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Review Article

Embalming with Formalin – Benefits and Pitfalls

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Abstract

Formalin is the most widely used preservative in anatomical embalming. It produces consistent results in terms of the prevention of autolysis and putrefaction at an affordable cost. It is a known carcinogen having several health hazards. Strict adherence to safety protocols is required to minimize its toxic effects. Regular monitoring of formaldehyde concentration in the dissection hall and embalming room will ensure remedial measures at the earliest. In the face of growing health concerns, several alternative preservatives are being studied. Apart from it, methods like cryopreservation, plastination can be used instead of embalming. Post-mortem toxicological analysis of formalin-fixed cadavers is of importance for forensic investigation. This article will review the overall advantages and disadvantages of formalin and its alternatives in the process of embalming and will guide the anatomists in choosing the proper options for the purpose.

Keywords: Formalin, Embalming, Preservative, Toxicity, Safety.

INTRODUCTION

Embalming is the process of artificial preservation of the human body following death. It entails the treatment of the dead body with a certain chemical solution (i.e. embalming fluid) which reduces the presence and growth of microorganisms in the body, temporarily halts organic decomposition by either autolysis or putrefaction, and restores the dead human body to a presentable physical appearance [1].

Indications for embalming

The indications for embalming are as follows:

1. When the body has to be transported to distant places for final disposition.
2. When the body is to be showcased for a funeral ceremony.
3. Preservation for academic purposes in the anatomical laboratory [1].

Desirable properties of an ideal embalming fluid

The properties of the ideal embalming fluid are as follows:

1. It should preserve the structures of organs and tissues for the long term with minimal shrinkage or distortion.
2. It should prevent excessive hardening while retaining flexibility and suppleness.
3. It should prevent tissue from desiccation or drying out.
4. It should resist fungal or bacterial infection.
5. It should have minimum chemical and environmental hazards.
6. It should preserve tissue and organ colour while minimizing discoloration [2].

General components of embalming fluid

The embalming fluid is made up of a combination of chemicals which includes preservatives, germicides, buffers, wetting agents, anticoagulants, dyes, vehicles, humectants, perfuming agents, and fungicides.

1. Preservatives: They react with cadaveric tissue proteins and form a cross-linked product that resists autolysis and putrefaction. E.g. Formalin.
2. Germicides: Kill microbes ie bacteria, viruses,

spores. E.g.: Phenol.

3. Buffers: They stabilize the pH of both embalming fluid and tissues are called buffers. E.g. Sodium borate.
4. Wetting agents: Lowers high surface tension of water & facilitates penetration and distribution of embalming fluids. E.g. Glycerine.
5. Anticoagulant: They maintain blood in a liquid state. E.g. Sodium citrate, sodium oxalate
6. Dyes: They simulate the natural coloring of tissues. E.g. Tetra bromo fluorescein (eosin).
7. Vehicle: Solvents that keep the ingredients in solution during passage through the vascular system of the body. E.g. Water.
8. Perfuming agents: Masks foul odour. E.g. Methyl salicylate (oil of wintergreen).
9. Fungicides: Prevents fungal growth. E.g. Thymol [1].

These groups of chemicals are combined in various proportions to produce the pre-injection, arterial, cavity, and cloth fluids. All of them contain formalin as a preservative. The formulation for the preparation of embalming fluid varies from institution to institution and also on other factors [2].

Pre-injection fluids

About 4–5 liters of pre-injection fluid is injected with drainage kept open to displace the blood from the vascular system.

Arterial fluids

About 10 liters of Arterial fluid is Injected into the vascular system through femoral arteries or the internal carotid artery until froth comes out of the natural orifices. The formal in strength ranges from 10 to 70 percent depending upon conditions such as dehydration, obese or edematous bodies or special conditions such as refrigerated bodies, burnt bodies, infants, and so on (Table 1 and Table 2) [2].

Table 1: Components of 1 litre of arterial fluid used for obese subjects

Preservative	Formalin Methanol	10% 55%
Anticoagulant	Sodium citrate	15 gram
Buffers	Sodium borate	15 gram
Vehicle	Water	15%
Wetting Agent	Glycerine	15%
Germicide	Phenol	5%
Dye	Eosin 1%	5 ml
Perfume	Oil of wintergreen	10 ml
Fungicide	Thymol	Few Crystals.

Table 2: Components of 1 litre of arterial fluid used for thin subjects

Preservative	Formalin Methanol	10% 55%
Anticoagulant	Sodium citrate	15 gram
Buffers	Sodium borate	15 gram
Vehicle	Water	10%
Wetting Agent	Glycerine	20%
Germicide	Phenol	5%
Dye	Eosin 1%	5 ml
Perfume	Oil of wintergreen	10 ml
Fungicide	Thymol	Few Crystals.

Cavity fluids

About 2 liters of cavity fluids is injected into body cavities.

Tank (immersion) fluid

It is used for immersing cadavers. Cetrimide can be added as anti-fungal to the tank solution. (Table 3).

Table 3: Components of 1 litre of tank fluid used for thin subjects

Preservative	**Formalin**	**15%**
Vehicle	Water	60%
Wetting Agent	Glycerine	20%
Germicide	Phenol	5%

Cloth fluid

Prevent drying of the area under dissection and isolated dissected part [2].

Formalin as a preservative

Preservatives are the agents which react with cadaveric tissue proteins to form an inert reaction product that resists autolysis and putrefaction for a longer period of time. Formalin (37% formaldehyde), as the commercial source of formaldehyde, is the most commonly used chemical for this purpose [1]. Commercially available formalin is 37 percent by weight or 40 percent by volume of formaldehyde gas in water. German scientist Wilhelm Von Hofmann first identified formaldehyde which is an extremely reactive colorless aldehyde gas at room temperature with an irritating pungent odor. It is highly inflammable in nature and is readily soluble in water, alcohol, and other polar solvents. Methanol is added to commercial formalin as a stabilizer to prevent its precipitation as paraformaldehyde. ln solutions with high formaldehyde concentration (>30%) formaldehyde tend to polymerize into long chains and thereby forms an insoluble white precipitate called paraformaldehyde. Formalin becomes acidic on storage due to the production of formic acid. Carbon dioxide from the air reacts with water to form carbonic acid, which when combined with formalin creates formic acid. Hence, a buffered solution of formalin is used [2].

Mechanism of action of formaldehyde in embalming
Effect on tissue and autolytic enzymes

Formaldehyde acts by cross-linking several proteins chemically by inserting a methylene bridge (-CH2-) between the nitrogen in the amino groups of adjacent proteins. The net result is the conversion of protein into a high molecular cross-linked latticework of inert solid materials that can no longer serve as food for bacteria or as a substrate of enzyme action. Autolytic enzymes being protein in nature are inactivated by the same mechanism. Other terms used to describe this include fixating, denaturing, and coagulating of protein [1, 2].

Effect on bacteria

Formalin achieves its bactericidal effects by cross-linking of either proteins or proteins with DNA in bacterial cell membrane and protoplasm. It results in cellular dysfunction and cell death. Formaldehyde is bactericidal, fungicidal, and insecticidal (in descending efficiency) and to some extent inactivates bacterial spores of some species. Formalin embalmed bodies may be affected by moulds or maggot infestation as it is less effective against them [1, 2].

Advantages of formaldehyde

The advantages of the formalin as an embalming component are as follows:

1. Completely biodegradable.
2. Inexpensive and exert germicidal action on a wide range of microorganisms.
3. Acts quickly.
4. A relatively small amount of formalin can preserve a large amount of tissue.
5. Formalin prevents the smell of body amines formed during putrefaction [1, 2].

Disadvantage of formaldehyde

1. Unpleasant irritating odour.
2. Hardening of tissue.
3. Discolorations of body parts
4. Formation of formic acid
5. It is a carcinogen [1, 2]

Formalin index

Embalming fluid is said to have a formaldehyde index, N, when 100 mL of fluid contains N-grams of formaldehyde gas at normal room temperature. The Occupational Safety and Health Administration (OSHA), formaldehyde standard, states that the acceptable exposure limit (PEL) for FA is 0.75 ppm of air measured as an 8-hour time-weighted average. A second PEL is included in the standard in the form of a short-term exposure limit of 2 ppm, which is the maximum exposure permitted over 15-minutes period [1]. Formaldehyde emission was measured during the course of dissection. The highest value was recorded during skin opening, followed by dissection in the subcutaneous plane. Emission level while muscle dissection came next. The lowest value was recorded when the inner body cavities were opened after about a few months [3].

Toxicity

In recent times there is an increasing awareness regarding the potential health hazards due to high-level formalin exposure in the workplace. At-risk groups are Medical students, academic and non-academic staff at the anatomy department. The route of exposure is primarily via the inhalation of formalin fumes present in dissection and embalming rooms. Another is by direct contact of formalin with skin or mucous membranes while handling the cadaver.

Effects of acute exposure

Irritating strong unpleasant odour, Running nose or rhinorrhoea, blocked or congested nose, redness of the eye, itching of the eyes, visual disturbance, excessive lacrimation or epiphora, dryness of nasal and pharyngeal mucosa, sore nose, sore throat, respiratory distress, heaviness in the head with visual disturbances, headache, nausea, syncope dizziness, abnormal fatigue, asthenia, cutaneous manifestations on exposed areas, gastrointestinal discomfort, and sleep disturbances [4-10].

Complications from chronic exposure

Skin disorders: White discoloration, drying, burning, erythema, edema, cracking, scaling, eczema, allergic contact dermatitis.

Reproductive disorders: Menstrual disorder, anaemia, spontaneous abortions, low birth weight babies, congenital anomalies.

Ocular disorders: Visual disturbances, discomfort, irritation of eyes, corneal clouding, blindness.

Airway disorders: Respiratory tract irritation, bronchial asthma. Gastrointestinal disorders: Nausea/vomiting, abdominal spasms, hemorrhage, gastric outlet obstruction (last two are late complications) [4-10].

The Carcinogenic potential of formaldehyde: In humans, a few types of malignancies have been associated with formaldehyde exposure like nasopharyngeal carcinoma, carcinoma of paranasal sinuses, leukemias (mainly myeloid), lymphomas, and cancer breast, cancer skin. The formaldehyde has been labeled as a cancer-causing agent by International Agency for Research on Cancer, in June 2004 [14].

Safety Measures

1. Mandatory wearing of personal protective equipment ie hand gloves, to prevent direct contact. Gloves should be changed often.
2. Wearing double gloves is highly recommended. Safety goggles, face masks, and lab aprons should be worn to protect the eyes, nose, mouth, and other parts of body from formalin fumes and accidental splashes.
3. Adequate number of Eyes and Hand Washing Stations should be installed in the dissection halls.
4. Taking out the cadavers from storage tanks at least 30 minutes prior to starting dissection will avoid exposure to peak levels.
5. Selective exposure of only that part of the body that is being dissected and periodical removal of fluid dripping collected in the body tray will help in minimizing the toxic effect of formalin.
6. Optimizing storage: Containers should be properly and distinctly labelled so as to prevent the unnecessary opening of them to take out cadavers or specimens.
7. Proper storage of Formalin in air-tight container. The lids of storage tanks should be tightly closed.
8. Leak prevention. Protective caps should be put on open connectors to prevent leakage of Formalin by dripping from the tap of the storage tank.
9. Cadavers taken out for teaching should be placed on a disposable sheet to collect the residual fluid draining from the specimen.
10. Ensuring adequate general ventilation through the planned construction of anatomy laboratory and dissection hall with regards to the number and location of windows and doors along with the provision of cross-ventilation. As formaldehyde is heavier than air, exhaust systems located at or near floor level, when combined with the introduction of uncontaminated air from the ceiling level, are an efficient method of ventilation.
11. Spilled formalin is absorbed in wet paper/fabric towels. Eliminate absorbed formalin towels in closed bags. Mop the floor with soapy water. Humidify the space of the room.
12. Installation of local exhaust ventilation with Perspex containment should be fitted.
13. Eating, drinking, and smoking in the workplace should be strictly prohibited [11-16].

Chemical Method of Reducing Formaldehyde emission from embalmed cadaver

Further reduction of formaldehyde exposure by treatment the formalin embalmed with a chemical agent which will capture formalin include Monoethanolamine (MEA)/Potassium permanganate or Sodium metabisulfite InfuTraceTM, solution [11-16].

Formaldehyde monitoring

Indoor formaldehyde should be monitored in

1. Cadaver storage room
2. Dissection theatre

Methods of monitoring airborne formaldehyde by portable direct-reading instruments for on-site analyses

1. Photometry 2. Fluorimetry 3. Electrochemical devices 4. lnfrared spectroscopy 5. Cavity ring-down spectroscopy 6. Mass spectrometry [17-27].

Low-formalin embalming technique

Using low concentration formalin (5–10%) instead of higher strength resulted in better preservation of cadavers in terms of retention of colour, maintenance of consistency of structures and keeping the luminal structures intact. It also reduced the exposure to formalin fumes to the faculty, staff, and students. Further reduction of the amount of applied formaldehyde below 1.5 percent endangers the quality of fixation and increases the risk for infections. [28,29]

Thiel's soft embalming solution

Professor W Thiel formulated an injection and an immersion solution using a minimum concentration of formaldehyde. In his method, the cadaver was first administered an intravascular injection of the injection solution followed by submersion in the immersion solution for a stipulated period. After it the cadaver is stored in a sealed container outside the tank, without preservative fluid.

The advantage of his method was that the cadavers were devoid of foul odor. The movement of body parts was very smooth and joints were very mobile. The color retention was excellent, the peritoneal cavity was very inflatable and can be used

for teaching and learning laparoscopic procedures (Table 4) [30].

Table 4: Basic composition of injection and immersion solutions (Thiel W, 1992)

Solution	Chemical	Quantity
Solution A		
	Boric acid	3 g
	Ethylene glycol	30 ml
	Ammonium nitrate	20 g
	Potassium nitiate	5 g
	Hot water	100 ml
Solution B		
	Ethylene glycol	10 ml
	4-chloro-3-methylphenol	1 ml
Injection Solution		
	Solution A	14300 ml
	Solution B	500 ml
	Formaldehyde	300 ml
	Sodium sulfate	700 g
Immersion Solution		
	Ethyleneglycol	10 ml
	Formaldehyde	2 ml
	Solution B	2 ml
	Boric acid	3 g
	Ammonium nitrate	10 g
	Potassium nitrate	10 g
	Sodium sulfate	7 g
	Hot water	100 ml

Risk of disease transmission even in formalin embalmed cadavers

Even the fixed cadavers do pose infection hazards to the handlers due to inadequacy of fixatives to inactivate the following infectious agents such as Mycobacterium Tuberculosis, Hepatitis B and hepatitis C, AIDS virus/HIV [31].

Formalin alternatives

Due to growing concern regarding the health hazards of formaldehyde, a safe and effective to it is being researched all over the world. Below are some proposed substitutes for formaldehyde.

1. Phenoxyethanol, based fixation ("Crosado" technique) provided a satisfactory level of muscle flexibility, softer tissue consistency, and better joint mobility as compared to formalin which causes extreme rigidity. Moreover, it is free from intrusive smell, unlike formalin. Limitations of the use of phenoxyethanol seem to be the requirement of a large amount of chemicals and its high pricing [32-36].
2. Natekar and DeSouza (2014) have described a soft embalming solution comprising of glutaraldehyde, water, methyl alcohol, glycerin, cetrimide, eosin, and eucalyptus oil. Glutaraldehyde is active over a wide pH range. It acts as a broad-spectrum disinfectant compared to formaldehyde [1, 31].
3. Hammer *et al*., (2012) reported a formaldehyde-free solution system consisting of ethanol, glycerine, and thymol. The ethanol-glycerine fluid is used as arterial fluid; following that, the corpses are submerged in ethanol. To preserve the condition of fixation at room temperature, a thymol-ethanol solution is utilized as a moistening solution [37].
4. Shi *et al*., (2012) suggested a solution which is a blend of acid, buffer solution, and cross-linking agent, Tetrakis (hydroxymethyl) phosphonium chloride, which acts as fungicide, stabilizer and fixative, respectively [38].
5. Recently, Al-Hayani *et al*., (2011; Bedir, 2009), suggested the use of shellac, a natural polymer derived from the hardened secretion of the lac insect(s). The soft parts were very flexible but there was slight brownish discolouration of the skin [39].
6. Polyhexamethyleneguanidine hydrochloride was used as an efficient preservative agent in place of formalin, by Anichkov *et al*., (2010, 2011) [40].
7. Goyri-O'Neill *et al*., (2013) recently published a study with an embalming solution combining diethylene glycol and mono ethylene glycol. This embalming solution produces good short-term preservation quality at the macroscopic level (up to 6 months). A histological

examination one month after injection demonstrated the greatest outcomes for a striated thigh muscle; the skin was likewise well maintained, but the buccal mucosa was not as well conserved [41].

8. N-vinyl-2-pyrrolidone (NVP) has been identified as a new substitute for formalin. NVP embalmed corpses successfully prevented decomposition and fungal proliferation. The bodies were supple and flexible and exhibited a wide range of motion in the glenohumeral, cubital, radiocarpal, interphalangeal, hip and temporomandibular joints compared to formalin-fixed cadavers. The subcutaneous fat was significantly decreased under the dermis, and the connective tissues were transparent, allowing the ligaments, cutaneous nerves, and veins to be clearly identified. The abdominal wall and visceral organs remained supple and elastic, similar to fresh cadavers. The lungs, liver, and gastrointestinal tract were easily separated and mobile in the thoracic and abdominal chambers [42].
9. Ionic liquids: They refer to a category of organic salts which remain liquid at room temperature. Tissues preserved in ionic liquids consistently demonstrated intact histological features, unaltered specimen colour, a mild pleasant smell, and no shrinkage. Moreover they did not emit toxic vapours and hence do not contribute to an occupational hazard [43].
10. Green or natural embalming fluids: They are composed of oil ingredients derived from gums and plant resins, a variety of spices, and certain alcohols used as vehicles for these preservatives; These embalming fluids do provide good preservation for 3–5 days, or possibly a week or longer but do not meet the need for anatomical preservation [1].
11. Cryo-preservation: Unembalmed cadavers can be preserved in deep freezers at -20 degrees Celsius for surgical dissections. Few colleges have such cadaveric laboratories equipped with deep freezing gear. The cadavers are then preserved at -4 degrees before the surgical workshops, at which point they are dissected at normal temperature. These unembalmed cadavers, which lack preservatives, cannot be used for anatomic dissections and must be disposed of by fire or deep burial [31].
12. Plastination It is a process of long-term permanent preservation of biological specimens in a life-like state. Fluids present in the biological specimen i.e. fat, water are replaced by synthetic materials such as silicone rubber, epoxy resin or polyester. The resultant plastination specimen is dry, odourless, durable, non-toxic, easily handleable, and serves as a unique teaching tool for anatomy, pathology, radiology, and surgery [44].

Toxicological analysis of formalin-fixed or embalmed tissues

When fresh biological specimens are unavailable, the study of frozen or embalmed tissues can make a substantial contribution to the forensic investigation of a case. Toxicologists should constantly keep in mind that the observed drug concentrations in embalmed and preserved tissues may change greatly from the original drug concentrations. Furthermore, various chemical compounds can be discovered as a result of the parent drug or substance's disintegration or change. In any event, a positive toxicological analysis points to exposure or consumption of a drug or substance before death and should be interpreted with caution.

A negative outcome, especially if it occurs outside of the time range of the toxin or drug by which it remains stable should not be considered. The stability of pharmaceuticals in formalin solution and the detection of reaction products are important topics in forensic toxicology that must be addressed throughout a forensic case investigation [45].

CONCLUSION

Regardless of its toxic effects, formalin remains a popular choice of tissue fixative because of its effectiveness, low cost, and consistent results. The students as well as the person involved in process of embalming should be taught regarding hazardous effects and methods to minimize them. We recommend a through follow up on the above mentioned preventive measures and precautionary guidelines so as to decrease chemical health hazards.

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