

Chapter 1 part 1

The little introduction of this course

- # histology : is the study of the tissues of the body and how these tissues are arranged to constitute organs .
- # Histology involves all aspects of tissue biology with the focus on how cells 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 such as collagen fibrils and the basement membrane .
- # Note : (The basement membrane : is a thin sheet of fibers which lines the cavities and surfaces of organs including skin) we will be discussed later ☺
- # The function of ECM : supporting the cells and the fluid that transports nutrients to the cells and carries away their catabolic products .
- # The cells produce the ECM and are also influenced and sometimes controlled by matrix molecules .
- # Cells and matrix interact extensively "with many components of the matrix recognized by the attaching to cell surface receptors "
- # The fundamental tissues of the body are each formed by several types of cell-specific association between cells and ECM .
- # Organs are formed by an combination of several tissues and the precise combination of these tissues allows the functioning of each organ and of the organism as a whole .
- # The small size of cells and matrix components makes histology dependent on the use of microscopes and molecular methods of study .

The preparation of tissues for study

- # The most common procedure used in histological research is the preparation of tissue sections or slices that can be studied with the LM (light microscope).
- # The ideal microscopic preparation is preserved so that the tissue on the slide has the same structure and molecular composition as it had in the body .
- # Note (simply Why do we cut the sample into several segments ? ----> so that the light beam can penetrate the sample because the sample is thick before cutting into slices .
- # The basic steps used in tissue preparation : cutting -->fixation-->dehydration---> clearing--> infiltration-->embedding-->trimming .

Fixation

to avoid tissue digestion by enzymes present within the cells (autolysis) or bacteria and to preserve cell and tissue structure(Small pieces of tissue are placed in solutions of chemicals that preserve by cross- linking proteins and inactivating degradative enzymes .

The fixation involves (one of ways included in our course) immersion in solutions of stabilizing or cross linking compounds called fixatives .
مركبات مثبتة أو رابطة .

هذا التقاطع مرأة أخرى وليس نفس التقاطع **Why do we cut the sample into several segments** ففي المرة السابقة ? because a fixative must fully diffuse through the tissues to preserve all cells .

- # One fixative used for LM is formalin (a buffered isotonic solution of 37% formaldehyde).
 - # another fixative used in the electron microscope is glutaraldehyde .
- > Both react with the amine groups (NH_2) of tissue proteins , preventing degradation .

Note : with the greater magnification and resolution of very small structures in the electron microscope , fixation must be done carefully to preserve ultra- structural details (التفصيات الدقيقة جداً في العينة) . Therefore we use a double fixation procedure , using a buffered glutaraldehyde solution followed by immersion in buffered osmium tetroxide . (Osmium tetroxide preserves and stains membrane lipids and proteins) .

Dehydration

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

the tissues are lost water by successive transfer through a graded series (سلسلة انتقالات متعاقبة للعينة) of ethanol and water mixtures , usually from 70% to 100% ethanol .

Clearing

This is (خطوة إزالة الماء يبعثها هذه الخطوة مباشرةً) followed by a hydrophobic clearing agent (such as xylene) to remove the alcohol. ----> as the solvent xylene infiltrates the tissues they become more transparent (undergo clearing).

infiltration

the fully cleared tissue is then placed in melted paraffin (in the oven at 52-60 degree) until it becomes completely infiltrated from the last substance

Embedding

the paraffin infiltrated tissue (impregnated tissue) is placed in a small mold with melted paraffin and allowed to harden (at room temperature) .

---> a hardened block containing tissue and paraffin is placed in an instrument called a microtome and sliced by the steel blade into thin sections . paraffin sections are generally cut at 1-10 Mm(micro meter) thickness (By steel blade) But By glass or diamond knives of ultra-microtome produce sections of less than 1 Mm for electron microscopy .

staining

most cells and extracellular material are completely colorless and to be studied microscopically sections must typically be stained (dyed) .

Cells components such as nucleic acids with a net negative charge (anionic) stain basic dyes and are termed basophilic BUT cationic components such as proteins with many ionized amino groups have affinity for acidic dyes and are termed acidophilic .

Of all staining methods, the simple combination of hematoxylin and eosin (H and E) is used most commonly .

hematoxylin produces a dark blue or purple color staining DNA or nucleus or other acidic structures such as RNA-rich portions of the cytoplasm and the matrix of cartilage .

Eosin produce pink staining other cytoplasmic components and collagen .

ملاحظة : جميع أنواع الأصباغ الأخرى لم يرد ذكرها بالمحاضرة وما تم إعطاؤها " في الأعوام الماضية " إلا بملخصات الدكتور فرج ورح احكي شوي ☺ عليها

DNA can be identified in nuclei using the" Feulgen" reactions in which deoxyribose sugars are hydrolyzed by mild hydrochloric acid followed by treatment with " periodic acid – Schiff" abbreviated PAS reagent (((this reaction is based on the transformation of 1,2-glycol groups present in the sugars

into aldehyde , which then react with Schiff reagent to produce a purple color

Note : polysaccharides constitute a heterogeneous group in tissue ☺ therefore--> because of their hexo- sugars contents , they can also be demonstrated by the PAS reaction . ALSO ---> The glycogen in the liver , striated muscle can be demonstrated by PAS reaction .

In many staining procedures certain structures such as nuclei become visible but other parts of cells remain color- free. (((in these cases we use a Counterstain to give additional information , it is usually a single stain that is applied separately to allow better recognition of nuclei and other structures . ((Very important info : Eosin is the counterstain to hematoxylin .))))

Lipids – rich structures of the cells are best revealed with lipid-soluble dyes and avoiding the processing steps that remove lipids such as treatment with heat, organic solvents or paraffin , the frozen sections are stained in alcohol solutions saturated with a lipophilic dye such as Sudan black which dissolves in lipid-rich structures of cells .

Metal impregnation techniques : using solutions of Silver salts is a common method visualizing certain ECM fibers and specific cellular elements in nervous tissue .

The whole procedure from fixation to observing a tissue in LM may take from 12hours to 2.5 Days ☺ depending on(the size of the tissue – fixative - embedding medium – the method of the staining) . the final step before microscope observation is mounting a protective glass cover-slip on the slide with clear adhesive .
