



TO STUDY THE EFFECTS OF VARIOUS FIXATIVES ON BRAIN TISSUES – A HISTOLOGICAL STUDY

Anatomy

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ABSTRACT

The fixation also in optical differentiation of cells and tissue constituents by altering their refractive indices in varying degrees. Present study aimed to find 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. The aim of 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 fixative for study of nuclear details is Bouin's fluid.

KEYWORDS

various fixatives, brain tissues, histological study.

INTRODUCTION

The aims of fixation 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]. 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 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. Ferdinand 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 itself 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].

MATERIAL AND METHOD

The present study was conducted in department of Anatomy, Maulana Azad Medical College and associated Hospital, New Delhi and Government Medical College Budaun on brain tissues. 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.

Tissue acquiring

The postmortem brain 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.

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

Tissues obtained, fixed were processed manually and 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

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	

The 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.

OBSERVATION AND RESULTS

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.

Staining

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

Nucleus: Best nuclear staining with distinctly visible chromatin pattern was seen in significant number of fields with Bouin's fluid (60). It was dark in appreciable number of fields with Bouin's fluid (90), Carnoy's fluid (85), and Zenker's fluid (90).

Vacuolization

Vacuolization was seen in more than half the fields with formalin (145), Buffered formalin (140), Bouin's fluid (140), and Carnoy's fluid (145). It was absent in many fields of Zenker's fluid (80).

Fixation artifacts

Fixation artifacts in the form of retraction spaces were found in most section with every fixative.

Table 1: Showing effects of various fixatives on brain tissues.

Parameter	10% Formalin	Buffered formalin	Bouin's fluid	Carnoy's fluid	Zenker's fluid
Retraction space					
Absent	0	0	0	50	80
Mild	0	5	100	90	70
Moderate	110	125	35	10	0
Severe	40	20	15	0	0
Disruption of cell membrane					
No Disruption	0	0	0	10	70
Mild Disruption	65	80	90	90	75
Moderate	75	70	110	50	5
Severe	10	0	0	0	0

Preservation of architecture					
Preserved	100	100	10	0	0
Preserved	50	45	130	55	20
Well preserved	0	5	10	95	130
Character of Staining					
Cytoplasm					
Light	90	95	55	15	0
Dark	60	55	95	135	150
Nucleus					
Light	90	95	55	15	0
Dark	55	40	90	85	90
Dark with Distinct Chromatin	5	10	60	15	0
Vacuolization					
Absent	5	10	10	5	80
Present	140	140	135	145	70
Marked	5	0	5	0	0
Fixation artefact					
Absent	0	0	0	0	0
Present	150	150	150	100	70

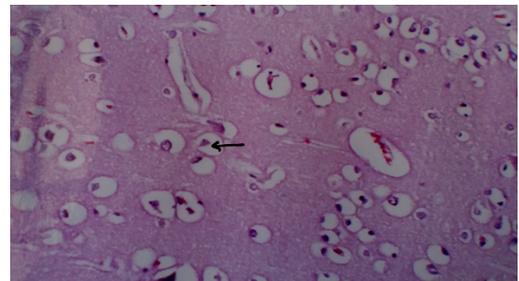


Figure 1: Formalin fixed brain tissue showing marked retraction spaces (with arrow, 40X, H&E).

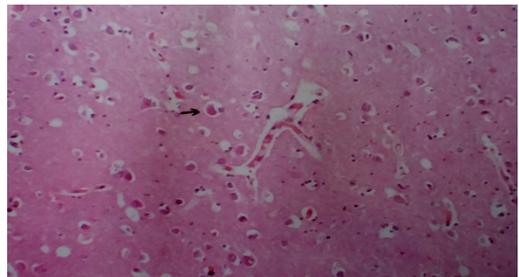


Figure 2: Brain tissue fixed in Bouin's fluid showing moderate retraction spaces (with arrow, 40X, H&E).



Figure 3: Brain tissue fixed in Zenker's fluid showing no retraction spaces (10X, H&E).

DISCUSSION

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. The cytoplasm was darkly stained with Bouin's fluid Carnoy's fluid and Zenker's fluid. Best nuclear stain with distinctly visible chromatin pattern was seen in significant number of fields with Bouin's fluid. 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.

CONCLUSION

There is no single fixative which can be considered as best fixative for all purposes.

The best fixative for study of nuclear details is Bouin's fluid.

Best fixatives for brain tissues are Carnoy's fluid and Zenker's fluid.

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