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Comparative Effects of 5% Formol Saline And 10% Formalin as Fixatives on Bovine Tissues to Determine the Level of Tissue Shrinkage

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Abstract
Tissue shrinkage is one of the disadvantages of formalin-based fixatives. This study compares the use of 5% formol saline and 10% formalin as fixatives on bovine tissues to determine the level of tissue shrinkage. There was relative shrinkage in the bovine tissue sections fixed in 10% formalin, in the heart appeared as spaces between muscle fibre bundles, liver as wider sinusoidal spaces, spleen within red and white pulps, kidney as widened convoluted tubules, cerebellum as spaces between the granular and Purkinje cell layers. Similar tissues fixed in 5% formol saline did not show signs of shrinkage.
Therefore, it is suggested that 5% formol saline could be a potential fixative in processing tissues for light microscopy. The finding of this research indicates that, 5% formol saline could have reduced the effects produced by 10% formalin concentration, while the fixative property was retained. However further studies are required using tissues from other animal species like poultry, laboratory animals like rats and mice.

Keywords: Bovine, Formol Saline, Shrinkage, Tissue

Introduction
In histopathology most tissues are fixed before they are examined microscopically. It follows that fixation is the foundation for the subsequent stages in the preparation of the sections, through to the making of a diagnosis [3]. Fixation is a part of histological technique used in preparation of permanent sections. It prevents tissue digestion by enzymes present in cells (autolysis) or by bacteria and preserves the structure and molecular composition of organs after removal from the animal's body [5]. The basic aim of fixation is to preserve protoplasm with the minimal alteration from the living state. This is achieved through coagulation of protoplasm, thus rendering it insoluble, and hardens the tissue so that sectioning is facilitated [4, 6].
When tissues are removed from the body, they undergo autolysis by the action of intracellular enzymes whose normal behaviour is altered, causing the breakdown of protein and eventual liquefaction. Bacterial action, are retarded by cold, greatly accelerated by keeping at 37 ºC and almost inhibited by heating the tissue to 57 ºC [1]. Autolysis is more severe in tissues which are rich in enzymes, such as liver, brain and kidneys and is less rapid in tissues such as elastic fibre and collagen. On microscopical examination, the cell nuclei of autolysed tissue may show pyknosis, karyorrhexis, or lysis and eventual disappearance (karyolysis) [1].
Formaldehyde is widely used as 4% solution (10% formalin) which is one of the best fixatives for light microscopy [5] or as formal (formol) saline that is a general purpose fixative which provides rapid and even penetration of tissues, and permits use of most staining and impregnation techniques [7]. Formalin fixation is associated with shrinkage (Smith and Bruton, 1978) [7], although this is reduced in 10% formol saline. Hence, 10% formol saline is generally recommended for routine fixation of tissues [2].
This study compares the use of 10% formalin and 5% formol saline as fixatives on bovine tissues to determine the level of tissue shrinkage.

2. Materials and Methods
2.1. Preparation of Fixatives
Nine hundred millilitres (900ml) of distilled water was measured using a clean measuring cylinder and transferred into a larger cylinder. One hundred millilitres of 40% formaldehyde (G. Koepcke and Co., Germany) was then measured and added to the distilled water to make it up 1000ml i.e 10% formalin [2]. The one litre solution was transferred into a Winchester bottle and labelled as 10% formalin. Nine hundred and fifty millilitres (950ml) of distilled water was measured using a measuring cylinder and transferred into a larger cylinder. Fifty millilitres of 40% formaldehyde was measured and added to make up 1000ml. Nine grams (9g) of sodium chloride (BDH Chemicals, England) was added to this solution to make 5% formol saline [2]. The solution was transferred into a Winchester bottle and labelled, as 5% formol saline.

2.2. Tissue Samples Collection
Tissues were collected from healthy cattle slaughtered at the Zaria abattoir in clean polyethylene bags and conveyed to the laboratory immediately. These included heart, liver, kidney, spleen, brain and lungs.

2.3. Fixation and Processing

Each tissue was cut 3mm thick in pair and placed in plastic recipients containing 15ml each of 10% formalin and 5% formol saline respectively and kept for at least 48 hours prior to processing, paraffin embedded sections were cut at 5µm thick, stained with haematoxylene and eosin (H&E).

3. Results and Discussion

3.1. Tissues fixed in 10% Formalin

Shrinkage was observed in the heart as spaces between muscle fibre bundles (Figure 1), liver as wider sinusoidal spaces (Figure 3), Spleen as shrinkage within red and white pulps (Figure 5), kidney as widened convoluted tubules (Figure 7), cerebellum as spaces between the granular and purkinje cell layers (Figure 9) and lung showed thin respiratory bronchiolar, alveolar ductular and alveolar walls (Figure 11).

3.2. Tissues fixed in 5% Formol Saline

There were no signs of tissue shrinkage observed in the heart (Figure 2), liver (Figure 4), spleen (Figure 6), kidney (Figure 8), cerebellum (Figure 10) and lung (Figure 12).

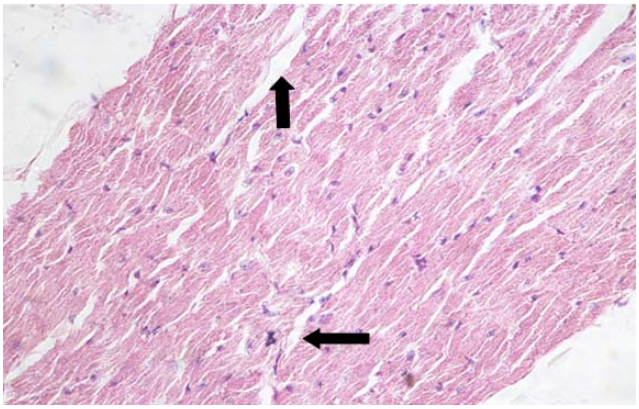

Fig 1: Photomicrograph of the bovine heart fixed in 10% formalin. Note shrinkage between myocardial fibres (arrows). H&E × 350

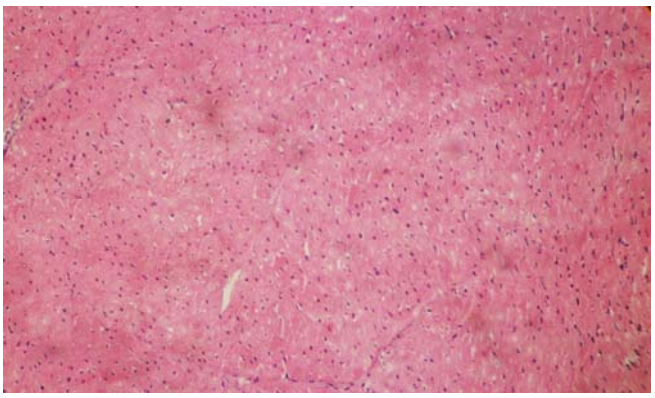

Fig 2: Photomicrograph of the bovine heart fixed in 5% formol saline.
Note absence of shrinkage between myocardial fibres. H&E × 350

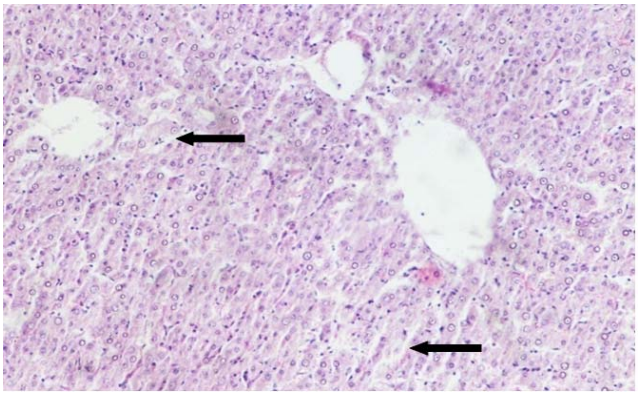

Fig 3: Photomicrograph of the bovine liver fixed in 10% formalin. Note relative widening of sinusoids (arrows). H&E × 350

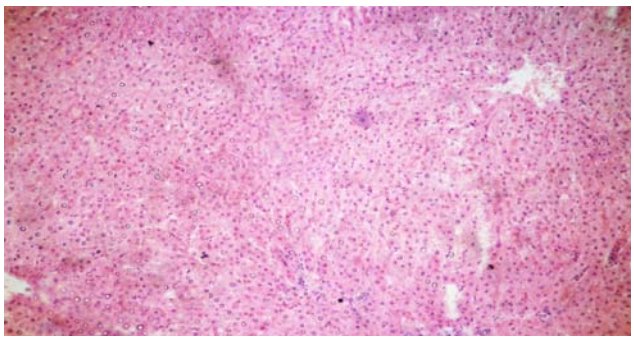

Fig 4: Photomicrograph of the bovine liver fixed in 5% formol saline.
The sinusoids are not widened. H&E × 350.

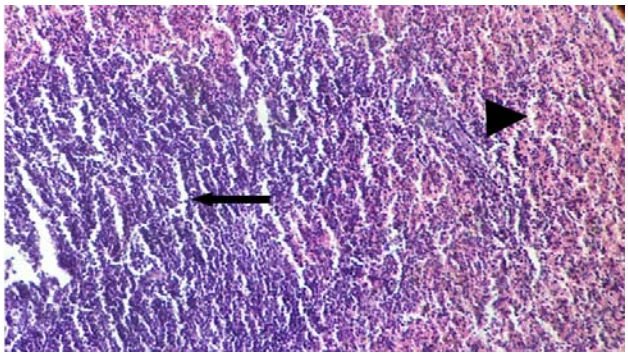

Fig 5: Photomicrograph of the bovine spleen fixed in 10% formalin. Note shrinkage in red (arrow head) and white pulps (arrow). H&E × 350

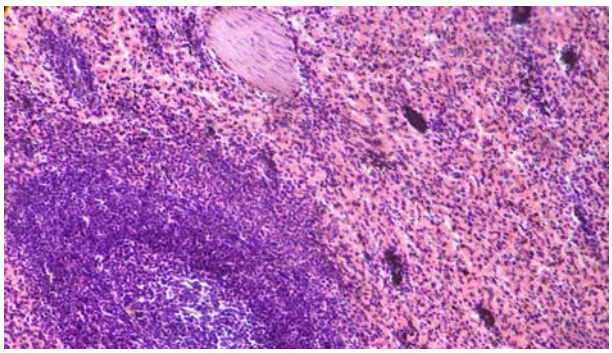

Fig 6: Photomicrograph of the bovine spleen fixed in 5% formol saline.
Note absence of shrinkage in red and white pulps. H&E × 350

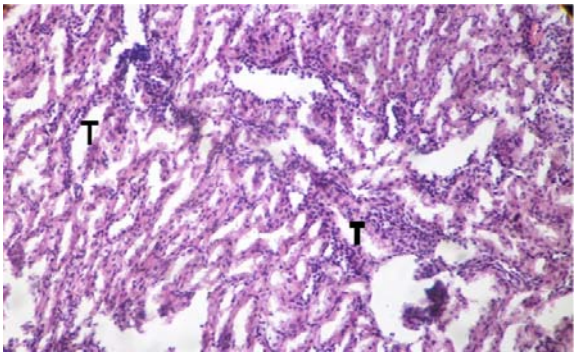

Fig 7: Photomicrograph of the bovine kidney fixed in 10% formalin. Note relative widening of convoluted tubules (T). H&E × 350

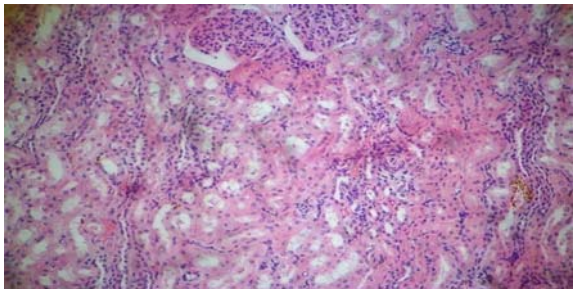

Fig 8: Photomicrograph of the bovine kidney fixed in 5% formol saline.
Note non widened convoluted tubules. H&E × 350

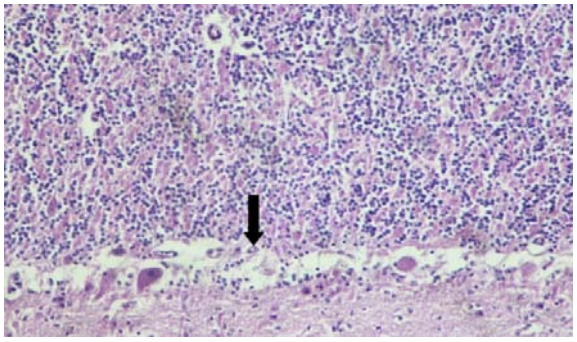

Fig 9: Photomicrograph of the bovine brain (cerebellum) fixed in 10% formalin.
Note shrinkage in the Purkinje cell layer (arrow). H&E × 350

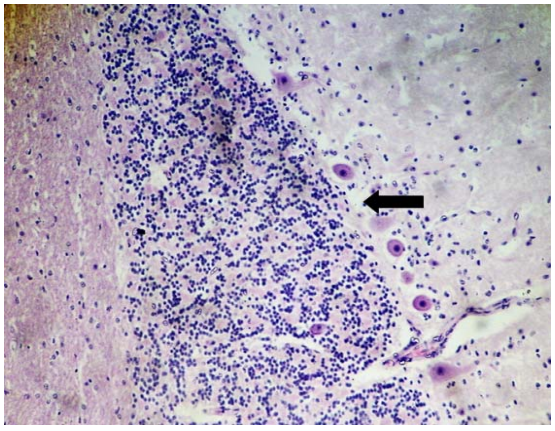

Fig 10: Photomicrograph of the bovine brain (cerebellum) fixed in 5% formol saline.
Note absence of shrinkage in the Purkinje cell layer (arrow). H&E × 350

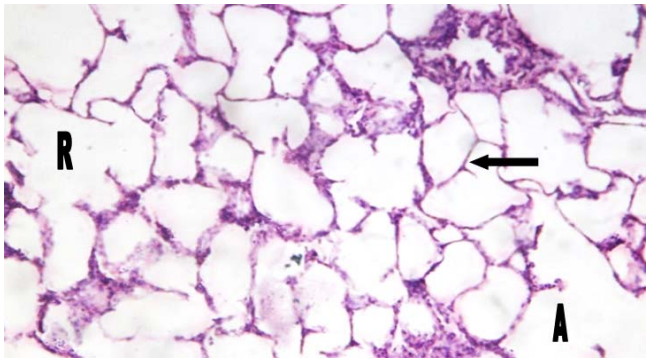

Fig 11: Photomicrograph of the bovine lung fixed in 10% formalin. Note thin walls of respiratory bronchiole (R), alveolar duct (A) and alveolus (arrow). H&E × 350

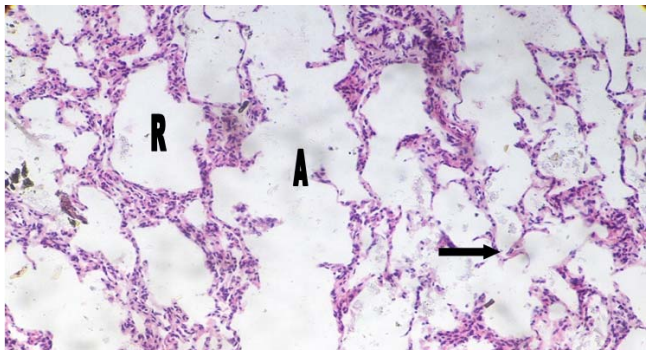

Fig 12: Photomicrograph of the bovine lung fixed in 5% formol saline.
Note thicker walls of respiratory bronchiole (R), alveolar duct (A) and alveolus (arrow). H&E × 350

Shrinkage appears as artefactual spaces in prepared tissue sections [8]. The reduced shrinkage observed in tissues fixed in 5% formol saline could be attributable to the sodium chloride concentration that is clinically similar to the osmolarity of blood which probably prevented excessive movement of fluid in or out of the tissues. Furthermore, formaldehyde coagulates protoplasm [8] and cross-links proteins [5]. Therefore, 5% formol saline could have reduced the effects produced by 10% formalin concentration, while the fixative property was retained.

Conclusion

Therefore, it is suggested that 5% formol saline could be a potential fixative in processing tissues for light microscopy. The finding of this research indicates that, 5% formol saline could have reduced the effects produced by 10% formalin concentration, while the fixative property was retained.

Acknowledgements

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References

1. Anthony SYL. Fixation and fixatives, Woods and Ellis. London, 2000.
2. Bush BM. Veterinary Laboratory Manual. William Heinemann Medical Books, London, 1975.
3. Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde fixation, in: The Theory and Practice of Histological Techniques, Bancroft, J.D. and Stevens, A.A, Eds., Churchill Livingstone, Edinburgh, 1990, 21-42.
4. Gurr E. Staining Practical and theoretical, Arrowsmith JW, Birtol, 1962, 471-485.
5. Janqueira LC, Carneiro J. Basic histology, 10th edition, Lange Medical Books McGraw-Hill, New York, 2003.
6. Leeson CR, Leeson TS. Histology, 3rd edition, Saunders WB, Philadelphia, 1976.
7. Smith A, Bruton J. A colour atlas of histological staining techniques, 1978.
8. Young B, Heath JW. Wheater's functional histology. 4th edition, Churchill Livingstone, Edinburgh, 2000.