Raghad F. T.

Histological techniques

Lecture 1

hestological sections → series of processes → examination

through
- LM — most common
  - the tissues must be sliced so the light can pass through them
    - thin, translucent sections
    - AS A RESULT
- EM

Step of preparation and examination of Hes. sections:

1. Sample collection

The tissue must be FRESH and very small (< 1cm so the fluids could enter the sample easily)

- Types of obtained tissues
  - Autopsy (the tissue is taken immediately after death).
  - Surgical procedure (the animal will stay alive).

- Methods: (how?)
  - under anaesthesia: ضع الكائن الحي تحت التخدير ثم اقتله
  - Decapitation: اقطع رقبته بحيث لا يشعر بالألم

2. Processing and sectioning

Will be discussed in details

processing → replacement of water by medium to make the tissue more rigid so the sectioning step could be operated properly. (adequate sectioning in another words)

the medium
- L.M:
  1. Paraffin
  2. Celloidin
  3. Freezing
- E.M → Resin

sectioning — cutting into slices
- L.M: Rotatory microtome
- E.M: Ultra microtome

(tome = knife)

(اسم الجهاز المستخدم للتقطيع)

3. Staining

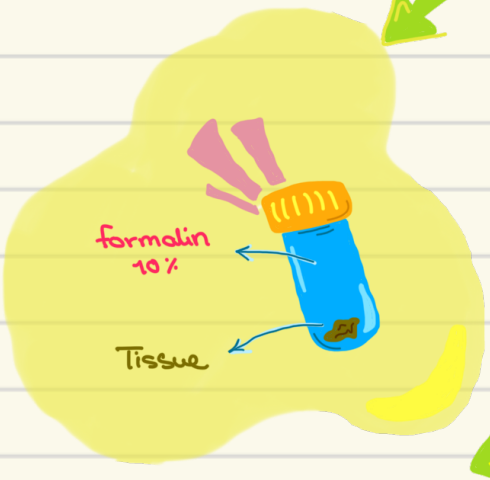
4. Microscopic Examination

Tissue processing for Paraffin method

most common method

1. Obtaining the tissue: fresh and small

RFT



2. Fixation

by formalin (formal saline) 10% * 24h

or other combinations of fixatives

why?
- to harden the tissue
- to save the cellular structure (natural state)
- to prevent enzymatic digestion
- to increase straining affinity

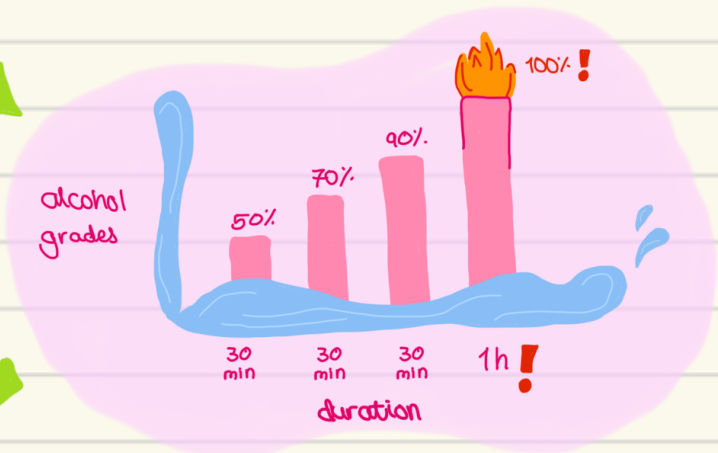
3. Dehydration

$-H_2O$

by alcohol

"ascending grades"

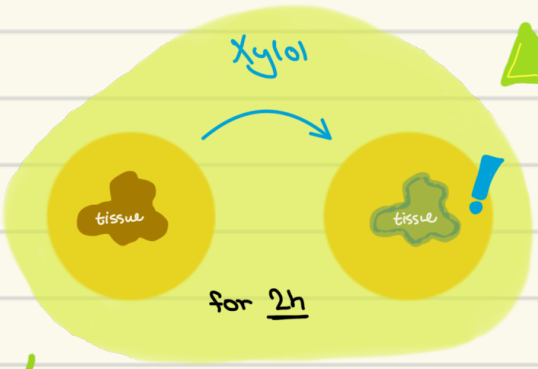
to prevent tissue shrinkage



4. Clearing

by Xylol

to replace alcohol



the tissue is TRANSLUCENT !

It's ready for being exposed to PARAFFIN

5. Impregnation

by melted soft PARAFFIN wax

In the oven 🔥

- to replace Xylol and harden the tissue from inside

the tissue

melted soft paraffin

This image does not represent the actual process, it is only meant to help you remember the key points.

for 2h!

6. Embedding

by melted HARD parffin

- to harden the tissue from Outside forming ✨ Paraffin BLOCK ✨
- make the section easy to cut.
- Can save the tissue for yeeeeeeeeears!

2025

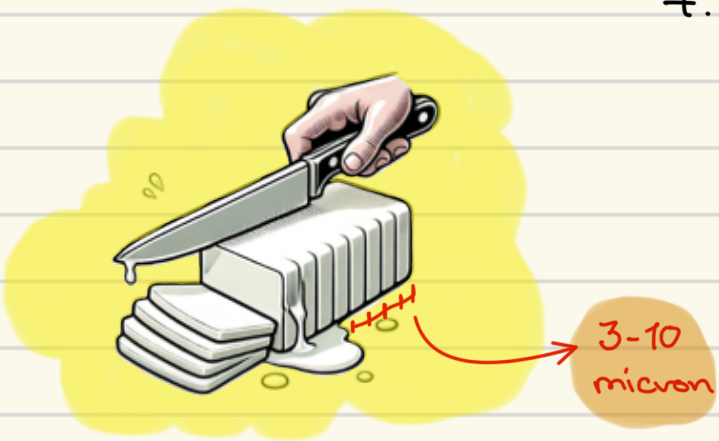
Paraffin BLOCK!

2000 YEARS LATER

Paraffin BLOCK!

7. Section cutting

by Rotatory microtrome



8. Mounting Sections

Attaching sections -permanently- to individual glass microscope slides.

The end ♥

Raghad F. T.

Processing for celloiden:

- used when the sample is at risk of breakage
- Embedded in celloiden.
- Cutted using sliding microtome.
- Used for large pieces of Samples (brittle materials) مواد هشة كالعظام

Freezing tichneque:

When to use?

- urgent diagnosis is needed (during surgery)
- during the study of sensitive enzymes or small molecules [Enzyme & Lipid staining]
  (Histochemical studies) لانه المواد الكيمياوية ممكن تتلفهم

Sample: Biopsy خزعة

Processing طريقة : biopsy rapidly frozen in (liquid nitrogen)

Sectioning: by a microtome called cryostat (subfreezing tempreture) تحت الصفر

Then? frozen sections are placed on slides for rapid staining and microscopic examination by a pathologist.

Preparation for Transmission electron microscope (TEM)

Same steps as LM but with some modifications

1. Sample : must be very small (1-3 mm)
2. Fixation : done by 2steps → Glutraldehyde then in osmic acid
   (instead of one step using formalin in LM)
3. Dehydration : in ascending grades of alcohol OR acetone
   (just alcohol in LM)
4. Clearing : Propylene oxide (xylole in LM)
5. Embedding : In epoxy resin (Paraffin in LM) epoxy = صلب
6. Sectioning : Ultra microtome with glass or dimond knives
   (50 - 100 nm) (3-10 micron in LM)
7. Staining : Salts of heavy Metals
8. Mounting and Examination : on copper grids (glass in LM)

Technique :	L/ M	E / M
Fixation	Formalin 10%	Glutaraldhyde 4% & osmic acid
Clearing	Xylol	Propylene oxide
Embedding	Paraffin	Epoxy
Cutting	By metal knife	By glass knife or diamond knife
Thickness	Up to 10 microns	(50 - 100 nm)
Staining	Depends on colour	Depends on contrast
Spreading	Upon glass slide	Upon cupper grids

Scanning electron microscope (SEM)

- More simple preparation
- The sample is in small as in TEM
- Sample is gold coated 3-D
- Scanning the external surface