3th Stage

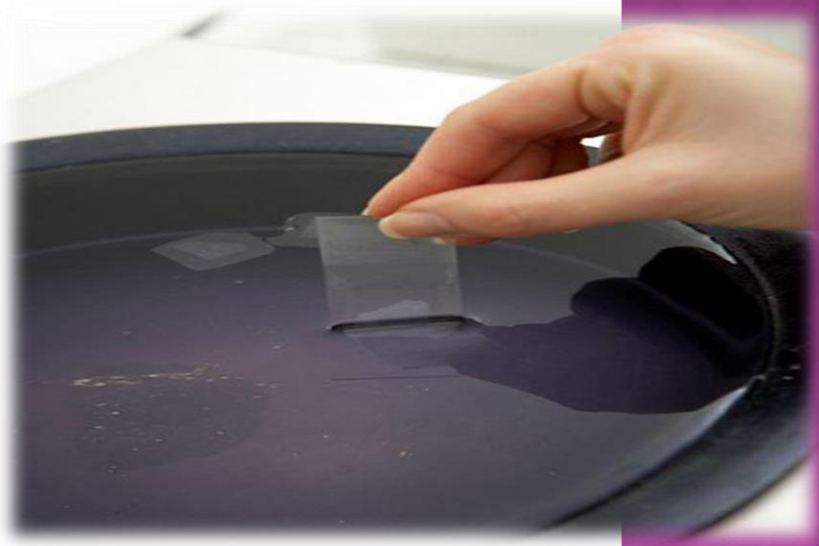
STAINING

Lab 8

M.SC. FADHELA NAFAA ALRAWI

TISSUE FLATTENING

- The sections are floated on a water bath, which allows them to relax and flatten out. This flattening step ensures that the tissue is properly aligned and free of wrinkles or folds, which could distort the tissue morphology and hinder accurate diagnosis or analysis.
- FLOATING &PICKING UP S CTIO: The ribbon is floated on the water surface heated at 40_ 45 C.
- The albumin material is placed to fix on the slide and then the strips are carried on the slides.



STAINING OF TISSUE SECTIONS WITH ROUTINE H&E STAIN

What is staining?

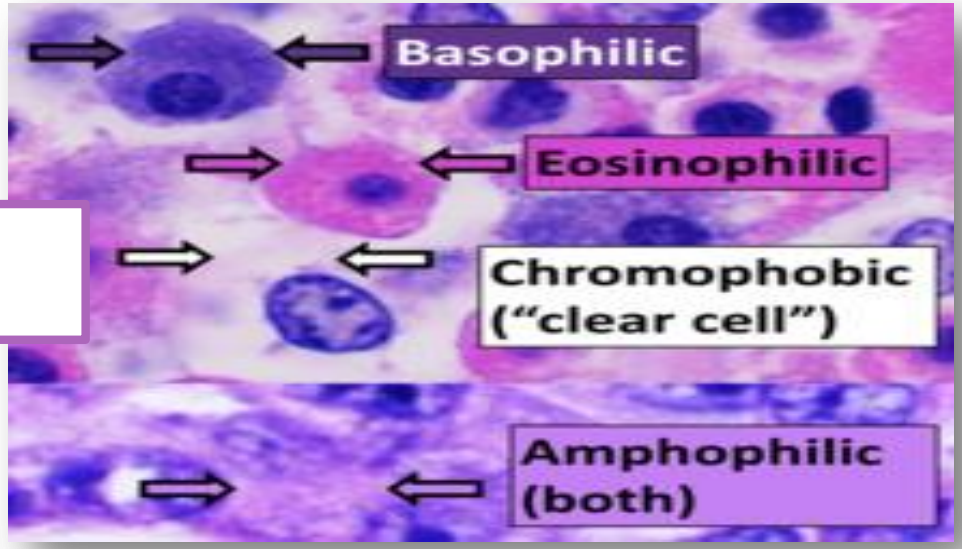
- Staining is a technique used to enhance contrast in samples, generally at the microscopic level.
- Stains may be used to define biological tissues, cell populations or organelles within individual cells.
- **HISTOLOGICAL STUDIES ARE USED IN:**

1. Forensic investigations
2. Autopsy
3. Diagnosis
4. In education

HEMATOXYLIN AND EOSIN STAIN (ROUTINE STAIN)

- Hematoxylin and Eosin (H & E) staining is the most common staining technique in histopathology.
- This stain used for display the general histological structure of tissue (routine diagnosis), and consist of two main stains (Hematoxylin stain and Eosin stain).
- Hematoxylin is a reasonably simple dye to make. The dye itself is extracted from the tree Haematoxylum campechianum. (Hematoxylin stains the parts of the cell which are basophilic).
- Eosin is the most commonly used counterstain that distinguishes between the cytoplasm and nuclei of cells. It is typically pink, (Eosin stains the parts of the cell which are acidophilic).

- Nucleus → Blue
- Cytoplasm → Red



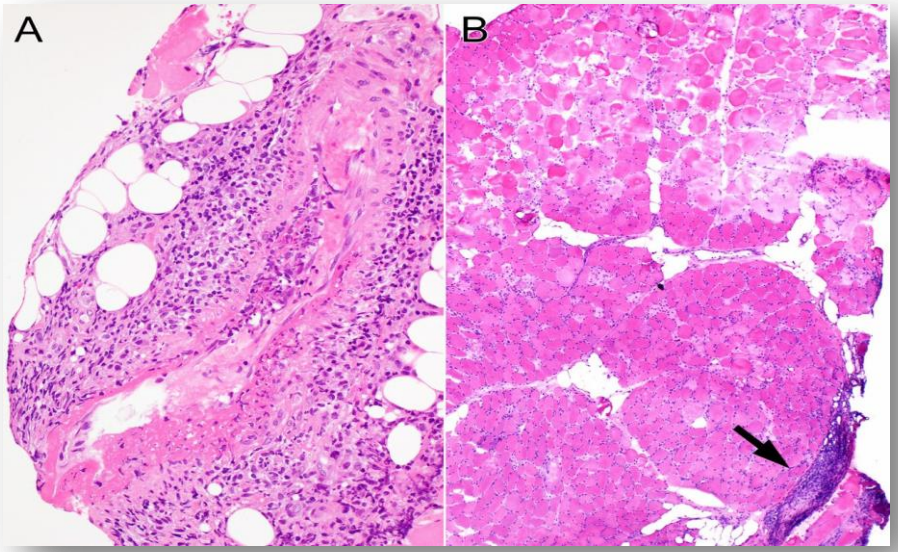
PROCEDURE

Process	Step	Solution	Time
Warming	Incubate at 56c°to melt paraffin and attach tissues to slide		5 min
Deparaffinize	1	Xylene 1	10 min
	2	Xylene 2	10 min
Rehydration	3	100 % alcohol	2 min
	4	90 % alcohol	2 min
	5	80 % alcohol	2 min
	6	70 % alcohol	2 min
Wash	7	Tap water	1 min
Stain (H)	8	Hematoxylin stain	8 min
Wash	9	Tap water	1 min
	10	Distal water	1 min
Differentiate	11	Acidic alcohol (1% HCl in 70% alcohol)	1-2 dips

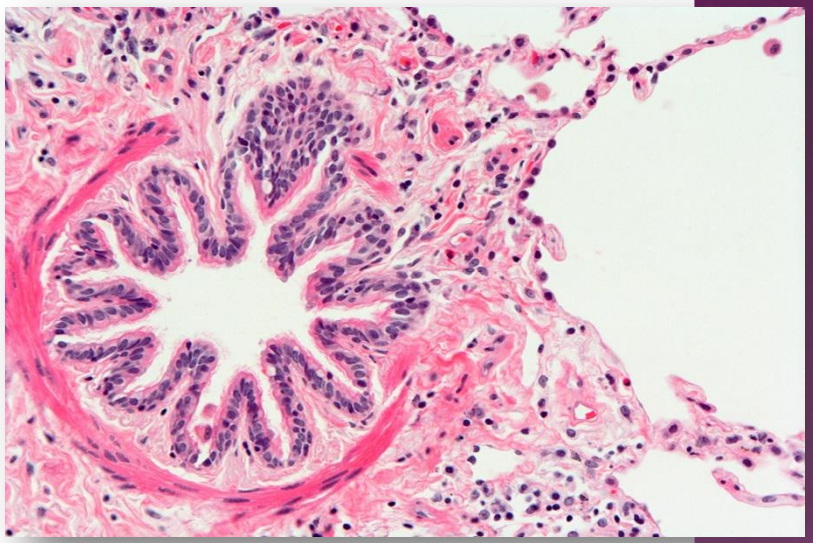
PROCEDURE

Counterstain (E)	12	Eosin stain	30 sec. to 1 min
Wash	13	Tap water	1 min
Dehydration	14	70 % alcohol	1 min
	15	80 % alcohol	1 min
	16	90 % alcohol	1 min
	18	100 % alcohol	1 min
Clearing	19	Xylene 1	1 min
	20	Xylene 2	1 min
Drying the slide	21	By a hot plate at (40C °)	2 hours
Mounting	22	Adding the amount of (Canada balsam or DPX) and then putting the cover slide on the tissue	
Examination	23	By microscope	

MICROSCOPE EXAMINATION



Necrosis of the gastrocnemius muscle



Human lung H&E staining


Thank You