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Research Article

An Anatomic Evaluation of Some Chemical Preservatives

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ABSTRACT

Objectives: Several attempts have been made at attenuating post-mortem putrescence. One of the earliest forms of embalming was mummification. Previous reports have emphasized superficial cosmesis with scant studies on microscopic changes. This treatise detailed the effects of some chemical reagents on gross and microanatomic preservation in Wistar rats.

Materials and Methods: This experimental study involved fourteen (14) Wistar rats assigned into control and test groups with 7 subgroups each. The selected animals were euthanized and subsequently infiltrated with chemical preservatives. The subgroups A and B were treated with water and 100% formalin respectively, while subgroups C-G were preserved with graded concentrations of formalin, alcohol, salt solution, phenol, and a combination of these chemicals. Subsequent on general observation at days 7 and 21, the brains and intestine of the rats were dissected, processed and analysed for histological changes.

Results: General observations revealed varying degrees of putrefaction with different reagents but with Formalin based fluids displaying excellent preservative qualities. Similarly, histological investigations showed graded and variable autolytic/degenerative changes concurring with gross assessment.

Conclusion: This study has demonstrated that Formalin based fluids are the most suitable chemical agents for post-mortem preservation.

INTRODUCTION

Several concerns involved in the use of human bodies as educational tools,[1-2] and issues arising for re-enhancing anatomical education by dissection have stimulated interests in post-mortem preservation.[3-4] A preservative is considered adequate when the tissue it preserves is maintained very close to normal.[4] Embalming, a form of preservation, involving the use of chemicals to avert decomposition,[5] was credited to ancient Egypt where embalming began with the practice of mummification and cryopreservation as a means of preserving the dead.[6] Mummification which also involved the removal of organs, dehydration, and emersion in natron, was performed by Egyptian priests in the period of the first Dynasty (3200 BC).[7] An investigation utilizing physico-chemical and microscopic properties of an ancient embalmed female discovered in Northern Greece revealed that various resins, oils, spices and other chemical components originating from coniferous and pistacia were commonly employed as preservatives at the period.[8] The Romans, initially did not practice embalming but embraced the culture of mummification in sarcophagus after Egypt was colonized in 30 B.C.[9] Europe as a continent, is home to a diverse spectra of spontaneous and anthropogenic mummies. Anthropogenic mummification is an unintentional act of preserving a body, that is mummified from natural causes.[10] Embalming spread from the ancient people of Africa and Asia to Europe with associated modifications.[11] The oldest known form of artificial embalming in Europe was recorded to have occurred in Osorno, Spain over 5000 years ago.[12] Human bodies were obviously embalmed in China with a liquid which preserved adequately but was not particularly well known.[13] Mummies from various Dynasties through China's history have however been exclusively considered to be accidental mummifications.[14]

During the middle age, embalming became more obvious and included immersion evisceration of the body in alcohol, and insertion the body with of preservative herbs.[15] This assertion was confirmed by the discovery in Nigeria of that large amounts of alcohol, potash, herbal leaf and Kernel oil constituting main ingredients of preservation by the Ogoni's of River State, Nigeria.[16]

When the practice of embalming in the ancient period could not fully solve the preservation of dead bodies as preservative failures abound, the Renaissance period anatomists and scientists developed new preservation techniques with consequent development of new embalming fluids, techniques and methods of preservation.[17-20] This culminated in the discovery of formaldehyde in 1863 and its subsequent adoption as the preservative of choice at the time.[21-23]

In a study conducted by Hayashi *et al*, it was demonstrated that Formalin inhibited the growth of most fungi and bacteria present in the pharynx, rectum and pleural fluids.[24] Hammer *et al*. infiltrated human cadaver with Ethanol, glycerin fixation and thymol conservation, and his findings revealed that the tissue embalmed closely resembled its natural state.[25] Results of a study directed at determining

the preservative effects of formalin based solution on ten undissected cadavers showed effective preservation for a period of about 5 years.[26] Similarly, Coleman and Kogan (1998) modified Logan's preservative procedure by replacing alcohol with isopropyl and excess amounts of sodium chloride and revealed that the resulting effects displayed excellent preservation with minimal structural distortions.[27-28] In another study where cadavers were embalmed with chemical preservatives of aliphatic alcohols, neither increase in skin resistance nor detachment of skin layers of the cadaver occurred but an improved joint flexibility with no significant change in colouration was observed.[29] In 2003, with the aid of ionic liquid, it was demonstrated that the tissues developed a pleasant smell, and that there was no colour change for approximately 2 years.[30] Similarly, cadavers embalmed with Thiel preservative method have been shown to produce tissues nearly identical to the living body both in colour and texture.[31-33]

It is general knowledge that Formalin based preservation utilized extensively for preservation in most Nigerian mortuaries prevent putrescence albeit issues with cosmesis. The explanation for the poor cosmesis is no doubt a consequence of the indifference to standardization in the use of chemical preservatives. While some use different grades of Formalin, others apply raw (stock 40% formaldehyde) 100% Formalin resulting in poor cosmesis and difficulties with prosection. By critical gross and microanatomic observations, this index study, was aimed at evaluating the effectiveness of some chemical preservatives commonly used in Anatomy for preservation and cosmesis, was conducted.

MATERIALS AND METHODS

The reagents used were obtained from Jemy Scientific Nig Analytical/Research Laboratory, Abraka, Delta State, RC 2368650. The experimental study involved 14 adult Wistar rats with average weight of 150gm obtained from the Animal house of Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka.

The animals were randomly assigned into 2 groups of 7 subgroups. A and B subgroups were the positive and negative control groups while subgroups C-G were the test groups. The animals were euthanized by standard procedures and subsequent infiltration of chemical preservative agents was performed.[34-35] The control group A (negative control) was treated with 10mls of water, control B (positive control) with 10mls of 100% formalin while subgroups C-F were perfused with 25% formalin, 100% isopropyl alcohol (absolute alcohol), saturated salt solution (66.67ml/KgBW), 50% phenol respectively at 66.67ml/kg/bw. Similarly, a combination of 10% Formalin, 100% Isopropyl alcohol (absolute alcohol), 50% Phenol and saturated table salt solution administered at 66.67ml/kg/bw in a proportion of 2.5:2.5:2.5:2.5. The chemicals were infiltrated into the Wistar rats until fully turgid. The animals in group 1 were studied for 7 days while the animals in group two (2) were observed till day 21. The intestines (descending colon) and Cerebri were subsequently dissected from each rat and then processed by standard techniques (5µm).[36] The processed tissues upon staining with haematoxylin and eosin, were examined and analyzed with a SCOPETEK DCM 500, 5.0 mega pixel compound microscope, model DN-1070.

RESULTS

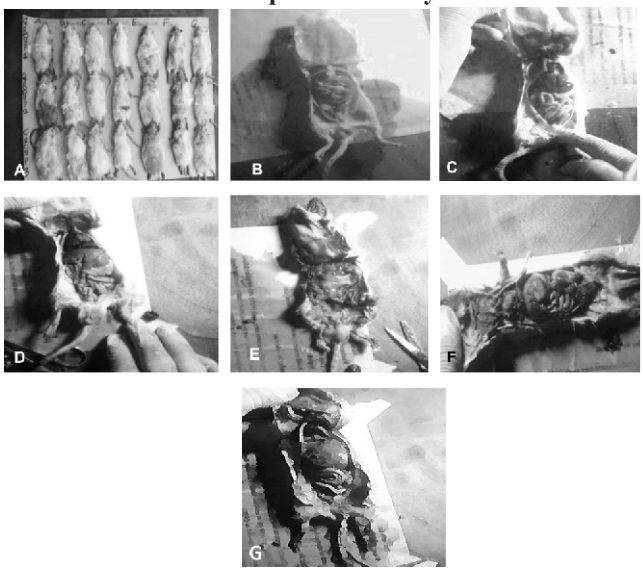
Observations on day 7

Gross findings (Pictures 1.1 – 1.7)

It was observed that a trepid odour emanated from the negative controls and rats preserved with saturated table salt solution. Maggots were seen to ravage most of the animals especially the abdominal region. Their skins were darkened and tissues revealed a generally soft and loose architecture.

The rats exposed to 100% formalin, 25% formalin, 100% Isopropyl alcohol (absolute alcohol), Phenol, and combined chemical solution showed mild skin colour changes in the tails, limbs and ears. Though, decomposition was absent, the test models displayed less flexibility in joints and general tissue firmness was observed. The animals exposed to the combined group of chemical preservatives however demonstrated soft and flexible joints. Similarly, absolute alcohol models were firm, rigid but maintained near intact skin colour.

Gross pictures at day 7



A) All rats at the first day. B)100% Formalin preserved rat . C) 25% Formalin preserved rat. D) 100% Alcohol preserved rat. E) Table Salt solution preserved rat. F) Phenol preserved rat. G) Chemical combination preserved rat.

Microscopic findings

As shown in micrographs 1.1, 1.3, 1.9 and 1.11 exposed to 100% Formalin, 25% Formalin, Phenol, and combined chemical solution respectively, sections of brain tissue (cerebrum) reveal several nerve cells (neurons) disposed within neuroglia together forming nuclei. The tissues possess five layers. Though few blood vessels are seen, the general architecture is well preserved.

Similarly, micrographs 1.2, 1.4, 1.10 and 1.12 are sections of the intestine exposed to 100% formalin, 25% formalin, phenol, and combined chemical solution respectively and reveal blunted mucosal villi lined by a single layer of columnar cells extending into intestinal crepts of

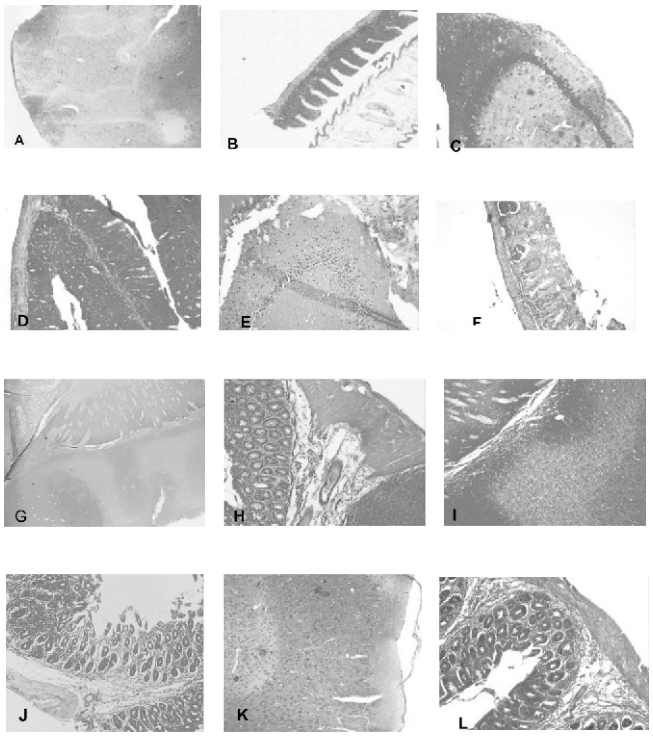
Lieberkuhn. The villi enclose cores of loose fibrovascular lamina propria which are separated from a thin submucosa by muscularis mucosae. The underlying muscularis propria are two muscle layered. The features above are in keeping with well-preserved intestinal tissue.

In micrographs 1.5 and 1.6, preserved with absolute alcohol, there are mild degenerative changes of astrocytic processes which is in keeping with mild nervous tissue degeneration. Though, in micrograph 1.6, sections of the intestine show minimal degenerative changes in the muscularis external layer of the tissue, the submucosa and mucosa are well preserved and the glands are intact.

Extensive degenerative changes involving all five layers of the outermost grey layer of the cerebrum is observed in the micrograph 1.8 (saturated salt preserved group). There is loss of cellular architecture with tissues and cells occurring in fragments. The nuclei and fasciculi of the inner white and grey mater are however normal. Features are in keeping with moderate degenerative changes.

Sections of the intestine in micrograph 1.7 show the mucosa lined by columnar epithelium, and blood vessels. There are degenerative changes in the muscularis propria.

Micrographs; Day 7



H and E x 100

A. section of brain, 100% formalin; B. Section of intestine, 100% formalin; C. section of brain, 25% formalin; D. section of intestine, 25% formalin; E. Section of brain, alcohol; F. section of intestine, alcohol; G. section of brain, salt solution; H. section of intestine, salt solution; I. section of cerebral cortex, phenol; J. section of intestine, phenol; K. section of cerebral cortex, combined chemical preservatives; L. section of intestine, combined chemical preservatives.

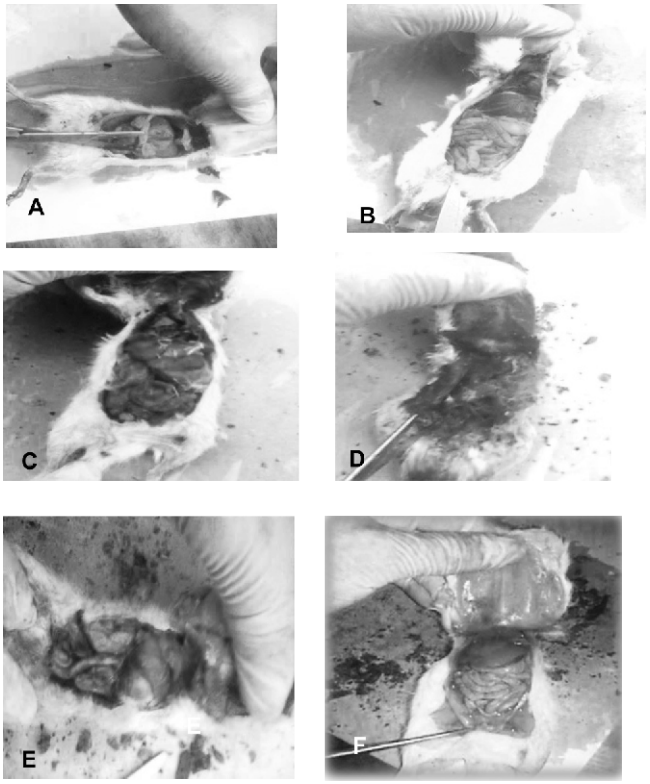
Observations at day 21

Gross features (Pictures 2.1-2.6)

The animals treated with 100% Formalin, 25% formalin and phenol were shown to maintain intact architecture but discoloration of skin in the tails, skin and limbs. The joints were less flexible and firmness was observed. Similarly, the Ethanol perfused animals displayed firmness with less flexible joints and colour was maintained. Though animals infiltrated with chemical combination were shown to be soft with flexible joints, skin discoloration in the tail and limbs was observed.

The gross observation revealed that salt solution preserved rats alone underwent autolysis with an offensive odour emanating from the rats, and appearance of maggots. The skin, limbs and tails were darkened but the joints were loosely applied to the rest of the body.

Gross pictures at day 21



A)100% Formalin preserved rat . B) 25% Formalin preserved rat. C) 100% Alcohol preserved rat. D) Table Salt solution preserved rat. E) Phenol preserved rat. F) Chemical combination preserved rat.

Microscopy

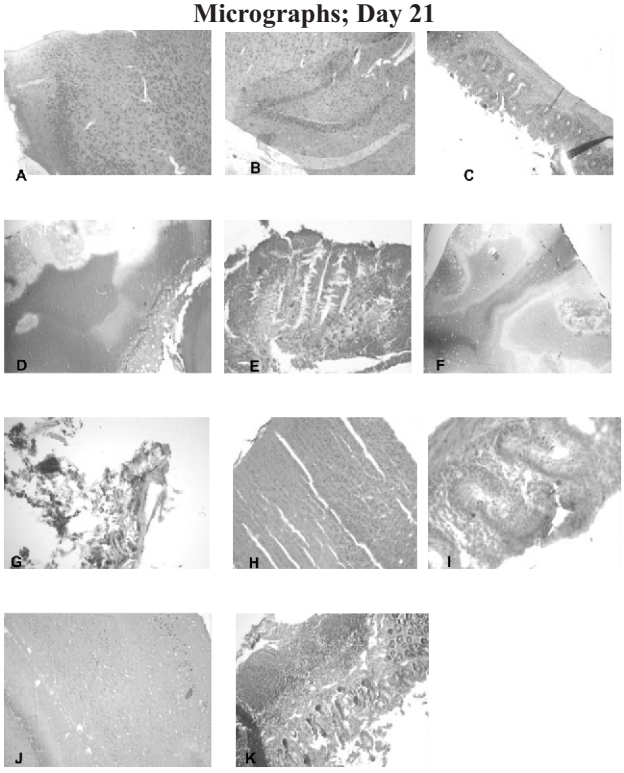
Micrographs 2.2, 2.2 and 2.10 are sections of cerebral tissues, exposed to 100% formalin, 25% formalin and a combination of chemical preservative reagents, at day 21. They show several nerves cell (neurons) disposed within neuroglia all together forming nuclei. The tissues are five layered. Few blood vessels are seen and the architectures are well preserved. Similarly, in micrographs 2.3, 2.9, 2.11, (exposed to 100% formalin, 25% formalin and a combination of chemical preservative reagents), blunted mucosal villi

lined by single layer of columnar cells are observed in the sections of intestines. The villi enclose cores of loose fibrovascular lamina propria which is separated from a thin submucosa by muscularis mucosae. The submucosa layers are seen and the underlying muscularis propria are two muscle layered. The features above are in keeping with well-preserved intestinal tissues.

More extensive degenerative changes are observed in the outer grey layer but minimal in the inner layer of brain in micrograph 2.4 (alcohol group). These features are in keeping with autolytic changes. In micrograph 2.5 (alcohol exposed group), sections of intestine reveal moderate degeneration in the submucosa and the intestinal glands. The muscularis propria layer is distorted. Generally, the features are in keeping with poorly preserved intestinal tissue.

In micrograph 2.6 (common table salt exposed group), there are moderate autolytic changes in the outer grey, inner white and innermost grey mater. Few blood vessels and nerve cells are seen. There is also loss of normal cellular architecture in the tissue. Similarly, the mucosa in micrograph 2.7 (common salt exposed group) displays a ghost outline representing the original luminal layer, and extensive loss of cellular outlines in the muscularis propria. Few food particles (undigested) are present in the lumen of the intestine. These features above are in keeping with moderate to extensive tissue degeneration.

Micrographs; Day 21



A. Section of brain Formalin 100%; B. section of brain, 25% formalin; C. section of intestine, 25% formalin; D. section of cerebral cortex, alcohol; E. section of intestine, alcohol; F. section of cerebral cortex, salt solution; G. section of intestine, salt solution; H. section of brain, phenol; I. section of the intestine, phenol; J. section of cerebral cortex, combined chemical preservatives; K. section of intestine,combined chemical preservatives

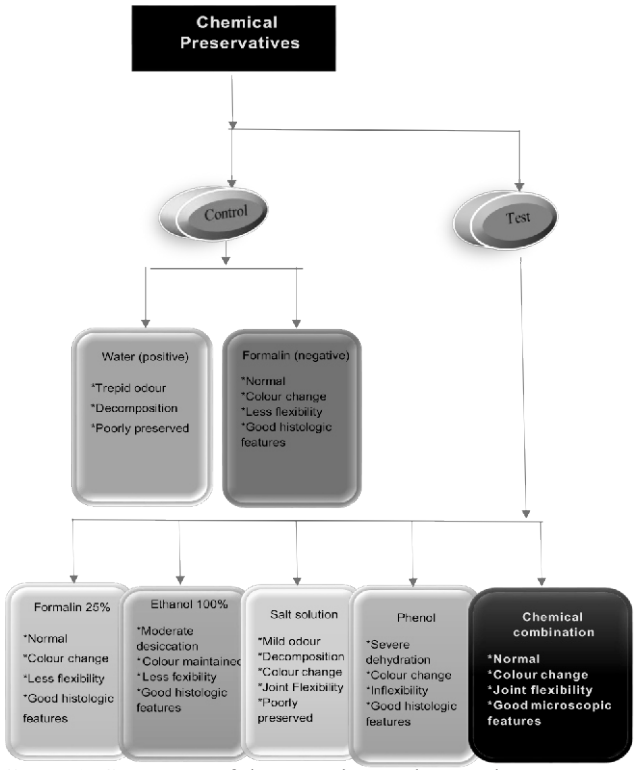


Chart 1: Summary of the experimental procedure.

DISCUSSION

Several attempts at achieving preservation and attenuating the rate of decomposition of human bodies following death have earlier been described.[37-38] This study was however an attempt at determining the most effective and proficient agent with both preservative and cosmetic properties.

By chemical cross linkages, formalin has been shown to convert proteins into inert cross linked lattices, resulting in extensive tissue dehydration with resultant antiseptic activity.[4,39-41] The result is rapid reaction with cellular components, cross-linking of several proteins, blood coagulation, tissue desiccation and dis-colouration, and constriction of capillaries.[4] The infiltrated with formalin in the index study demonstrated suppressed putrefaction confirming previous studies that formalin possessed excellent preservative properties.[25,42-46] Hubbell *et al*. opined however that formalin embalmed animals did not exhibit many qualities of living organs especially colour, flexibility and pliability.[47] This was clearly evident in the gross observation of Formalin perfused rats as noted in the current study. Histological findings further revealed intact cellular components which supported Hess' assertion that Formalin preserved tissue without distortion of the cellular structures.[46]

Rapid dehydration resulting in hardening of tissue in cadavers embalmed with alcohol derivatives had earlier been reported.[48] In the current study, the tissues assumed firmness and the joints were less flexible in animals infiltrated with Ethanol in both early and late test groups. This was contrary to the findings in another study where the models preserved with alcohol displayed flexible joints.[25] The possible reason for this dissimilarity could be due to the

differences in graded concentration of Ethanol employed in the index study. In another study, alcohol was shown to have a wide range of antiviral and antifungal effects in the short run which was in tandem with our observation as normal architecture was maintained.[49] Histologic assessment however revealed that Ethanol poorly preserved tissue integrity.

Phenol (a carbolic acid) is colourless liquid which is known to deactivate enzymes within the cell and influence cell permeability.[4] This agent easily dissolves into tissues owing to its lipophilic nature. It quickly dehydrates tissues, a feature that discourages bacterial and viral growth. It further denatures and precipitates protein compounds as was further confirmed in the index study where Phenol displayed preservative properties in rats of both groups.[50] Laboratory animals embalmed with phenol displayed marked tissue firmness with a colour change in the tail and the limbs as was earlier noted by Richin's *et al*.[51]

Coleman and Kogan argued that high common salt content in tissue prevented dehydration and that cadavers were adequately preserved with solution of this agent.[28] We have however demonstrated that though common salt solution attenuated desiccation due to its hygroscopic properties, preservation was generally temporary. It is therefore suggested that frequent administration of salt solution would likely improve the preservative properties of this reagent.

In a study conducted by Bradbury and Hoshino, a moderate degree of flexibility and adequate degree of firmness for dissection was highlighted following the treatment with some chemical preservatives.[5] From this present study, the animals perfused with formalin, phenol, alcohol, and salt combination were well preserved. The index report is closely related to previous reports that demonstrated soft and moderate flexibility in the joints.[52-53]

This study has underscored the use of formalin and formalin based fluids for post mortem preservation, and also demonstrated the effectiveness of alcohol in persevering skin colour. It has further established that both low and high concentrations of formalin displayed very similar preservative activity and we therefore encourage the use of diluted formalin in tissue preservation. We have further highlighted that though phenol possesses effective preservative properties, colour changes and joint stiffness are drawbacks.

Conflict of interest

There is no conflict of interest regarding this study.

Ethical Approval

The design was approved by the Ethical Committee of the Department of Human Anatomy and Cell Biology, Delta State University, Abraka with reference number DELSU/CHS/ANA/68/44.

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