THE PROLONGED STORAGE OF DONOR CORNEAS BY GLYCERINE DEHYDRATION

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MUCH INTEREST WAS BEING SHOWN IN KERATOPLASTY IN THE MID 40S, HOWEVER, its development was impeded by an overwhelming obstacle—the demand for donor corneas greatly exceeded the available supply. Eye banks were being organized, however, the term "bank" was a misnomer and remains a misnomer today. Transplantation of donor corneas is best done within 48 hours after enucleation, with 72 hours being the time limit beyond which endothelial changes may make the eye unusable, except for a lamellar graft.

In an effort to develop a means of preserving the cornea for a longer period of time, researchers worldwide became interested. Most methods involved freezing, with varying amounts of glycerine, which was accidentally found to be effective in protecting the cells against damage on freezing and thawing.

No practical method was found which would preserve the cornea for use in penetrating grafting, as viability of the endothelium could not be maintained.

McNair and King[1] preserved cat corneas by dehydration in a mixture of 15% glycerine and in a frozen mixture of alcohol-dry ice. The corneas were then sealed in a vacuum and stored for as long as 4 months. After they were rehydrated with isotonic saline, all tissues were clear when used as lamellar grafts in cats.

King[2] carried the work further by dehydrating human corneas in 95% commercial glycerine, without freezing, and sealing the tube, maintaining a vacuum. The tubes were stored at room temperature. Preserved corneas were used in over 50 patients for lamellar grafting, and the results were considered equal to those obtained with fresh material. The oldest cornea had been stored at room temperature for 2 years.

The details of preserving the cornea by this method are simple, but do

require a technician. The donor cornea is excised together with a 4 mm rim of adjoining sclera and is placed in a sterile Pyrex test tube containing 5 ml of 95% sterile glycerine and 30 minims of antibiotic mixture. The tube was then connected to the glass manifold of a closed dehydration system. A vacuum pump removes the air and water from the unit and dehydration is complete when a baseline pressure of 5 μ is reached, which takes about 8 hours. The glycerine is rendered anhydrous and has replaced the tissue fluid. The specimen tube is hermetically sealed above the glycerine by using a gas-oxygen torch. The tube is stored at room temperature after it is labeled with pertinent information.

When the cornea is needed for lamellar keratoplasty, the tube is opened under sterile conditions by using an electric tube cutter, and the glycerine is decanted off. The cornea is placed in a medicine glass containing enough antibiotic solution to cover the tissue which, when it releases no more glycerine, is rehydrated. This takes about 10 to 15 minutes. The rehydrated cornea is fixed in a special clamp, and the lamellar graft is dissected in the usual manner and is transplanted using the same technique as for a fresh graft.

This research solved the need for corneal tissue for a lamellar graft, and it was recommended that eye banks maintain a supply of dehydrated donor corneas for use as lamellar grafts for emergencies and patch grafts. It was thought that fresh corneal tissue kept at 4° should be made available only for use in penetrating keratoplasty. Twenty years ago, a lamellar graft was considered more useful by many surgeons and was preferred to the penetrating technique. It was a safer operation with wide application and indications including optical, tectonic, therapeutic, cosmetic, and for certain miscellaneous conditions.

The dehydration in glycerine method of preservation was modified in 1962 by King and associates[3] using a desicant to produce dehydration. It is simple and because of the ease of storing without vacuum at room temperature and transporting worldwide with no special precautions, it is an advance still being applied today. It is recommended that corneas stored for no more than 10 years be used because of autolysis which has appeared in some corneas after that time.

Donor corneas preserved by the older method of dehydration in glycerine and sealed in a vacuum were used to improve or restore vision when the opacification was superficial. They can be applied to replace diseased corneal tissue in chronic keratitis and to improve the corneal configuration as a preparatory graft preceding a penetrating transplant.

A number of preserved corneas were labeled and stored for varying periods of time. Successful lamellar grafts were obtained with donor

corneas in storage at room temperature in a vacuum from 2 months to 10 years.

The purpose of this report is to describe a successful case of lamellar grafting using a preserved donor cornea stored at room temperature for almost 23 years.

The donor cornea was excised from the enucleated eye of a 70-year-old woman who had died in May 1961 from a coronary thrombosis. The cornea was dehydrated in glycerine and stored in a tube in a vacuum and kept on a shelf at room temperature.

A female patient who was 67 years of age had suffered from chronic uveitis for over 20 years and had developed a dense band keratopathy with recurring periods of pain the left eye. The visual acuity was light perception only in each eye. The left eye was hypotensive with early peripheral corneal vascularization. There was no improvement by medical therapy, and it was believed that a large lamellar graft was indicated for tectonic and therapeutic purposes. The operation was performed in November 1983.

A 10 mm trephine was used to include most of the thickened host cornea with a deep lamellar dissection.

The test tube containing the dehydrated cornea was opened, and the cornea was teased into a medicine glass containing a mixture of antibiotic solutions (Neosporin). It was allowed to rehydrate for several hours, at which time the glycerine had been replaced by the antibiotic-saline solution.

The donor cornea was then placed on a special clamp to hold the cornea, the 10 mm trephine was used, followed by a lamellar dissection of about 0.6 mm in depth. The graft was then sutured in the host corneal defect by means of multiple appositional sutures of 10-0 nylon. The operation was concluded by using a pressure dressing to maintain apposition of the donor graft and the host cornea.

The donor cornea remained translucent for 3 weeks and then cleared becoming transparent. Some striae, or streak-like areas of nonclearing persisted 6 months postoperatively.

There is now one cornea from a 75-year-old man remaining in storage which was dehydrated in July 1961. It will be kept for several more years and eventually be used to see if it still achieves clarity.

The intent of this presentation is to share with you this amazing result: that a cornea stored in glycerine for over 23 years can still achieve a high degree of clarity when it is used for a lamellar graft.

REFERENCES

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2. King JH Jr: Keratoplasty: Experimental studies with corneas preserved by dehydration. *Trans Am Ophthalmol Soc* 1956; 54:567-609.
3. King JH Jr, McTigue JW, Meryman HT: A simple method of preservation of corneas for lamellar keratoplasty. *Am J Ophthalmol* 1962; 53:445.

DISCUSSION

DR DONALD J. DOUGHMAN. The name of Doctor John Harry King has always been synonymous with eye banking and all its altruistic ideals. Besides his well known achievements in corneal surgery and eye banking in the United States, his founding and continuing leadership of the International Eye Foundation has meant prevention of blindness and sight restoration of vision to perhaps hundreds of thousands of men, women, and children in developing nations. I salute you Doctor King and I am pleased to discuss your paper.

The use of glycerine-preserved corneas for lamellar keratoplasty has allowed eye banks to utilize nonviable tissue, especially for emergency surgery. Although the use of lamellar keratoplasty in the surgery of the pathologic cornea has been replaced almost entirely by penetrating keratoplasty using viable donor cornea, the availability of glycerine-preserved corneas for emergency use in corneal thinnings and perforations has been important and in many cases crucial to saving such eyes. The use of tectonic or patch grafts using glycerine-preserved corneas has become a common procedure in most corneal surgeons practices. In addition, there are a few surgeons who use the lamellar keratoplasty for such conditions as keratoconus or as mentioned by Doctor King, in thin recipient corneas this can be used as a preparatory graft prior to penetrating keratoplasty (Table).

More recently, lamellar keratoplasty has become an important technique in refractive surgery. Frozen lenticules ground to a hyperopic power have been used in keratophakia. However, due to the technical problems in grinding lenticules, as well as performing the surgery itself, this procedure has limited application at the present time. However, epikeratophakia appears to have a broader application due to the relative simplicity of the surgery, its apparent reversibility, and the fact that commercially prepared lyophilized lenticules are now available from commercial sources. This is an important advance in corneal surgery that will undoubtedly play a significant role in the correction of ametropia in the future. However, the introduction of commercial laboratories buying and selling donor tissue to produce these lenticules introduces new strains on our donor supply and potential ethical problems, especially if viable tissue that is needed for penetrating keratoplasty is "sold" to the highest bidder for refractive surgery.

Doctor King and co-workers have introduced glycerine preservation as a method of long-term corneal storage. Although glycerine will not preserve endothelial viability, a condition needed for penetrating keratoplasty, cryopreservation and

TABLE: INDICATIONS FOR LAMELLAR KERATOPLASTY

Optical
1. Reis-Bückler dystrophy
2. Salzmann's nodular degeneration
3. Mucopolysaccharidosis
4. Climatic keratopathy
5. Superficial leukomata
6. Keratoconus
7. Band keratopathy??
Tectonic
1. Corneal thinnings
2. Corneal perforation
3. Thin recipient cornea before penetrating keratoplasty
Therapeutic
1. Pterygium
2. Bowen's disease
3. Squamous cell carcinoma
Cosmetic
1. Dermoid
2. Scars

34° organ culture have been developed that will allow long-term storage of viable corneal tissue prior to penetrating keratoplasty.

Cryopreservation, due to its technical complexity and costs, has limited application in a few centers. Organ culture storage of corneas as it has been developed by our group at the University of Minnesota promises to provide sterile, viable, and thin corneal tissue that can be stored for at least 30 days. Our first report on this subject was presented to this organization in 1974. As of March 1, 1984, we have performed 702 corneal transplants using this method and believe that organ culture is now proven to a safe, effective new method of storing donor corneal tissues for at least 1 month. The method has been simplified and a quarantine method of isolating and culturing the media developed so that we can now assure sterile tissue that is viable and thin, easy to transport, and to use. We expect this system will soon be commercially available.

In summary, Doctor King's work on glycerine preservation has been an important chapter in eye banking. It has helped stimulate many of us in our own research into better methods of donor cornea storage. It was a pleasure to hear his report here some 29 years after his initial paper on this technique.