary cells are cells that are isolated directly from animal tissues and organs. They are extracted using mechanical or chemical methods, such as mechanical dissociation or enzymatic digestion. These cells represent different types of cells present in the original tissue.

- **Primary cell extraction**

Primary cells are extracted from animal tissues and organs by mechanical or chemical dissection. In mechanical dissection, the tissue is cut into small pieces using blades or scissors. In chemical dissection, enzyme solutions are used to digest the extracellular material and release the cells.

After the primary cells are extracted, they are cultured in suitable nutrient media to stimulate their growth and reproduction. These primary cells are the basis for animal tissue culture and its various applications.

❖ **Cell line :**

After the first operation subculture of cells, primary cultures are called cell line or subclone cell lines derived from primary cultures have a specific life span. As the passage of cells continues, cells with a high capacity for growth and division become dominant, resulting in growth with a uniform genetic pattern and phenotypic characteristics in the developing cell population..

❖ **Cell lineage**

It is the cell line resulting from selecting cells from a parental cell line culture by cloning or another method. Here we find that certain characteristics and features appear in these cells, and these cells can grow as a single layer or as suspensions.

- A collection of cells in a laboratory culture is called homogeneous population if the cells in the culture are of homogeneous origin.
- A group of cells in agriculture is called heterogeneous population if the cells in the culture were heterogeneous.
- A homogeneous group of cells that originated from a single parent cell.So it is these cells are identical genetically.

❖ **growth-limited cell lines (finished) and continuous growth cell line**

Normal cells have a limited ability to grow and divide.) That is, they undergo a specific number of cell divisions (until they reach a certain age at which they lose the ability to divide and reach the stage of senescence. Such cells are called finite cell lines. However, some cell lines may become immortal by the process of genetic transformation, which may occur automatically or be induced using specific viral or chemical methods. Cell lines become continuous cell lines after the process of genetic transformation, which gives them the characteristic of continuous division.

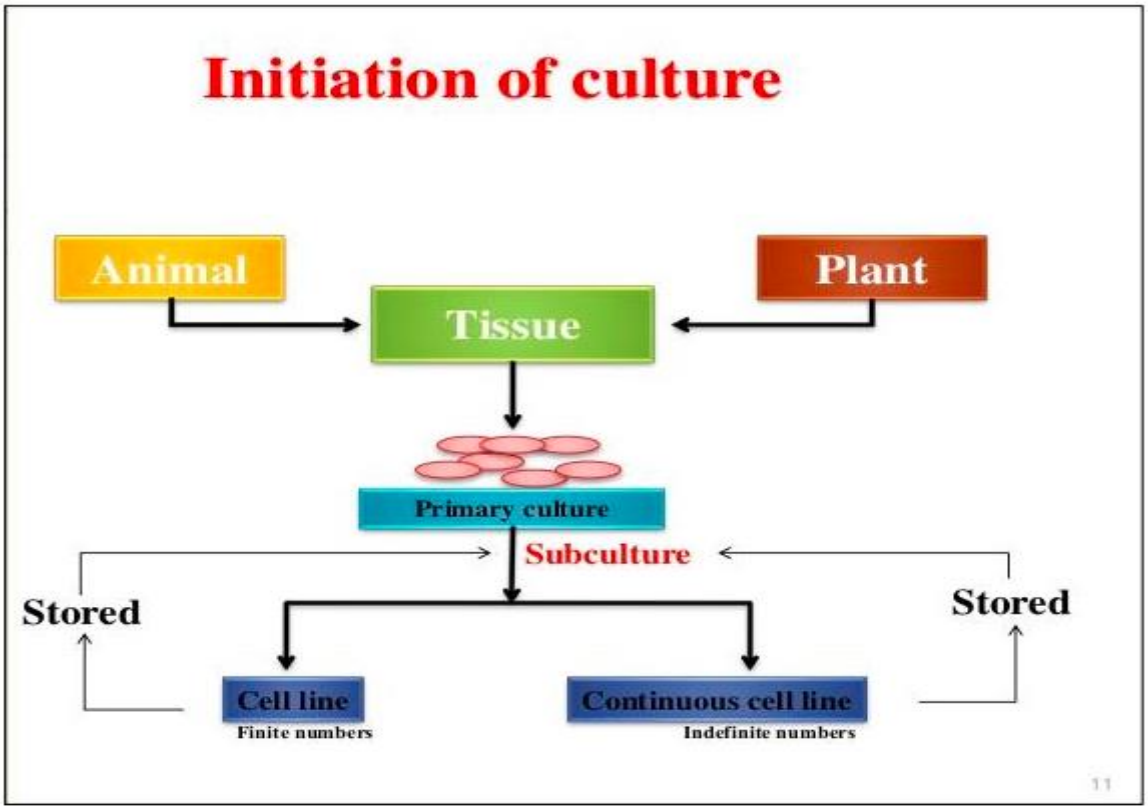


Figure 01 : Shows how to set up cell farms.

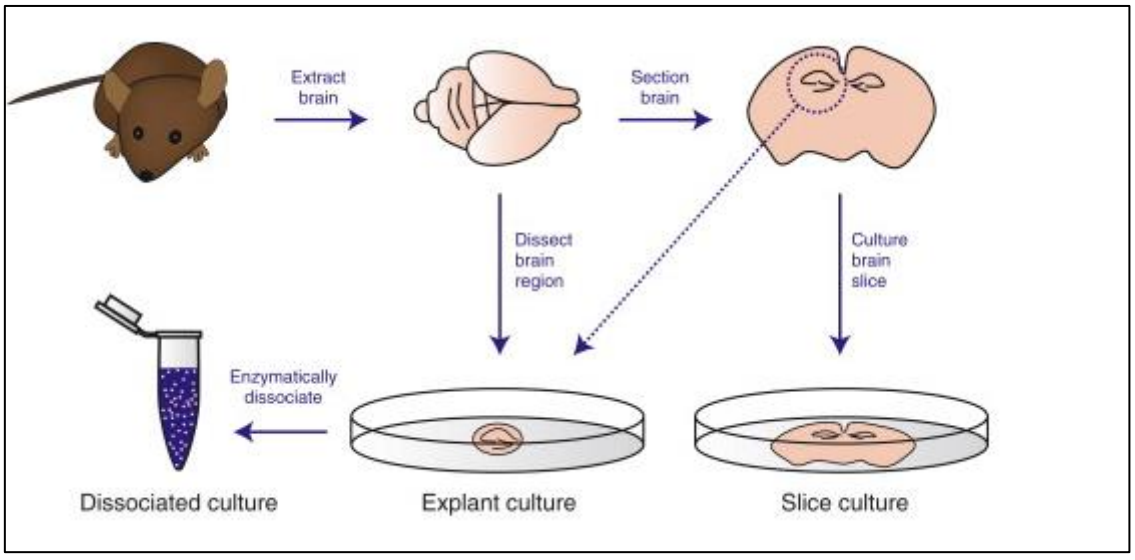


Figure 02 : Primary cell culture

1. **secondary cell culture**

Secondary cells come from primary cell cultures. They are then grown in fresh culture medium. These cells grow longer and better than primary cells.Cells are transferred to secondary cultures via enzymatic digestion. They are then washed and resuspended in appropriate volume.

Secondary cell cultures are used in viral and immunological research. They provide an ideal environment for cell growth and proliferation. This makes them a valuable tool in research.

❖ **Characteristics of secondary cells**

- Grow longer than primary cells
- It needs constant feeding to ensure sustainable growth.
- They are transferred to secondary farms by enzymatic digestion.
- Widely used in viral and immunological research.

1. **Animal tissue culture techniques**

Animal tissue culture is carried out in a sterile environment and media at its various stages, because the cells and tissues taken from the animal's body and isolated from its organs lose their immune resistance and ability to defend against infection and inflammation, and therefore it is necessary to prevent germs, viruses, fungi, etc. from reaching the culture medium. The process of removing tissue samples to be cultured is carried out with sterile, precise and decisive dissection or surgical tools and in small laboratories that are entered through a special passage to prevent air currents and contamination. The culture incubator, sterilized by ultraviolet rays, is supplied with filtered air and researchers wear masks on their faces. The equipment, tools, glassware or plastics used must also be clean and well sterilized.

It is worth noting that the discovery of antibiotics antibiotics such as penicillin, streptomycin, mycostatin, etc., and their use in culture media have enabled scientists to diversify and deepen their experiments instead of focusing on delicate and complex sterilization processes.

2. **Animal tissue culture media**

Since animal cells and tissues are heterotrophicheterotrophs, that is, they must derive their entire food from the medium in which they live. It is essential that the medium contains all the elements and materials necessary for their life. There are two basic types of tissue culture media, which are :

1- Natural culture media that include nutrients derived from biological fluids, such as blood plasma or serum and embryonic extracts. This type of media is generally preferred for cell growth and proliferation because it is taken from animal bodies, but its exact chemical composition is difficult to determine.

2- Artificial culture media, the components of which are carefully selected by the researcher, to include amino acids, peptides, glycosides, lipids, vitamins, etc.

3. **Animal tissue culture methods**

It is generally preferable to use transparent glass containers and plates that enable the researcher to follow the development of the tissue culture under a microscope or magnifying glass. There are a variety of methods, including :

1- Tissue culture in hanging drop or chamber wet :

A drop of the medium is placed the culture is placed in the middle of a sterile glass plate in which the tissue biopsy to be studied is immersed. Then this plate is carefully turned over onto another special plate with a concave hole in the middle (Figure03).

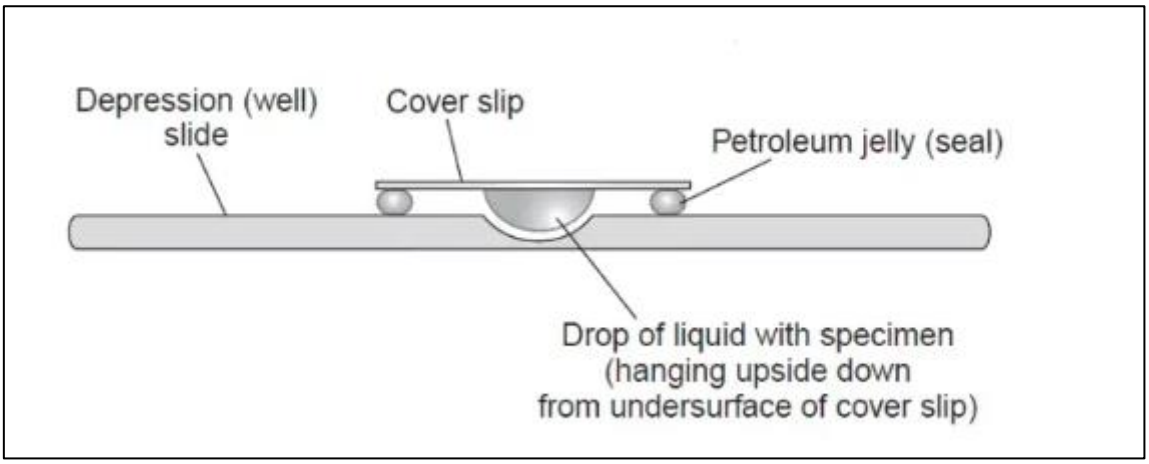


Figure 03 : Tissue culture in hanging drop

In this case, the culture is placed suspended from the ceiling of a small humidified glass chamber, where it can be studied directly under a microscope, and transferred to a warmer at the appropriate temperature. Re-cultivation is easy in this way, as the culture medium is free of the products of cellular metabolism.

2- Tissue culture in a watch glass :

It differs from the previous method in that the tissue biopsy can remain floating on the surface of the culture medium and the surrounding air can be inhaled. Here, the sterile watch glass is placed in a petri dish contains cotton moistened with distilled water to maintain proper humidity. A balanced mixture of plasma and embryonic extract is placed at the bottom of the watch glass, and the tissue to be cultured is placed on top of it. Then the Petri dish is covered and placed in the humidifier (Figure04).

Agriculture the first basically follows the study of the cellular units that make up the tissue in a particular organ, while the second is concerned with studying the differentiation of the organ and the mutual relationships that exist between it and the various organs in the body.

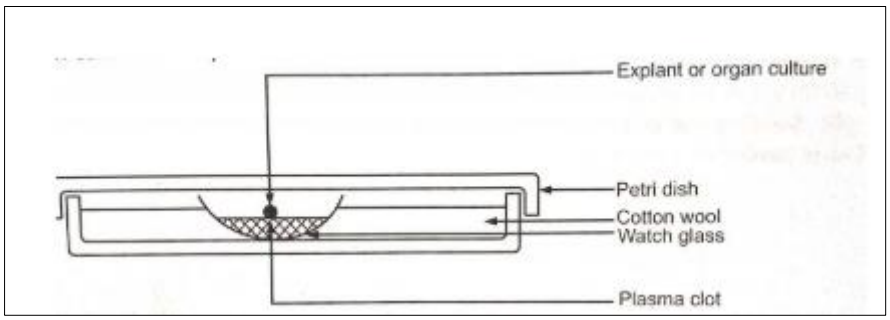


Figure 04 : Tissue culture in a watch bottle

3.- method Trowell :

It is more complex, but it has the advantage of being able to change the culture medium without moving the cultured tissue, and it is also possible to change the gas atmosphere of the culture room. It was modified by Shen placed the study tissue on a special filter paper that floated on the surface of the culture medium.

4- Wolf's method and Haffen:

It uses a gelatinous mixture.gélose with a suitable nutrient medium with a drop of penicillin dilute. The cultured tissue is placed in a thin vitelline membrane (Figure05).

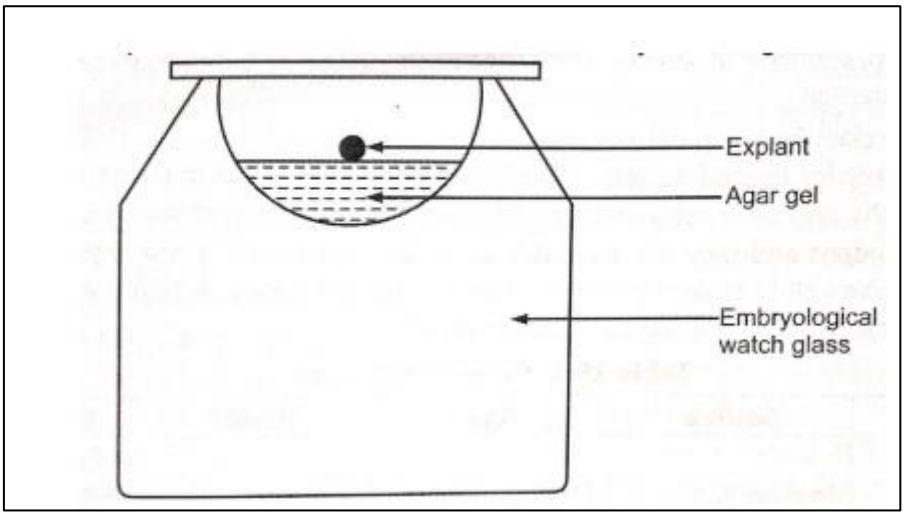


Figure 05 : Wolff's method of tissue culture

5. Replantation

These processes are essential in monitoring the successive stages of tissue culture, as it has been observed that after the success of the first stage of tissue culture, the cells multiply and form a mass surrounding the initial biopsy. Therefore, it is necessary to renewIn the middle of agriculture regularly every 72 or 96 hours to maintain the vitality of the cultured cells and rid their surroundings of waste by replanting them in a new nutrient medium. In addition, tissue culture is sometimes required to obtain a pure cell line called monoclonal, and here it is necessary to select the appropriate cells under the microscope, isolate them, then transfer them to a new medium and repeat their cultivation to maintain their safety, activity, growth and regular reproduction.

6. Applied results of animal tissue culture

- ✓ The benefits of animal tissue culture are many and include different biological domains. It has enabled in-depth studies in cell biology, as the tissue culture can be transferred and placed under the microscope, especially the phase-contrast microscope, that allows viewing transparent cell organelles and observing the movement of chondrosomes and chromosomes during cell division and other stages.
- ✓ The researchers were able to chèvremont and frédéric, and others installed a microscopic cinematograph and recorded minute details of different stages of the life of living cells. These methods helped to determine the chromosomal formula of cells and to conduct comparative studies to find out cases of chromosomal abnormalities in a number of disease states in animals and humans.
- ✓ Researchers are able to conduct a number of experiments that are difficult to conduct in the living body, especially in humans, including, for example, placing tissue and cell cultures in media containing drugs or toxins in varying amounts and observing their effect on the life of the cultured cells.
- ✓ As for cancer, tissue culture methods have played a major role in clarifying the differences between benign and malignant tumors and revealing the causes, materials and pollutants that cause cancer or induce tumor formation. At the same time, research is being conducted to investigate the appropriate drugs and amounts of radiation treatments that help stop the spread of cancer in laboratory animals or in tissue cultures of tumors taken from different organs of patients, such as the breast, cervix, prostate, liver, etc.
- ✓ As for the importance of tissue culture in technology modern biotechnology at the end of the twentieth century, research into embryonic tissue transplantation, especially stem cells, began, which opened new horizons for addressing many vital issues, including intractable infertility

problems in animals and humans, and resolving the issue of tissue compatibility in transplantation members?

7. Agricultural environments

It is the nutritional medium used in tissue culture, in which different cells are grown for a specific purpose. The goal may be to obtain cell products, continue dividing until we obtain cell growths, or continue until we obtain a complete embryo.The environment is considered the main factor in cell and tissue culture, as it works to maintain the life of the cell through its various components.

1. Components of the agricultural environment :

1-Basic Mineral Salts 2- Vitamins 3-Amino acids 4-Carbohydrates5- Hormones 6 -Distilled water

2. Functions of each component of the agricultural environment

First : Basic Mineral Salts

It is a group of inorganic salts and is divided into :

Major elements : It consists of seven basic elements as follows : nitrogen, phosphorus,-Potassium-Calcium -Sodium -Magnesium -Sulfur.

Trace elements : These are the elements that are needed in very small quantities, not exceeding a few.Milligrams they are also called archaeological elements, which are : iron.-Manganese –Zinc and others have been proven that these elements, they act as enzyme activators.

Second : Vitamins

Vitamins act as cofactors in enzyme systems and are required in very small amounts.

Third : Amino Acid

The most important amino acids used in tissue cultures are :

Histidine, vcystin, orginine, lysine, leucine, iso leucine, phenylalinine, methionine, tryosine, tryptophane, thretonine, glutamine, valine

Fourth : Carbohydrates

Every living organism needs a source of energy to complete all vital processes within the organism. Accordingly, every food medium needs sugars as a source of carbon and energy, such as glucose. Sucrose is the most commonly used sugar and they are usually used at a concentration of 2-5% in the food medium.

Fifth : Growth organizations

There are organic substances that regulate growth and development called natural hormones.

Growth stops

It is usually used in the case of maintaining a certain breed for a period of time from 6 to12 months

Growth inhibitors

These hormones are added to the environment to slow down cell growth, and all vital processes occur very slowly, and their goal is to prevent cells from differentiating.

Growth promoters

In general, it is necessary to add one or more of these compounds to aid growth.

TD03 :Tissue culture

Orientation Lesson 02: Plant Tissue Culture

Secondly: Plant tissue culture

Plant tissue culture techniques are considered one modern biotechnologies play an important role in serving humanity, especially in the field of plant production. Tissue culture means the growth of different cells, tissues or plant parts in glass containers.And sometimes-plastic containers containing artificial nutrient environments consisting of the nutrients that the plant needs, and this is done under completely sterile conditions. The containers, with the environment and plant material they contain, are called (tissue culture), and the cultures are kept in incubators whose temperatures and lighting can be controlled according to the appropriate needs of the plant.

1. Types of plant tissue cultures:

Given the important role of plant tissue culture techniques in various basic and applied research and studies that are then implemented at the commercial level, the term plant tissue culture is a common term, and it is useful to use the termsmore specifically and specialized in serving different fields:

1-Agriculture Plant: This means planting seedlings and whole plants.

2-Agriculture members: This means planting plant organs such as leaves, roots, etc.

3-Agriculture callus: It means the culture of undifferentiated cells that formed on the wounded parts of the explants. The callus is an undifferentiated mass and its growth is unspecialized. This mass may be brittle or cohesive depending on the type of plant it was taken from and the type of culture medium it lives on. The callus is taken and broken up by mechanical or enzymatic methods, then the cells are cultured on the same culture medium but in liquid form and in shaking incubators.

4-Agriculture the fetus: It means culturing separated embryos, whether they are fully developed or incompletely developed.

5-Agriculture suspended cells: It means culturing cells individually or in the form of very small cell clusters in a liquid nutrient medium.

6-Agriculture protoplast: This means the culture of cells without walls. Protoplasts are prepared from any part of the plant, and it is preferable to prepare them from plant parts in which the middle layers are thin, such as the parenchyma of leaves, or to use cells in fast-growing areas. Some enzymes are used to remove the cell walls, and protoplasts are grown in nutritional media similar to those used for plant cells, but by providing osmotic support to prevent them from bursting due to the absence of walls.

7- Anther or haploid culture: This means culturing the entire anther with pollen grains inside it, or culturing pollen grains. In this case, the cells contain half the original number of chromosomes of normal or somatic cells. These cultures are used for educational purposes or to produce some special materials are also important in plant breeding programs.

2. Stages of plant propagation using tissue culture techniques:

1-Stage selection and preparation of mother plants: Selection and preparation of stock plants

2-Stage obtaining a sterile tissue culture: Establishment of an aseptic culture

3-Stage tissue duplication: Multiplication of the tissue

4-Stage laboratory root formation and conditioning: In vitro Rooting and Conditioning

5- Formation roots outside the lab and the pen: In live Rooting and Acclimatization

3. Exposed tissue cultures or organ cultures

It means planting fully formed plant parts or those in the beginning of their formation, such as the growing tips of the stems, roots, leaf primordia, or flowers, and pushing these parts to complete their growth in the same manner as if they were connected to the mother plant. This section includes a number of types :

3.1Meristems Culture

It is the isolation of a very small part of the growing tip with only one or two leaf primordia and planting it in a specific nutritional medium to give one plant. This method is used to obtain plants free of viral diseases. The theoretical basis for this is that the virus spreads in the vascular tissues of the plant and that the growing tip shydrate active is characterized by I am emptyha from the virus.It is also used in the propagation of plants such as orchids.(Figure 01).

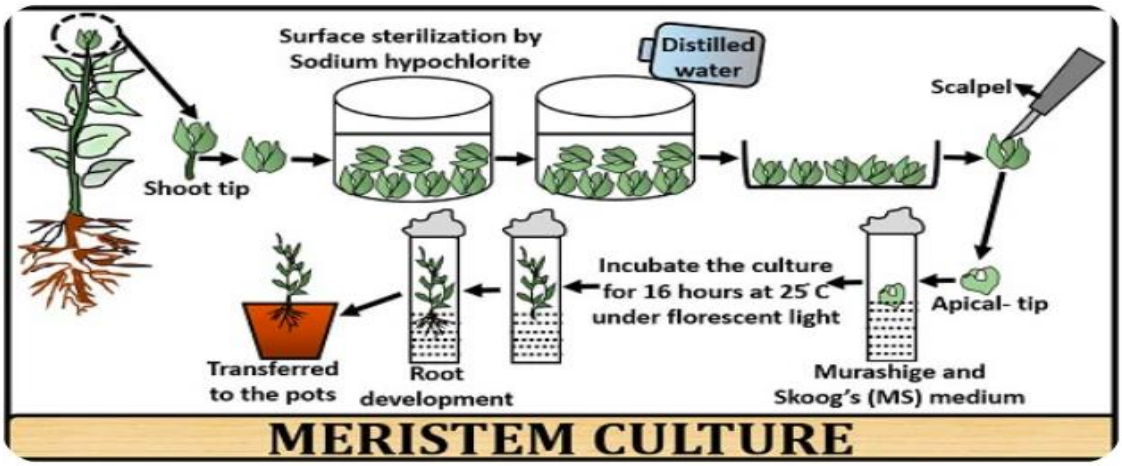


Figure 01 : Planting meristematic tops

3.2Shoot Tip Culture

It includes the meristematic tip with a few leaf primordia in a nutrient medium to produce one or more plants. It is required that they be from buds previously formed on the mother plant. It is also considered one of the methods of preserving the rootstocks genetic (Figure 02).

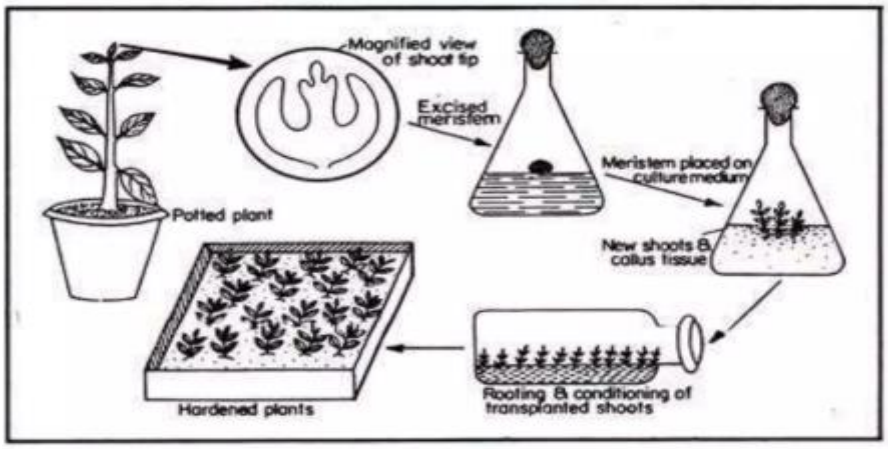


Figure 02 : Growing tip cultivation

3.3 Stem nodes culture

In it, the stem is divided into several parts, each of which contains a node.Buds one or more buds grow into a stem, and the growing tip and stem node methods are used for commercial propagation of many plants (Figure 03).

Figure 03 : Stem Nodes Culture

3.4Leaves Culture

What is meant by it ? for the beginnings of the leaves and their cultivation in a nutrient medium, and thus it is possible to follow the different developmental stages that the leaves go through under controllable intermediate conditions in it(Figure 04).

Figure 04 : Planting leaves

3.5 Roots Culture

Where the roots are separated and planted in the growth medium, and this method is often used to obtain effective medical materials in the laboratory(Figure 05).

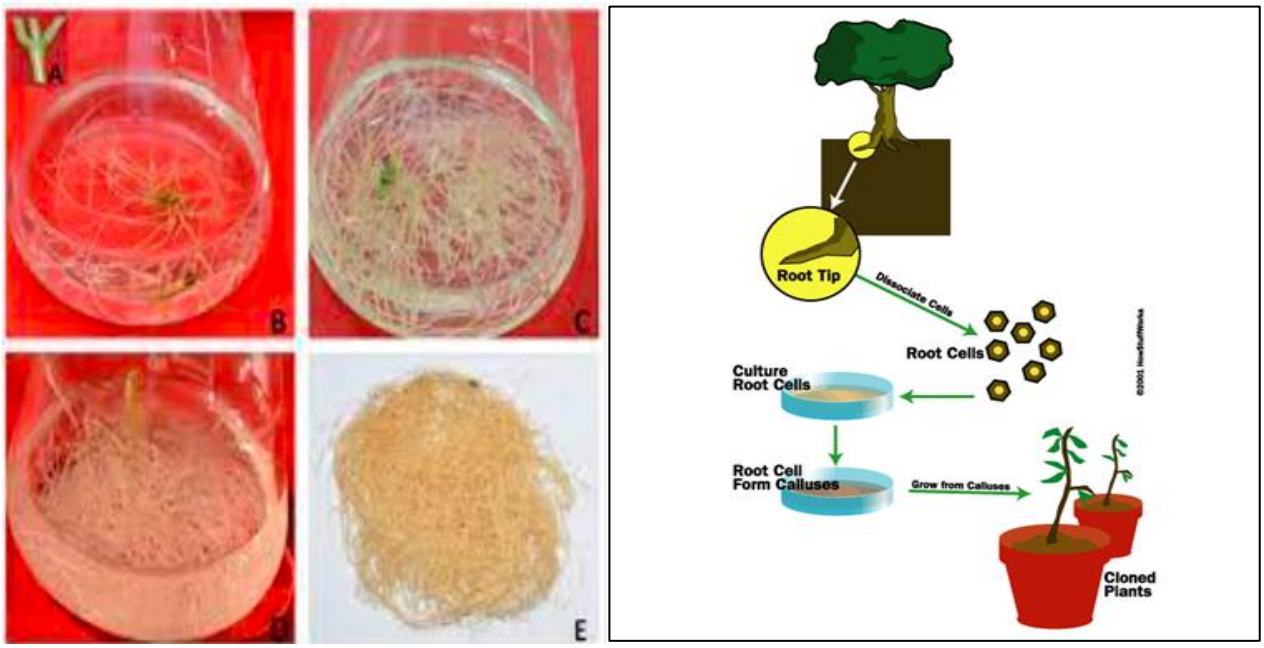


Figure 05 : Root planting

3.6 Embryos Culture

It is divided into two sections according to the embryonic stage :

1. Mature Embryo Culture

It is summarized in separating the embryo from the seed with the aim of planting it in a nutritious environment to overcome the problem of dormancy (Figure 06).

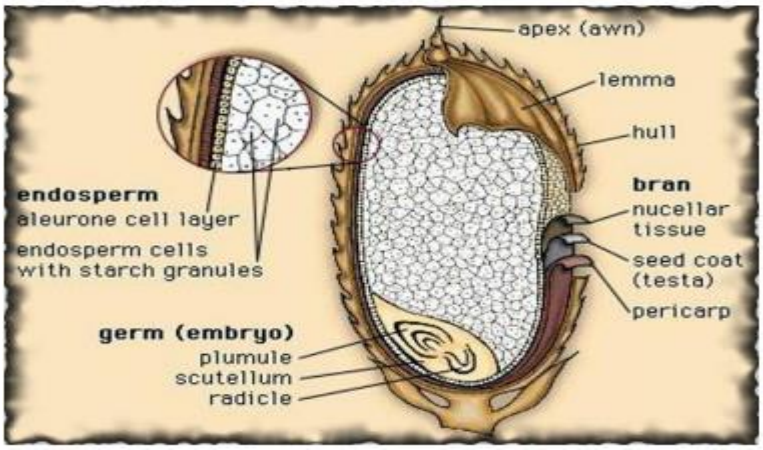
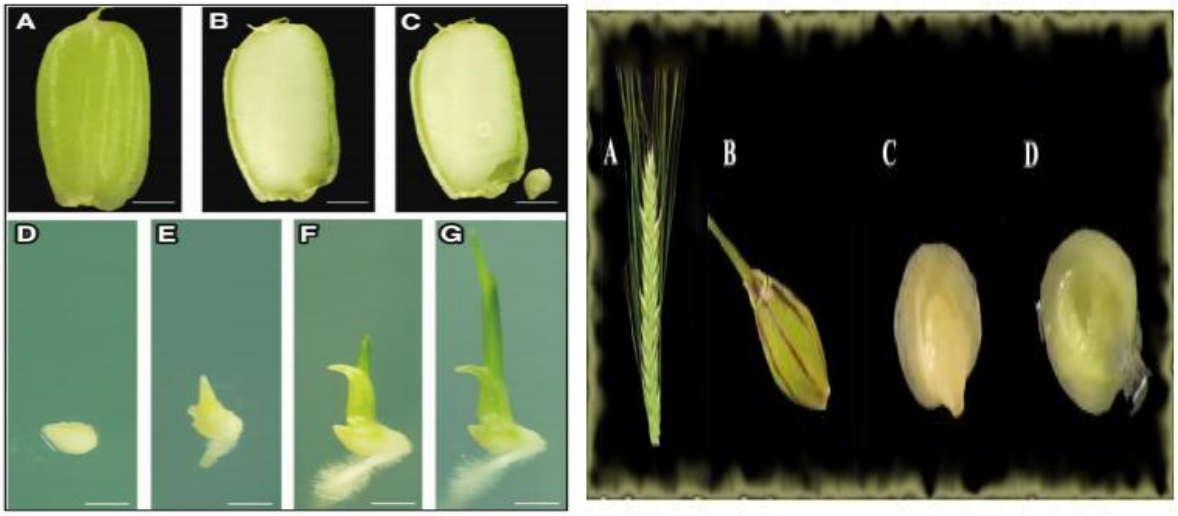


Figure 06 : Mature Embryo Culture

2. Immature Embryo culture

Here the embryo is separated at an early stage immediately after fertilization in a period ranging between 11-23 a day after pollination, this method is used to obtain distant hybrids that are impossible to obtain or produce by other methods.Normal and the success of this process depends on the speed of separating the embryo once fertilization occurs. This is called (Embryo Rescue) the process of rescuing the embryo before the miscarriage occurs (Figure 07).



Embryo culture technique.A) Surface sterilization of 7 immature seeds. (B) The seed can be distinguished after removing the seed coat. The white endosperm can be distinguished. And the yellow fetus (lower right).C) Seed after embryo isolation. (D) Embryo at 24 h after transfer to embryo rescue medium. (E) Embryo germination at 48 h after embryo rescue. (F) Seedlings at 72 h after embryo rescue. (G) Seedlings at 96 h after embryo rescue. Scale bars, 1mm.

Figure 07 : Culture Embryo Immature

3.7Ovules and Excised Flowers cultures

Only the ovaries or the entire flower are separated and planted in the nutrient medium in order to overcome some problems resulting from fertilization, such as the flowers falling before the embryo is fully formed, as well as to cause reproduction laboratory(Figure 08).

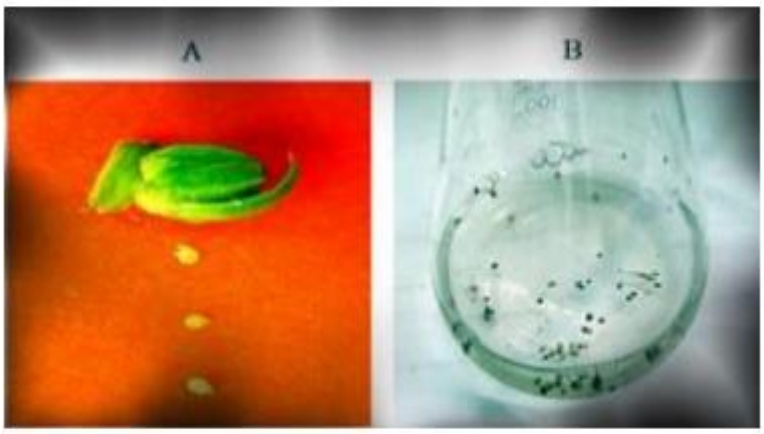


Figure 08 : Ovules and Excised flowers Culture

3.8Anthers Culture

In it, the anther containing pollen grains or pollen mother cells are planted to form individuals with a single genetic makeup, where the transcription and translation of the gene are stopped.yen the cells responsible for the formation of the gamete phase and the activation of the transcription and translation of genes responsible for the formation of tissues kharmful. Instead of pollen grains forming,the mother cell of pollen grains directly forms haploid embryos(Direct Regeneration) or tissues callus reveals embryos or branches(Indirect Regeneration) pollen grains may be planted to same purpose and called(Pollen grain culture microspore)

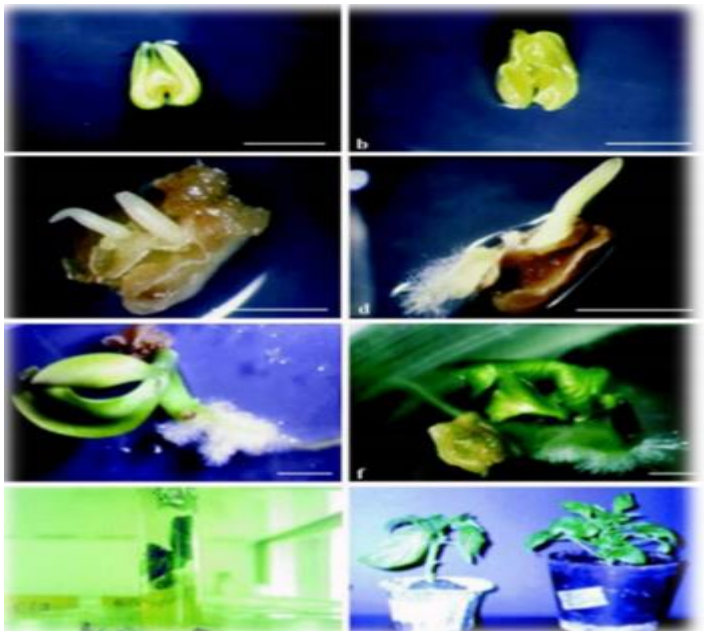


Figure 09 : Anthers Culture

4. Uses (Goals) Tissue culture

-Propagation of plants that are difficult to propagate by conventional methods or plants that are threatened with extinction on a commercial scale.
- Production of virus-free plants.
-Preservation of genetic resources (germplasm conservation)
- Production of many organic compound and serums.
- Obtaining whole plants of genetically modified cells.

-Increasing genetic variations by taking advantage of chromosomal changes resulting from tissue culture, or what is known as : Somaclonal Variation

- Obtaining somatic hybrids using protoplast fusion technique.

TD 04 : The wall Cellular

All cells of the plant kingdom are surrounded by non-living membranes or walls, and the shape, size, and composition of the cell's tissues are determined by the presence of these walls.The thickness of the walls varies from one cell to another, as the thickening system is a characteristic of different cells. For example, parenchyma cells have a thin cell wall, while the cells that control the distribution of water in the tissues of the woody part have a thicker wall.

1. Gross structure of the cell wall

Three layers (which differ from each other in many characteristics, including the percentage of water, the chemical composition, and some physical characteristics) characterize the cell wall. These layers are:

1.1 Middle lamella

Also called the interstitial material.Intercellular substances are composed of calcium and magnesium pectinate. In woody elements, they contain lignin in addition to pectinate. These substances are decomposed by the pectinase enzyme and are described as amorphous or isotropic, so they have no effect on polarized light.

1.2Primary cell wall

It is the first part of the wall that is added by the cell protoplast above the middle plate. When it is added, the cell is still in a state of superficial and volumetric growth. It is composed of cellulose and non-cellulose materials are polysaccharides and pectic substances. Due to the presence of cellulose, the primary wall is optically active or anisotropic, because cellulose is composed of fibers arranged in a way that helps to deflect light through them. Cellulose exists in two types: crystalline, in which the fibers are parallel, and amorphous, in which the fibers are not parallel. The percentage of amorphous cellulose is large, so it remains flexible and undergoes reversible changes.

1.3Secondary cell wall

This wall is added to the primary wall from the inside after the cell reaches its final size and growth stops, it surrounds dead cells, and is composed of cellulose and non-cellulosic materials, such as lignin and suberin is free of pectin, and due to the presence of cellulose, the secondary wall is described as optically active or anisotropic, and the large proportion here is for crystalline cellulose, as it constitutes 90% of the total cellulose, and undergoes irreversible changes.

Table -1: comparisonBetween primary and secondary wallIn plants.

secondary wall	The first wall	The attribute
Added after cells stop growing	It is formed in the early stages of cell growth.	Formation
It consists of unequal amounts of polysaccharides. Cellulose makes up 80% of the wall.	It consists of equal amounts of polysaccharides.	Ingredients for polysaccharides
regular	irregular	Cellulose microfiber arrangement
Rigid and inflexible	Non-rigid flexible	Hardness
Vascular tissues such as wood	parenchymal tissue	The tissues in which it is found

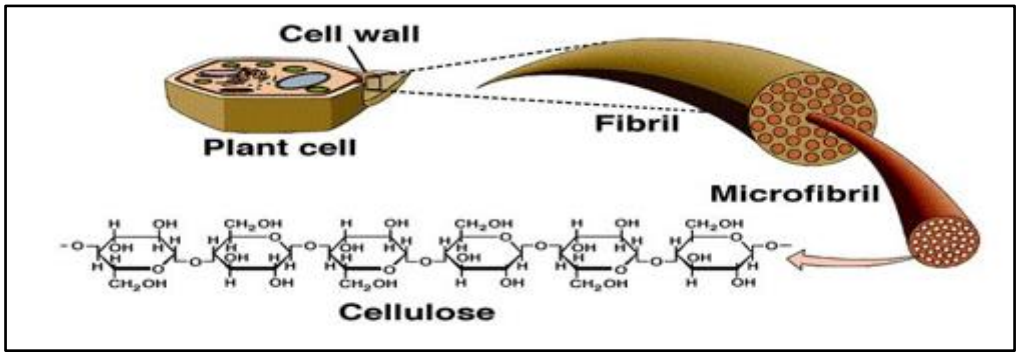


Figure 01: Chemical composition of the cell wall

Note:

When the middle plate is not distinct from the primary and secondary walls, it is called the composite middle plate.In this case, the compound lamella is either three-layered or five-layered.The simple middle plate is the one that was formed first before the wall Primary.

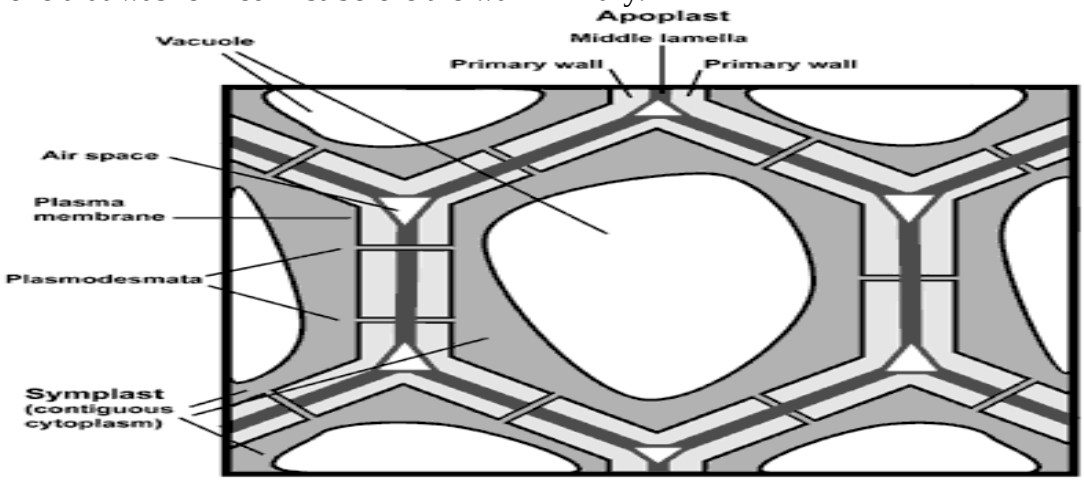
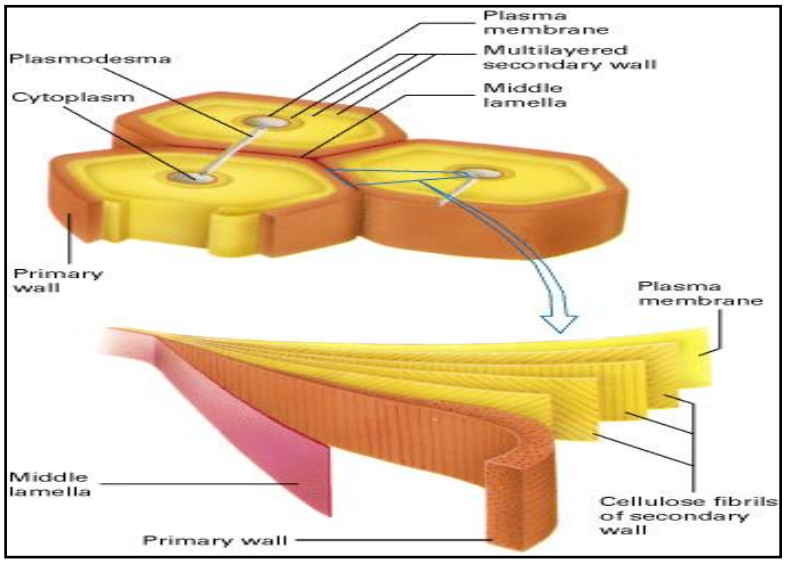


Figure 02: Cross section of plant cell wall



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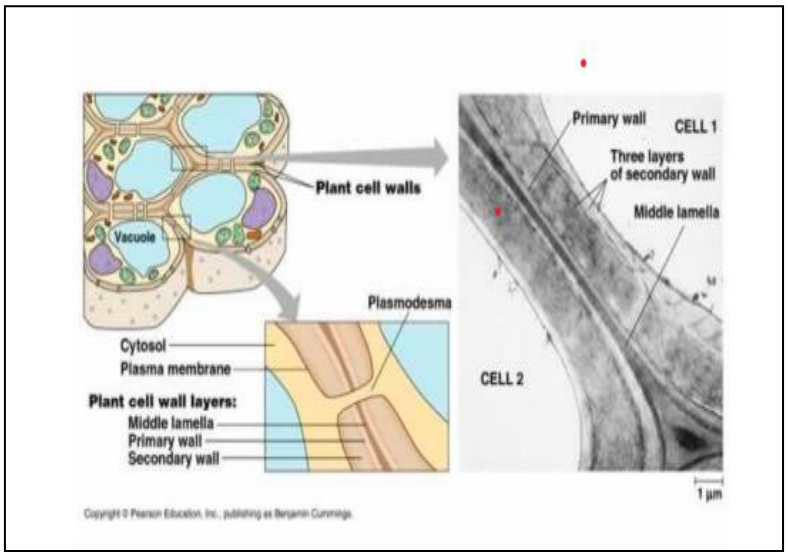


-B-

Figure 03: Illustration of cell wall components (A-B)

2. Pits

They are cavities or depressions in the Secondary walls they usually appear in primary walls as thin areas that arise from the uneven addition of primary wall materials to the middle plate. These areas are called pit fields (Elementary primarypit fields).Pits appear in pairs in the walls of adjacent cells and are separated from each other by a thin membrane, composed mainly of calcium pectate.

2.1Pit components

The pit consists of three parts:

1. **Pit membrane**: It is a thin membrane consisting of the middle lamella and a small part of the primary wall.
2. **Pit cavity**: The depression lies between the membrane and the cell cavity.
3. **Pit aperture**: It is the opening at the end of the cavity of the fovea where it meets the cavity of the cell.

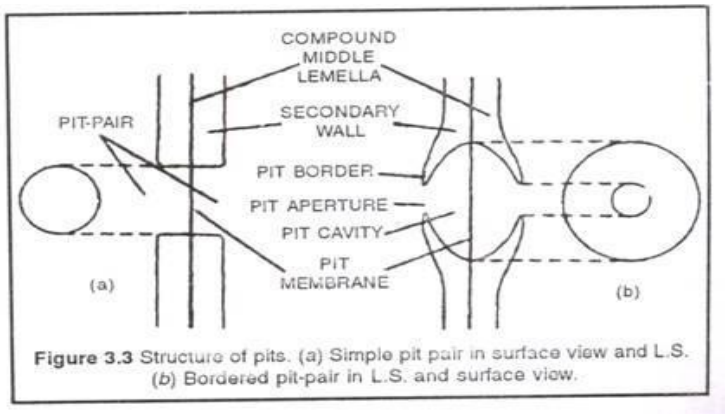


Figure 04: Pit components

2.2 Type pit pits

1. Primary pit fields

When it expands as a result of the growth of protoplasts, it looks like a rosary in the view.Sideways, and plasma bonds pass through it plasmodesmata.

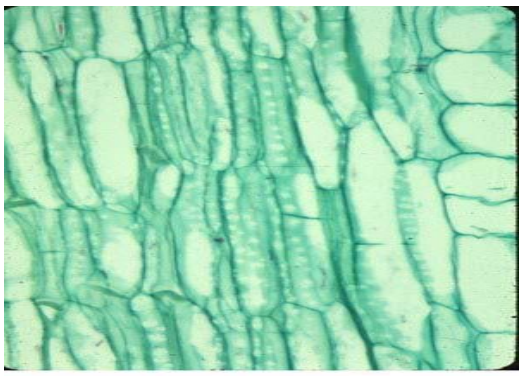


Figure 05: Primary pit

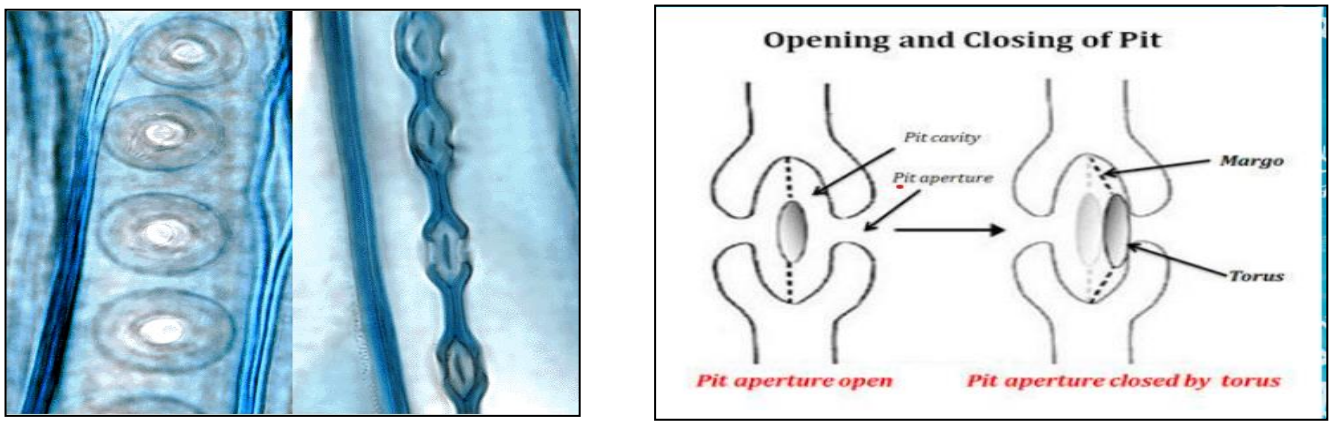


Figure 06: Bordered pits

2. Simple pits

It is found in the secondary walls, it has a diameter ofhomogeneous, it features pit components three, it is found in parenchymal cells thickened and some wood and fibre elements.

3. Bordered pits

It differs from simple tapping by the presence of borders and Torus (How are the borders formed? What is meant by Torus?)

These pits are found in the gymnosperm order. (Coniferals, Gnetales, Ginkgo ales)

4. Ramiform or Branched pits

It is characterized by increased thickness of the secondary wall and the pits are deep in the form of channels branching out, it is found in the fruits of the pear pyrus fruit (Figure 07).

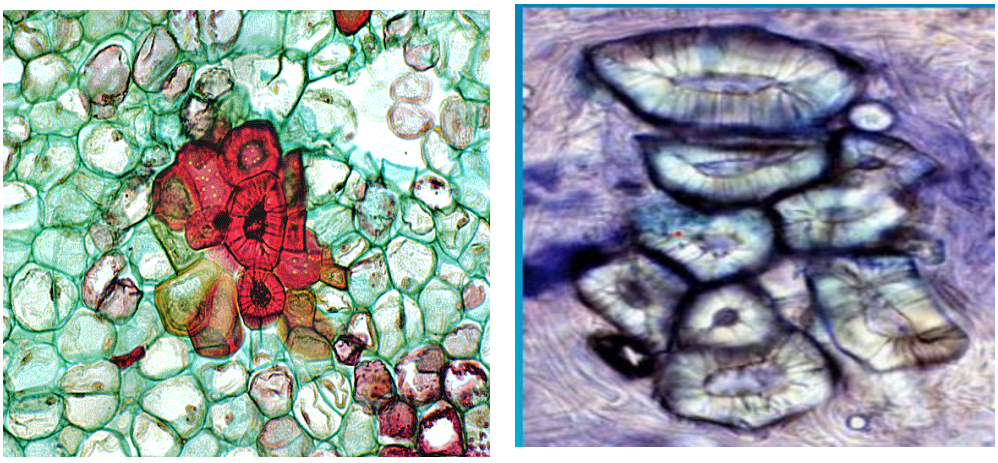


Figure 07: Branched or channeled pits

Note:

Margo: A term used to describe the thin wall surrounding the bed.

2.3Pit combination

Pits are often found in pairs between adjacent cells and paired clicks are calledpit pair, it is of the following types:

1- Simple pit pair

In it, a simple click on one side is coupled with another simple click on the other side, as in parenchymal cells thickened rice.

2 - Bordered pit pair

In it, a groove braided on one side is coupled with another groove braided on the other side, as in wooden elements.

3 - Semi or half-bordered pit pair

Here, a double click on one side is coupled with a simple click on the other side.The other, as in the partition walls between a conductor element and a parenchymal cell.

4- Pitting compound unilaterally

In it, a notch on one side is coupled with more than one notch on the other side, as in the branched notches in the fruits of the pear.

5 - Blind pits

Coupling a click from one side to an interspace.

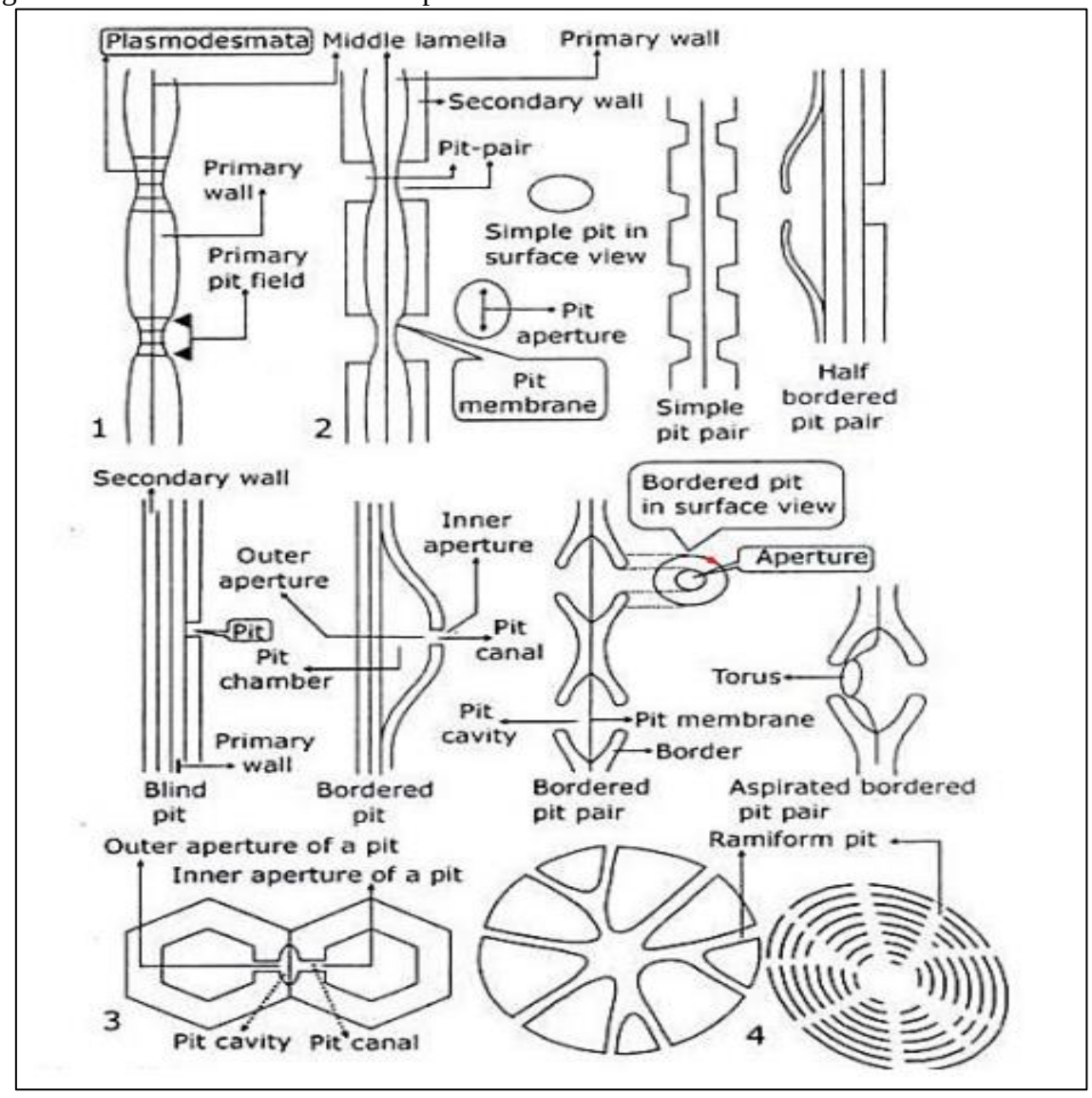


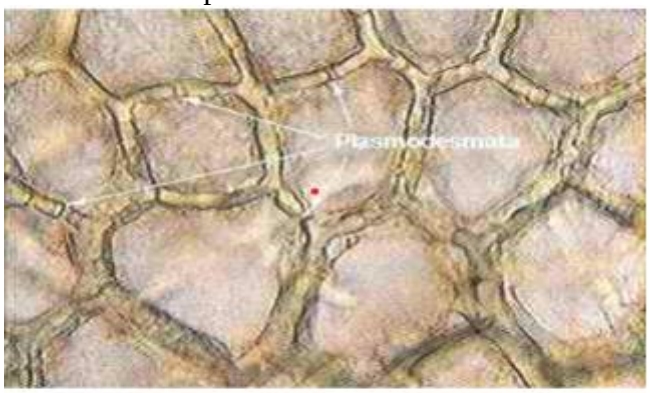
Figure 08: Pits coupling forms

As mentioned earlier, cells appear as a single unit, connected to each other by fine threads called plasma membranes plasmodesmata are defined as: threads cytoplasmic and a thin layer that passes through the openings in the vicinity of the cell to connect the protoplasts of adjacent cells. It is alive and has a protoplasmic nature evidence for this is:

Found in cells the snake, it is dyed with the same dyes cytoplasm, it is interrupted in case of plasmosis permanent, its interactions are positive with oxidation enzymes and it is similar for cytoplasmIn that.

Note:

These threads can be observed in the endosperm of the date kernel.*Phoenix dactylifera.*



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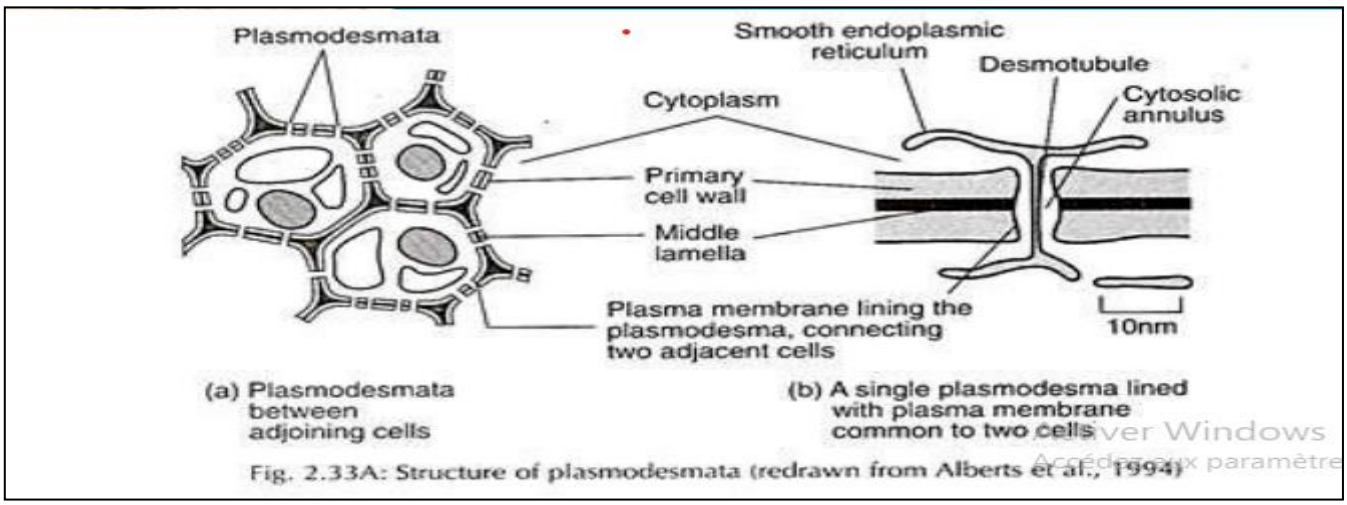

-B-

Figure 09: Illustration of plasma bonds (A and B)

3. Pitting

The pecking order in the cell wall is an example of a ladder arrangement.Scalarifomand mutual tappingalternate pitting and opposite pitting.

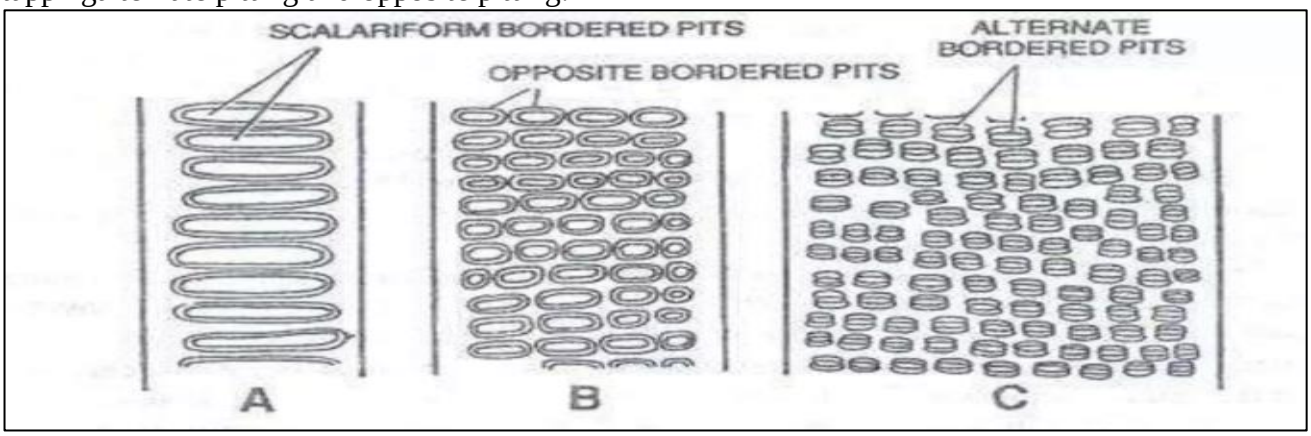

Figure 10: Pitting shapes

4. Cell wall functions

1- Mechanically assisting plant cell by giving it hardness and durability.
2- Resistance to bulging pressure mozzie for the cell.
3- Helping in cell growth by some proteins present in the wall composition that give flexibility to the primary cell wall.
4- The porosity of the wall allows the passage of dissolved materials through the cell wall and mediates the exchange of ions between the cell and its surroundings.
5- To set and sustainability appearance cell.
6- Protection against pathogens and environmental factors.

5. How the cell wall is formed

Cell wall formation begins after the final phase (Telophase) for division, a cytoplasmic plate is formed between the two nuclei in the middle of the cell, and pectic materials are deposited in it, and it is then known as the middle plate, which consists of calcium and magnesium pectate, and is deposited on both sides of the middle plate (Middle lamella) a thin layer of cellulose between the two cells resulting from division and forming the primary or primary wall then other materials such as lignin are deposited on the two thin cellulose layers lignin and the superin is thus formed.Secondary wall for plant cell.

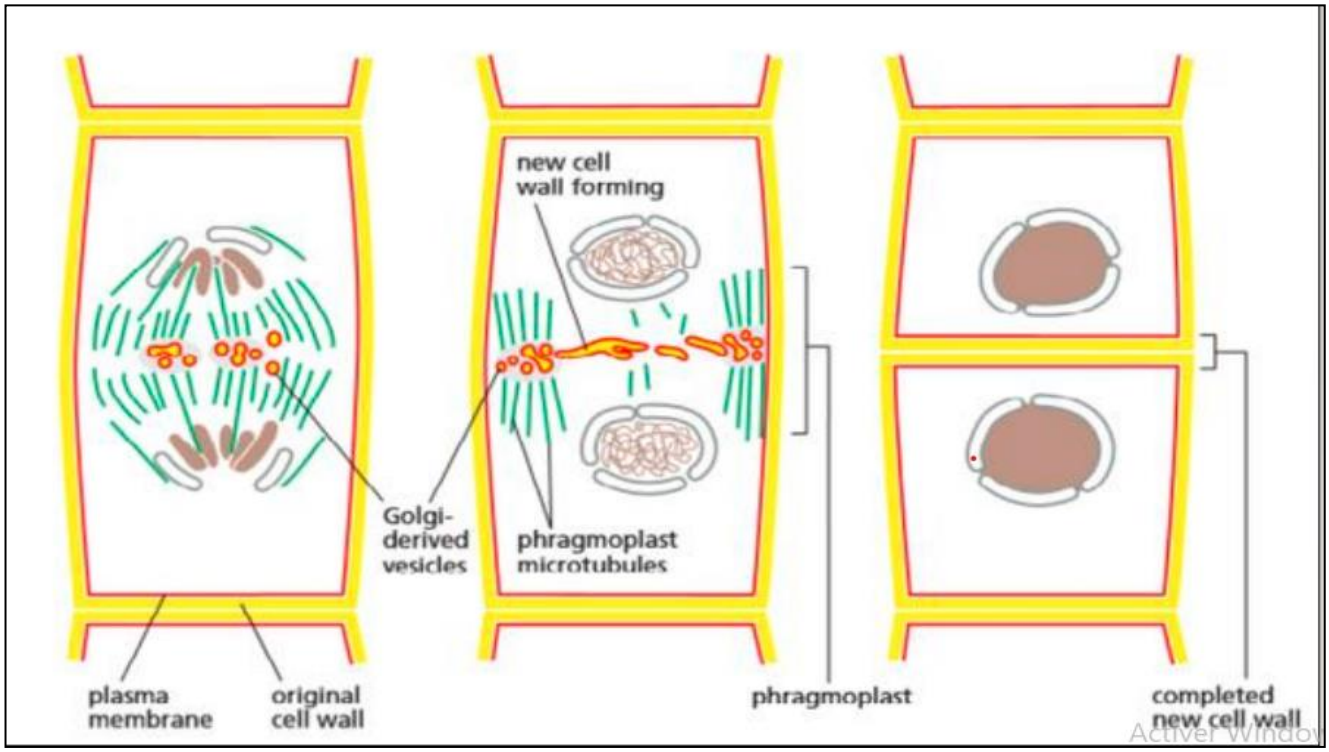

Figure 11: Stages of cell wall formation

TD05 : Protein synthesis in eukaryotes and prokaryotes Cell biology

Reminder of the gains

-The genetic information carrier is the part that carries the genetic information present in the nucleus, which is the DNA.

-Genetic information is in the form of genes in the DNA molecule, and the gene is a specified sequence of nucleotides.

-Gene expression is the expression of genetic information by a gene in the form of a specific protein, which is the source of the individual's phenotype at its various levels (Molecular cellular organic).

1. Protein synthesis site

-Protein synthesis takes place in cells at the cytoplasmic level.

-The transmission of genetic information from the nucleus to the cytoplasm, the site of protein synthesis, is ensured by another type of nucleic acid called messenger (ARNm).

2. **Chemical composition of ARNm**

They are identified according to the total and partial hydration products as :

A-Hydration College

It is done at high temperature using NaOH and results in : 4Types of nitrogen bases are :

- Pyrimidine bases (two rings) :(U) Uracil-(C) Cytosine.
- Purine bases (single ring) :(G) Guanine-(A) Adenine.
- Five carbon sugar is ribose sugar C5H10O5
- Phosphoric acid H3PO4

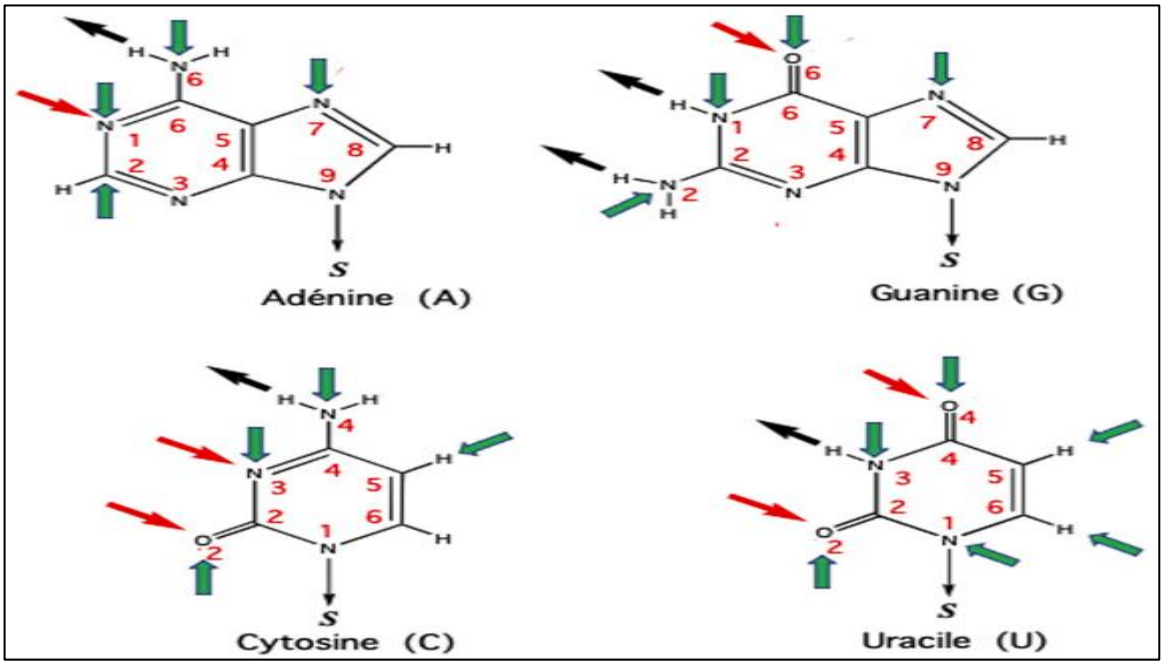

Figure 01 : Ingredients Chemical for ARNm

B.Partial hydration.

It is done using specific enzymes of the type ARNase and results in :

- Nucleotides : representtoIn the bonding of phosphoric acid with ribose sugar with a nitrogenous base, and depending on the type of base, we find 4 Types : Adenosine-guanosine-Xanthosine-Uridine.
- Multiple Nucleotide : It consists of a few nucleotides being linked together.
- Nucleosides : It is a bond of ribose sugar with a nitrogenous base.

C.ARN Structure

It consists of a single chain of nucleotides linked together by phosphate ester bonds between the ribose sugar and the first cleotide, which side ? '3 with phosphoric acid of the pro-nucleotide side 5' so the series always starts at the end 5'and it ends in the end'3.

- **Comparison between DNA and ARN**

ARN	DNA	comparison
It consists of one chain.	It consists of two helically twisted chains.	Structure
Regular ribose	deoxyribose	Sugar
Cytosine, the Guanine, Uracil, Adenine	Cytosine, the Guanine, thymine, Adenine	Nitrogen bases
Nucleus and Cytoplasm.	Nucleus only (in eukaryotes))	the site

3. Genetic information cloning

- It takes place in the nucleus and during it the biosynthesis of the molecule takes place ARNm based on the DNA.

3.1The basic elements for the cloning process to occur :

- Gene (The original genetic information on a molecule DNA).
- Enzyme ARN polymerase.
- 4Types of Nucleotides in the composition ARN
- Energy ATP

3.2 Stages of You will clone

The cloning process goes through three stages :

a.Bitter Launch suit :

Enzyme is associated ARN polymerase starts at the beginning of the gene and removes the winding and opens the two DNA strands after breaking the hydrogen bonds between the nitrogenous base pairs. The enzyme begins reading the sequence of bases on one of the two DNA strands. (The series (cloned) and nucleotide binding approval for her to install a series of ARN, where it is located Nnucleotides ARN vs DNA nucleotides by complementarity of nitrogenous bases.

b.Elongation phase :

Enzyme is transmitted ARN polymerase travels along the gene to read the information on the DNA molecule and binds the ARN nucleotides according to their sequence in the transcribed DNA strand, leading to the elongation of the ARN molecule.

c.Stagethe end :

The enzyme reaches the end of the gene and elongation stops ARNm separates from DNA, the enzyme separates, and the two DNA chains close.

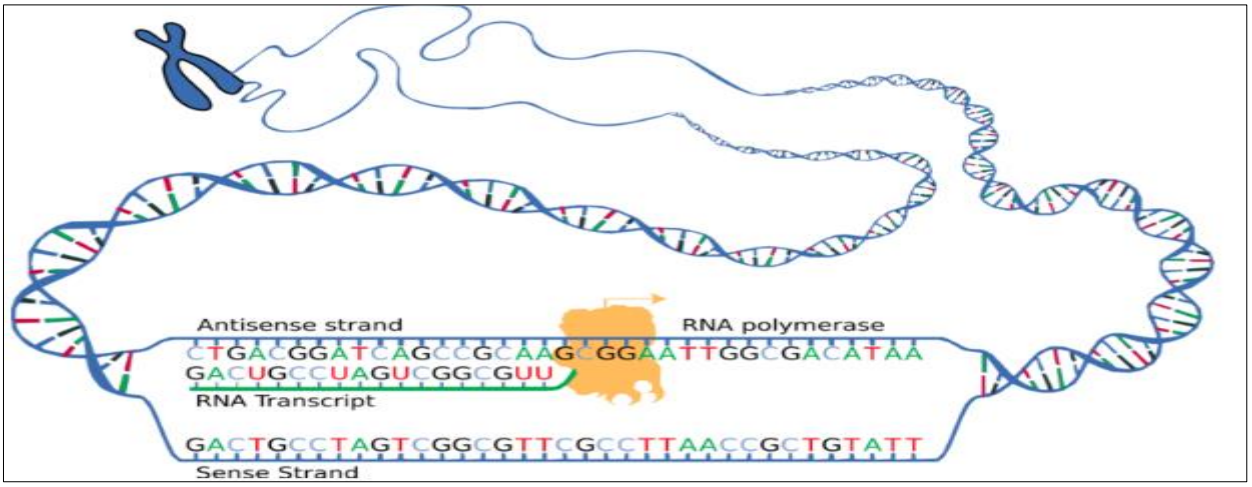

Figure 02 : Stages Cloning

-Several molecules of the enzyme move from the start site of transcription to the end site and thus several molecules are copied ARNm at once.

-The direction of cloning is always from the end '5 to 3'in ARNm.

-The cloned chain is in the opposite direction to the original chain ARNm, while the non-transcribed strand is in the same direction as ARNm.

3.3 Maturation The ARNm after transcription

-Genes in eukaryotes are characterized by (DNA) containing non-significant segments (introns) and significative segments (exons).

-Immediately after cloning, it is formed ARNm has the same length gene (number Nucleotides) It consists of functional and non-functional pieces and is called primary ARNm (immature, precursor).

-At the kernel level, non-functional segments are deleted and functional segments are linked to the first ARNm is processed by deletion and splicing enzymes to form mature ARNm that is shorter in length and consists of only functional segments to be transferred to the cytoplasm and translated into a specific protein.

- The called this process maturation ARNm.

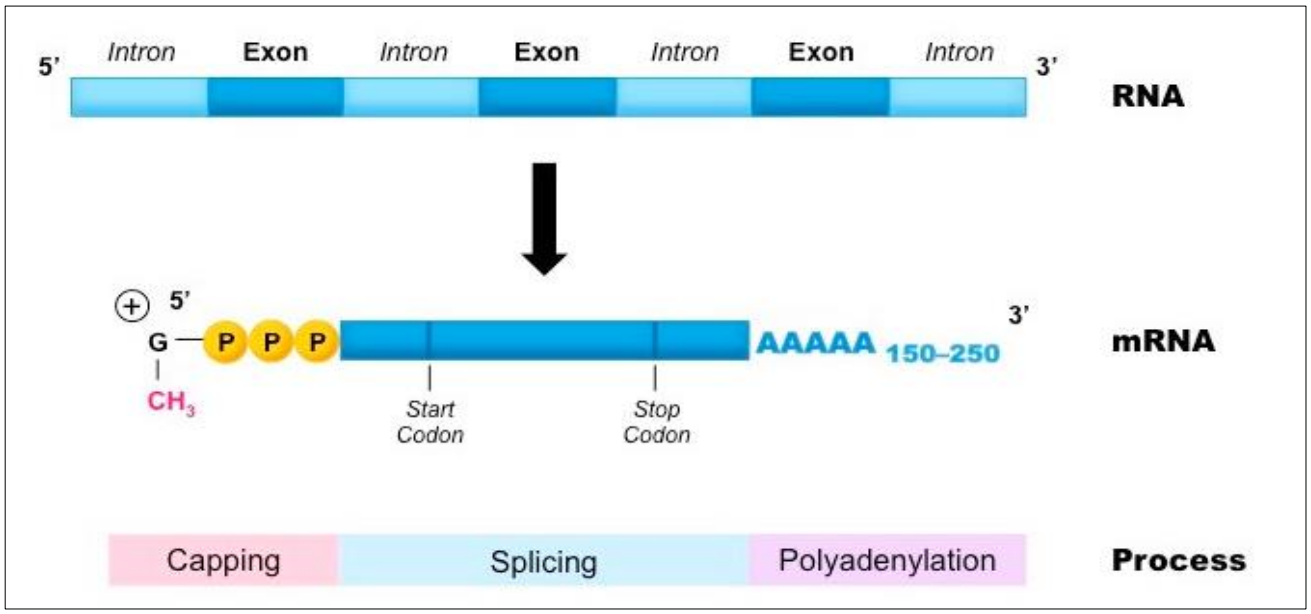

Figure 03 : Maturation ARNA messenger after the process of You will clone.

-Maturity phenomenon ARNm is not found in prokaryotic cells, it is characteristic of eukaryotic cells only.

-In prokaryotes, all parts of the gene are functional segments and are directly produced from them mature ARNm.

4. Translation

-It is a nuclear language conversion. ARNm (nucleotide sequence) to protein language (amino acid sequence) at the cytoplasmic level, using the genetic code dictionary.

-Genetic information is copied by code especially : It is called the genetic code, and the unit of the genetic code is a triplet of bases called a codon that codes for a specific amino acid in a protein.

-The nuclear language represented by 4 letters of nitrogenous bases is transformed into a protein language represented by 20 words of amino acids according to the following relationship :

A^B=C -----> 4^3 = 64

a. Represents the number of nitrogenous bases.

b. Is the number of bases in the codon.

c.Number of code types.

-The number of words in the nuclear language (64) is more than the number of words in the protein language (20), and this indicates the existence of the synonymy property, several codons can code for the same amino acid.

-The genetic code table shows that 61 codons out of a total of 64 code for amino acids, the most prominent of which is the start codon.AUG codes for methionine, and there are 3 nonsense codons that do not code for any amino acid : UAG, UAA, and UGA, and they are called codons stop.

Table 01 : The Code Genetic

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC : Phe UUA, UUG : Leu	UCU, UCC, UCA, UCG : Ser	UAU, UAC : Tyr **UAA Stop** **UAG Stop**	UGU, UGC : Cys **UGA Stop** UGG Trp	U C A G
C	CUU, CUC, CUA, CUG : Leu	CCU, CCC, CCA, CCG : Pro	CAU, CAC : His CAA, CAG : Gln	CGU, CGC, CGA, CGG : Arg	U C A G
A	AUU, AUC, AUA : Ile AUG Met	ACU, ACC, ACA, ACG : Thr	AAU, AAC : Asn AAA, AAG : Lys	AGU, AGC : Ser AGA, AGG : Arg	U C A G
G	GUU, GUC, GUA, GUG : Val	GCU, GCC, GCA, GCG : Ala	GAU, GAC : Asp GAA, GAG : Glu	GGU, GGC, GGA, GGG : Gly	U C A G

The genetic code was deciphered thanks to the experiment he conducted the world Nirenberg

4.1 Stages Translation

4.1.1 Site of protein synthesis in cytoplasm

-Translation takes place at the level of the polysome, which is in the cytoplasm or connected to the active endoplasmic reticulum.

4.1.2Polyribosome definition

- It represents the association of a number of ribosomes with a single molecule of ARNm where each ribosome produces a polypeptide chain, and the more ribosomes are linked, the more protein is produced.

4.1.3Patterns of RNA involved in protein synthesis

-Three types of proteins are involved in protein synthesis ARN is : ARNm the Messenger, ARNt carrier, Ribosomal ARN.

4.1.4 Structure and components of the ribosome

The ribosome consists of two subunits that are formed at the level of the nucleus :

-**Under unit Major** : It consists of 31 types of proteins.And two types from ARNr (23S.5S), it contains two link sites.ARNt has an A site and a P site and contains a tunnel for the peptide chain to exit.

-**Under the unit agglutinate** : It consists of 21 types of proteins and ARNr (16S), it has a site for linking the ARNm allows the ribosome to slide and move on the ARNm molecule.

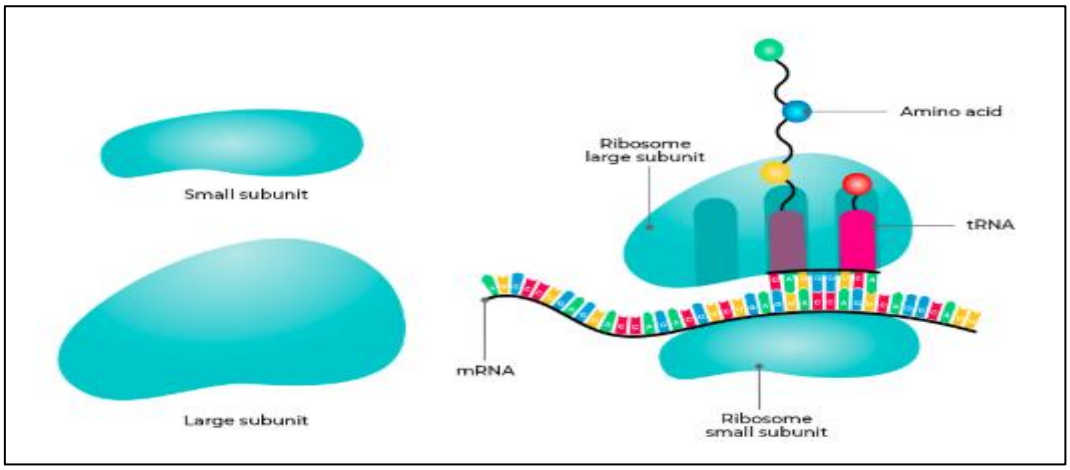

Figure 04 : Ribosome components and structure

4.1.5 Structure of the ARNt :

It consists of a chain of nucleotides that twist to take a specific spatial shape and includes two main sites :
-Amino acid binding site : allows amino-acid binding.
-The anticodon site recognizes the codon in ARNm.

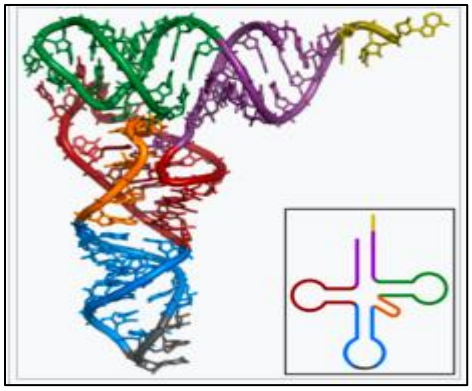

Figure 05 : The spatial structure of ARN

4.1.6Activate amino-acids

It consists of linking the amino acid to its ARNt is synthesized by a specific enzyme with the availability of ATP energy and is carried out according to the following stages:
- Verify ARNt and amino acid on their respective attachment sites in the specific enzyme.
-It is forming complex (Enzyme, amino acid, in the presence of ATP, the amino acid and ARNt are linked.
- Separates ARNt binds to the amino acid, and the enzyme is released.

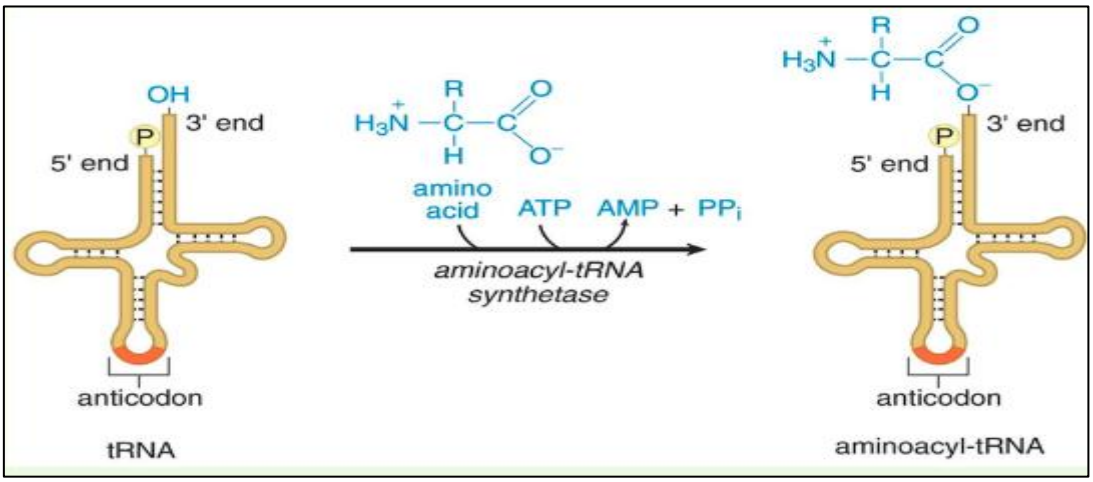

Figure 06 : Amino acid activation stages

5. Stagestranslation occurrence

5.1 Launch phase :

- Verify ARNm is located on the subunit, then methionine ARNt is located in the P site of the ribosome and the ARNt recognizes the start codon AUG in the ARNm via anticodon.
- They are linked under the larger unit to form the launch complex.
- It is positioned ARNt of the second amino acid in the a site of the ribosome according to the second codon of ARNm, a peptide bond is formed between the first and second amino acids.

5.2Elongation phase

The ribosome moves a codon on the ARNm, leading to the separation of the first ARNt from its amino acid and from the P site, and the site of the second ARNt carrying the dipeptide changes from the A site to the P site, and the A site becomes empty to receive a new ARNt carrying a third amino acid, so a peptide bond is formed between the third acid and the previous dipeptide, and thus the same steps are repeated and the peptide chain is elongated by an amino acid in each step.

5.3 Final stage :

- The ribosome reaches one of the stop codons on the ARNm.

The formed peptide chain separates and the last ARNt is separated from the two ribosomal subunits, and the first amino acid (methionine) is removed from the peptide chain.

- The ribosome can repeat the cycle and form another polypeptide chain.

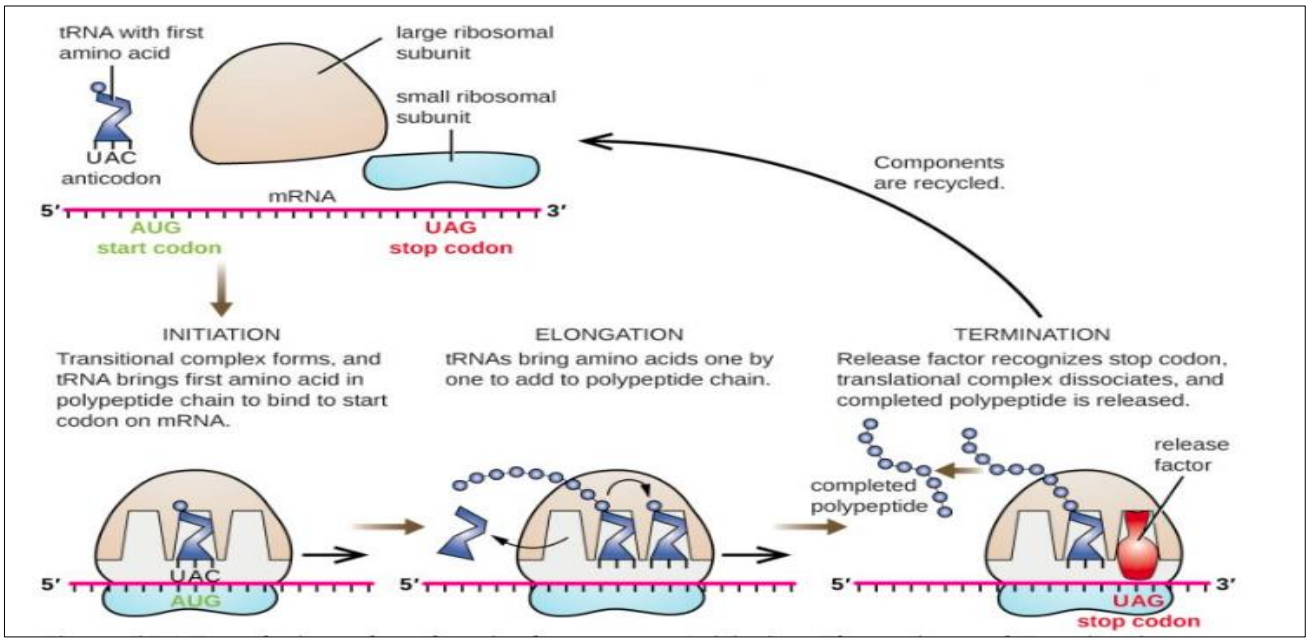

Figure 07 : Stages of translation process

6. The fate of protein after its synthesis

- Synthesis of proteins at the level of the polysome, which are found free in the cytoplasm or connected to the endoplasmic reticulum.
- It is transported by means of transitional vesicles to the golgi apparatus, where it matures and these proteins are classified, stored and packaged in vesicles.
- Vesicles transport the protein to its place of action and activity.
- In prokaryotes, translation can begin before the end of replication (Absence of nuclear envelope + formation directly mature ARNm.)

-In eukaryotes, transcription and translation cannot occur in the same place (Presence of nuclear envelope + maturation process (primary ARNm).

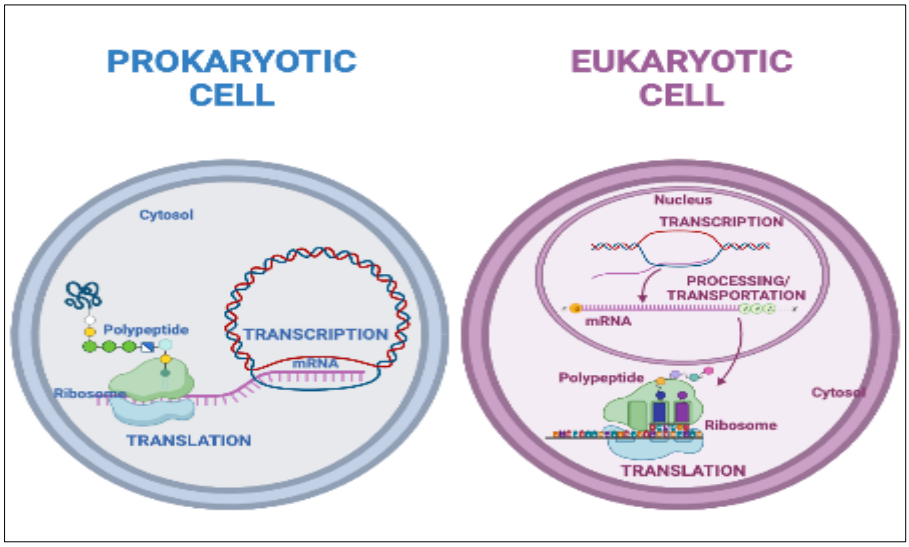

Figure 09 : Drawing Schematic diagram of protein synthesis (in eukaryotes and prokaryotes)

TD 06 : Plastids

Of the cell organelles that swim within cytoplasm the Plastids.

1. Definition of Plastids :

They are cytoplasmic organelles with a double membrane, characteristic of all plant cells, except fungi, and take different shapes, colors and sizes. It has its own genetic material, which codes for the production of many enzymes and proteins necessary for its functions, and allows it to self-replicate according to the cell's needs.Plastids are distributed in the cytoplasm along with the particles the chondria.

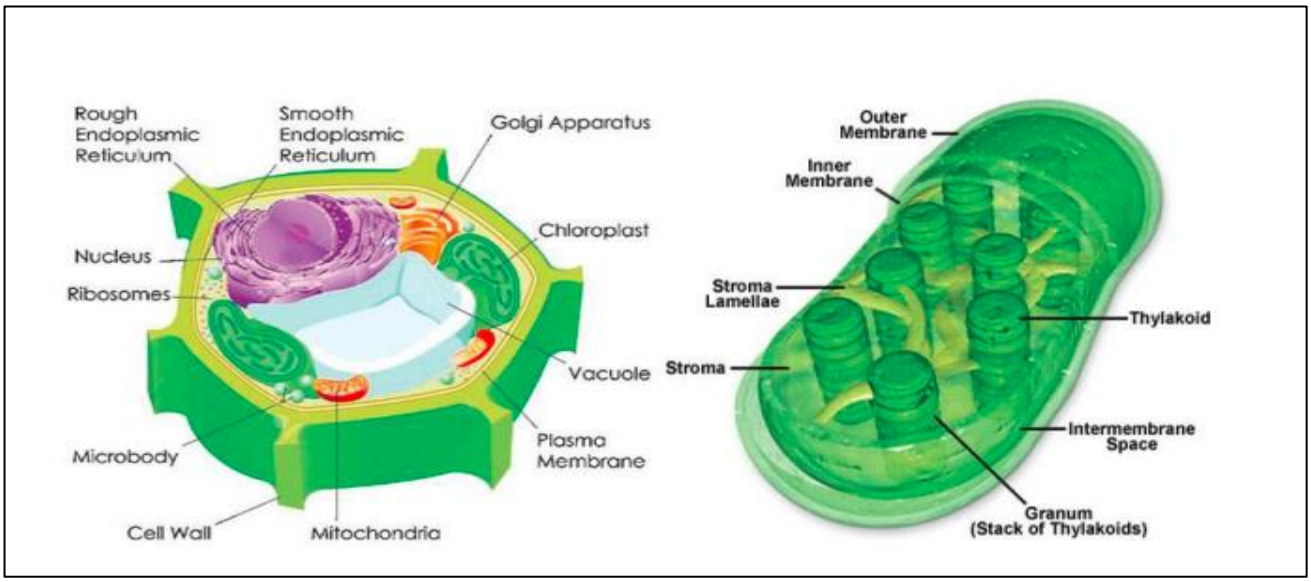

Figure 01 : General structure of the green Plastid (Chloroplasts)

a. **Her job** : Responsible for activities related to food production and storage, many of which are Photosynthesis.
b. **Origin** : All these types of makers descend and differentiate from a common, undifferentiated and unspecialized ancestor.It is the primary or precursor of the primary Plastids proplaste that are distributed into chromophores starchy and green, depending on the cell type it contains (root-leg-papers-Buds) .This differentiation is in many cases a reversible or two-way differentiation according to the following diagram.

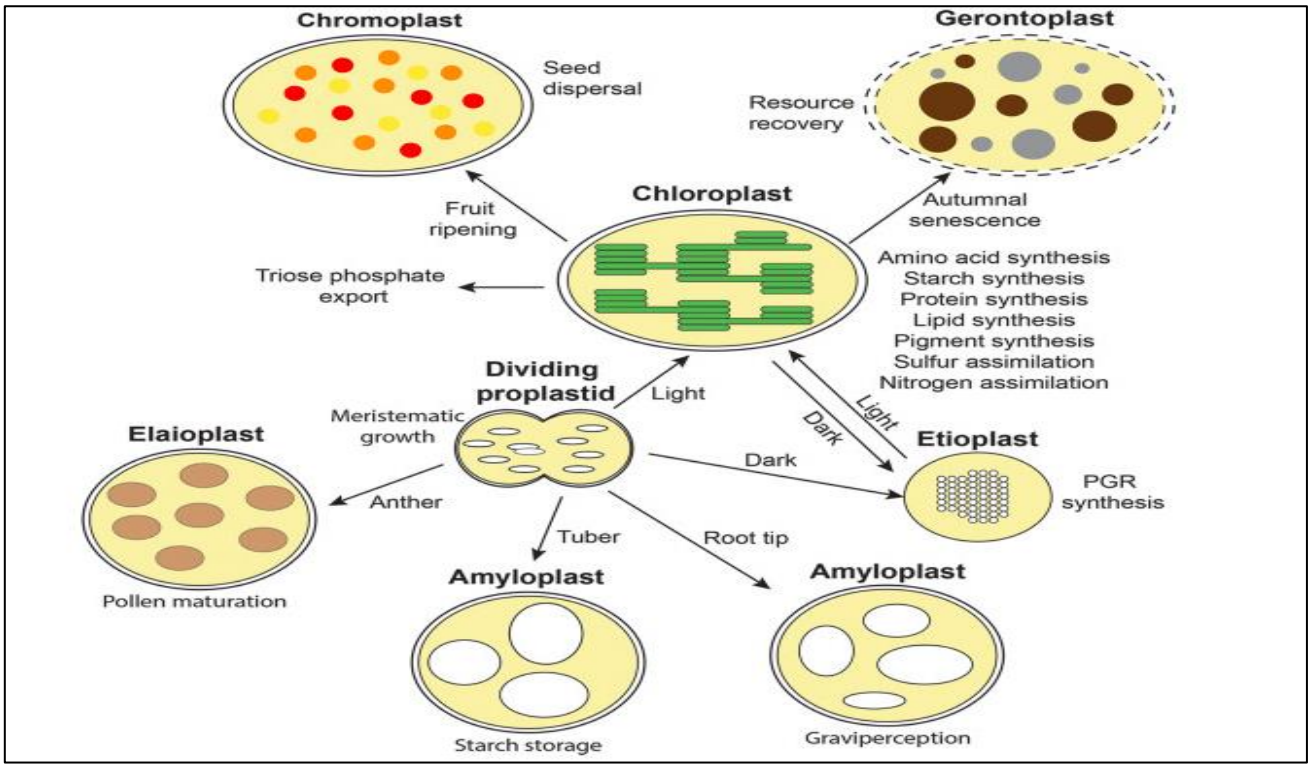

Figure 02 : Two-way reversible differentiation of different types of Plastids

- **primary Plastid**

It is the simplest and least differentiated of the blastocysts and is the blastomere that differentiates into other blastocysts as we have seen and is found especially in cells meristematic.Besides the origins of biophotonics (chondria) but it islarger than it a little bit.

2. Types of Plastids :

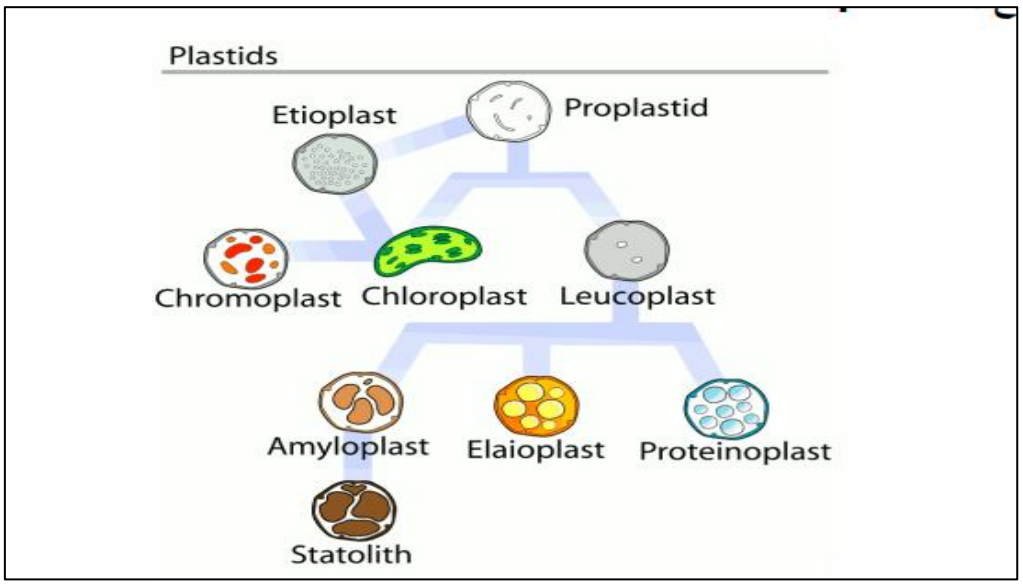

Figure 03 : Different types of Plastids

2.1 Leucoplasts :

These are the non-pigmented organelles which are colourless. Leucoplasts are usually found in most of the non-photosynthetic parts of the plant like roots. They act as a storage sheds for starches, lipids, and proteins depending on the need of the plants. They are mostly used for converting amino acids and fatty acids.

2.1.1 Types of white Plastids : Leucoplasts are of three types :

- **A- Amyloplasts** : are greatest among all three and they store and synthesize starch.

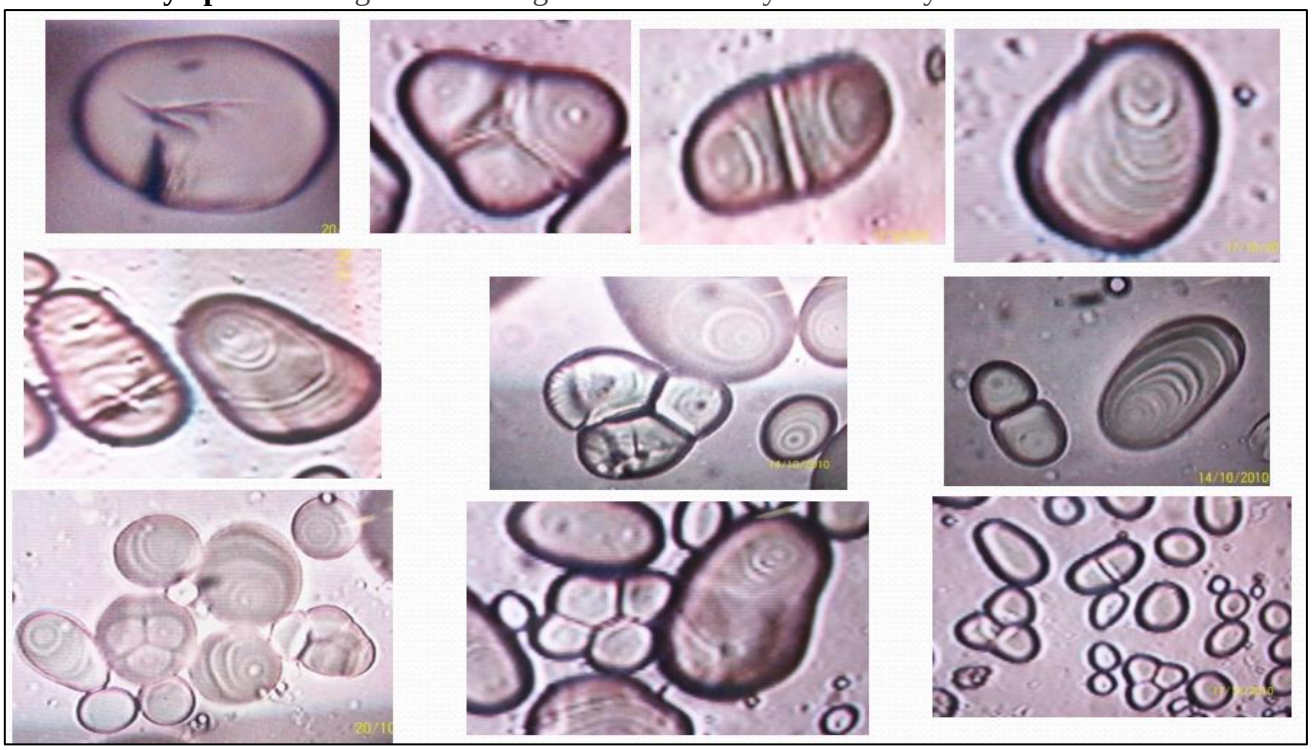

Figure 04 : Different forms of Amyloplasts

B- Proteinoplasts : help in storing the proteins that a plant needs and can be typically found in seeds.

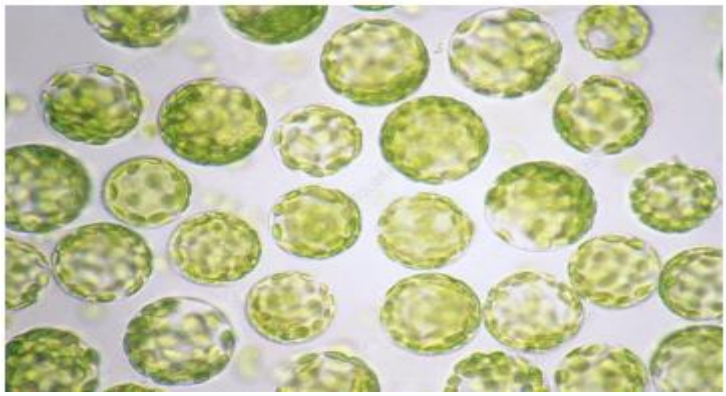

Figure 05 : Proteolytic Plastid (Proteinoplasts)

C- Elaioplasts -Elaioplast helps in storing fats and oils that are needed by the plant.

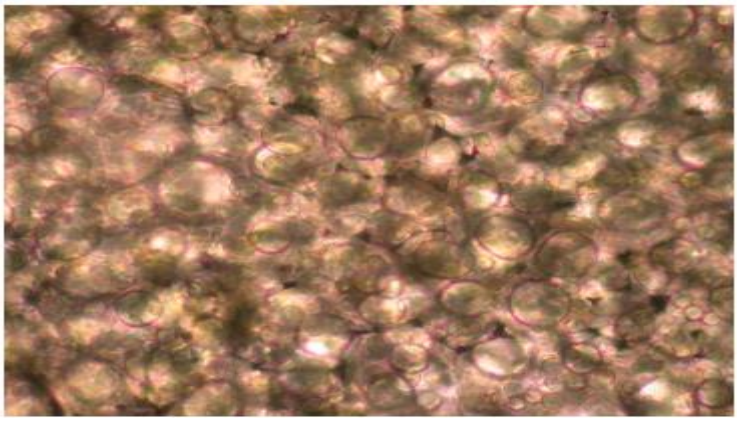

Figure 06 : Oil Plastids (Elaioplasts)

Note : When the **Leucoplasts** are exposed to light, they will turn into **Chromoplasts,** which means they have the potential to develop and carry out photosynthesis.

2.2 Chloroplasts : it is found in many plant cells, especially green tissues (leaves).

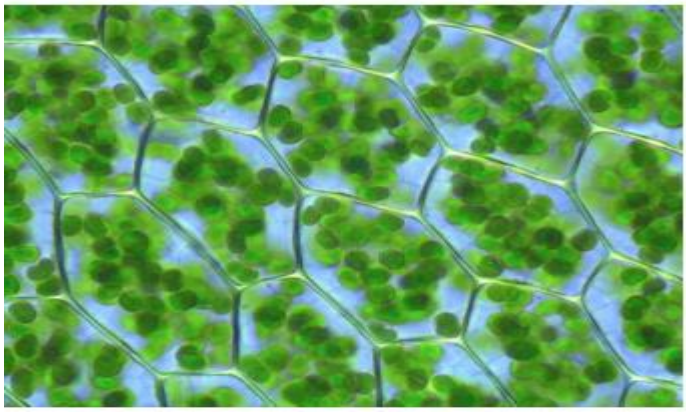

Figure 07 : Green Plastids (Chloroplasts)

Chloroplasts are distributed in the cytoplasm of higher plants.And green lichens it may be lenticular or elliptical in shape, it produces chlorophyll which is important in the process of photosynthesis (hence it is green in colour), it is particularly distinguished in the cells of the green parts of the plant.

Chloroplasts can transform during development into other types of chloroplasts, where their pigments are broken down. This happens in late summer, and the leaves turn red and then yellow.

The green maker body is surrounded by a sheath and two membranes ((Internal and external), the external is smooth.The inner space of the green maker is filled with a gelatinous substance we call the stroma in it :

-Ribosomes and nucleic acids DNA and RNA.

-All enzymes necessary for photosynthesis.

-Water, fats, proteins and minerals : Magnesium, phosphorus, calcium, iron, sodium, copper and no potassium.

There is also a third membrane within the stroma, which is arranged in parallel sheets parallel to the longitudinal axis for the Plastids , in one direction and forms flat bags called with Thylakoïdes, which are stacked on top of each other to form grana (grana)singular (Granum)

The membrane contains the Thylakoide Chlorophyll is a substance that absorbs light.

The chloroplast body contains (40-60) granules, the total of which is called grana means granules.

Granum : she meeting of a number of pillars cystsfrom (2-100) a bag to form a granum.

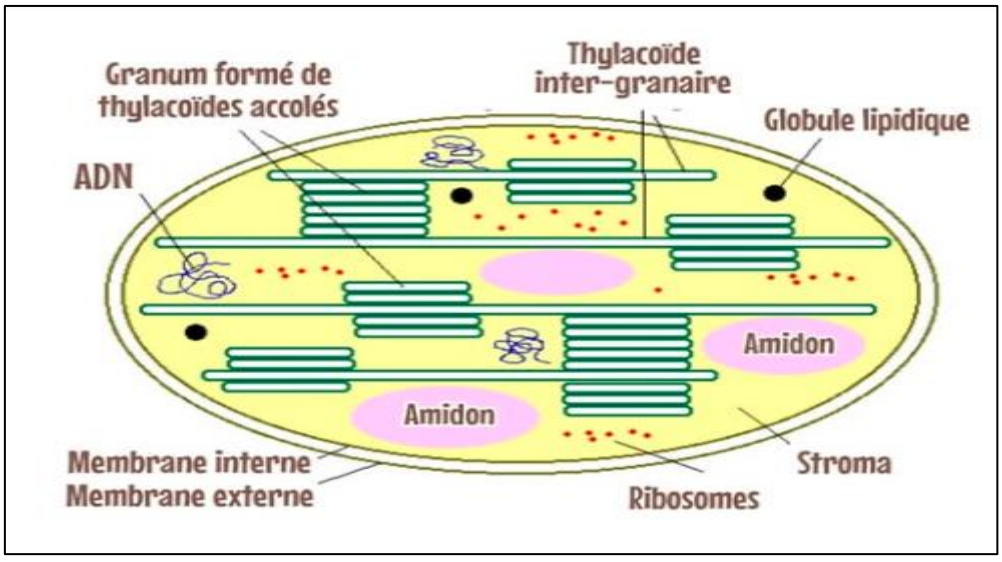

Figure 08 : Theinstallation Flour for Chloroplasts

- **Most of the synthetic particles :**
- By direct division of a spore as in lichens.
- In higher plants, particles arise from the growth of small precursor vesicles that then differentiate. They reproduce by fission and budding.

Chlorophyll pigments appear green in solution and black in solid state (chlorophyll pigments). Chlorophyll is a group of highly colored substances. Chlorophyll pigments are not soluble in water, but are soluble in organic solvents (Like ethre and alcohol).The chlorophyll pigments are found within the cysts and are never found in the internal environment of the phytosome.

2.3 Chromoplasts : Like the saplings found in tomato plant tissue.

Chromoplasts are found in fruits, flowers, and old leaves. They arise from chloroplasts, and their colors vary according to their pigment content.

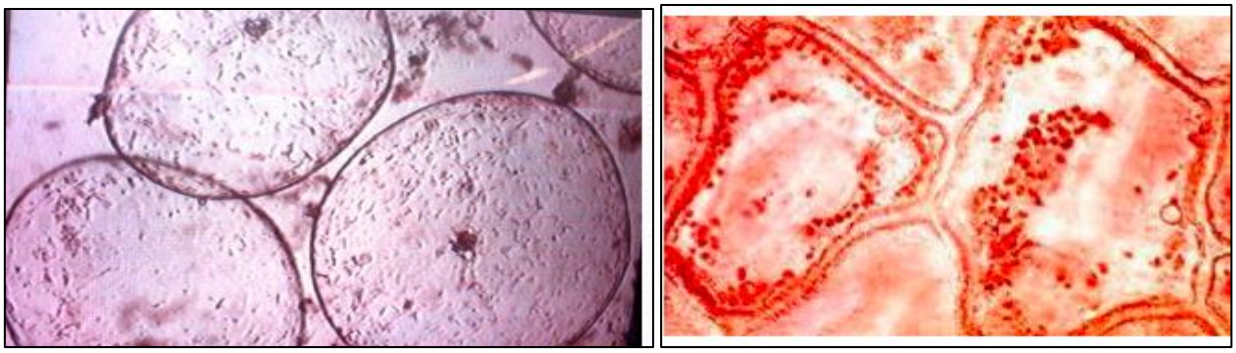

Figure 09 : Color Plastids (Chromoplasts)

The importance of color Plastids lies in the fact that they are :

- The place where dyes are Plastids and stored in the plant.
- Responsible for attracting insects that contribute to the transfer of pollen.

Chromoplasts appear as small, transparent vesicles containing small red needles or dots inside them, composed mainly of the red carotenoid pigment lycopene, which is responsible for the color of tomatoes.Chromogenic particles may burst, dispersing needle-shaped crystals of lycopene within the cytosol.

Exercise series

Exercise 01 :

Answershort And accurately on next questions.

1. What is the role of each of the stages shown in the table ? The next :

Stage	The role
(Inclusion)	
(Fixation) Installation	
(Dehydration)	
(Coloration)	
(Rehydration)	

2. Why is fixation done quickly immediately after taking the sample ?
3. Why is water removed from samples when examining them with a transmission electron microscope (MET) ?
4. Are water removed from samples prepared for examination with a scanning electron microscope (MEB) ? Explain your answer.
5. Arrange the stages in chronological order.techniquec ryocapture (preparation of molds). Decapage – e - Metal shading d - Freezing c - Mold insulation b- Cryofracture –a

Exercise 02 :

1. Researchers took a piece of mouse liver and ground it (crushed it) in an isotonic medium. An experiment was conducted on the grinding extract homogeneity result, first centrifugation process at speed 1000 g. The nuclei present in the precipitate were recovered and the supernatant was centrifuged at a speed of (10,000 g).) .I repeated centrifugation process four times to isolate different cellular components

a.Why was the grinding (crushing) done in an isotonic medium ?

b.What is this type of centrifugation called ?

c.Why were not all the cellular components found in the first sediment ?

d.The sediment resulting from the second centrifugation at 10,000 g contains mitochondria, lysosomes and peroxisomes. How do we explain the presence of the three cellular components in the same precipitate ?

e.What type of centrifugation can be used to separate organelles present in the same sediment ?

Exercise 0 3 :

Cell culture was performed in the laboratory in a medium containing thymidine labeled with tritium (a radioactive precursor). Taking cell collections and then processing them using radiographic technology self, in succession in time T1 and T2. Time T1 dense granules are observed at the level of the photographic emulsion above the cytoplasm and nucleus. At time T2 all dense granules are concentrated above the nucleus.

a.State the three main stages in which cell sections are processed by autoradiography.

b.What is the chemical nature of thymidine ?

c.How do we explain the appearance of dense granules above the cytoplasm and nucleus at time T1 ?

d.How do we explain the accumulation of radioactive thymidine in the nucleus at time T2 ?

e.In this experiment, what is the purpose of using radioactive thymidine ?

Exercise 04 : Complete the following sentences

1. The scanning electron microscope allows observation of ... objects with ……... Therefore, sample preparation no requires a stage.....no stage .....

2.Transmission electron microscope allows MET note .....

3. Apply technology of cryocapture is generally done with a microscope ... and is used to highlight.....and... ....

4. Immunofluorescence technology depends on the reactions of .....

5.The method of metal shading is to spray a thin layer of .... And .....

Exercise 05 : Short answer questions

Answer the following questions

1.Why **?** Is the sample dewatered before being immersed (embedded) in paraffin ?

2. After completing the sections, how is the paraffin removed from the samples ?

3- To remove water from the cells, we use .....

4. Transmission electron microscope allows MET note .....

5. Scanning electron microscope allows(MEB) allows the object to be observed..... . is done use it to study.....

6-Uses dark background light microscopy MO to black background note ……

Exercise 06 : Mark the correct answer(s).

1- Whereas Related to separating power

a.It is the maximum distance that must separate two points in order for them to be distinguished.

b.It is the minimum distance that must separate two points in order for them to be distinguished.

c.It is the average distance that must separate two points in order for them to be distinguished.

d- All answers are wrong.

2. During fractionation by differential centrifugation, cellular components are precipitated in a precise order. What is it ? Microsomes, then mitochondria, then nuclei

b.Nuclei then microsomes then mitochondria

c.Nuclei then mitochondria then microsomes

d.Mitochondria then nuclei then microsomes.

3. What are the correct statements for both the transmission electron microscope and the ordinary light microscope ?

a.Specimens are generally subjected to a process of fixation, cutting and then contrast lifting.

b.Observation is made by sendingtransmission

c.After fixation, the sample undergoes a dewatering process dehydration

d.Sections are made by super-sectioning the microscope ultra microtome

4- Regarding the differential centrifugation

a.The unit of sedimentation coefficient is m./1s

b.The unit of sedimentation coefficient is.(s) svedberg

c.The sedimentation rate depends on one indicator, which is the size (the volume).

d.Particles settle in bands at their own density.

5. Regarding cell adhesion :

a.The molecules contribute toIntegrins in cell/basal plate adhesion.

b.An immunoglobulin molecule canN-CAM can interact via its extracellular region with another N-CAM molecule.

c.The molecules formIntegrins homophilic bonds with fibronectin molecules.

d.The molecules formIntegrins heterophiles bind to fibronectin molecules.

Exercise 07 :

The researchers took two pieces of mouse liver.

The first piece was immediately immersed in a large amount of fixative fluid (Bouin's fluid) and then subjected to several successive steps in order to prepare tissue sections. Before microscopic observation, the obtained sections were stained with Hematoxylin-Eosin

1.What is the benefit of installing with bowen fluid ?

....

2. What is the importance of coloring by Hematoxyline-Eosine?..... ……..

Exercise 08 : Tick the correct answer(s).

1. Observing a sample under a light microscope requires : a. In order : taking the sample (preliminary) removing the Water, install and then embed.inclusion. b. Inclusion in resine. c. Spraying a thin layer of platinum on the surface of the sample. d. Inclusion in paraffin after dehydration.	3. Regarding the mold preparation technique : a. Sublimation is carried out after cryofracture. b. Sublimation is carried out after metal shading. c. Allows the study of the internal surfaces. d. Sample sections must be very thin.
2. In the so-called immunofluorescence technique, it must be Second antibody used : a. Produced by the same species that produced the primary antibody. b. Labeled with a fluorochrome so that it can be detected. c. Directed against the cellular component to be studied. d. Directed specifically against the primary antibody.	4. Which of the following substances are used as tissue fixatives ? Uranyl acetate a. Araldite b. Formol c. Glutaraldéhyde d. Xylene e.

References

1. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Membrane proteins. In Molecular Biology of the Cell. 4th edition. Garland Science.
2. Callen J-C. *Biologie Cellulaire En 30 Fiches*, Dunod, 2009.
3. Karp G. *Cell And Molecular Biology, Concepts And Experimrnts*, 4e Ed., John Wiley and Sons, 2005
4. Lodish H., Berk A., Matsudaira P., Kaiser C.A., Krieger M., Scott M.P.,Zipursky S.L., Darnell J.E. *Molecular Cell Biology*, 5e Ed., W.H, Freeman And Co, New-York, 2004.
5. Roland J-C., Callen J-C. *Atlas De Biologie Cellulaire*, 6e Ed., Dunod, 2007
6. B. Albert, A. Johnson, J. Lewis, M. Raff, K. Roberts Et P. Walter, 2011- Biologie Moleculaire De La Cellule. Ed. Lavoisier, Paris, 1601p.
7. Marc Maillet, 2006- Biologie Cellulaire. Ed. Elsevier Masson, Paris, 618p.
8. Socle Commun L1 : Sciences De La Nature Et De La Vie Page 13
9. Arms , (1988) Biologie (tome II), Université, Bruxelles.
10. Bassaglia, (2013) biologie cellulaire Ed Malonie
11. Boujard, (2018) biologie cellulaire Ed Dunod.
12. Boujard, (2012) biologie cellulaire et moléculaire Ed Donud
13. Bruno ,(2011) biologie cellulaire en 80 fiches Ed Dunod
14. Callen, (2005) Biologie cellulaire des molécules aux organismes Ed Dunod. PP 433-435
15. Favro, (2011).Biologie cellulaire UE2 page 174
16. Maillet ,(1981) Biologie Cellulaire Edition Masson.
17. Racano ;Rigo, (2014) Biologie cellulaire Ed Dunod.
18. Thiry, (2014) Biologie cellulaire : exercices et méthodes Ed Dunod
19. Woese ; Fox, (1977) Phylogenetic structure of the prokaryotic domain: The primary kingdoms, Vol.74, pp 5088-5090.

1. ميساء محمد الراوي. 2014. أساسيات علم الخلية والأنسجة، لطلبة السنة أولى في العلوم الصحية. الصيدلة والأحياء باللغتين العربية والانجليزية. مكتبة دار الموسوعة للنشر والتوزيع.
2. مكرم ضياء شكارة. 2015. علم الخلية، دار الميسرة للنشر والتوزيع. الطبعة السابعة، ص294.
3. محمود حباوي حماش. 2021. علم الخلية، المكتبة الزراعية الشاملة، ص300.
4. خناق محمد، زبيري صليحة. 2008. محاضرات مقرر الخلية. المدرسة العليا للأساتذة. القبة (الجزائر). ص 102
5. عبد الحسين الفيصل.2000. الخلية التركيب الدقيق والوظائف. الأهلية والتوزيع. الطبعة الأولى. المملكة الاردنية (عمان). ص.309
6. محمد يوسف السباعي. 2013. علم الخلية. دار جامعة للطباعة والنشر. جامعة عدن (اليمن). ص 107

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