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Anatomy

To Study the Effects of Various Fixatives on Liver – A Histological Study

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Abstract: The fixation also in optical differentiation of cells and tissue constituents by altering their refractive indices in varying degrees. This is of value in the microscopic examination of cells and tissue. The present study aimed to find the best fixative for a particular organ, so that the best histological section details can be produced. We studied the effect of five different types of fixatives. An essential part of all histological and cytological techniques is preservation of cells and tissues as they naturally occur. To accomplish this, tissue blocks, sections or smears are usually immersed in a fixative fluid, although in the case of smears, merely drying the preparation acts as a form of preservation. The aim of the current study is to see the effect of the following fixatives namely 10% formalin, Buffered 10% formalin, Bouin's fluid, Zenker's fluid, Carnoy's fluid on liver tissues and to observe the optimum result in a particular fixative in H&E sections. There is no single fixative which can be considered as best fixative for all purposes. Best fixatives for architectural preservation are Carnoy's fluid and Zenker's fluid. Best fixative for study of nuclear details is Bouin's fluid.
Keywords: Various fixatives, Liver, histological study.

INTRODUCTION

Fixation aims at the maintenance of cells and tissues in a life like state as much as possible. The microscopic examination of cells and tissues require treatment of the tissue must be capable of the withstanding further steps in the laboratory without any change.

Since the initial use of fixative by Hippocrates in 400bc [2, 3] many new substances and techniques for cell and tissue fixation have been introduced [1]. There are number of fixatives available and many combinations are advocated for a particular purpose or a particular organ. This chaos was put into order and now fixative are classified into coagulant and non-coagulants [4]. The purpose of fixation of tissue is [5]

- To prevent of fixation of tissue of cells.
- To arrest bacterial decomposition and putrefaction.
- To coagulate the tissue components.
- To modify the tissues so that it can withstand the deleterious effects of the various stages in the preparation of sections.
- To leave the tissue in a condition this facilitates differential staining with dyes and other reagents.

This is of value in the microscopic examination of cells and tissue [6]. The present study aimed to find the best fixative for a particular organ, so that the best histological section details can be produced. We studied the effect of five different types of fixatives. An essential part of all histological and cytological techniques is preservation of cells and tissues as they naturally occur. To accomplish this, tissue blocks, sections or smears are usually immersed in a fixative fluid, although in the case of smears, merely drying the preparation acts as a form of preservation. The fixatives employed prevent autolysis by inactivating lysosomal enzymes, and they stabilize the fine structure, both inside and between cells, by making macromolecules resistant to dissolution by water and other liquids. Fixatives also inhibit the growth of bacteria and mold that give rise to putrefactive changes. The most commonly used fixative for histopathology is a 4% aqueous solution of formaldehyde, often called 10% formalin because it is made by tenfold dilution of formalin. For about 50 years this fixative has also included inorganic salts to maintain a near neutral pH and an osmotic pressure similar to that of mammalian extracellular fluid.

History of fixation started as early as 400 BC, when Hippocrates discussed the biological effects of mercury and alcohol as a fixative [2, 3] since then a number of fixation substances were introduced. Much attention was focused on developing fixation that would

preserve cell and tissue constituents in as close to life like state as possible while allowing them to undergo further preparative procedures without change [7]. However the systematic study of the fixation began only in the latter half of the nineteenth century [8]. Ferdinard Blum has been credited as the first person to use formaldehyde as a tissue fixation [9]. ''formalin'' is the solution of formaldehyde gas (approx.40%) in water. Formaldehyde is commonly used as a 4 per cent solution that comes out to be 10 per cent formalin, for tissue fixation [10]. 10% formalin is the most widely used fixative in histology either by if self or in various mixtures. In fact to date buffered formalin is the most widely used universal fixative because it preserves a wide range of tissues and tissue components [8]. The aim of the current study is to see the effect of the following fixatives namely 10% formalin, Buffered 10% formalin, Bouin's fluid, Zenker's fluid, Carnoy's fluid on liver tissues and to observe the optimum result in a particular fixative in H&E sections.

MATERIALS AND METHODS

The present study was conducted in department of Anatomy, Maulana Azad Medical College and associated Hospital, New Delhi and Government Medical College Budaun. A comparative study of various fixatives was undertaken. The five different fixatives namely 10% formaline, Bouin's fluid, Carnoy's fluid and Zenker's fluid were used. The liver tissues pieces were taken for study.

Tissue acquiring

The postmortem tissues were collected within 6 hours of death of person from routine autopsies done in the mortuary, department of forensic Medicine Maulana Azad medical college, New Delhi. The care was taken not to include organ in which any pathological changes was expected. The liver tissue mentioned above was obtained three times from different autopsies. Tissues were equally divided in to five parts to be fixed in five fixatives.

Fixation

The tissues acquired were kept in fixation for at least 24 hours to get adequate fixation for each type of fixative.

Formulae for fixatives used

Formalin

40% formaldehyde	100ml
Tap water	900ml

Buffered 10% formalin

40% formaldehyde	100ml
Distilled water	900ml
Sodium dihydrogen phosphate monohydrate	4gm
Disodium hydrogen phosphate anhydrous	6.5gm

Carnoy's fluid

Absolute ethanol	60ml
Chloroform	30ml
Glacial acetic acid	10ml

Bouin's fluid

Saturated aqueous picric acid solution	75ml
40% formaldehyde	25ml
Glacial acetic acid	5ml

Zenker's fluid

Distilled water	950 ml
Potassium dichromate	25gm
Mercuric chloride	50gm
Glacial acetic acid	50gm

Tissue processing

Table-1: Showing schedule of tissue processing

Chemical	Time(hrs)
Acetone50%	3
Acetone70%	Overnight
Acetone90%	1
Acetone90%	1
Acetone100%	1
Acetone100%	1
Xylene	1
Xylene	1
Paraffin wax	Over night
Paraffin wax	1

Tissue obtained and fixed were processed manually using the following schedule

The paraffin blocks were made after cutting, the section was stained with Hematoxylin and Eosin stain. The ten section cut from each block.

Staining

The standard Haematoxylin and Eosin stain for paraffin section were dewaxed and hydrated through graded alcohols to water. The fixation pigments were removed, if necessary. Stained with Hematoxylin for 20 min and differentiated in 1 % acid alcohol (1% HCL in 70% alcohol) for 5-10 sec. Washed well in tap water until section were blue(25 min). Stained in 1% eosin for 2 min and dehydrated in acetone. Cleared in Xylene and mounted in DPX mountant.

Microscopic examination

Since 10 sections were cut from three sets of a particular tissue, a total of 30 slides were studied for each tissue fixed in particular fixatives. Five fields were studied from each section, thus a total of 150 field of each tissue were studied in a particular fixatives. The following parameters were noted in each field.

Tissue shrinkage

Due to differential shrinkage of various tissue constituents there is formation of pericellular reaction space. Thus the measure of tissue shrinkage is retraction

space, which is seen in brain tissue fixation. Retraction space examination is described below.

Retraction Space
Space around the cell seen only in brain tissue fixation
Absent: Not present
Mild: Mild reaction space
Severe: Severe reaction space
Disruption of cell membrane
No disruption: Not present
Mild Disruption: less than one third of Cytoplasmic border is disrupted
Severe: more than two third of Cytoplasmic border is disrupted

Preservation of architecture
Preserved: Architecture not preserved:
Preserved: Architecture preserved to a significant extent
Well preserved: Architecture is totally preserved

Character of staining
Cytoplasm
Light: Light cytoplasm
Dark: Dark cytoplasm
Nucleus
Light: lightly stained nucleus
Dark: Darker nucleus but chromatin detail not visible
Dark with distinct Chromatin

Vacuolization
Absent: Not present
Present:
Marked: vacuolization

Fixation artifacts
Fixation artifacts include retraction space and formalin pigment.
Absent: Not present
Present: Present

Fixation profoundly affects histological and immunohistochemical staining,technicians, pathologists and research workers must therefore decide on the most appropriate method. Aspects to consider are temperature, size of the storage container, volume ratio, salt concentration, pH and incubation time.

OBSERVATIONS AND RESULTS

The present study was conducted in the department of Anatomy Maulana Azad Medical College and associated hospitals on liver were acquired from routine autopsies under going in the department of forensic Medicine, Maulana Azad Medical College. Each of the three sets of tissue were preserved in five different fixatives ie. 10% Formalin, Buffered 10% formalin, Bouin's, Carnoy's fixative and Zenker's Fixative. Ten sections were cut from each set of five different tissues in a particular fixative and five fields were studied from each section. Therefore total of 150 fields were studied of each tissue. The effects of various fixatives on liver are tabulated in table 2.

Disruption of cell membrane: Disruption of cell membrane was moderate in significant number of fields with formalin (90), Buffered formalin (90) and Bouin's fluid (100). It was predominantly mild with Carnoy's fluid (95) and Zenker's fluid (105). This is shown in figure 1.

Preservation of architecture: The architecture was predominantly ill preserved with Formalin (110) and buffered formalin (105), as compared to predominantly well preserved with Carnoy's fluid (100) and Zenker's fluid (150). It was appreciably preserved with Bouin's fluid (1500) Figure 2.

Staining

The cytoplasm was darkly stained with Bouin's fluid Carnoy's and Zenker's fluid.

Nucleus: Best nuclear staining with Bouin's fluid (50). It was dark in appreciable numbers of field with Bouin's fluid (100), Carnoy's fluid (140) Figure 3.

Vacuolization was seen in more than half the fields with formalin (150), Buffered formalin (150), Bouin's fluid (140) and Carnoy's fluid (150). It was absent in many fields of Zenker's fluid (60) Figure 4.

Fixation artefact

No obvious fixation artefact was found on section study with any fixative.

Table-2: Showing effects of various fixatives on liver tissues

Parameters	10% Formalin	Buffered formalin	Bouin's fluid	Carnoy's fluid	Zenker's fluid
Retraction space	Nil	Nil	Nil	Nil	Nil
Absent	Nil	Nil	Nil	Nil	Nil
Mild	Nil	Nil	Nil	Nil	Nil
Moderate	Nil	Nil	Nil	Nil	Nil
Severe	Nil	Nil	Nil	Nil	Nil
Disruption of cell membrane					
No Disruption	0	0	0	5	45
Mild Disruption	55	60	50	95	105
Moderate	90	90	100	50	0
Severe	5	0	0	0	0
Preservation of architecture					
Preserved	110	105	0	0	0
Preserved	40	45	150	50	0
Well preserved	0	0	0	100	150
Character of Staining					
Cytoplasm					
Light	100	90	50	10	0
Dark	50	60	100	140	150
Nucleus					
Light	100	90	0	0	0
Dark	50	60	100	140	150
Dark with Distinct Chromatin					
Vacuolization					
Absent	0	0	0	0	60
Present	150	150	140	150	90
Marked	0	0	0	0	0
Fixation artifact					
Absent	0	0	0	0	0
Present	0	0	0	0	0

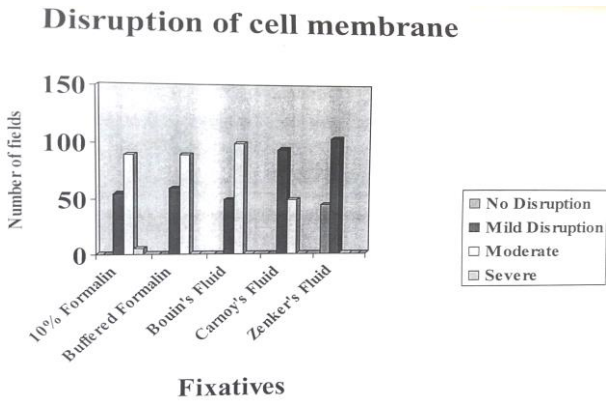

Fig-1: Bar diagram showing disruption of cell membrane with various fixatives

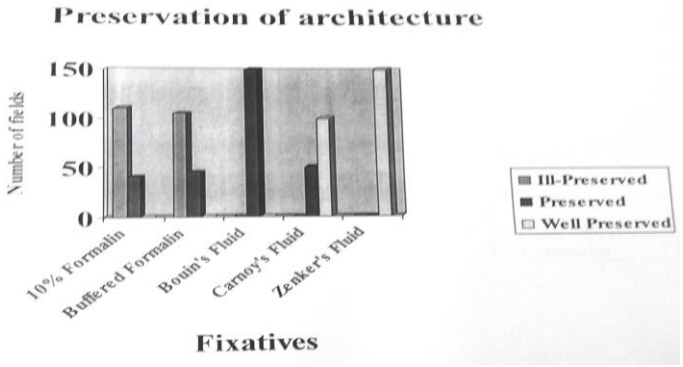

Fig-2: Bar diagram showing preservation of architecture with various fixatives

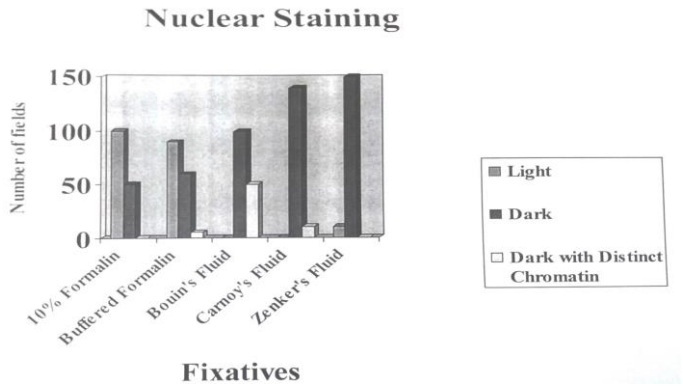

Fig-3: Bar diagram showing nuclear staining with various fixatives

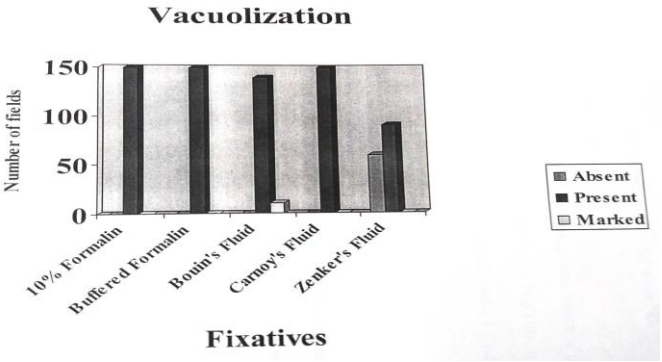

Fig-4: Bar diagram showing vacuolization with various fixatives

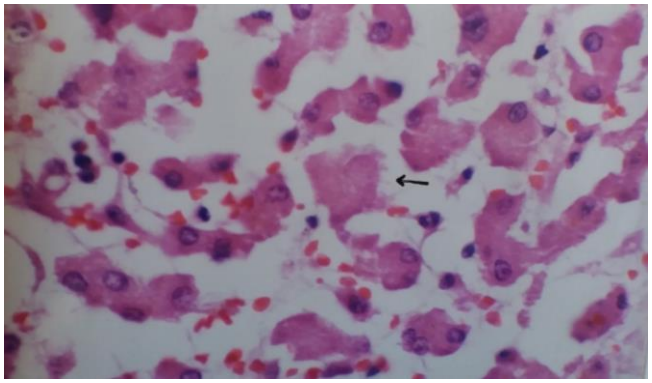

Fig-5: Formalin fixed liver tissue showing the severe disruption cell membrane (with arrow, 40X, H&E)

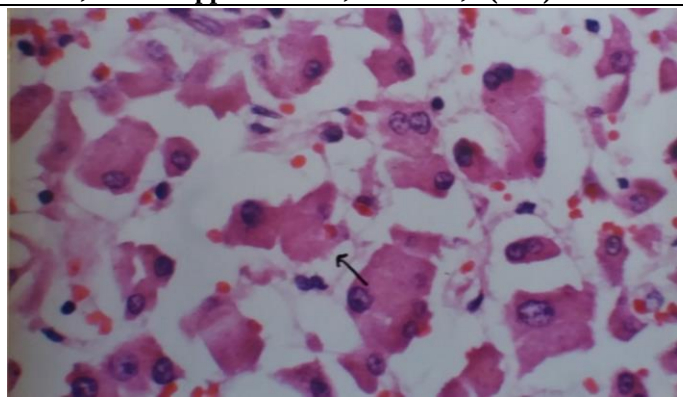

Fig-6: Liver fixed in buffered formalin showing the moderate disruption cell membrane (with arrow, 40X, H&E)

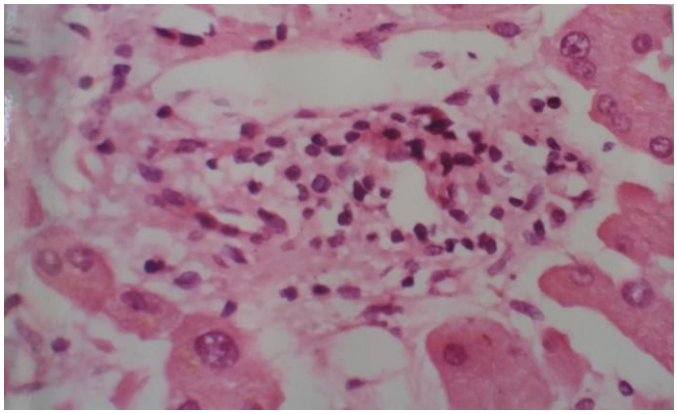

Fig-7: Liver fixed in Carnoy's fluid showing preserved cells and architecture (40X, H&E)

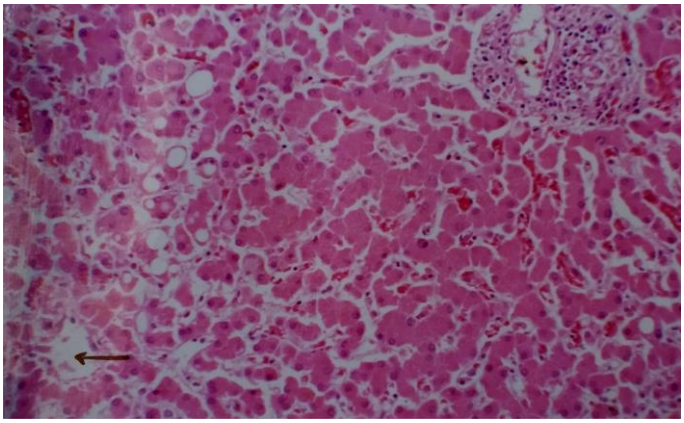

Fig-8: Liver fixed in Zenker's fluid showing well preserved architecture (with arrow 10X, H&E)

DISCUSSIONS

Disruption of cell membrane

Disruption of cell membrane was minimal with carnoy's fluid and Zenker's fluid. It was much more with formalin fixation whether buffered or not buffered.

Preservation of architecture

Architecture was best preserved with Carnoy's fluid and Zenker's fluid. It was not satisfactory with formalin fixative.

Staining

Cytoplasmic

The cytoplasm was darkly stained with Bouin's fluid Carnoy's fluid and Zenker's fluid.

Nucleus

Best nuclear stain with distinctly visible chromatin pattern was seen in significant number of fields with Bouin's fluid.

Vacuolization

Vacuolization was seen in more than half the fields with formalin, buffered formalin Bouin's fluid and Carnoy's fluid. It was absent in Zenker's fluid.

CONCLUSIONS

- There is no single fixative which can be considered as best fixative for all purposes.
- Best fixatives for architectural preservation of liver are Carnoy's fluid and Zenker's fluid.

- Best fixative for study of nuclear details of liver is Bouin's fluid.

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