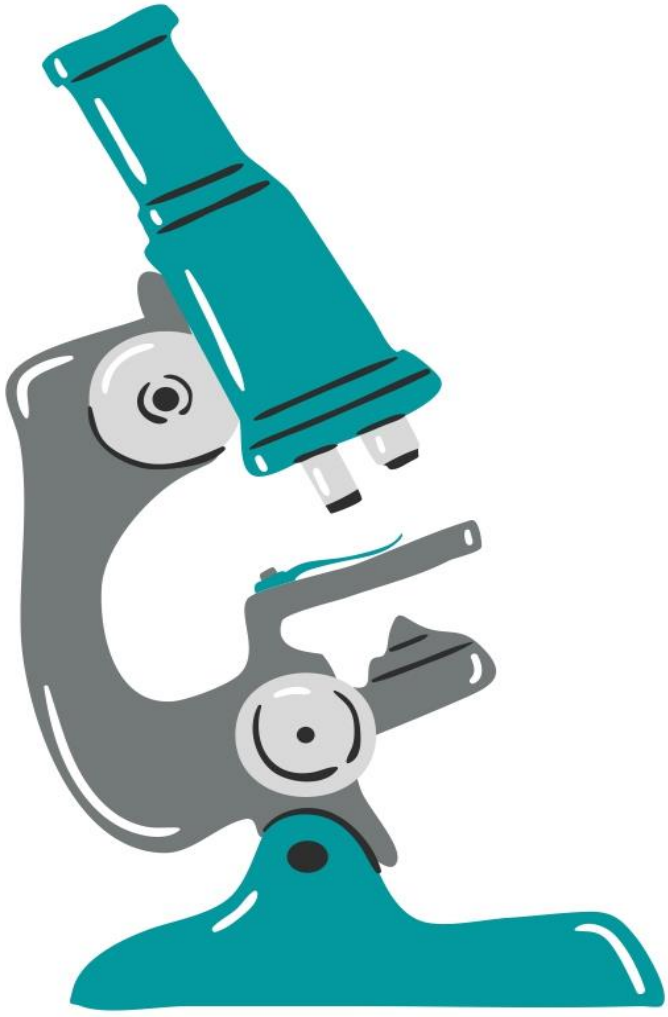
Sheet no. **1**

Histology



Writer: Zaid Altaweel
Corrector: Raghed Alasaly
Doctor: Ghada Abu el Ghanem

Human Histology

Some definitions

- **Cells : the basic structural and functional units of an organism**
- **Tissues : groups of cells and the materials surrounding them that work together to perform a particular function**
- **Organs are composed of two or more different types of tissue , they have specific functions and usually have recognizable shapes**
- **A system consists of related organs performing a function**

Group of organelles ⟶ **Cell**

Group of cells ⟶ **Tissues**

Group of tissues ⟶ **Organs**

Group of organs ⟶ **System**

Group of system ⟶ **Organism**

- **Histology**
is the study of the tissues of the body and how these tissue arrangedto constitute organs

This subject involves all aspects of **tissue** biology, with the focus on how cell's structure and arrangement optimize functions specific to each organ

Tissues have two interacting components: **cells and extracellular matrix** (ECM)

- The ECM consists of many kinds of macromolecules, most of which form complex structures, such as collagen fibrils.
- The ECM supports the cells and contains the fluid transporting nutrients to the cells and carrying away their wastes and secretory products.
- Cells produce the ECM locally and are in turn strongly influenced by matrix molecules.
- matrix molecules bind to specific cell surface receptors that span the cell membrane and connect to structural components inside the cells.
- this is how the cell and the ECM function together in a well-coordinated matter.

- ***NOTE*** *// The nature of cells are almost similar, so what makes the difference in physical properties such as, hardness, is the extracellular matrix for Example: smooth muscle tissue has extremly small ECM so it's hard tissue But, like a blood have large ECM (fluid)*

- **Histology** is the study of the tissues of the body and how these tissues are arranged to constitute organs

- For Example : The skin is an organ made up of tissues. The outer part of the skin that we can touch is the epithelial tissue. underneath the epithelium: there's connective tissue

Histology studies one tissue at a time of each organ.

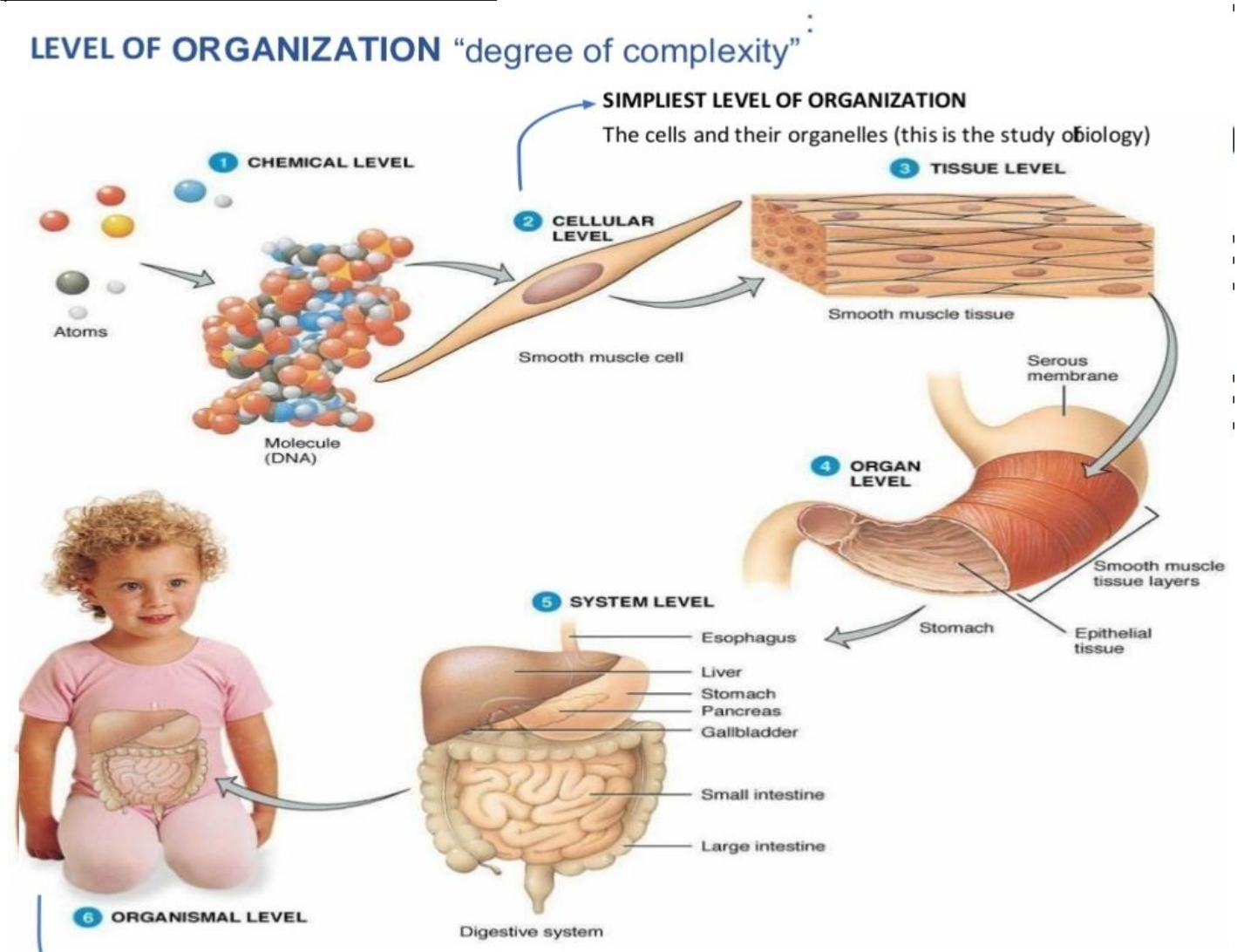
- For example: the stomach's lining is made of epithelium, the middle layer is a different layers of smooth muscles, and the outside part of the stomach is made of connective tissue.

Basic tissues in our body:

1-Epithelial tissue
2-Connective tissue
3-Muscular tissue
4-Nervous Tissue

Techniques used in Histology

1- Light microscopy
2- Electron microscopy

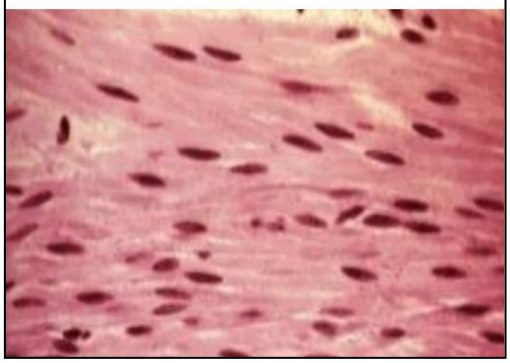


Preparation of tissues for study

- The most common procedure used in histologic research is the preparation of tissue slices BECAUSE Most tissues and organs are too thick , thin translucent sections are cut from them.

OF COURSE // The ideal microscopic preparation is preserved so that the tissue on the slide has the same structural features it had in the body.

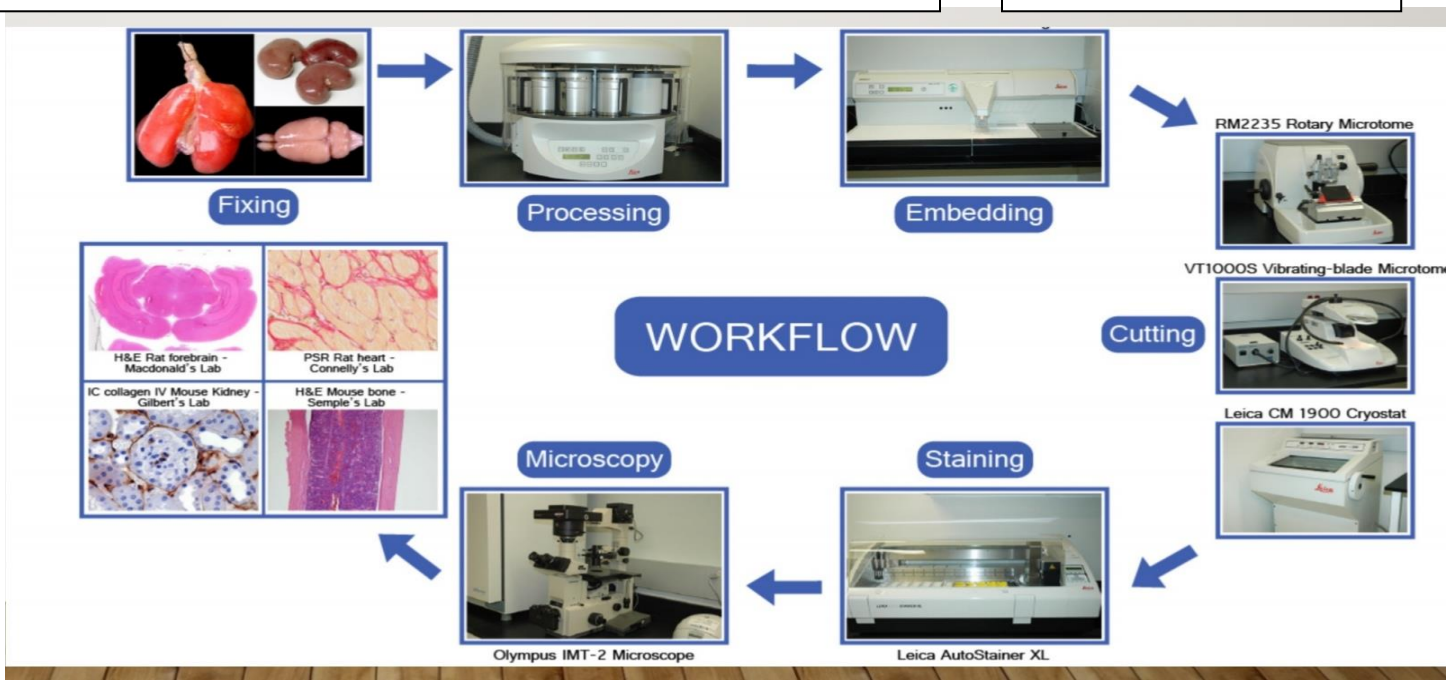
HOW DO WE SET THIS IMAGE ?



We got this image by a series of processes known as TISSUE PROCESSING.

- General outlook : We take a sample of a tissue, then the specimen (sample) goes through certain conditions, afterwards we put it in a paraffin(wax) block, then we cut it, put it on glass slides, stain it, and then it́s ready to be used and we can look at it by the microscope.

NOTE // if the tissue sample is left on the bench it becomes useless and we need to throw it away.



- **Fixation**: (the most important step)

The whole preparation process Exists in the last part of the sheet

- pieces of tissue are placed in solutions of chemicals that cross-link proteins and inactivate degradative enzymes, which preserves cell and tissue structure (for example Formaldehyde for **light microscope** LM, Gluteraldehyde for **electron microscopy** EM).

DETAILS //

when the tissue leaves the body the blood supply stops therefore the oxygen supply stops, the cell starts degrading itself throughout enzymes, this is known as autolysis so we need to prevent autolysis from happening to avoid destruction of the tissue structure. as a result we immediately fix the sample tissue once we take it or we can leave in the fridge, the low temperature is not suitable for the enzymes to function.

-There are chemical materials called fixatives that we use on the tissue sample for fixation. they prevent the autolysis by cross-linking protein; the fixatives connect the amino acids of the proteins in the tissue which protects them from degrading.. This helps us to see the tissue as close as possible to how it actually looks in our body..

-- The most widely fixative used in **light microscope** is **formaldehyde** added at certain concertation and pH level, usually a buffered isotonic solution of 37% formaldehyde known as (formalin) is used as a fixative. * *The most widely fixative used in **electron microscopy** is Gluteraldehyde , it react with the amine groups (NH2) of proteins, preventing their degradation by common proteases, also cross-links adjacent proteins, reinforcing cell and ECM structures

-Electron microscopy provides much greater magnification and resolution of very small cellular structures and fixation must be done very carefully to preserve additional "ultrastructural" detail so we use glutaraldehyde.

-to get this result using electron microscopy glutaraldehyde-treated tissue is immersed in buffered osmium tetroxide, why? Because this solution preserves and stains cellular lipids as well as proteins.

Tissue Processing

Processing is a serial steps of dehydration done by a machine to wash the water completely out of the tissue by gradually replacing water with alcohol, and then impregnate liquid paraffin wax maintain the cell structure to and shape , as well as reserve the cell architecture, preventing any indentations or shrinkage from happening & increasing the shelf life of the specimen.

DETAILS // We can do Tissue Processing by several steps include:

1- **Dehydration**: The tissue is transferred through a series of increasingly concentrated alcohol solutions, ending in 100%, which removes all water.

2- Clearing : Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium (e.g. Xylene for LM & propylene oxide for EM)

the fixed tissue must undergo dehydration by having its water extracted gradually by transfers through a series of increasing ethanol solutions, ending in 100% ethanol. The ethanol is then replaced by an organic solvent miscible with both alcohol and the embedding medium.

3- Infiltration: Replacing the clearing agent (inside the cell) with a material that can be ready for the next step which is Embedding.

Note: Dehydration, clearing and infiltration together are called tissue processing.

Note : in infiltrating sample tissue the temperature of melted paraffin is at 52"-60°C

-**Embedding the tissue**: -embedding is molding the tissue sample by hardening the material that the tissue was infiltrated with (e.g. **paraffin** wax for LM & plastic resin for EM) this is important to support biological tissue. the embedding center consists of a hot surface that maintains the paraffin wax in a liquid form and a cold surface to solidify the paraffin wax into a mold. after tissue processing the tissue sample is placed at the hot surface area and then moved quickly to the cold surface are to thicken and solidify the paraffin into a block that surrounds the tissue sample. this helps to manipulate the sample and easily cut it.

*- electron microscope : we use **resin**.*

*- light microscope :we use **paraffin**.*

Cutting the tissue sample: -tissue sample is sectioned by a device named microtome, it cuts the sample to a very thin sections but with a manageable thickness.

-Embedding materials include paraffin, used routinely for light microscopy, and plastic resins, which are adapted for both light and electron microscopy.

-paraffin sections are at 3-10 um thickness for light microscopy.

-the microtome forms a paraffin ribbon while cutting the sample, that contains 4-5 sections of -the paraffin ribbon is moved to a warm water bath that helps to soften the paraffin and easily tissue.

-The wax supporting the tissue is removed after picking up the tissue using a glass slide, and the tissue is stained using **dyes** .

Staining. Most cells and extracellular material are completely colorless, and to be studied microscopically tissue sections must be stained (dyed). Methods of staining have been devised that make various tissue components not only conspicuous(l) but also distinguishable from one another. Dyes stain material more or less selectively, often behaving like acidic or basic compounds and forming electrostatic (salt) linkages with ionizable radicals of macromolecules in tissues. Staining depends on the components of the cells in the tissue sample. For general staining we use a combination of Hematoxylin & Eosin stain (H&E).

Hematoxylin stains DNA and RNA rich components of the cell + the matrix of cartilage and give them a dark blue or purple color, on the other hand eosin stains other cytoplasmic structures and collagen pink. if we need to distinguish a particular structure in our sample we need to use a special stain.

Dyes are classified as Basic (have a positive charge) & Acidic (have a negative charge).

General stain: to give overview of cells structure in the tissue. cell components with a net negative charge such as nucleic acids have an affinity for basic dyes (BASOPHILIC) -cationic components cell components such as proteins with ionized amino group stain more readily with acidic dyes (ACIDOPHILIC)

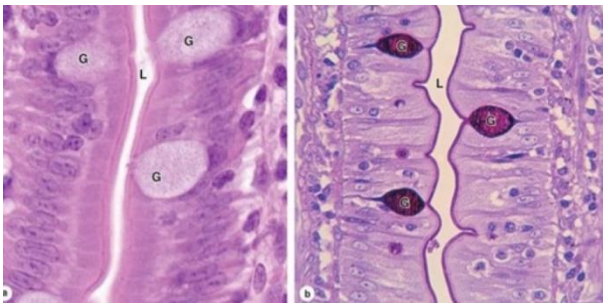
Special stain: to distinguish parts -If I am looking at a particular structure like Specific type of cell/organelle/blood vessels we use a special stain

-for example PAS (periodic acid-schiff stain reaction) is used to distinguish carbohydrates in the cell; it stains polysaccharides and carbohydrate-rich tissue structure with purple or magenta.

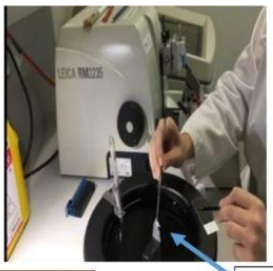
IN SHORT // Most cells and extracellular material are completely colorless, and to be studied microscopically tissue sections must be stained (dyed).

- Dyes stain material more or less selectively either acidic or basic.
- Cell components with a net negative charge have an affinity for basic dyes (BASOPHILIC)
- Cationic components stain more readily with acidic dyes and are termed (ACIDOPHILICE)

- H&E STAIN - PAS STAIN

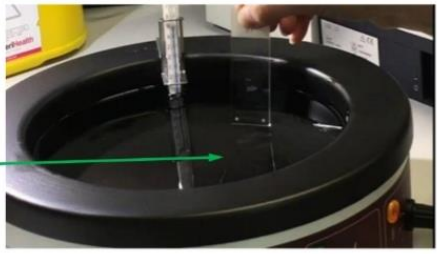


Hematoxylin	Eosin
Basic dye Has positive charge Will stain negative (basophilic) structures BLUE Examples: DNA, RNA, ribosomes, GAGs	Acidic dye Has negative charge Will stain positive (acidophilic, eosinophilic) structures PINK Examples: proteins, collagen, cytoplasm, mitochondria, secretory granules



Paraffin

Tissue



Staining

Good Luck!