Faculty of Applied Science

Medical Analysis Department

Micritechnique (MA505)

Lecture 2

TISSUE FIXATION

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M.Sc. Biomedical Science Blood Science

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Learning outcome

- By the end of this lecture, students will be able to:
- Define tissue fixation and explain its importance in histopathology.
- Explain the purposes and principles of fixation.
- Identify major factors affecting fixation quality.
- Classify common fixatives and describe their mechanisms.
- Explain formalin: properties, uses, advantages, and limitations.
- Compare advantages and limitations of common fixatives.

Outlines

- Introduction to tissue fixation
- Purpose of fixation
- Principles and mechanisms
- Factors affecting fixation
- Types of fixatives
- Formalin
- Advantages and limitations of common fixatives
- Class activity
- References

What Is Tissue Fixation?

- Fixation is the process of preserving tissue as close as possible to its original structural and molecular condition.
- It minimizes post-mortem changes after tissue removal.
- It is the first major step in routine histological tissue processing.
- Good fixation supports good processing, sectioning, staining, and diagnosis.

Why Does Tissue Need Fixation?

- After tissue removal, autolysis begins because intracellular enzymes remain active.
- Bacterial decomposition may occur.
- Cellular structures can lose their normal shape .
- Soluble components may diffuse away.
- Fixation stabilizes tissue before significant deterioration occurs.

Purpose of Fixation

- Preserve tissue morphology and architecture.
- Prevent or minimize autolysis.
- Prevent putrefaction and bacterial decomposition.
- Stabilize proteins and cellular structures.
- Provide appropriate tissue consistency for processing and sectioning.
- Preserve selected chemical components.
- Facilitate subsequent staining and microscopic examination.

Principles of Fixation

- Fixatives stabilize tissue components and reduce degradation.
- Different fixatives act through different chemical mechanisms.
- Major mechanisms include protein cross-linking, coagulation/precipitation, and denaturation.
- The fixative must penetrate the entire tissue specimen.
- Fixation proceeds from the surface toward the center.

Protein Cross-Linking

- Cross-linking fixatives form chemical bonds between tissue proteins.
- Formaldehyde is the most important routine example.
- Cross-linking stabilizes proteins and helps maintain tissue architecture.
- Excessive cross-linking can reduce accessibility of some antigens.

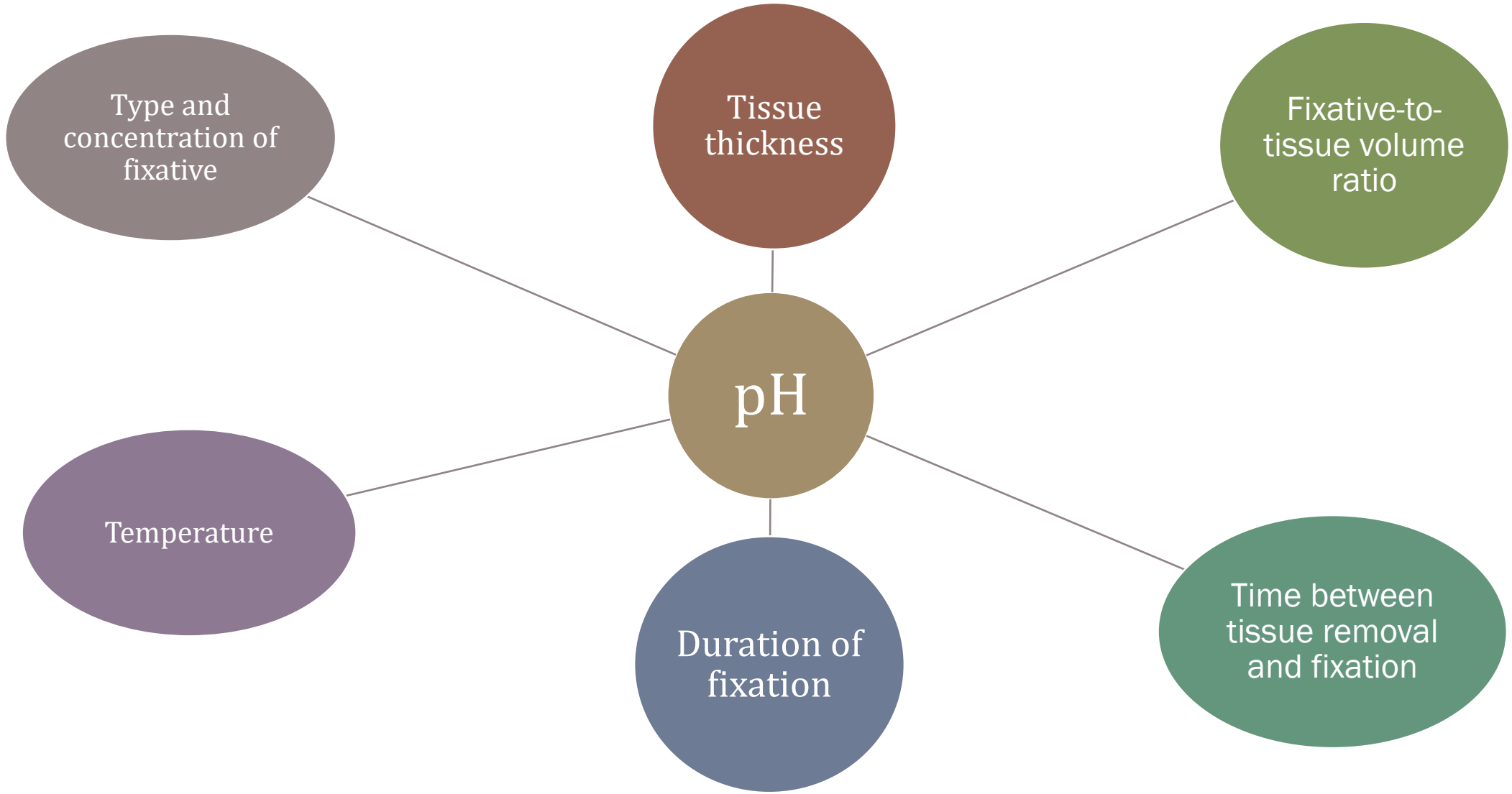
Coagulating / Precipitating Fixatives

- Alcohols such as ethanol and methanol precipitate or coagulate proteins.
- They can act rapidly.
- They are useful in selected cytological and blood-film applications.
- However, they may cause shrinkage, hardening, or cellular distortion.

Fixative Penetration

- Fixation begins at the tissue surface and progresses inward.
- Thin tissue pieces are fixed more rapidly and uniformly.
- Thick specimens may develop a poorly fixed or autolysed center.
- Appropriate specimen size is therefore critical for successful fixation.

Factors Affecting Fixation



Tissue Thickness

- Thin tissue → faster penetration and more uniform fixation.
- Thick tissue → slower penetration.
- Poor penetration can allow autolysis in the center.
- Routine specimens should be cut to an appropriate thickness before fixation.

Concentration & Duration

- Insufficient concentration may produce inadequate fixation.
- Excessive concentration can alter tissue chemistry and morphology.
- Under-fixation may cause autolysis and poor staining.
- Over-fixation may cause excessive cross-linking and tissue hardening.
- Fixation time should be appropriate for tissue size and fixative.

Temperature, pH & Volume

- Temperature influences the rate of chemical fixation reactions.
- Extreme temperatures can damage tissue.
- pH affects preservation, chemical reactions, and staining characteristics.
- Routine formalin is commonly buffered near neutral pH.
- A commonly taught practical principle is about 10:1 fixative volume to tissue volume

Types of Fixatives

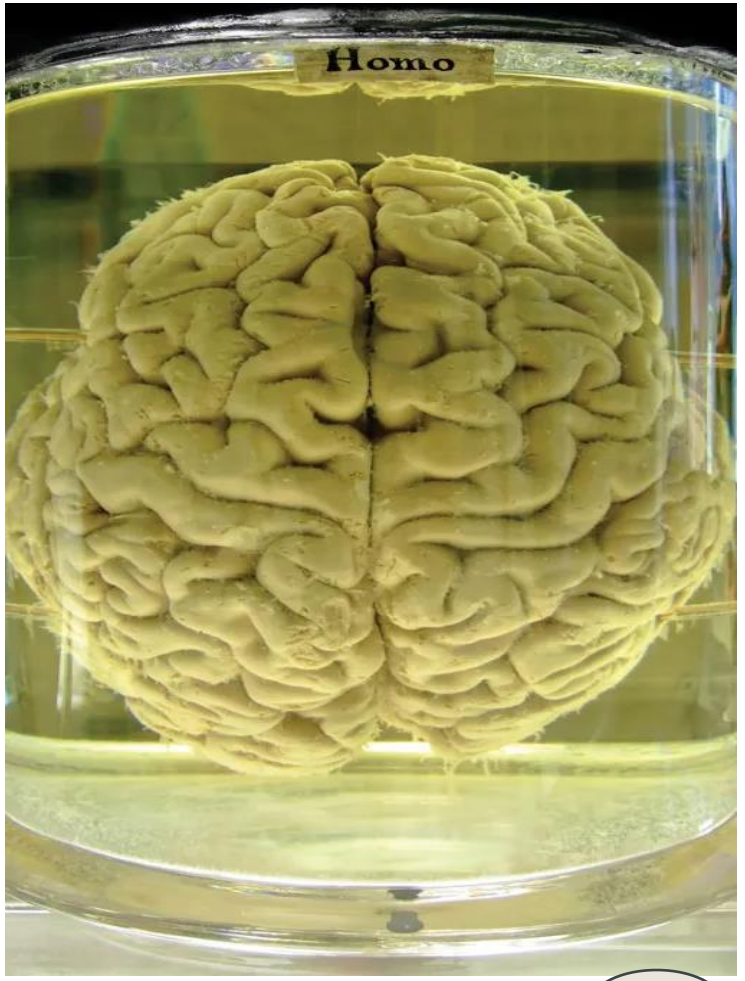
- By mechanism:
  - • Cross-linking: formaldehyde, glutaraldehyde
  - • Coagulating/precipitating: ethanol, methanol, acetone
- By composition:
  - • Simple fixatives: one principal fixing agent
  - • Compound fixatives: two or more chemical components

Common Fixatives

- 10% Neutral Buffered Formalin – routine histology
- Glutaraldehyde – electron microscopy / ultrastructure
- Ethanol – selected cytology applications
- Methanol – blood films and cytology
- Bouin's solution – excellent morphology in selected tissues
- Carnoy's solution – rapid fixation in selected cytological applications

What is Formalin?

- Formalin is an aqueous solution of formaldehyde.
- 10% Neutral Buffered Formalin (10% NBF) is widely used in routine histopathology.
- 10% formalin contains approximately 4% formaldehyde.
- It provides a useful balance of morphology, availability, cost, and staining compatibility.



How Formalin Works

- Formaldehyde reacts with tissue proteins and other cellular components.
- It produces chemical stabilization mainly through cross-linking.
- Cross-linking helps preserve:
  - Nuclear morphology
  - Cytoplasmic morphology
  - Tissue architecture

Advantages of Formalin

- Good routine morphological preservation.
- Relatively good penetration when tissue is appropriately sized.
- Inexpensive and widely available.
- Compatible with routine H&E staining.
- Compatible with many special stains.
- Suitable for routine paraffin processing.
- Well established in diagnostic histopathology.

Limitations of Formalin

- Cross-linking may mask some antigens.
- Prolonged fixation can increase cross-linking and tissue alteration.
- Some soluble substances and lipids may be lost during processing.
- Formalin pigment may occur under inappropriate conditions.
- Formaldehyde is hazardous and requires appropriate laboratory safety measures.

Safety When Handling Formalin

- Use appropriate personal protective equipment.
- Work with adequate ventilation or local exhaust/fume extraction.
- Avoid skin and eye contact.
- Keep containers properly labeled and closed.
- Follow institutional chemical waste-disposal procedures.
- Know the laboratory's exposure and spill-response procedures.

Common Fixatives: Comparison

- **10% NBF**: excellent routine morphology; limitation – cross-linking.
- **Glutaraldehyde:** excellent ultrastructure; limitation – poor penetration and strong cross-linking.
- **Ethanol:** rapid fixation; limitation – shrinkage and hardening.
- **Methanol:** useful for blood films; limitation – protein precipitation/cellular distortion.
- **Bouin's solution:** excellent selected morphology; limitation – picric acid and compatibility/disposal concerns.
- **Carnoy's solution:** rapid fixation; limitation – tissue shrinkage and lipid extraction.

Under-Fixation vs Over-Fixation

Under Fixation	Over Fixation
Incomplete preservation	Excessive cross-linking
Autolysis	Tissue hardening
Poor morphology	Reduced accessibility of some molecules
Poor staining	Potential immunohistochemical problems
Possible diagnostic difficulty	

Question bank

- What is tissue fixation?
- Why should tissue be fixed as soon as possible after removal?
- What is autolysis?
- Name three factors affecting fixation.
- What is the main mechanism of formaldehyde fixation?
- What is 10% Neutral Buffered Formalin?
- Give two advantages and two limitations of formalin.

Discussion Case

- A large tissue specimen is placed directly into formalin without being appropriately trimmed.
- Question:
- What problem is likely to occur in the center of the specimen?
- Why does this happen?
- How could the laboratory improve fixation quality?

References

- Bancroft, J. D., & Gamble, M. (Eds.). (2019). Theory and practice of histological techniques (8th ed.). Elsevier.
- Carson, F. L., & Cappellano, C. (2020). *Histotechnology: A self-instructional text* (5th ed.). American Society for Clinical Pathology Press.

Next lecture

- Tissue Processing