DONOR CORNEA FOR KERATOPLASTY* **TROPICAL FACTORS INFLUENCING ITS USABILITY AND VIABILITY**

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IN keratoplasty, concepts regarding the effects of various influencing factors like climatic circumstances, the time interval between death and removal, the cause of death, the interval between removal and use, and the bacterial flora, on the usability and viability of donor human corneas have not been adequately emphasized in the literature. The scanty reports so far are mostly from countries with moderate climatic variations and near identical conditions. The purpose of this paper, therefore, is to report on these various factors that may have an important bearing on the viability and usability of donor corneas in such tropical conditions as exist in India. The report is based on observations made on 775 donor human eyes processed in this unit during the four years since 1960; 291 of these eyes were used for transplantation surgery.

Materials and Methods

Most of the eyes were removed either from unclaimed bodies or at medico-legal post-mortem examinations. None of the bodies was kept under refrigeration and no local medication, prior to enucleation, applied to the conjunctival sac to protect the cornea. Aseptic enucleation was performed at varying intervals after death. Only those eyes were removed in which the corneas were found to be macroscopically clear.

Immediately after enucleation the eyes were irrigated with sterile normal saline solution, then treated with antiseptic and antibiotic solution, and preserved at 4–6° C. refrigeration temperature in autoclaved glass containers, with a moist cotton pad underneath the eyeball. The corneas were examined every 24 hours and observations were made, with particular reference to the transparency of the cornea and the tension of the eyeball, until the cornea was used for surgery, or up to 96 hours, when the eye was otherwise disposed of.

Bacteriological studies were made on a series of 102 donor eyeballs. The first saline wash immediately after enucleation was culturally studied. A second saline wash was made after immersion of the eyeball in an antibiotic for 15 minutes immediately before use or disposal of the eye. This second saline wash was similarly studied bacteriologically to determine the most effective antibiotic solution.

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To standardize our observations of the corneal condition and clinical usability of the cornea for surgery, the following criteria were based on the number of folds (F) in Descemet's membrane and degree of tension (T) felt with a glass rod:

A		
F-0	Transparent cornea	} Suitable both for penetrating and lamellar surgery
F-1 +	Few fine folds	
F-2 +	Deep folds in central cornea	} Suitable for lamellar grafting only
F-3 +	Folds involving two-thirds of cornea	
F-4 +	Folds all over the cornea	} Unsuitable for keratoplasty
B		
T. good	Normal tension	
T-1 -	Slightly low tension	
T-2 -	Moderately low tension	
T-3 -	Very low tension with concave cornea	

Effects of Seasonal Climatic Variations and Interval between Death and Enucleation on Corneal Usability

Limits of time interval between death and enucleation have been rather arbitrarily stated. That eyes or donor grafts should be removed within one to two hours or immediately after death has been emphasized by McLean (1948), Barraquer (1949), Bock (1950), and Arruga (1956). Bock has suggested, however, that enucleation can be done up to 12 hours after death, but only if the body is kept under refrigeration. Paufigue, Sourdille, and Offret (1948) are of the view that this limit can be extended up to 24 hours, provided that the lids are properly closed and bacterial flora inhibited by local antibacterial drugs during refrigeration of the body. They are emphatic, however, that eyes must be enucleated within six hours if the above conditions cannot be fulfilled.

A comparative study of 116 donor eyes removed at varying intervals after death from bodies not refrigerated prior to enucleation, and having remained exposed to extreme climatic effects, has provided interesting observations about the influence of these factors on the usability of donor corneas. As all such eyes were preserved after enucleation under identical circumstances at a temperature of 4-6° C., the variations noted in the corneal condition during the observation period of 96 hours were found to be directly related to the effects of the climatic variations to which the bodies remained exposed prior to enucleation. This study was made on two groups of donor eyes. The winter group included eyes obtained in the three coldest months, the temperature in the shade varying from 40 to 60° F., and the summer group comprised eyes obtained in the three hottest months with temperature variations between 90 and 110° F. The relative atmospheric humidity during the hot summer months varied from 19.5 per cent. to 40.3 per cent. and in the cold winter months from 51.3 per cent. to 67.2 per cent.

The following table (Table I) indicates the percentage of eyes which were clinically viable according to the standards stated above, the observations being recorded at the end of every 24 hours up to 96 hours since removal.

TABLE I
CLINICAL VIABILITY IN WINTER AND SUMMER
IN RELATION TO INTERVAL BETWEEN DEATH AND REMOVAL OF EYES

Interval since Death	No. of Eyes	Percentage Viable after							
		24 hrs		48 hrs		72 hrs		96 hrs	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
<i>Winter Group—48 eyes</i>									
Less than 6 hrs	19	19	100.0	18	94.7	9	47.3	3	15.7
6–12 hrs	6	6	100.0	5	83.3	1	16.6	—	—
12–24 hrs	23	16	69.5	8	34.7	2	8.7	—	—
<i>Summer Group—67 eyes</i>									
Less than 6 hrs	21	17	80.9	9	42.8	3	14.2	2	9.5
6–12 hrs	14	11	78.5	6	42.8	1	7.1	—	—
12–24 hrs	32	14	43.7	3	9.3	—	—	—	—

It may be noted that there is a direct relationship of the usability of cornea to the time interval since death in both the winter and the summer groups. The more important observation, however, is that clinical usability of the cornea is much more prolonged in winter as compared to summer months. Whereas 34.7 per cent. of eyes removed more than 12 hours after death were still viable at the end of 48 hours of preservation in winter, only 9.3 per cent. of the corresponding group in summer were viable and usable. Similarly, although 12 out of the 48 eyes (25 per cent.) were viable at the end of 72 hours in winter, only 4 out of 67 (5.9 per cent.) were viable at the end of 72 hours in the summer group.

It can be stated that it is not necessarily the time interval since death which is important but the influencing factors, on which the viability of the cornea depends, so that a certain percentage of the corneas of eyes removed even up to 24 hours after death can remain viable up to 72 hours of preservation or more. These observations should therefore encourage a more optimistic approach to the usability of donor material in countries with extreme climatic variations, where refrigeration of bodies after death is impracticable and where the local circumstances do not permit removal of eyes within the conservative time limits mentioned in the literature.

Effects of the Cause of Death on the Viability of the Cornea

Hünemohr (1956) observed that the best results of keratoplasty are obtained by using donor eyes from persons who have died suddenly.

We have studied 251 unused donor eyes from the point of view of the effects of the known causes of death on the corneal transparency. The following table (Table II) gives the observations up to 96 hours of preservation.

TABLE II
VIABILITY IN RELATION TO KNOWN CAUSE OF DEATH

Cause of Death	No. of Eyes	Viable at the End of							
		24 hrs		48 hrs		72 hrs		96 hrs	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
Sudden death	115	99	86.0	65	56.5	29	25.1	6	5.2
Chronic ailment	77	53	68.8	25	32.4	4	5.2	—	—
Drowning	22	15	68.2	6	27.2	—	—	—	—
Burns	37	25	67.6	10	27.0	—	—	—	—

It is evident that the cornea remains transparent in a greater percentage, and for a much longer period, in cases of sudden death than in deaths after chronic ailments. Burns and drowning provided a poorer material, because not one of the 59 eyes had a transparent cornea after 48 hours, whereas the corneas of 39 out of 192 eyes were still usable after a corresponding interval in the series of sudden deaths and chronic ailments. The relatively shorter viability of cornea in cases of drowning and burns is probably due to oedema of the cornea and autolytic changes in its histochemistry. The donor eyes removed from bodies in cases of burns and drowning will therefore need to be used earlier.

Bacteriological Study of Donor Eyes and their Sterilization

Intestinal bacterial flora are known to migrate post mortem to other anatomical situations in the human body. Bacterial contamination of the conjunctival sac may occur from this source, from the nasal and oral cavities, from the fact of being exposed, and also from the environmental sources of infections. The bacterial flora may be of a different nature in different climatic conditions.

In this study in tropical conditions, 102 donor eyes were studied from bodies which remained inadequately cared for and exposed to environmental contamination. The following table (Table III) indicates the different organisms that were identified from the first saline wash (referred to earlier), which was subjected to cultural investigations.

TABLE III
BACTERIOLOGICAL STUDY OF 102 DONOR EYES AT REMOVAL

Organisms Identified	No.	Per cent.
<i>Escherichia coli</i>	78	76.48
Pathogenic staphylococci	8	7.84
Non-pathogenic staphylococci	8	7.84
<i>Streptococcus haemolyticus</i>	2	1.96
<i>Pseudomonas aeruginosa</i>	2	1.96
Sterile	4	3.92

Only 4 out of 102 eyeballs were found to be sterile. The most common organism was *Escherichia coli*, 76.48 per cent.

To find the best means of neutralizing this contamination these eyeballs were treated with three types of antibiotics during the preservation period before making the second wash for cultural study. The following table (Table IV) gives the results of this study.

TABLE IV
EFFECT OF ANTIBIOTICS ON BACTERIAL FLORA OF DONOR HUMAN EYES

Organisms	Percentage of Sterile Cultures after use of					
	Crystalline Penicillin		Streptomycin + Penicillin		Ne-Ba-Sulf*	
	No. of eyes	Sterile (Per cent.)	No. of eyes	Sterile (Per cent.)	No. of eyes	Sterile (Per cent.)
Pathogenic staphylococci	8	8 (100.0)	—	—	—	—
<i>Escherichia coli</i>	21	5 (23.8)	12	12 (100.0)	33	33 (100.0)
<i>Pseudomonas aeruginosa</i>	—	—	1	—	1	1 (100.0)

* Neomycin sulphate, bacitracin, and sulphacetamide

It may be noted that although penicillin sterilized all eyes against staphylococci, it was effective in only 5 out of 21 eyes with *E. coli* contamination. This partial action may be due to the effectiveness of penicillin on some strains of *E. coli* and not on others, and supports the view expressed by Drill (1958). The addition of streptomycin to penicillin produced a 100 per cent. sterilizing effect against *E. coli*.

Similarly, Ne-Ba-Sulf (Pfizer-Dumex) also completely sterilized the third group of donor eyes. It may be added that not one recipient eye in a series of 291 keratoplasties performed to date has been lost because of infection from the donor corneal button.

How Soon after Enucleation must a Donor Eye be used?

Most corneal surgeons would prefer to use donor corneas as soon as possible after enucleation. Stocker (1954) has suggested that eyes stored at a temperature of 4–6° C. should be used within 24 hours. Paton (1955) observed that an eyeball enucleated immediately after death from an adult can be preserved for up to 3 days and still be useful for surgery. Filatov (1945) and Barraquer (1949) have stated that a preservation of 12 to 48 hours is preferable to immediate use after enucleation, because during preservation the biological entity is reduced and therefore its antigenicity is less harmful.

In this series 281 eyes have been clinically studied to assess the effect on the transparency of grafts of time interval between removal of the eye and its use. The following table (Table V) indicates this relationship.

TABLE V
TIME INTERVAL SINCE REMOVAL—RESULTS OF SURGERY

Eyes used Within	For Penetrating Keratoplasty (162 eyes)			For Lamellar Keratoplasty (119 eyes)		
	No.	Improved (Per cent.)	No Improvement (Per cent.)	No.	Improved (Per cent.)	No Improvement (Per cent.)
24 hrs	66	24 (36·4)	42 (63·6)	45	33 (73·3)	12 (26·7)
48 hrs	71	34 (47·8)	37 (52·2)	48	39 (81·2)	9 (18·8)
72 hrs	17	7 (41·2)	10 (58·8)	16	12 (75)	4 (25)
96 hrs	8	4 (50·0)	4 (50·0)	7	3 (42·9)	4 (57·1)
After 96 hrs	—	—	—	3	2 (66·7)	1 (33·3)

Results of corneal transplantation surgery depend on several factors which are common to all cases. In this series of 281 keratoplasties, 8 donor eyes for penetrating surgery and 10 for lamellar surgery were used after as long as 72 hours, and the results were in no way worse than those used within the accepted time interval of 24 to 48 hours (Paton, 1955). It is therefore suggested that donor corneas preserved at a temperature of 4–6° C. can be used for as long as 72 to 96 hours after enucleation. These observations also support the view of Billingham and Rycroft (1955) that at present there is no better criterion for clinical judgement than experience in deciding whether the corneal tissue is or is not suitable for providing a graft. If this is true of eyes removed from bodies in tropical conditions, the period of clinical viability of donor corneas should be still longer in circumstances where there is no extreme climatic variation to which the bodies are exposed before the eyes are enucleated.

Summary

(1) The influence of extreme climatic variation on the clinical viability of donor corneas has been studied. It has been observed that the cornea remains viable for a longer period in winter than in summer.

(2) It is reported that the clinical viability of donor corneas is directly related to the time interval between death and enucleation.

(3) Corneas of eyes removed in cases of sudden death remain viable for longer periods, while cases where death has occurred from drowning or burning provide poorer donor material.

(4) *E. coli* is the commonest contaminating organism on donor human eyes in tropical climates. Ne-Ba-Sulf (Pfizer) is found to be most effective for neutralizing this contamination.

(5) On the basis of the experience of 281 keratoplasties, it is suggested that donor eyes can provide good corneal buttons for a much longer interval after enucleation than is generally stated in the literature, and that the best criterion for accepting the donor material is not the time interval but the clinical condition of the cornea.

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