Reports

Excision of the donor cornea instead of enucleation. SALME VANNAS.

Shortage of corneal grafts is common in many countries. The method presented here has been developed to relieve this situation and make it easier, especially from the psychological point of view, to obtain a license for excising corneas from cadavers. An 11 mm. corneal button is excised from the donor eye in situ with a Draeger electric rotor trephine and the donor eyes remain apparently normal looking. These excised corneas without sclerocorneal rims are placed in adequate storage media for long-term cryopreservation according to Capella, Kaufman, and Robbins or for short-term storage according to McCarey and Kaufman. The preliminary results are promising and the technique seems not to be too delicate or complicated to be carried out by an experienced eye bank technician. It has improved our possibilities for storing donor corneas and for collecting many tissue-typed corneas in our eye bank.

The present law relating to tissue transplantation came into force in 1957. Under this law, a donor eye can be removed for transplantation purposes if it has been bequeathed to the eye bank or if it is known that the dead person or his relatives do not oppose the removal of tissues. Permission has to be obtained from the relatives and, in practice, this has led to a shortage of corneas for emotional reasons.

The valuable technique of long-term cryopreservation was introduced here two years ago and, recently, another method for short-term storage but the increase in the number of corneas in our eye bank has been slow. For donor tissue typing a broad selection of corneas is required if a good match is to be obtained for bad-risk recipients. The ever increasing need for tissue-typed corneas has led us to modify our procedure and during the last six months we have removed only corneal tissue from the donor. Emotionally, the relatives view removal of the cornea alone more favorably than enucleation. The same applies to the hospital staff who have to ask the relatives for permission.

Methods. When the cornea is to be removed, a lid retractor is placed on the donor eye. Bacterial and fungal cultures are prepared from the limbal area. The eye is irrigated with BSS (Alcon). As an antibiotic prophylaxis, chloramphenicol 0.5 per cent with polymyxin-B sulfate 0.1 per cent in a mixed collyrium is instilled once into the cornea.

The corneas are removed with an electrical

Fig. 1. Equipment for removing donor cornea from cadaver eye in situ.

Fig. 2. A, Draeger rotor trephine with perforated edge for microscopic surgery. B, the first streams of aqueous (arrows) indicate the right moment to cut off the power supply with the pedal.
Fig. 3. A, Descemetocoele after recurrent herpetic ulcer in a heavily vascularized cornea. B, Clear graft 4 months after keratoplasty. A cryopreserved cornea excised from the donor eye in situ was used.

Draeger rotor trephine\(^3\) (Klein). The eye is held with Castroviejo forceps with 0.5 mm. teeth. The body of the trephine has a 12 volt electric motor connected to a 12 volt transformer and is controlled with a pedal especially designed for this purpose (the rotor trephine is usually used in connection with our operating microscope which has all the necessary power supplies).

This trephine needs (almost) no pressure because of its construction. Operating loops are used during the procedure and enable the technician to see when the first streams of aqueous escape from the anterior chamber through the perforated edge of the trephine (Figs. 1 and 2). At this moment the power supply is cut off with the pedal and the motor stops instantly. At this point the cornea is usually completely detached from the surrounding tissue. Special care has to be taken to prevent the cornea from rotating with the trephine, as this might injure the endothelium. Preferably, if the cornea is not completely free at this point, the adhering bridge can be cut carefully with curved Vannas scissors.

The cornea is transferred to the appropriate storage medium with fine fixation forceps, or a fine suture. It is lifted into a solution exchanger apparatus for cryopreservation,\(^4\) the storage procedure being started immediately in a portable ice box at 4° C. If more than six hours has elapsed since death, the cornea is placed in a tube containing modified tissue culture medium for short-term storage\(^2\) and this is placed in the portable ice box at 4° C. (The latter medium is used if the cornea is to be grafted immediately.) The corneas are transported directly to the cornea laboratory, which usually takes not more than ten minutes. During the actual operation the button is excised from the endothelial side with a Brightbill-Polack-Slappay punch.\(^4\)

**Results and comments.** This method has already proved very useful. The endothelium, as examined in the biomicroscope, seems to be well preserved except when the cornea started spinning with the trephine. However, that can easily be noticed at the time of removal. If there is uncertainty regarding the endothelium a new cornea can be obtained from the eye bank within a few minutes. After the button has been punched, the remnants of the excised cornea are studied in light and electron microscopy for the viability of the endothelium. Details of these results will be published later.\(^5\)

The clinical results are illustrated by an interesting case: Patient RL had a recurrent herpetic ulcer and a descemetocoele developed (Fig. 3). Keratoplasty was mandatory to save the eye. The donor cornea had been excised from a cadaver eye in situ with the rotor trephine six weeks earlier and cryopreserved. It shared two HL-A antigens with the recipient. Cortisone and immunel were given postoperatively. Recovery was excellent and the graft has remained completely clear for four months. VA 0.8 (+ 4.5 d cyl. + 3.5 d ax. 70°).

The technique for removing a donor cornea described here seems to be no more delicate for an experienced eye bank technician than for detaching it with a sclerocomeal rim. When combined with short-term storage it seems very useful as a routine. Corneal grafts removed with a rotor trephine seem to be equally suitable for long-term cryopreservation and will make it considerably easier to collect tissue-typed corneas.

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From the Department of Ophthalmology, University of Helsinki, Helsinki, Finland. Supported by a grant from the Silmasatio. Submitted for publication Dec. 16, 1974. Reprint requests: Dr. S. Vannas, Department of Ophthalmology, Haartmaninkatu 4C, Helsinki 00290, Finland.

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REPORTS


Several immunologic tests were used to compare the soluble antigens of cultured and normal bovine corneas. Results of fluorescent-antibody tests on normal corneas confirmed our earlier observation that the epithelium contained little serum protein, and that stroma and epithelium shared an antigen not found in bovine serum. Immunelectrophoresis, immunodiffusion, and fluorescent-antibody tests performed on extracts prepared from normal and cultured corneas indicated that corneas absorbed serum proteins during the incubation period. We also noted that very little intact epithelium remained on corneas cultured in the presence of calf serum. The extract prepared from cultured corneas did not contain the strongly antigenic protein present in extracts of normal cornea or epithelium. The implications of these findings are discussed.

Our previous report described the soluble antigens extracted from bovine corneas. In addition to serum proteins, a protein was found in extracts of whole cornea and of corneal epithelium that was not found in bovine serum. This strongly antigenic protein was similar in distribution and electrophoretic mobility to a protein described earlier by Holt and Kinoshita and to a protein described by Whiteside, Hamada, and Manski. The present experiments were designed to determine the antigenic nature of an extract prepared from cornea that had been cultured in vitro and to compare this extract with that prepared from normal corneas.

Materials and methods.

Culture methods. Bovine corneas, with a small rim of sclera, were cultured in 60 by 15 mm. plastic culture dishes containing Eagles' minimal essential medium (MEM), supplemented with 10 per cent heat-inactivated (56° C, 30 minutes) calf serum (CS), penicillin, and streptomycin. The cultures were maintained at 37° C. in an atmosphere of 95 per cent air, 5 per cent CO2. The medium was changed twice weekly. At the end of three weeks, the corneas that were free of bacterial or fungal contamination were frozen. Some corneas were cultured in MEM that contained 10 per cent normal heat-inactivated rabbit serum (NRS) instead of CS. We prepared a section of a representative cornea for routine histologic examination and a section for use in fluorescent-antibody studies.

Preparation of corneal extracts. Soluble proteins were extracted from normal and cultured corneas as described previously. We concentrated the material to a protein concentration of at least 20 mg. per milliliter (Biuret). In one experiment, the culture medium was removed at each medium change and concentrated to approximately one-fourth its original volume.

Immunodiffusion and immunelectrophoresis. These procedures were carried out as described previously. We tested the extracts from normal corneas (CA) and cultured corneas (CaC) against antiserum to bovine serum (anti-BS), anti-CA, anti-CaC, and anti-bovine gamma-globulin (BCG). These antisera were prepared in rabbits by an initial intravitreal injection of 0.1 ml. of the material. A second intravitreal injection was given one week later. Booster intravenous injections were given at intervals in order to maintain the serum antibody titer at a high level. Hemolytic antibody titer of anti-CA and anti-CaC (against BCG-coated erythrocytes) were greater than 1:256. The concentrated tissue culture medium was tested in immunelectrophoresis studies against anti-CA and against anti-BS.

Fluorescent antibody (FA) studies. Sections of cultured and normal corneas were fixed in the solution described by Wolman and Behar and stained according to the technique described by Carver and Goldman. The sections were stained with the direct method, using fluorescein-conjugated goat anti-rabbit IgG (FL-RG) and fluorescein-conjugated goat anti-bovine IgG (FL-BG). Both antisera were obtained from Miles Laboratory, Elkhart, Ind. For indirect FA tests, we first reacted the sections with anti-BS, anti-CA, anti-BGG, or anti-EPI, and then with FL-RG.

Results.

Appearance of cultured corneas. Bovine corneas that had been cultured for three weeks were edematous and opaque. Their pink color indicated that they had absorbed the culture medium (containing phenol red) during the incubation period. Microscopic examination of stained sections (he-