World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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# Preservation of muscle tissue with a formaldehyde-free borax solution for gross anatomy lessons

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World Journal of Advanced Research and Reviews, 2022, 16(03), 390–395

Publication history: Received on 01 November 2022; revised on 07 December 2022; accepted on 10 December 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.16.3.1341

## Abstract

It is known that formaldehyde has serious toxicological effects on medical students, laboratory workers and environmental health. Recently various scientists search for a safe preservation fluid instead of the formaldehyde. One of them and the famous one is the Thiel's embalming solution. However, it has been realized that it causes deterioration in muscle tissues. It was determined that this deterioration was caused by the boric acid. In the present study, previously formalin fixed muscle tissues were stored into the alkali form of boron during 40 days. At the end of the trial the appearance of the borax stored cadavers was quite similar to the formalin stored cadavers. In cross-sections taken from the muscle tissues, no deterioration could observe. Additionally, there was no putrid odor; on the contrary, a slight level of formaldehyde odor. In microbiological tests, there was a slight insignificant increase in the total number of bacteria in borax solutions. As a result, it was concluded that borax could be used as a cadaveric storage solution, and it can be more appropriate to use borax instead of boric acid in Thiel's solution.

**Keyword:** Borax; Formaldehyde; Muscle Tissue; Preservation

## 1. Introduction

Embalming is a chemical process applied to the body after death to purify the body from microorganisms and make it last for a long time. It has been tried to protect bodies in this way for about 5000 years [1, 2]. Recently, embalming is performed after people and animal's death, and their bodies are examined as cadavers in medical schools for the education of students. As the cadaver will be purified from microorganisms as a result of embalming, disease transmission to students and trainers will be prevented and the tissue preserved for many years without deterioration [2, 3]. In terms of education, a cadaver is desired to have the softness of tissue that a living body has. This softness is tried to be achieved in the solutions applied for the fixation. When evaluating the quality of a cadaver, it should get full marks from criteria such as hardness, color, odor, and structure. The closer these criteria are to the real tissue, the higher the quality of cadaver detection [4].

The most up-to-date and valid method used to make a cadaver of a body is the form of fixation using a chemical called formaldehyde. The formaldehyde has antibacterial and fungicidal properties thus cadavers can be stored for many years without spoiling, although they turn into a slightly harder tissue. However, formaldehyde is an important adverse effect on the skin and respiratory tract [5]. Furthermore, the international cancer research unit has declared formaldehyde a 1st class carcinogen [6]. For this reason, various solutions are sought that can be used as cadaver storage solutions instead of formaldehyde [5].

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In addition to formaldehyde, glycerine, ethyl alcohol, and phenol are also used in the fixation of cadavers. It has been found that fixations with these chemicals do not cause stiffness in the tissues and joint movements [5]. The formaldehyde performs the fixation process by forming a cross-link between nucleic acid and proteins through methylene bridges formed between reactive groups. Alcohol, on the other hand, separates water from the proteins in the tissues and causes the formation of protein-aqueous clots, and performs the fixation process. However, excessive shrinkage and microscopic deterioration were observed in tissues kept in alcohol for a long time [7, 8]. Chung et al.[8], reported that the buffered ethanol fixation increased biomolecular protection, and as a result of histomorphological examinations, it succeeded in obtaining findings similar to formaldehyde fixation. It has been observed that the staining intensity weakens in the histological stainings as the fixation time increases in the tissues fixed in formaldehyde, whereas a very good staining intensity is obtained in the buffered ethanol fixation solution regardless of time [7, 8, 9]. Furthermore, it has been stated that cadavers can preserve their red color by adding food additives with bacteriostatic properties such as nitrite and nitrate to the storage solution [4].

Boron is a mineral that is taken into the body with daily food and beverages. It is abundant in nature in rocks, soil, coal, and seawater. Boron is used in glass, detergent, ceramics, and fertilizer industries [10]. Boron, which also has antimicrobial properties, has been tested in various fixing solutions before. The most well-known of these methods is Thiel's method, which includes cadaver fixation solution. This solution, prepared by a scientist named Thiel under his name, has come to the fore as an accepted solution instead of formaldehyde over the years [5, 11]. Thiel solution is prepared by combining many different chemicals such as boric acid, ethylene glycol, ammonium nitrate, potassium nitrate, and water [5]. However, as a result of the subsequent examination of the cadavers, it was seen that the Thiel solution caused degenerations in muscle tissues. It has been revealed as a result of various studies that the cause of these degenerations is boric acid [12, 13, 14].

For this reason, in this study, it was investigated by using color, odor, microbial growth, and histological methods whether the muscle tissues fixed in formaldehyde in the non-acidic form of boron could be stored and solve the degeneration problem.

## 2. Material and methods

The present study was carried out on beef muscle tissue taken from the slaughterhouse. In the study, sodium tetraborate decahydrate (Sigma S9640) and 10% formaldehyde solution in 2 different concentrations (1% and 5%) were prepared. The muscle tissue was sliced into 21 parts and placed in 10% formaldehyde for fixation. One week later, the slices were removed from the formaldehyde solution, and 7 parts of muscle tissue were kept in 1%, and another 7 parts of muscle tissue were kept in 5% borax solutions. The remaining 7 pieces were kept in the same formaldehyde solution. The solutions were kept at room temperature and removed after 40 days for visual texture examination. The cadavers were photographed and documented, and they were also examined in terms of odor. Later, the muscle slices were sliced, subjected to routine histological tissue follow-up, and embedded in paraffin. Sections of ~5 microns were taken and stained with Hematoxylin dye and examined with a light microscope in terms of possible degenerations in muscle tissues. The width of the nearly 100 muscle bundles was measured on the cross-sections with M-Shot digital imaging system.

Meanwhile, microbiological examinations were carried out on the solutions. The 1 ml of samples were added to 9 ml of sterile peptone water. Then, serial dilutions were prepared from a 1:10 diluted sample, and sowing was done instead of the medium. In the samples, total aerobic mesophilic bacteria count [15], the count of psychrophilic bacteria [16], lactic acid bacteria [17], Enterobacteriaceae [18], and yeast/mold count [19] were performed.

## 3. Results

No color change or deterioration was observed during the waiting period of the cadavers, which were removed from formaldehyde and kept in borax solutions. In the examinations made after the cadavers were removed from the borax solution, there was no visual difference from the cadavers kept in the formaldehyde solution (Figure 1). At this stage, after photographing, examinations were made as hardness by palpation. In their hardness examination, they had a slight hardness like cadavers soaked in formaldehyde. As a result of the odor examination, no bad odor related to any deterioration in the borax solution was found. However, there was a faint smell of formaldehyde from the cadavers.

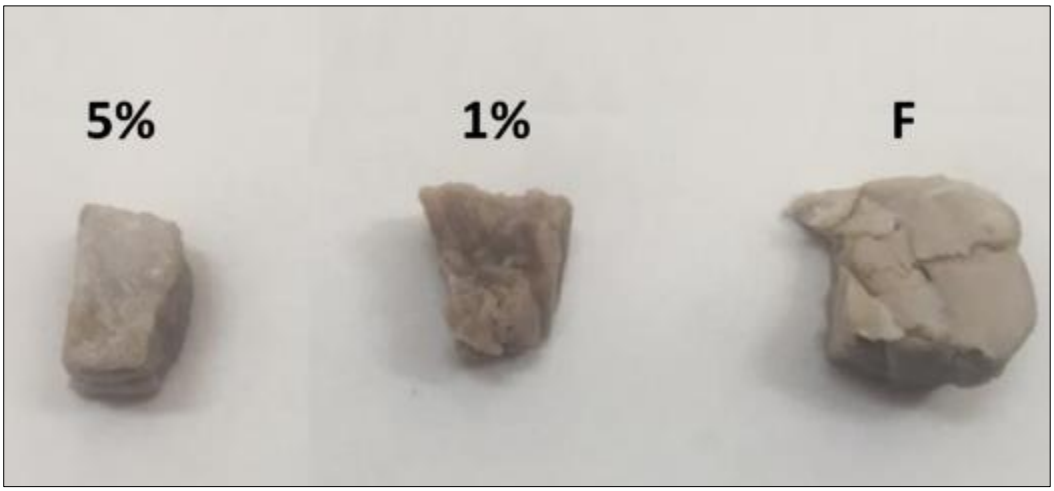


**Figure 1** Photographing the cadavers at the end of the storage process. F: Formaldehyde, 1% Borax solution, 5% Borax solution. There was no visual difference between the cadavers

As a result of histological examination of the sections of paraffin-embedded cadavers, no degenerative findings were found in terms of muscle structures among the cadavers kept in formaldehyde, 1%, and 5% borax solutions (Figure 2 and 3).

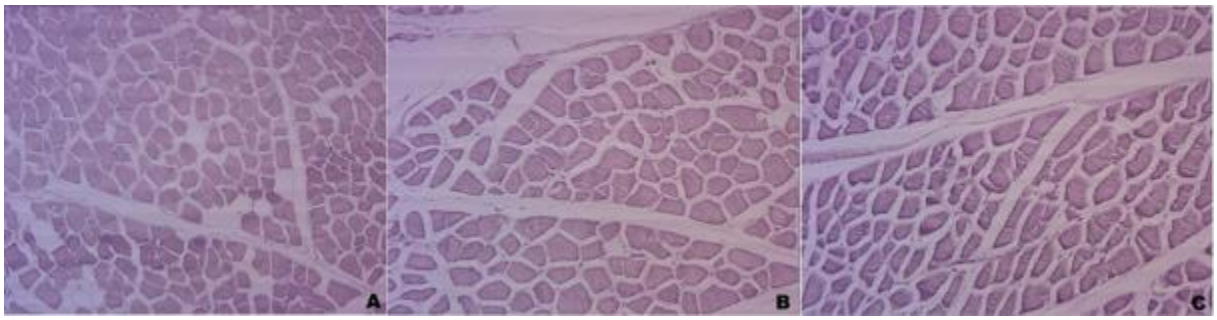


**Figure 2** Staining of samples taken from the surfaces of cadavers. No degenerative disorders were encountered (x10). A. 10% formaldehyde solution B. 1% Borax solution C. 5% Borax solution

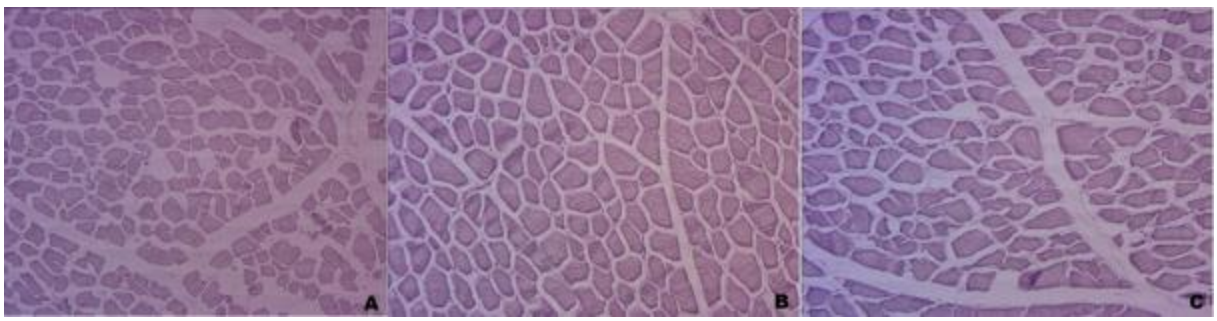


**Figure 3** Staining of samples taken from mid parts of cadavers. No degenerative disorders were encountered (x10). A. 10% formaldehyde solution B. 1% Borax solution C. 5% Borax solution

The mean width of the muscle bundles in the borax solutions is quite similar to the formalin solutions (Table 1).

**Table 1** The mean width values of the muscle bundles on the cross-sections. The mean values of the groups quite similar to each other. µm-micrometer

| Measurement place | Group | mean |
|---|---|---|
| Superficial | Formaldhyde | 82 µm |
| | %1 borax | 82 µm |
| | %5 borax | 81 µm |
| Mid | Formaldhyde | 87 µm |
| | %1 borax | 87 µm |
| | %5 borax | 86 µm |

As a result of microbiological cultivation, there was an insignificant growth in the total number of bacteria in 1% boron solution, which did not cause any degeneration. No changes were encountered in other parameters examined.

**Table 2** The result of the microbiological cultivation. The insignificant growth in the total number of bacteria in 1% boron solution was observed (cfu/ml)

| | pH | TAMBC | PBC | LAB | Enterobacteracea | Yeast/Mold |
|---|---|---|---|---|---|---|
| Control | 3.95±0.08 | $4.0x10^2$ | <1.0x10 | <1.0x10 | <1.0x10 | <1.0x10 |
| 1% | 8.78±0.12 | $8.0x10^2$ | <1.0x10 | <1.0x10 | <1.0x10 | <1.0x10 |
| 5% | 9.23±0.07 | $2.0x10^2$ | <1.0x10 | <1.0x10 | <1.0x10 | <1.0x10 |

TAMBC: total aerobic mesophilic bacteria count, PBC: psychrophilic bacteria count, LAB: lactic acid bacteria

## 4. Discussion

Examining cadavers and performing applications on them in medical schools is a very important issue, especially for branches of science such as anatomy, pathology, and surgery [5, 20]. It is an important problem for these branches of science to preserve the tissues without losing their softness, color, and flexibility as in the original cadaver [5, 21]. In this type of science, this problem has been overcome by the application of chemical called formaldehyde [5]. Thanks to its antimicrobial feature, formaldehyde can be safely detected after death in animal and human bodies, and it can be stored in formaldehyde solutions for years without spoiling, even if it hardens a little and loses color [4]. However, various problems such as reproductive problems, dermatitis, and respiratory tract problems have emerged over time in people who come into contact with formaldehyde [5, 20]. The biggest of these problems is cancer. The World Health Organization has classified formaldehyde as the number 1 carcinogen [6].

In parallel, scientists have tried different solutions [21]. The most well-known of these is Thiel's embalming solution [5]. A study conducted in 2011 revealed that Thiel solution is known in more than half of the medical laboratories in the world [22]. Unlike formaldehyde fixation, thanks to the Thiel solution, it has been reported that the tissues are closer to their original state, and problems such as color loss and hardness are not encountered [5]. However, it was later determined that various deteriorations were observed in the muscle tissues of cadavers in Thiel's cadaver solution [12, 13, 14]. One of the chemicals that make up Thiel's embalming solution is boric acid. Boric acid is an acidic form of boron and is classified as a protective agent. Due to its bactericidal and fungicidal properties, it is used safely in many areas, from agriculture to industry. As a result of in-depth examinations made by scientists, it was found that the degenerative problems that occur in muscle tissues over time are caused by the boric acid in the solution [12, 13, 14].

Thiel solution has been tried in many studies until today. In one of these studies, cadavers fixed in Thiel solution were examined for upper and lower urinary tract endoscopy training, and it was found that the ureter and urethral mucosa were very similar in color and structure to the original cadaver, and preserved all the anatomical features of tissues [23]. The usability of human cadavers fixed with Thiel solution for kidney operations in surgical training has also been investigated in various studies. As a result of these studies, it was stated that the cadavers fixed with Thiel solution are very similar to the original tissue, preserve their anatomical features, and therefore can be used in surgical training [24, 25]. Furthermore, it has been reported that in addition to Thiel fixation, intraventricular formaldehyde injection will be more functional on brain tissue in order to make the fixation made with the Thiel method in the brain tissue more

similar to the original tissue [26]. On the other hand, when the detection of muscles and tendons was examined, it was observed that the Thiel solution was unsuccessful. It has been reported that the reason for this is the problems in the collagen fiber/collagen network mechanism due to boric acid-induced denaturation. It has been stated that boric acid causes breakdown and degeneration in muscle proteins due to being a corrosive chemical [12, 14, 27].

## 5. Conclusion

In this study, the borax solution revealed beneficial preservation findings. Borax, which is the alkaline form of boron, was tried on the muscle tissue, and very successful results were obtained. When the cadavers fixed in formaldehyde were kept in borax, it was observed that there was no difference in texture such as visual and odor between them and formaldehyde. Moreover, no microscopic degenerative findings were encountered. In addition, the borax solution revealed very successful results in terms of microbial growth, and no microbial growth that could cause degeneration was seen. In conclusion, it is thought that testing the alkaline form of boron instead of boric acid in future studies may yield more positive results.

## Compliance with ethical standards

### *Acknowledgments*

This manuscript does not require IRB/IACUC approval because there are no human and animal participants. The data, information and documents presented in this article were obtained within the framework of academic and ethical rules

### *Disclosure of conflict of interest*

The authors declares no potential conflict of interest**.**

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