KERATOPLASTY

EXPERIMENTAL STUDIES WITH CORNEAS PRESERVED BY DEHYDRATION *

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A LITTLE MORE than a century ago, the idea of corneal transplantation was described by Dieffenbach (1), the father of modern plastic surgery, as an "audacious fantasy." Today, this surgical operation is an accepted and practical procedure with wide application in ophthalmology. The indications, contraindications, and selection of cases are fairly well understood. Surgical technics have been developed almost to the point of standardization. Despite these advances, the progress of keratoplasty is still retarded by an overwhelming obstacle—the demand for donor corneas exceeds the available supply.

The purpose of this report is to present a new method of corneal preservation, which may alleviate the shortage of donor corneal material, and to consider its applications for keratoplasty.

PENETRATING KERATOPLASTY

The indications for penetrating keratoplasty have been clearly defined for many years. These include corneal leukoma, blood staining of the cornea, inactive interstitial keratitis, certain dystrophies (Groenouw), and keratoconus. The operation is usually employed for conditions which effect marked diminution of vision

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or blindness. The visual results are often dramatic. The technic is precise, and demands experience and dexterity on the part of the surgeon. Complications during and after the operation are not uncommon.

LAMELLAR KERATOPLASTY

Nonpenetrating, or lamellar, keratoplasty is indicated less frequently in corneal conditions which result in diminished vision. If the leukoma is superficial, its replacement by a layer of clear cornea can be expected to result in improved vision. If the opacification includes the deeper stroma and endothelium, lamellar keratoplasty is not beneficial. This technic can be applied as a primary procedure to improve the anatomical and physiological status of the tissues in certain instances of severe corneal disease or vascularization, and should be followed later by the penetrating method to obtain better vision. Such conditions would include interstitial keratitis with activity and neovascularization following corneal trauma or disease.

The uses of lamellar keratoplasty have recently been greatly extended by the experiences of European ocular surgeons. Paufique, Sourdille, and Offret (2) have emphasized the therapeutic indications. Franceschetti (3) also revived therapeutic transplantation and improved the technic in order to apply it to greater advantage. Although not in total agreement with these authors, Castroviejo (4) felt that lamellar keratoplasty was justified for treating certain corneal conditions, such as Mooren's ulcer, recurrent herpetic, rosacea, and neuroparalytic keratitis, when these were not responsive to other therapy. Paton (5) stated that the most interesting development in keratoplasty, and the least known in this country, is the use of the nonpenetrating corneal transplant for therapeutic purposes.

Whereas the main purpose of penetrating keratoplasty is to improve vision, the indications for lamellar keratoplasty are more numerous. Franceschetti (6) lists these as tectonic, esthetic, and therapeutic, as well as optical. The tectonic type is an attempt to restore or improve the anatomical or architectural structure of the cornea in preparation for an optical graft later. This is applicable to densely vascularized leukomata, such as those resulting

from chemical burns, and to very thin corneas in advanced keratoconus. It may be useful to stop the progress of a "malignant" pterygium instead of employing buccal mucosa or skin. Lamellar keratoplasty may be an "eye-saving" procedure when employed as an emergency operation for a ruptured descemetocele (7). The technic is rarely indicated for esthetic or cosmetic purposes.

The therapeutic indications for lamellar keratoplasty offer the widest field of application for grafting. Paufique (8) stated that therapeutic grafting "is a primary indication for keratoplasty and is at least as useful as optical grafting, if not more so. It actually amounts to a preventive treatment of corneal blindness." Therapeutic lamellar keratoplasty offers a means of treating progressive corneal diseases or chronic and recurrent conditions which do not respond to other therapy. The rationale of this treatment is not fully explained. Paufique (8), however, is in agreement with Filatov, whom he quotes as attributing a trophic action to corneal grafting as a part of his theory of "biogenic stimulants." The new cornea appears to stimulate a remarkable clearing of the diseased cornea surrounding the graft. Filatov, quoted by Paufique (8), recommends the use of small lamellar grafts near a diseased penetrating graft as a treatment. Paufique, however, does not feel that this procedure is always successful.

The therapeutic indications for lamellar keratoplasty are now agreed upon by most authorities (4, 6, 8) to include: (1) keratitis caused by a neurotropic virus (herpes simplex or zoster), the so-called disciformis, and "metaherpetic" keratitis, (2) interstitial keratitis, luetic or tubercular, or (3) recurrent keratitis of indefinite etiology. Franceschetti (6) feels that the aim of the operation is to "hinder the evolution of a corneal disorder."

The use of corticosteroids has probably reduced some of the indications for therapeutic transplants, as witnessed by the marked effect of prednisone and prednisolone (7) in metaherpetic keratitis. The misuse of these drugs in herpetic keratitis (dendritic) can be deleterious, however, and may result in a neurotropic keratitis requiring corneal transplantation to effect a cure.

Therapeutic grafting in more than forty cases was reported from France by Hugonnier-Clayette (9) in 1949. In England, Black, Foster, Rycroft and Romanes, Lister, and Hobbs (10–14) have

noted the value of lamellar keratoplasty for numerous corneal diseases. In this country Paton (15) and others (4) have published their experiences with this technic.

Lamellar grafting is also useful in traumatic keratitis, postoperative dystrophic keratitis, and ulcerative keratitis. This technic produced good results in 90 percent of the cases of hereditary keratitis (Groenouw, Haab-Dimmer) in Paufique's (8) experience. He also recommends it in fatty dystrophy of the cornea, in primary chronic edema, and for rosaceous keratitis.

Lamellar keratoplasty is a much safer procedure than penetrating keratoplasty, and should therefore be preferred where benefit can be expected. The perforating technic is usually applicable for the same conditions, and may give similar results, but not without greater risk to the patient. Lamellar should be the operation of choice in nervous, unruly individuals, in aphakia, in one-eyed persons, and in the presence of nystagmus. Transparent grafts and favorable functional results were obtained in 75 percent of 270 lamellar grafts performed by Paufique (8). He aptly states that "if the lamellar graft loses its clarity, then the perforating graft is sure to fail." Every ocular surgeon should have a knowledge of the value of this simple technic, and should investigate its wide applications.

THE NEED FOR DONOR MATERIAL

The problems of obtaining donor corneas for keratoplasty have seriously impeded the progress and development of corneal surgery. A few years ago, the number of corneal transplantations was limited because of lack of training in operative technic and ignorance of the indications concerned. Donor material was adequate from freshly enucleated eyes. The experiences and teachings of such surgeons as Franceschetti, Paufique, Thomas, Castroviejo, and Paton have stimulated wide use of keratoplasty throughout the world, and many ocular surgeons are now well versed in the surgical technic and in evaluating the selection of cases. The indications for penetrating transplantation have become rather limited and static. Lamellar grafting, however, is constantly being given wider application. Although its uses from the optical and preparatory standpoints probably do not compete with penetrating

grafts, lamellar keratoplasty as a therapeutic agent makes this technic far more valuable to a greater number of patients in the treatment and prevention of corneal blindness.

STORAGE OF FRESH CORNEAS

For several years the supply of donor corneas has been inadequate to the demand, and the situation has recently become critical. The dependence upon the procurement of fresh corneas from enucleated eyes has been superseded by the use of cadaver material. Filatov (16) claimed that such material preserved at temperatures of 4° C. to 6° C. for as long as fifty-six hours was as good as fresh cornea. Fine (17), in a controlled experiment with animals, disputed this by showing that corneas preserved for twenty-four hours at low temperatures gave results which approached fresh material, but that after forty-eight hours the chances for successful transplantation were jeopardized.

"Eye banks" were established throughout the world in order to make grafts available to surgeons when needed. The system of obtaining and storing eyes varies in many countries. In England, the Corneal Grafting Act was passed by Parliament in 1952 (18) to permit eyes to be removed from the dead when objection had not been previously made by the deceased or his relatives. Eyes are removed within ten hours after death and are sent to an eye bank in liquid paraffin (18). A culture is taken and the eyes are then transferred to a container holding a mixture of liquid paraffin, streptomycin, and penicillin. This is refrigerated at 4° C. until the eye is needed, at which time another culture is taken. Successful grafts have been obtained from eyes preserved for as long as three weeks by this method.

The French Eye Bank (19), established in 1948, began operation in 1950. Eyes are made available through regional centers to all of France and North Africa. Modification of a law was necessary to permit the removal of eyes from cadavers. This method of supply is supplemented by eyes from donors who have signed pledges.

In 1945, Paton and Breckenridge pioneered in the establishment of an eye bank for the United States (20). Many hospitals are affiliated with this bank to furnish eyes from enucleations and donors. Branches of The National Eye Bank of New York City

have been established in other cities, such as Boston and Chicago. Eyes are preserved in a moist sterile glass container fitted with a ground glass top. They rest on a cotton dental roll saturated with aqueous merthiolate and the entire container is refrigerated at 6° C. Paton (21) states that an adult eye enucleated immediately after death (within six hours) will remain in good condition for as long as three days. Children's eyes and those of premature infants must be used within twenty-four hours. The best results are obtained from adult eyes, regardless of the donor's age. Eyes of stillborn infants are not desirable because of technical difficulties experienced with the malleable and delicate tissues. The donor material is carefully selected and classified as to age, sex, race, cause of death, and condition of the eye, and this information is transmitted to the surgeon (22).

Numerous other independent eye banks have recently been organized throughout the United States. Holt (23) has stated that the term "bank" is a misnomer, since transplantation should be done within forty-eight hours; eyes stored for longer periods are not suitable for use. Stocker (24) feels that transplantation should be performed within twenty-four hours after the eye has been removed from the donor, since endothelial changes occur after this period in cold storage. This is not as important when the cornea is to be employed for a lamellar graft (25), because the endothelium and Descemet's membrane are not used.

PREVIOUS STUDIES IN CORNEAL PRESERVATION

Although eye banks serve an excellent purpose, the demands for corneal material still overshadow the supply. Despite recent advances in keratoplasty, the operation is frequently limited because donor corneas are not available at the exact time they are needed. The need is often urgent because of the increased usage of lamellar grafting for therapeutic purposes.

The storage life of corneas is limited under the present methods of refrigeration, in which the temperature is above the tissue's freezing point. If an eye is not used within a few days, it must be discarded and therefore a supply cannot be accumulated.

Many investigators have attempted experimentally to preserve corneas. Smelser and Ozanics (26), Leopold and Adler (27), and

Katzin (28) employed rapid freezing in liquid nitrogen. Smelser and Ozanics (26) also froze corneas in isopentane chilled with liquid nitrogen to expedite the freezing process. The entire donor eye was plunged into the liquid nitrogen and left there from one to two hours. In four cases, eyes were stored in the freezing medium from three to four days. When eyes were removed from the liquid nitrogen, they were allowed to remain at room temperature for a few minutes until the cornea thawed sufficiently to permit the transplant to be cut. The authors state that the grafts were as good as or better than fresh cornea as far as operative technic was concerned. The corneal epithelium loosened easily and was soon lost. Grafts with the frozen cornea were uniformly clear until the fourth day. After this, even though they healed well, they began to cloud, and two months after operation all grafts were translucent or opaque. These investigators concluded that freezing corneas in this manner appears to preserve the tissue in as nearly a normal condition as possible. However, the corneas were presumably no longer living, although the tissue was clear and not changed structurally. They concluded that it was necessary to use viable tissues to produce clear, enduring corneal transplants, and that the corneal stroma of a clear graft retains its identity and is not replaced by the host for a long time. Leopold and Adler (27) and Katzin (28) dried the grafts *in vacuo* over P_2O_5 at 40° C. after freezing. In most cases they were reconstituted by isotonic sodium chloride solution. These investigators concluded that frozen cornea never regains its normal transparency when transplanted (27). Bajenova (29) had previously reported that the cornea can survive a temperature of −3° C. for nine days and maintains its viability in all cases.

The preservation of other living cells at low temperatures has been the subject of extensive investigations. Polge and his coworkers (30) treated fowl spermatozoa with glycerine-saline solution prior to freezing and found that they retained their motility after thawing. Skin, blood, and endocrine tissue (31) have also been successfully preserved by this method. The survival of tissues pretreated with solutions of either glycerine or sugars and frozen for long periods of time at very low temperatures was also reported by Fleming (32).

Smelser (33), stimulated by these successes, repeated his experiments on preserving corneas for transplantation. Donor corneas were immersed in sterile solutions of 20 percent glucose or 20 percent glycerine from fifteen to twenty minutes. They were then placed on small aluminum foil carriers, and were either slowly frozen in a dry ice-chilled chamber, or quickly frozen by immersion in liquid nitrogen. They were stored in the dry ice chamber or nitrogen until used (within two to twenty-four hours). They were rapidly thawed by placing in a warm solution (40° C.) of either the glucose or glycerine in which they had been pretreated. Slowly frozen corneas gave opaque grafts in every instance, while even in the best cases the rapidly frozen material yielded only translucent grafts. Poor healing of the grafts was the major cause of failure in using frozen corneas.

In England, in 1954, Eastcott and his co-workers (34) reported success in the preservation and transplantation of frozen human corneal tissue. Lamellar and full-thickness cadaver corneas were covered with 15 percent glycerine in Ringer solution (pH6) in small sterile bottles. They were allowed to stand at room temperature for one hour, and the solution was then decanted, leaving only enough to cover the graft. The bottle was closed by a tight screwtop and immersed for several minutes in a mixture of carbon dioxide and alcohol at −79° C. The bottle was then stored at the same temperature by placing it in a dry container with solid carbon dioxide (dry ice) held in a special box (Perspex). The entire chamber was then stored within a standard commercial deep-freeze. When needed, the cornea was thawed by immersing the bottle in a water-bath at 40° C. Fresh Ringer solution was then substituted and the bottle taken to the operating room where the cornea was transferred to normal saline solution containing 1000 units of penicillin per milliliter immediately before its use as a graft. After thawing, the lamellar and full-thickness grafts appeared normal in every way. The epithelium was loosened in two cases. Of the twelve grafts transplanted, five were lamellar and seven were full-thickness. The storage time of the lamellar grafts was from three days to nine months, and the results were successful in all instances. The postoperative course was the same as for fresh corneas. The full-thickness grafts were stored from five days to

seven months, and when transplanted these were considered successful in two patients and partially successful in three patients. Their postoperative behavior differed from fresh grafts in that clearing occurred much later. The investigators concluded that lamellar grafts can be stored indefinitely by this method, and will give results equal to those obtained with fresh corneal material. They do not advocate the routine use of frozen full-thickness grafts, however, and feel that further study is necessary to discover the reasons for failure.

In this country, McPherson and his colleagues (35) performed experimental studies on the viability of fresh and frozen rabbit corneas. The success obtained clinically by Eastcott and his associates (34) with preserved corneas pretreated by glycerine and saline seemed to prove that this method maintained viability of the tissues after freezing. McPherson's group (35) showed that excellent migration of epithelial and fibroblastic cells occurred in tissue culture of fresh cornea within forty-eight hours. When frozen without glycerine protection, the cornea showed no migration of cells in 28 percent of the cultures, and retarded migration with severe cell injury in the remainder. Corneas which were frozen after soaking in dilute glycerine showed a slight delay in cell migration on tissue culture, but were later indistinguishable from fresh corneas. Iliff (36), in 1954, prepared a human cornea by following Eastcott's method (34). It was kept frozen for about two hours, and was then used as a graft in a penetrating keratoplasty operation. The postoperative course was stormy, but a final corrected visual acuity of 20/20 was obtained.

McNair and King (37) felt that the technic of freezing corneas after soaking in the glycerine-saline mixture could be carried a step further by drying the tissue in a vacuum. They felt that the success achieved by storing corneas in a 15 percent glycerine and saline mixture at low temperatures (34) was the result of the protective action of the glycerine on the tissue cells rather than the freezing. The entire excised corneas of cats were placed in a glass tube container with just enough 15 percent glycerine and isotonic saline mixture to cover the tissue. The container was then immersed in a thermos containing a mixture of crushed solid carbon dioxide (dry ice) and alcohol for cooling purposes only.

This tube, with its mixture covering the cornea, was connected to a vacuum pumping system and the water was removed, leaving the tissue in approximately 100 percent glycerine. The process was simplified and expedited later by employing 80 percent glycerine and isotonic saline to begin with, so that less water had to be removed in the dehydration process. When dehydration was complete, the corneas covered by the glycerine were stored in the container in a vacuum with an airtight ground glass stopper. They were kept at room temperature, and remained clear and transparent as long as they were stored, which was for a maximum period of four months. The corneas were rehydrated in isotonic saline and were used as grafts at intervals of one week to four months after preservation. Nonperforating lamellar grafts from 5 mm. to 7 mm. in size were successful in all the eleven cats used in the experiments. Two corneas were placed in 95 percent commercial glycerine and were stored without dehydration at plus 4° C. They were transplanted after one month. When they were rehydrated they did not absorb the isotonic saline as well as the dehydrated corneas. The postoperative course was stormy, and final clearing did not occur until six weeks after grafting. The end result was a slight diffuse clouding rather than complete transparency. It was concluded that grafts dehydrated in glycerine and stored in vacuum without refrigeration were suitable for lamellar keratoplasty. Corneas stored in glycerine without dehydration at plus 4° C. were not sufficiently protected from autolysis.

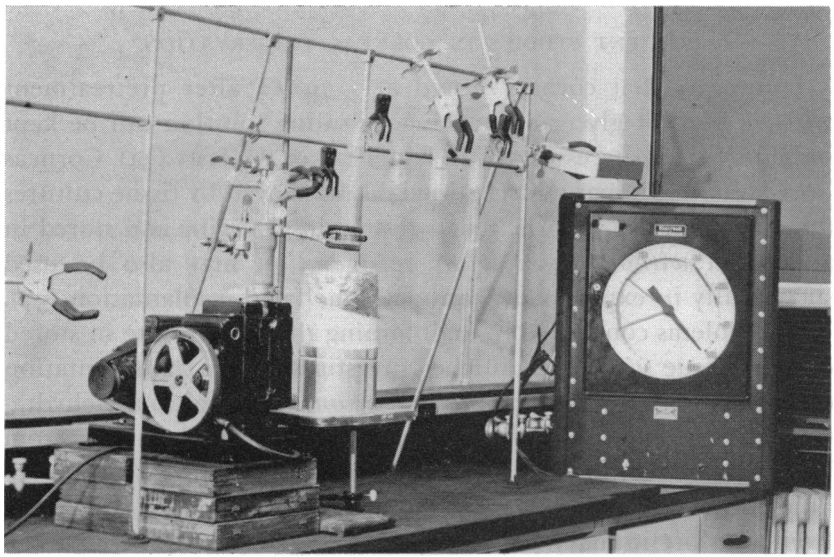
Bonhoure (38) preserved a human cornea for nineteen days by lyophilization. The method is not described. However, the corneal surfaces took on a grayish-white granular appearance and the cornea became opaque in its entirety. The cornea was lyophilized four days after enucleation. At the time of operation it was rehydrated in serum for forty-five minutes and regained its "flexibility and almost normal transparency." The cornea was used as a lamellar graft following the removal of an extensive pterygium by keratectomy. The graft was not sutured in place but was covered by amniotic membrane and crossed sutures. It healed well and became transparent, and the author felt that it retained its viability.

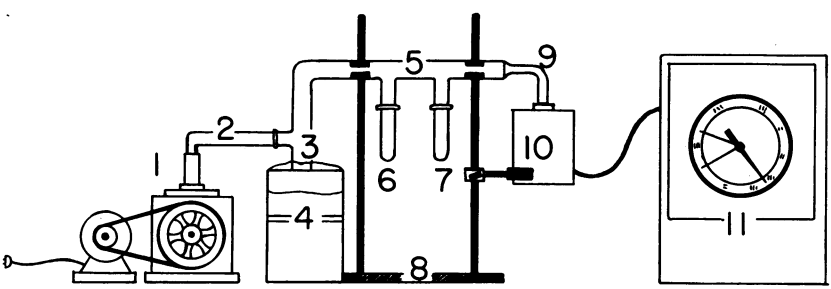
PRESENT STUDIES IN CORNEAL PRESERVATION

It appears that corneas stored at —79° C. after pretreatment with 15 percent glycerine in Ringer's saline solution can be kept indefinitely for successful use in lamellar keratoplasty (34). Corneas preserved in this manner remain viable as proved by tissue cultures (35) on animals. Cat corneas dehydrated in glycerine and stored in a vacuum *without freezing or refrigeration* may also be used successfully in experimental animal lamellar transplantation (37). The problems concerned in maintaining the frozen state in stored corneal tissue stimulated further investigation on transplantation of corneas *preserved without refrigeration.* It was felt that dehydration of corneas in 80 percent glycerine removed the water from both the solution and the cellular fluids and replaced the tissue fluid with approximately 100 percent glycerine. We concluded from our preliminary experiments that glycerine protected the cellular structure and maintained viability with cellular metabolism at a standstill. It was thought that a technic of dehydration similar to lyophilization could be developed in which the most concentrated glycerine solution available (commercial 95 percent) could be employed, eliminating the necessity of removing the fluid from a weaker solution.

APPARATUS AND METHOD OF PRESERVATION

The apparatus employed is a manifold vacuum system modified from that used to freeze-dry arterial segments for an artery bank (39). The equipment is simple, and can be assembled on an ordinary table or desk top. It consists of a Pyrex manifold with two ground glass outlets (Figure 1). (More may be used if necessary.) Two metal ring stands are used to support the manifold. A brass manifold can be employed, but glass has the advantages of allowing observation, being easy to clean, and permitting checking for air leaks. The manifold connects to a vacuum pump by way of a condensation vapor trap and hose connection. The closed bottom of the glass vapor trap is surrounded by a Dewar flask holding a mixture of dry ice and alcohol (95 percent) slush (—72° C.). This bath will usually last from twenty-four to thirty hours. It acts as a





1 - PUMP, CENCO HYPERVAC 4

2 - CONNECTING HOSE

3 - VAPOR TRAP

4 - DEWAR FLASK SURROUNDING TRAP

5 - PYREX MANIFOLD

6, 7 - SPECIMEN TUBES

8 - SUPPORTING RING STANDS

9 - CONNECTING HOSE

10 - PIRANI VACUUM GAUGE

11 - RECORDER

FIGURE 1. (ABOVE) PHOTOGRAPH OF DEHYDRATING EQUIPMENT; (BELOW) LINE DRAWING OF DEHYDRATING APPARATUS

coolant bath, which functions as a cold trap to remove condensable vapors (water) from the system. Vapors are drawn by the vacuum pump to the bottom of the trap where they condense and freeze. The pump must be protected from the water vapor evaporated from the specimens. The mechanical vacuum pump removes most of the air from the system and it is not necessary to increase the pressure by a diffusion pump. A vacuum gauge is attached to the manifold system. A "secondary" Pirani gauge, with a scale of 0 to 1,000 microns, is employed, and this is connected to an electronic recorder to obtain continuous vapor pressure records. Following the studies of Sauvage and his co-workers (39), the system is pre-evacuated by plugging the outlets in the manifold, starting the pump, and determining the maximum degree of vacuum which can be attained. A base-line pressure, without specimen tubes attached, was therefore determined, and this was found to be about 5 microns on the Pirani gauge.

ANIMAL EXPERIMENTATION

Technic of Preservation. Full-grown cats and dogs with normal eyes were sacrificed, and the corneas excised under sterile conditions.

1. Corneas are placed in a small sterile Pyrex test tube with a ground glass opening and with enough 95 percent commercial glycerine to completely cover the tissues. The glycerine has been previously sterilized by passing it through a Seitz filter.

2. The outlets of the manifold system are plugged and the motor pump started. After the base-line pressure is determined for this dry system, the pump is stopped. The outlet plugs are removed and the specimen tubes are rapidly connected to the ground glass outlets which are greased with "high vacuum" grease.[1]

3. The pump is again started and, because the vacuum system has already been primed, the system is cleared of air in several minutes.

4. During the early part of the dehydration run the specimen tubes are immersed in a dry ice-alcohol bath. This prevents the temperature of the specimen from rising too high in case there are

[1] High-vacuum grease, No. 43, manufactured by Dow Corning Corp., Midland, Michigan.

air leaks or extraneous water in the system when it is being evacuated.

5. No further attention is necessary while the system is operating, and the original base-line pressure is usually reached in about five hours. The procedure is begun in the late afternoon, however, and the apparatus is allowed to run until the next morning. Thus, although the end point of the dehydrating cycle has been reached, the system is allowed to continue for several hours longer (Figure 2). If the apparatus is efficient and has been pre-evacuated, the specimen and its glycerine solution become the

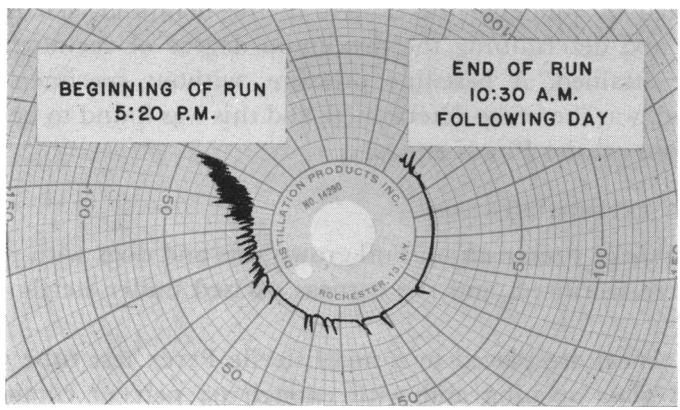


FIGURE 2. A TYPICAL PIRANI RECORDING

only source of additional gases entering the system unless an air leak is present. The pressure, as recorded on the Pirani gauge, is then a reliable index of the amount of gas being drawn from the specimens. When the base-line pressure is reached, there is little if any water left in the specimens. Occasionally, toward the end of the run, several small bubbles are noted surrounding the cornea. If the hand is cupped about the specimen tube to create heat, these gas bubbles will rise above the fluid level and be evacuated from the tube. This heat is continued until no further gas bubbles are visible. If the pressure during dehydration, as recorded on the Pirani recorder, does not continue to drop, or rises suddenly, the system should be checked for air leaks. This is done by placing a spark coil against various parts of the manifold and specimen

tubes. If the system is intact, a faint bluish glow is produced; if there is a leak, a dense bluish flash is noted.

6. The specimen tubes are hermetically sealed off near their attachment to the manifold by using a gas-oxygen torch, and the run is terminated.[2] In order to prevent spontaneous cracking, the ends of the tubes are carefully annealed to remove stress lines. Cement or wax is then coated over the sealed ends as a precautionary measure to maintain the vacuum in the event that a crack should occur. The spark coil is again placed near the specimen tube to verify the presence of a vacuum.

7. The tubes are labeled with pertinent information concerning the donor, length of time after enucleation, and the date of dehydration. They are then placed upright in a rack and stored at room temperature. The animal corneas rest on the bottom of the tube and are folded slightly toward the endothelial surface.

Technic of Transplantation. Preserved corneas were used for transplantation at varying intervals in the normal eyes of full-grown cats and dogs. The animals were anesthetized with intravenous pentobarbital and the eye was prepared for operation under sterile conditions. The top of the tube containing the preserved corneas was cracked off and the tissue, after most of the glycerine was decanted, was "poured" into a Petri dish or a medicine glass half filled with a sterile solution of antibiotic-sulfadiazine mixture.[3] The glycerine could be seen to diffuse rapidly from the transparent normal appearing cornea. In about fifteen or twenty minutes the tissue appeared opaque, but otherwise assumed the physical properties of the fresh state. The saline and antibiotic-sulfadiazine solution had apparently replaced the glycerine in the cellular structure.

Lamellar Keratoplasty. 1. Sutures were used to retract the animals' eyelids. The globe was fixed by placing sutures through the episclera near the limbus, above and below.

2. A corneal trephine, modified by the author (40), was employed. This consists of a Gradle-Schiötz trephine holder, amended

[2] The first specimens prepared were closed with a ground glass stopper and sealed with wax.

[3] A stock solution containing crystalline penicillin, 1 million units; streptomycin, 1 Gm.; and soluble sulfadiazine, 2.5 Gm.; dissolved in 1,000 cc. of sterile normal saline.

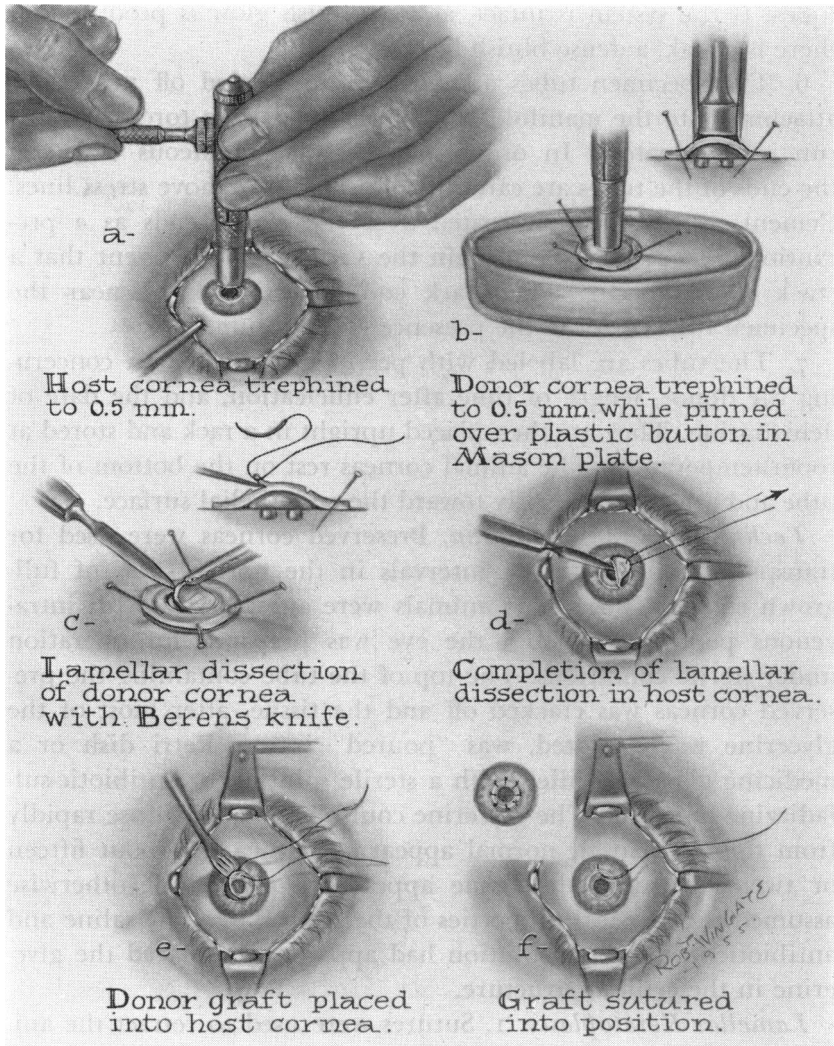


FIGURE 3. THE TECHNIC OF LAMELLAR KERATOPLASTY

to take a trephine blade with a guard, similar to the Katzin type (Figure 3a). The regular Gradle-Schiötz trephine has a fixed guard which is not suitable for partial penetrating keratoplasty. A trephine, with a guard that can be regulated for various depths, can be used for lamellar or penetrating transplantation. The usual trephine, rotated between the thumb and index finger of one hand,

tends to wobble and may not produce the clearcut edge desired for the donor disc or the host's corneal window. The Gradle-Schiötz handle offers the advantage of stability in that it is held and steadied by the left hand while the right hand is used to turn the side shaft which rotates the trephine blade.

The desired diameter of the trephine blade is selected and the trephine guard is set at 0.5 mm. to 0.6 mm. (or any depth required). The trephine is exactly centered and is turned until the cornea is penetrated to the guard. A small amount of fluorescein

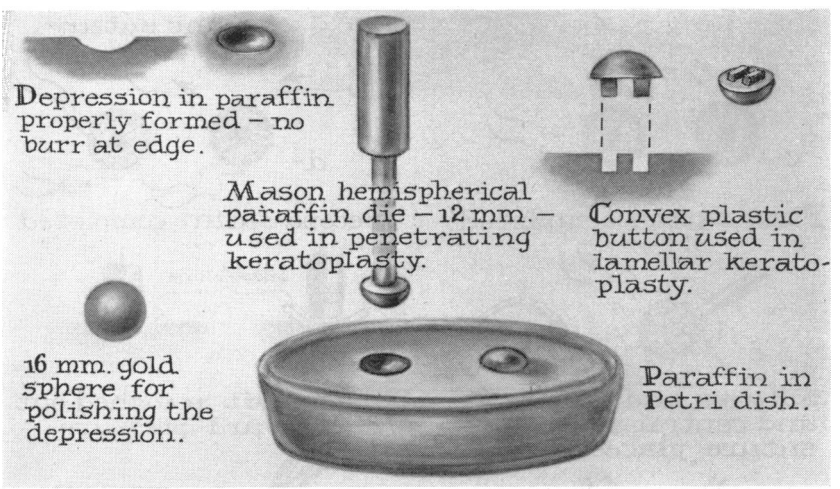


FIGURE 4. MASON PARAFFIN PLATE FOR USE IN TREPHINING DONOR CORNEA

solution on a cotton swab is used to touch the cornea to outline the trephine mark (Figure 3a).

3. The rehydrated donor cornea is removed from the solution in the Petri dish with a fenestrated spatula. It is placed upon a convex plastic button embedded in a Mason plate,[4] with the endothelial surface down. It is more difficult to dissect the lamellar graft from an excised donor cornea. Therefore, it is fixed by pinning the edges in the wax around the plastic button. The same

[4] Mason plate was developed by Gertrude I. Mason, R.N., for keratoplasty. This consists of a Petri dish half filled with paraffin, sterilized in an autoclave, and allowed to harden. A sterilized convex plastic button is embedded in the paraffin before it hardens. This is used in fixing the donor cornea in lamellar keratoplasty. Several concave depressions are made in the wax, using sterile convex brass molds, to hold the cornea for penetrating keratoplasty (Figure 4).

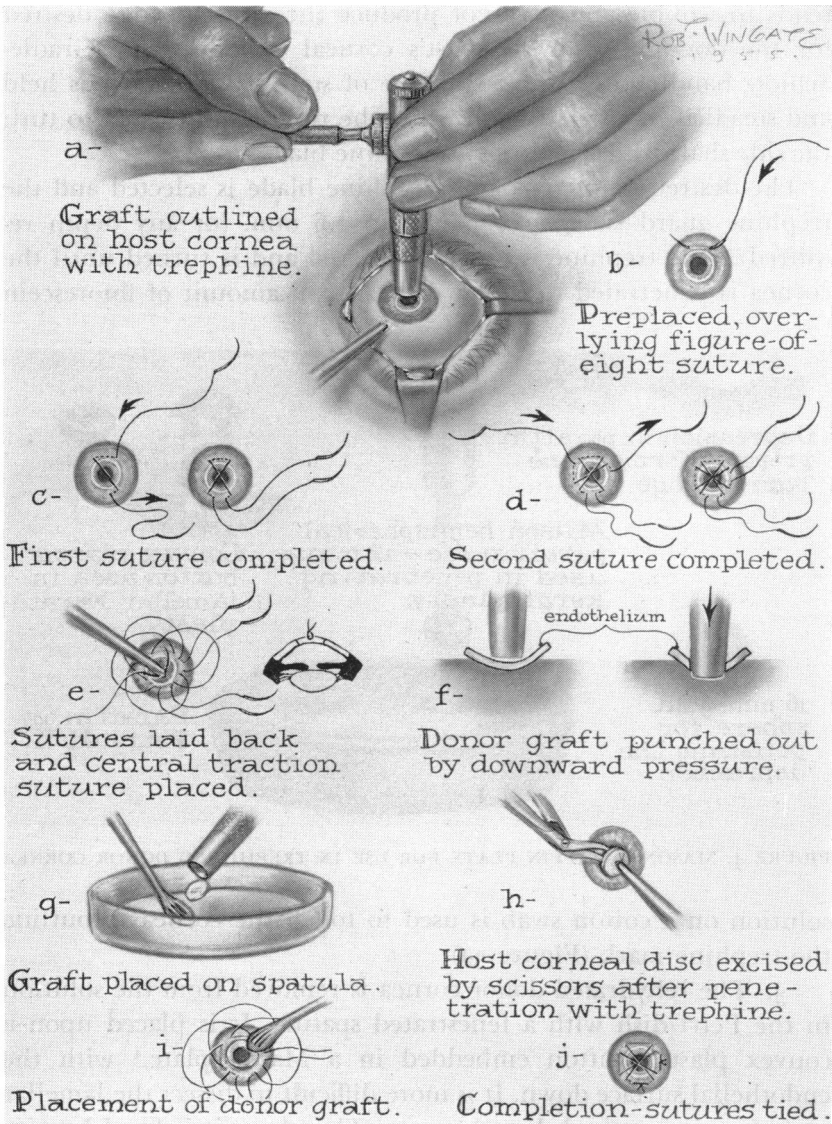


FIGURE 5. THE TECHNIC OF PENETRATING KERATOPLASTY

trephine, with the guard regulated to the depth used for the host cornea, is centered on the donor cornea and is rotated until the guard is reached (Figure 3b).

4. A Paufique knife, Berens knife, or a keratome is slipped under the edge of the outlined graft, freeing it, so that a suture can be placed through the edge for traction during the dissection. The needle should enter from the epithelial surface, since this suture is used for the first appositional suture later. If desired, more sutures can be placed in the edges as the dissection progresses (Figure 3c).

5. The dissection is continued in the same plane of the lamellas so that an even disc is removed. A Gill knife or Desmarres dissector may also be useful.

6. The outlined disc is removed from the host cornea in the same manner (Figure 3d) as described in paragraphs 4 and 5.

7. The donor cornea is transferred to the defect in the host cornea and the first suture, previously placed in the graft, is carried through the edge of the host cornea. Additional border-to-border sutures are taken, one opposite the other, until the graft is secured in position (Figures 3e, 3f).

8. Atropine sulfate solution, 1 percent, is instilled and the lid sutures are tied to close the eye.

9. Penicillin, 150,000 units, is given intramuscularly immediately after the operation and is repeated again in two days.

10. The lid suture is severed on about the fourth day, if it is still present. Atropine sulfate, 1 percent, and oxytetracycline ointment are then applied daily.

Penetrating Keratoplasty. 1. The trephine of the selected size, 5 mm. or 6 mm., has the guard set at about 0.1 mm. It is centered on the animal's cornea and is turned slightly to outline the graft (Figure 5a). This circular area is stained with fluorescein solution. If preplaced sutures are employed, they are inserted in a figure-of-eight fashion after the manner of Paton (15) (Figures 5b, 5c, and 5d). The sutures are then withdrawn beyond the outlined graft (Figure 5e). In this animal study, appositional sutures were used in the majority of cases. A central suture, tied with a loose knot, is applied to be used later for traction.

2. The preserved donor cornea is hydrated as previously described for lamellar keratoplasty. It is transferred to the Mason

plate into a suitably sized concave depression in the paraffin, with the endothelial surface up.

3. The same trephine previously used to outline the graft on the host eye now has the guard moved back to expose at least 2 mm. of the blade. The trephine is carefully centered over the cornea and the graft is *punched out* without rotating the blade, in the manner described by Amsler (41) (Figure 5f). This results in a clean-cut disc without injury to the endothelium. The donor disc usually remains in the trephine and is released onto a spatula by tightening the guard (Figure 5g).

4. The trephine guard is released beyond 2 mm. and the trephine is carefully placed over the previously outlined disc on the host cornea. The assistant fixes the eye, while the operator applies both hands to the trephine, as previously described. The trephine is rotated back and forth until perforation into the anterior chamber is revealed by a gush of aqueous humor or by displacement of the iris. The trephine is then immediately removed.

5. The central corneal suture is grasped with forceps and the cornea is excised by using curved Katzin scissors, which are directed to produce a vertical cut through the cornea and are kept towards the limbal side of the trephine incision (Figure 5h).

6. The donor cornea is transferred by a spatula to the host corneal window and is teased into position (Figure 5i). Four to eight appositional sutures are used to fix the graft if overlying sutures are not employed (Figure 5j). (If the nictitating membrane tends to cover the graft edge, it is incised and retracted or is excised.)

7. The lids are closed by lid sutures, and the same postoperative care is carried out as that described for the lamellar technic, except that solutions are employed instead of ointments for the medications. Any remaining sutures are removed in ten or twelve days.

8. In those cases where vascularization appeared after about one week, corticosteroids (hydrocortisone or prednisolone) were applied topically.

Results: Lamellar.—All grafts in the nine animals became clear and transparent within four weeks after operation. They remained transparent and in most cases were indistinguishable from the

good eye for the entire period of observation, which was from three months to one year. The postoperative course and healing did not differ from that observed when fresh cornea was used. All grafts, for about one week, retained the whitish opaque appearance which occurred upon rehydration. After this, clearing was rapid and complete clarity occurred in three to four weeks after transplantation (Figure 6). There was no instance of vascularization, and no infections occurred. The longest storage period for the preserved corneas was seven months (Table 1).

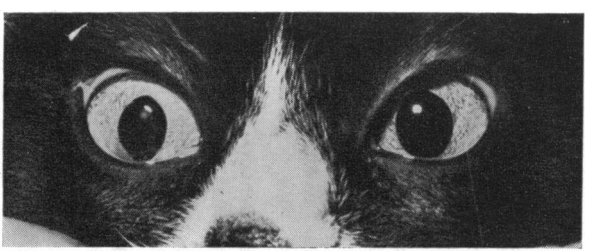


FIGURE 6. POSTOPERATIVE LAMELLAR TRANSPLANTS IN CAT
Right eye, three weeks postoperative. Left eye, one year postoperative from previous experiment (37).

Penetrating.—Five grafts became transparent or translucent in the peripheral area after two to six months, but the remaining three transplants resulted in totally opaque corneas. The tissues healed well in the usual time. Neovascularization occurred in two instances, and synechias to the grafts were present in these cases. Most of the grafts which became opaque did so within three to four weeks, while the remainder started to clear, became slightly translucent, but never became completely transparent. The five which cleared in the periphery did so after two to six months. There were no infections (Table 2).

Comment. Cats were preferred as experimental animals for several reasons. The cornea of the cat is thick, and this facilitates the technic of keratoplasty. The secondary aqueous humor is not as fibrinoid as that of the rabbit. It has been noted by others (24) that, although rabbits are the usual animals employed in experimental corneal grafting, they are not too suitable for these studies. A rabbit's tissue regenerative powers are apparently much greater than a human's and an opaque corneal graft may become clear after months of observation. This disadvantage may also apply to cat cornea. The five transplants of the penetrating type which

TABLE I. LAMELLAR GRAFTS

ANIMAL	OPERATION		STORAGE TIME	COURSE AND RESULTS
	Date	*Type*		
Cat 785	Nov. 23, 1954	Lamellar, 7 mm., 8 appositional sutures (right eye)	11 days. Dehydrated, Oct. 28, 1954	Gradual clearing, total transparency in 3 weeks.
Cat 809	Dec. 9, 1954	Lamellar, 6 mm., 9 appositional sutures (right eye)	6 weeks. Dehydrated, Oct. 28, 1954	Slight cloudiness with complete transparency, end of 4 weeks.
Cat 3940	Dec. 16, 1954	Lamellar, 6 mm., 6 appositional sutures (right eye)	7 weeks. Dehydrated, Oct. 28, 1954	Transparent, 3 weeks.
Dog 1855	Mar. 24, 1955	Lamellar, 6 mm., 8 appositional sutures (left eye)	2 weeks. Dehydrated, March 10, 1955	Transparent, 3 weeks.
Dog 861	April 7, 1955	Lamellar, 6 mm., 8 appositional sutures (left eye)	4 weeks. Dehydrated, March 12, 1955	Transparent, 3 weeks.
Cat 1640	April 13, 1955	Lamellar, 6 mm., 6 appositional sutures (left eye)	5½ months. Dehydrated, Oct. 28, 1954	Gradual clearing, total transparency, 1 month.
Cat 3921	May 5, 1955	Lamellar, 6 mm., 6 appositional sutures (left eye)	6½ months. Dehydrated, Oct. 28, 1954	Transparent, 3 weeks.
Cat 3921	May 12, 1955	Lamellar, 6 mm., 6 appositional sutures (right eye)	Approx. 7 months. Dehydrated, Oct. 28, 1954	Transparent, 1 month.
Cat 3952	May 12, 1955	Lamellar, 7 mm., 8 appositional sutures (right eye)	Approx. 7 months. Dehydrated, Oct. 28, 1954	Complete transparency, 3 weeks.

TABLE 2. PENETRATING GRAFTS

ANIMAL	OPERATION		STORAGE TIME	COURSE AND RESULTS
	Date	*Type*		
Cat 803	Nov. 18, 1954	Penetrating, 5 mm., 8 appositional sutures (right eye)	3 weeks. Dehydrated, Oct. 29, 1954	Graft generally cloudy first 4 months. No vascularization. End result, 6 months, dense central scar 2 mm. wide, periphery translucent.
Cat 740	Dec. 2, 1954	Penetrating, 5 mm., 4 appositional sutures (right eye)	5 weeks. Dehydrated, Oct. 28, 1954	Central graft cloudy, periphery transparent after 6 months.
Cat 3972	Feb. 10, 1955	Penetrating, 6 mm., 6 appositional sutures (right eye)	3½ months. Dehydrated, Oct. 28, 1954	Gradual clearing of graft. Small central nebulas after 6 months.
Cat 3972	Feb. 10, 1955	Penetrating, 5 mm., 4 appositional and overlying figure-of-eight sutures (left eye)	3½ months. Dehydrated, Oct. 28, 1954	Central graft opaque, clearer periphery, but not transparent after 6 months.
Dog 1854	Mar. 24, 1955	Penetrating, 6 mm., 8 appositional sutures (left eye)	2 weeks. Dehydrated, March 10, 1955	No clearing. Graft opaque with vascularization after 3 weeks. Synechias present to graft in third of circumference.
Cat 1184	April 7, 1955	Penetrating, 6 mm., 6 appositional sutures (left eye)	3 months. Dehydrated, Jan. 10, 1955	Cloudy in 1 week; opaque after 3 weeks. Small area of neovascularization, with synechias to fourth of circumference.
Cat 1641	April 13, 1955	Penetrating, 5 mm., 6 appositional sutures (right eye)	5½ months. Dehydrated, Oct. 28, 1954	Gradual opacification with opaque graft after 1 month.
Cat 3945	May 5, 1955	Penetrating, 6 mm., 4 appositional and overlying figure-of-eight sutures (right eye)	6 months, 1 week. Dehydrated, Oct. 28, 1954	Gradual clearing of graft periphery. Central area cloudy after 2 months, periphery transparent.

became clear in the periphery after several months of observation may have resulted from an unusual regenerative power not applicable to the human cornea. The postoperative care of cats is more satisfactory than that of many other animals. They are easy to handle and they do not claw at the eye. The cornea of the dog is very thin and malleable, and is not as suitable technically as is that of the cat. The cornea of the dog does not become opaque after rehydration, however. A dog will usually irritate the eye during the postoperative period. The monkey's cornea is ideal, technically, and also from the regenerative standpoint. They are not satisfactory for a study such as this, however, because of the expense involved and the difficult postoperative care.

Even though it was obvious from these experiments that penetrating keratoplasty was not attended with the success of the nonpenetrating lamellar technic, certain other factors should be considered. Penetrating transplantation is more difficult to perform and is more prone to operative and postoperative complications. Postoperative care in animals is far from ideal. When fresh corneas were transplanted in our control animals, success occurred in only one third of the cases. This is in keeping with the experiences of others with rabbits (27).

HUMAN APPLICATION

Method of Preservation. Enucleated human eyes with healthy corneas and eyes removed under sterile conditions within three hours after death were used in the human application of these experiments. The corneas were excised by making a Graefe knife incision including one-half the corneal circumference and completing the remainder with scissors. The tissues were immediately placed in small sterile glass tubes containing enough 95 percent commercial glycerine (sterile) to completely cover them. The tubes were connected to the outlets on the manifold of the dehydrating apparatus within one to five hours following enucleation. The same equipment and method of dehydration were employed as previously described for the use of animal cornea. After dehydration, the tubes were properly labeled and stored upright at room temperature for preservation until needed. The cornea rested on the bottom of the tube, but, unlike the animal cornea, it

retained its conformity and did not fold upon itself (Figure 7).

Technic of Operation. At the time of operation, the tube is opened under sterile conditions and the cornea is placed in a Petri dish containing the saline and antibiotic-sulfadiazine solution. After about thirty minutes, the cornea releases no more glycerine and is rehydrated. Unlike animal cornea, it does not become opaque, but remains transparent with only a slight haze.

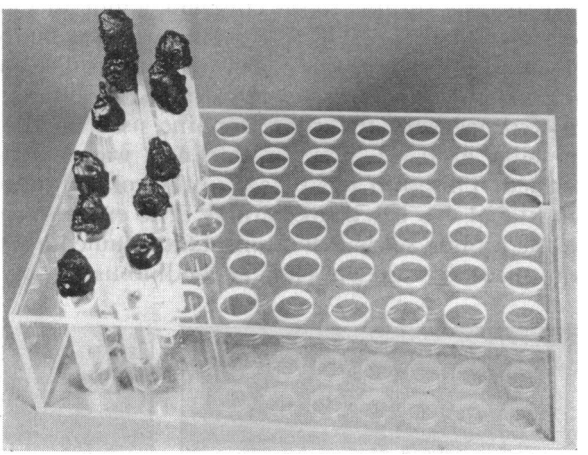


FIGURE 7. RACK HOLDING TUBES OF PRESERVED CORNEAS

The technic for lamellar and perforating keratoplasty was the same as that described for the animal experiments (Figures 3 and 5). A self-retaining lid speculum was used instead of lid sutures. Overlying figure-of-eight sutures were used for the perforating graft.

Indications. The accepted indications were followed in performing lamellar transplantation on five patients. Only one eye was available for the penetrating operation because of limitation of patients and certain medical legal aspects.

CASE REPORTS

CASE 1. A forty-year-old Negro man was admitted to the hospital on March 13, 1955, with a diagnosis of recurrent, right pterygium, with right visual acuity reduced to 20/400. The left eye was normal. There had been four operations for pterygium on the right eye, in 1950, 1951, 1952, and 1953. Examination revealed an extensive vascular "malignant" pterygium which extended over the nasal limbus to slightly be-

yond the mid-pupillary area. The cornea was deeply scarred beneath the growth and for several millimeters on each side (see color plate).

Operation: Lamellar keratoplasty was performed on the right eye on April 6, 1955, using local infiltration anesthesia. An 8 mm. trephine, with the guard fixed at 0.6 mm. in depth, was placed over the pterygium including the apex. This covered the entire growth on the cornea and part of the base. The pterygium and corneal tissues were dissected on the plane of the lamellas leaving a semicircular defect. The body of the growth adjacent to the limbus was excised, and the conjunctiva was sutured to the episclera, 3 mm. to 4 mm. back, by the bare-sclera method (42) (Figure 8). This area was badly scarred and densely vascularized. The donor cornea, which had been dehydrated in glycerine two days before, was rehydrated. An 8 mm. area was outlined on this cornea with the same trephine used on the patient's eye, and a lamellar graft 0.6 mm. in thickness was dissected as previously described. This graft was placed in the corneal defect and secured by appositional 6–0 black silk sutures. The excess corneal graft was excised at the limbus, so that the transplant assumed a semilunar shape. It was also sutured to the episclera at the limbus.

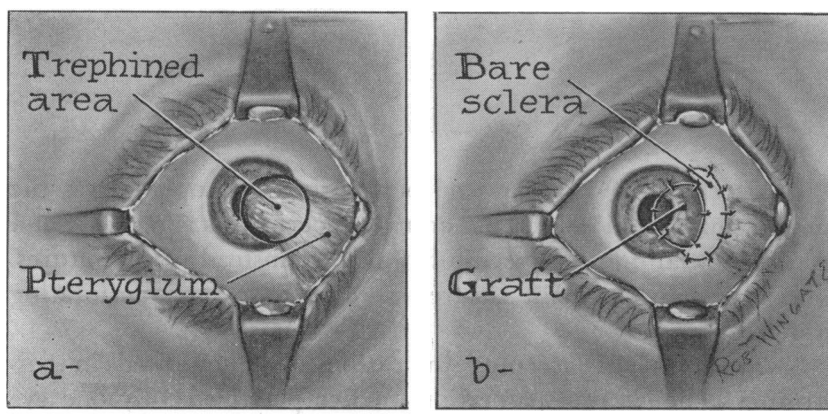


FIGURE 8. CASE 1; PARTIAL LAMELLAR KERATOPLASTY
a: Operative technic, area of pterygium trephined; b: Operative technic, partial lamellar keratoplasty.

Course: The graft healed well and remained transparent. The anterior chamber was immediately visible under the graft throughout its entirety. There was a slight haziness, however, from the scarred cornea. On the tenth postoperative day, when the sutures were removed, several fine vessels were noted entering beneath the graft at the limbus. At this time prednisone was administered orally, 5 mg. four times a day for one week, then 5 mg. twice daily. Prednisolone alcohol, 0.5 percent solu-

tion, was instilled topically every two hours. This therapy was continued for three weeks, and resulted in much improvement in the neovascularization. Two larger vessels at the nasal limbus were then subjected to beta irradiation with a total of 2,600 REP (Roentgen equivalent physical) to each area over a period of ten days. One month after operation, the graft was well healed and was transparent except for some haziness at the limbus where it had been irradiated. There was no evidence of vascularization following this and the vision improved to 20/100.

Comment: The indication for keratoplasty in this case was therapeutic, and was done to inhibit the progression of a massive multiple recurrent pterygium. It was realized that a graft encroaching upon the pupillary center would not allow much improvement of vision. A repeat keratoplasty at a later date is planned, with the graft properly centered, either penetrating or lamellar, for optical purposes.

This was the first human cornea subjected to the glycerine-dehydration preservation process. Even though the storage time was only two days, it was considered important to determine the success or failure of such a graft (see color plate).

CASE 2. A twenty-three-year-old white man was admitted to the hospital on April 13, 1955, with a diagnosis of chronic recurrent vascularizing keratitis of the left eye. Vision in the left eye was 12/200. The condition was the result of an accidental carbide of lime burn to the left eye in 1948. The patient was disabled at frequent intervals because of pain and photophobia, and the usual methods of therapy did not benefit the condition. The ocular tension was normal. The left eye was markedly blepharospastic. There was moderate circumcorneal injection, and generalized scarring of the cornea reached the interstitial area. Neovascularization was extensive, and involved the entire corneal circumference with superficial and deep vascular activity. Corneal staining was not prominent. Beta irradiation was applied to six different areas at the limbus over a period of eight weeks, with a total of 2,600 REP to each area of vascularization.

Operation: On May 2, 1955, lamellar keratoplasty was performed on the left eye. A 7 mm. trephine, with the guard adjusted to 0.5 mm., was centered over the cornea. There was considerable bleeding as the vascularized tissues were penetrated, and these areas were touched lightly with a hand battery cautery. A cornea which had been preserved for one month was used to obtain an equal size lamellar donor graft by the usual method. This tissue was normal in appearance and was transparent. The graft was secured in the host cornea by ten 6–0 mild chromic catgut sutures (see color plate).

Course: The postoperative course was uneventful, and healing was as rapid as with fresh corneal tissue. All sutures were absorbed by the

twelfth day, except for one which required removal. By the third week, blepharospasm had disappeared, and the patient felt more comfortable than he had for many months prior to the operation. The graft remained transparent, and the surrounding cornea showed marked improvement in clarity. Several small areas of neovascularization were apparent by slit-lamp biomicroscopy on about the fourth postoperative week. Local instillation of prednisolone alcohol solution, 0.5 percent, for two weeks, improved the vascularization. Four treatments were then administered with beta irradiation to several areas of vessel growth, with a total of 2,600 REP to each area. The condition of this patient's eye was dramatically improved by the operation. At the end of two months (June 30, 1955), the visual acuity in the left eye had improved to 20/80 (see color plate). At the time of this writing (eight months after operation), the cornea is still quiet and transparent.

Comment: This patient's corneal condition represented the classical indication for a therapeutic lamellar transplant. Catgut sutures were used because it was felt that the removal of silk sutures would be difficult and might cause additional trauma to the markedly inflamed blepharospastic eye.

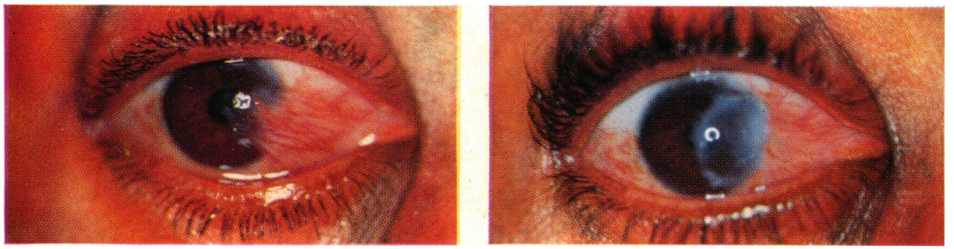
This case proved that a human cornea could not only survive the glycerine-dehydration processing, but could also be preserved in a vacuum for thirty days and could then be used with success as donor material for lamellar keratoplasty.

CASE 3. A twenty-five-year-old white man was admitted to the hospital on May 17, 1955. He was suffering from recurrent pterygiums of both eyes. Two operations had been performed on the right eye in 1944 and 1951. The left eye had had three operations, two in 1951 and one in 1953. Postoperative beta irradiation, dosage unknown, had been given to the left eye elsewhere. Corrected visual acuity was 20/20 in each eye. The left pterygium encroached upon the nasal cornea about 4 mm., and showed much scarring and vascularization.

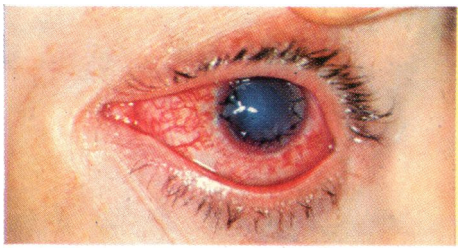
Operation: On May 25, 1955, the pterygium on the left eye was removed by the bare-sclera technic. A strip of scarred vascularized cornea about 2.5 mm. wide, 10 mm. in length, and 0.5 mm. in depth, of semicircular shape, was removed adjacent to the nasal limbus over the base of the growth. A double-bladed corneal knife was used to make the incision, and the tissue was dissected with a Paufique knife (Figure 9). A strip of glycerine-dehydrated cornea similar in size was prepared after the tissue was rehydrated. The preserved cornea had been stored for five weeks. The strip was sutured in place in the corneal defect by interrupted 6–0 black silk sutures.

Course: Healing was uneventful, and the sutures were removed at the end of two weeks. At this time the graft was in good position and was transparent. The lower end, however, was slightly elevated above

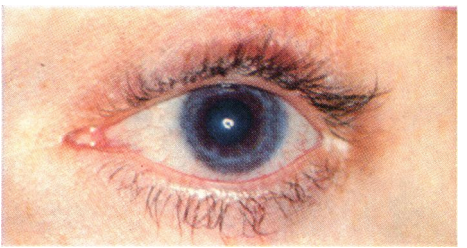
CASE I. BEFORE AND ONE MONTH AFTER OPERATION



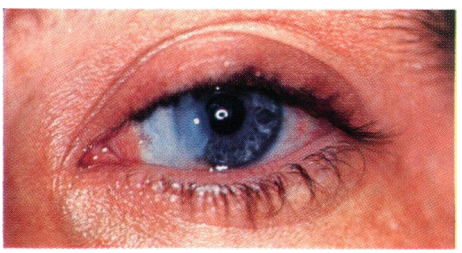
POSTOPERATIVE APPEARANCE OF FOUR CASES



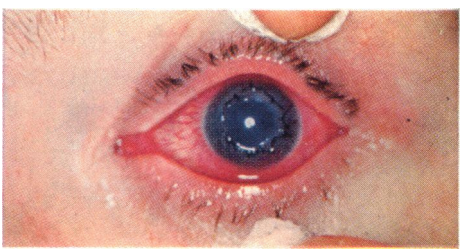
CASE 2. TEN DAYS



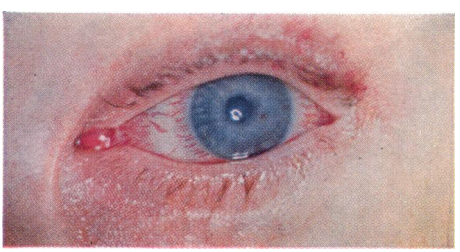
CASE 2. TWO MONTHS



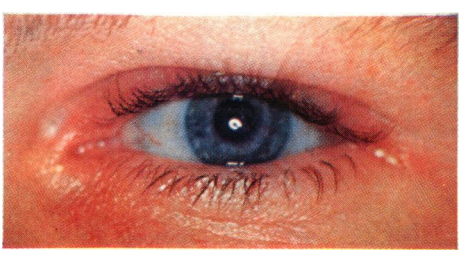
CASE 3. TWO MONTHS



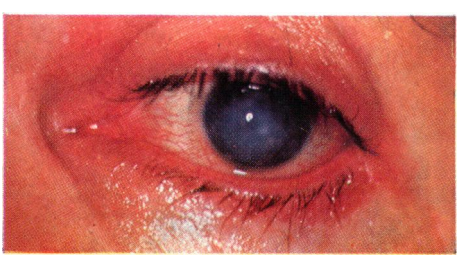
CASE 4. TEN DAYS



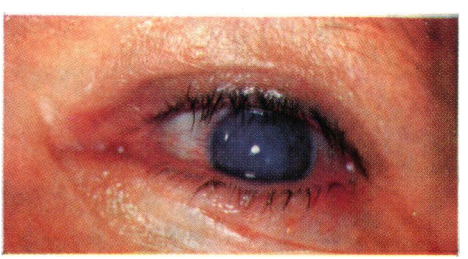
CASE 4. THREE WEEKS



CASE 4. SIX MONTHS



CASE 5. ONE MONTH



CASE 5. TWO MONTHS

the host cornea. At the end of three weeks a small new vessel was noted approaching the graft at the lower end. Beta radiation was applied to this area on four occasions for 23 seconds each time, and 650 REP each treatment, with a total of 2,600 REP. The neovascularization decreased during the next few weeks, and at the end of two months the raised lower edge of the graft became smooth (see color plate). The graft was clear and no recurrence of the pterygium was noted after six months of observation.

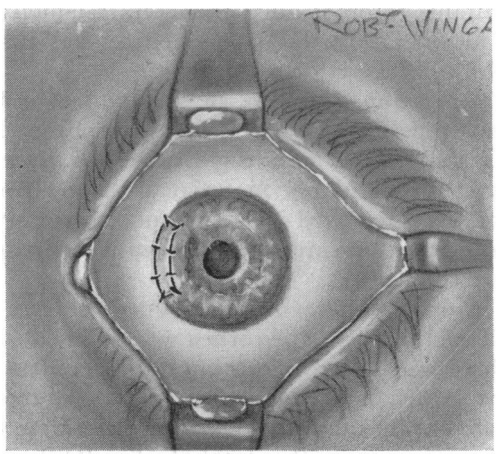


FIGURE 9. CASE 3; OPERATIVE TECHNIC, PARTIAL LAMELLAR GRAFT

Comment: The therapeutic application for limited lamellar keratoplasty was considered advisable to prevent repeated recurrence of a vascular pterygium. Mucous membrane and skin transplants frequently employed for this condition often result in a poor cosmetic appearance (42). The use of corneal tissue is not popular, mainly because of its unavailability.

CASE 4. This patient was a twenty-five-year-old white man who had had normal vision and no ocular complaints until September, 1954, when the right eye became inflamed and painful. The left eye became involved two weeks later. He was admitted to our hospital as a transfer from another hospital on September 30, 1954, and a diagnosis of bilateral atypical dendritic keratitis was made. The condition became quiescent after prolonged therapy, which included cauterizations, butazoladine, and retrobulbar alcohol injections. Upon discharge from the hospital on December 8, 1954, there was no staining, but both corneas showed deep infiltration and the vision in the left eye was reduced to 20/100. The patient was readmitted to the hospital in February, 1955, with a recurrence of symptoms in both eyes. Disseminated superficial eroded areas in both corneas were revealed by

staining. After conservative therapy for one week, the right cornea took no stain, but the left continued to ulcerate in the central area. Vision in the right eye was 20/25 and in the left 20/100. There was neovascularization entering both upper corneas. Both exhibited dense infiltration of the metaherpetic type. Topical application of prednisone and prednisolone (7) for three weeks resulted in marked improvement in both corneas. Because of prolonged hospitalization for frequent recurrences of the keratitis, and the continued vascularization and infiltration, which was more marked in the left eye, numerous consultants recommended therapeutic lamellar keratoplasty for this eye. The operation was postponed several times because of the unavailability of a fresh cornea.

Operation: On May 26, 1955, lamellar keratoplasty was performed on the left eye, using a 7 mm. trephine. The depth of the graft was 0.5 mm. A two-months-old preserved glycerine-dehydrated cornea was used for the donor material. The host cornea was prepared and the graft was removed in the manner previously described. The rehydrated cornea was sutured in place with interrupted 6–0 black silk sutures (see color plate).

Course: The postoperative course was uneventful and the graft cleared rapidly. The cornea was well healed, viable, and transparent in three weeks (see color plate). Vision after six weeks was 20/80 in the eye which had been operated on. At this time, three areas of neovascularization were noted, and these were treated by beta radiation on four occasions with a total of 2,600 REP to each area. After six months of observation, the left eye remained quiet and the patient was asymptomatic (see color plate). The grafted cornea was transparent throughout its depth, but deep opacification was still present in the host cornea. Scattered small areas of neovascularization approached the graft in several places, but were not seen to penetrate it. Six months following the lamellar keratoplasty, a 6 mm. perforating graft was performed within the well-healed lamellar graft. Healing was normal and visual acuity of 20/20 with correction was obtained.

Comment: Therapeutic lamellar keratoplasty was indicated to retard the activity of this severe recurrent viral keratitis. A donor cornea, which had been preserved for two months, fulfilled this purpose and healed in the manner of a fresh graft. The visual acuity was slightly improved. The physiology of the cornea, however, was returned to a more normal state in preparation for the optical penetrating corneal transplantation which was done later.

CASE 5. A fifty-one-year-old white man had been under treatment for a year for recurrent corneal ulceration of suspected viral etiology in the left eye. There had been numerous dendritic ulcers and a low-grade glaucoma which resulted in a metaherpetic keratitis with chronic

central ulceration. The cornea was densely opacified and the iris was barely visible. About two months prior to the admission, the left cornea was treated with topical prednisone and prednisolone solutions and ointments (7). Much clearing of the cornea was effected, and the anterior chamber and iris became clearly visible. The central ulcer became clean, and about the middle of April, 1955, a descemetocele was noted. The patient (a physician) was advised to enter the hospital at once, but he delayed for several days. On the morning of April 27, 1955, he was admitted as an emergency case because of rupture of the descemetocele. The anterior chamber was collapsed, and the perforation was noted in an ulcerated area occupying 5 mm. by 5 mm. in the central cornea. An emergency lamellar transplant was performed at once, using a 7 mm. fresh donor graft. Recovery was uneventful, and the graft was clear and without ulceration when the patient was discharged on May 28, 1955. There was a central leukoma where the perforation had occurred. On June 1, 1955, superficial ulceration was noted in the central area of the graft and a 2 mm. hypopion was present. The patient was readmitted to the hospital and was given intensive antibiotic therapy with chloromycetin, orally, and polymyxin B, intramuscularly. Marked improvement occurred, and within one week the hypopion disappeared. Medication was gradually diminished as the corneal ulceration in the graft subsided. On June 20 the central defect became more severe and rupture occurred in an area slightly below the original perforated descemetocele. The inferior half of the graft appeared ulcerated and necrotic. The anterior chamber was flat.

Operation: Fresh donor corneal material was not obtainable, and the patient was operated on about five hours following perforation with the use of a preserved cornea. A rehydrated glycerine-dehydrated cornea which had been stored for 42 days was used to obtain a 7 mm. donor graft. The host cornea was trephined in an area about 2 mm. below the previous fresh graft in order to include the ulcerated cornea and some clear host tissue. Eight 6–0 black silk interrupted sutures were employed to secure the graft. The anterior chamber was reformed by an air injection.

Course: The graft healed well, and when the sutures were removed in two weeks the donor tissue was transparent, but the host cornea underlying it was cloudy. A mucopurulent secretion grew *Staphylococcus aureus* on culture, which was resistant to all antibiotics. One month after keratoplasty, the donor cornea remained clear, but several superficial staining areas were present in the lower half (see color plate). This gradually disappeared with local instillation of 4.5 percent Gantrisan solution. The patient was discharged from the hospital two months after the operation (see color plate). After six months, the follow-up examination showed the eye to be quiet, and there was no corneal ulceration present. The graft was transparent and the

cornea upon which it was placed was scarred. The ocular tension remained normal. Vision was hand motion only in the left eye.

Comment: This patient suffered a severe viral keratitis with a protracted course, which is often the cause for total destruction of an eye. The use of stored cornea for keratoplasty saved the eye, which otherwise would have been lost. Fresh corneal tissue was not available at the time it was needed for the emergency operation. It was deemed advisable to perform lamellar rather than penetrating transplantation because of the presence of mixed infection and the tendency to ocular hypertension. After the eye has been quiet for a sufficient period of time, perforating keratoplasty should be done in order to improve the vision.

CASE 6. A twenty-one-year-old white man was admitted to the hospital on April 28, 1955. He was suffering from pain and photophobia in the right eye, which had become progressively more severe over the

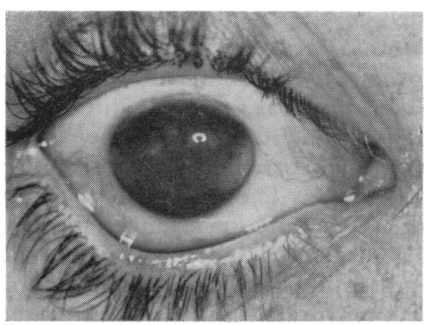


FIGURE 10. CASE 6
Preoperative condition, phthisis bulbi.

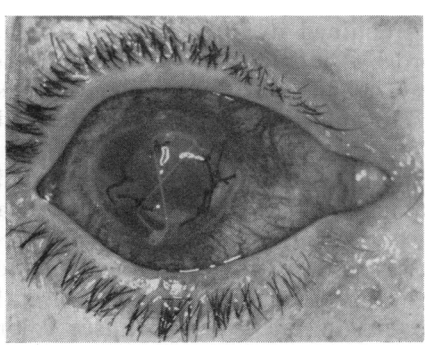


FIGURE 11. CASE 6
Postoperative appearance after one week.

past few years. A severe lacerating wound to the right eye had occurred in 1942, and there had been poor light perception since that time. The patient was referred for enucleation. The right eyeball appeared phthisical and was enophthalmic. A band-shaped opaque keratopathy

TABLE 3. HUMAN CASES GRAFTED WITH PRESERVED CORNEA

Patients	*Type of Operation*	*Indications*	*Storage Time*	*Result*
1	Lamellar, 8 mm., 0.6 mm. depth (right eye)	Recurrent active "malignant" pterygium	2 days	Therapeutic success. Transparent graft with haziness over incision and at limbus where beta radiation was used.
2	Lamellar, 7 mm., 0.5 mm. depth (left eye)	Chronic recurrent keratitis with vascularization following lime burn	30 days	Therapeutic success. Transparent graft with marked improvement of surrounding cornea.
3	Lamellar, strip, 2.5 mm. wide, 10 mm. long, 0.5 mm. depth (left eye)	Recurrent active pterygium	37 days	Therapeutic success. Transparent with no vascularization into graft.
4	Lamellar, 7 mm., 0.5 mm. depth (left eye)	Recurrent viral keratitis (metaherpetic)	56 days	Therapeutic success. Loss of symptoms, less vascularization, and no ulceration.
5	Lamellar, 7 mm., 0.5 mm. depth (left eye)	Recurrent viral keratitis (metaherpetic) chronic ulceration; emergency, ruptured descemetocele	42 days	Therapeutic success. Eye saved, with final elimination of symptoms.
6	Penetrating, 6 mm. (right eye)	Band keratopathy (phthisis bulbi)	42 days	Clear graft with dense pink membrane over inner surface separated from main graft.

was present in the inter-palpebral area. The pupil was occluded and drawn up. The anterior chamber was deep. Moderate ciliary injection was noted, but there was no neovascularization (Figure 10). The patient agreed to a penetrating corneal transplantation with preserved donor corneal tissue, as an experimental procedure.

Operation: Penetrating keratoplasty was performed on May 3, 1955, using a 6 mm. trephine. The technic previously described was followed, with preplaced figure-of-eight overlying silk sutures. The graft was punched from rehydrated corneal tissue which had been preserved for six weeks. The horizontal cross suture was cut at the time of trephination, and the cornea was secured by six additional interrupted 6–0 black silk sutures (Figure 11).

Course: The graft remained in good position and healed well. The sutures were removed on the fourteenth day, and the donor tissue appeared to be clearing, although the iris was not visible through the graft. Six weeks after the operation, the graft, by slit-lamp examination, was clear and transparent throughout its entirety. The thickness appeared normal, but a whitish, opaque mass or membrane was present behind the graft in the anterior chamber. Deep neovascularization entered this membrane from above and below. This tissue was not apparent before the operation, and grossly it did not seem to involve the endothelium. The globe was enucleated on June 21, 1955, for the purpose of pathology studies.

Comment: This eye was not an ideal one in which to test the viability of a full-thickness corneal transplant with preserved human donor material. It offered the only opportunity, however, to employ the procedure. The graft healed well, and there was no unusual host reaction except for the presence of the connective tissue membrane behind the graft which might not have occurred in a more suitable host eye (Table 3).

HISTOPATHOLOGY

HISTOLOGY

Cornea preserved in glycerine without dehydration.—An adult human cornea was placed in a vial covered by 95 percent sterile glycerine. The tube was sealed *without dehydrating* the specimen and was stored at room temperature for six months. The tissue was prepared for microscopic examination in the usual manner and was stained with hematoxylineosin. The epithelium and endothelium were intact. The section resembled normal fresh cornea except for the fact that the cell nuclei were smaller and darker upon staining under high power and they had a questionable pyknotic appearance.

Cornea preserved by dehydration in glycerine and not rehydrated.—An adult human cornea was dehydrated in 95 percent glycerine and stored in a vacuum for five months. It was *not rehydrated* but was placed first in absolute alcohol (a dehydrating agent) for several hours, then in chloroform for several hours, and was finally impregnated with paraffin. The epithelium, under miscroscopic examination, appeared irregular, thinner, and generally more compressed. The stromal corpuscles were paler upon staining and the lamellas appeared closer together. The nuclei of the endothelial cells were somewhat darker staining than usual.

Cornea preserved by dehydration in glycerine and rehydrated.—Another adult human cornea, after dehydration in 95 percent glycerine, was stored in a vacuum for five months. It was *rehydrated* by fixing in 10 percent formalin for several hours and then by washing in tap water for two hours. This was followed by the usual laboratory preparation, with final impregnation in paraffin. Microscopic examination showed the epithelium and endothelium to be intact. The epithelial cells were thicker than usual, and the nuclei of the endothelial cells were more elongated than were those in fresh cornea. The stromal lamellas were slightly irregular, and fewer corpuscles were visible in the outer third of the stroma.

PATHOLOGY

Animal cornea with healed lamellar transplant of graft previously preserved by dehydration in glycerine.—The right eye of a cat was enucleated (No. 809, Table 1) which had had a lamellar graft performed eight months earlier. The grafted cornea had been dehydrated in glycerine and stored for six weeks prior to the operation. The microscopic appearance of the section was similar to that of other successful lamellar grafts. The epithelium was somewhat thickened, and the outer half of the stroma appeared more cellular and deeper staining. The endothelium was intact and appeared normal. There was no vascularization.

Animal cornea with healed penetrating transplant of a graft previously preserved by dehydration in glycerine.—The right eye of a cat (No. 803, Table 2) which had received a penetrating transplant nine months previously was enucleated. The graft had been dehydrated in glycerine and was kept in storage for three weeks

before operation. The epithelium and outer two thirds of the stroma were normal. The inner third of the stroma showed dense deposition of pigmented cells with scarring and some vascularization. The endothelium was present, but was technically stripped from most of the graft, and in the intact area only occasional nuclei were noted.

Human cornea with healed lamellar transplant of graft previously preserved by dehydration in glycerine.—A circular section of cornea, 7 mm. in diameter, was removed from the left eye of a patient who was operated upon for a penetrating transplant. The button of cornea removed included a lamellar graft performed six months previously with tissue dehydrated in glycerine and stored for two months before operation (Case 4, Table 3). The specimen consisted of the entire disc of cornea containing the healed lamellar graft. The epithelium was intact over half the specimen and this area showed marked irregularity. The other half was replaced by scar tissue in which active fibroblastic proliferation was evident. No significant inflammatory reaction was noted, however. The cornea was scarred along a line corresponding to the middle of the stroma, and there was slight neovascularization present. The outer two thirds of the stroma was paler staining, and the stromal fibers were more swollen and less sharply outlined than in the inner zone. The nuclei in this area were also paler upon staining and were more indistinct. Descemet's membrane and the endothelium showed no significant changes.

The tissue was consistent with the appearance of a normal healed lamellar transplant, with slight neovascularization.

Human cornea with healed penetrating transplant of a graft previously preserved by dehydration in glycerine.—The right eye was enucleated from a patient who had been operated upon with a 6 mm. penetrating graft seven weeks earlier (Case 6, Table 3). The donor material had been dehydrated in glycerine, and was stored for forty-two days before transplantation. Microscopic examination revealed a traumatic cataract, extensive posterior synechias, a cyclitic membrane, and advanced degeneration of the retina. A broad band of scar tissue extended from the inner surface of the cornea at the margin of the grafted portion across the anterior surface of the iris to the margin of the graft on the op-

posite side. Between this band of fibrovascular tissue on the anterior surface of the iris and the posterior surface of the grafted corneal segment, there was a small space remaining in the anterior chamber which was filled with serosanguinous exudate. The epithelium of the graft appeared normal, but beneath this was a compressed layer of dark staining cells of new-formed fibrous tissue. The stroma appeared normal under this layer, but the graft was not as thick as the surrounding host cornea. Descemet's membrane was present in the graft, but was not continuous with the same membrane on the host cornea. The endothelium was not intact, but a few scattered endothelial cells were present on the grafted section. The inner layers of the host cornea extended into the band of scar tissue passing behind the donor cornea.

Comment: The corneal tissue preserved in 95 percent glycerine without dehydration apparently had undergone some autolysis and its viability, from the microscopic appearance, was questionable. The cornea preserved by dehydration in glycerine and not rehydrated seemed to contain less tissue fluid, as shown by its compressed character. Cornea stored after dehydration in glycerine, and then rehydrated, more closely approximated the cellular structure of normal tissue. The lamellar transplants showed excellent healing, and were consistent in appearance with successful nonpenetrating grafts. Both penetrating transplants healed, but were not successful grafts. Lack of viability or partial absence of the endothelium could have accounted for at least one of the failures. The unsuccessful human graft could have been the result of a poor recipient cornea, loss of donor endothelium, or possibly an inadequate approximation of the transplant. The smaller thickness of the graft may have been the result of too little rehydration of the previously dehydrated cornea. The new layer of connective tissue which formed behind the graft, and which bore some characteristics of corneal stroma, has been mentioned by other authors (27) in describing unsuccessful transplants.

COMMENT

Bajenova's (29) extensive work on the viability of corneas after storage at low temperatures proved that freezing begins at $-5°$ C. The percentage of growth on tissue culture is reduced as the tem-

perature is lowered, so that at −10° C. tissue viability is preserved for only two days and scanty growth is observed in 60 percent of the cases. At −20° C. for seven days, there is more abundant tissue growth. This occurs, however, in only 30 percent of the cases. He explains this better growth as being a result of the rapid freezing at a very low temperature, which causes the formation of fine ice crystals rather than the solid ice block which occurs in slower freezing. The latter freezing method produces cracks upon thawing, which tends to distort the tissue architecture.

Smelser and Ozanics (26) concluded that their failure to obtain clear grafts with frozen rabbit cornea was caused by the absence of viability of the tissue. They felt that the use of living tissue was necessary for success. Katzin (28), in his studies, concurred in this opinion.

Sedan (43) and Stocker (24) have proved that the corneal endothelium is rapidly damaged by cold storage. It is agreed by all authors that the endothelium on a graft must be intact, healthy, and undisturbed in order to produce a successful result in penetrating transplantation.

Some dehydration always accompanies prolonged freezing of the cornea (29) and the fluid is not regained upon thawing. This may not be injurious to the tissue, since cornea dried to a constant weight in a desiccating oven for three hours loses 76.3 percent of its initial weight. Filatov and Bajenova (44) showed that this dried cornea retains its viability. These same investigators claimed excellent results when transplanting such corneas after preservation at 2 to 4° C.

Eastcott and his group (34) felt that the failure of full-thickness grafts preserved by freezing after pretreatment with glycerine-saline solution was probably the result of damage to the delicate endothelium from the freezing process. McPherson and his coworkers (35) proved that animal cornea preserved by the glycerine-freezing method retained viability in a significantly greater number of tissue cultures than corneas frozen without prior glycerine treatment. In addition to mechanical cell damage from the formation of ice crystals in the tissue, Smith (45) and Lovelock (46) felt that when saline was employed it also exerted a deleterious effect. Salt becomes concentrated in the solution in which the cells

are suspended at some point between 0 and −40° C. It was suggested by Lovelock that this concentrated salt solution affected the integrity of the cell by its action upon lipids and lipoproteins of the cell membrane. He felt that glycerine, by its hygroscopic properties, prevented the formation of intracellular ice crystals by partially removing water prior to freezing. In addition, the glycerine may have a "buffering action" against the sudden increase in electrolyte concentration. The rapid action of glycerine in clearing edematous corneas has been noted by Cogan (47) and others (24).

As previously mentioned, corneas may be partially dried (44) and still remain viable. Complete dehydration, as practiced in the lyophilization preservation process applied to other tissues, cannot be used for the cornea. Because the cornea consists of about 81 percent water, not enough cohesive structure remains and the tissues will powder. In the method herein reported, when the cornea is dehydrated in glycerine, the water which is withdrawn is replaced by the glycerine. Although cellular metabolism is brought to a standstill, the cellular structure of the tissue is left undisturbed. Freezing is, therefore, unnecessary and the tissue remains preserved at room temperature. Glycerine compounds have wide commercial application in protecting fluids from freezing and from boiling. Our corneas remained at temperatures varying from 66° F. to 99° F., and were carried to the laboratories and operating rooms in the investigator's coat pocket.

The advisability of storing only the cornea rather than the whole eyeball has also been emphasized by others (48, 49).

These experiments have proved that lamellar keratoplasty performed with donor material preserved by dehydration in glycerine is successful. The dehydration process and the replacement of the tissue fluids by glycerine apparently do not affect the viability. The failure of the full-thickness grafts to remain clear may be the result of many factors. The endothelium may be damaged by the processing, so that it cannot prevent aqueous humor from infiltrating the donor cornea. The endothelium may not be capable of maintaining its function of regulating the nutritional exchanges between the cornea and the aqueous humor. It is possible that, in rehydrating the graft slight edema, even though temporary, may prevent perfect apposition of the posterior corneal layers with the

host's tissues, so that aqueous humor seeps into the graft. Technical difficulties in performing penetrating keratoplasty in animals may have accounted for some failures. Not enough cases were available to properly evaluate the penetrating procedure in humans.

SUMMARY AND CONCLUSIONS

Penetrating keratoplasty has a limited use for the average ophthalmologist (50). Therefore, this technic of corneal surgery is usually confined to certain ophthalmic centers. Nonpenetrating or lamellar keratoplasty, however, has wide application from the therapeutic aspect, and it becomes a necessity for every ophthalmologist to recognize its value. The physiology of the cornea can often be restored in recalcitrant corneal disease by lamellar keratoplasty, thus reducing morbidity.

The increased demands for donor cornea have exceeded the availability and this has greatly retarded the progress of corneal surgery. The solution to this problem is dependent upon proper utilization of donor eyes and the development of a satisfactory method of preservation of corneas. Until recently no method has been devised which will preserve the excised cornea in a viable state for an indefinite period of time. The process of freezing causes cell damage and jeopardizes the chances of retaining tissue viability (26, 27, 28). When the cornea is pretreated with 15 percent glycerine in Ringer's solution and then rapidly frozen, it can be preserved for an indefinite time (34, 35). Lamellar grafts of human cornea stored for as long as nine months have been uniformly successful. Full-thickness grafts with the same material have not always yielded clear transplants, and their routine use is not advocated. This method of preservation is a notable advance in conserving corneal tissue and in making it available when needed for lamellar keratoplasty. The technic of processing the tissue is not complicated. Storage at a very low temperature, however, requires special care and equipment, which may not be available under all circumstances. It poses a problem for shipment, and transportation to foreign countries would not be feasible.

A method for preserving corneas by dehydration in glycerine is herein reported. It has proved successful in lamellar keratoplasty in all animal and human experiments to which it has been applied. Corneas preserved and stored as long as seven months resulted in

transparent grafts in all of the nine animals' eyes used for the experiments. In the five human patients, the results of the lamellar grafts were considered equal to those obtained when fresh donor material was used. Penetrating keratoplasty, utilizing these preserved grafts, has not offered the same degree of success, and further studies are necessary. Five of the eight grafts in animals became clear or translucent in the periphery, but no graft could be considered a success. Only one human eye was available for the penetrating technic, and the outcome of the operation must be considered a failure.

The process described for preparing corneas for storage is a simple one, which requires very little equipment or expense. The grafting material remains viable for an indefinite period, and is kept in vacuum sealed tubes without refrigeration, at room temperature. The corneas can be shipped in the tubes, protected against breakage only, to any distance desired.

Lamellar keratoplasty is now receiving greater attention than it has in the past. It is said to be the least known and the most interesting development in keratoplasty (5). By virtue of its therapeutic applications, its usefulness is generally much greater than is the penetrating technic. Both methods of keratoplasty have clearly defined indications, and it is not probable that one will replace the other. The demands for cornea for the lamellar operation will, however, outweigh those needed for the penetrating type.

The supply of donor cornea could be made constant, and wastage could be diminished, if eye banks would consider utilizing the method of preservation which we employ. At present it appears that fresh corneal tissue from eyes enucleated within twenty-four hours and preserved at 4° C. to 6° C. should be made available only for use in penetrating keratoplasty. Eyes not requested within twenty-four hours can be preserved by dehydration in glycerine and stored indefinitely for use in lamellar keratoplasty. The cornea can be shipped in vacuum tubes to any distance without refrigeration.

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