THE USE OF PRESERVED OCULAR TISSUES FOR TRANSPLANTATION*

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MANY MODERN TECHNIQUES in ocular surgery require the use of donor eye tissues for transfer to the living eye. These materials may be obtained from eye banks when the operation is an elective one. Often, when eye tissues are required for an emergency, they are unavailable to the average ophthalmologist. A true eye bank should be able to furnish tissues to any eye surgeon at any time in any location. This ideal is nearing attainment as it is now possible to preserve virtually any eye tissue and to store it indefinitely at room temperature.

CORNEA

Many unsuccessful experimental attempts were made to preserve corneas before Eastcott and his co-workers (1) reported their method of preserving human corneas in England. This work followed the technique used by other investigators to preserve other living tissues, such as sperm, skin, blood, and endocrine tissues. The corneas were pretreated for one hour with 15 percent glycerine in Ringer's solution. They were then frozen by immersing the container in a mixture of carbon dioxide and alcohol at —79° C. The bottle was placed in a special dry container surrounded by solid carbon dioxide (dry ice) and stored in a standard deep-freeze. After thawing, and just before grafting, the cornea was transferred to normal saline with penicillin. The results were successful in all lamellar transplantations but not for full-thickness penetrating grafts. The longest period of storage was nine months. McPherson and his colleagues (2), working in the United States, proved the viability of rabbit corneas preserved by this method as shown by tissue culture.

McNair and King (3) preserved cat corneas by dehydration in a mixture of 15 percent glycerine and isotonic saline mixture. The

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corneas were then sealed in a vacuum and stored without refrigeration for as long as four months. After rehydration with isotonic saline, all tissues were clear when used as lamellar grafts.

King (4) felt that glycerine protected the cellular structure of the cornea and maintained viability with cellular metabolism at a standstill. He carried the work further by dehydrating corneas in 95 percent commercial glycerine, sealing the tube, maintaining a vacuum, and storing at room temperature. Nine animal corneas stored for a maximum period of about seven months and five human corneas, the oldest having been preserved for almost two months, were successfully used for lamellar grafts. The penetrating grafts could not be considered a success in animals, and they were not properly evaluated in humans.

Preserved corneas have now been 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 two years. Viability studies by tissue culture have not been done. Clarity after transplantation, however, is practical proof of viability if living tissues are necessary.

The method of preservation and the surgical technique have been modified since the original work was reported. Instead of excising the cornea from the donor eye at the limbus, it is now removed together with a 4 mm. rim of adjoining sclera. This offers the advantages of supporting the cornea and maintaining its form during the dehydration process; it results in less edema of the donor cornea during dehydration and it facilitates the technique of removing the donor graft at the time of operation.

The excised cornea is placed in a sterile Pyrex test tube containing 5 c.c. of 95 percent glycerine and 30 minims of antibiotic mixture.* The tube is then connected to the glass manifold of the closed dehydration system. A vacuum pump removes the air and vapors from the unit, and dehydration is complete when a base-line pressure of 5 microns is reached, which takes about 8 hours. The specimen tube is then hermetically sealed well above the glycerine by using a gas-oxygen torch. The tube is labeled with pertinent information and stored at room temperature.

When the cornea is needed for lamellar keratoplasy, the tube is opened under sterile conditions by means of an electric tube cutter. The glycerine is decanted off, and the cornea is placed in a medicine glass containing enough antibiotic solution* to cover the tissue. The cornea is then allowed to rehydrate for about 10 to 15 minutes. The

*Neosporin® solution, Burroughs Wellcome & Co., Inc., Tuckahoe, N.Y.

method of storage in a sealed test tube has a disadvantage for the surgeon who does not own an electric cutter, since the tube is difficult to open without a considerable amount of filing. In current studies, test tubes are being replaced by small vaccine-like vials which will be easier to open, allow more efficient closure, and should reduce storage space.

The rehydrated cornea is fixed in a special clamp and the graft is dissected in the usual manner (5).

There is no doubt that corneas can be preserved indefinitely for lamellar keratoplasty either by freezing after pretreatment with 15 percent glycerine or by dehydration in concentrated glycerine and storage at room temperature. The latter method is obviously to be preferred from the standpoint of economy of storage and shipment.

In certain types of keratitis, when a lamellar graft is required for therapeutic purposes, a preserved cornea appears to be preferable to a fresh one. With the preserved cornea, there is less postoperative inflammation of the eye and more rapid clearing of the cornea. This may indicate less antigen-antibody reaction, possibly because some of the tissue proteins have been removed during the dehydration process. The trophic or clearing effect on the surrounding diseased cornea appears greater and there is less vascularization of the preserved graft. Rehydration of the donor tissue in antibiotic mixtures provides concentrated local therapy to the diseased host cornea. Other medications can be added to the rehydrating solution to enhance the therapeutic effect of the graft, for example, the corticosteroid prednisolone is of value in active interstitial keratitis (not in herpetic keratitis).

Preserved corneas could be held in reserve at all hospitals for emergency use in corneal injuries, chemical burns, ruptured ulcers, and descemetoceles.

At this time, thirty preserved corneas have been mailed to eye surgeons in all areas of the United States, Central America, and Europe for use in lamellar keratoplasty. Two corneas, one preserved for twelve months and the other for eighteen months, were sent by regular mail to Barcelona, Spain. Barraquer (6) reports:

> The two preserved corneas arrived in good condition and have been utilized, one of them for a total (11 mm.) perforating graft in a case of purulent fusion of the anterior pole of the eye, due to staphylococci. The graft took very well. The infection disappeared and the eye has been saved. At present, there is a good luminous projection and perception. The graft is clouded and a bit translucent in the center and there has not been any vascularization. The ocular tension is normal.

The other cornea was utilized in a case of serpiginous ulcer, perforated. We performed a perforating keratoplasty of 6 mm. The graft is well adapted and quite transparent, without vascularization, even though there are some anterior synechiae, since the eye had been perforated during several days.

Taking in account the good results, you would oblige me by sending some more preserved corneas, as well as your human preserved vitreous.

These results have encouraged us to perform a 6 mm. penetrating graft with a preserved cornea which had been stored for eight months. The graft was done on an aphakic eye and is clear at this time, one month after operation.

VITREOUS

Preservation of the vitreous has not been practised in the past because the need did not exist. The successful use of vitreous implantation in treating complicated retinal detachments has now increased the demands for human vitreous (7). Storage of pooled fluid vitreous by ordinary refrigeration does not appear to be the best method of preservation.

Paufique and Moreau (8) feel that the passage of fresh vitreous through a needle alters its physical and chemical composition. They have advised the use of lyophilized vitreous to alleviate this, by reducing the volume of the gel without altering its chemical properties. These investigators did not propose lyophilization primarily as a method of storage. They have transplanted vitreous by placing the lyophilized powdered substance in the eye with a specially constructed cannula and following this by the injection of 1.5 c.c. of saline solution or distilled water. In an eye without a lens, they introduced the powdered vitreous through the pupil with a curette after the diseased vitreous was aspirated.

In our studies, the vitreous is not pooled but is removed from individual human eyes within 72 hours after enucleation, after the cornea is excised. The lens is removed, and if the hyaloid membrane has not ruptured, it is incised. A 10 c.c. syringe without a needle is placed into the eye and the vitreous is aspirated. Usually 2 or 3 c.c. of vitreous can be obtained. In a young donor the gel is often too thick to be drawn into the syringe, in which case it is removed by a spatula. The vitreous is transferred to a sterile Pyrex tube containing 30 minims of antibiotic solution (Neosporin solution) or sodium sulamyd, 10 percent. The tubes are immersed in a container of alcohol–dry ice

slush for about fifteen minutes until the vitreous is frozen. They are then removed and are connected to an apparatus similar to that used for the rapid freeze-drying of arterial segments. The system is designed for maximum diffusion and speed in drying. The tubes are kept immersed in containers filled with solid carbon dioxide during the first hour of the run. The manifold system is evacuated by a high vacuum pump connected to a diffusion pump. The silvered trapping apparatus on a table is surrounded by liquid nitrogen as a cooling agent to reduce the vapor pressure of water to a minimum. While the drying process is going on, the traps are kept filled with liquid nitrogen. The run lasts about ten hours and it may be necessary to fill the traps a second time during the run. When the vacuum is maintained at 1 to 4 microns on a Pirani gauge, the tubes are sealed by an oxygen torch at the thickened section below the joint and *well above* the vitreous. The tubes are stored at room temperature until needed.

Vitreous can be preserved by lyophilization with the powder sealed in a vacuum in a tube, and can apparently be stored indefinitely.

When vitreous is dehydrated after the introduction of antibiotic solution, it remains a whitish crystalline powder. With a 10 percent sodium sulamyd solution, it takes on a pinkish color. Lyophilization of vitreous alone results in a slightly yellowish powder which is more closely adherent than that previously described. Rehydrated lyophilized vitreous resembles normal vitreous in consistency and transparency. Vitreous is species specific, and heterogenous transplantation results in coagulation.

We have implanted vitreous following operations for retinal detachment by using a concentrated form. The powder obtained from 3 c.c. of vitreous was rehydrated with 1 c.c. of normal saline. This was injected through a knife-needle (Amsler) inserted through the sclera. There were no adverse effects to the eye, and a firm pressure was maintained at the outset. The vitreous probably undergoes further hydration in a period of time within the eye.

Preserved lyophilized vitreous has been implanted in twelve patients with retinal detachment and has been used in four instances as a vitreous transplant. All cultures taken on this material after storage have been sterile. Dried stored vitreous appears to act as well as fresh vitreous and has the following advantages:

1. It is readily available.
2. It is more viscous when rehydrated than is refrigerated pooled vitreous.

3. It can be used in concentrated form or even powdered form to undergo further rehydration within the eye over a more prolonged period, thus maintaining its pressure effect.

4. The inclusion of antibacterial agents assures sterility rather than depending upon the vitreous to be "self-sterilizing."

5. The inclusion of antibacterial agents may be beneficial in a diseased eye.

CONJUNCTIVA

The use of autogenous conjunctiva is the ideal means of supplying tissue to replace defects following injury, removal of extensive pterygiums, symblepharon, or tumors. This may be accomplished by transplanting conjunctiva from the same eye, by sliding flaps, or by a pedicle graft. An autogenous free graft may be taken from either eye by utilizing either the bulbar or the palpebral conjunctiva.

When enough conjunctival tissue cannot be obtained from the same patient, other tissues may be employed. Labial or buccal mucous membrane is the most commonly used. Epidermal grafts are also advised for an extensive defect, such as for restoration of an obliterated socket. Amniotic membrane has been advocated by some surgeons as a substitute for a conjunctival graft.

Fresh cadaver conjunctiva has been successfully grafted (9, 10) after four days of storage in a saline-dextrose mixture at 2° C.

Substitutes for conjunctiva are used by necessity, and although they may be satisfactory from a functional standpoint, they are often undesirable cosmetically. We have been able to preserve conjunctiva by several methods of dehydration. The most satisfactory technique appears to be that of dehydration in glycerine. This is a slower procedure than lyophilization, and the tissue is somewhat more fragile after rehydration. Conjunctiva preserved by freeze-drying after pretreatment with 15 percent glycerine becomes very hard and brittle. It resembles normal conjunctiva in appearance and consistency when it is reconstituted from the dry state.

After the eyeball is enucleated, the bulbar conjunctiva is excised in as large an area as is possible, taking only the conjunctival layer and avoiding the subconjunctival tissue. A suture is placed through the epithelial surface to aid in handling and in identifying this surface later. The tissue strip is then rolled upon a plastic rod and placed in a sterile Pyrex test tube. When the dehydration process is completed, the tubes are sealed in a vacuum and stored at room temperature.

When the conjunctiva is needed, the tube is opened under sterile conditions. The tissue is transferred to a shallow dish where it is covered with antibiotic mixture and allowed to rehydrate for 15 to 30 minutes before use.

Preserved dehydrated homogenous donor conjunctiva heals well, and when it is transplanted in animals it assumes the appearance of fresh homogenous or autogenous conjunctiva. Lyophilized conjunctiva heals more slowly and causes more tissue reaction during the healing period. Preserved human conjunctiva is being stored, but as yet there has been no opportunity for its use.

Preserved conjunctiva would offer the advantages of being immediately available for use following injury with loss of tissue, and it could be substituted for mucous membrane in the Denig method of treating severe chemical burns of the eye. It could be used wherever fresh conjunctiva is required.

SCLERA

Ectasia and staphyloma of the sclera which require repair are often operated upon by primary suturing or by resection and suturing. After excision, a staphyloma may be reinforced by sliding additional Tenon's capsule over the defect. The grafting of the patient's own fascia lata over a staphylomatous area has been reported in several cases of scleromalacia perforans (11). Progressive myopia has been treated by combining a 180 degree vertical scleral resection with the insertion of a band of sclera 12 mm. wide placed horizontally from the medial to the lateral rectus muscles around the posterior pole of the eye. This shortens the globe and strengthens the posterior pole, thus avoiding further stretching (12).

It would be possible and preferable to use cadaver sclera in many operations requiring the use of a tissue to reinforce or replace the sclera. Its availability, however, would pose a problem in furthering this type of surgery.

Sclera can be preserved by dehydration in glycerine, or by freeze-drying, and stored indefinitely in vacuum-sealed tubes at room temperature. The latter method is more rapid and is preferred. The tissue is rehydrated before use by immersing it in a saline-antibiotic solution for 15 to 30 minutes.

We have removed full-thickness circular discs and strips of sclera from animal eyes and have repaired the defects with preserved stored homogenous sclera. The donor sclera is sutured in place with inter-

rupted silk sutures and covered by a piece of preserved conjunctiva. After healing, the transplanted tissues cannot be distinguished from normal conjunctiva and sclera.

LENS

In 1956, Cavka (13) reported the successful transplantation of a cadaver lens to the eye of a 32-year-old patient without light perception and from which a cataract had been removed. There was no irritation of the eye and the intraocular pressure remained normal. The transplanted lens was reported to be perfectly clear six months later.

Hull (14) transplanted fresh kitten lenses to cats and all the transplants became opaque.

We have experimentally transplanted fresh homogenous lenses in rabbits, cats, and dogs. Most lenses became opaque several days after operation. The lens of one dog remained transparent for three weeks. There are great technical difficulties in removing animal lenses because of the strongly adherent zonules which must be ruptured mechanically, a process which results in considerable trauma. Barraquer's recent discovery of zonular lysis, by means of alpha tryptic enzyme, is now being applied and may allow more accurate evaluation of lens transplantation (6).

Efforts to preserve the lens by freeze-drying and by dehydration in glycerine have proved fruitless. The lens has been stored immersed in vitreous, sealed in a vacuum tube, and kept at 6° C. for as long as three months and has maintained its transparency. Studies are continuing in an attempt to preserve and transplant lenses.

The use of amniotic membrane to replace conjunctiva (15) has been advised by some researchers. This membrane has also been used to cover a corneal graft in the same manner in which Thomas employs egg membrane. Amniotic membrane has been preserved by freeze-drying.

We have used preserved human corneas as corneal caps to cover a corneal graft.

In conclusion, it is felt that new developments in the preservation of eye tissues will greatly advance the progress of eye surgery. Corneas, vitreous, conjunctiva, and sclera may be made available to any eye surgeon in any location when the ideal eye bank becomes a reality. Surgical techniques, now confined to large hospital centers, could then be performed whenever and wherever they are required.

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DISCUSSION

DR. FREDERICK W. STOCKER. Dr. King's paper is a very timely one, and I greatly enjoyed reading it. He puts the situation squarely before us when he states that although we have a number of eye banks in this country they really are merely collecting and distributing agencies rather than banks. While most eye banks limit themselves to corneal tissue, Dr. King has

extended his investigation to other ocular structures which should be available at all times for emergency use.

Dr. King's methods of preservation are vacuum dehydration for the cornea and freeze-drying for vitreous, conjunctiva, and sclera. The great advantage of these methods is that the material so processed can be stored at room temperature over long periods of time. Whether the tissue preserved by these methods retain their viability does not seem to be of great importance if they are used only for tectonic purposes. With regard to the cornea, we do not know as yet whether donor tissue treated in this manner will be the most suitable material for perforating grafts where perfect clarity of the graft is desired for optimal visual results. We shall come back to this point later.

Since Shafer (Tr. Am. Acad. Ophth., 61:194–200, 1957) has revived and revised the method of vitreous implantation for the treatment of retinal detachment first described by Deutschmann in 1910 (Graef, Arch. f. Ophth., 74:206, 1910), a demand for stored vitreous has developed. It has been shown that vitreous can be stored in the refrigerator for many months. At our hospital, vitreous has been used successfully that had been stored in this manner for five months. This would seem to make it unnecessary to develop other methods of preservation. Yet, according to Paufique and Moreau, quoted by Dr. King, lyophilized vitreous might even be preferable to fresh vitreous. Also, the theory that vitreous is self-sterilizing when stored in the refrigerator has been severely questioned (Ted Suie and Stanley Stroufe, Tr. Am. Acad. Ophth., 61:530, 1957). Therefore, the possibility of adding an antibiotic with Dr. King's method might constitute another advantage. Since the essayist has successfully used vitreous preserved in this manner in humans, the method should be ready for general evaluation by other surgeons.

There is no doubt that it would be of great importance if conjunctival tissue were readily available for grafting in cases of severe injuries, in particular, chemical burns. True, steroid therapy has made conjunctival grafts unnecessary in many cases. But there are still instances which call for grafting procedures.

The same remarks apply to scleral grafts.

Both conjunctival and scleral grafts have been used successfully in animals by the author. If these results can be duplicated in humans, the preserved tissues might become of great value in the future.

Where corneal grafts are concerned, the evaluation of preserved tissue for its suitability is more complicated. Through the courtesy of Dr. King we have been able to test rabbit corneas stored by his method for viability in tissue culture. In contrast to tissues preserved by other methods, discussed in another paper in this volume (pp. 217–238), no growth could be obtained from any of the three layers of the cornea. Also the graft became cloudy and was edematous on rehydration, a fact already mentioned by Dr. King. Since Dr. King has reported excellent results with lamellar grafts, it must be

assumed that, for this type of grafting, it is not necessary for the donor tissue to be viable. The proliferation of the recipient cornea probably does readily replace the graft. After all, even after a simple keratectomy, without graft, a fairly normal cornea may be regenerated. Dr. King reports that perforating grafts in animals have not been successful with his material. He mentioned, however, that Dr. Barraquer in Spain had used two of these corneas for penetrating grafts and considers them successful. It was very interesting to hear that Dr. King, giving up his reluctance to use his material for perforating grafts, only a few days ago performed a corneal transplant using a donor cornea processed by his method and that the graft was clear after a few days. Certainly more extended experience should be available before it would be possible to evaluate these results. The combination of research with tissue culture and clinical experience might finally resolve the most interesting question of whether it is at all necessary for the corneal tissue to be viable when used for perforating grafts.

With regard to preserving the lens for transplantation, I personally have my doubts whether it is worth further investigation. After all, the presently used method of dealing with cataracts is one of the most satisfactory in the whole field of medicine. What more can you wish for than to be able to restore function to the point where, as demonstrated by one of our eminent members, ocular surgery is possible.

In conclusion, I should like to say that Dr. King has made real progress in the field of preserving ocular tissues, and he is to be congratulated on this. While there are still some points to be clarified where perforating corneal grafts are concerned, it is to be hoped that Dr. King's work will help to get the ball rolling toward better methods of preservation of corneal tissue in this country.

DR. RAMÓN CASTROVIEJO. I am very grateful to Dr. King for having permitted me to read his paper before presenting it to the Society and also for having given me some material to be used both in corneal transplantation and for vitreous implants. I was so eager to use his corneal material for a case that would give the best opportunity to evaluate its use that I have not been able yet to use it for lamellar grafts. One of these donor corneas was prepared for a lamellar graft, but the cornea of the recipient perforated and I was hesitant to use it for a penetrating graft. Dr. King seems to have shown by his experience that lamellar grafts will be just as successful when using his preserved material as when using a fresh one. A few days ago I was faced with a difficult situation. A patient who had undergone a total penetrating keratoplasty for a severe corneal ulcer ready to perforate was developing the same situation in the graft. I was in a hurry to save the eye at least structurally, but I could not secure a donor eye. Therefore, I used one of Dr. King's preserved corneas. The donor cornea after it was hydrated looked edematous and quite cloudy when it was implanted in the eye of the patient, but it sealed the eye thus improving it at least structurally. The

following day, when the eye was examined, I was surprised to find out that the preserved cornea had regained complete transparency. It looked exactly the same as if fresh material had been used. Only seven or nine days had elapsed from the time of the operation and it is very early to tell what the final result will be, but it proves that at least for emergencies the preserved material of Dr. King will be invaluable. Possibly it will also be invaluable for all purposes if it is proven to be as successful in penetrating keratoplasties as it seems to be in lamellar ones. I have used his lyophilized vitreous in a case of retinal detachment and have not observed any difference in the postoperative behavior of this case as compared with those where fresh vitreous was used. If lyophilized vitreous proves to be as effective as fresh vitreous, this will also be a very important contribution to ocular surgery. I have not had any experience with conjunctiva or sclera but this may prove also to be a great addition to our surgical stock. I am extremely grateful to Dr. King for his kindness in supplying me with his preserved material and I sincerely hope that his contribution will prove to be as outstanding as the initial successes already reported seem to indicate.

DR. WILLIAM JOHN HOLMES. In my opinion the use of human preserved cornea for transplantation is a significant milestone in ophthalmology. In areas where donor cornea is not available, where there are few if any Eye Banks, as in most parts of Asia, and where the incidence of eye disease is very high, the use of preserved ocular tissue may make the difference between sight and blindness. Donor corneas may not be available because of the beliefs of the people. Among many of the Buddhists in India life is considered merely one phase of existence to be followed by re-birth, possibly is a better form, until perfection is eventually reached. The untouchable (Harajan) does not want to depart to the Great Beyond and start life with one or both of his eyes missing. Elsewhere in the Orient, among the Taoists in China and Okinawa, who are ancestor worshipers, an ancestor who had one or both of his eyes removed after he died might not command the respect that is due him.

Last year two young American ophthalmologists travelled to American Samoa to take care of the ophthalmologic needs of that community. According to their report the commonest cause of unilateral blindness was corneal scarring. If they had had a handful of Dr. King's preserved stored corneas with them, they could have restored the sight of many people. Lastly, if Dr. Tom Dooley, author of *Deliver Us from Evil* and *The Edge of Tomorrow*, and ophthalmologists serving in underdeveloped areas of the world could be taught the technique of keratoplasty and be supplied with a handful of preserved stored corneas, they might restore the sight of many blind people and accomplish a great deal in furthering international friendship and understanding.

DR. JOSEPH A. C. WADSWORTH. Since detachment of the retina rarely occurs without disease of the vitreous, it appears to me that preserved vitreous may

be far more beneficial than the normal or pooled vitreous. By inserting this more concentrated vitreous within the vitreous body and re-expanding it and thereby increasing the cushioning effect of the vitreous body, we may have a more prolonged contact of the retina to the treated area. If this proves to be true, it may obviate the need for performing such difficult and dangerous operations as are now advocated.

DR. KING. Dr. Stocker's remarks on determining the viability of preserved corneal material by tissue culture are very apropos. We have not cultured our preserved corneas mainly because of lack of facilities and lack of funds for this highly specialized technique. It is generally accepted that lamellar grafts do not have to be viable whereas in penetrating grafts the endothelium must be living. If it now proves true that successful penetrating grafts can be done with our corneas, we must assume that at least in some instances the endothelium remains viable or that viable tissue is not required for a penetrating graft. This, of course, would revolutionize the current ideas on tissue preservation. Regarding tissue cultures, Dr. Stocker has only had two rabbit corneas which were preserved by us and we hope we will be able to study more of these later. Clarity of the graft after transplantation could be construed to be practical proof of viability if a living tissue is necessary. It is known that viability in arterial grafts is not only unnecessary, but is actually undesirable.

No doubt better methods of preserving corneas will be developed. We feel that at least we have "broken the ice" and have proved that eye tissues can be preserved. There is no question concerning the value of preserved corneas for lamellar keratoplasty and preserved vitreous when this material is needed for transplantation or implantation. The real break-through in preservation of tissue research has come with the use of glycerin in preservation. As you know this was an accidental observation in England in 1949 when Parkes noted the remarkable properties of glycerin in protecting fowl spermatozoa against very low temperatures. Many years have been spent since then attempting to prove why it is effective. Some of the advantages of glycerin are:

1. It protects the cell organoids by preventing the formation of free water. It does not, however, prevent the formation of free ice crystals.
2. It protects the cells against damage on freezing and thawing by functioning as a salt buffer.
3. It has low molecular weight.
4. It is miscible with salt solutions.
5. Glycerin is non-toxic even in concentrated forms.
6. It permeates cells freely.

In answer to Dr. Castroviejo's remarks regarding the slowly developing clarity of his preserved full-thickness graft, this has been our observation from the early phases of this research with lamellar grafts. They remain translucent for four to six days and then almost suddenly become clear.

This was especially true when we rehydrated the graft for about 30 minutes. Now, with rehydration of only 10 to 15 minutes the grafts are often clear within one to two days. They also clear more rapidly now that we include a scleral rim with the donor cornea. Dr. Castroviejo's cornea was about a year and a half old and had been excised at the limbus.

Dr. Rychener has just told me that donor eyes are often available in remote areas and are not always needed. We are most anxious to have this material sent to us for preservation and in turn we will supply preserved tissues to such areas for their use when needed. Most human eyes which were stored for these studies were sent from the eye banks in Buffalo, Rochester, and Schenectady, New York, which have been most cooperative.