بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

HISTOLOGY

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

MID | Lecture 1

# Introduction to histology pt.1


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**Reviewed by :** Amal Al-khatib

# Human Histology

**REFERENCE**: JUNQUEIRA'S BASIC HISTOLOGY, TEXT AND ATLAS, 15TH EDITION, BY ANTHONY L. MESCHER , CHAPTER 1.

# COURSE OBJECTIVES

- DESCRIBE THE STRUCTURE AND FUNCTION OF MAJOR TISSUE TYPES.
- UNDERSTAND THE RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION IN HISTOLOGICAL SAMPLES.
- IDENTIFY TISSUES AND ORGANS UNDER THE MICROSCOPE.
- APPLY HISTOLOGICAL KNOWLEDGE TO CLINICAL AND PATHOLOGICAL CONTEXTS.

# HISTOLOGY

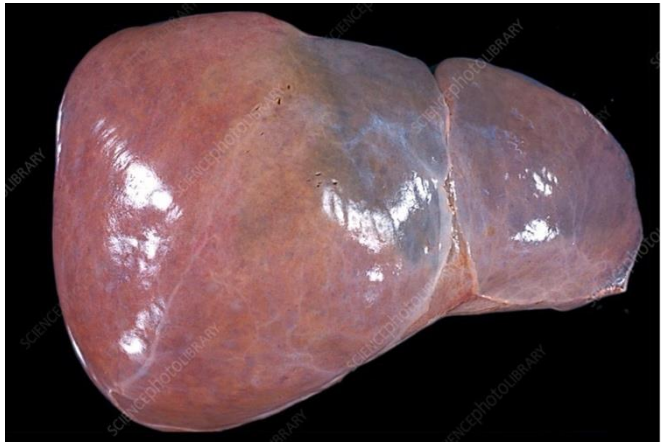
- MICROSCOPIC ANATOMY!
- *HISTO= WEB OR TISSUE*
- *LOGOS= STUDY*
- THE STUDY OF CELLS AND THE EXTRACELLULAR MATRIX

# Histology

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

After putting the glass slide we can use these two lenses after we came on the powerful of the microscope and then we can see the architecture of the liver (هدول الخلايا كيف شكلهم تحت المايكروسكوب)


This will be seen in anatomy



This is a glass slide (the carrier) which the piece will be loaded on .
We will take this carrier and put it under the microscope

# Topics

Introduction to Histology and Microscopy

In histology We have four major types of tissues ( Epithelial ,Connective , Nervous and Muscle), all these types incorporate together in a specific way to give us organs , the topics that will be discussed in this course are

1) Introduction to histology & microscopy techniques
2) The cell: structure and organelles Epithelial tissue
3) Connective tissue
4) Cartilage and bone تحت مظلة الـ connective tissue
5) Nervous tissue
6) Muscle Tissue

What is the function of the GI system?
It's depends on what we are talking about, the GI system begin with the oral cavity and end with the anus , the GI system concerned with ingestion of food , breaking of food ( digestion) , absorption , eventually the things that we don't need it it's discarded.

# Level Of Organization

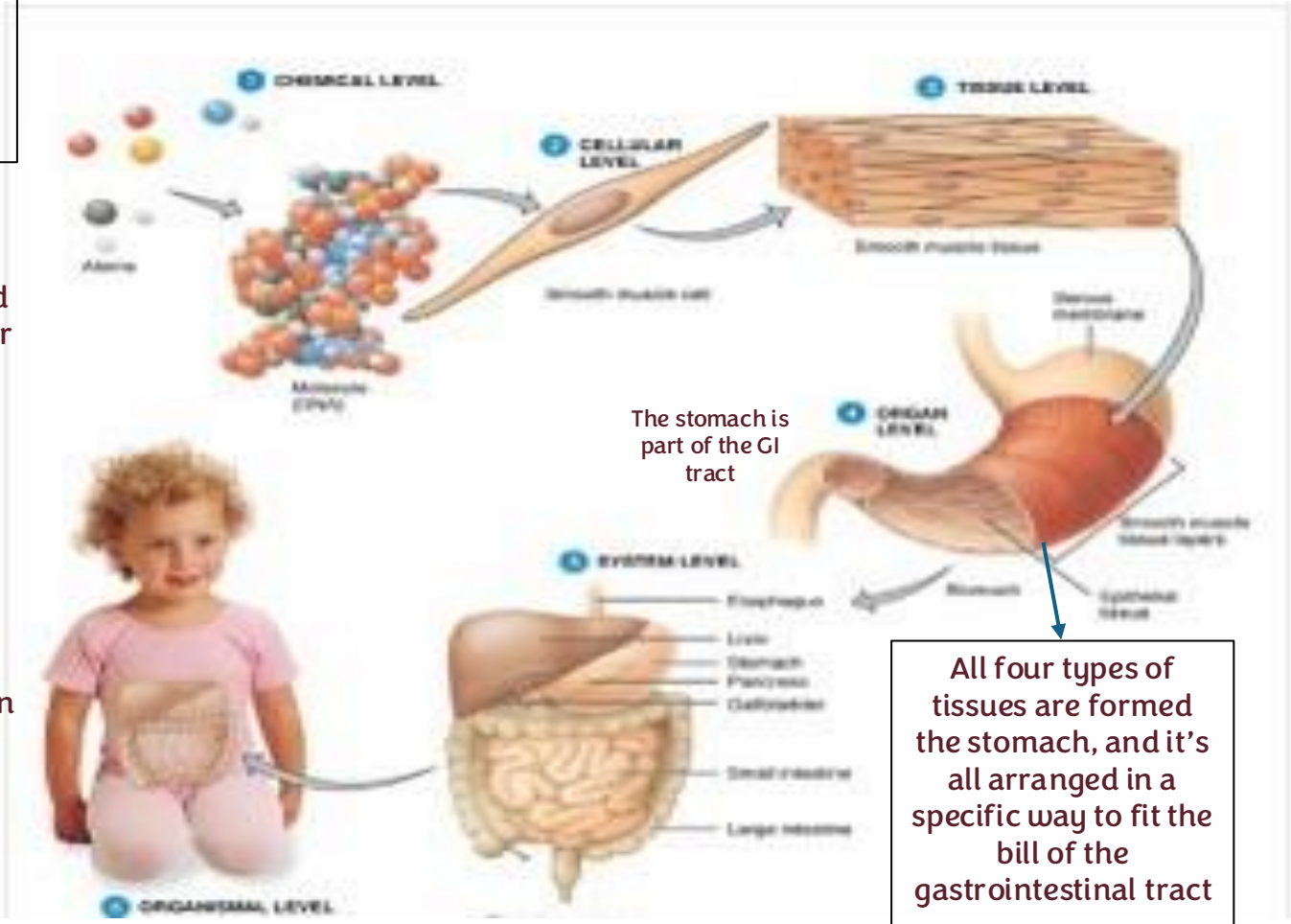
In the GI system we have from the beginning all the way down we have different types of tissues, because everywhere in the GI system there is a different function, but collectively the GI tract do digestion and absorption, each of these tissues have a specific function:

*** Epithelial tissue is involved in protection and secretion

*** The connective tissue is involved in binding different tissues together (supporting)

*** The muscle tissue, anything we want it to move , I need to put muscle tissue there, it undergoes lengthening and shortening

***The nervous tissue, if the muscle needs to contract and relax , it would not be able to do it on its own , it's require a signal from nervous tissue. The nervous tissue acts as the conductor (maestro) that coordinates these events . So , you will see nerves reaching every single aspect in the human body




The stomach is part of the GI tract

All four types of tissues are formed the stomach, and it's all arranged in a specific way to fit the bill of the gastrointestinal tract

The stomach is a muscular sac that protects itself with epithelial tissue. The epithelial tissue provides a barrier and secretes substances, but it can't survive on its own, it needs support from underlying connective tissue. At the same time, the stomach performs digestion, including mixing and moving the food

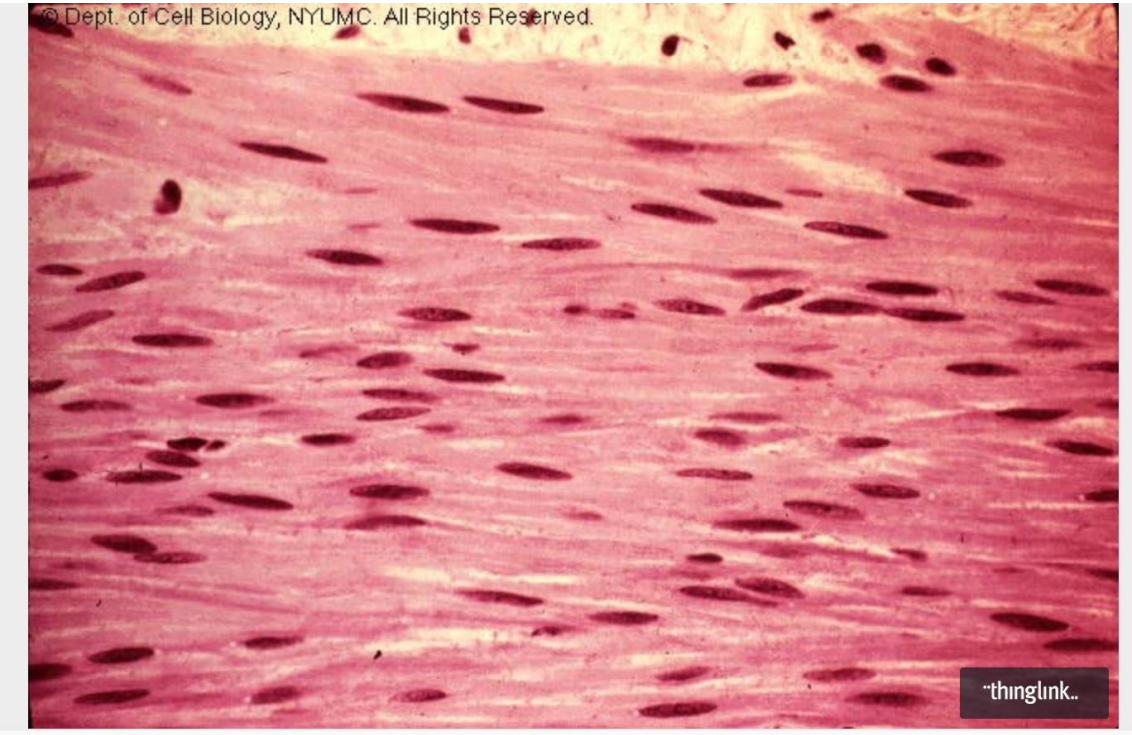
This mixing happens through the contraction and relaxation of the stomach muscle. However, muscle tissue don't contract on its own , it requires signals from nervous tissue. Therefore nervous tissue is present in the stomach to coordinate and control these muscle contractions, acting as the "maestro" of stomach movements. Although the arrangement isn't as simple as that , the general organisation of the stomach layers in common sense follows this pattern. Connective tissue act as a binding or supporting agent , presenting everywhere between the different layers and surrounding them externally

Let's take another example: the liver . The liver is an accessory organ in the GI system. It has a long list of very important functions , including secretion . Unlike hollow organs , the liver doesn't have a lumen because it's a gland . This means that the liver produces and releases substances as part of its function ( we will study the detailed functions next year in the GI tract )

How is it organised ? Since it performs secretion , it's mainly composed of many cells scattered throughout the organ , which is typical for glands . Therefore, we find secretory cells . Usually secretory cells are epithelial cells . These cells need structural and nutritional support , so we find connective tissue surrounding and supporting them . There is no muscular layer because the liver is gland and doesn't require contraction to perform its function .

We took the different examples because not all organs contain the four tissues in all structures. In stomach, muscle tissues is essential because need muscle contraction to mix and move the food . In contrast the liver doesn't require muscle con for its main function . The liver cells are specialised to synthesise and secret substances. Since their function is production and secretion, they don't need a muscular layer to perform their role .
Instead , the liver is mainly composed is secretory epithelial cells supporting internally and externally by connective tissue. The connective tissue forms the structural framework and surrounds the organ . In addition, there is another tissue component (we will discuss it later)

# What is this?
# How did we get it?



How we reach the final step ? (Loaded the piece in the glass slide and see the tissues under the microscope)
It's a complex process , let's take an example for clarification:

When we buy meat , we don't leave it at room temperature. If we plan to cook it today or tomorrow, we will put it in the refrigerator, but if we want to keep it for a longer time , we freeze it. Why do we do that? Because we want to preserve it. The same concept Appleton tissues in histology, we can't simply cut a tissue sample and leave it at room temperature then place it directly on a glass slide and examine it under the microscope.

There are several reasons for that. First of all, the tissue will start to degrade . After cell death, autolysis (الخلية بتحلل حالها بالأنزيمات اللي طالعين منها) occurs-enzymes are released and begin to digest the cells themselves. As a result, the normal architecture of the tissue is lost , then we can no longer properly visualise the tissue under the microscope , it would appear disorganised and indistinct . Therefore, proper preservation (fixation) is essential before microscopic examination.

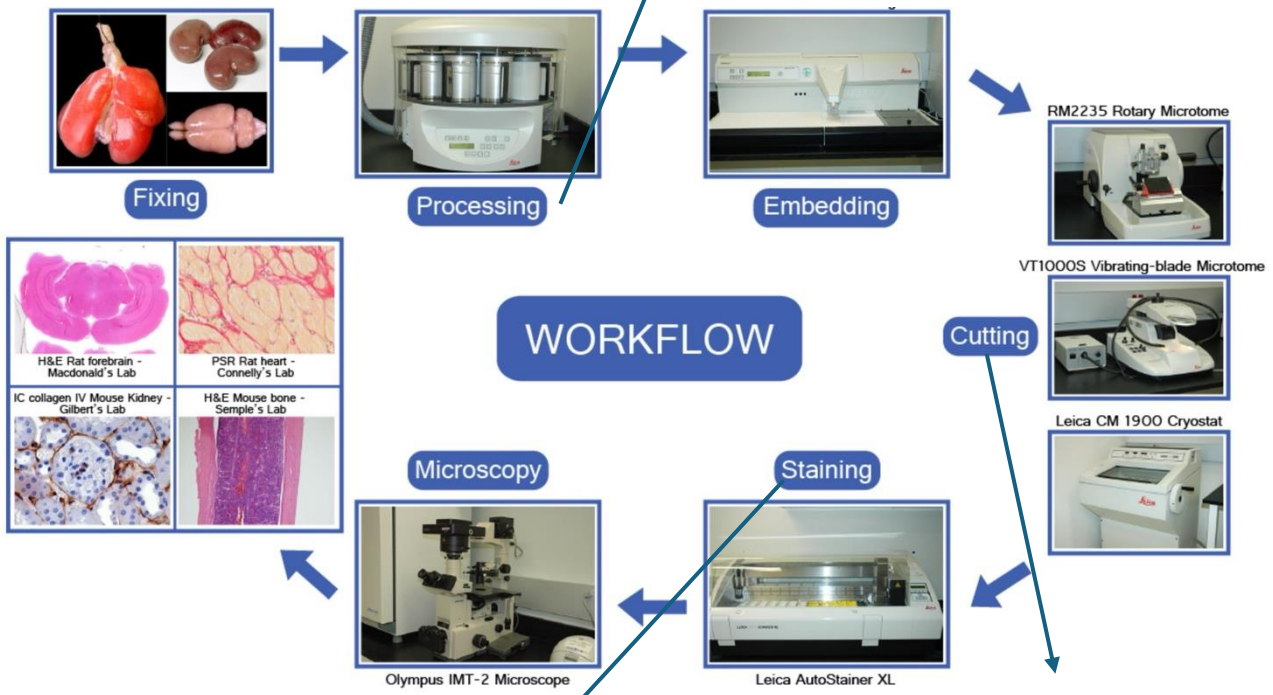
Of course, we can't stay in the freezer and work there just to prevent tissue degradation, we need a smarter method to preserve tissue for a long period of time. Therefore, we fix it (بنحنطها) .
Fixation involves placing the tissue in a solution that cross-links proteins. After cell death , enzymes recognise specific protein sites, bind to them , become activated, and start breaking down the tissue . These target sites are mainly proteins (antigenic sites) .

By cross linking the proteins during fixation, we block these binding site . As a result, enzymes can't bind or function properly, which prevents tissue breakdown. In this way, the tissue architecture is preserved for microscopic examination.

# Tissue Processing For Histology

We need to remove the water because if it remains the tissue would go back to hydration
Which may interfere with preservation and embedding
Then when we remove the water (dehydration) from the tissue, it undergoes shrinkage and its shape changes because the water is gone. To restore a staple structure ,we perform embedding


https://www.youtube.com/watch?v=4DJm4NLECQs




Wax (the one around end and inside )
The wax penetrates inside the tissue and inside the cell

Of course, it isn't enough just to put the tissue on the slide . If we place it there without coloring it , it will be colorless and hard to visualize so we will perform staining. After that, the tissue is ready , and we place it under the mic. Then we can visualize the sample that we have prepared

Then we want to cut the tissue using a microtome . The microtome allows us to obtain extremely thin sections , measured in micrometeorites . Initially , the sections can be up to 10 micrometeorites thick , it could be thicker , but the image won't be clear . Therefore, we need to cut the tissue into extremely thin sections and place them on a glass slide

Here is a mnemonic to help : Fix Dead Cells In Every Tissue

# Tissue Preparation For Light Microscopy

After cutting the tissue, we place it in a small plastic carrier because the pieces are tiny. If we put them directly in the solution without the carrier, it would take a very long time to find them . Therefore, we use the carrier to make it was to handle and move the tissue

Should occur as soon as possible to prevent degradation

- **Fixation**: small pieces of tissue are placed in solutions of chemicals that cross-link proteins and inactivate degradative enzymes, which preserves cell and tissue structure.

Formaldehyde or formalin for example but the commonly used is formaldehyde

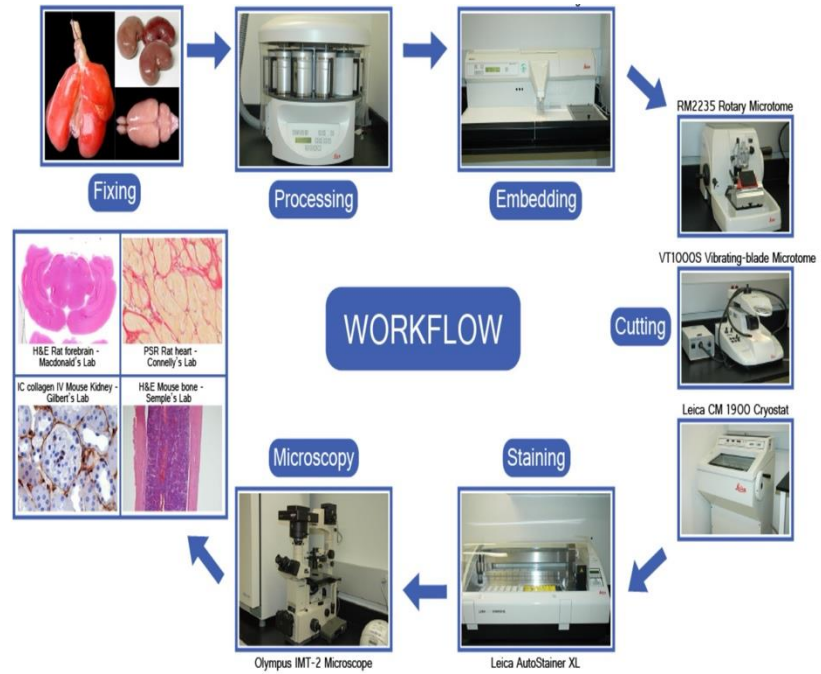
(After fixation, we perform tissue processing, which requires specific amount of time , you can't overdo it , or under do it , and the timing depends on the size of the tissue and instructions of the manufacturer designated time is completed , we proceed with the next steps of processing)

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

The most important result of processing is that we remote water from the tissue. This is essential so that we can later embed the tissue in wax

- **Clearing**: alcohol is removed in organic solvents in which both alcohol and paraffin are miscible.

Replace alcohol with a substance ( organic solvent) compatible with paraffin wax



Here is a mnemonic to help : Fix Dead Cells In Every Tissue

# Tissue Preparation For Light Microscopy

This involves placing the tissue in another substance which in this case is wax. As a result, the tissue is surrounded and supported by the wax

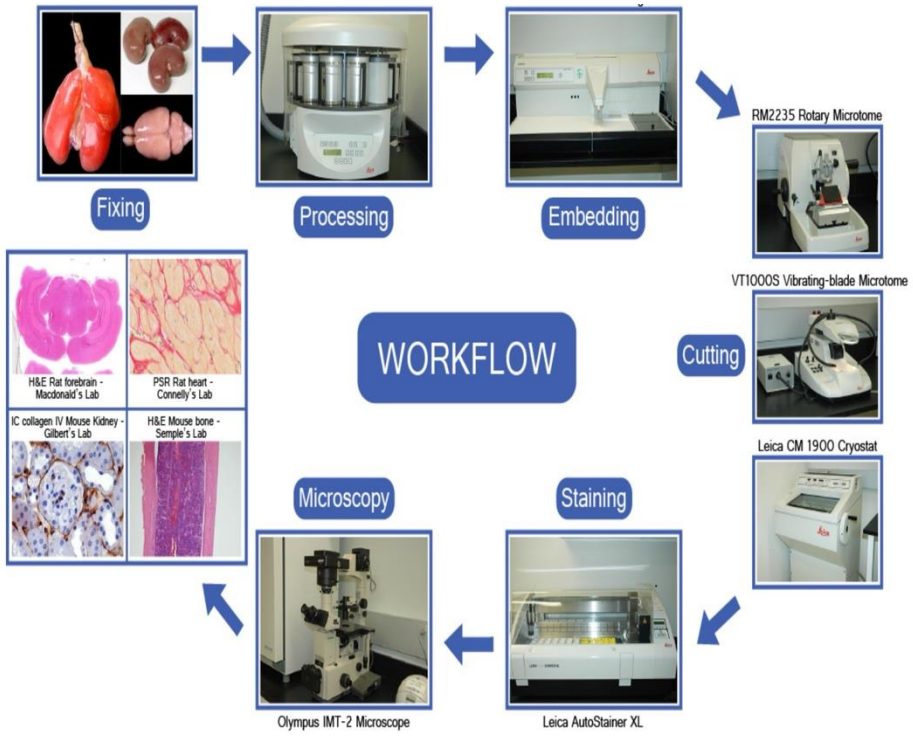
Support the tissue externally and create the block that can be sliced

Wax penetrates the tissue replacing the organic solvent = support the tissue internally

- **Infiltration**: the tissue is then placed in melted paraffin until it becomes completely infiltrated with this substance.
- **Embedding**: the paraffin-infiltrated tissue is placed in a small mold with melted paraffin and allowed to harden.
- **Trimming**: the resulting paraffin block is trimmed to expose the tissue for sectioning (slicing) on a microtome.

The paraffin block is brittle, so, it will be carried in a plastic cassette to support it while the suctioning to form a very thin ribbon

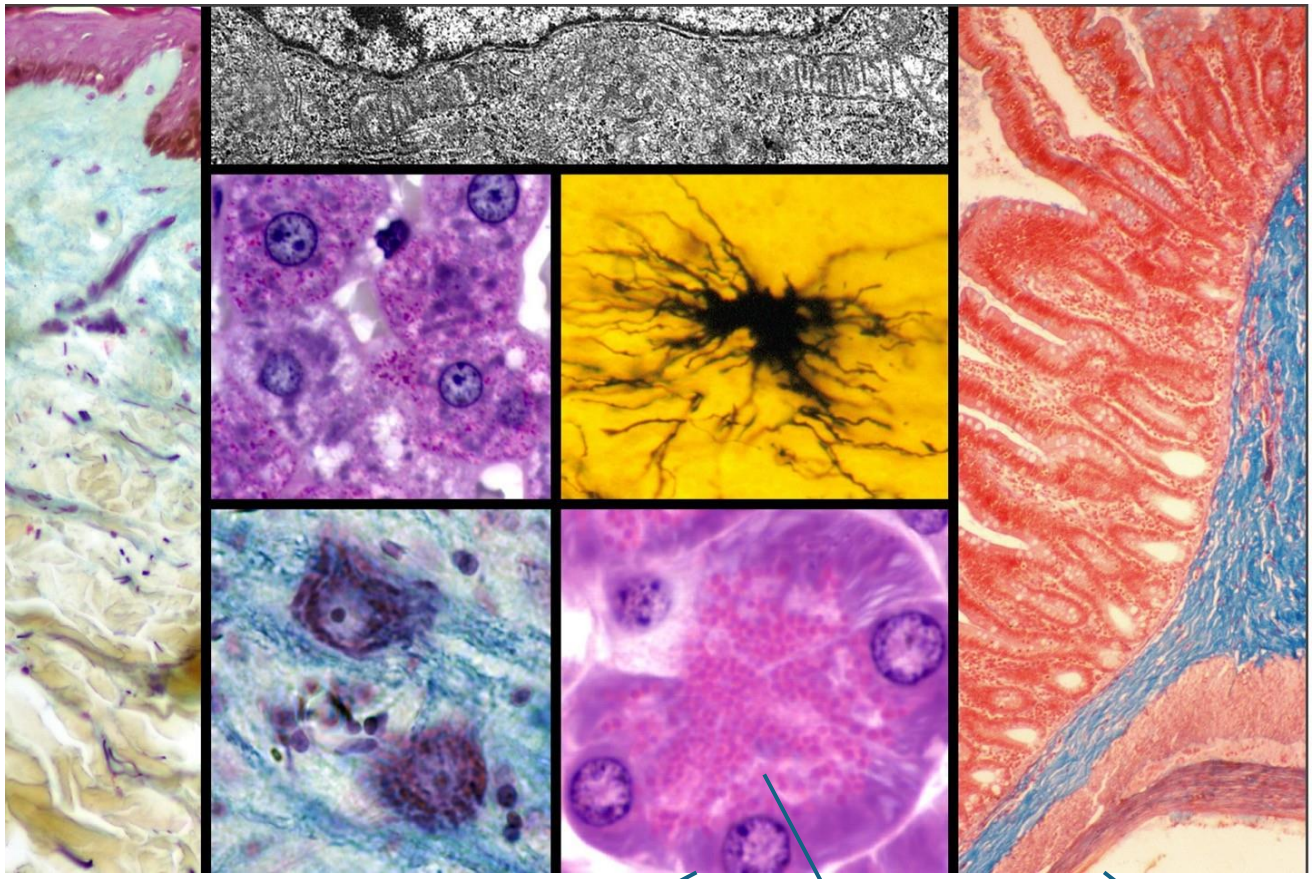
After getting the ribbon, we place it in a warm water bath to be straightened and o warm the wax a little bit so it can adhere to the warm glass slide. Then we remove the hydrophobic wax supporting the tissue and rehydrate it so it's ready to be stained with the hydrophilic stains




***Wax is relatively hard at room temperature, so we need to warm it up to convert it into a liquid. Only in it's liquid form can the wax penetrate and surround the tissue sample. If it is remains hard , it won't be able to reach the details of the tissue.

# STAINING

***You can distinguish the plasma membrane because , as we mentioned, we add solvents and alcohol during preparation. The plasma membrane is made of lipids, which will be dissolved by these solvents , unless you target specific structures on the membrane that you want to recognise



These are cells and we recognise them by their nuclei . Just by looking at this image, we can see that the cells look similar to each other . Therefore, they form a single tissue because of their similar appearance. (The cell consists of a nucleus and cytoplasm, all enclosed by a membrane)

The pinkish around the nuclei is the cytoplasm

Sooth muscle

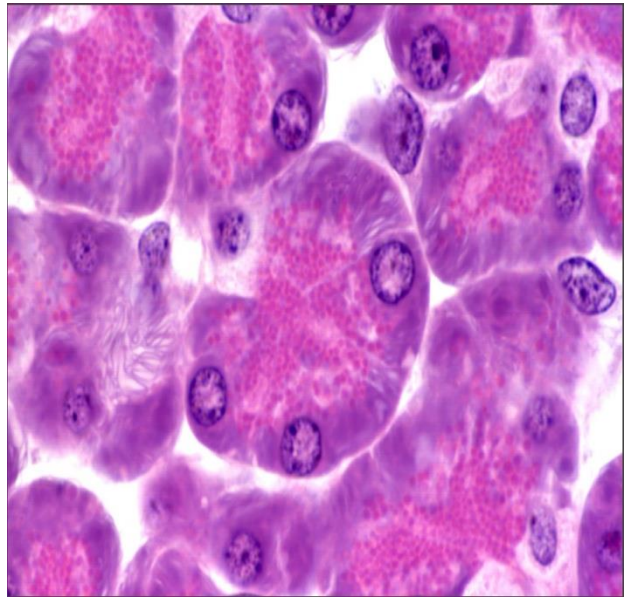
# Staining And Stains

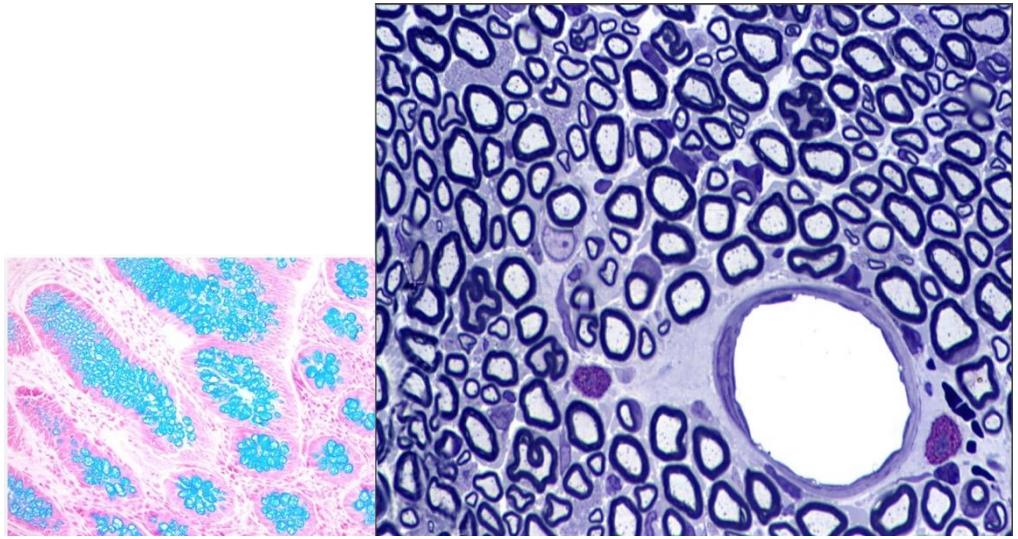
- Most cells and extracellular material are completely colorless!
- Dyes forming electrostatic (salt) linkages with ionizable radicals of macromolecules in tissues.
- Cell components such as nucleic acids with a net negative charge (anionic) have an affinity for basic dyes and are termed **basophilic.**
- Cationic components, such as proteins with many ionized amino groups, stain more readily with acidic dyes and are termed **acidophilic**.

# Staining and Stains

- Basic dyes include toluidine blue, alcian blue, and methylene blue.
- **Hematoxylin** behaves like a basic (bluish) dye, staining basophilic tissue components.
- DNA, RNA, and glycosaminoglycans: ionize and react with basic dyes do so because of acids in their composition
- Acid dyes: **eosin** (pinkish) , orange g, and acid fuchsin stains mitochondria, secretory granules, and collagen are acidic.

Secretory granules



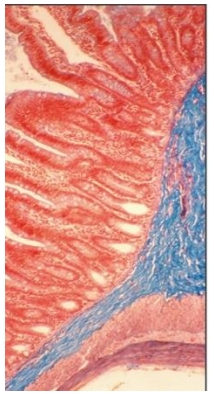


Section of a nerve

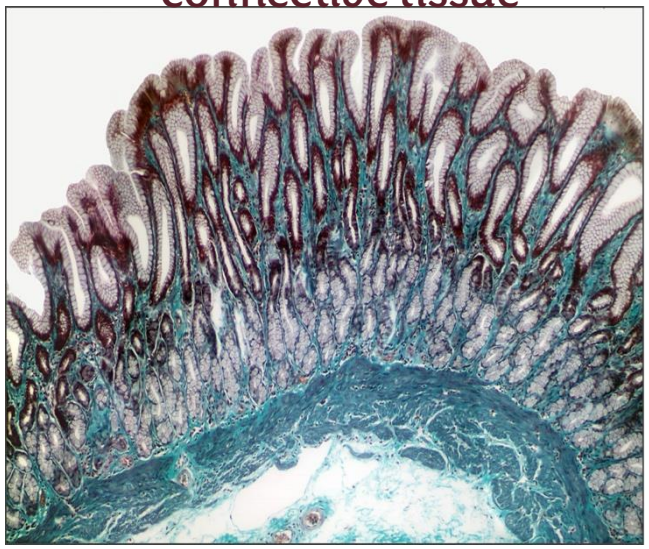
# Staining and Stains-special stains

Tri : three colors which are blue ( including turquoise), brown and red

- **Trichrome** stains allow greater distinctions among various extracellular tissue components, e.g., Masson trichrome.

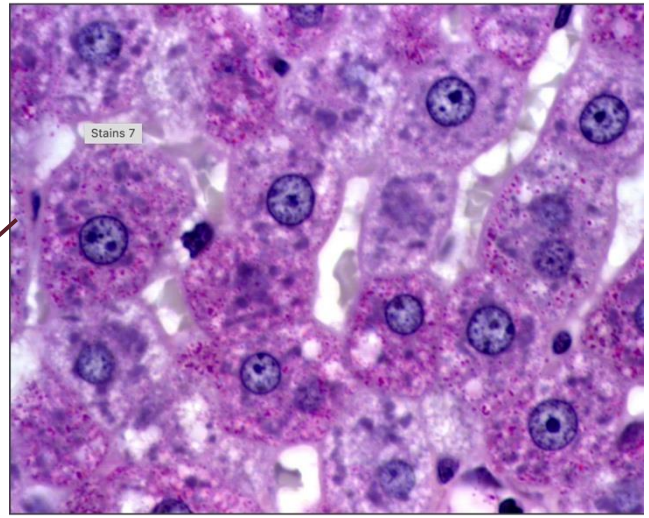


Mason trichrome in a connective tissue



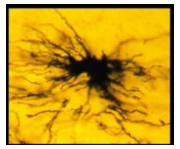
- **The periodic acid-Schiff (PAS)** reaction utilizes the hexose rings of polysaccharides and other carbohydrate-rich tissue structures and stains such macromolecules distinctly purple or magenta.

(Looks like the H & E stains but it differs in the existence of more clumps of a purplish molecules)



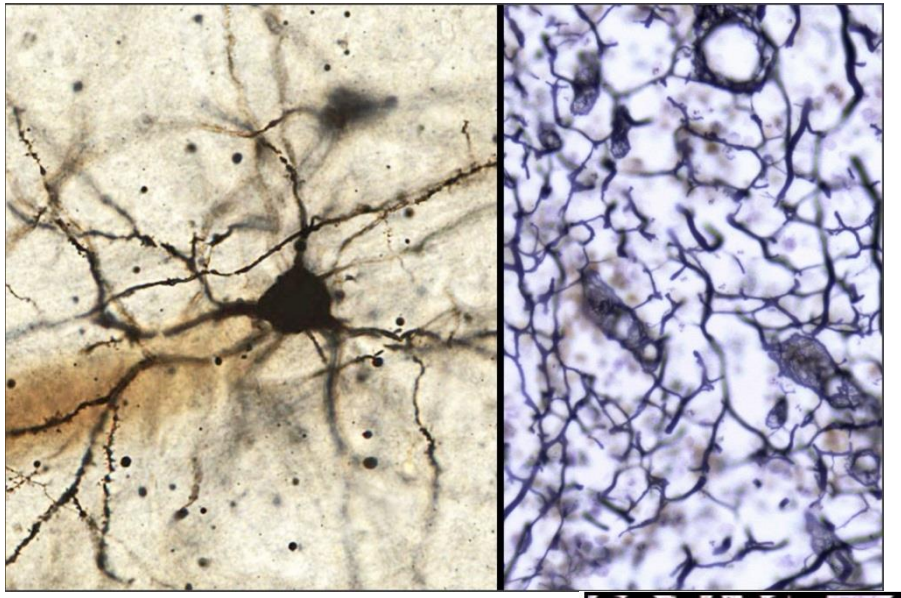

Liver tissue

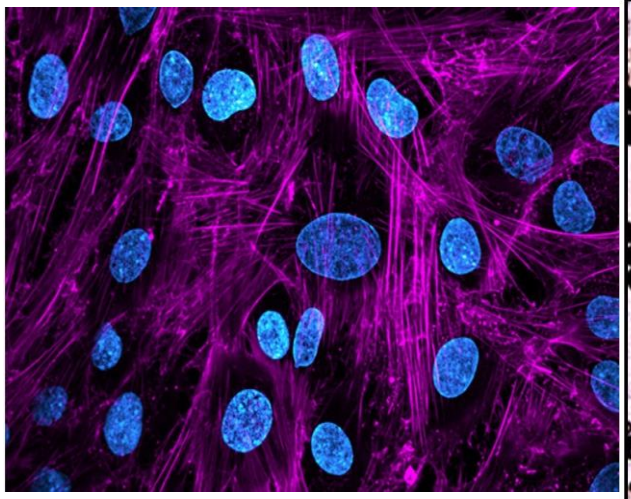
# Staining and Stains-special stains

- **Sudan black**: lipid-soluble dyes --satins lipids; avoiding the processing steps that remove lipids, such as treatment with heat and organic solvents which can be useful in diagnosis
- **Metal impregnation:** less common methods. Using solutions of silver salts to visual certain ECM fibers and specific cellular elements in nervous tissue. 
- **Immunostaining**: immunofluorescence and immunohistochemistry.

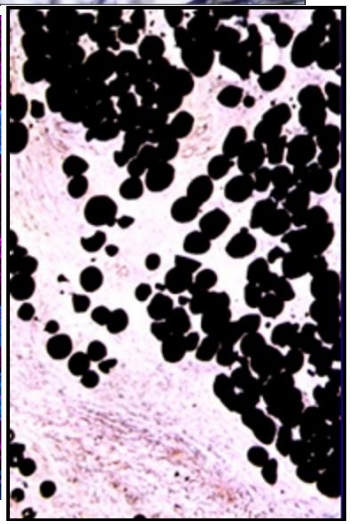
Further illustration :
Antigen : the target protein ( we want to find)
Antibody : the one that targets and specified to bind with the antigen and nothing but it
Then we add a specific label ( enzyme for example ) that will bind to the antibody and the label must be fluorescent so it can illuminate to distinguish the existence of the antigen if it was there

Metal impregnation



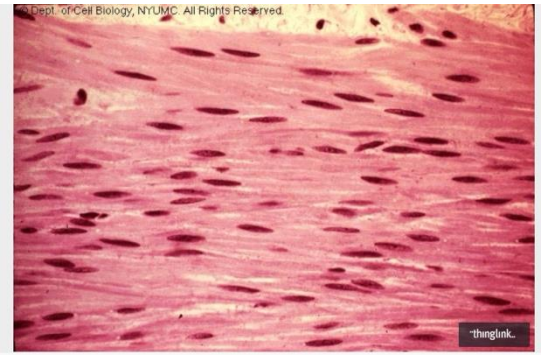



Immunostaining

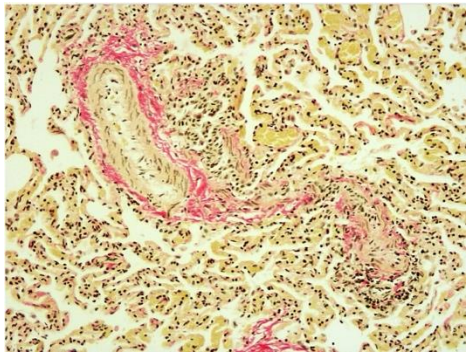


Sudan black

# Examples Of Commonly Used Histological Stains

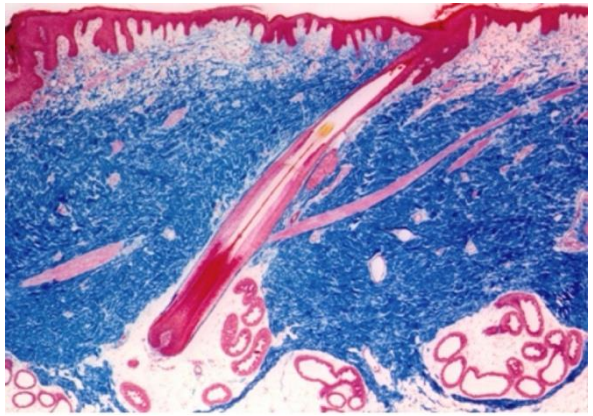



- Hematoxylin and eosin (H&E): nucleus/blue, cytoplasm/pink



- Van Gieson method: collagen/pink, muscle/yellow.

- Trichrome method: three color system to emphasize support fibers: connective tissue/blue, cytoplasm/pink, nuclei/dark brown.



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Corrections from previous versions:

| Versions | Slide # and Place of Error | Before Correction | After Correction |
|---|---|---|---|
| V0 → V1 | | | |
| V1 → V2 | | | |

# رسالة من الفريق العلمي:

جددوا النوايا ، ولا تنسونا من صالح دعائكم في هذه الأيام المباركة