

Histology
Sheet **No.**
1

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Human Histology

Reference: Junqueira's Basic Histology, Text and Atlas, 15th edition, By Anthony L. Mescher , Chapter 1.

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

-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** (Tissues: groups of cells and the materials surrounding them that work together to perform a particular function)
- The body is composed of several systems.
- Each system has a number of organs.
- The organs in each system share the same function and share some of the tissues together but they have different proportions of each tissue.
- So, the organ itself is composed of a lot of tissues.
- 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.

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

-**CELL**: the basic structural and functional units of an organism

-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.

Basic tissues in our body:

1-Epithelial tissue

2-Connective tissue

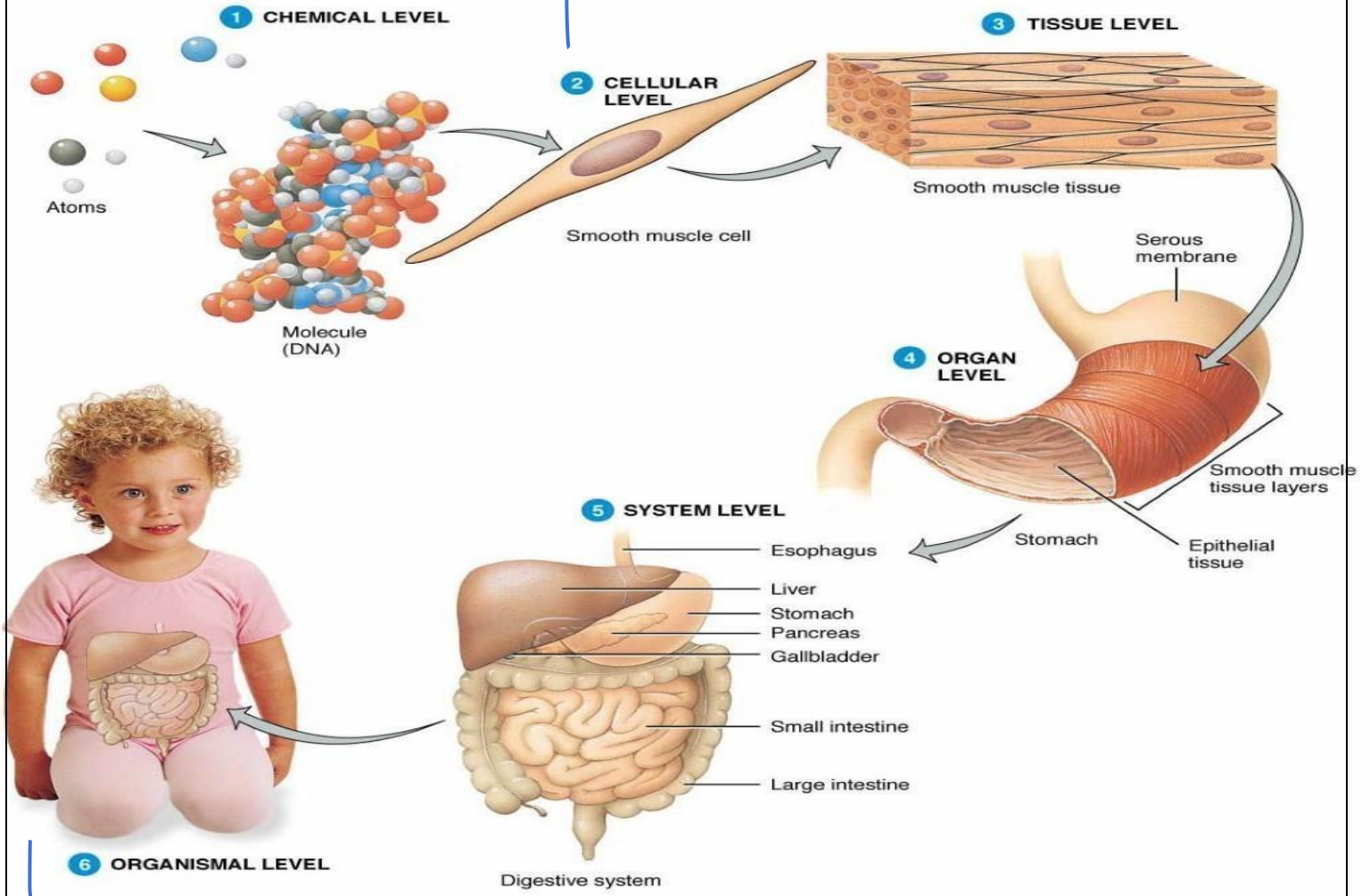
3-Muscular tissue

4-Nervous Tissue

LEVEL OF ORGANIZATION “degree of complexity”:

SIMPLEST LEVEL OF ORGANIZATION

The cells and their organelles (this is the study of biology)



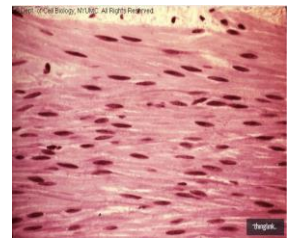
HIGHEST COMPLEX LEVEL

Which is the whole human body.

HOW DID WE GET THIS IMAGE?

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

This is a stained smooth muscle under the light microscope.

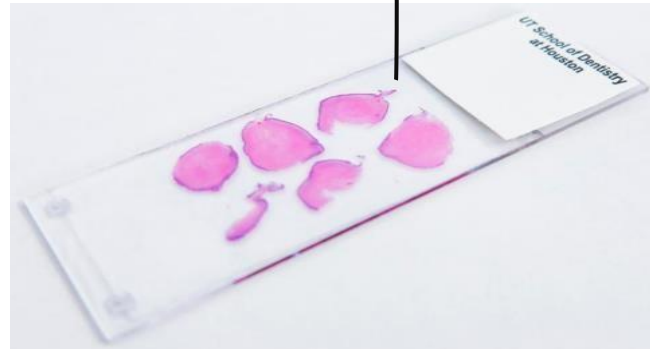


TISSUE PROCESSING FOR HISTOLOGY (for regular light microscope)

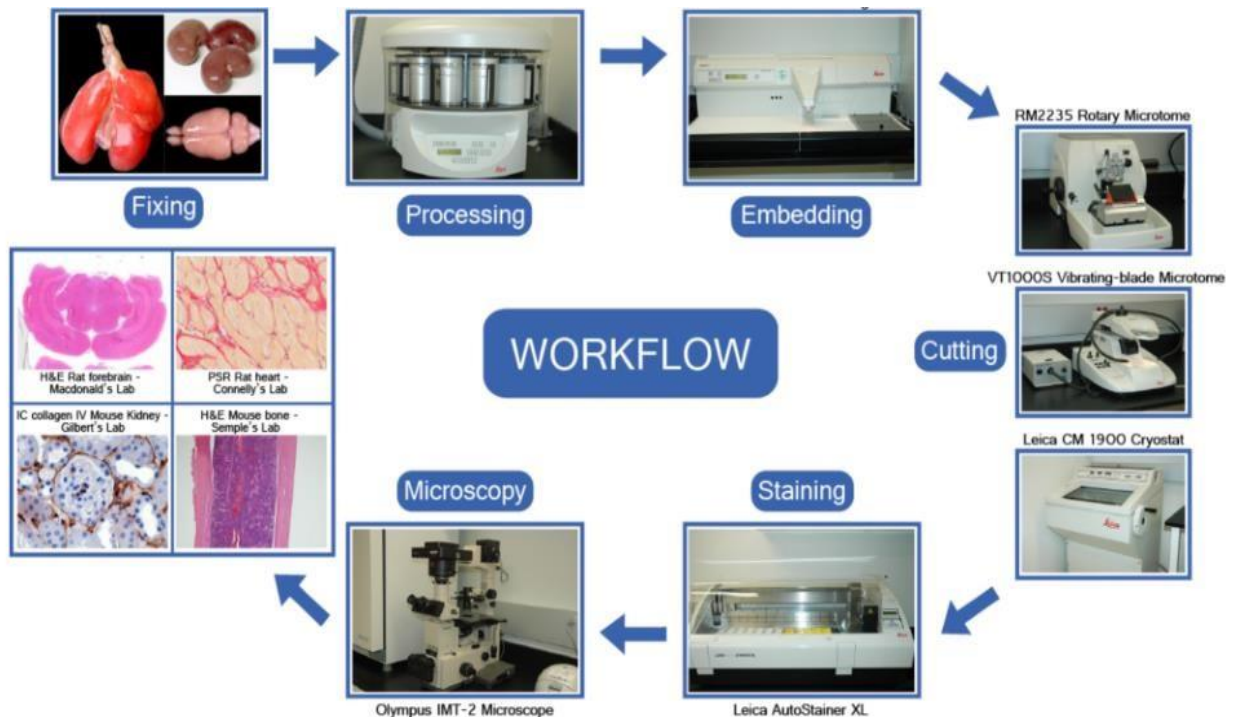
General outlook:

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, and put it on glass slides, stain it, then its ready to be used and we can look at it by the microscope.

Tissue stained with Hematoxylin and Eosin stain:



TISSUE PREPERATION STEPS:



1-Fixation of the tissue (the most important step):

-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**.

- 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.

****Side note:** if the tissue sample is left on the bench it becomes useless and we need to throw it away.

-There are materials called **fixatives** that we use on the sample or tissue for fixation.

*The most widely used in light microscope is **formaldehyde** added at certain concentration and pH level but **glutaraldehyde**, a fixative used for electron microscopy, 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 used glutaraldehyde.

-The **fixatives** prevent the autolysis by make **cross-linking** for protein; the fixatives connect the amino acids of the proteins which protects them from degrading.

This helps us to see the tissue as close as possible to how it actually looks in our body.

2. 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 to maintain the cell structure to preserve the cell architecture, this helps to **visualize** the tissue accurately.

-paraffin is liquid because it is easier to control.

- the impregnation of the tissue replaces water with paraffin wax, this maintains the cell shape preventing any indentations from happening or shrinkage as well as increasing the shelf life of the specimen.

مثل البالون عندما يتم نفخه وفيه هواء ، من الممكن أن يتغير شكله مع مرور الوقت ولكن لو وضعنا بداخله ماء فإنه سيبقى محافظاً على شكله وبنفس المبدأ سوف نخرج الماء من الخلية ونضع مكانها برفافين

We can do it 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)

3-Infiltration: Replacing the clearing agent (inside the cell) with a material that can harden to support biological tissue (e.g. paraffin wax for LM & resin for EM)

3-Embedding the tissue:

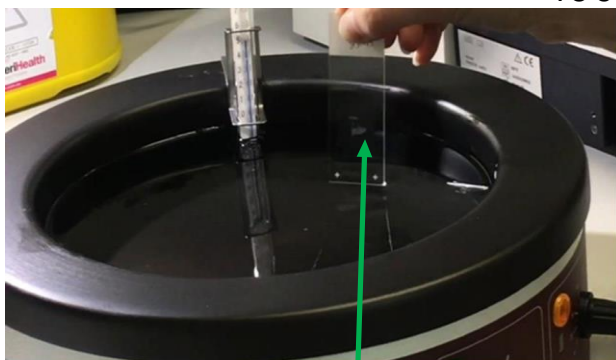
- at the embedding center the tissue sample is molded.
- the embedding center consists of a hot surface that maintains the paraffin wax in a liquid form and a cold surface to solidify the paraffin wax into a mold.
- after tissue processing the tissue sample is placed at the hot surface area and then moved quickly to the cold surface are to thicken and solidify the paraffin into a block the surrounds the tissue sample.
- this helps to manipulate the sample and easily cut it.

4-Cutting the tissue sample:

- tissue sample is cut by a device named **microtome**, it cuts the sample to a very thin sections but with a manageable thickness which is 4-5 micrometers.
- the microtome forms a **paraffin ribbon** while cutting the sample, that contains 4-5 sections of tissue.
- the paraffin ribbon is moved to a **warm** water bath that helps to soften the paraffin and easily pick up the tissue using a glass slide.



To see test the organs or



Tissue



Paraffin

3. 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 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
- The wax supporting the tissue is removed after picking up the tissue using a glass slide, and the tissue is stained using dyes
- Staining depends on the components of the cells in the tissue sample.
- For general staining we use Hematoxylin & Eosin stain.
- If we need to distinguish a particular structure in our sample we need to use a special stain.

**general stain : to give overview*

- 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 **ACIDOPHILIC**

**special stain : to distinguish parts(If I am looking at a particular structure like : Specific type of cell/organelle/blood vessels)*

-light microscope :we use paraffin.

-electron microscope :we use resin.



Staining

Thank you