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HISTOLOGICAL EFFECTS OF VARIOUS FIXATIVES ON SMALL INTESTINE

Anatomy

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ABSTRACT

The fixation is a critical step in the preparation of histological sections by which biological tissues are preserved from decay, thereby preventing autolysis or putrefaction. 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. The five different fixatives namely 10% formaline, Bouin's fluid, Carnoy's fluid and Zenker's fluid were used. The small intestine pieces were taken for study. We studied the effect of five different types of fixatives on small intestine namely 10% formalin, Buffered 10% formalin, Bouin's fluid, Zenker's fluid, Carnoy's fluid and to observe the optimum result in a particular fixative in H&E sections. The best fixatives for architectural preservation are Carnoy's fluid and Zenker's fluid. Best Fixatives for small intestine are Carnoy's fluid and Zenker's fluid.

KEYWORDS

various fixatives, small Intestine, histological study.

INTRODUCTION

Fixation terminates any ongoing biochemical reactions, and may also increase the mechanical strength or stability of the treated tissues. The broad objective of tissue fixation is to preserve cells and tissue components. Many techniques have been developed which are designed to preserve the structural integrity of a specimen so that it can be viewed microscopically. The process through which cell structure is preserved is called fixation. Since cells rapidly deteriorate after a tissue has been removed from the body, achieving adequate fixation is often the most difficult task confronting a histologist. "Artifacts" are changes to the original structure of cells and tissues that arise from tissue deterioration and from the fixation process itself. Thus, a skilled histologist employs techniques that minimize the formation of artifacts in different types of tissues, and has is the ability to distinguish artifacts from normal cell structures.

The cells and tissues require treatment of the tissue for microscopic examination must be capable of the withstanding further steps in the laboratory without any change. 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 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.

MATERIALAND METHOD

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 small intestine 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 small intestine 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

Tissues obtained and fixed were processed manually.

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.

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

OBSERVATION AND RESULTS

Disruption of cell membrane
Disruption of cell membrane was moderate in significant number of fields with formalin (60) , Buffered formalin (70) and Bouin's fluid (50). It was predominantly mild with Carnoy's fluid (85) and Zenker's fluid (50).

Preservation of architecture
The architecture was predominantly ill preserved with Formalin (65) and buffered formalin (60), as compared to predominantly well preserved with Carnoy's fluid (90) and Zenker's fluid (130). It was appreciably preserved with Bouin's fluid (105).

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

Nucleus: Best nuclear staining with distinctly visible chromatin pattern was seen in significant number of fields with Bouin's fluid (90). It was dark in appreciable number of field with Bouin's fluid (110), Carnoy's fluid (80) and Zenker's fluid (95).

Vacuolization:
Vacuolization was seen in more than half the fields with formalin (145), Buffered formalin (140), Bouin's fluid (140) and Carnoy's fluid (135). It was absent in many fields of Zenker's fluid (100).

Table 1: Showing various fixatives effects of on small Intestine.

Parameter	10% Formalin	Buffered formalin	Bouin's fluid	Carnoy's fluid	Zenker's fluid
Retraction space					
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	15	90
Mild Disruption	65	70	90	85	50
Moderate	60	70	50	45	10
Severe	25	10	10	5	0
Preservation of architecture					
Ill Preserved	65	60	15	0	0
Preserved	75	60	105	110	20
Well preserved	10	30	30	90	130
Character of staining					
Cytoplasm					
Light	95	85	50	15	5
Dark	55	65	100	135	145
Nucleus					
Light	75	70	50	50	40
Dark	65	65	10	80	95
Dark with Distinct Chromatin	10	15	90	20	15
Vacuolization					
Absent	15	10	10	1	100
Present	135	135	140	125	50
Marked	10	5	0	10	0
Fixation artefact					
Absent	0	0	0	0	0
Present	0	0	0	0	0

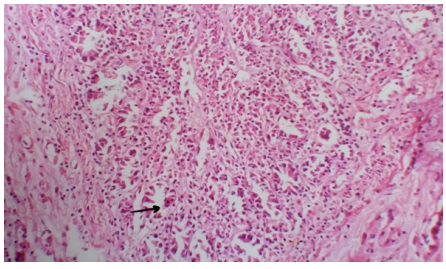

Figure 1: Small intestine fixed in formalin showing ill preserved architecture and glands (with arrow, 10X, H&E).

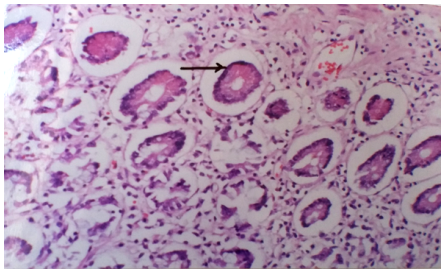

Figure 2: Small intestine fixed in Bouin's fluid showing ill preserved architecture and glands (with arrow, 40X, H&E).

DISCUSSION

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.

CONCLUSION
The best fixatives for architectural preservation are Carnoy's fluid and Zenker's fluid. Best Fixatives for small intestine are Carnoy's fluid and Zenker's fluid. The proper fixation of tissue is essential to ensure the highest level of specimen evaluation. There is no single fixative which can be considered as best fixative for all purposes.

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