Department of biology

2025-2026

((Theoretical Histological))

Stage (-3-)

LEC- ((2))

Tissue preparation

By

Assistant Professor Ahmed Jamil Al-Taie

Histology:- Is the science deals with study of the fine detail of biological cells and tissues using microscopes to look at specimens of tissues that have been carefully prepared using special processes called "histological techniques".

PREPARATION OF TISSUES FOR STUDY: The most common procedure used in the study of tissue is the preparation of histological sections or tissue slices that can be examined under the light microscope. tissues are examined via a light that is transmitted through the tissue. Because most tissues and organs are too thick for light to pass through them must be sectioned to obtain thin, translucent sections and then attached to glass slides before they can be examined .

Aim of tissue processing to:

1- Preserve microscopic components of tissue.

2- make them hard so that very thin section (3-4 micron) can be mad

The basic steps used in tissue preparation for histology:

1. Tissue fixation Slide preparation begins with fixation of your tissue specimen. This is a crucial step in tissue preparation, and its purpose is to prevent tissue autolysis and putrefaction. For best results, your biological tissue samples should be transferred into fixative immediately after collection. Although there are many types of fixative, most specimens are fixed in 10% neutral buffered formalin. The optimum formalin-to-specimen volume ratio should be at least 10:1 (e.g., 10 ml of formalin per 1cm^3 of tissue). This will allow most tissues to become adequately fixed within 24-48 hours.

The Purpose of tissue fixation are:

A. to inhibit autolytic enzymes and kill microorganisms of decomposition .

B. to preserve tissue as nearly as possible in its original form.

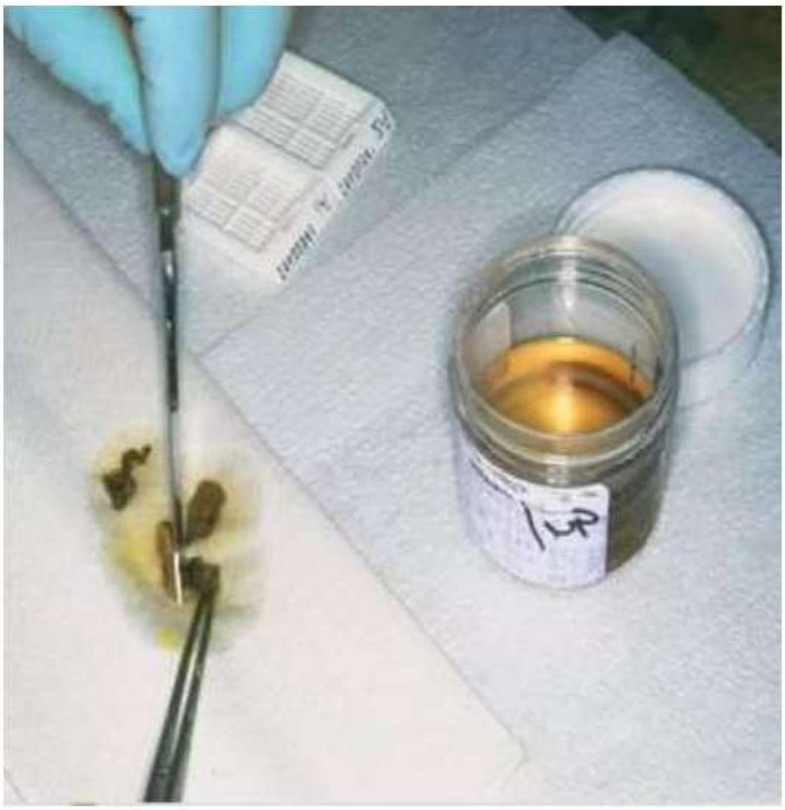
C. to protect tissues against subsequent damage during embedding.

D. to give tissue a texture which permits easy sectioning

E. to render the various constituents receptive of the proposed stains.

Types of Fixatives :

- Buffered normal saline
- 10% formalin
- Suza, Bouin, Zenker solution
- Formaldehyde or Glutradhyde
- Osmium tetraoxide
- Potassium permanganate.



2. Specimen transfer to cassettes

After fixation, specimens are trimmed using a scalpel to enable them to fit into an appropriately labelled tissue cassette. Specimens should not be so big that they fill the cassette. The filled tissue cassettes are then stored in formalin until processing begins.

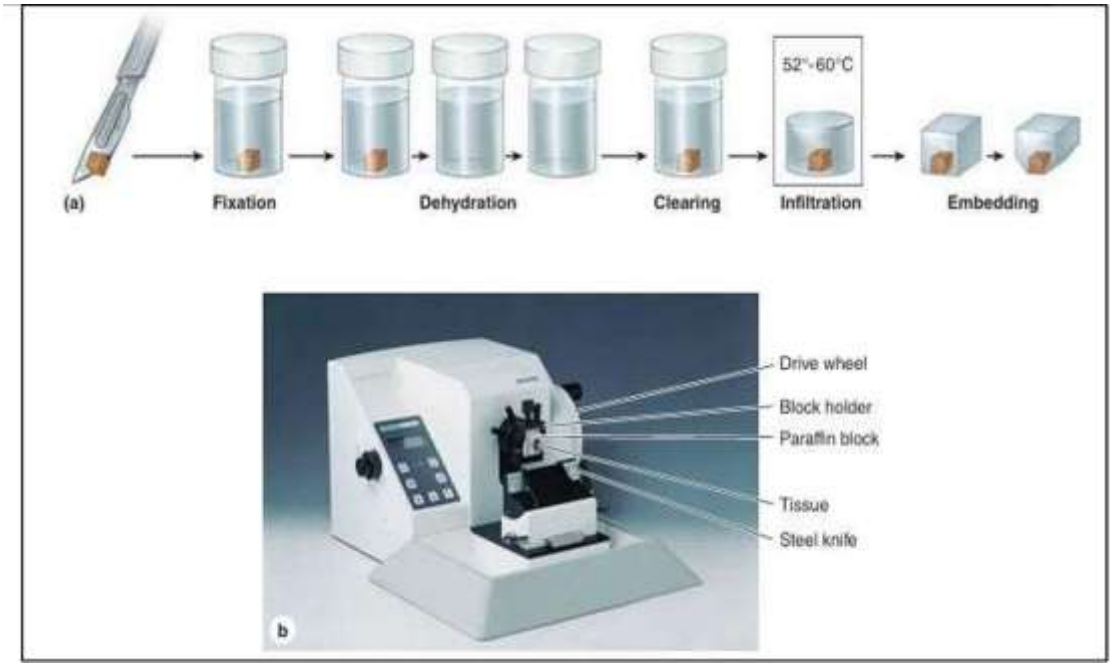
3. Tissue processing processing tissues into thin microscopic sections is usually done using a paraffin block, as follows:

- **Dehydration:** is the first step, which involves immersing your specimen in increasing concentrations of alcohol to remove the water and formalin from the tissue.

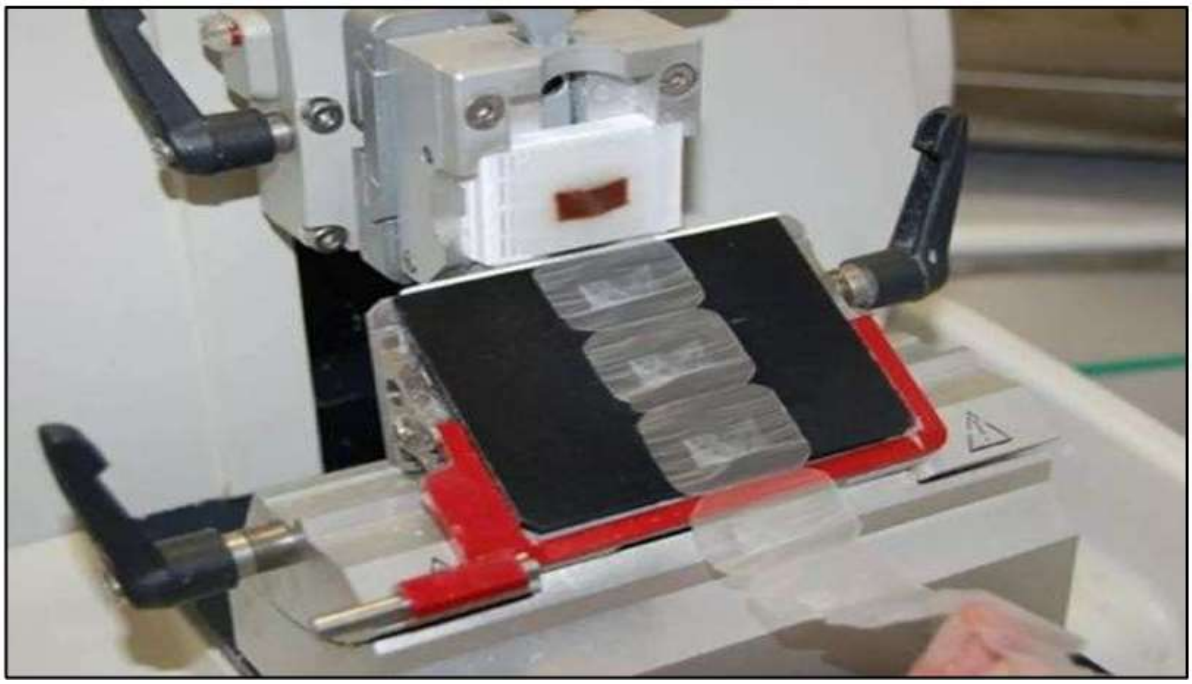
- **Clearing**: is the next step, in which an organic solvent such as xylene is used to remove the alcohol and allow infiltration with paraffin wax.

• **Embedding**: is the final step, where specimens are infiltrated with the embedding agent – usually paraffin wax. The tissue becomes surrounded by a large block of

molten paraffin wax . Once the block solidifies, it provides a support matrix that allows very thin sectioning.



4. Sectioning Your tissue specimen is now ready to be cut into sections that can be placed on a slide. Wax is removed from the surface of the block to expose the tissue. Blocks are chilled on a refrigerated plate or ice tray for 10 minutes before sectioning. A microtome is used to slice extremely thin tissue sections off the block in the form of a ribbon. The microtome can be pre-set to cut at different thicknesses, but most tissues are cut at around 5 µm.



5. Mounting: sections spread on the hot plate and mounted on glass slides. - Sections mounted on metal grids.

A. 40 C^{o} water bath

1. Flattens paraffin section

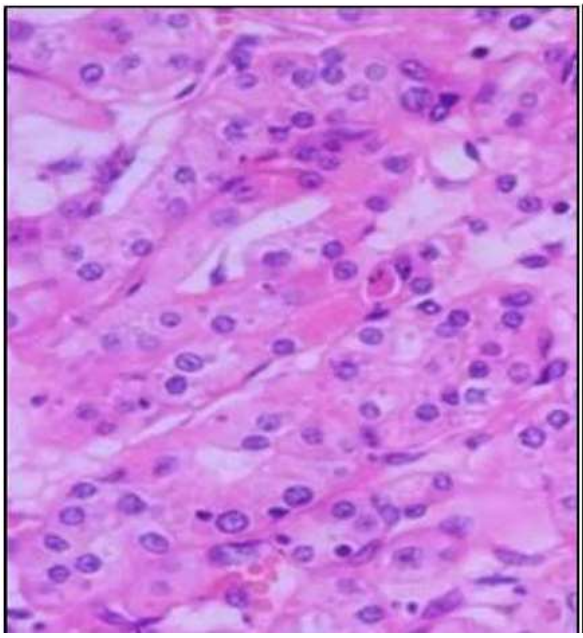
2. Permits mounting on slide

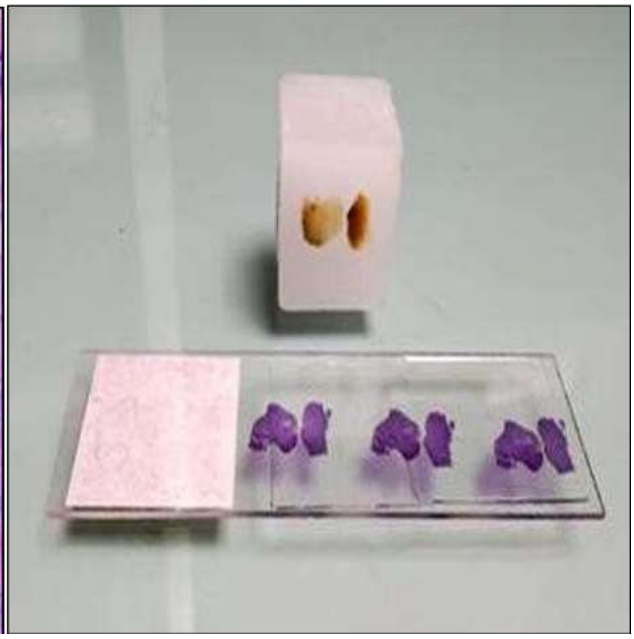
B. Gelatin & albumin

C. Glass slides

D. Oven / air dry

6. Staining: most cells are transparent, and appear almost colourless when unstained. Histochemical stains (typically haematoxylin and eosin) are therefore used to provide contrast to tissue sections, making tissue structures more visible and easier to evaluate. Following staining, a cover slip is mounted over the tissue specimen on the slide, using optical grade glue, to help protect the specimen.





Listed below are the stains that were used to prepare the slides and their specific staining:

characteristics.

A- Hematoxylin and Eosin (H&E) Stain

- Nuclei stain blue
- Cytoplasm stains pink or red
- Collagen fibers stain pink
- Muscles stain pink

B- Masson's Trichrome Stain

• Nuclei stain black or blue black

• Muscles stain red

• Collagen and mucus stain green or blue

• Cytoplasm of most cells stains pink

C- Periodic Acid-Schiff Reaction (PAS)

• Glycogen stains deep red or magenta

• Contents of goblet cells in digestive organs and respiratory epithelia stain magenta red.

• Basement membranes and brush borders in kidney tubules stain positive, or pink.

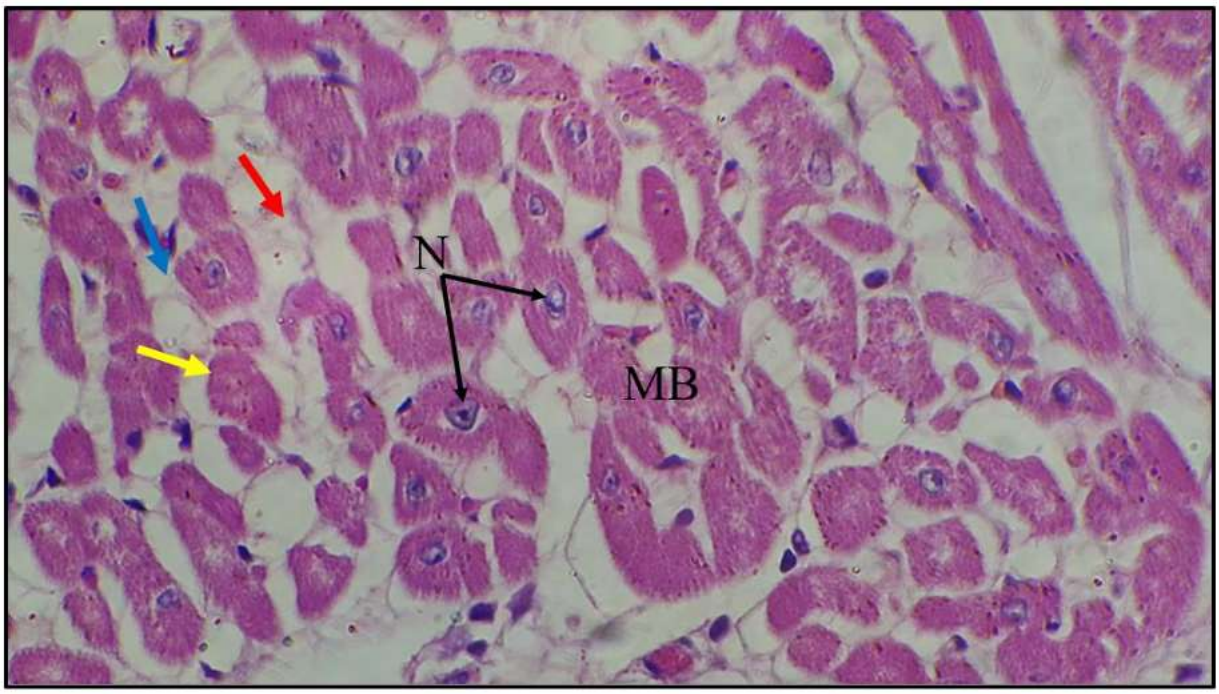


Figure (4-58) Histological cross-section of the left ventricle myocardium at six months of sheep lamb showed: muscle fibers bundles (MB), perimysium (blue arrow), epimysium (red arrow), endomysium (yellow arrow), nucleus (N) (H & E stain 40X).

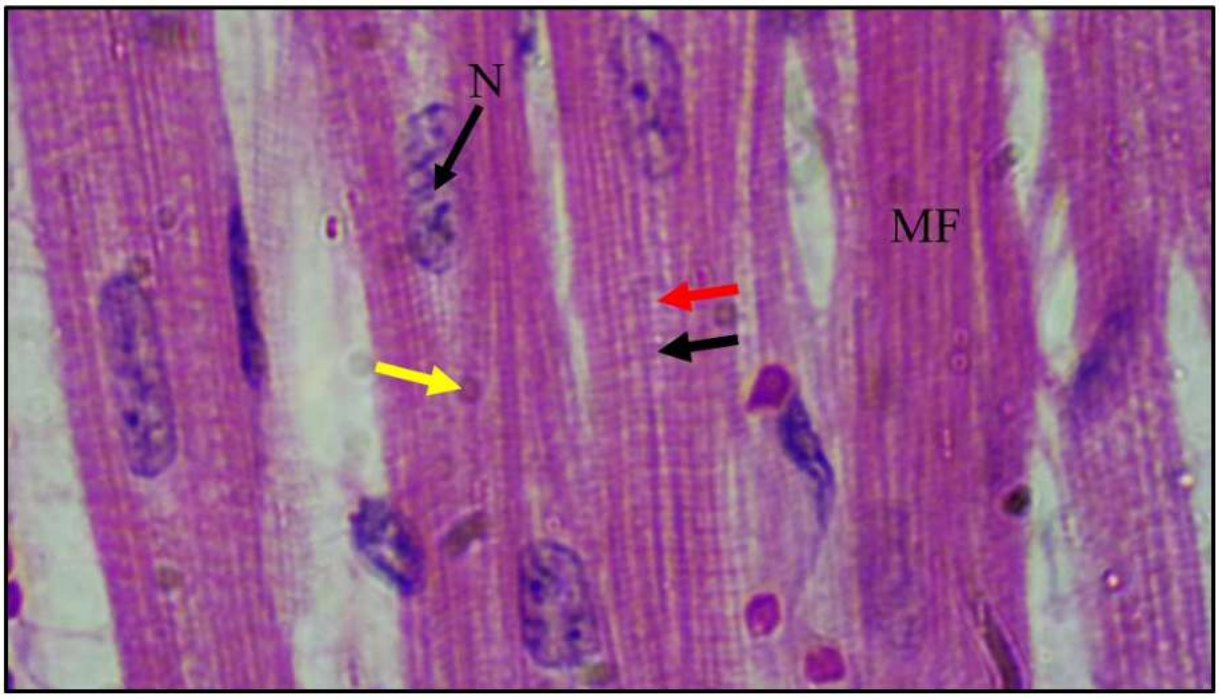
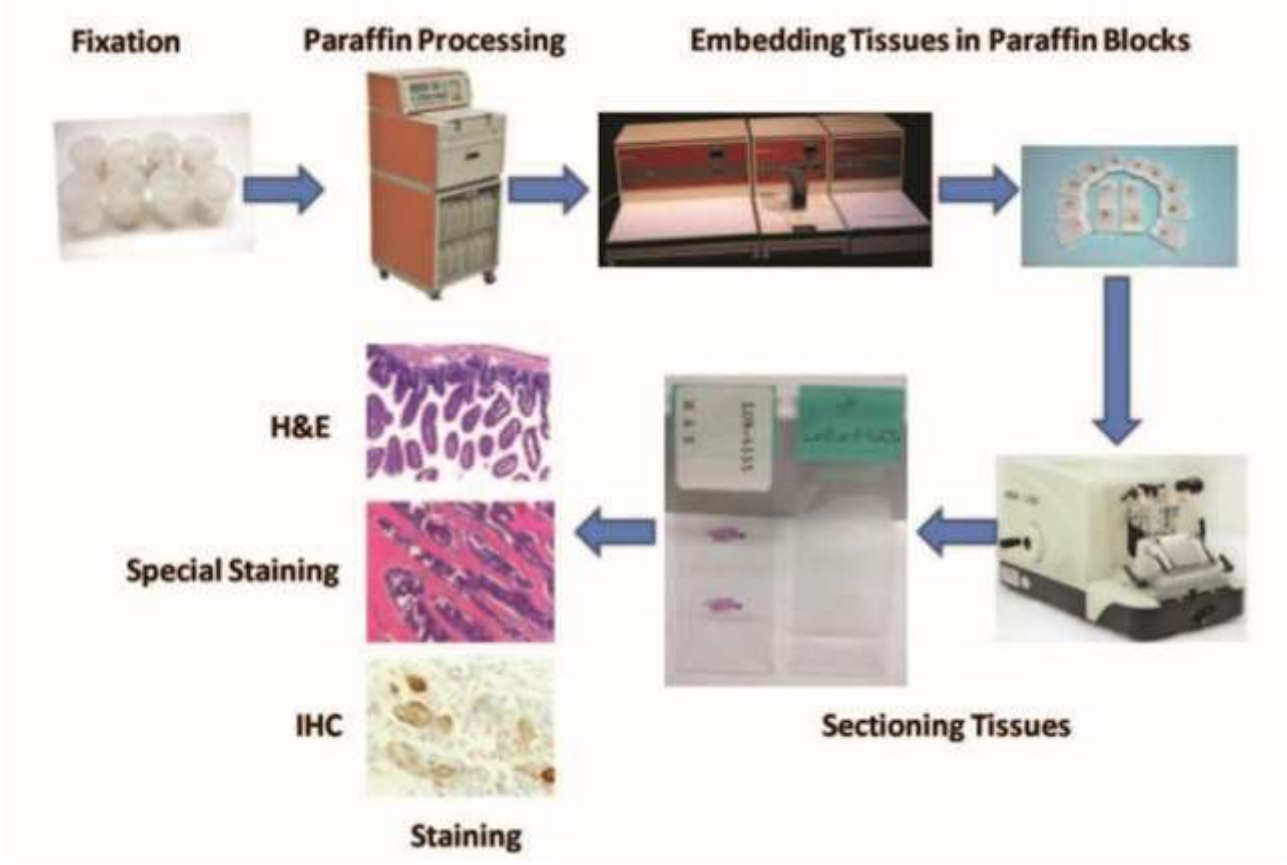


Figure (4-60) Histological longitudinal section of the left ventricle myocardium at six months in sheep showed: muscle fibres (MF), nucleus (N), intercalated disc (yellow arrow), A-band (black arrow), I-band (red arrow) (H & E stain 100X).



Registration:

•Lab.no, Date, Name of patient, age, Sex, Occupation, address, reffereing doctor

Gross examination:

•Includes description of the specimen : weight , dimensions , color , texture , cut section , followed by photography