

PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

Preservative for Human and Animal Eyes

I, YASU HARU KUWAHARA, a citizen of Japan, of 27-1 Shinano-machi, Shinjuku-ku, Tokyo, Japan, do hereby declare the invention for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

THIS INVENTION relates to the preservation of human and animal eyes. More particularly, this invention is concerned with a liquid composition for preserving eyes *ex vivo* with the retention of their optical properties, and the preparation of the said liquid composition.

In the prior art, several methods have been made to effect the preservation of eyes *ex vivo* with the retention of their optical properties. Fixation with formalin and immersion in liquid paraffin or blood serum are well known. Freezing or freeze-drying of eyes, as well as their storage in a moist chamber, have also been proposed. None of these methods, however, is very satisfactory. For instance, the probability of success in transplanting a corneal graft which has been stored at 4°C. in a moist chamber is found to be approximately 10/23. Where a corneal graft stored at 4°C. in liquid paraffin is used the probability of success is approximately 24/46. Freezing methods which have been considered promising up until the present are also unsatisfactory because they cause undesired penetration of the aqueous humour through the corneal endothelium to the parenchyma until the cornea becomes cloudy. Furthermore, vacuum-dried transplants unavoidably suffer from swelling and opacification of the cornea when it is regenerated. In order to remove the physical troubles accompanying the freezing method, it has been proposed to carry out stepwise freezing in a glycerine or gelatine medium. However, such a proposal is worthless if maintaining the optical properties of the corneal tissue is a serious problem.

[Price 4s. 6d.]

We have noted that although the prior art techniques might be somewhat satisfactory for the preservation of corneal grafts to be used in lamellar keratoplasty, they are of no use in preserving corneal grafts to be used in penetration keratoplasty. Further, we have noted that the prior art techniques deal mainly with physical dehydration of corneas (as in freeze-drying or glycerine treatment) or with physical insulation from water (as in storage in various oils). If successful corneal transplants are desired, not only in lamellar keratoplasty but also in penetration keratoplasty, it is essential to maintain the optical properties of corneal tissues at the required level. To realize this, we have investigated the physiology of the eye *in vivo* at some length. From our extensive studies, we have found that a liquid preservative for corneal grafts can be obtained by addition of certain macromolecular substances and vitamin C (1-ascorbic acid) to a liquid composition which may be considered as a liquid model of the aqueous humour. For convenience, such a liquid composition will hereinafter be referred to as an artificial aqueous humour.

The present invention provides a new liquid composition which is useful as a preservative of human and animal eyes and corneas to be used in corneal transplants including both lamellar keratoplasty and penetration keratoplasty.

In accordance with the present invention, there is provided a liquid preservative for human and animal eyes, which comprises an artificial aqueous humour containing a sodium or potassium keratosulphate, chondroitin-4-sulphate or chondroitin-6-sulphate and vitamin C.

The term "keratosulphate" is used herein to describe a complex polysaccharide comprising an approximately equimolar amount of each of N-acetyl glucosamine, galactose and sulphuric acid, "chondroitin-4-sulphate" de-

scribes a mucopolysaccharide derived from chondroitin where the sulphate residue is attached to the 4-position of the galactosamine residue; in "chondroitin-6-sulphate" this sulphate residue is attached to the 6-position of the galactosamine. In all these natural mucopolysaccharides, sulphation occurs only on the galactosamine residue and not on the other saccharide derived moiety, glucuronic acid.

Unexpectedly it is found that the respective calcium salts of the acidic mucopolysaccharides are not effective for the purpose of the present invention. Addition of suitable mucopolysaccharides makes possible maintaining the activity of corneal cells, preventing swelling of the cornea so that it may be kept transparent. The amount of acidic mucopolysaccharides added can vary within the range from 0.75 to 1.25 g., preferably 1.0 g., per 100 ml. of the artificial aqueous humour.

The vitamin C which is added together with the acidic mucopolysaccharide will usually be in an amount from 0.02 to 0.2 g., preferably 0.1 g., per 100 ml. of the artificial aqueous humour. Use of vitamin C in the liquid composition of the present invention is not only useful for stable preservation of the cornea, but is also effective in accelerating cure of trauma which may occur during transplantation.

The liquid preparation of the invention is far more effective in the preservation of corneal grafts than a similar solution to which an acidic mucopolysaccharide and vitamin C has not been added. For example, whereas Ringer's solution *per se* is effective for preservation of corneal grafts only for a very short time, say one or two hours, Ringer's solution to which a mucopolysaccharide and vitamin C have been added is capable of preserving corneal grafts for 8 to 10 hours, with good results in subsequent planting. When such a solution having a composition specified later is adapted as an artificial aqueous humour in combination with the above-indicated two components, it allows successful preservation of corneal grafts for over seven days. At present, ophthalmologists who are concerned with corneal transplanting set as a goal the preservation of corneas for seven days, since the indicated period is sufficiently long to make it possible to transport a donated eye to any required place. Accordingly, the present invention is epoch-making in view of its realization of the preservation of donor eyes for seven days. Another great advantage of the present invention is that corneas stored by using a liquid composition of the invention allow a success as high as 95% in penetrating keratoplasty, while those stored by any one of the prior methods assure a probability of success of only 50% or less.

The term "artificial aqueous humour" used

herein means a liquid having a composition similar in effect to natural aqueous humour. Such a liquid may be a known aqueous humour substitute, occasionally used in various physiological tests. Accordingly, Ringer's solution is an artificial aqueous humour which can be used in this invention. After thorough studies of physico-chemical properties of true aqueous humour, however, we have found that the most successful preservation of eyes can be obtained by using, as an artificial aqueous humour, an aqueous solution defined below:

Component	Strength	
Na ⁺	129—157 mM/l	80
K ⁺	3.5—3.7 mM/l	
Ca ⁺⁺	1.12—1.18 mM/l	
Mg ⁺⁺	1.16—1.24 mM/l	
Cl ⁻	121.6—129.2 mM/l	
PO ₄ ⁻⁻⁻	0.485—0.515 mM/l	85
SO ₄ ⁻⁻	1.16—1.24 mM/l	
HCO ₃ ⁻	21.9—23.3 mM/l	
Glucose	5—20 mM/l	
Alkali metal salt of 1-glutamic acid	0.25—0.5 mM/l	90

The above solution may be prepared as follows: Suitable electrolytes which are capable of providing the respective ion components, i.e. sodium chloride, potassium chloride, calcium chloride, potassium dihydrogen phosphate, sodium hydrogen carbonate, potassium hydrogen carbonate, magnesium sulphate, sodium sulphate, etc., are dissolved in appropriate amounts into re-distilled sterile water, and then glucose and alkali metal 1-glutamate, e.g. sodium or potassium 1-glutamate, are added to the resulting solution. Care should be taken in the sequence of addition of individual components to avoid the possible formation of any precipitate. Passage of carbon dioxide gas is preferable for this purpose. Although the exact roles of individual components are not fully clear, it is assumed that glucose serves as an energy source and alkali metal 1-glutamate serves as an ion-carrier for corneal cells. Sodium glutamate is preferable as the alkali metal 1-glutamate because of its good solubility in water.

The aqueous mixed salt solution thus obtained has an approximately equal composition to that of human aqueous humour and is very useful for the purpose of the present invention. Besides the specified components, true human aqueous humour further contains 1-aspartic acid, 1-cysteine and lower molecular weight proteins. These materials may be added to the said mixed salt solution if the total osmotic pressure of the solution is not influenced thereby. Into this solution is then dissolved 0.75—1.25 W/V% of a sodium or potassium keratosulphate, chondroitin-4-sulphate or chondroitin-6-sulphate,

with 0.02—0.2 W/V% of vitamin C.

The following Examples illustrate the invention.

EXAMPLE 1

14.03 g. of sodium chloride, 3.865 g. of sodium bicarbonate, 0.462 g. of potassium chloride and 0.136 g. of potassium dihydrogen phosphate are dissolved in 2000 ml. of re-distilled sterile water at room temperature. 100% CO₂ gas is blown into the resulting solution until it is saturated. To the CO₂-saturated solution, 0.339 g. of CaCl₂ · 2H₂O and 0.592 g. of MgSO₄ · 7H₂O are added portionwise. Then the solution (2 l.) is mixed with 3.6 g. of glucose and 73.6 mg. of sodium 1-glutamate at room temperature. The resulting clear solution has the following physico-chemical properties:

Molar ionic concentration

Na ⁺	143 mM/l
K ⁺	3.6 mM/l
Ca ⁺⁺	1.15 mM/l
Mg ⁺⁺	1.2 mM/l
Cl ⁻	125.4 mM/l
HCO ₃ ⁻	22.6 mM/l
PO ₄ ⁻⁻⁻	0.5 mM/l
SO ₄ ⁻⁻⁻	1.2 mM/l

Total Osmotic Pressure
309

The solution (2 l.) thus prepared is mixed with 20 g. of sodium keratosulphate (hereinbefore described) (1.0 W/V %) and 2.0g. of vitamin C (0.1 W/V %). The resulting clear solution has a pH value of 7.4 under quasi-physiological conditions, i.e. under 5% CO₂ and 95% O₂. It can be stored in a plastic bottle (e.g. polyethylene) at 0°C. under aseptic conditions.

A cornea, together with a part of its plura, is cut from a human eye extracted six hours after death. The cornea is then immersed in the above-prepared liquid preservative in an amount of about 50 ml. per eye. After storage in an ice box at 0°C. for seven days, this cornea is used in penetrating keratoplasty. The transplanted cornea does not show cloudiness six months after operation. Visibility of the corneal cells is also found to be normal by inspection of oxygen consumption or tissue culture.

EXAMPLE 2

The same procedures used in Example 1 are repeated except that the sodium keratosulphate used in Example 1 is replaced by an equal amount of sodium chondroitin-4-sulphate. The maximum period for preservation of monkey's eye in this liquid preservative, during which period corneal transplantation in penetrating keratoplasty can be made with successful results is ten days.

EXAMPLE 3

The same procedures used in Example 1

are repeated except that the sodium keratosulphate used in Example 1 is replaced by an equal amount of sodium chondroitin-6-sulphate or potassium chondroitin-4-sulphate (hereinbefore defined). In the resulting two clear solutions, one of which contains sodium chondroitin-6-sulphate and the other potassium chondroitin-4-sulphate, human eyes are preserved at 0°C. for seven days. At the end of that period, microscopic observation of the eye shows that no detrimental change occurs in their corneal structures.

WHAT WE CLAIM IS:—

1. A liquid preservative for human and animal eyes, which comprises an artificial aqueous humour containing a sodium or potassium keratosulphate, chondroitin-4-sulphate or chondroitin-6-sulphate and vitamin C.

2. A liquid preservative as claimed in Claim 1, containing the acidic mucopolysaccharide and vitamin C in the amounts of 0.75 to 1.25 parts by weight and 0.02 to 0.2 parts by weight, respectively, per 100 parts by volume of said artificial aqueous humour.

3. A liquid preservative for human and animal eyes, which comprises an aqueous solution defined below:

Component	Strength
Na ⁺	129—157 mM/l
K ⁺	3.5—3.7 mM/l
Ca ⁺⁺	1.12—1.18 mM/l
Mg ⁺⁺	1.16—1.24 mM/l
Cl ⁻	121.6—129.2 mM/l
PO ₄ ⁻⁻⁻	0.485—0.515 mM/l
SO ₄ ⁻⁻⁻	1.16—1.24 mM/l
HCO ₃ ⁻	21.9—23.3 mM/l
Glucose	5—20 mM/l
Alkali metal salt of 1-glutamic acid	0.25—0.5 mM/l

in which is further dissolved 0.75 to 1.25 W/V% of a sodium or potassium keratosulphate, chondroitin-4-sulphate or chondroitin-6-sulphate, with 0.02 to 0.2 W/V% of vitamin C.

4. A method for the preparation of a liquid preservative as claimed in Claim 3, which comprises dissolving sodium chloride, sodium bicarbonate, potassium chloride and potassium dihydrogen phosphate in re-distilled sterile water, saturating the resulting solution with carbon dioxide gas, dissolving into the saturated solution calcium chloride and magnesium sulphate, adding glucose and sodium 1-glutamate to the resulting solution, and then further adding thereto the acidic mucopolysaccharide and the vitamin C.

5. A liquid preservative for human and animal eyes as claimed in claim 1 substantially as described.



with 0.02—0.2 W/V% of vitamin C.

The following Examples illustrate the invention.

EXAMPLE 1

- 5 14.03 g. of sodium chloride, 3.865 g. of sodium bicarbonate, 0.462 g. of potassium chloride and 0.136 g. of potassium dihydrogen phosphate are dissolved in 2000 ml. of re-distilled sterile water at room temperature.
- 10 100% CO₂ gas is blown into the resulting solution until it is saturated. To the CO₂-saturated solution, 0.339 g. of CaCl₂ · 2H₂O and 0.592 g. of MgSO₄ · 7H₂O are added portionwise. Then the solution (2 l.) is mixed
- 15 with 3.6 g. of glucose and 73.6 mg. of sodium 1-glutamate at room temperature. The resulting clear solution has the following physico-chemical properties:

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	K ⁺	3.6 mM/l
	Ca ⁺⁺	1.15 mM/l
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	Cl ⁻	125.4 mM/l
25	HCO ₃ ⁻	22.6 mM/l
	PO ₄ ⁻⁻⁻	0.5 mM/l
	SO ₄ ⁻⁻⁻	1.2 mM/l

Total Osmotic Pressure

309

- 30 The solution (2 l.) thus prepared is mixed with 20 g. of sodium keratosulphate (hereinbefore described) (1.0 W/V %) and 2.0g. of vitamin C (0.1 W/V %). The resulting clear solution has a pH value of 7.4 under quasi-
- 35 physiological conditions, i.e. under 5% CO₂ and 95% O₂. It can be stored in a plastic bottle (e.g. polyethylene) at 0°C. under aseptic conditions.

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- 45 cornea is used in penetrating keratoplasty. The transplanted cornea does not show cloudiness six months after operation. Visibity of the corneal cells is also found to be normal by inspection of oxygen consumption or tissue
- 50 culture.

EXAMPLE 2

- The same procedures used in Example 1 are repeated except that the sodium kerato-
- 55 sulphate used in Example 1 is replaced by an equal amount of sodium chondroitin-4-sulphate. The maximum period for preservation of monkey's eye in this liquid preservative, during which period corneal transplantation in penetrating keratoplasty can be made with
- 60 successful results is ten days.

EXAMPLE 3

The same procedures used in Example 1

are repeated except that the sodium kerato-

sulphate used in Example 1 is replaced by an equal amount of sodium chondroitin-6-sul-

65 phate or potassium chondroitin-4-sulphate (hereinbefore defined). In the resulting two clear solutions, one of which contains sodium chondroitin-6-sulphate and the other potas-

70 sium chondroitin-4-sulphate, human eyes are preserved at 0°C. for seven days. At the end of that period, microscopic observation of the eye shows that no detrimental change occurs in their corneal structures.

WHAT WE CLAIM IS:—

1. A liquid preservative for human and animal eyes, which comprises an artificial aqueous humour containing a sodium or potassium keratosulphate, chondroitin-4-sul-

75 phate or chondroitin-6-sulphate and vitamin C.

2. A liquid preservative as claimed in Claim 1, containing the acidic mucopolysaccharide and vitamin C in the amounts of 0.75 to 1.25 parts by weight and 0.02 to 0.2 parts

80 by weight, respectively, per 100 parts by volume of said artificial aqueous humour.

3. A liquid preservative for human and animal eyes, which comprises an aqueous solution defined below:

90

Component	Strength
Na ⁺	129—157 mM/l
K ⁺	3.5—3.7 mM/l
Ca ⁺⁺	1.12—1.18 mM/l
Mg ⁺⁺	1.16—1.24 mM/l
95 Cl ⁻	121.6—129.2 mM/l
PO ₄ ⁻⁻⁻	0.485—0.515 mM/l
SO ₄ ⁻⁻⁻	1.16—1.24 mM/l
HCO ₃ ⁻	21.9—23.3 mM/l
Glucose	5—20 mM/l
100 Alkali metal salt of 1-glutamic acid	0.25—0.5 mM/l

in which is further dissolved 0.75 to 1.25 W/V% of a sodium or potassium keratosul-

105 phate, chondroitin-4-sulphate or chondroitin-6-sulphate, with 0.02 to 0.2 W/V% of vitamin C.

4. A method for the preparation of a liquid preservative as claimed in Claim 3, which

110 comprises dissolving sodium chloride, sodium bicarbonate, potassium chloride and potassium dihydrogen phosphate in re-distilled sterile water, saturating the re-

115 sulting solution with carbon dioxide gas, dissolving into the saturated solution calcium chloride and magnesium sulphate, adding glucose and sodium 1-glutamate to the resulting solution, and then further adding thereto the acidic mucopolysaccharide and the vitamin

120 C.

5. A liquid preservative for human and animal eyes as claimed in claim 1 substantially as described.



6. A liquid preservative for human and animal eyes prepared by the process of claim 4. 6. tive as claimed in any of claims 1 to 3, 5 or 6.

7. A method for preserving human and animal eyes which comprises storing said grafts under sterile conditions in a liquid preserva-

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