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Cadaver preserving methods – is it possible to do anything better?

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Abstract

It is impossible to imagine learning anatomy without properly fixating cadavers. Thanks to accurate preservation techniques, students can differentiate anatomical structures by their sight and touch. The formalin method was first described almost 150 years ago, and as such it may seem primitive, however it is effective and easy to use, which is why it is still in use today. Despite formaldehyde's bactericidal, fungicidal, and insecticidal properties contact with vapors of embalming solutions such as formalin may have a negative health effect. Skin drying, eczema, allergic contact dermatitis and lowered red blood cells (RBCs) and platelets are only a few symptoms that may occur as a result of spending long periods of time in places where cadavers are stored.

Due to formalin's features, other techniques were invented. Thiel's method is also well known; after liquid application, tissues remain their natural color as the method is non-irritating and almost odorless. With the rise of technology, more modern methods of cadaver preservation were developed as alternative to formalin, such as the use of N-vinyl-2-pyrrolidone (NVP), which is commonly implemented in Japan and Brazil, and the Modified Larssen solution (MLS), which gives the possibility to reduce formalin concentration without losing the effectiveness of tissue preservation.

The aim of this work is to develop an integrated approach to cadaver preservation through the analysis of frequently used preservation techniques. This is a considerable issue because first-year students of medicine and related faculties encounter the preserved material while learning anatomy.

In comparison to more modern methods, formalin seems to be the worst choice due to worse joint flexibility, tissue colour and structure and, most importantly, its cancerogenic action.

Running title: Cadaver preserving methods

Keywords: cadaver, cadaver preservation, formaldehyde, formalin, embalming, anatomy

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Introduction

Effective learning of anatomy in the dissecting room is based on the use of cadavers, thanks to which the presented structures have a real appearance and unchanging course. We can distinguish muscles, nerves or vessels not only by their color, but also by their macroscopic structure and our sense of touch.

The use of cadavers in teaching anatomy would not be possible without their proper preparation and preservation, because fresh specimens carry a high risk of transmitting infectious diseases and can be stored only for a short time span.

The almost archaic method based on the use of formalin solution, which appeared in the mid-1890s, is still in use [1]. It is a method that may seem to be primitive, but it is effective and easy to use, which is why it is still in use today. It is worth emphasizing that the use of this technique carries the risk of cancer development. Its scientifically proven carcinogenic effect on humans is the basis for the recommendations of many anatomical societies to reduce its use [2].

Thiel's method is also well known, its first descriptions appearing in 1992. The procedure is based on ammonium and potassium nitrate, boric acid, and ethylene glycol [3].

During the development of technology used in medicine and referred sciences, more modern methods were developed. These include the technique using N-vinyl-2-pyrrolidone, which is commonly implemented in Japan and Brazil, and the Modified Larssen solution (MLS) method, which gives the possibility to reduce formalin concentration without losing the effectiveness of tissue preservation.

The aim of this work is to develop an integrated approach to cadaver preservation through the analysis of frequently used preservation techniques, especially because of the negative impact of formaldehyde on respiratory tract [4]. The main advantage of this study the possibility to change the most commonly used method of cadaver embalming in Poland and surrounding European countries. Future investigations are necessary to validate the conclusions that can be drawn from this study.

Cadaver preserving methods

Formaldehyde method

Formalin-based solutions have been used since the discovery of formaldehyde by August Wilhelm von Hoffman in 1863. Formalin is a saturated (35-40%) aqueous solution of formaldehyde [5]. Its first description as a fixating fluid was introduced by Blum in 1893 [6]. Its simplicity in use and accessibility has made formalin-based solutions the most used preservation method [7].

Not only do the preserved cadavers need to be of high quality, but they also must be harmless to the persons exposed. Human tissues preserved with formalin-based solutions tend to have mediocre quality. Properties of fresh tissues, such as softness, flexibility and color, are diminished. Moreover, preferred imaging techniques for quick analysis of cadaver (ultrasound, radiography) are also reported to be of lower quality [8].

Despite formaldehyde's bactericidal, fungicidal and insecticidal properties [9], some opportunistic bacteria, such as *Staphylococcus epidermidis, Gardenella vaginalis, Gemella morbilorrum,* can be cultured from previously preserved cadavers by this method [10]. This may indicate that formalin may be insufficient for prevention from infectious diseases.

Apart from this, contact with vapors of embalming solutions such as formalin may have a negative effect on the health of persons in contact with formalin-treated cadavers, such as medical students, anatomy lecturers and anatomy laboratory workers.

The research work by Khaliq et al. revealed short-term effects on FVC of medical students after their first exposure in a gross anatomy laboratory [4].

Although mild transient bronchoconstriction in subjects of Khaliq's study returned to normal after twenty-four hours, decreases in dynamic lung function could occur over a longer period of time [11].

Anatomy laboratory staff members are especially endangered. Skin drying, eczema, allergic contact dermatitis and lowered RBCs and platelets counts appeared in formalin-exposed stuff employed at Alexandria Faculty of Medicine [12].

N-vinyl-2-pyrrolidone method

The method which uses N-vinyl-2-pyrrolidone (NVP) as an embalming medium has recently been reported to be a safe and effective application for cadavers' embalmment [13].

NVP solution is commercially available and sold under the name "Preserve" consisting of 100% NVP and <0.1% N,N'-dibutyl-phenylenediamine. NVP was also described by Haaf in 1985 [14] as an organic compound with uses in cosmetics and artificial teeth. Tissue fixative properties of NVP are an effect of intracellular water replacement and polymerization into PVP (poly-vinylpyrrolidone) [15].

Diluted Preserve solution was infused into cadavers' arteries using a peristaltic pump [16]. The final concentrations of NVP were 10% of the cadaver's body weight, apart from the first study [13], where concentrations were either 4.0%-5.5%, 10.0%-10.5% or 21.5%. Following infusion, the bodies were immersed in 30% ethanol solution [14,15] or 5% "Preserve" solution [13], sealed in a plastic bag and stored at 4 °C or room temperature.

The NVP method allowed for long-term preservation for up to 37 months with no signs of decomposition [17].

Cadavers following NVP fixation appeared intact, tissues remained soft and pliable, comparable to the

living tissues, with only the keratinous layers of the skin partially peeled off, subcutaneous fat mostly missing and the orbit, intercostal spaces and abdomen depressed [13,17]. Moreover, the range of motion of joints was similar to physiological and significantly greater than in the formalin-fixed cadavers, with no resistance during movement.

Subcutaneous tissues, such as tendons and ligaments, blood vessels, nerves and visceral organs, such as heart, lungs and intestines were easily dissected or manipulated, pliable, elastic and identifiable [15-17].

Additionally, the velocity of airflow through excised larynx and vocal fold vibrations were equivalent to living humans, resulting in voiced sounds produced by NVP-embalmed larynges [18].

Unfortunately, the brain tissue was described as too soft and easily damaged when manipulated. Also, in some cases, soft tissues become harder than expected for unknown reasons [15].

Thiel's method

In 1992, Walter Thiel published an innovative method for embalming bodies. Since then, this method has been improved by the author [19] and also by other researchers [20-22]. The Thiel method consists of basic, tissue-specific and container solutions with the main ingredients being ammonium and potassium nitrate, boric acid, ethylene glycol. First, the corpse is injected with a basic and tissue-specific mixture. Second, it is placed in a container solution for 2-3 months of submersion. Afterwards, cadavers can be stored submerged in containers or in plastic bags [3,20].

The main advantage of Thiel's method is the „lifelike" appearance of preserved tissue. Essentially its realistic color, composition and permanent malleability makes this method unique. Furthermore, the product is non-irritating and almost odorless [21-24]. Blood vessels are well-preserved, flexible and collapsed. Additionally, infusion of blood vessels with colored reagents impacts higher visibility and solidity of small arteries [25,26].

In addition, this technique proved its usefulness for teaching surgical skills such as those used in urological, thyroid and oral surgeries, thanks to the increased flexibility and realistic tissue appearance, in contrast cadavers embalmed in formalin [24,27].

However, some studies note that while macroscopic presence is boosted, the microscope investigation shows some connective tissue damage. Also, the preservation of peripheral nerves might be worse whereby they stand out less compactly [21,22,26]. Furthermore, Thiel's method is a high-maintenance solution; there are a lot of chemical substances needed due to its multi-step preparation. As well, this method requires experienced personnel and an adequate work environment to perform. Hammer et al. and Niels et al. note that agents used in this method are incendiary, corrosives, toxins or oxidative reagents - generally harmful for humans. Additionally, this fact makes the reagents hard to store safely [3,20].

Modified Larssen Solution (MLS) method

In accordance with the current trend of progressive resignation from the use of 10% formalin (F10) for fixing cadavers in favor of other methods, e.g., mentioned in the article, the modified Larssen method should be mentioned. In addition to the widely-used Thiel method it is possible to find descriptions of an interesting method that has one basic advantage – its price.

The MLS method was first described by Sampaio in 1989 [28]. The original recipe was modified in subsequent years by Guimaraes da Silva et al. [29] by adding glycerol and reducing the concentration of formaldehyde, which is significant during long exposure while in dissection room.

The former solution of Sampaio from the Hospital Cochim in Paris was composed of 500 g sodium chloride, 900 g sodium bicarbonate, 1000 g chloralhydrate, 1100 g sodium sulfate, and 500 mL of F10 and 1L distilled water. Sampaio used one part of this solution with five parts of distilled water [28]. Guimaraes da Silva's modification was prepared by mixing 100 mL F10, 400 mL glycerol, 200 g chloral hydrate, 200 g sodium sulfate, 200 g sodium bicarbonate, 180 g sodium chloride and 2 L distilled water. The working solution was made from one part concentrate and three parts distilled water, mixed at room temperature and stored. [29,30].

Immediately after saline perfusion, the vascular system was cleansed with a modified Larssen solution (volume equal to about 5% of cadaver weight). Then the jugular vein was ligated and a volume of modified Larssen solution corresponding to 10% of cadaver weight was perfused by gravity flow (10–15 mL/min) to preserve the cadaver; the carotid artery was ligated at completion. After fixation and between each class use, each cadaver was stored in a plastic bag. These were hung from iron S-hooks, in a walk-in freezer and were stored an average of 4 months. [28-30].

The use of this solution was described in 2004 at the University of Sao Paulo [29] during preparation of dog cadavers for practical surgical skills. For a year, the authors of the study made 97 preparations, each of them was used for training from 1 to 4 times. None of them lost flexibility in the joints, which is important when simulating a surgical procedure. Desquamation was present on some cadavers after passing 2-4 weeks.

Okan and Servet [30] have extended previous courses and provided cadavers fixed by the MLS technique for 252 trainees. Throughout the course, comparisons were made of these cadavers with the standard F10 fixation technique. Skin color re-

mained the same after MLS perfusion. Moreover, deep tissues such as muscles, fascia, nerves and vessels also had a realistic appearance. No disturbing and irritating odor with F10 was observed in this method. Joint flexibility was similar to previous courses described in papers, meaning the MLS method was significantly better than F10 – e.g., 140 degrees for elbow flexion for MLS to 30 degrees for F10 [30].

Comparison of two different concentrations of MLS (10% and 20%) showed no significant differences [31]. Both ensure good preservation of tissues without signs of putrefaction.

Discussion

It is worth paying attention to cadaver embalming methods because first-year students of medicine and related faculties come into contact with the preserved material while learning anatomy. Therefore, the use of specific techniques should not result from patterns established over the centuries, but a deliberate and well-planned action.

The method using a 10% formaldehyde solution seems to be the easiest to use. A popular explanation is that formalin is cheap, but actually only makes up a small percentage of total cadaver supply [20]. Unfortunately, many universities are not adapted to the use of innovative methods of cadaver embalming, which is why methods adequate to the equipment possessed are used.

It cannot be forgotten that the International Agency for Research on Cancer classifies formalin in 'group 1', i.e., cancerogenic to humans [32].

The methods mentioned in the article, such as the Thiel method or MLS, can effectively replace the well-known F10. The implementation of these methods will not only reduce the harmful effects of formalin but will also allow to achieve results otherwise impossible to obtain with formalin.

Conclusions

From the brief overview above, the key findings emerge: Formalin solution should be replaced whenever possible by alternative methods due to its harmful effects on the human body. These techniques, such as N-vinyl-2-pyrrolidone, the Thiel method and Modified Larssen technique offer many advantages such as joint flexibility, color and tissue texture. These characteristics lead us to suspect the use of formalin in upcoming years.

Ethical approval
The conducted research is not related to either human or animal use.

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Conflict of interest
All authors declare that they have no conflict of interest.

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