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(71) Applicant and
(72) Inventor: **DUNPHY, Brian, W.** [US/US]; 235 Castletown Road, Timonium, MD 21093 (US).

(74) Agents: **ROTHWELL, G., Franklin** et al.; Rothwell, Figg, Ernst & Manbeck, P.C., 1425 K Street, N.W., Suite 800, Washington, DC 20005 (US).

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(54) Title: FORMALDEHYDE-FREE AQUEOUS TISSUE PRESERVATION COMPOSITIONS

(57) Abstract: This invention relates to tissue preservative compositions which do not contain formaldehyde (a known carcinogen) or glutaraldehyde, and which also do not contain alcoholic solvents or compounds such as acetic acid which pose hazardous waste disposal problems and potentially are irritants to persons exposed to tissue preservative compositions in their work. Three compositions useful for embalming human remains and a composition for use in histological preservation are disclosed. The compositions disinfect and preserve animal tissues and remains, yet avoid the use of dangerous or potentially dangerous and noxious compounds.

FORMALDEHYDE-FREE AQUEOUS TISSUE PRESERVATION COMPOSITIONS

BACKGROUND OF THE INVENTION

1. Technical Field

This invention relates to the field of tissue preservation compositions used in mortuary science and taxidermy for the preservation of animal remains, including human remains.

2. Description of the Background Art

Since the turn of the century, practitioners of mortuary science and taxidermy have relied upon formaldehyde in high concentration to preserve and disinfect human and animal remains. During this same period, histologists and anatomic pathologists likewise have used solutions containing formaldehyde, albeit in much lower concentrations, to preserve tissue for examination. While formaldehyde is an excellent tissue preservative which disinfects and preserves tissue (primarily by cross-linking proteins) and is both readily available and inexpensive to use, repetitive exposure to high levels of formaldehyde vapor has been linked to cancer, particularly nasopharyngeal cancer and some brain cancers and leukemias. In light of such findings, workplace formaldehyde vapor exposure has become subject to regulation by government regulatory authorities including the U.S. Department of Labor and the Occupational Health and Safety Administration.

Workers in mortuary science settings, including embalmers, handlers of human remains and students in a variety of settings, as well as practitioners of taxidermy are routinely exposed to very high concentrations of formaldehyde vapors. Whereas in anatomic pathology laboratories and the like, where pathologists,

researchers and students may be exposed to formaldehyde-containing solutions or vapors (formalin) containing between 3.5% to 4.0% formaldehyde in water, embalmers and other mortuary science workers using traditional tissue preservation compositions are exposed consistently to vaporous formaldehyde in concentrations of over 25% and up to near saturation in water (40%). Repeated exposure to such levels of formaldehyde vapors has been associated not only with carcinogenesis but with other syndromes, including a pulmonary pathologic condition known as embalmer's lung.

In more recent years, alternatives to formaldehyde have been proposed, in particular 1,5 pentanedial, more commonly referred to as glutaraldehyde. See, for example, U.S. Patent No. 3,912,809. As is the case with formaldehyde, glutaraldehyde is an effective tissue preservative and disinfectant. The vapors of glutaraldehyde, however, are quite noxious, and its safety concerning human exposures in high concentrations or as a possible human carcinogen has not yet been established.

In addition to the dangers of formaldehyde to workers exposed to it, however, tissue preservative solutions such as embalming fluid contain other ingredients, in particular volatile ingredients such as alcohol and acetic acid which not only may have unpleasant odors but are considered hazardous materials for disposal and environmental health purposes, increasing the costs of using these compositions.

Thus, a need exists for an improved tissue preservation compositions that will satisfactorily preserve human and animal remains, and prevent and/or control the growth and propagation of pathogenic microbes in tissue, and that will at the same time greatly reduce, if not eliminate, the environmental workplace hazards and disposal problems associated with currently available tissue preservative compositions. Accordingly, there is a need in the art for tissue preservative compositions and methods of

tissue preservation which are free of the toxic and other hazardous materials problems associated with formaldehyde- and glutaraldehyde- containing compositions and composition relying on alcohol solvents or harsh, volatile acids, such as acetic acid, but which also are effective at preserving animal tissues.

SUMMARY OF THE INVENTION

The present invention therefore provides a formaldehyde-free tissue preservative composition which comprises ethanedial and a polar aprotic solvent in aqueous solution, wherein said composition does not contain acetic acid and does not contain alcohol. Also disclosed are such compositions which further contain one or more of a polymer such as polyethylene glycol, a humectant such as ethylene glycol, propylene glycol or glycerol, an antimicrobial agent such as 1-hexadecylpyridinium chloride, a chelating agent such as EDTA, a buffer or buffer system and a surfactant. In preferred compositions the polar aprotic solvent is DMSO.

Most highly preferred compositions comprise the following ingredients:

1. 1. sodium alkyl sulfonate (2%)
   2. ethanedial (14%)
   3. EDTA (0.08%)
   4. DMSO (0.0275%)
   5. protease enzyme (Papain) (0.03%)
   6. water (83.9%)

2. 1. ethanedial (28%)
   2. polyethylene glycol (2.5%)
   3. ethylene glycol (0.3%)
   4. 1-hexadecylpyridinium chloride (0.06%)
   5. DMSO (0.275%)
   6. EDTA (0.08%)
   7. anhydrous monosodium phosphate (0.4%)
   8. anhydrous disodium phosphate (0.65%)

9. methyl paraben (0.06%)
10. propyl paraben (0.02%)
11. water (67.2%)

C. 1. ethanedial (5.0%)
2. DMSO (0.0275%)
3. 1-hexadecylpyridinium chloride (0.007%)
4. methyl paraben (0.075%)
5. propyl paraben (0.025%)
6. water (94.9%)

or

D. 1. ethanedial (3.75%)
2. polyethylene glycol (2.5%)
3. ethylene glycol (0.75%)
4. DMSO (0.0275%)
5. anhydrous monosodium phosphate (0.4%)
6. anhydrous disodium phosphate (0.65%)
7. water (91.9%)

The invention also provides methods for embalming an animal body which comprise administering composition(s) such as those described above to the body. Preferred methods include administering one or more of compositions A, B, and C listed above to the body. The most preferred method involves administering a composition A, composition B and composition C, in that order, to the body.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The tissue preservative compositions of this invention include a pre-injection fluid, an arterial injection fluid and a cavity fluid. A tissue preservative composition useful for histology also is provided. Each of these fluids is free of formaldehyde and/or glutaraldehyde and of acetic acid and alcohol, and relies instead on aqueous solutions of ethanedial (also referred to as glyoxal) which includes a small amount of a polar aprotic solvent such as dimethylsulfoxide for tissue preservation.

Preferred methods for preserving animal, including human, remains according to the invention involve sequential or simultaneous use of three different compositions, a pre-injection fluid composition, a tissue preservation composition and a body cavity composition. Each solution contains an aqueous solution of ethanedial, a stable aldehyde, and an aprotic solvent, preferably dimethylsulfoxide (DMSO).

The pre-injection fluid comprises an aqueous solution containing ethanedial a polar aprotic solvent, a proteolytic enzyme, a surfactant and, optionally a chelating agent. Pre-injection fluids generally function to clear and cleanse the circulatory system in preparation for the administration of the tissue preservative composition and are contacted with the remains to be preserved prior to an arterial tissue preservation composition. The tissue preservative composition (also referred to herein as an arterial injection fluid when for use in preserving human remains in the field of mortuary science) of the invention is an aqueous solution containing ethanedial, a long-chain polymer and a polar aprotic solvent. This tissue preservative solution may be optimized for preservation of bulk tissues by injection into the circulatory system such as in mortuary science, through soaking of tissue as in the taxidermy arts, or for use as a tissue fixative suitable for histological use to preserve tissue samples for examination such as by a pathologist.

In the field of mortuary science as well as the other uses described above, the arterial injection or tissue preservation fluid of the invention preserves, disinfects and hardens tissue remains without the use of formaldehyde or glutaraldehyde and without acetic acid or alcohol. A body cavity fluid according to the invention is an aqueous solution containing ethanedial, a polar aprotic solvent and a quaternary ammonium surfactant. This cavity fluid preserves and hardens human organic tissue in situ or in suitable and appropriate sealed containers, and disinfects human body cavity spaces without the use of formaldehyde, glutaraldehyde, acetic acid or alcohol. Tissue preservation fluids lacking these volatile components result in a work

environment without the type of dangerous fumes and vapors associated with traditional tissue preservation and embalming fluids, and in fluids which possess fewer environmental dangers and high-cost hazardous materials disposal problems. It has been discovered that tissue preservation compositions can be highly effective, even without the dangerous and noxious components relied upon by prior art compositions.

For methods according to the invention, these tissue preservative compositions may be used collectively or individually to effect the preservation of human or animal remains and prevention and/or control of the growth and propagation of pathogenic microbes in such remains. Preferably, for embalming of human remains, the pre-injection composition will be injected into the human remains first, the arterial injection (tissue preservation) composition injected second and the cavity fluid injected last. Each of the fluid compositions preferably is provided as aqueous solutions in purified, distilled or deionized water, which may be diluted with tap water for use.

1. Pre-Injection Fluid Composition

The term pre-injection is used herein to denote a composition that typically is administered into the circulatory system of a human or animal body as an initial part of an embalming process, prior to the injection of the tissue preservation composition. The major role of pre-injection fluids is to clear clots and other obstructions from the circulatory system (primarily the vascular system), although pre-injection fluids may have and preferably do have some tissue preservation and disinfection properties. Alternatively, a pre-injection composition may be mixed with and administered at the same time as the main tissue preservation composition. In this case, the mixed composition is more properly referred to as a co-injection fluid.

The pre-injection composition according to this invention is an aqueous solution which contains a biodegradable anionic or nonionic surfactant (such as sodium lauryl sulfate or the like), a proteolytic enzyme (a protease, such as papain), a chelating agent (such as disodium ethylene diaminetetracetic acid (EDTA) and the like), a polar aprotic solvent (for example dimethylsulfoxide (DMSO)) and a stable aldehyde, ethanedial. Optionally, an antimicrobial agent such as, for example, 1-hexadecylpyridinium chloride or benzalkonium chloride, also is included. The pre-injection composition desirably has an acidic pH, desirably a pH of about 3.0 to about 5.0, preferably about pH 3.5 to about 4.5 and most preferably about pH 4.

The protease is particularly useful for aiding in the removal of fibrin deposits and clots within the vascular system of the remains. Although a variety of commercially available protease enzymes, such as any that are known in the art, may be used in compositions according to this invention, preferred compositions contain papain or a mixture of bromelain and papain. These enzymes can be derived from fungal sources and therefore provide the desirable enzymatic activity at a reasonable cost.

The pre-injection composition serves several functions as part of the process of embalming human remains. In addition to conditioning water used to physically flush vascular lumae and tissue interstices by chelating divalent cations found in some tap water, the fluid helps dissolve intravascular clots and fibrin deposits and remains, loosens atherosclerotic deposits (plaques) and cleanses the vascular system to create a patent circuit for the subsequent injection of a tissue preservative composition or compositions. Although not wishing to be bound by any particular theory, it is believed that dimethylsulfoxide assists flow of ethanedial from the tissue interstitial spaces through cellular membranes into the cells so that proteins residing within the cell membrane and intracellular proteins are

rendered insoluble and fixed. This composition also is believed to begin the process of tissue hardening through protein crosslinking. The detergent action of the surfactant in the composition accelerates the removal of blood vessel obstructions. The flushing action of the fluid further helps to remove tissue metabolic wastes, as well as exogenous substances such as therapeutic drugs, drug metabolites and chemotherapeutic agents.

Preferred pre-injection compositions contain an anionic or nonionic surfactant in amounts of about 1.0% to about 3.0% by volume, more preferably about 1.5% to about 2.5% by volume and most preferably about 2% by volume. Preferred surfactants are sodium alkyl sulfate surfactants such as sodium lauryl sulfate (SDS) and the like. Ethanedial preferably is present at about 12% to about 16% by weight of the total composition, more preferably about 13% to about 15% by weight and most preferably about 14% by weight. A chelating agent such as for example disodium ethylenediaminetetraocetic acid (EDTA) or ethyleneglycoltetracetic acid (EGTA) preferably is present at about 0.06% to about 0.10% by weight, more preferably at about 0.07% to about 0.09% by weight and most preferably at about 0.08% by weight.

Dimethylsulfoxide (DMSO) is a preferred polar aprotic solvent. The polar aprotic solvent preferably is present at about 0.025% to about 0.030% by volume of the total composition, more preferably at about 0.026% to about 0.029% by volume and most preferably at about 0.0275% by volume. A protease enzyme also is included in the composition. The amount present will depend on the activity of the enzyme. Commercial protease enzyme preparations such as papain, bromelain or a combination thereof are preferred, and may be present at about 0.02% to about 0.04% by weight, more preferably at about 0.025% to about 0.035% by weight and most preferably at about 0.03% by weight. If desired, an antimicrobial agent may be included in this composition, for

example 1-hexadecylpyridinium chloride, benzalkonium chloride or any known antibacterial agent. The remainder of the composition in preferred embodiments is water or other inert ingredients. Colorants, perfumes or any other such compounds may be included if desired. Preferably the water in the composition is purified water, distilled water or deionized water, however any water may be used so long as it does not deleteriously affect the composition.

For use as a pre-injection fluid (that is use prior to application of a separate tissue preservative composition), the solution described above is diluted 1:8 with tap water and injected into the vascular system to commence flushing. Alternatively, the composition may be co-administered with a tissue preservative solution. Preferably, undiluted pre-injection composition is added to the tissue preservative composition (see below) and the resulting mixture then is diluted with tap water as desired for administration as a co-injection fluid. The ratio of pre-injection and tissue preservative compositions in this co-injection composition can vary according to the judgement of the user, however preferable co-injection solutions contain about 1:3 pre-injection composition to tissue preservative composition. The mixed solution then preferably is diluted 1:8 with tap water to produce an injectable co-injection composition.

The pre-injection or co-injection composition may be injected into blood vessels by pressure or by gravity feed. The amount of pre-injection fluid to be used will depend upon the amount of remains to be embalmed or otherwise preserved, and other factors known in the art and considered to be routine.

2. Tissue Preservative Fluid Composition

The tissue preservative composition according to this invention functions to preserve and disinfect tissues, including

crosslinking proteins in the tissue without the use of formaldehyde. The preservative composition arrests the natural autolytic process, prevents or retards the growth of microorganisms in situ, stabilizes the tissue by crosslinking proteins and maintains tissue hydration by creating equilibration of intracellular/extracellular osmotic pressures.

Preferably, the tissue preservative composition according to this invention contains a low molecular weight humectant (for example 1,2-ethanediol (ethylene glycol), 1,2-propanediol (propylene glycol), 1,2,3-propanetriol (glycerol; glycerine) and the like, or a mixture thereof), a polymer of ethylene glycol (polyethylene glycol; PEG), a polar aprotic solvent (for example dimethylsulfoxide (DMSO) and the like), an antimicrobial agent (for example a quaternary ammonium salt antimicrobial agent such as 1-hexadecylpyridinium chloride, or benzalkonium chloride, or methyl paraben or propyl paraben and the like), a chelating agent (for example a salt of ethylene diaminetetracetic acid (EDTA) and the like) and a stable aldehyde, ethanedial.

Buffers preferably form part of the tissue preservation composition. Any suitable buffer which is capable of maintaining the working solutions of the tissue preservative composition at or near neutral pH (pH about 6.8 to about 7.2, or preferably about 7.0) are contemplated for use with the compositions and methods of the invention. Additionally, coloring agents may be added to the arterial injection fluid, if desired, to obtain the desired cosmetic effect, i.e. imparting a life-like hue to embalmed human tissue remains. A preferred coloring agent is Eosin Y dye (tetrabromofluorescein; D&C red No. 22), which may be added in an amount ranging from 0.1% to 0.5% by weight, preferably 0.25% by weight.

The polyethylene polymer (preferably polyethylene glycol) of the composition preferably has an average molecular weight above 1000, preferably above 5000 and generally preferably between 7000

and 9000. Most preferably, the polyethylene glycol has an average molecular weight of about 8000, however average molecular weights of 10,000, 12,000 or higher may be used. Commercially available polyethylene glycol preparations typically contain a distribution of polymer chains of varying length and hence molecular weights, thus referring to an average molecular weight is common. Any of the commercially available preparations are suitable for use with the compositions and methods of the invention, with the following guidelines. Polyethylene glycol polymers of substantially lower average molecular weights than 1000 generally are not rigid or solid enough to provide the desired physical properties and so are not suitable for use with the invention. Polyethylene glycol polymers having average molecular weights substantially greater than 9000, for example 10,000, 12,000 or higher may be used, but these compounds typically are more expensive than the lower molecular weight compounds and do not function substantially better. Therefore, polyethylene glycols having an average molecular weight greater than about 12,000 to about 15,000 are not preferred.

A buffer pair consisting of monobasic sodium phosphate (anhydrous) and dibasic sodium phosphate (anhydrous) at a pH of about 6.8 to about 7.2 is preferred, however any buffer with a strength capable of maintaining the working pH of the solutions at or near neutral pH may be used. A preferred antimicrobial composition for prevention of growth of molds and fungi is a 3:1 ratio mixture of methyl paraben and propyl paraben.

Preferred tissue preservation compositions contain ethanedial in amounts of about 24% to about 32% by weight, more preferably about 26% to about 30% by weight and most preferably about 28% by weight. A polyethylene polymer preferably is present in amounts of about 2% to about 3% by weight, more preferably about 2.25% to about 2.75% by weight and most preferably about 2.5%. Humectant, for example ethylene glycol,

propylene glycol, glycerol or a mixture thereof forms part of the composition, preferably in amounts of about 0.2% to about 0.4% by weight, more preferably about 0.25% to about 0.35% by weight and most preferably about 0.3% by weight. An antimicrobial composition such as 1-hexadecylpyridinium chloride or benzalkonium chloride, preferably is present is present in amounts of about 0.05% to about 0.07% by weight, more preferably in amounts of about 0.055% to about 0.065% by weight and most preferably in amounts of about 0.06% by weight. A polar aprotic solvent such as dimethylsulfoxide preferably is present at about 0.025% to about 0.03% by volume, more preferably about 0.026% to about 0.029% by volume and most preferably about 0.0275% by volume. A chelating agent such as EDTA or EGTA preferably is present at about 0.06% to about 0.10% by weight, more preferably at about 0.07% to about 0.09% by weight and most preferably at about 0.08% by weight. A buffer is present in an amount sufficient to maintain the pH of the working solution at the desired pH. Many such buffers and buffer systems are known in the art and are contemplated for use with this invention. Preferred buffers include anhydrous monosodium phosphate in amounts of 0.35% to about 0.45% by weight and most preferably about 0.04% by weight in conjunction with anhydrous disodium phosphate in amounts of 0.6% to about 0.7% by weight and most preferably about 0.65% by weight.

Preferred compositions contain a combination of methyl paraben and propyl paraben. When present, methyl paraben is desirably present in amounts of about 0.5% to about 0.7% by weight, preferably about 0.55% to about 0.65% by weight and most preferably about 0.6% by weight. Propyl paraben, when present, is desirably in amounts of about 0.1% to about 0.3% by weight, preferably about 0.15% to about 0.25% by weight and most preferably about 0.2% by weight. The remainder of the composition in preferred embodiments is water or other inert

ingredients such as colorants, perfumes and the like. Preferably, the water in the composition is purified water, distilled water or deionized water, however any water may be used so long as it does not deleteriously affect the function of the composition.

For use as an arterial injection fluid, this composition is desirably diluted 1:8 with tap water prior to injection, however the strength of the solution may be modified according to routine methods depending on the exact circumstances for tissue preservation. Arterial injection fluid generally is injected into selected blood vessels by pressure injection or by gravity feed. Alternatively, the composition is injected directly into local tissue sites by hypodermic syringe. The amount of tissue preservative fluid to be used will depend upon the amount and type of tissue to be preserved and other conventional factors. The tissue preservative composition preferably is injected into the body or tissue through a suitably large and patent blood vessel (usually an artery), while a suitably large and patent blood vessel (usually a vein) is prepared as a drain for the distributed fluid. The fluid, under mild positive pressure or by gravity flow, is distributed throughout the tissue by flow through continually subdivided vasculature to the interstitial spaces between cells. The fluid enters cells by diffusion.

Although not wishing to be bound by any particular theory, the process by which the inventive composition preserves, disinfects and stabilizes tissue in situ occurs as follows. The presence of a small amount of a polar aprotic solvent (such as DMSO) assists movement of the fluid components into the cells by increasing cellular permeability. It is believed that low molecular weight constituents of the tissue preservative composition enter the cells by traversing the cell membrane by passive osmosis.

Once across the membrane, intracellular proteins are rendered inactive and insoluble through crosslinking by the stable aldehyde of the composition, the reaction occurring between carbonyl groups of the aldehyde and the amino groups of proteins. After this reaction, the proteins are fixed and cannot be acted upon by proteolytic enzymes released by cellular lysosomes. The reaction of carbonyl functional groups of ethanedial and amino groups of proteins occurs simultaneously in proteins residing in the cell membrane so that the membrane remains intact and does not disintegrate or dissolve due to autolytic enzymatic activity. As the fixation progresses, intracellular water tends to move into the more hypertonic extracellular environment, while low molecular weight glycols such as 1,2-ethanediol, 1,2-propanediol or 1,2,3-propanetriol traverse the cell membrane in the other direction. This proceeds until an equilibrium is reached in which both water and low molecular weight glycols are evenly distributed intra- and extracellularly. In time, secondary bonding or ethanedial-protein complexes occurs through establishment of methylene bridges, weak non-ionic, non-covalent bonds between adjacent fixed molecules. This contributes to the rigidity which occurs in fixed tissues.

It is believed that the presence of DMSO allows the larger molecular weight PEG polymer to enter the cell membrane. This is an important step in the tissue preservation process of the invention because polyethylene glycol reinforces the structure and architecture of the membrane, preventing it from collapsing or shrinking due to loss of elasticity caused by the crosslinking of proteins in the membrane.

The compositions according to the invention possess bactericidal activity. An antibacterial agent such as 1-hexadecylpyridinium chloride preferably confers this bactericidal activity. Concentrations of 1-hexadecylpyridinium chloride or benzalkonium chloride present in the diluted working solutions

(about 0.0075%) are sufficient to kill a broad range or microorganisms, including most bacterial pathogens potentially encountered in human remains. Ethanedial, as well, has been demonstrated to kill most common bacterial pathogens which are encountered in human remains, in both vegetative and spore form. While polyethylene glycol is an effective mold inhibitor, a mixture of methyl paraben and propyl paraben confers demonstrated antiphycotic and antymycotic activity to the composition in diluted (working) strength.

A chelating agent is present in the inventive compositions to sequester the divalent cations that are present in some tap water and which may cause undesirable performance in tissue preservative solutions. These divalent cations (such as Mg^{++}, Ca^{++} and Fe^{++}) are important cofactors necessary for bacterial enzymatic activity, therefore their removal improves the bacteriostatic activity of the composition.

Optionally, a dye such as tetrabromofluoroscein, also referred to as Eosin Y or D & C red No. 22, may be added to the composition as a colorizing agent for cosmetic reasons and as an aid in tracking flow of the preservative composition through the tissue being preserved.

Buffers which maintain the hydrogen ion concentration near a neutral pH are important to the overall function of the composition because a sufficient supply of hydrogen ions is needed for adequate formation of secondary bonds or methylene bridges between adjacent fixed tissues, as discussed above.

3. Body Cavity Composition

The body cavity composition of this invention is an aqueous solution of ethanedial, a polar aprotic solvent (such as DMSO), an antimicrobial composition (such as 1-hexadecylpyridinium chloride) and a mixture of methyl paraben and propyl paraben. This solution functions to disinfect evacuated body cavities and

to preserve removed gross organic tissue returned to the body for interment in the embalming process. During the embalming of human remains, certain body cavities such as the peritoneal and thoracic cavities are evacuated of the natural fluids which fill these spaces. Removal of the fluid is necessary to deny microorganisms a rich media in which to grow. Once evacuated of the natural body fluids, the cavities must be filled with a suitable fluid to prevent growth of microorganisms.

Upon death of an organism, normally saprophytic bacteria, especially those capable of endospore formation, may migrate out of the gut and into the peritoneal cavity where they begin to digest tissue with deleterious effect. Of particular concern to embalmers is the process of purging, where gas liberated from tissue putrefaction infiltrates tissue membranes, causing discoloration of the skin and swelling. The bacteria most usually associated with the formation of ptomaines (cadaverine and putrescine) are those of the genus *Clostidium*. These bacteria possess decarboxylase enzymes, which break down amino acids, forming ptomaines and carbon dioxide gas. The Clostridia bacteria particularly are capable of endospore formation, making them difficult to kill with many bacteriocidal agents, including 1-hexadecylpyridinium chloride, which easily kills most bacteria encountered in tissue cavities at a concentration of 1:15,000 or less. Endosporic bacteria, however, are killed by ethanedial in a matter of several hours.

Organs sometimes are removed from the body at autopsy for examination. After examination, the organs are returned for placement into the body at interment. Returned organs generally are placed in a suitable sealed plastic bag, which is placed in the chest cavity, and the chest is sewn tightly closed. Clearly, the prevention of tissue putrefaction and gas production is critical in this environment. Therefore, a powerful disinfectant

and preservative composition is needed to preserve and disinfect these organs.

Using the body cavity preservative solution of the invention, tissues are fixed by ethanedial, which cross-links intracellular and extracellular proteins. The flow of the fixative into cells is assisted by an aprotic polar solvent such as DMSO. Cell preservation is accomplished through the insolubilization of proteins, which prevents degradation of the proteins by naturally occurring enzymes released by lysosomes upon termination of life.

The body cavity preservation composition of the invention will not prevent shrinkage or loss of tissue fluid content, which occurs when cell membranes are altered by fixation, however the physical appearance of the tissues removed at autopsy is of secondary importance, while prevention of autolysis is critical. Denial of a rich media in which microorganisms thrive is likewise important. Bacterial growth is prevented or greatly retarded by ethanedial and 1-hexadecylpyridinium chloride; mold and fungi are inhibited by a mixture of methyl paraben and propyl paraben. This composition also is maintained at an acidic pH which is not compatible with most biologic life, for example a pH of about 2 to 4. *Clostridium perfringes*, an organism closely associated with tissue putrefaction and gas formation, requires a neutral or slightly alkaline pH for growth and therefore is inhibited further by the pH of composition.

The body cavity composition may be injected directly into evacuated body cavities, used as an external packing saturant material or may be added directly into containers holding gross organs prior to sealing. The amount of the body cavity solution to be used depends upon the amount of tissue to be embalmed and other conventional factors.

Preferred body cavity compositions for preservation and disinfection of tissues contain ethanedial in amounts of about 4%

to about 6% by weight, more preferably about 4.5% to about 5.5% by weight and most preferably about 5%. DMSO preferably is present in amounts of about 0.025% to about 0.03% by volume, more preferably is present at about 0.026% to about 0.029% by volume and most preferably about 0.0275% by volume. An antimicrobial agent such as 1-hexadecylpyridinium chloride preferably is present at about 0.006% to about 0.008% by weight, more preferably about 0.0065% to about 0.0075% by weight and most preferably about 0.007% by weight.

For control of fungal growth, preferred compositions contain a mixture of methyl paraben and propyl paraben. Methyl paraben is preferably present at about 0.065% to about 0.08% by weight, more preferably present at about 0.065% to about 0.078% by weight and most preferably present at about 0.075% by weight. Propyl paraben is preferably present at about 0.02% to about 0.03% by weight, more preferably present at about 0.022% to about 0.028% by weight and most preferably present at about 0.025% by weight. The remainder of the composition in preferred embodiments is water or other inert ingredients such as colorants, perfumes, buffers and the like. Preferably the water in the composition is purified water, distilled water or deionized water, however any water may be used so long as it does not deleteriously affect the function of the composition.

4. Tissue Fixation Composition for Histology

The tissue preservative solution according to the invention for use in the field of histology is an aqueous solution of ethanedial, a polar aprotic solvent (such as DMSO), a long-chain polymer (such as polyethylene glycol having an average molecular weight of about 7000 to about 9000 Daltons), a humectant (such as 1,2-ethanediol, 1,2-propanediol or 1,2,3-propanetriol) and a buffer sufficient to stabilize the pH of the solution at a pH of about 6.8 to about 7.2 or preferably about 7.0 (such as a

combination of monobasic and dibasic anhydrous phosphate salts, for example monosodium phosphate and disodium phosphate).

The purpose of the histology tissue fixative is to prevent autolysis of tissue removed from the body, so that the tissue remains as it exists in the natural, *in situ* state to the extent possible. Tissue samples which are taken from the body during surgery, in biopsy or in autopsy, are processed and stained for microscopic examination. For accurate examination and diagnosis, it is of paramount importance that the tissue be fixed or rendered free of autolysis as soon as possible after collection. In tissue analysis, a fixative must be uniformly effective in penetrating the tissue from the outside inward, so that all cells of the tissue are preserved.

In embalming, arterial fluid is provided for diffusion into individual cells from interstitial spaces under mild positive pressure. In tissue fixation for histology, the fixative solution must enter cells passively through penetration of tissue thickness without outside pressure. This process may require several hours or days to accomplish and is a function of tissue thickness and composition. For example, simple epithelial tissue becomes fixed rapidly compared to tissues high in fat content such as breast, colon and brain tissue. The mode of action of tissue fixation with the tissue fixative of the invention is believed to be similar to that described above for the tissue preservation (arterial injection) composition with the exception that the time required for adequate tissue fixation required by tissue immersion is longer than that required in pressure tissue embalming. The fixation process may be accelerated by using heat (for example microwave energy) and negative pressure (vacuum) to induce more rapid movement of fixative into the tissue.

Preferred tissue fixture compositions for histology contain ethanedial in amounts of about 3.5% to about 4.5% of the total composition by weight, more preferably about 3.6% to about 4.0%

by weight and most preferably about 3.75% by weight. Polyethylene glycol preferably is present at about 2% to about 3% by weight, more preferably about 2.25% to about 2.75% by weight and most preferably about 2.5% by weight. A humectant, for example ethylene glycol, propylene glycol or glycerol preferably is present at about 0.5% to about 1% by weight, more preferably about 0.6% to about 0.9% by weight and most preferably about 0.75% by weight. An aprotic polar solvent such as DMSO preferably is present in amounts of about 0.025% to about 0.03% by weight, more preferably about 0.026% to about 0.029% by weight and most preferably about 0.0275% by weight.

Buffer compositions are included in preferred histology tissue preservation compositions as discussed above to maintain solutions at or near neutral pH. A preferred buffer system includes anhydrous monosodium phosphate (preferably at about 0.35% to about 0.45% of the total compositing by weight, more preferably at about 0.37% to about 0.42% by weight and most preferably at about 0.4% by weight) and anhydrous disodium phosphate (preferably at about 0.6% to about 0.7% of the total composition by weight, more preferably about 0.62% to about 0.68% by weight and most preferably about 0.65% by weight). The remainder of the composition is water or other inert ingredients such as colorants, perfumes, and the like. Preferably, the water in the composition is purified water, distilled water or deionized water, however any water may used so long as it does not deleteriously affect the function of the composition.

Although the present invention has been described in connection with certain preferred embodiments and specific compositions and ingredients, it is not so limited. Variations and modifications within the scope of the claims will be apparent to those knowledgeable in the mortuary science and tissue preservation fields and technologies. The following examples are

provided to illustrate further the most preferred embodiments of the invention.

EXAMPLES

Example 1. Pre-Injection Tissue Preservative Composition.

Ingredient	Amount
Sodium alkyl sulfonate	2%
Ethanedial	14%
EDTA	0.08%
DMSO	0.0275%
Protease enzyme (Papain)	0.03%
Water	83.9%

Example 2. Injectable Tissue Preservative Solution for Embalming.

Ingredient	Amount
Ethanedial	28%
Polyethylene Glycol (MW 7k-9k)	2.5%
Ethylene Glycol	0.3%
1-Hexadecylpyridinium chloride	0.06%
DMSO	0.0275%
EDTA	0.08%
Monosodium phosphate (anhydrous)	0.4%
Disodium phosphate (anhydrous)	0.65%
Methyl paraben	0.06%
Propyl paraben	0.02%
Water	67.2%

Example 3. Body Cavity Tissue Preservative Composition for Embalming.

Ingredient	Amount
Ethanedial	5.0%
DMSO	0.0275%
1-hexadecylpyridinium chloride	0.007%
Methyl paraben	0.075%
Propyl paraben	0.025%
Water	94.9%

Example 4. Tissue Preservative Composition for Histological Study.

Ingredient	Amount
Ethanedial	3.75%
Polyethylene Glycol (MW 7k-9k)	2.5%
Ethylene Glycol	0.75%
DMSO	0.0275%
Monosodium phosphate (anhydrous)	0.4%
Disodium phosphate (anhydrous)	0.65%
Water	91.9%

Claims:

1. A formaldehyde-free tissue preservative composition which comprises ethanedial and a polar aprotic solvent in aqueous solution.

2. A formaldehyde-free tissue preservative composition which comprises ethanedial and a polar aprotic solvent in aqueous solution, wherein said composition does not contain acetic acid and does not contain alcohol.

3. A composition according to claim 1 wherein said polar aprotic solvent is dimethylsulfoxide (DMSO).

4. A composition according to claim 1 which further comprises a polymer.

5. A composition according to claim 4 wherein said polymer is polyethylene glycol having an average molecular weight of about 7000 to about 9000.

6. A composition according to claim 1 which further comprises a humectant.

7. A composition according to claim 6 wherein said humectant is selected from the group consisting of ethylene glycol, propylene glycol, glycerol and a combination thereof.

8. A composition according to claim 1 which further comprises an antimicrobial agent.

9. A composition according to claim 8 wherein said antimicrobial agent is 1-hexadecylpyridinim chloride.

10. A composition according to claim 1 which further comprises a chelating agent.

11. A composition according claim 10 wherein said chelating agent is ethylenediaminetetracecetic acid (EDTA) or a salt thereof.

12. A composition according to claim 1 which further comprises a buffer.

13. A composition according to claim 1 which further comprises a surfactant.

14. A formaldehyde-free tissue preservative composition which consists essentially of sodium lauryl sulfate, ethanedial, EDTA, DMSO, a protease enzyme and water.

15. A formaldehyde-free tissue preservative composition which consists essentially of ethanedial, polyethylene glycol, ethylene glycol, 1-hexadecylpyridinium chloride, DMSO, EDTA, a buffer, methyl paraben, propyl paraben, water, and optionally a dye.

16. A formaldehyde-free tissue preservative composition which consists essentially of ethanedial, DMSO, 1-hexadecylpyridinium chloride, methyl paraben, propyl paraben and water.

17. A formaldehyde-free tissue preservative composition which consists essentially of ethanedial, polyethylene glycol, ethylene glycol, DMSO, a buffer and water.

18. A method of embalming an animal body which comprises administering to said body a formaldehyde-free tissue preservative composition according to claim 1.

19. A method of embalming an animal body which comprises administering to said body a formaldehyde-free tissue preservative composition according to claim 15.

20. A method of embalming an animal body which comprises:

(a) administering to said body a composition according to claim 14;

(b) administering to said body a composition according to claim 15; and

(c) optionally administering to said body a composition according to claim 16.

AMENDED CLAIMS

RBE

PCT/DK 02/00074

AMENDED CLAIMS
[received by the International Bureau on 27 June 2002 (27.06.02);
original claim 1 replaced by new claim 1;
remaining claims unchanged (1 page)]

PCT/EP/04153

AMENDED CLAIMS
[received by the International Bureau on 14 July 2003 (14.07.03);
original claims 1-32 ammended]

PCT/US03/08683

AMENDED CLAIMS
[received by the International Bureau on 21 July 2003 (21.07.03);
original claims 1-20 replaced by new claims 1-22]

PCT/US01/12376

AMENDED CLAIMS
[received by the International Bureau on 12 Septembre 2001 (12.09.01);
original claims 1 and 3-6 replaced by new claims 1 and 3-6; original
claim 2 cancelled; remaining claims unchanged (2 pages)]

PCT/IB0100580

AMENDED CLAIMS
[received by the International Bureau on 9 October 2001 (09.10.01);
original claims 4-9 amended; ; original claims 1-3 cancelled; new claim 1 added:
claims 4-13 renumbered as claims 2-11; remaining claims unchanged (5 pages)]

JP01/01475

AMENDED CLAIMS
[received by the International Bureau on 31 July 2001 (31.07.01);

Claims:

1. A formaldehyde-free, non-alcoholic tissue preservative composition which comprises ethanedial, a polar aprotic solvent, a low molecular weight humectant, a polymer of ethylene glycol, an antimicrobial agent, and a chelating agent in aqueous solution, and optionally also comprises one or more of a buffer, a coloring agent, methyl paraben and propyl paraben, wherein said tissue preservative composition does not comprise acetic acid.

2. A formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 wherein the polar aprotic solvent is dimethyl sulfoxide, wherein the low molecular weight humectant is ethylene glycol, propylene glycol, glycerol or a mixture thereof, wherein the antimicrobial agent is 1-hexadecylpyridinium chloride or benzalkonium chloride and wherein the chelating agent is EDTA, EGTA or a salt thereof.

3. The formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 which comprises ethanedial, dimethyl sulfoxide, a low molecular weight humectant, a polymer of ethylene glycol, an antimicrobial agent, a chelating agent, a phosphate buffer, methyl paraben and propyl paraben.

4. The formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 which is diluted with water prior to use.

5. The formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 wherein said polymer of ethylene glycol has an average molecular weight of about 7000 to about 9000 atomic mass units.

6. The formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 which comprises 16-32% ethanedial.

7. The formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 which comprises 24-30% ethanedial.

8. The formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 which comprises 28% ethanedial.

9. The formaldehyde-free, non-alcoholic tissue preservative composition of claim 1 which consists essentially of 24-32% ethanedial, 0.3% low molecular weight humectant, 2.5% polyethylene glycol, 0.0275% dimethyl sulfoxide, 0.6% 1-hexadecylpyridinium chloride, 0.08% chelating agent, a buffer sufficient to maintain the composition at a pH of 6.8-7.2, methyl paraben, propyl paraben, water and optionally a coloring agent.

10. A formaldehyde-free, non-alcoholic tissue preservative composition which comprises ethanedial, dimethyl sulfoxide, an antimicrobial agent, methyl paraben and propyl paraben in aqueous solution and which does not comprise acetic acid.

11. A formaldehyde-free, non-alcoholic tissue preservative composition which consists essentially of sodium lauryl sulfate, ethanedial, EDTA, DMSO, a protease enzyme and water.

12. A formaldehyde-free, non-alcoholic tissue preservative composition which consists essentially of ethanedial, polyethylene glycol, ethylene glycol, 1-hexadecylpyridinium chloride, DMSO, EDTA, a buffer, methyl paraben, propyl paraben, water, and optionally a dye.

13. A formaldehyde-free, non-alcoholic tissue preservative composition which consists essentially of ethanedial, DMSO, 1-hexadecylpyridinium chloride, methyl paraben, propyl paraben and water.

14. A formaldehyde-free, non-alcoholic tissue preservative composition which consists essentially of ethanedial, polyethylene glycol, ethylene glycol, DMSO, a buffer and water.

15. A formaldehyde-free, non-alcoholic tissue prservative composition which comprises ethanedial, DMSO,

polyethylene glycol, a low molecular weight humectant, a buffer and water.

16. A formaldehyde-free, non-alcoholic tissue preservative composition of claim 15 which comprises 3.5% to 4.5% ethanedial, DMSO, polyethylene glycol, ethylene glycol, a buffer sufficient to stabilize the pH of the composition at pH 6.8-7.2 and water.

17. A method of preserving the tissues of an animal body which comprises contacting said tissues with a formaldehyde-free, non-alcoholic tissue preservative composition claim 1 of any of claims 1-16.

18. A method of preserving the tissues of an animal body which comprises

(a) contacting said tissues with a formaldehyde-free, non-alcoholic tissue preservative composition of any of claims 1-9; and

(b) optionally contacting said tissues with a formaldehyde-free, non-alcoholic tissue preservative composition of claim 10.

19. The method of claim 18 which further comprises, prior to step (a), contacting said tissues with a fluid composition which comprises sodium lauryl sulfate, ethanedial, EDTA, dimethyl sulfoxide, a protease enzyme and water.

20. A method of embalming an animal body which comprises administering to said body a formaldehyde-free, non-alcoholic tissue preservative composition of any of claims 12-14.

21. A method of embalming an animal body which comprises:

(a) administering to said body a pre-injection fluid composition;

(b) administering to said body a composition according to claim 12; and

(c) optionally administering to said body a composition according to claim 13.

22. A method of preserving tissues for histological

examination which comprises contacting said tissues with a formaldehyde-free, non-alcoholic tissue preservative composition of claim 15 or claim 16.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/08683

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 679 333 A (BRIAN WILLIAN DUNPHY) 21 October 1997 (1997-10-21) * the whole document *	1-20
A	US 5 091 174 A (WILLIAM A. LEMBERGER) 25 February 1992 (1992-02-25)	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 May 2003	30/05/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Fort, M

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 03/08683

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5679333 A	21-10-1997	NONE	
US 5091174 A	25-02-1992	NONE	