STUDENT ESSAY

A comparison of different embalming techniques.

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INTRODUCTION

There are many ways to preserve a body, but not all of these are appropriate for the education of students who are studying anatomy through cadaveric dissection. For example, fresh-frozen cadavers are useful for teaching surgical trainees, but their short 'shelf-life' post-thawing prevents their use for dissecting over the course of an entire semester. A further example is plastination; though this technique is fantastic for permanently preserving bodies, the rigidity and dryness of the tissues limits their use for teaching.

As such, an alternative preservation method is to embalm the bodies. Embalming is a chemical process used to preserve a deceased person's body and delay its decay in death. This most commonly tends to be for funeral purposes, so that the bereaved may view their loved one before cremation or burial. However, more robust embalming methods are also used to provide cadavers to universities, for students to learn the subject of anatomy through dissection and prosection. Embalming cadavers enables the bodies to be hygienically stored and studied for long periods of time; this makes the most of the donors' generosity by maximising the amount of teaching that each cadaver can provide. Although cadavers have a great role in surgical and clinical training, this essay will focus on their use for anatomical education, and the variety of embalming methods currently available for this purpose.

FORMALIN EMBALMING

Traditionally, embalming for academic anatomical purposes is performed using formalin-based techniques.[1] Formalin is an aqueous solution of methanol and formaldehyde gas dissolved in water, with various strengths depending on its purpose.[2,3] Routinely in anatomy departments, embalming fluid is made up of ~10% formalin, where the undiluted formalin itself is made up of formaldehyde (~37%) and methanol (~10%) dissolved in water.[2,3] Other chemicals may subsequently be added to the formalin, commonly ethanol and phenol, to produce the final commercial embalming fluid.[3,4]

Formalin penetrates the cadaveric tissues through diffusion, once the body has been intravascularly infused with the embalming solution and left to fix for around a month.[3] The formalin preserves the tissues in two phases: dehydration followed by fixation.[3,5]

In the aforementioned first phase, dehydration of the body by the alcohol hardens the tissues and cellular membranes,[3] giving formalin-embalmed cadavers (FECs) resistance against decomposition.[6] In the second phase, the formaldehyde is hydrated by the water in which it is dissolved, resulting in the formation of methylene glycol, which then polymerises.[3] These polymer molecules may depolymerise within the body, but both the hydrated and dehydrated forms of formaldehyde serve to fix and harden the tissues.[3] Formaldehyde readily inserts cross-links between various functional groups found on protein macromolecules within the tissue; namely, these are stable intermolecular methylene bridges linking polypeptide chains.[3,5,7,8] This results in changes within the physiology of the tissue as well as the reactivity of the cells, making the tissues stiffen and become inflexible.[3,5]

Formalin also serves as a preservative through its content of the antimicrobial chemicals formaldehyde and methanol.[9,10,11,12] Through the action of denaturing proteins and nucleases, the alcohol can prevent bacterial activity and the resulting tissue decay.[11] The formaldehyde, on the other hand, exerts genotoxic effects on bacteria, for example by causing DNA to covalently bind with other molecules and proteins.[12,13,14] This results in the modification of crucial cell components, inhibiting bacterial function and therefore leading to microbial dysfunction.[13]

Although this process of fixation usually takes around a month to complete, there are many factors that can alter this timeframe, including but not limited to: temperature, volume of solution, or surface area of the specimen.[3]

The rationale for using formalin includes its ubiquity, its low cost, and its ability to maintain the cadaveric tissues with minimal physical distortion over long periods of time.[15] However, the FEC does not come without its pitfalls. As a known carcinogen and allergen, formaldehyde can generate both acute and chronic adverse effects on the health of students and anatomy department personnel respectively; this is caused mainly through the inhalation of chemical fumes, but may also occur through contact with the skin.[1] The short term effects include: dermatitis, headaches, and both ocular and airway irritation, not to mention formaldehyde's pungent odour.[1,4] Long term exposure to formaldehyde is likely to be a factor in the development of: asthma, corneal clouding, congenital malformations, and malignancies. [1,4,9,12. Another drawback to formalin is its tendency to heavily discolour the tissues, rendering the technicolour palette of a fresh cadaver a monochrome spectrum of brown.[4] Finally, as aforementioned, formalin hardens the tissues, reducing their pliancy; these tissues are also more prone to desiccation, making delicate structures brittle. Both of these factors can make dissecting the hard-fixed FEC far trickier, if inflexible and fragile anatomical structures cannot be readily handled and manoeuvred.[16] In order to bypass the challenges of formalin, the optimal embalming technique would similarly be cheap, antimicrobial, and easy to perform, but would also be safer, as well as better at retaining the visual and physical properties of a fresh body.[6,15] More modern alternatives to formalin explore these properties in order to improve the quality of cadavers, thereby increasing their educational value for anatomy students. Table 1 provides a general summary of the advantages and disadvantages of the FEC, as well as the following alternative cadavers mentioned in this essay, which a university anatomy department may consider when thinking about implementing new embalming methods.

THIEL EMBALMING

Devised in 1992, Walter Thiel's soft embalming method produces a lifelike cadaver with excellent retention of tissue colour and flexibility.[4,17,18,19,20] The Thiel embalming method involves two separate applications of differing embalming fluids: intravascular administration, followed by full-body submersion into a large tank of preservation solution.[18] The Thiel-embalmed cadaver (TEC) remains submerged in this embalming tank for between one and six months before it is removed and kept in storage until dissection.[4,5,18] Like FECs, the TEC can be stored in a plastic bag, outside of refrigeration.[20]

The Thiel embalming solutions contain various chemicals, including boric acid for disinfecting the tissues; ethylene glycol to maintain tissue pliability; different nitrate and sulphate salts to aid fixation; and the alcohol 4-chloro-3-methylphenol to serve as a disinfectant.[5,9,18,21]

	FEC	TEC	PBEC	EGEC	SPC	NEC
Odour	offensive	disputed	inoffensive	inoffensive	inoffensive	unknown
Flexibility	inflexible	very flexible	flexible	inflexible	flexible	flexible
Colour	discoloured	lifelike	discoloured	lifelike	lifelike	lifelike
Refrigerated storage required	no	no	no	yes	no	yes
Full-body submersion required	no	yes	no	yes	no	no
Toxic	yes	yes	no	no	unknown	yes
Fire hazard	no	yes	yes	yes	no	unknown
Cost	very low	high	low	low	very low	unknown

Table 1 *Summary of the broad factors that a university anatomy department may consider when choosing the most suitable embalming method for their cadavers. Some of these directly contribute to the quality of anatomical teaching, whereas others focus more on the practical aspects of running a dissection course within an anatomy department.*

There is still a small amount of formaldehyde within the Thiel embalming solutions, but the final concentration is less than 2%, and so the TEC lacks the acrid aroma of the FEC.[5,18,20,22] There is seemingly no consensus regarding the overall smell of the TEC; some papers claim that the TEC has little odour,[17,20,22] but others disagree.[4,23,24]

It has been posited that the boric acid within the Thiel embalming fluid is responsible for the characteristic flexibility of the joints within the TEC, due to its corrosive and denaturing effect on myofibres.[25,26] Interestingly, borates and boric acid were being used to preserve bodies even in Pharaonic Ancient Egypt,[26] and also serve as an antimicrobial within the tissues.[23] Due to this elasticity of soft tissues within the TEC, along with the lifelike colouration, not only does the body resemble *in vivo* anatomy more accurately than the FEC, it's also easier to dissect, since anatomical structures are more readily manoeuvred for improved visibility and accessibility.

Interestingly, one study has demonstrated that even rigid and desiccated formalin-embalmed prosections can be revived by subsequent submersion into Thiel embalming fluid, which may restore some flexibility into the tissues for easier dissection.[19] This could help to recycle cadaveric specimens which are already in use within anatomy departments, therefore providing a greater number of teaching tools for students, as well as lengthening the lifespan of the prosections. However, this particular study only submerged two specimens, and so further research is required into the reliability of this revival technique.

Despite its many assets, the Thiel embalming method still presents its own challenges. Due to the necessity for full-body submersion within embalming tanks, university anatomy departments must have both the space and the funds for the installation of this new infrastructure.[18,20] It has therefore been estimated that the TEC may cost up to twenty times more than the FEC.[4] Further, whilst the Thiel embalming solution only contains a minute amount of toxic formalin, it does contain larger proportions of some flammable and poisonous chemicals, and therefore poses different risks to the anatomy department.[4,8] Finally, a number of studies note that the Thiel embalming method is very complex and therefore requires expensive, specialised equipment and specially-trained anatomy staff.[4,18,23,24]

PHENOXYETHANOL-BASED EMBALMING

Similarly to Thiel embalming, the phenoxyethanol-based (PB) embalming technique also produces a flexible cadaver, with long term preservation and less toxic properties.[24,26,27] However, unlike Thiel embalming, this method involves only the intravascular perfusion of the PB-embalmed cadaver (PBEC), without the need for sizeable immersion tanks.[18,24] The PB embalming fluid is made up mainly of ethanol (~60%), and also contains glycerine (~15%), water (~15%), phenoxyethanol (~7%), and a small amount of formaldehyde (~2%).[24,28]

The phenoxyethanol serves as a preservative through its broad-spectrum antimicrobial activity.[26,29] The mechanism for this action is twofold, causing irreversible cellular injury.[30] Firstly, phenoxyethanol increases the permeability of microbial membranes to potassium ions, thereby acting as a bactericide by directly inhibiting the synthesis of microbial nucleic acids.[29] Secondly, phenoxyethanol is also thought to prevent energy metabolism in bacteria by inhibiting malate dehydrogenase and disrupting the proton gradient.[30]

One recent paper studying the PBEC reported the cost of embalming solutions required per body to be $61.49.[24] Whilst the exact costings of the FEC and the TEC were not available, as well as including the running costs of the anatomy department and cadaveric processing alongside the price of the PB embalming solutions alone, it seems that like the FEC, the PBEC will be a cheaper option to produce than the TEC. A further similarity to the FEC is the brown-yellow discolouration of the PBEC.[24] The PBEC is also stored without refrigeration within a plastic bag, like both the FEC and TEC.[20,24] Little literature could be found regarding the smell of the PBEC, but one paper claims the odour to be mildly fruity.[31]

However, one necessity that could limit the use of this embalming method is the requirement for explosion-proof storage and preparation facilities owing to the high ethanol content in the preservation fluids.[24] One paper has claimed that the TEC also poses a risk of explosion,[32] though this conclusion is not widely found throughout the literature.

ETHANOL-GLYCERINE EMBALMING

Ethanol-glycerine (EG) embalming solutions have been developed to preserve a body, resulting in a soft-fixed EG-embalmed cadaver (EGEC). Application of the EG embalming fluids include intravascular infusion of a 2-10% glycerine solution (depending on the body's habitus),[23] followed by full-body submersion of the body into ethanol for a period of around one month.[23,33] After submersion, the EGEC is refrigerated and a solution of ethanol and thymol, a known fungicide, is used to refresh the body and maintain its moisture.[6,7] This is to prevent desiccation when at room temperature, for example during dissection classes. Also, as aforementioned, alcohol has a dual role of both dehydration and antimicrobial protection.

Much like the TEC, the EGEC maintains lifelike flexibility and colouration in death.[33] It has also been reported to have an inoffensive odour.[23] One notable pitfall for the EGEC is that, unlike the FEC, TEC, or PBEC, it must be refrigerated for storage.[23,33] This would incur a higher cost for long term retention through powering large refrigeration units, as well as setting up such infrastructure which would be likely required in anatomy departments where FECs have been the cadavers of choice until now. Further, like the PBEC, this embalming method also requires an explosion-proof room due to the high ethanol content in the preservation fluids.[23,33] Unfortunately, similarly to the FEC, the EGEC is also relatively inflexible.[34,35]

SATURATED SALT PRESERVATION

Like boric acid, salt has been used historically for embalming.[8] As such, it is perhaps unsurprising that there have been endeavours to revive this and use salt as a novel embalming technique, leading to the emergence of the saturated salt method. A saturated salt preservation solution used in one paper consisted of 20kg sodium chloride dissolved in 25L of fluid.[36] The fluid consisted mainly of water, but also contained 4L of isopropanol alcohol, 1L of formaldehyde, and smaller quantities of both phenol and glycerine.[36] This was intravascularly infused into the body, and then the salt-preserved cadavers (SPCs) were stored in sealed bags at room temperature, like FECs, TECs, and PBECs.

It is thought that salt has various roles in preserving tissues. One function of salt is to draw the water out of cells, thereby causing osmotic shock to bacteria which results in either necrosis or extremely retarded growth.[37] Through the same osmotic mechanism, salt draws water out of the cells within the tissues, reducing the amount of intracellular free water available for both chemical reactions and the aforementioned microbial growth to occur.[37]

As a cheap and ubiquitous substance, salt has other advantages too; its ability to retain extracellular fluid within the tissues prevents desiccation, and maintains a flexible salt-preserved cadaver (SPC).[8,36] However, this could be a double-edged sword, resulting in an oedematous and swollen body, which could render dissection tricky and also perhaps more dangerous if using sharp tools within a slippier environment.[8] This fluid retention can be overcome through prolonged storage of cadavers, since it has been observed that over a period of months, water may be lost gradually;[8] however, this strategy would be restricted by the anatomy department's space and infrastructure for long term cadaveric storage. Finally, whilst not as effective as formalin, using a saturated salt solution has been shown to provide sufficient antimicrobial protection against tissue degradation by bacteria.[8] It seems that the SPC is cheap and uncomplicated to produce, like the FEC, but beneficially, the SPC is safer and more lifelike too. However, the SPC does still contain some formaldehyde.[36]

NVP EMBALMING

Recently, the organic compound N-vinyl-2-pyrrolidone (NVP), a precursor molecule to water-soluble polymer polyvinylpyrrolidone (PVP), has been identified to have antiseptic, preservative, and fixative effects on cadaveric tissues.[34,38] Intravascular infusion with NVP-based embalming fluids can produce a flexible, soft-fixed cadaver with minimal microbial activity.[34,38] The embalming solutions can vary in strength, with NVP concentrations varying between 4% to 21.5%.[38] The NVP-embalmed cadaver (NEC) is then placed into a sealed plastic bag containing 5L of 5% NVP preservation solution for refrigerated storage.[34,38]

NVP enters the cells of the body and replaces water, resulting in tissue fixation through polymerisation into its macromolecule PVP and cross-link formation using free radicals.[34,38] There is little literature either on NECs or the mechanism of NVP in tissue preservation and fixation, but perhaps this could be an area for future research.

Whilst the NEC remains flexible (depending on NVP concentration) and retains its colour, it has been found that the connective and subcutaneous lipid tissues become transparent, the latter thought to be due to lipolysis;[38] this can be useful in the differentiation of similar looking structures, but does not represent the appearance of *in vivo* anatomy. The keratinous dermal layers of the NEC also frequently peel,[34,38] resembling the 'skin slip' of a decomposing corpse. Further, whilst the NEC contains no formalin, NVP can potentially cause mild irritation to the eyes, skin, and airways, as well as serious damage to the eyes;[34] therefore, appropriate personal protective equipment would have to be supplied to all students and staff. However, other positive aspects of the NVP embalming solutions include the fact that it does not require specialist equipment, and results in soft fixation.[38] The cost of processing an NEC could not be found.

CONCLUSION

Although formalin remains the ubiquitous preservation technique for cadavers in anatomy departments due to its low cost and reliability, there are various alternative embalming methods available. However, due to higher costs, elaborate processes, and great infrastructural demands, it is easy to see why FECs remain the main cadaveric tools available in academia. Although this essay has been in no way exhaustive of the available embalming methods, it shows that different techniques have diverse strengths and weaknesses. Considering the individual advantages and disadvantages of the embalming methods, it is this author's opinion that the SPC appears, at least superficially, to be the most realistic replacement for the average, busy anatomy department; perhaps there are other factors not covered in this paper that are preventing its emergence, or maybe it's a case of tradition being hard to shift. Nevertheless, it is promising to see research into safer and reasonably practicable alternatives to formalin, in order to keep improving anatomical education for students across the world. Further studies from independent institutions comparing embalming methods would be useful in determining the most suitable and realistic techniques for cadaveric preservation.

REFERENCES

1. Elshaer NSM, Mahmoud MAE. 2017. Toxic effects of formalin-treated cadaver on medical students, staff members and workers in the Alexandria Faculty of medicine. *Alexandria Journal of Medicine*, 53(4): 337-343.

2. Takayasu T. 2013. Toxicological Analyses of Medications and Chemicals in Formalin-Fixed Tissues and Formalin Solutions: A Review. *Journal of Analytical Toxicology*, 37(9): 615-621.

3. Thavarajah R, Kazhiyur Mudimbaimannar V, Elizabeth J, Krishnamohan Rao U, Ranganathan K. 2012. Chemical and physical basics of routine formaldehyde fixation. *Journal of Oral and Maxillofacial Pathology*, 16(3): 400-405.

4. Kennel L, Martin DMA, Shaw H, Wilkinson T. 2017. Learning anatomy through Thiel- vs. formalin-embalmed cadavers: Student perceptions of embalming methods and effect on functional anatomy knowledge. *Anatomical Sciences Education*, 11(2): 166-174.

5. Liao X, Kemp S, Corner G, Eisma R, Huang Z. 2015. Elastic properties of Thiel-embalmed human ankle tendon and ligament. *Clinical Anatomy*, 28(7): 917-924.

6. Pal AK, Bhanarkar UP, Ray B. 2022. Embalming with Formalin – Benefits and Pitfalls. *Scholars International Journal of Anatomy and Physiology*, 5(3): 70-77.

7. Hammer N, Löffler S, Feja C, Sandrock M, Schmidt W, Bechmann I, Steinke H. 2012. Ethanol-glycerin fixation with thymol conservation: A potential alternative to formaldehyde and phenol embalming. *Anatomical Sciences Education*, 5(4): 225-233.

8. Hayashi S, Naito M, Kawata S, Qu N, Hatayama N, Hirai S, Itoh M. 2016. History and future of human cadaver preservation for surgical training: from formalin to saturated salt solution method. *Anatomical Science International*, 9: 1-7.

9. Janczyk P, Weigner J, Luebke-Becker A, Kaessmeyer S, Plendl J. 2011. Nitrite pickling salt as an alternative to formaldehyde for

embalming in veterinary anatomy—A study based on histo- and microbiological analyses. *Annals of Anatomy*, 193(1): 71-75.

10. Kalathenos P, Russell NJ. 2003. Ethanol as a food preservative. In: Russell and Gould (editors). *Food Preservatives*. 196-217.

11. Connell J, Chaseling J, Page M, Wright K. 2018. Tissue preservation in extreme temperatures for rapid response to military deaths. *Forensic Science International: Genetics*, 36: 86-94.

12. Yu R, Lai Y, Hartwell HJ, Moeller BC, Doyle-Eisele M, Kracko D, Bodnar WM, Starr TB, Swenberg JA. 2015. Formation, Accumulation, and Hydrolysis of Endogenous and Exogenous Formaldehyde-Induced DNA Damage. *Toxicological Sciences*, 146(1): 170-182.

13. Denby KJ, Iwig J, Bisson C, Westwood J, Rolfe MD, Sedelnikova SE, Higgins K, Maroney MJ, Baker PJ, Chivers PT, Green J. 2016. The mechanism of a formaldehyde-sensing transcriptional regulator. *Scientific Reports 6,* article 38879.

14. Lu K, Ye W, Zhou L, Collins LB, Chen X, Gold A, Ball LM, Swenberg JA. 2010. Structural Characterization of Formaldehyde-induced Cross-links Between Amino Acids and Deoxynucleosides and Their Oligomers. *Journal of the American Chemical Society*, 132(10): 3388-3399.

15. Coleman R, Kogan I. 1998. Correspondence: An improved low-formaldehyde embalming fluid to preserve cadavers for anatomy teaching. *Journal of Anatomy*, 192(Pt 3): 443-446.

16. Darras KE, de Bruin ABH, Nicolaou S, Dahlström N, Persson A, van Merriënboer J, Forster BB. 2018. Is there a superior simulator for human anatomy education? How virtual dissection can overcome the anatomic and pedagogic limitations of cadaveric dissection. *Medical Teacher*, 40(7): 752-752.

17. Humbert M, Micault E, Moreau S, Patron V, Bois J, Hitier M. 2022. The advantages of modified Thiel technique in head and neck surgical anatomy teaching. *Surgical and Radiologic Anatomy*, 44: 345-352.

18. Ottone NE, Vargas CA, Fuentes R, dS M. 2016. Walter Thiel's Embalming Method. Review of Solutions and Applications in Different Fields of Biomedical Research. *International Journal of Morphology*, 34(4): 1442-1454.

19. Hunter A, Eisma R, Lamb C. 2014. Thiel embalming fluid - A new way to revive formalin-fixed cadaveric specimens. *Clinical Anatomy*, 27(6): 853-855.

20. Eisma R, Lamb C, Soames RW. 2013. From formalin to thiel embalming: What changes? One anatomy department's experiences. *Clinical Anatomy*, 26(5): 564-571.

21. Thiel W. 2002. Supplement to the conservation of an entire cadaver according to W. Thiel. *Annals of Anatomy*, 184(3): 267-9.

22. Balta, J. Y.; Lamb, C. & Soames, R. W. 2015. A pilot study comparing the use of Thiel- and formalin-embalmed cadavers in the teaching of human anatomy. *Anatomical Sciences Education*, 8(1):86-91.

23. Hammer N, Löffler S, Bechmann I, Steinke H, Hädrich C, Feja C. 2014. Comparison of modified thiel embalming and ethanol-glycerin fixation in an anatomy environment: Potentials and limitations of two complementary techniques. *Anatomical Sciences Education,* 8(1): 74-85.

24. Crosado B, Löffler S, Ondruschka B, Zhang M, Zwirner J, Hammer N. 2020. Phenoxyethanol-Based Embalming for Anatomy Teaching: An 18 Years' Experience with Crosado Embalming at the University of Otago in New Zealand. *Anatomical Sciences Education,* 13(6): 778-793.

25. Benkhadra M, Bouchot A, Gerard J, et al. 2011. Flexibility of Thiel's embalmed cadavers:

the explanation is probably in the muscles. *Surgical and Radiologic Anatomy*, 33:365–368.

26. Brenner E. 2014. Human body preservation – old and new techniques. *Journal of Anatomy*, 224(3): 316-344.

27. Frølich KW, Andersen LM, Knutsen A, Flood PR. 1984. Phenoxyethanol as a nontoxic substitute for formaldehyde in long-term preservation of human anatomical specimens for dissection and demonstration purposes. *The Anatomical Record,* 208(2): 271-278.

28. Nicholson HD, Samalia L, Gould M, Hurst PR, Woodroffe M. 2005. A comparison of different embalming fluids on the quality of histological preservation in human cadavers. *European Journal of Morphology*, 42(4-5): 178–184.

29. Dréno B, Zuberbier T, Gelmetti C, Gontijo G, Marinovich M. 2019. Safety review of phenoxyethanol when used as a preservative in cosmetics. *Journal of the European Academy of Dermatology and Venereology,* 33(S7): 15-24.

30. Langsrud S, Steinhauer K, Lüthje S, Weber K, Goroncy-Bermes P, Holck AL. 2016. Ethylhexylglycerin Impairs Membrane Integrity and Enhances the Lethal Effect of Phenoxyethanol. *PLOS One*, 11(10): e0165228.

31. Jochebed SR, Thenmozhi MS. 2020. Phenoxyethanol as a substitute for formaldehyde in the preservation of anatomical specimens. *International Journal for Research Trends and Innovation*, 5(1): 2456-3315.

32. Barua M. 2021. Is thiel embalming worth the risk? *The American Journal of Surgery*, 222(2): 459.

33. Hammer N, Löffler S, Feja C, Bechmann I, Steinke H. 2011. Substitution of Formaldehyde in Cross Anatomy Is Possible. *Journal of the National Cancer Institute*, 103(7): 610-611.

34. Nagase M, Nagase T, Tokumine J, Saito K, Sunami E, Shiokawa Y, Matsumura G. 2022. Formalin-free soft embalming of human cadavers using N-vinyl-2-pyrrolidone: perspectives for cadaver surgical training and medical device development. *Anatomical Sciences International*, 97: 273-282.

35. Burns DM, Bell I, Katchky R, Dwyer T, Toor J, Whyne C M, Safir O. 2018. Saturated Salt Solution Cadaver-Embalming Method Improves Orthopaedic Surgical Skills Training. *The Journal of Bone and Joint Surgery*, 100(15): pe104.

36. Hayashi S, Homma H, Naito M, Oda J, Nishiyama T, Kawamoto A, Kawata S, Sato N, Fukuhara T, Taguchi H, Mashiko K, Azuhata T, Ito M, Kawai K, Suzuki T, Nishizawa Y, Araki J, Matsuno N, Shirai T, Qu N, Hatayama N, Hirai S, Fukui H, Ohseto K, Yukioka T, Itoh M. 2014. Saturated Salt Solution Method: A Useful Cadaver Embalming for Surgical Skills Training. *Medicine*, 93(27): pe196.

37. 2010. Preservation and Physical Property Roles of Sodium in Foods. In: Henney, Taylor, Boon (editors). *Strategies to Reduce Sodium Intake in the United States.* 91-118.

38. Haizuka Y, Nagase M, Takashino S, Kobayashi Y, Fujikura Y, Matsumura G. 2018. A new substitute for formalin: Application to embalming cadavers. *Clinical Anatomy*, 31(1): 90-98.