

## **PRACTICAL -2**

# PREPARATION OF THE FIXATIVE





# HISTOLOGICAL FIXATIVES



# \* TYPES OF HISTOLOGICAL FIXATIVES:

## ❖ 10% Formal saline ----formaldehyde (40%) - 1Ltr

□ 8.5 gm -----sodium chloride

□ 900 ml -----tap water

□ Formaldehyde-----100ml

- To avoid the formation of formic acid, marble chips (calcium carbonate) should be added to
- neutralize the solution.

## ❖ **10% FORMAL SALINE**

- Wear appropriate protective clothing eg. laboratory coat, gloves and goggles.
- Weigh out 900g of sodium chloride and dissolve in warm water. Unscrew cap on porthole of formalin mixing tank (beneath cut-up bench).
- Place a funnel in the front porthole of the formalin mixing tank and pour sodium chloride solution into the main tank.
- Replace screw cap on porthole. Using the sealed pump attached to the formalin mixing tank , add 10 liters of formaldehyde to the main mixing tank.
- Turn on the mains water tap (adjacent to the tank) and fill the formalin mixing tank to the 100 liter mark, using the line on the tank wall.
- When mixed , using the sealed pump attached to the formalin mixing tank, pump 50 liters into the top tank where the solution is ready for use. 10% formal-saline solution can be dispensed from the tap with the red knob on the cut-up bench.



❖ **10% Formal Calcium..1ltr:**

- ❑ 100 ml-----formaldehyde (40%)
- ❑ 20 gm-----calcium acetate or calcium Chloride
- ❑ 900 ml-----water
- ❑ This fixative is widely used. The addition of calcium chloride preserves phospholipids whereas the addition of calcium acetate has the advantages of buffering the solution.

❑ **Bouin's fluid - fixation time 6 hours.**

- ❑ Saturated aqueous solution of picric acid - 75ml
- ❑ Glacial acetic acid - 5ml
- Thin blocks are fixed for 6-24 hours and then transferred to 70% alcohol. The yellow color of picric acid is an advantages with very small biopsies; it should be removed from section before staining by alcohol.



❖ **Carnoy's fluid - fixation time 1-3 hours.**

- Ethanol - 60ml
- Chloroform - 30ml
- Glacial acetic acid - 10ml
- Fixed tissue should be processed immediately or transferred to 80% alcohol.
- This is a rapid fixative. Generally tissues contain some fat which hinders and slow down
- the penetration of fluids. The chloroform dissolves the fat and allows the penetration of the fixatives.

❖ **Zenker's fluid - fixation time 4-24 hours.**

- Distilled water - 950ml
- Potassium dichromate - 25g
- Mercuric chloride - 50g
- Glacial acetic acid - 50g
- Fixed tissue should be washed overnight in running tap water before processing.





# Fixation:

- Fixation is:

- ◆ A chemical process by which biological tissues are preserved from decay, thereby preventing autolysis or putrefaction. Fixation terminates any ongoing biochemical reactions, and may also increase the mechanical strength or stability of the treated tissues.
- To achieve good histology it is important to fix fresh material correctly.
- Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles (e.g., nucleus, endoplasmic reticulum, mitochondria).



## ➡ How to fix..!?

- The most common fixative for light microscopy is **10% neutral buffered formalin**. Tissues should be fixed in 10 % neutral buffered formalin (**NBF**) and placed in a plastic container of suitable size with a wide neck and a tight fitting lid.
- It is important to use the correct amount of fixative for the size of the sample. Ideally a block of tissue approximately 2x1x1cm should be placed in a pot containing approximately 50-60 ml of fixative.
- Larger samples can be fixed provided they are placed in a container in which the volume of fixative is approximately 10 times the volume of the tissues.
- **NOTE...** This 100ml container is ideal for most samples. Containers with narrow necks .Should be avoided because it is difficult to remove samples which may have been squeezed in when still fresh.



# aims of fixation:

1. Is the preservation of shape, relationship and chemical constituents of the tissue.
2. Tissues which have been fixed should not change shape or volume during the subsequent procedures.
3. A condition which subsequently allows clear staining of sections.
4. Tissues should be as close to their living state as possible without loss or rearrangement.

Fixative usually acts to disable intrinsic biomolecules—particularly proteolytic enzymes which otherwise digests or damages the sample.

A fixative typically protects a sample from extrinsic damage.

Fixatives often alter the cells or tissues on a molecular level to increase their mechanical strength or stability.





# important factors involved in fixation are:

**Hydrogen ion concentration (ph)** – satisfactory fixation occurs between ph 6-8.

**Temperature**– fixation of surgical specimens is arrived out at room temperature.

**Concentration** – determined by factors of custom, cost effectiveness and solubility.

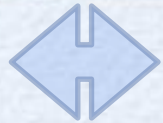
**Penetration** – of fixatives into tissue is clearly an important phenomenon.

**Duration**- allow primarily fixation in buffered formalin to proceed for 2-6 hours during the day of specimen is obtained.

**Osmolality**- the effects of altering the osmolality of fixatives has been investigated.



# Fixation artifacts:



Example of fixatives can generate artifacts:

- **Formalin pigments** is a well known artifacts produced under acid conditions.  
To overcome this problem formaldehyde may be generated fresh paraformaldehyde from powder.
- **Picric acid fixative:** It preserves glycogen well but causes considerable shrinkage of tissues.
- **Mercuric chloride** penetrates poorly and produces shrinkage of tissues.  
Mercuric containing fixatives have been recommended for fetal brain. Tissues fixed with mixture containing mercuric chloride contain black precipitates of mercury.
- **Hypertonic solutions** give rise to cell shrinkage.
- **Isotonic fixatives produces** swollen cells and poor fixation.
- **Prolonged fixation** in formaldehyde is known to cause shrinkage and hardening of tissues.



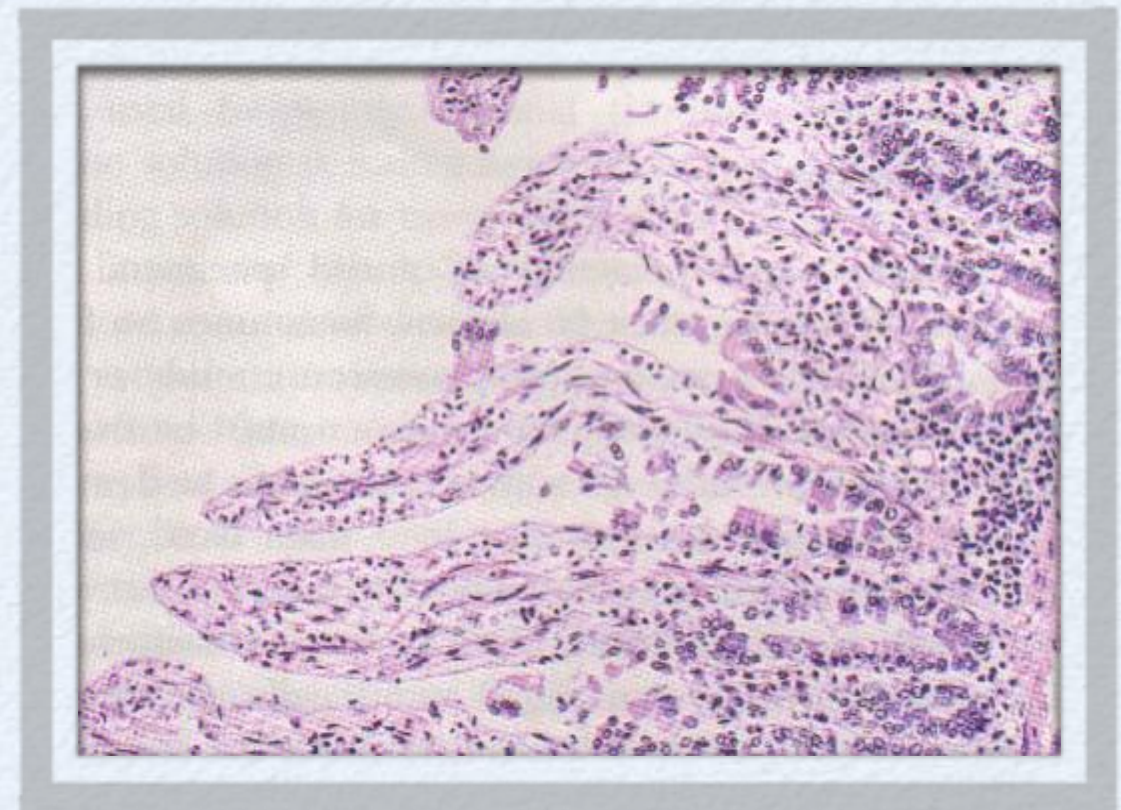
# ★ you should know:

- **Formal saline** is a fixative frequently recommended for histological. An advantage of this fixation is more rapid and glycogen is better preserved.
- **Alcoholic fixative:** alcohols penetrate rapidly in combination of other fixatives and so maybe used to increase the speed of tissue rapidly.
- **Methanol** is also used for the presentation of smears.
- **Carnoy's fixatives:** is recommended if nucleic acid. It is rapid in action and maybe used for urgent biopsy specimens for paraffin processing for 5 hours.





Small intestine well preserved



Autolyzed Small intestine  
 Carson Book, Page 5, Image 1-2  
 Notice how is missing the epithelium



# ★ Frozen section:

Is a rapid way to fix and mount histology sections. It is used in surgical removal of [tumors](#), and allow rapid determination of margin (that the tumor has been completely removed).

It is done using a refrigeration device called a [cryostat](#).

The frozen tissue is sliced using a [microtome](#), and the frozen slice are mounted on a glass slide and stained the same way as other methods.







1- [http:// www.youtube.com/ watch?feature=player\\_detailpage&v=KnMdSgd5mts](http://www.youtube.com/watch?feature=player_detailpage&v=KnMdSgd5mts)

2- [http:// www.youtube.com/ watch?feature=player\\_detailpage&v=1z5j12q2PAw](http://www.youtube.com/watch?feature=player_detailpage&v=1z5j12q2PAw)

3- [http:// www.youtube.com/ watch?feature=player\\_detailpage&v=QEPMAzBfEg](http://www.youtube.com/watch?feature=player_detailpage&v=QEPMAzBfEg)

4- [http:// www.youtube.com/ watch?feature=player\\_detailpage&v=Xv3MdHjpmkc](http://www.youtube.com/watch?feature=player_detailpage&v=Xv3MdHjpmkc)