بسم الله الرحمن الرحيم



Histology - Lecture 1 Histology-Lecture 1 Histology Intro



Written by : Mayar Khader Shorouq Matalkah Written by : Tala Alali Reviewed by : Mayar Khader

Human Histology

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

TOPICS TO BE COVERED

- 1. OVERVIEW
- النسيج الطلائي (الحرشفي) EPITHELIUM (الحرشفي)
- 3. CONNECTIVE TISSUE النسيج الضام
- 4. CARTILAGE
- 5. BONE
- 6. MUSCULAR TISSUE
- 7. NERVOUS TISSUE

The main types of tissues:-1-The epithelium tissue 2-The connective tissue 3-Muscular tissue 4- Nervous tissue

Note-the bone and cartilage tissues are a special type of the connective tissue



- 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 constitute organs.
- Tissue is composed of cells and ECM (extracellular matrix)

Level Of Organization



Many types of tissues are arranged in a way to perform a function that is anticipated from that particular organ

How did we get this image?



 Stained smooth muscle tissue

Tissue Processing For Histology

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Tissue Preparation For Light Microscopy

- For Example if we want to test a specific drug on kidney, at first we test that drug on cells, then on an animal. Like فأر التّجارب
- **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. If we leave the cells without fixation the the tissue digestion by enzymes within the cells (outolysis) or bacteria.
- Dehydration: the tissue is transferred through a series of increasingly concentrated alcohol solutions, ending in 100%, which removes all water. (For preservation purposes)
- After Dehydration, the tissue loses its shape ; that's why we need the Wax in the next step ;)
- Clearing: alcohol is removed in organic solvents in which both alcohol and paraffin (Wax) are miscible.
 - Wax here

replaces Water

Tissue Preparation For Light Microscopy

- **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.

*Note : We can store the sample for hundreds of years ! (This, of course, if its not exposed to a chemical change.

 As it's light Microscopy; Light only pass through a thin medium, Sooo the tissue we want to study must be <u>THEN</u>. (7-9) μm

 We stain them , And the exciting part : to the LMS & visualize them .

Additional Notes from the Doctor ③

•You have to separate the specimens so they doesn't mix together.

•Histopathology : Histology, can do pathological analysis for the tissue.

A video which will help you to understand

- <u>https://youtu.be/4DJm4NLECQs?si=EQ9TEw518uZlEBJ9</u>
- Because a fixative must fully diffuse through the tissues to preserve all cells, tissues are usually cut into small fragments before fixation to facilitate penetration and better ensure tissue preservation.
- Pieces of organs being to be treated as soon as possible after removal from the body to avoid autolysis . We can but it in the fridge for short period of time . (The point is important, expected it in the exam)
- One fixative widely used for light microscopy is formalin, a buffered isotonic solution of 37% formaldehyde. (المصدر من formaldehyde الدكتورة ما حكت النسبة بس ذكرت)
- In dehydration, water is extracted from the fixed tissues by successive transfer through a graded series of ethanol and water mixtures, usually from 70% to 100% ethanol.
- The very thin sections are placed on glass slides and stained for light microscopy .

STAINING & MICROSCOPES

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**.
- Basic dyes include toluidine blue, alcian blue, and methylene blue.
- Hematoxylin behaves like a basic 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**, orange g, and acid fuchsin stains mitochondria, secretory granules, and collagen are acidic.

Staining And Stains

Staining is essential in microscopy because most cells and tissues are naturally colorless, making it difficult to distinguish their structures and organelles. Different stains help visualize and identify various cellular components

There are simple stains, which are commonly used, and more advanced, specialized stains that are expensive and specific to certain tissue types. For example, if a cancer patient's tissue sample is examined, a routine stain like Hematoxylin and Eosin (H&E) is used first. If more detail is needed, special stains are applied to highlight specific structures

Hematoxylin and Eosin (H&E) is the most commonly used general stain. Stains work by binding to ionizable molecules in cells, which carry a charge. This charge difference allows different stains to highlight different parts of the cell

In H&E staining :

* The nucleus appears bluish-purple because DNA and RNA are negatively charged and attract the basic dye Hematoxylin

* The cytoplasm appears pink because many cytoplasmic components are positively charged and bind to the acidic dye Eosin

Some cytoplasmic regions may also contain negatively charged molecules, making them stain more basophilic (blue-purple). The nucleus, however, will always show a distinct basophilic staining due to its high nucleic acid content -like a pancreas tissue-

Staining And Stains-special stains

- **Trichrome** stains allow greater distinctions among various extracellular tissue components, e.g., Masson trichrome. We use it usually when we study connection 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.
- Sudan black: lipid-soluble dyes satins lipids; avoiding the processing steps that remove lipids, such as treatment with heat and organic solvents (or alcohol effect) 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.
 Like the one used to visualise reticular fibres (we use silver)
- **Immunostaining**: immunofluorescence and immunohistochemistry.

Antigen-Antibody specificity

H&E

PAS STAINING!



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Similar to H&E , but more details are shown , a line is present on cells containing carbohydrates

Staining And Stains-special stains

Another type of stain is Toluidine Blue, which is used for nerve tissue sections. It helps distinguish the myelinated parts of the nerve, which cannot be clearly seen with Hematoxylin and Eosin (H&E) staining. Since H&E does not highlight myelin effectively, Toluidine Blue is used instead

Another special stain is Alcian Blue, which is used to identify a specific type of protein called mucin (or mucus). Mucin is a sticky, gel-like substance found in glands and certain epithelial tissues. H&E staining does not highlight mucin, so Alcian Blue is used to specifically stain and identify it

Examples Of Commonly Used Histological Stains

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

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

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







For any feedback, scan the code or click on it.

Corrections from previous versions:

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

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