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Anatomy

HISTOLOGICAL STUDY- THE EFFECTS OF VARIOUS FIXATIVES ON KIDNEY

Dr Ved Prakash — Associate Professor, Department of Anatomy, Govt. Medical College Budaun, U.P. India.

Dr. Muktyaz Hussein — Assistant Professor, Department of Anatomy, Govt. Medical College Budaun, U.P. India. *Corresponding Author

ABSTRACT Fixation is the critical step in processing tissues. Following tissue removal from the body, autolysis begins and proceeds more or less quickly depending on many factors, including the level of enzymes or microorganism present in the tissue. We studied 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 the current study is to see the effect of 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 : histological study, kidney, various fixatives.

INTRODUCTION

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]. The purpose of the various fixatives is stabilization of those enzymes and other tissue proteins and disabling microorganisms, thereby arresting autolysis, with the goal to preserve the tissue as close to the in vivo state as possible. Fixatives can be classified in different ways depending on their mechanism of action. 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]. An essential part of all histological and cytological techniques is preservation of cells and tissues as they naturally occur.

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 percent solution that comes out to be 10 percent 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.

MATERIAL 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 kidney 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.

Fixation

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

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.

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.

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

The effect of various fixatives on kidney are tabulated in table1

Disruption of cell membrane

Disruption of cell membrane was moderate in significant number of fields with formalin (70), Buffered formalin (80) and Bouin's fluid (40). It was predominantly mild with Carnoy's fluid (80) and Zenker's fluid (40).

Preservation of architecture

The architecture was predominantly ill preserved with formalin (75) and buffered formalin (70) as compared to predominantly well preserved with Carnoy's fluid (95) and Zenker's fluid (135). It was appreciably preserved with Bouin's fluid (110).

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 (80). It was dark in appreciable number of fields with Bouin's fluid (55), Carnoy's fluid (65), and Zenker's fluid (90).

Vacuolization was seen in more than half the fields with formalin (140), Buffered formalin (145), Bouin's fluid (145), and Carnoy's fluid (120). It was absent in many fields of Zenker'sfluid (110).

Fixation artifacts: No obvious Fixation artifact was found on section study with any fixative.

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

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	5	0	10	100
Mild Disruption	60	55	105	80	40
Moderate	70	80	40	60	10
Severe	20	10	5	0	0
Preservation of architecture					
Ill-Preserved	75	70	10	0	0
Preserved	55	40	110	55	15
Well preserved	20	40	30	95	135
Character of Staining					
Cytoplasm					
Light	95	30	40	5	0
Dark	85	70	110	145	150
Nucleus					
Light	80	75	15	55	50
Dark	60	55	55	65	90
Dark with Distinct Chromatin	10	20	80	30	10
Vacuolization					
Absent	10	5	5	30	110
Present	120	140	135	120	40
Marked	20	5	10	0	0
Fixation artefact					
Absent	0	0	0	0	0
Present	0	0	0	0	0

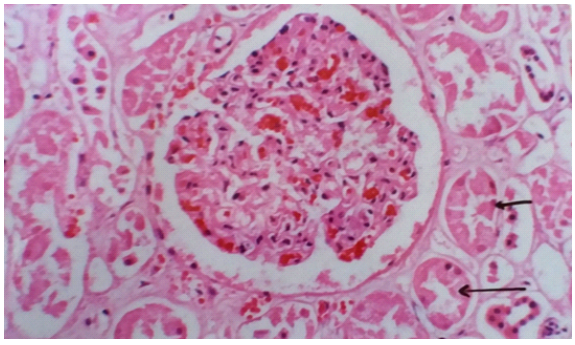

Figure 1: Kidney fixed in Zenker's fluid showing well preserved architecture and tubes (with arrow, 40X, H&E).

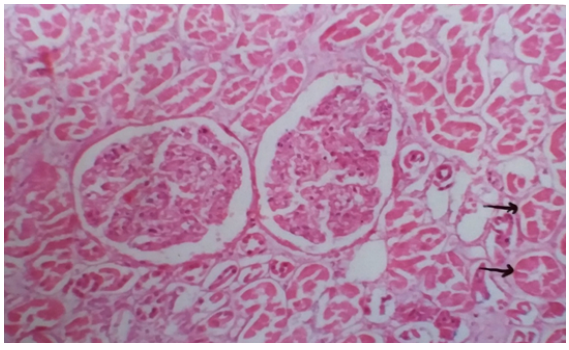

Figure 2: Kidney fixed in Carnoy's fluid showing well preserved architecture and tubes (with arrow, 40X, H&E).

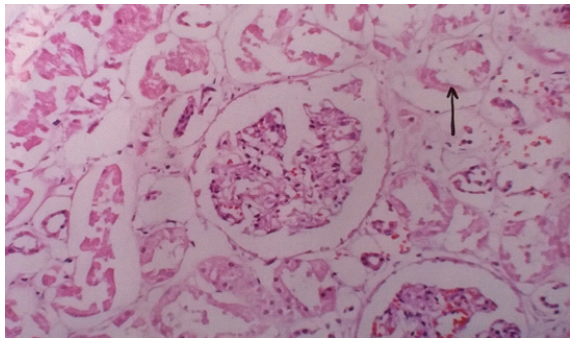

Figure 2: Kidney fixed in buffered formalin showing well preserved architecture and tubes (with arrow, 40X, H&E).

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.

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 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

Best fixatives for kidney are Carnoy's fluid and Zenker's fluid.

Best fixatives for architectural preservation are Carnoy's fluid and Zenker's fluid.

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

REFERENCES

1. Hopwood D Cell and tissue fixation. Histochem Journal 1985; 17:389-442.
2. Feeney Burns L. The early years of research. Prog Clin Biol, Res1985; 1903-23.
3. Fox CH, Johnson FB, Whiting J Roller PP. Formaldehyde fixation .J Histochem Cytochem. 1985; 33:845-853.
4. Baker JR. Principles of Biological Microtechnique.London:Methuen.1960.
5. Carleton. Fixation. In: Drury RAB, Wallington EA eds Histological Technique 5th edn. New York: Oxford University press. 1980; 41.
6. Carleton. Fixation. In: Drury RAB, Wallington EA eds Histological Technique 5th edn. New York: Oxford University press. 1980;43
7. Leong ASY, James Cl Thomas AC. Handbook of surgical pathology New York Churchill Livingstone. 1996pp8 viii 321.
8. Srinivasan M SedmakD, JewellS. Effect of fixatives and tissue processing on the content and integrity of nucleic acid. Am J pathol 2002; 161:1961-1971.
9. Puchtler H, Meloan SN. On the chemistry of formaldehyde fixative and its use on Immunohistochemical reaction. Histochemistry. 1985, 82; 201-204.
10. David Hopwood. Fixation and fixatives In: Bancroft JD, Steven A, Turner D Reds. Theory and practice of Histological Techniques. Fourth edn. Tokyo: Churchill Liingstone. 1996:35.
11. Pearse AGE eds Theory and practice and Applied .4th edn. Edinburgh: Churchill Livingstone.1980.
12. David Hopwood. Fixation and fixatives. In. Bancroft JD, Steven A, Turner DR eds. Theory and practice of Histological Techniques. Fourth edn. Tokyo: Churchill Living stone.1996; 29.
13. Baker JR. Principle of biological micro techniques. A study of fixation and dyeing. 1st edn. London: Methuen and co ltd.1958.
14. PenttilaA, Mcdowell EM, Trump BF.Effect of fixation and post fixation treatment on volume of injured cells. Journal of Histochem and Cytochem 1975; 22:251-270.
15. Sabatini LDBensch K, Barnett RJ.Cytochemistry and electron microscopy. The preservation of cellular ultra structure and enzyme activity by aldehyde Fixation. Journal of cellular Biology 1963; 17:19-58.
16. Trump BF, Ericsson JLE. The effect of the fixative solution on the ultra structure of cells and tissues. A comparative analysis with particular attention to the proximal convoluted tubule of the rat kidney. Laboratory investigation 1965; 14:1245-1325.
17. Joh son WD.Lang M, Johnson MT. Fixatives and method of fixation in selected tissues of the laboratory rat. Clin toxicol1978; 12:583-600.
18. Stickland NC.Adetailed analysis of the effects of various fixatives on animal tissue with particular reference to muscle tissue. Stain technol 1975; 50:255-264.
19. Hopwood D Slidders W, Yeaman GR. Tissue fixation with phenol formaldehyde for routine Histopathology. Histochem Journal 1989; 21:228-238.
20. David Hopwood. Fixation and fixatives. In. Bancroft JD, Steven A, Turner DR eds. Theory and practice of Histological Techniques. Fourth edn. Tokyo: Churchill Living stone.1996; 36.