2-Oct-2024

INTRODUCTION

Histology:
Branch of anatomy deals with the study of tissue.
Greek words
Histo — Tissue
Logia — study

⇒ Autolysis:
→ own bacteria
⇒ Fixator:
→ To maintain
of sample

Tissue:
Cell combines to make tissue.
Human — Epithelial, connective, Nervous & Muscular tissue. divided into

Fresh tissue → Fixator → 3-8 microones → Glass slide → Staining → Microscope

⇒ **TECHNIQUES:**

1- Preparing the tissue:
→ Necessary to inform about it to the patient.
→ Tell his/her about purpose e.g diagnosis & treatment.

2- Tissue acquisition: (the way of getting sample)
→ Important step

Endoscopy: a camera insert through mouth to see the tissue in internal body & remove the effected tissue's part for test.

3- Fixation:
Tissue after removal — Fixative (10% Formal saline)
tissue immersed 10 times.

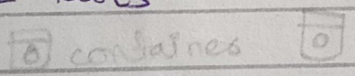
Normal Flora
Beneficial Bacteria

Why fixatives are used?
→ Prevent autolysis & putrefaction. on skin.
→ Prevent tissue from osmotic shock.
→ Distortion and shrinkage.

Fixation containers:
→ Jars or bottles with screw top.
→ Suitable capacity

→ **Large Specimens**
Any Sample
Blood, urine, Pus, stool, tissue
Large specimens — not squeezed into small
~~Inadequate~~ fixation — allow autolysis
Large → wrap in rubber sheet.

→ **Bulky Specimens**
Large tumors → cancer
Spleen — iv organ
bisect with sharp knife placed in
↓ fixative
2 Parts



→ **Solid Organs:**
→ Cut slices
→ Not thicker than 5mm.

Brain:
→ Uncut Brain
→ Pass thread through vessels
organ gently lowered to bucket.

Hollow Viscera:
→ Stomach & intestine
→ Cut along their length

Small biopsies:

→ Place first on filter paper.

→ then put in solution.

→ **PRINCIPLE OF FIXATIVES:**

→ Denaturation of Protein

→ Coagulation of Protein (Forming of insoluble gel like substance)

→ Dehydration (Removal of water from cell)

→ Inhibition of Autolysis (Prevent self-digestion)

→ Inhibition of Proteolysis (Prevent Protein breakdown).

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TYPES OF FIXATIVES

Three main types of fixatives.

(i-) ⇒ **Primary Fixatives:**
→ Single Fixative in solution
e.g → absolute ethanol or 10% Formaline

(ii-) ⇒ **Compound Fixatives:**
→ Consists of two or more Fixatives in solution.
e.g → Zenker's, Helly's & Bouin's fixatives.

(iii-) ⇒ **Cytological Fixatives:**
→ Preserve cellular structures or inclusions
↓
e.g mitochondria
→ Penetration & allow tissue to be cut easily.

⇒ PROPERTIES: (Ideal Fixative)

→ Prevent autolysis & bacterial decomposition.
→ Preserve tissue in their natural state & fix components (Protein, Carbohydrates & fats).
→ Make cellular components insoluble to reagents used in tissue processing.
→ Preserve tissue volume.
→ Avoid excessive hardness of fixed tissue.
→ Allows enhanced staining of tissues.
→ Non-toxic & non-allergic for user.
→ Not very expensive.

⇒ Amount of fixing Fluid:
⇒ 10-20 times the volume of specimen.

⇒ **Classification of fixatives:-**
Fixatives are classified on the basis of three main criteria:
⇒ Action on proteins
⇒ Types of fixative solution
⇒ Use

{ Action on proteins Fixatives can have two main actions on Protein i.e may be coagulant or non-coagulant fixatives }.

→ Tissue Fixatives:
(i) Buffered Formal Saline
(ii) Buffered Glutaraldehyde
(iii) Zenker's formal saline
(iv) Bouin's Fluid

→ Cytological Fixatives:
(i) Ethanol
(ii) Methanol
(iii) Ether

→ Histochemical Fixatives:
(i) Formal Saline
(ii) Cold acetone
(iii) Absolute alcohol

COMMON FIXATIVES

1. → Formaline: (Routine Fixative)
Formaline is a 40% solution of formaldehyde in water.
Usage: Commonly diluted to 10% - 15% with normal saline or calcium chloride.
How it works? Preserve the tissue by forming an insoluble gel with protein.
→ Preventing them from Precipitating
→ Maintain natural form
→ Donot form solid

Benefits:
→ Preserve elements including fats Eg Phospholipids.

2- Buffered Formalin:
Buffered Formaline is a 10% formal saline with neutral pH. (7)
Usage: Regular formaline can become acidic, causing crystals that mess up staining. → calcium oxalate.
How it forms? (10% Formal Buffered Saline)
→ 10 ml Formalin (Pure)
→ 0.4 g sodium dehydrogen Phosphate
→ 0.65 g disodium hydro Phosphate
→ Add normal saline to reach 100 ml.
Advantages: → Keep PH neutral.
→ Help to preserve tissue for better staining.
→ Tissue can be left in fixative for long Period (1 year)
→ No haematin (haemotin) crystals are formed.

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CHEMICALS USED DURING FIXATION

⇒ Ethyl Alcohol (90-100%)

Precipitates: Albumin and globulin (not nucleo-proteins).

Effects: Shrinks and hardens tissues.
Destroy mitochondria

Compatibility: Can't be used with chromic acid or osmium tetra-oxide.

Preservations: keeps glycogen, helpful for histo-chemistry (like glycogen & Iron).

⇒ Mercuric Chloride

Concentration: Used as saturated (70%) or half-saturated solution.

Properties: * Rapid Penetration.
* Precipitates Proteins.
* Fixes chromatin effectively, improving staining.

Usage: * Rarely used alone.
* Valuable for nuclear fixation.

⇒ Picric Acid

Concentration: Saturated aqueous solution (1%)

Properties: * Poor Penetration
* Causes shrinkage but does not harden tissues.

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Preservation: Preserves glycogen and most other elements.
Staining: Does not effect staining results.
Usage: Not typically used alone.

⇒ Chromic Acid

Chromic acid is a strong chemical used to preserve tissues.
⇒ It is often mixed with other chemicals like in Zenker's Solution.
⇒ It helps keep most parts of the tissue but makes it harder to see the nucleus clearly.
⇒ It doesn't work well with formalin or alcohol because its too reactive.
⇒ It can desolve chromatin, which affects nuclear visibility.

oxidizing agent → Which accept electrons
Chromatin → In nucleus, where chromosomes present.

Bleaching

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⇒ Potassium Dichromate

⇒ Potassium Dichromate is used as a 2-3% aqueous solution.

⇒ It is weak oxidizing agent.

⇒ Dissolve chromatin

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⇒ The material in the nucleus that forms chromosomes.

⇒ It work well for fixing the Cytoplasm but poorly for nucleus.

↓

⇒ The part of the cell outside the nucleus.

⇒ Osmium Tetraoxide (Osmic Acid)

⇒ Osmium Tetraoxide is used as a 2% aqueous solution.

⇒ It is expensive & unstable quickly turning into irritating vapours.

⇒ It is a strong oxidizing agent but does not penetrate tissues well.

⇒ It preserves fat & creates a black precipitate (solid) of osmium dioxide with unsaturated Fats.

⇒ It also preserves fine cell details like Golgi Apparatus

↓

⇒ A part of cell involved in processing & Packaging Proteins.

⇒ B-5 Fixative

⇒ B-5 Fixative is used for fixing lymph nodes.

⇒ Zenker's Solution

⇒ Zenker's Solution is used for bone marrow trephine biopsy

↓

⇒ A procedure to take a sample of bone marrow.

⇒ For studying Negri bodies

↓

⇒ Structures found in certain viral infections.

FACTORS AFFECTING FIXATION

⇒ Size and Thickness: Larger & thicker tissues take longer to fix.

⇒ Mucus or Blood: Tissues with a lot of mucus or blood fix slowly.

⇒ Fatty tissues: Fatty tissues also fix slowly.

⇒ Agitation: Moving the tissue around speeds up fixation.

⇒ Temperature: keeping the temperature around 60°C speeds up fixation.

⇒ Time: Smaller samples fix in a few hours, while larger ones need 8-12 hours after being cut open.

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CHAPTER

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