PROLONGED DONOR CORNEA PRESERVATION IN ORGAN CULTURE: LONG-TERM CLINICAL EVALUATION

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INTRODUCTION

ENDOTHELIAL CELL IMPORTANCE IN MAINTENANCE OF NORMAL CORNEAL TRANSparency has been clearly established, especially since Stocker's classic treatise on this subject.[1] Since the human endothelial cell has limited, if any, regenerative capacity, the success of penetrating keratoplasty in human beings depends upon transplanting an adequate amount of functioning donor corneal endothelium. Therefore, any method of interim donor storage (storage from the time of death of the donor to its transplantation in the recipient), hereafter called donor storage, must maintain endothelial viability.[1] Refrigeration at 4 C of the whole globe is the traditional method of donor storage. The progressive loss of endothelial cells at this low temperature, however, compels most surgeons to use donor corneal tissue stored in this manner as soon as possible. The chronic shortage of donor tissue forces the patient to be on call for emergency surgical treatment when donor eyes become available. Besides creating anxiety for the patient, this situation inconveniences the surgeon, who often must operate at night or on weekends with inexperienced operating room personnel. This results in poor use of personnel, hospital beds, and operating rooms. In other cases when both the surgeon and patient are unavailable, precious donor tissue goes to waste. For these reasons, ways have been sought to prolong donor storage time to allow elective corneal transplantation surgery.

This author and his colleagues have been interested in 37 C organ culture of the cornea not only as a way to prolong the interim storage time of human donor corneas but also as a possible way to immunologically modify corneal tissue, a phenomenon that had been reported using other organs and tissues.[2] (Because the five-layered architecture and histology of the cornea is maintained during incubation, it is appropriate to refer to this as organ culture rather than tissue culture.) This thesis presents data from a long-term clinical study of 114 penetrating kerato-

plasties using organ culture preserved donor corneal tissue between May 1974 and July 1979, which were done by this author. Emphasis is on evaluation of the safeness of this method regarding control of microbial contamination and on its efficacy as judged by the long-term fate of these corneal transplants compared with corneal transplants reported in the literature using other storage methods.

HISTORICAL REVIEW AND BACKGROUND OF DONOR STORAGE METHODS

INTRODUCTION

In 1872, Henry Powers reported experimental corneal transplants in animals and human beings. He was the first to recognize that homologous corneal tissue was essential for success.[3] In 1906, Zirm[4] reported the first successful penetrating keratoplasty in man, using fresh tissue from a boy whose eye had just been removed for sequelae following a penetrating scleral injury. For the next 30 years, all human penetrating keratoplasties were performed using clear corneas from living donors whose eyes had been removed for posterior segment pathologic conditions. This made the surgeon dependent upon a sporadic and scarce supply of donor tissue. At the same time, advances in surgical techniques and instrumentation, as well as the introduction of antibiotics in the 1930s and corticosteroids in the 1940s improved the prognosis of corneal transplantation. These factors were associated with a growing demand for donor tissue. In 1937 Filatoff[5] introduced the use of 4 C refrigerated cadaver eyes as a source of donor material, which increased the number of available eyes and created a need for interim storage. This led to the establishment of eye banks to collect, process, and distribute tissue.[6] However, sufficient numbers of cadaver eyes still are not always available because of social, legislative, and religious barriers.

It was recognized early that if a method could be devised to prolong the storage time, wastage due to "outdating of tissue" could be reduced and the number of available corneas could be increased. Many methods were tried including freezing at −79 C,[7] dehydration,[8] freeze-drying,[9] desiccation,[10] and embedding in paraffin.[11] Although corneas stored by these methods can be used for lamellar keratoplasty for which endothelial viability is not critical, the results have been poor and unpredictable with penetrating keratoplasty.[12] Therefore, until recently, refrigeration at 4 C of cadaver eyes remained as the best storage method for corneas destined to be used in penetrating keratoplasty.

In the past 15 years three alternative methods of corneal preservation have been introduced to increase the storage time: (1) cryopreservation (1965), (2) McCarey-Kaufman medium, ie, M-K medium (1974), and (3) 37 C organ culture (1975). These methods employ cryoprotective solutions or tissue culture media and are technically more complicated than whole globe storage at 4 C. With the alternative methods, the cornea with a 2- to 3-mm scleral rim is removed from the globe and is the only part of the eye that is preserved. Although there is a potential for endothelial damage caused by lens-iris touch if the anterior chamber collapses during removal of the corneoscleral segment,[13] this technique has the advantage of removing the endothelium from postmortem aqueous, which may be toxic.[14] A corneoscleral segment requires posterior trephination at the time of surgical treatment to obtain a donor button, a technique with which many surgeons are unfamiliar. However, posterior trephination yields more endothelial cells with less damage at the cut edges than the anterior trephination technique.[15]

What characterizes the ideal storage method? Fjordbotton has listed the following criteria.[8] It should (1) be readily and easily done, (2) leave the cornea in its most nearly normal state, (3) provide suitable tissue for either lamellar or penetrating keratoplasty, (4) insure sterility, (5) allow indefinite storage under readily available facilities, and (6) have transportability. To these I would add (1) maintain endothelial viability indefinitely, (2) allow assessment of endothelial viability, and (3) be inexpensive. With these criteria in mind, the current status of each storage method in use today will be discussed.

4 C REFRIGERATION

The term "4 C refrigeration" is often used interchangeably with "fresh." In this paper, fresh tissue refers to a cornea removed from a living donor and immediately transplanted to a recipient without interim storage. Since 1937, 4 C refrigeration has been the standard storage method against which all other methods must be compared. It meets most of the ideal storage method criteria in that it is technically easy to perform, requires only a standard household refrigerator as equipment, allows the eye, packed with ice in an insulated container, to be transported readily, rarely results in infections, leaves the cornea in a nearly normal state, and is relatively inexpensive.

Its major disadvantage is limited storage time because of progressive endothelial cell death at 4 C. The limit of this storage duration is not known. It has been reported to be from 24 to 96 hours.[16-26] Experi-

mental studies by Van Horn and Schultz[21] demonstrate maintenance of normal endothelial cell ultrastructure up to 90 hours except for some reversible mitochondria changes. Clinically, Filatoff[27] and Barraquer-Moner[28] have stated that corneas stored from one to three days enhance graft survival. It is assumed that at 4 C storage endothelial cell death occurs in a linear fashion proportional to postmortem time; therefore, most surgeons use 4 C refrigerated tissue as soon as possible (usually within 24 hours). However, Saleeby[20] has reported an 83% transplant success rate with corneas from 4 C refrigerated cadaver eyes used 50 to 80 hours postmortem in 148 cases followed three to seven years. This may indicate that if cell death is proportional to postmortem time, the rate is much slower than previously assumed. It might also mean that cell death is not linear but exponential, with most cell death occurring only after prolonged storage times. It could also mean that, although a large number of endothelial cells may perish at 4 C, the remaining viable cells are able to maintain graft function. In this regard, Bourne and Kaufman,[29] utilizing clinical specular microscopy, found as few as 13% of cells remaining in some clear and thin grafts.

Many other variables probably play a role in endothelial cell survival at 4 C refrigeration. Cause of donor death, donor age, circumstances of donor death, and length of postmortem time are all implicated.[19] As with duration of postmortem storage, the importance of these various factors is unknown. Abbott and Forster[30] recently reported that donor age and postmortem time are not significant factors in determining graft success. All these factors could be ignored if there were a way to assess endothelial viability during storage. Unfortunately, with this method or any other method used today, this is not possible. In spite of all that is stated and published, the storage time limit of 4 C refrigeration is not known. Because of many surgeons' long experience with this method, 4 C refrigeration enjoys their wide acceptance, confidence, and trust, and each surgeon sets his own limits of tissue acceptability.

CRYOPRESERVATION

Although cryopreservation was studied experimentally and clinically by others,[16,31-37] Capella, Kaufman, and their associates[38-41] were the first to report a series of successful penetrating keratoplasties using donor corneas that had been preserved by freezing in a series of special cryoprotective solutions. With this method, the storage is at −79 C, completely suspending metabolism of the cornea.

The most recent review of corneal cryopreservation indicates a trans-

plant success rate equal to that with storage by 4 C refrigeration.[42,43] Storage duration of a year or longer is possible and is the major advantage of this system.

Cryopreservation has many disadvantages. First, it is technically complicated, requiring a well-trained technician, expensive equipment, and the use of young donor tissue (under 50 years of age) having postmortem times of less than six hours.[39] This severely limits the number of donor eyes that can be stored in this manner. Also, thawing the tissue is a complex, critical, and often capricious procedure that may irreversibly damage the endothelial cells. Because there is no way to recognize endothelial damage should it occur during thawing, these transplants may fail. In addition, thawing must be done at the time of surgical intervention, thereby limiting its transportability. Because of these disadvantages, use of cryopreservation is limited to a few centers.

STORAGE IN MEDIA

Introduction.—Various media have been used to store the cornea with varying results. Burki[44] introduced the use of liquid paraffin at 3 to 6 C and obtained satisfactory clinical results with corneal grafts after three days' storage (43.5% clear grafts).[11] However, endothelial damage that occurred when removing the cornea from paraffin, as well as the limited storage time, diminished paraffin's usefulness. Blood was used as early as 1911 as a storage medium.[45] Stocker[46] has shown that storing corneas in autologous serum for as long as 101 hours results in 63.30% to 77.35% clear grafts. Geeraets et al[47] have also successfully used serum for short-term storage of human corneas. Other media that have been studied include artificial aqueous humor for seven days,[48] a "nutrient" media for 14 days,[49] and various saline solutions for from seven to ten days.[16]

Tissue culture has been used since 1936[31] as a viability test of the various cell layers in the cornea; Archer and Trevor-Roper[50] suspended whole eyes in tissue culture medium and noted clear corneas after 96 hours of storage, but they did not report any transplants using these eyes. The literature on corneal tissue culture is related to its use as a measure of viability and not as a storage medium.[51,52] Lamellar grafts of tissue-cultured corneas were used experimentally by Messier and Hoffman[53] in 1949 for studies of wound healing.

In the early 1970s, the use of tissue culture media as a method of donor cornea storage was introduced.

M-K Medium.—This method, first reported in 1974, was developed by McCarey and Kaufman[54]; hence the acronym "M-K." With this method, the corneoscleral segment is immersed in TC-199 medium and stored at 4 C. Dextran is added to a final 5% concentration as an osmotic agent to thin the cornea, and an antibiotic solution of either streptomycin-penicillin or gentamicin is added to control bacterial contamination. McCarey and Kaufman[54-56] demonstrated that rabbit corneal endothelium remained ultrastructurally and functionally intact for up to 14 days when stored in M-K medium. Further laboratory studies confirmed the viability of human endothelium[23,55,57] stored in M-K medium for at least four days. With human corneas, McCarey et al[58] believe that elapsed time from death of the donor to placement in M-K medium is important, an interval less than six hours being ideal. Clinical studies demonstrate that transplant results using corneas stored in M-K medium are as good as 4 C refrigeration or cryopreserved tissue when stored for up to 96 hours.[59-61] Many surgeons have accepted this method not only because of the longer duration of storage but also because the tissue remains thin, clear, and of normal texture. With this method, transportability can be easily accomplished using a standard styrofoam insulated eye bank container packed with ice around the vial containing the cornea in M-K medium.

Does M-K medium extend the viability beyond that of 4 C refrigeration? There are ultrastructural studies showing human endothelial cell population is markedly reduced by 72 hours' storage in M-K medium.[55] Another study compared corneas in the intact globe at 4 C with M-K medium-stored corneas, and no ultrastructural differences could be detected up to 48 hours' storage time.[34,59] When the isolated cornea stored at 4 C in a moist chamber was compared with a cornea stored in M-K medium, no ultrastructural differences were noted at 138 hours (6.75 days) of storage time.[26] After seven to 14 days of storage, human corneal endothelium in M-K medium at 4 C shows severe ultrastructural damage.[62,63] However, there is ultrastructural and clinical evidence that 4 C refrigeration and M-K medium are similar and both may be used beyond 48 hours with good results. To date there has not been a randomized prospective clinical trial comparing results of transplants using corneas stored 48 hours or longer in M-K medium with corneas similarly stored at 4 C refrigeration. Only a study such as this can show whether or not M-K medium actually prolongs viability over 4 C refrigeration.

Quality control of the medium has caused problems. In April 1979, a letter from F.S. Brightbill, MD, was sent to many corneal surgeons

alerting them to a pH problem with the medium. (At that time an abrupt decision was made by the manufacturer to stop making M-K medium, creating a supply problem for most surgeons.) Because human corneal endothelium is easily damaged in an acid or alkaline environment, quality control of pH, as well as other factors, is critical. Research to improve media quality by changing its composition[63] or by adding constituents such as hydrocortisone[64,65] continues.

There have been disturbing reports of endophthalmitis following the use of M-K medium,[66,67] despite the use of antibiotics in the medium. Is it rational to use antibiotics when the medium is stored at 4 C? The antibiotics used are only effective against metabolizing organisms. Because metabolism is inhibited at 4 C, the presence of these antibiotics in the medium is of little value. Therefore, use of M-K medium requires meticulous care in decontamination of the donor eye before placement in the medium and good sterile technique during preparation and handling of donor tissue.

Organ Culture Incubation.—From the preceding discussion it can be seen that all donor storage methods have limitations causing them to fall short of the ideal storage method. This led to investigations by this author and others of 37 C organ culture as a storage method for donor corneas.

LABORATORY STUDIES

Ultrastructural Studies.—Maintenance of human endothelial ultrastructural integrity for 21 to 35 days in organ culture has been reported,[68-70] and there is unpublished data that extends these findings to 120 days' organ culture (Fig 1).

Although there is moderate intercellular edema, epithelial ultrastructure is also maintained for at least 35 days' organ culture.[70,71] After storage times longer than 40 days, epithelial overgrowth onto the endothelial surface may be seen (Fig 2). This occurs when there is endothelial cell damage, confiming the work of Yanoff,[72] who showed that in vitro epithelial-endothelial interactions are dependent upon the presence and viability of each layer. If the endothelium is damaged or absent, epithelial overgrowth onto Descemet's membrane is uninhibited.

Accumulation of glycogen often occurred in the epithelial, stromal, and endothelial cells after 11 days' organ culture,[70] and although some degeneration of stromal cells toward the center of the stroma was seen, most stromal cells appeared ultrastructurally intact up to 35 days' organ culture.[70,71]

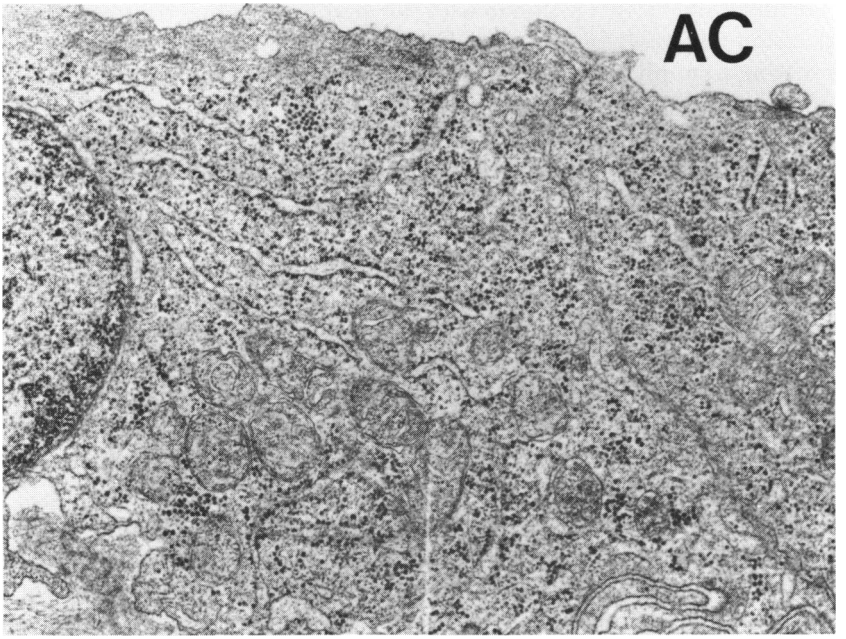

FIGURE 1
Human corneal endothelium from 69-year-old donor, stored 120 days in 37 C organ culture. Cells contain rough endoplasmic reticulum, abundant mitochondria and ribosomes. Note normal nuclear and posterior plasma membranes. AC = Anterior chamber (×20,510).

Endothelial Wound Healing Studies.—Electron microscopic studies of paired human organ culture corneas demonstrated a complete layer of ultrastructurally intact endothelial cells by transmission (TEM) and scanning electron microscopy (SEM), whereas fellow corneas in moist chamber storage at 4 C had areas of endothelial cell lysis and disruption. This indicated that a process of endothelial cell repair occurred during organ culture incubation.[68,69]

To test this hypothesis, the endothelium of 12 pairs of human corneas was injured using a freeze-thaw technique.[73] A circular 4-mm endothelial wound was made and the cornea placed in organ culture for from one to 21 days. By SEM a layer of larger endothelial cells was detected completely covering the defect by seven days (Fig 3). The mechanism of repair appeared to be a process of enlargement and spreading. No mitoses were observed, and the cells that covered the defect were approximately four times the size of the normal surrounding cells. The TEM showed ultrastructurally intact endothelial cells with normal intracytoplasmic organelles.

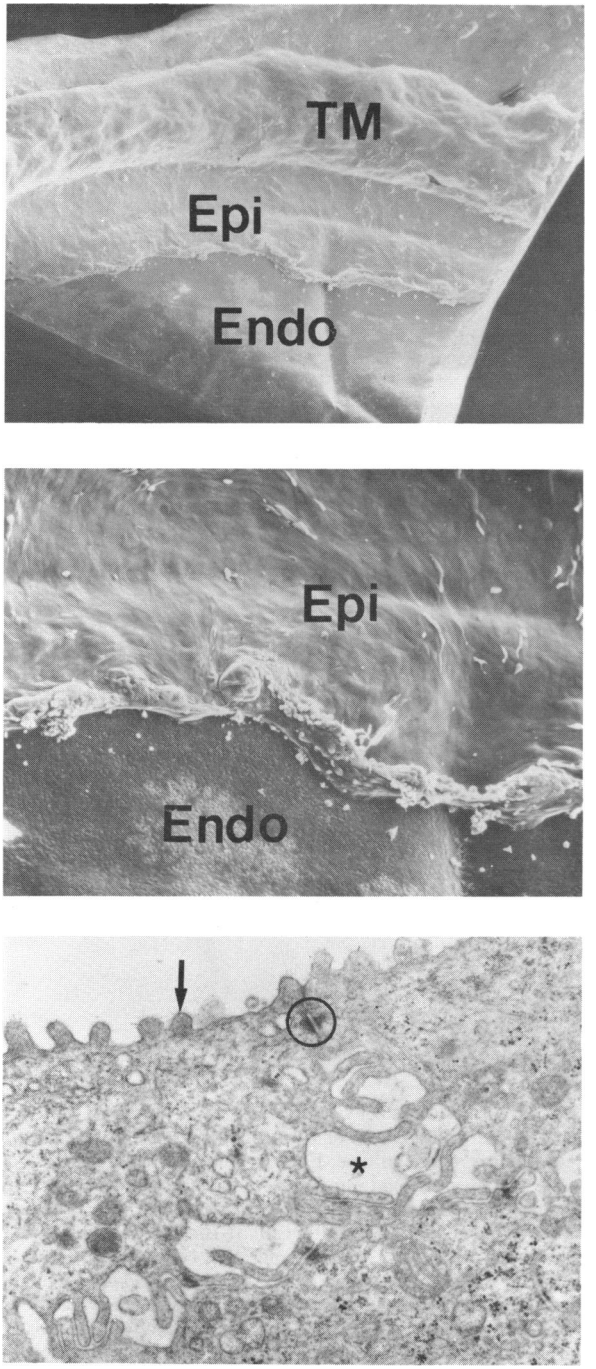

FIGURE 2
Top, Endothelial surface of 29-year-old human cornea in 37 C organ culture for 62 days. Note overgrowth of epithelium (Epi) onto trabecular meshwork (TM) and onto endothelial (Endo) surface (×15). *Center*, Advancing edge of epithelium (Epi) on endothelial (Endo) surface as eeen at top (×75). *Bottom*, Epithelium seen at top and center. Note surface microplicae (arrows) and desmosomes (circle) characteristic of epithelial cells. Intercellular spaces (*) are dilated (×15,000).

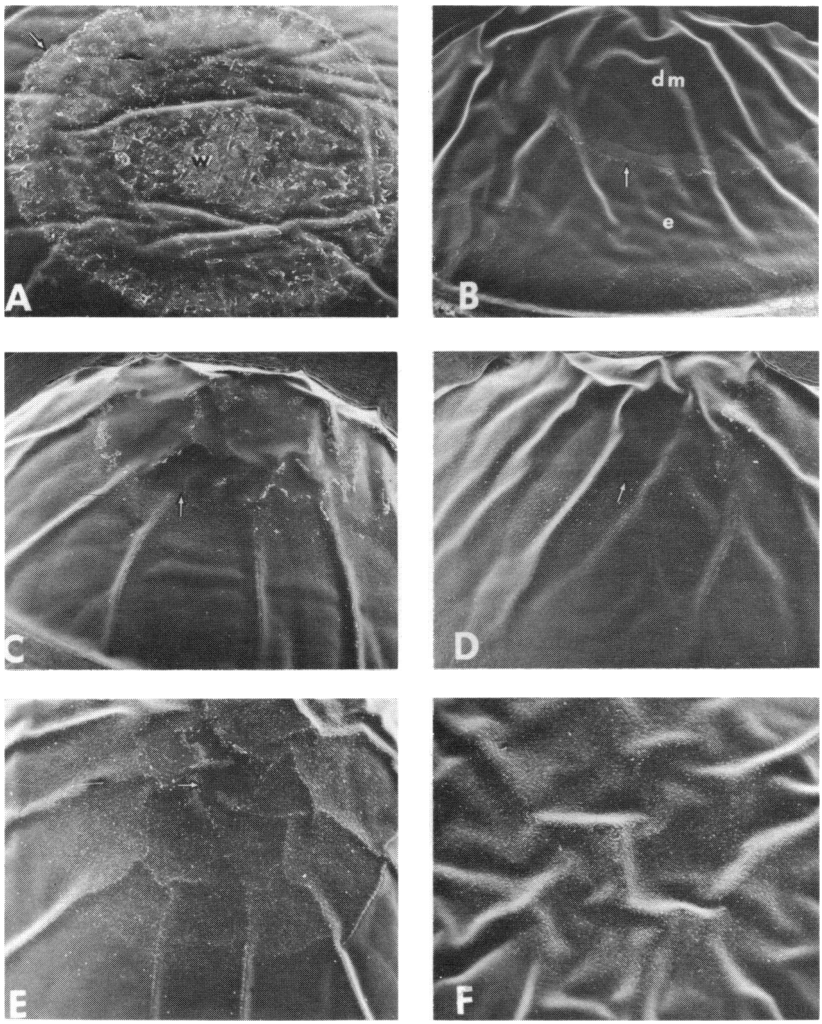

FIGURE 3

A: Circular endothelial wound (W). All cells in area of wound (area) have been destroyed (×20). B: Wounded area after 24 hours' organ culture. Endothelial cells (e) outside area of wound are normal. Cells at the margin of wound (arrow) are degenerated cells (×20). C: Endothelial wound after 24 hours' organ culture. Endothelial cells at margin of wound are moving centrally. Arrow indicates edge of migrating cells (×20). D: Endothelial wound after 72 hours' organ culture. Arrow indicates leading edge of migrating cells that continue to advance centrally as in C (×20). E: Endothelial wound after four days' organ culture. It is almost completely covered by endothelial cells. Arrow indicates small area of Descemet's membrane that remains uncovered (×20). F: Completely healed wound after seven days' organ culture (×20).

An additional 12 pairs were similarly wounded and their rate of deturgescence studied by chamber diffusion studies after one to three weeks' organ culture. Whereas fresh wounded corneas deturgesced only 2.8% of their original thickness, the cultured corneas deturgesced 31% of their original thickness at a rate of 76μ/hr. There was no difference in the rate or amount of deturgescence between one and three weeks' organ culture. This anatomic and functional endothelial regeneration in vitro in corneas from donors as old as 85 years is in contrast to human endothelial regeneration in vivo, where regenerative capacity is said to decline with age.[74] Although no mitoses were seen, autoradiographic studies utilizing tritiated thymidine to label DNA are needed to detect mitoses.[75]

Preliminary unpublished autoradiographic data using wounded rabbit cornea are available. The rabbit cornea was used because of its well-known ability to regenerate endothelium by mitoses in vivo.[74] However, in vitro freeze-thaw wounds in the central rabbit cornea are covered by endothelial cells without evidence of mitoses by autoradiography. This may be the result of an absence in the media of mitogenic factor needed for DNA synthesis as seen in studies with cultured lens epithelium,[76] cultured rabbit skin epithelial cells,[77] and cultured rabbit uterine endometrium.[78] Growth factors are present in the aqueous humor of inflamed rabbit eyes[79]; these are probably absent in organ culture medium, which may explain the lack of mitoses in cultured rabbit cornea.

Harding et al[80] pointed out that variables such as age, species, temperature, hormones such as insulin, concentration and freshness of serum in the medium, and type of culture system may play roles in the control of cell division in the epithelium of the cultured ocular lens. It is likely that some or all of these may play a role in the way the endothelium regenerates in vitro. Experimentally, the importance of the organ culture model is that it enables the researcher to control these many variables and to define what factors may be important in human endothelial regeneration. The clinical importance of these findings is that the endothelium of corneas stored in this manner may be of better quality because of this dynamic process of endothelial repair that occurs during storage.

Physiologic Studies.—The human organ cultured cornea swells to 1,200μ during organ culture. Chamber perfusion studies of human organ cultured corneas demonstrate rapid thinning of these corneas from 1,200μ to 800μ after three hours' perfusion.[73] Transmission electron microscopic studies demonstrate ultrastructurally intact cells after

TABLE I: ORGAN CULTURE MEDIA

Minimum essential media (Eagle's)
Earles' salts without L-glutamine
L-glutamine—1% final concentration
Decomplemented calf serum—10% final concentration
Penicillin, 100 units/cc
Amphotericin B, 0.25μg/cc
Gentamicin, 100μg/cc

these perfusion experiments. Therefore, just as thinning in temperature reversal studies indicates viable endothelium,[81] thinning of the organ cultured cornea during perfusion indicates viable endothelium.

There is unpublished data using various perfusion media, namely, T-3, TC-199, and KEI. The best endothelial preservation and function (stromal dehydration) is found using Minimum Essential Medium (MEM) as described in Table I.

Biochemical Studies.—To investigate glucose metabolism, glucose and lactate concentrations were measured in the medium for up to 35 days' organ culture.[70] Changing the medium twice a week resulted in glucose concentrations falling from 110 mg/dl to 30 mg/dl and lactate concentrations rising from 7 mg/dl to 84 mg/dl between changes. The pH ranged from 7.22 to 7.28, which indicated adequate buffering capacity of the media, even with the elevated lactate concentration. By day 12, glucose uptake and lactate release decreased by approximately 50%. Glucose uptake stabilized at this level, but lactate release continued to fall. By day 25, it was 20% of its original rate. Electron microscopic studies revealed normal endothelial, stromal, and epithelial cells that contained glycogen granules. This data demonstrated maintenance of adequate but reduced rate of glucose metabolism during organ culture.

In another study, the activity of corneal cytoplasmic and lysosomal enzymes from five pairs of stillborn fetus corneas was measured, and showed maintenance of normal enzyme activity after eight days' organ culture.[82] After 22 days' organ culture there was a marked increase in lysosomal enzyme activity, suggesting in vitro protein synthesis. Cytoplasmic enzyme levels were decreased, correlating well with the decreased rate of glucose metabolism.[70]

Temperature Studies.—In an attempt to find the ideal storage temperature for organ cultured corneas, endothelial ultrastructure at 37 C, 4 C, and room temperature (24 to 27 C) in organ culture medium was compared.[83] Four degrees centigrade was associated with ultrastructural damage as early as 48 hours using MEM alone, MEM with 5% dextran, or M-K medium. However, human corneas could be left at

room temperature in MEM for as long as seven days or at 37 C for as long as ten days and remain ultrastructurally and physiologically normal. Others have recently found maintenance of ultrastructural integrity after seven days' storage of human corneal endothelium in organ culture, whereas endothelial cell death occurred in the fellow cornea in M-K medium at 4 C.[62] Successful corneal transplants have been done using corneas initially stored at 37 C and then reduced to room temperature for as long as 72 hours prior to transplantation. Successful transplants have been performed by other surgeons in the United States using organ cultured corneas where ambient temperature prevailed during shipping for up to 48 hours. Therefore, transportation of organ cultured corneas is possible at ambient room temperature without special temperature controls.

Immunologic Studies.—Immune modification occurring during organ culture has been reported with a variety of rat and mice tumor allografts,[84-86] cultured lymphocytes,[87] ovarian allografts,[2,88] thyroid allografts[89] and xenografts,[90] and rabbit vitreous cells.[91] However, Summerlin's report of increased survival of organ cultured skin allografts in mice[92] and humans[92,93] could not be confirmed by Ninnemann and Good.[94] Investigations into the effect of organ culture on corneal immune rejections have involved experimental xenograft and allograft models as well as studies of the antigenic composition of organ culture corneas.

Experimental Organ Cultured Corneal Xenografts.—Preliminary studies indicated immune modifications of chicken-to-rabbit intralamellar endothelial xenografts after four weeks' organ culture.[95] This work was expanded to include greater numbers of chicken xenografts as well as guinea pig and human xenografts to rabbits.[96] Three- to four-week organ cultured chicken and guinea pig corneas transplanted in rabbits had significant delayed rejection times compared with control corneas. In addition, 22% of the four-week organ cultured chick xenografts did not reject. Organ cultured human-to-rabbit xenograft rejection was not delayed. This indicated species specificity. Histologically, organ cultured nonrejected xenografts and organ cultured xenografts with delayed rejection times were hypocellular with a decrease or absence of donor epithelium. This suggests that prolonged survival of xenografts after organ culture represents reduced antigenicity secondary to donor hypocellularity. Another explanation for organ culture modification of the immune response is the loss of "passenger" lymphocytes.[89] These cells are postulated to be responsible for the afferent arc of the immune rejection response. However, the role of this cell in

corneal graft rejection is unknown.

Another xenograft study using nonviable tissue made hypocellular by repeated freeze-thaw technique or viable tissue with epithelium removed showed delayed rejection times similar in magnitude to organ cultured tissue xenografts, supporting the hypothesis that organ cultured corneal xenograft modification may be related to hypocellularity.[97] Contrary to these findings, Benezra and Sachs[98] could not demonstrate immunologic modification of short-term (two to 14 days) organ cultured bovine to rabbit intralamellar corneal xenografts.

Gospodarowicz and Greenburg[99] recently reported that cultured bovine corneal endothelium readily coated and adhered to organ cultured cat corneas that had been previously denuded of endothelium. Seven of eight cultured cat corneas coated with cultured bovine endothelial cells and transplanted back into the original donor cat (autologous for stroma and epithelium, xenologous for endothelium) remained clear for at least five months. The mechanism of why these cultured xenogenic endothelial cells were not rejected is unknown. However, if this finding is supported by other investigators, the clinical implications are significant.

Extrapolation of results from experimental xenograft studies to allograft immunology may not be warranted in view of Silverstein and Khodadoust's warning that such grafts carry with them a variety of species' specific antigens which may introduce many variables that have nothing to do with allograft immunology.[100]

Experimental Organ Cultured Corneal Allografts.—The rabbit cornea swells to ten times its normal thickness during organ culture. With these swollen corneas the endothelium is damaged during surgical treatment, and only 30% of grafts clear after experimental penetrating keratoplasty using organ cultured corneas.[101] Therefore, an experimental penetrating keratoplasty model in rabbit studies of immune modification should not be used. Studies of experimental organ cultured allografts have been done using an intralamellar keratoplasty model. Because the allograft response in rabbits is weak and variable, all studies have also used either "second set" studies[102] or prior sensitization of the recipient with skin grafts from the donor to enhance immune rejection. Studies to date show no evidence of immune modification in the previously sensitized recipient using corneas cultured up to four weeks. However, the "second set" rejection of the organ cultured cornea is delayed in some groups when compared with fresh controls, but because of variation in results between the groups studied, no conclusions can be drawn until these experiments are repeated.

Jamblatt et al[103] recently showed that cultured rabbit endothelial cells regained their physiologic function when replaced in the rabbit eye. Rabbit corneas denuded of endothelium were seeded with a monolayer of cultured allogenic endothelial cells in organ culture. After short-term incubation these corneas were transplanted to recipient rabbits. Although only six of 27 grafts remained clear, none of the 21 that failed did so because of immune rejection.

Antigenic Composition of Organ Cultured Bovine Corneas.—Although Benezra and Sachs[98] found no immunologic modification of two-week organ cultured bovine to rabbit xenografts, others[104] have demonstrated that three-week organ cultured bovine corneas lack a strong antigenic protein present in normal bovine corneas. Although this is caused in part by loss of epithelium during organ culture, the antigen was absent from the stroma as well. It is likely that during organ culture, loss of soluble antigens known to accelerate heterograft rejection occurs.[105]

Effect of Autologous, Homologous, or Heterologous Serum in the Organ Culture Media.—Stocker[17] and Kuwahara[106] found that storing the donor cornea in recipient (autologous) serum modified immune graft reaction. Geeraets et al[47] reported similar findings using homologous serum. Bovine organ cultured corneas absorbed the serum protein present in the media during organ culture.[104] There appears, therefore, to be a theoretic advantage in using homologous or autologous rather than heterologous serum in the media. This hypothesis was tested in four-week organ cultured chicken-to-rabbit intralamellar xenografts.[96] Although autologous and heterologous serum did delay the xenograft reaction when compared with the fresh control group, homologous serum did not. Comparing autologous with heterologous serum, there was no difference in delayed rejection time between the two groups. Therefore, there was no immunologic advantage using autologous vs heterologous serum in this model.

Clinical Studies.—The only clinical report using organ cultured cornea for penetrating keratoplasty was a preliminary study reported in 1975.[107] Forty-one cases followed up longer than six months and 22 cases followed up less than six months using donor corneas with an average storage duration of 13 days in organ culture (range 2 to 35 days) were reported. Sixty-six percent of the long-term cases were clear and 34% failed. The length of storage time could not be associated with graft failure or immune rejection. Immune graft rejection occurred in seven recipients, five of which went on to graft failure. However, this was less

than would have been expected based upon incidence of graft rejection reported with other studies.[108] Wound separation at the time of suture removal was the major complication that was increased over other reports of penetrating keratoplasty using corneas stored by 4 C refrigeration, cryopreservation, or M-K medium.

Bourne et al[109] reported clinical specular microscopic findings of 14 clear organ cultured penetrating keratoplasties from four days to 2½ years postoperatively.[109] Donor corneas that had been stored up to five weeks prior to transplantation had a mean endothelial cell density of 1,548 cells/mm^2, showing that endothelial cells survive organ culture storage conditions and subsequent transplantation as well as storage by other methods.

MICROBIOLOGY OF DONOR EYES

A major concern regarding any method of storage is microbial contamination of donor tissue that could be transferred to the recipient, causing vision-threatening endophthalmitis or life-threatening systemic infections in the recipient. Previous studies of donor eye contamination have shown positive cultures in frequencies ranging from 50%,[6,110] 61%,[111] and 85.3%,[112] to 100%.[113] In addition, transmission of fatal CNS viruses such as rabies[114] and Cruetzfeldt-Jakob disease[115] via donor cornea has been responsible for raising even more concern about the sterility of donor tissue.

Endophthalmitis following penetrating keratoplasty is rare. In fact, before 1976, no cases of reported endophthalmitis following penetrating keratoplasty could be found in the literature. In 1976 a paper delivered at the Scientific Session of the Eye Bank Association of America reported that 18 cases of endophthalmitis occurred in 13,249 transplants between 1974 and 1976.[116] Also at that meeting, a paper reporting a case of *Pseudomonas aeruginosa* endophthalmitis following the use of corneal tissue stored in M-K medium was presented.[67] LeFrancois and Baum[66] reported *Flavobacterium* endophthalmitis following penetrating keratoplasty in tissue stored in M-K medium. Experimental studies of M-K medium contamination show that *Staphylococcus aureus* can survive in the medium even with antibiotics added,[117] and clinically there has been a report of *Staphylococcus aureus* contamination of M-K medium resulting in a postoperative donor corneal infection.[118] Khodadoust and Franklin[119] recently reported two cases of fulminating *Pseudomonas aeruginosa* endophthalmitis in two recipients following penetrating keratoplasty using a pair of donor corneas from

the same donor that had been stored in 4 C refrigeration.[119] The donor had died of Hodgkin's disease complicated by septicemia. Shaw and Aquavella[120] also reported pneumococcal endophthalmitis following penetrating keratoplasty using 4 C refrigeration donor eyes.

Many studies have been performed on various ways to sterilize the donor globe. One method is to flush the surface of the eyeball with topical antiseptic or antibiotic drops.[121] There are many studies in the literature documenting this technique as being inadequate.[110,117,121] Another method is saline irrigation of the globe followed by topical antibiotics. There is good evidence that vigorous saline irrigation is effective in removing surface microorganisms.[117,121,122] The third and best method is to immerse the globe in antibiotic solution.[121] This, in conjunction with removal of all excess adnexal and conjunctival tissue and vigorous saline irrigation, is the most effective of all methods, especially for bacteria.[117,121] A recent paper suggests that increasing the thimerosal concentration in Neosporin solution from 0.001% to 0.01% kills all fungi as well as bacteria if the globe is immersed for five minutes.[122]

MICROBIOLOGY OF ORGAN CULTURE STORAGE

Incubating corneas at 37 C in enriched tissue culture media greatly increases the potential for growth of bacteria and fungi that might be present on the donor cornea or may be introduced into the media from the environment or by personnel. This biohazard is well known to all working with tissue culture systems and is a major concern when using 37 C incubated organ culture tissue as a method of long-term preservation.

There are three areas of concern regarding organ culture as a method of donor storage with respect to sterility control: (1) decontamination of the donor globe before placement in organ culture, (2) maintenance of sterility during organ culture, and (3) assurance of sterility of the donor cornea at the time of transplantation. Decontamination of donor globes was discussed earlier and is part of the microbiologic investigation reported in this thesis.

As to sterility control during organ culture, the culture media used initially consisted of penicillin, 100 units/ml, streptomycin, 100 units/ml, and amphotericin B, 0.25μg/ml. The incidence of contamination was approximately 1.5 contaminated cultures out of approximately 60 cultures per month. Fungal contamination was more common in the summer months or when inexperienced technicians changed the media. Beginning in September 1976, we replaced streptomycin with gentami-

cin, 100μg/ml, and continued to use the same dose of penicillin and amphotericin B in the culture media. The experience with contamination since September 1976 will be reported in this thesis.

We have always used a terminal quarantine procedure to check sterility of the cornea at the time of transplantation. The initial routine included the following: Forty-eight hours prior to transplantation, a final change of medium was performed. As a sterility check, samples of the medium from that final medium change were streaked on blood agar and Sabouraud's media. The petri dish with the donor cornea was then closed and stored at 37 C until opened by the surgeon in the operating room at the time of surgical treatment. If the microbiologic media grew organisms or if the media in the petri dish containing the cornea became turbid or changed its pH as evidenced by a change in the phenol red indicator, the cornea was discarded.[107] In January 1976, in order to thin the corneas prior to transplantation, this routine was modified by placing the donor corneas into M-K medium containing penicillin, 100 units/ml, streptomycin, 100μg/ml, and amphotericin B, 0.25μg/ml, at the final medium change 48 hours prior to surgical treatment. Incubation was continued at room temperature or 37 C for 48 more hours. Sterility check procedures were carried out as described previously. However, M-K medium contains no phenol red indicator so that turbidity of the medium was the only visual check possible with the vial containing the donor cornea. In all cases, samples of the unused medium and corneoscleral rims were frozen and stored for possible future recall.

In May 1976, an organ cultured cornea was transplanted into a 76-year-old woman with diabetes for bullous keratopathy. At the time of surgical treatment, the sterility checks were negative and the M-K medium containing the donor cornea appeared clear. The postoperative course was complicated by smoldering inflammation. Two months postoperatively a fluffy ball-like mass appeared on the posterior cornea (Fig 4) and an aqueous tap revealed *Torulopsis glabrata*. Culturing the residual donor rim and medium in which the donor cornea had been stored revealed abundant growth of *Torulopsis glabrata* after 24 hours incubation. The details of this case have been reported.[123] Sterility checks of other residual donor rims and media from previous transplants revealed no other instances of contamination, nor did any other clinical cases appear with bacterial or fungal infection. Because of this one case of fungal endophthalmitis that was apparently missed by the sterility checks, the terminal sterility procedures were changed Sept 1, 1976 (see Microbiologic Investigations following).

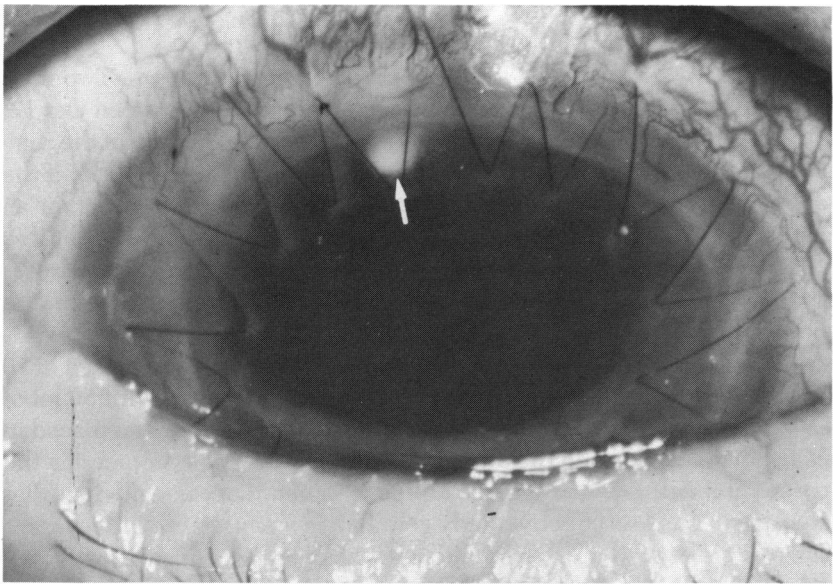

FIGURE 4
Round, white fluffy mass (arrow) on endothelium at host-graft junction two months postoperatively. Anterior chamber tap showed *Torulopsis glabrata*.

MATERIALS AND METHODS

MICROBIOLOGIC INVESTIGATIONS

To determine the role of some factors that may be involved in contamination of organ cultured corneal tissue, the following studies were performed: (1) identification of the type and frequency of donor eye contamination when it arrives at the Eye Bank; (2) comparison of the effectiveness of immersing the globe in Neosporin or gentamicin ophthalmic solutions to decontaminate donor eyes (Each milliliter of the Neosporin ophthalmic solution contained polymyxin B, 5,000 units; neomycin sulphate, 2.5 mg [equivalent to 1.75 mg neomycin]; gramicidin, 0.025 mg; alcohol, 0.5%; and thimerosal, 0.001%. Each milliliter of the gentamicin sulphate ophthalmic solution contained gentamicin sulphate equivalent to 3.0 mg gentamicin, disodium phosphate, monosodium phosphate, sodium chloride, benzalkonium chloride as a preservative and buffered to a pH of 7.0.); and (3) determination of the effectiveness of gentamicin, penicillin, and amphotericin B in prevention of contamination of the medium during organ culture incubation. In addition, the effectiveness of the new terminal sterility check procedure initiated Sept 1, 1976, was investigated.

Donor Eye and Organ Culture Medium Contamination.—Two hundred thirty eyes (115 pairs), received by the eye bank between Oct 17, 1977, and June 21, 1979, were studied. Eyes were not used if at the time of death the donor was septic (temperature greater than 101 F) or if the donor had septicemia, leukemia, lymphoma, Hodgkin's disease, or an immune deficiency disease. The intact globes were removed from the bottles that had been stored at 4 C and placed on sterile gauze. Excess conjunctiva as well as other adnexal tissue was trimmed back from the limbus at least 5 mm. A calcium algonate swab moistened with trypticase soy broth (TSB) was rolled around the limbus 360° and placed in 10 ml TSB broth. Both globes were then vigorously flushed with 50 ml of sterile saline. In the early part of the study, one of the pair was placed in Neosporin solution and the other in gentamicin solution, immersing the cornea and anterior sclera three minutes. When it became obvious that Neosporin was less effective, only gentamicin was used for both eyes for the rest of the study. After immersion, the eyes were flushed with 5 ml saline to remove residual antibiotic and the limbus was recultured, placing the swab in TSB broth to which 0.1% grobox was added to inhibit carryover of the antibiotic. The TSB broth with swabs was then incubated for two weeks or until growth appeared. All positive cultures were streaked on tryptose blood agar base (TBAB) agar plates and incubated until adequate growth appeared for identification by standard diagnostic microbiologic procedures. After removal from the posterior segment, the corneoscleral rims were placed in organ culture medium as described following.

To study the incidence of contamination during organ culture, these same 230 were observed during organ culture. If contamination of the medium occurred, identification of the organism was performed as described previously.

Organ Culture Method.—All organ cultured procedures are performed using sterile technique in a vertical laminar flow hood as previously reported[107] (Fig 5). The cornea with a 2- to 3-mm scleral rim is carefully sectioned without loss of anterior chamber from the anterior segment, as described by McCarey.[13] The corneoscleral segment is placed epithelial side down in a sterile Falcon tissue culture dish containing fresh medium (see Table I for constituents of medium) and incubated for 45 minutes at 37 C. The corneoslceral segment is then transferred into three separate washes of medium and dipped gently five times in each petri dish. In a fourth petri dish it is placed epithelial side down and completely covered with medium (Fig 6). This last dish is placed in a water-jacketed incubator at 37 C with an atmosphere of 5%

FIGURE 5
Technician working at laminar flow hood in organ culture room.

CO_2, and 95% filtered air and 100% humidity (Fig 7). The medium is changed three times a week.

Terminal Sterility Procedure.—Based upon the findings of Lindstrom et al[83] that organ cultured corneas remain metabolically active and ultrastructurally intact in a closed system for ten days at 37 C, the donor cornea is placed in 60 ml of medium without penicillin, gentamicin, or amphotericin B in a closed system, ie, tube closed with a cap and medium left unchanged, at 37 C for at least seven days prior to surgery (Fig 8). In addition, one medium change prior to terminal storage, the antibiotics and amphotericin B are removed to avoid antibiotic carryover that could inhibit microbial growth and mask contamination. Ten milliliter samples from the next-to-the-last medium change and the terminal 60 ml in which the donor cornea is stored are inoculated into

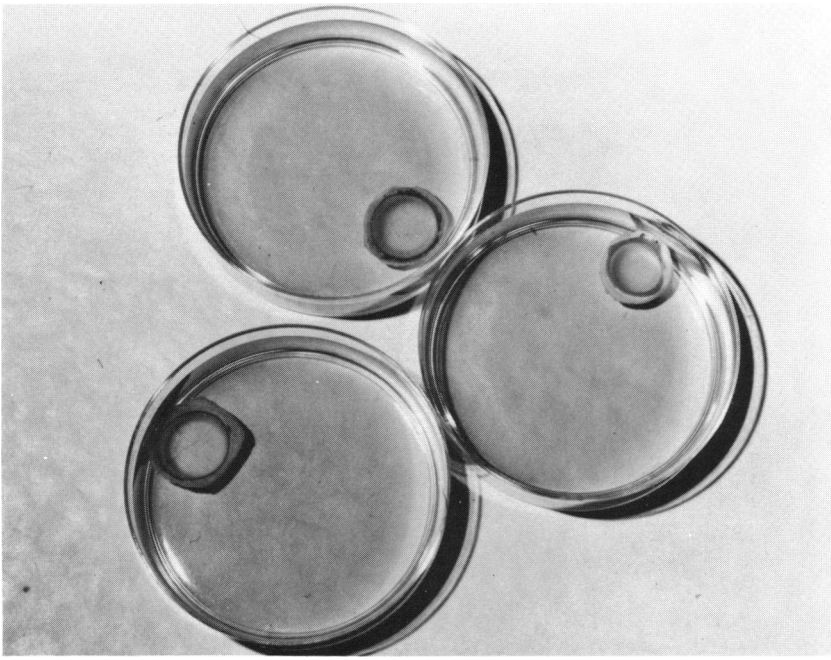

FIGURE 6
Petri dishes, each containing a human cornea in organ culture medium.

diagnostic media for anaerobic and aerobic bacteria as well as fungi and yeast by the hospital diagnostic microbiologic laboratories according to sterility check protocol already established for parental solutions and serums. If any of the diagnostic media become contaminated or if the medium in which the donor corneas are placed shows evidence of turbidity or change of pH, the cornea is discarded prior to the surgical procedure. To thin the corneas preoperatively, the corneoscleral segment is placed in M-K medium with penicillin, 100 units/ml, gentamicin, 100μg/ml, and amphotericin B, 0.25μg/ml, 18 hours prior to the surgical procedure and stored at 4 C (Fig 9).

CLINICAL STUDIES

From May 20, 1974, to July 30, 1979, the author performed 124 penetrating keratoplasties in 116 patients using donor corneas stored by the 37 C organ culture method previously described. Ten patients were lost to follow-up or died and were removed from the series, leaving 114

FIGURE 7
Technician placing petri dishes containing human corneas in incubator.

penetrating keratoplasties in 104 patients. These were not consecutive cases. Due to a three-month moritorium placed on organ cultured grafts following the case of *Torulopsis glabrata* endophthalmitis,[123] only 4 C refrigeration (four cases) or M-K medium (13 cases) stored corneas were used during that time. In addition, five M-K stored corneas have been used during this five-year period when operating room time became available within 24 to 48 hours, a time too short for the terminal sterility check procedure needed to use organ cultured corneas. With these exceptions, the 114 grafts were consecutive, not selected, cases.

Recipient diagnoses are listed in Tables II and III. All recipients were assigned to a prognostic group according to Pollack's classification.[124] All cases were seen by the author or by another ophthalmologist with a follow-up time of not less than six months.

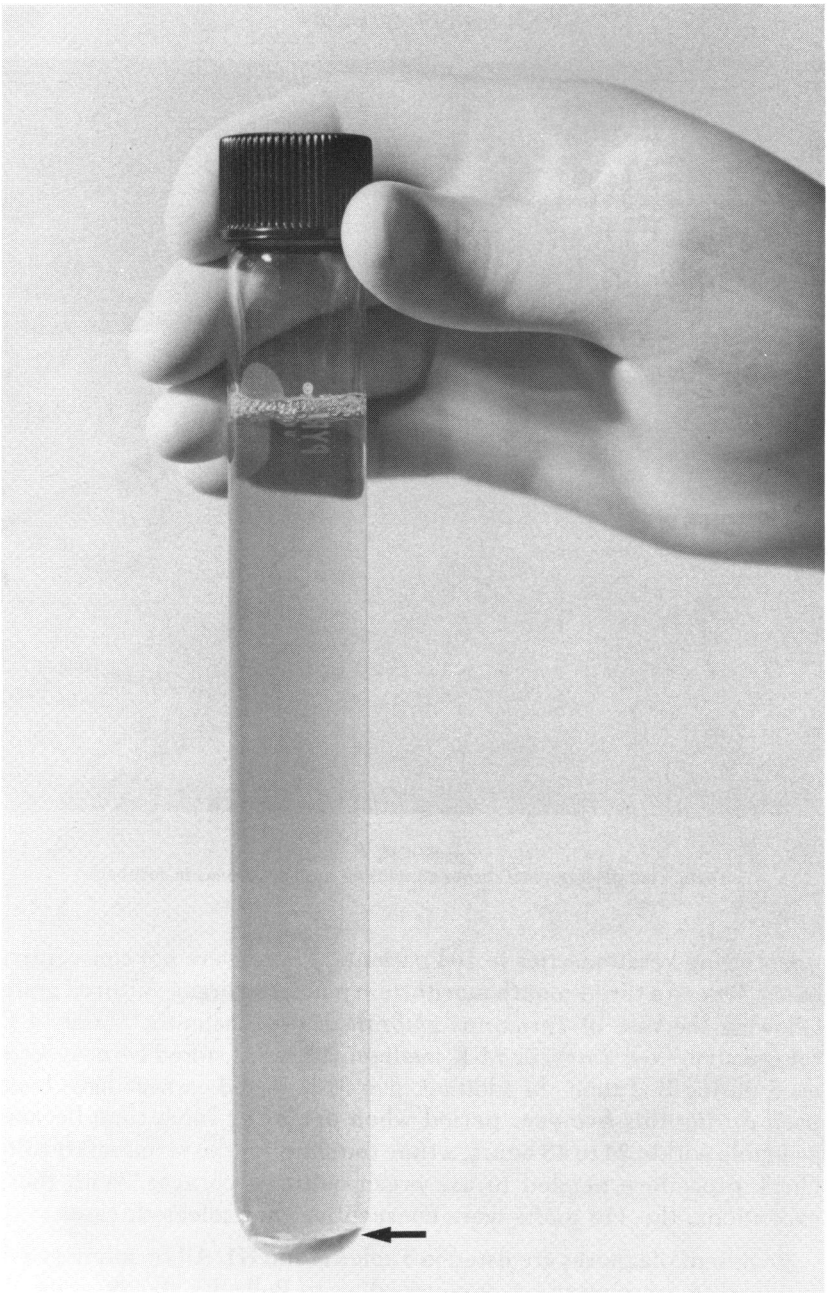

FIGURE 8
Cornea (arrow) in closed vial during terminal sterility check.

TABLE II: ORGAN CULTURED CORNEAL TRANSPLANTS: SUCCESSFUL RECIPIENT DATA

Preoperative Diagnosis	Prognosis*		
	F†	I‡	U§
Keratoconus			
Uncomplicated	18		
Vascularized recipient cornea		1	
Regraft: irregular astigmatism		1	
Aphakic bullous keratopathy			
Uncomplicated	17		
Failed graft, etiology?		1	
Thick with band keratopathy		1	
3 immune rejections in fellow eye		1	
Fuchs' dystrophy			
Localized	5		
Generalized		14	
Generalized with dry eye			1
Pseudophakos bullus keratopathy			
Intraocular lens removed		3	
Intraocular lens retained or replaced		3	
Herpes simplex keratitis			
Active thinning or perforated			3
Inactive scar	4		
Failed keratoplasty			
Immune rejection			2
Persistent epithelial defect		1	
Etiology unknown		1	
Familial dystrophy	7		
Leukoma			
Thin—vascularized cornea			2
Traumatic—scarred and vascularized		2	
Miscellaneous			
Fungal ulcer, healed and quiet	1		
Corneal cyst	1		
Neuroparalytic keratitis			1
Chemical burn			1
Total	53 (58%)	29 (31%)	10 (11%)

*Pollack's classification.[124]
†Favorable.
‡Intermediate.
§Unfavorable.

Donor data are shown in Table IV. The time interval between death of the donor and placement of the corneoscleral segment into organ culture has been divided into enucleation time, ie, time from death of donor, to enucleation and postenucleation time, ie, time between enucleation and placement in organ culture. All donor corneas came from eyes donated to the eye bank and were removed by resident ophthalmologists or trained morticians.

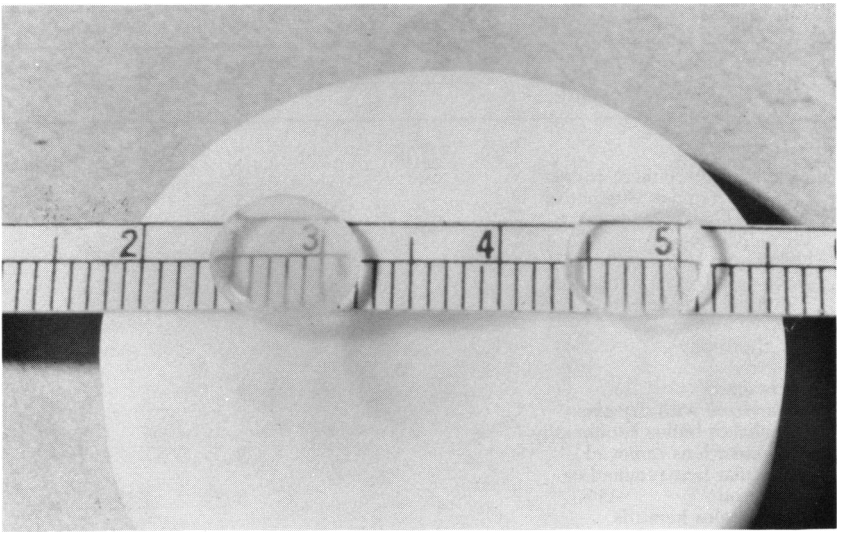

FIGURE 9
Corneal button on left after 15 days' organ culture. It is approximately 1.2 mm thick and opaque. Fellow cornea on right, organ cultured 15 days then placed in M-K medium at 4 C for 18 hours. Its thickness is approximately 0.8 mm and is clear.

Processing, preparation of the eyes, and organ culture storage procedures were performed as described previously. Terminal sterility procedures were performed as described previously on all corneas transplanted. Since January 1976, corneas were stored at 4 C in M-K medium 18 hours preoperatively in order to thin the cornea. Since this last step may cause a microbial contamination, the corneas are stored at 4 C to inhibit microbial replication should contamination occur.

Preoperatively, patients received topical 3% gentamicin every four hours prior to surgery. Cycloplegics were used in aphakic and combined procedure patients. No mannitol or acetazolamide (Diamox) was used.

At the time of the surgical procedure, the corneas were removed from the medium, placed endothelial side up on a polytetrafluoroethylene (Teflon) block, and a corneal button trephined from the endothelial surface using a corneal trephine press as described by Bourne.[125] The size of the buttons ranged from 7.0 to 9.5 mm. For the last two years of this study, donor buttons in aphakic and combined penetrating keratoplasty-cataract extraction procedures were 0.5 mm larger than the recipient bed. The donor button was sutured in place with continuous or interrupted 10-0 monofilament nylon suture. Donor epithelium that remained after culture was left in place. The unused corneoscleral rim

TABLE III: ORGAN CULTURED CORNEAL TRANSPLANTS: FAILED RECIPIENT DATA

Preoperative Diagnosis	Prognosis*		
	F†	I‡	U§
Aphakic bullous keratopathy			
Generalized		5	
Postoperative silicone replacement of vitreous with corneal touch			1
Shallow anterior chamber, 2 prior penetrating keratoplasties			1
Chemical burn			2
Previous immune rejection			5
Leukoma			
Traumatic with shallow anterior chamber			2
Anterior cleavage syndrome			1
Herpes simplex			
Perforation			1
Vascularized cornea		2	
Radiation keratitis			1
Epithelial downgrowth			1
Total	0	7 (23%)	15 (77%)

*Pollack's classification.[124]
†Favorable.
‡Intermediate.
§Unfavorable.

and residual media were returned to a sterile container and stored at −70 C for possible future recall. Prior to trephination, five minutes of digital massage was applied to the globe to lower intraocular pressure in all eyes except those with threatened or actual perforation.

All penetrating transplants combined with cataract extractions or those performed in aphakic patients were done with a single scleral support ring. α-chymotrypsin in a 1:7,500 dilution was used for zonulysis. Anterior vitrectomy was performed in most aphakic and some combined cataract-penetrating keratoplasty procedures. Acetylcholine was used for pupillary constriction in phakic patients. All surgical procedures were performed by the author using either local or general anesthesia.

Postoperatively, the patients had daily slit-lamp examinations while hospitalized, then weekly for two visits followed by progressively longer intervals from four to six weeks until all sutures had been removed. All patients received topical dexamethasone, cycloplegics, and antibiotics postoperatively. Cycloplegics were usually discontinued at two months. Topical antibiotics and dexamethasone were used until all sutures had been removed. Aphakic patients were kept on one drop of

dexamethasone indefinitely unless contraindicated. Some received systemic corticosteroid postoperatively if severe inflammation was diagnosed. Systemic antibiotics were not used.

Corneal thickness measurements, using the Mishima modification of the Haag-Streit pachometer,[126] external photographs, and Mackay-Marg tonometry, were performed by technicians and nurse-clinicians trained in these procedures on a periodic basis. Clinical specular microscopic examination was performed on selected patients in a manner described by Bourne and Kaufman.[127]

Suture removal was performed by the author when vessels reached the edge of the graft, when the sutures loosened, or when wound healing appeared complete as judged by the density of the surgical scar. Grafts were classified as successful or failed on the basis of the last examination done by the author or referring ophthalmologist.

An immune reaction was diagnosed when edema suddenly appeared in a graft that had been clear at least three weeks postoperatively and was associated with iritis, keratic precipitates, or an endothelial rejection line as described by others.[108,128] In addition, immune rejection was diagnosed if, in a previously quiet eye, an anterior segment inflammation without corneal edema occurred that subsequently responded to topical or systemic corticosteroids or both. In all cases, immune rejection was diagnosed only after all other possible causes of inflammation were eliminated.[108]

Refractions were performed and final vision measured by technicians at least three weeks after final suture removal.

RESULTS

MICROBIOLOGIC STUDIES

Of the 230 eyes in the study, 152 (66%) were contaminated with 176 organisms (Table V). Neosporin sterilized only 36% of the eyes whereas gentamicin sterilized 78%. The number of eyes sterilized with gentamicin is larger because when it became obvious early in the study that Neosporin was less effective, it was discontinued. Thereafter only gentamicin was used. Although gentamicin was more effective in decontaminating donor eyes, especially gram-positive organisms (coagulase-negative staphylococcus, *Staphylococcus aureus*, and α-streptococcus), it did not totally eliminate them. In addition, *Pseudomonas aeruginosa* and *Proteus mirabilis* organisms were still present on a significant percentage of the globes following gentamicin immersion. Neosporin

TABLE IV: ORGAN CULTURED CORNEAL TRANSPLANTS: DONOR CORNEA DATA

	Total Series (N = 114)			Successful (N = 92)			Failed (N = 22)		
	Average	SD	Range	Average	SD	Range	Average	SD	Range
Duration in organ culture (days)	16.5	6.5	3-35	16.5	5.8	3-29	16.6	8.9	3-35
Donor age (yr)	35.3	17.7	5-71	35.4	17.1	5-68	32.4	20.9	5-71
Postmortem time enucleation time (hr)	3.8	3.8	1-26	3.5	3.8	1-20	4.9	4.1	1-26
Postenucleation time (hr)	6.4	5.0	0-24	6.2	4.5	0-24	7.4	6.6	0-16
Follow-up time (mo)	31.0	19.0	6-63	28.8	18.8	6-62	40.5	17.6	6-63

TABLE V: DONOR EYE MICROBIAL CONTAMINATION: EFFECTIVENESS OF ANTIBIOTIC IMMERSION

Organism	No.	% of Total Eyes	Neosporin Immersion			Gentamicin Immersion		
			Before	After	% Change	Before	After	% Change
Coaguloase-negative Staphylococcus	81	35.2	23	16	−30	58	10	−83
Staphylococcus aureus	10	4.3	3	3	0	7	1	−86
Diphtheroids	28	12.2	9	2	−78	19	1	−95
α-streptococcus	15	6.5	7	4	−43	8	3	−62.5
Pseudomonas aeruginosa	8	3.5	4	2	−50	4	2	−50.0
Proteus mirabilis	7	3.0	2	0	−100	5	3	−40.0
Enterococci	4	1.7	1	2	+50	3	2	−33.3
Enterobacter aerogens	3	1.3	. . .	. . .	. . .	3	1	−66.6
Bacillus sp	2	0.9	. . .	. . .	. . .	2	0	−100
Serratia rubidea	2	0.9	1	0	−100	1	1	0
Ser marcescens	1	0.4	1	0	−100	. . .	. . .	. . .
Acinetobacter	1	0.4	. . .	. . .	. . .	1	0	−100
Lactobacillus sp	1	0.4	. . .	. . .	. . .	1	0	−100
Actinomyces	1	0.4	. . .	. . .	. . .	1	0	−100
Klebsiella oxytoca	2	0.9	1	1	0	1	0	−100
K pneumoniae	1	0.4	. . .	. . .	. . .	1	0	−100
β-streptococci	1	0.4	1	1	0	0	0	. . .
Candida albicans	5	2.2	2	4	+50	3	2	−33.3
C tropicalis	1	0.4	1	1	0	. . .	. . .	. . .
Torulopsis glabrata	2	0.9	. . .	. . .	. . .	2	0	−100
Total	176	76.66	56	36	−36	120	26	−78

TABLE VI: MICROBIAL CONTAMINATION: 37 C ORGAN CULTURE

Culture No.	Organisms Present before Organ Culture*	Organism Isolated during Organ Culture	Day Detected
1	*Candida albicans*	*C albicans*	4
2	*C tropicalis* *Klebsiella oxytoca*	*C tropicalis*	8
3	Coagulase-negative staphylococcus	Coagulase-negative staphylococcus	11
4	Coagulase-negative staphylococcus	Coagulase-negative staphylococcus	8
5	*Staphylococcus aureus*	*Corynebacterium*	218
6	Coagulase-negative staphylococcus	*Alternaria* sp	10
7	None	Coagulase-negative staphylococcus	14
8	None	*Achromobacter* sp	32
9	None	*Penicillium* sp	9
10	None	*Sporothrix schenckii*	11
11	None	*Candida albicans*	15

*Present on donor globe after antibiotic immersion.

was ineffective against yeast organisms, whereas gentamicin had some effect.

Four of the corneoscleral segments contaminated after the antibiotic immersion subsequently became contaminated with the same organism during organ culture in spite of penicillin, gentamicin, and amphotericin B in the culture medium (Table VI). Since in these cases the duration of organ culture was eleven days or less, and since these organisms were present on the donor globe after immersion in antibiotic, it is likely this contamination was carried over from the donor eye and not introduced during the organ culture process. Seven additional corneoscleral segments became contaminated after at least ten days' organ culture with organisms not present on the globe after antibiotic washing. These presumably occurred because of environmental contamination or contamination by laboratory personnel. Therefore, for this series of 230 nonseptic eyes studied over 20 months, 4.7% (0.55 per month) became contaminated during prolonged organ culture storage.

Table VII shows the results of the terminal sterility culture. One hundred eight donor corneas were processed between Sept 1, 1976, and Aug 1, 1979. Twelve (11%) had positive cultures in one of the media samples sent to the diagnostic microbiologic laboratories. In none did the media in the vial containing the donor cornea show evidence of contamination. Cases 6 and 9 through 12 were recultured with samples of the media from the vial with the donor cornea and were sterile. In cases 4 and 8, vials with the donor corneas were from the same donor and were inadvertently discarded before additional samples were

TABLE VII: TERMINAL STERILITY CULTURE RESULTS: NEXT TO LAST MEDIA CHANGE

Culture No.	Organism	Day Detected	Organ Culture Duration (day)*	Comment
1	CDC† group II K-1	2	11	+Growth rim and media‡
2	CDC group II K-1	3	16	+Growth closed vial
3	CDC group II K-1	4	18	+Growth closed vial
4	Coagulase-negative staphylcoccus	3	9	Discarded
5	Propisubacterium sp	7	13	−Growth rim and media‡
6	*Penicillium* sp	3	6	−Growth closed vial
7	Aerobic *Actinomyces*	16	7	−Growth rim and media‡
8	Coagulase-negative staphylococcus	3	9	Discarded
9	Coagulase-negative staphylococcus	3	9	−Growth closed vial
10	Coagulase-negative staphylococcus	4	19	−Growth closed vial
11	Anaerobic gram-negative rod§	1	6	−Growth closed vial
12	*Penicillium* sp	5	3	−Growth closed vial

*Days in organ culture before terminal sterility check.
†Center for Disease Control.
‡Transplants performed without postoperative infection.
§Organisms discovered before identification.

taken. Case 1 cultured CDC group II, K-1 organism in the remaining donor rim and the media. Due to mislabeling of samples in the laboratories, the surgeon was not notified of this contamination until after the cornea had been transplanted. Fortunately there was no evidence of postoperative infection. Cases 2 and 3 showed the same organism and showed positive growth in the vial containing the donor cornea. These corneas were not transplanted. In case 5, an anaerobic *Propionibacterium* species was reported to the surgeon after the surgical procedure. Reculturing of the residual corneoscleral rim and medium showed no evidence of growth, and the patient's postoperative course was uneventful. In case 7, an aerobic actinomyces was reported 16 days after the culture was initiated and eight days following surgical intervention. Reculturing the donor rim and media showed no evidence of growth, and the postoperative course of this transplant was uneventful. Contamination in this case was believed to occur in the microbiologic laboratory. Therefore, although contamination occurred in seven of the media samples taken from the next-to-the-last media change and five of the samples taken from the last media change, none of the donor corneas showed evidence of contamination at the time of surgical intervention (pH change or turbidity). In those three cases where transplants were performed, one of the residual media samples from surgical treatment

was contaminated. However, in these cases no postoperative endophthalmitis occurred.

CLINICAL STUDIES

In this series of 114 penetrating keratoplasties, donor corneas were stored by organ culture incubation at 37 C for an average of 16.5 days with a range from 2 to 35 days (Table IV). Of the 114 transplants, 92 remain clear and 22 have failed. There is a statistically significant difference in length of follow-up time, the clearer group being followed up an average of 28.8 months and the failed group an average of 40.4 months. All grafts that failed were clear for at least four weeks before failing. Therefore, in this series, no cases of primary graft failure occurred.

Donor age, enucleation time, postenucleation time, and duration of organ culture storage did not differ between the clear and the failed grafts, which indicates that in this study, these factors were not important to the success of penetrating keratoplasty. Of the 92 clear grafts, 53 (58%) are in a favorable preoperative prognostic category, 29 (31%) are in an intermediate, and 10 (11%) are in an unfavorable preoperative prognostic category (Table II). Of the 22 failed grafts, 77% (15) are considered to have an unfavorable prognosis, whereas intermediate prognosis comprises 23% (7) of the failed grafts (Table III). There are no favorable prognosis cases in the failed group.

The visual results are tabulated in Table VIII. Accurate data were available on 61 of the 92 successful penetrating keratoplasties. Only the best final visual acuity was used for this analysis and only after all sutures had been removed and the corneal curvature stabilized. Seventy-five percent (46) of these 61 transplants obtained 20/40 or better vision. Seven (11.5%) have a visual acuity between 20/50 and 20/100, five of which are aphakic with cystoid macular edema. Eight (13.5%) have a visual acuity worse than 20/100. Seven are aphakic, six with macular degeneration and one with optic atrophy. The remaining patient in this group is phakic with a dense cataract.

Four grafts are considered marginally successful because of increased thickness or the presence of peripheral donor corneal edema. In one, the postoperative donor thickness has ranged from 0.65 to 0.70 mm for four years, yet the visual acuity has remained 20/30. Of the three with peripheral corneal edema, one was done for advanced Fuchs' dystrophy. Nonprogressive peripheral edema appeared at the sixth postoperative month. The patient is 1½ years postoperative with a central corneal

TABLE VIII: SUCCESSFUL ORGAN CULTURED CORNEAL TRANSPLANTS: VISUAL RESULTS

Distance Vision	No.	Type	Comment
20/40 or better	22	Phakic	
	24	Aphakic	2 Macular degeneration
20/50 to 20/200	2	Phakic	1 Cataract 1 Amblyopic
	5	Aphakic	5 CME*
20/200 or worse	1	Phakic	Cataract
	7	Aphakic	6 Macular degeneration 1 Optic atrophy

*Cystoid macular edema.

thickness of 0.57 mm and a visual acuity of 20/60 caused by cystoid macular edema. The other two transplants were done for advanced aphakic bullous keratopathy. In one, peripheral corneal edema appeared 2½ years postoperatively (Fig 10) and has remained stable over 18 months' observation. In the other, peripheral corneal edema developed six months postoperatively, and no progression has been noted after three months. Both have 20/40 vision. In these four grafts, the intraocular pressures have been well controlled and there has been no evidence of graft rejection.

In the first two years of this study, organ cultured tissue was used without thinning it preoperatively in M-K medium. This resulted in thick donor corneas stained pink with phenol red indicator (Figs 9 and 11, top). Suturing was not a problem, nor did the sutures loosen as the cornea thinned. By 24 hours all corneas were without the pink color. Phakic grafts were usually thin by 24 to 48 hours. However, aphakic grafts remained thick (Fig 11, center). By the second postoperative week, the clarity and thickness of aphakic and phakic grafts appeared the same (Fig 11, bottom).

To improve visualization of the anterior chamber during surgical intervention, we began placing the donor cornea in M-K medium 16 hours preoperatively in January 1976 (Fig 9). Postoperatively, the phakic grafts were thin and clear by 24 to 48 hours. However, there was still a delay in deturgescence in aphakic grafts similar to that seen before using M-K medium.

This delay in postoperative thinning of aphakic grafts can be seen in the pachometry values. Figure 12 plots distributions of corneal thickness with time postoperatively and divides the transplants into aphakic and phakic transplants. The phakic transplants are thinner than the aphakic transplants for the first eight weeks postoperatively. For the first four weeks this is statistically significant. There is also a statistically significant increase in thickness of aphakic over phakic transplants at ten

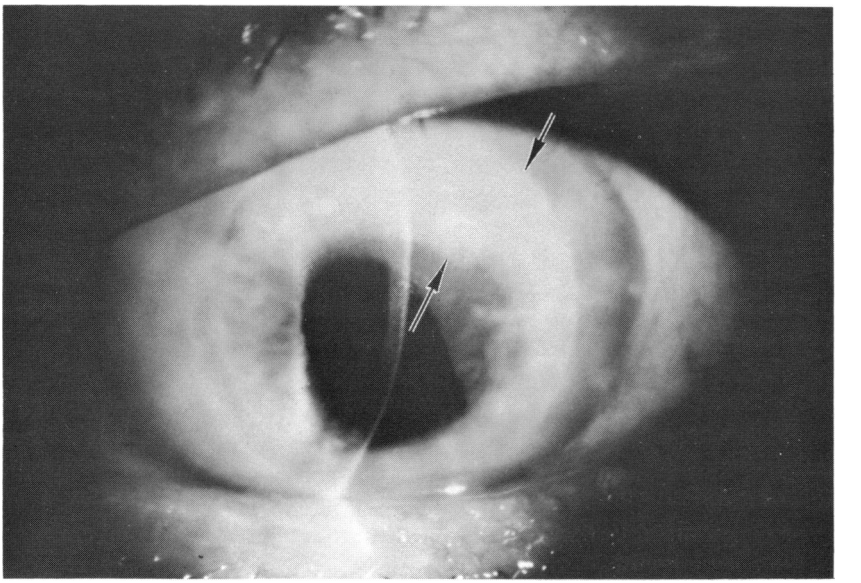

FIGURE 10
Nonprogressive peripheral corneal edema (arrows). Central corneal thickness 0.57 mm. Visual acuity 20/40.

weeks and four months. Postoperative pachometry values for corneas thinned in M-K medium prior to surgical treatment were similar to organ cultured donor corneas without preoperative thinning in M-K medium.

Specular microscopic data of organ cultured corneal transplants in this series are seen in Table IX. The appearance of the central graft endothelium from patients 5, 14, and 22 are seen in Fig 13. We did not have a specular microscope available until the last six months of the study. In addition, frequent breakdowns of the instrument reduced the amount of data we could gather. Table IX includes previous data published by Bourne et al.[109] The mean endothelial cell count was 1,598.5 cells/mm^2. Comparing those corneas with cell counts above 1,700 cells/mm^2 with those below 1,700 cells/mm^2, we find no statistically significant difference with regard to donor age, cornea thickness, postmortem time, or organ culture duration. There was, however, a statistically significant difference ($P > .01$) in postoperative follow-up time between these groups. Seventeen corneas with 1,700 cells/mm^2 were measured an average of 10.12 months postoperatively, whereas 14 with less than 1,700 cells/mm^2 were measured an average of 25.5 months. Six patients

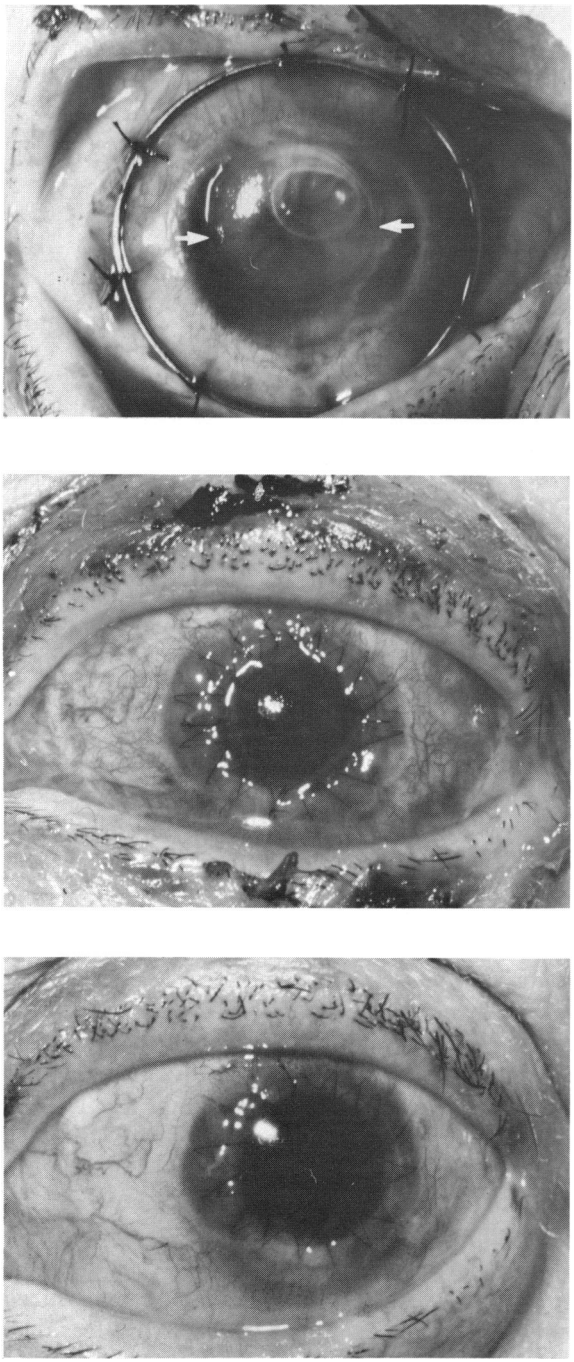

FIGURE 11

Top, Donor cornea organ cultured 18 days shown in recipient bed (arrows) prior to suturing. Note donor button is thick and opaque. Preoperative diagnosis is aphakic bullous keratoplasty. *Center*, Same eye 48 hours postoperatively. Donor cornea remains slightly cloudy and thick (corneal thickness 0.78 mm). *Bottom*, Same eye two weeks postoperatively. Donor cornea is thin and clear (corneal thickness 0.58 mm).

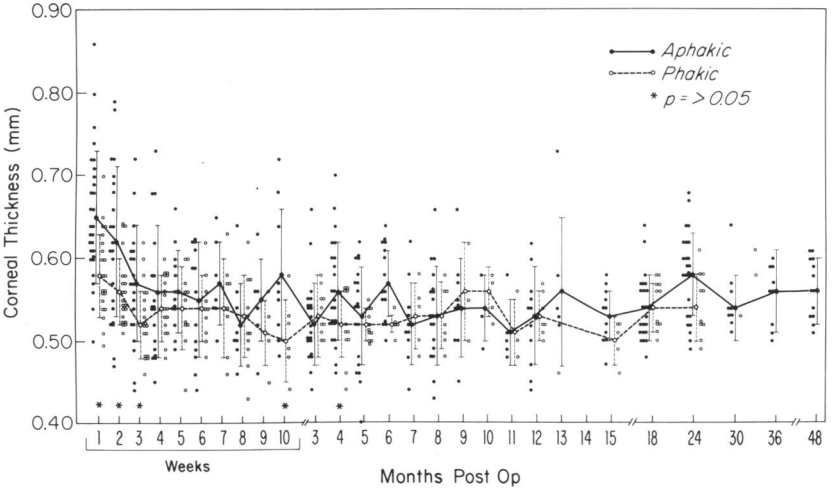

FIGURE 12
Postoperative corneal thickness measurements in clear organ cultured penetrating keratoplasties.

had repeated specular microscopic measurements of central endothelial cell density 34 to 54 months postkeratoplasty. Patient 7 lost 52% of the cells that were present when measured 32 months previously. However, three rejection episodes (all reversed) had occurred during this time. The other five lost an average of 21% (range 8% to 42%) over an average of 32 months (range 27 to 35 months) since the initial measurements. Patient 25 had 401 cells/mm^2 when initially measured 23 months postoperatively. At 32 months postoperative, the graft clouded without apparent cause, presumably due to endothelial failure.

Table X lists the causes of graft failure. In this series, the largest number of failures was due to immune graft rejection. The next leading cause was uncontrolled glaucoma. Of the six grafts that failed due to uncontrollable glaucoma, four did so in spite of cyclocryotherapy; in the two remaining cases, patient compliance with glaucoma treatment was inadequate.

Of the two grafts that failed because of wound dehiscence, one occurred in a patient who had had radiation keratitis with secondary fungal infection. An emergency transplant was done for threatened perforation. The graft remained clear but with a persistent epithelial defect for eight months. Approximately two days after suture removal the wound separated, resulting in graft failure in spite of wound repair. The other occurred in a patient who had had a penetrating keratoplasty through a conjunctival flap that had been performed one year earlier for threat-

TABLE IX: ORGAN CULTURED CORNEA: POSTOPERATIVE SPECULAR MICROSCOPIC DATA

Patient	Endothelial Cells/sq mm	Patient Age (yr) at Keratoplasty	Donor Age (yr) at Keratoplasty	Mo After Operation	Corneal Thickness, mm	Time from Death to Storage, hr	Time in Organ Culture, days	Diagnosis
1	2,926	66	11	4	0.60	11	12	Failed graft
2	2,686	70	11	4	0.51	11	12	Fuchs' dystrophy
3	2,558	83	13	7	0.54	1	17	ABK*
4	2,132	76	55	9	0.58	9	15	Fuchs' dystrophy
5	2,111	86	50	18	0.54	8	18	Fuchs' dystrophy†
	1,934			45	0.51			
6	2,079	82	22	4 days	0.66	11	10	Fuchs' dystrophy
7	2,003	64	20	12	0.65	1	7	PBK‡
	965			45	0.61			3 Rejection episode-reversed
8	1,975	83	52	4	0.55	10	14	Fuchs' dystrophy†
9	1,948	65	47	3	0.54	7	18	Fuchs' dystrophy†
10	1,934	79	14	8	0.44	1.5	22	Granular dystrophy
11	1,868	71	59	8	0.54	11	11	Fuchs' dystrophy†
12	1,860	74	52	4	0.62	10	17	Fuchs' dystrophy†
13	1,852	69	17	9	0.62	12	13	Fuchs' dystrophy†
14	1,835	70	36	2	0.48	3	17	Interstitial keratitis
	1,499			37	0.52			
15	1,736	62	21	16	0.52	10	24	ABK*
16	1,539	66	60	15	0.51	4	18	ABK*
17	1,377	55	36	3	0.48	1	19	Fuchs' dystrophy
	793			34	0.50			
18	1,307	47	44	17	0.51	2	12	Herpes scar
19	1,288	80	54	19	0.49	15	7	Fuchs' dystrophy†
	992			51	0.56			
20	1,280	76	44	9	0.51	14	29	ABK*
21	1,042	72	19	27	0.59	7	18	Fuchs' dystrophy†
22	1,039	70	68	21	0.53	16	9	ABK*
	872			54	0.60			
23	905	31	63	11	0.48	3	26	Keratoconus with chronic rejection
24	818	61	42	6	0.55	10	23	Descemetocele, herpes, and intraocular lens
25	401	82	51	23	0.58	26	35	ABK*
				32				Graft failed

*Aphakic bullous keratopathy.
†Combined procedure.
‡Pseudophakos bullous keratopathy.

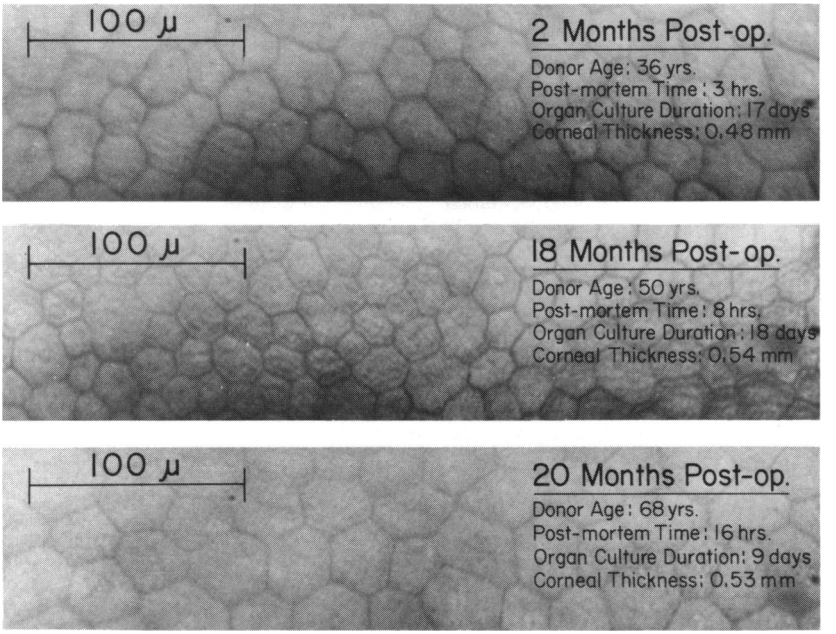

FIGURE 13
Central corneal endothelium of three clear organ cultured grafts as photographed with clinical specular microscope.

ened perforation due to herpes simplex keratouveitis. After penetrating keratoplasty, a persistent epithelial defect was treated with a continuous-wear soft contact lens. Three months postoperatively a *Staphylococcus aureus* stitch infection occurred. After removal of the suture the wound gradually separated and the cornea became opaque.

The failed graft due to epithelial downgrowth occurred in a patient who had had phacoemulsification for a traumatic cataract. Corneal edema developed, and a penetrating keratoplasty was performed by another surgeon using organ cultured corneal tissue 12 months after the phacoemulsification procedure. Although the cornea was initially thin and clear, one month postoperatively a fistula in the recipient cornea was noted 2 mm from the graft-host junction at the 12-o'clock position. One week later, a wavy line suggestive of epithelial downgrowth was noted moving down the endothelium of the donor cornea from the 12-o'clock to the 6-o'clock position. Multiple attempts to seal the fistula with tissue adhesive, sutures, cryotherapy, and a small (2-mm) penetrating keratoplasty were unsuccessful. After three months the fistula was removed by regrafting the cornea with an 8-mm organ culture donor button. Histologic examination of the

TABLE X: ORGAN CULTURED CORNEA: CAUSES OF FAILED KERATOPLASTIES

Cause	No. (%)
Immune rejection	8 (36.4)
Uncontrolled glaucoma	6 (27.3)
Persistent epithelial defect	3 (13.6)
Wound separation	2 (9.0)
Postoperative retinal detachment	1 (4.5)
Epithelial downgrowth	1 (4.5)
Unknown	1 (4.5)

recipient corneal button showed multiple layers of epithelium on the endothelium. The second penetrating keratoplasty remained clear for three months. The fistula did not recur. However, the eye gradually became soft, the cornea became thick and cloudy, and the graft was considered failed three months after the second transplant. Histologic examination of the frozen residual corneoscleral segments remaining after both penetrating keratoplasties showed no evidence of epithelial overgrowth onto the endothelium of the donor corneas.

The one graft failure following retinal detachment surgery was in a patient who had suffered severe anterior segment disruption secondary to a battery explosion. At the time of keratoplasty, eight months postinjury, an anterior vitrectomy was performed. Retinal detachment was diagnosed four weeks postkeratoplasty and was successfully repaired. However, retinal detachment recurred three days postretinal reattachment and was considered inoperable because of massive preretinal proliferation. The graft remained clear for nine months, then gradually clouded without specific cause.

The postoperative complications in this series are listed in Table XI.

Of the 39 patients in whom glaucoma developed postoperatively, 35 were aphakic and four were phakic. None of the phakic patients had glaucoma before surgical treatment, whereas 30 of the aphakic patients had controlled glaucoma before surgical treatment. Almost 90% of the aphakic patients required glaucoma control postoperatively. All the grafts that failed postoperatively because of the glaucoma were aphakic. In all phakic cases, treatment was stopped when sutures were removed (two cases) or topical corticosteroids discontinued (two cases). Medical control was maintained in aphakic patients in all cases except where the toxicity or side effects occurred (seven cases).

Of the 33 immune rejections, eight went on to fail (Table X). Comparing organ culture duration, age of donor, or postmortem times, no statistically significant difference could be found between those grafts that had immune

TABLE XI: ORGAN CULTURED CORNEA: POSTOPERATIVE COMPLICATIONS

Type	No. (%)
Glaucoma	39/114 (34.2)
Aphakic	35/39 (89.7)
Phakic	4/39 (11.3)
Immune rejection	33/114 (28.9)
Synechia to graft	24/114 (21.1)
Successful	18/92 (19.6)
Failed	6/22 (27.3)
Immune reaction	7/31 (22.6)
Aphakic grafts	20/70 (28.6)
Phakic grafts	4/42 (9.5)
Cataract	5/42* (11.9)
Wound separation	11/114 (9.6)
Epithelial defect	10/114 (8.8)
Infections	6/114 (5.5)
Herpes simplex	4/10† (40.0)
Stitch abcess	2/114 (1.8)
Endophthalmitis	1/114 (0.9)

*Total phakic cases.
†Total herpes simplex cases.

rejection episodes and those grafts that did not. In addition, no differences could be found between those rejected grafts that resolved and those that failed. However, the size of the graft was significantly larger in 22 grafts that had rejection episodes (7.95 mm ± 0.31 mm) than in 69 grafts that did not have rejection episodes (7.75 mm ± 0.33 mm) ($P > .01$). (This includes grafts with accurate data available; mean = ± 1 SD).

Peripheral anterior synechiae to the graft were seen in 21.1% of the transplants. This ranged from 9.5% (4) for the phakic grafts to 28.6% (20) for aphakic grafts. Except for the low incidence in phakic grafts, these differences were not statistically significant for any of the subgroups including graft with immune rejection.

In the five phakic patients in whom cataracts developed postoperatively, all were first noted between 8 and 27 months postoperative. Four of the five were slowly progressing peripheral cortical spokes that have not interfered with vision. There were postoperative complications in three, which could have accounted for the cataract, high intraocular pressure in one, chronic rejection episode requiring intensive oral and systemic corticosteroids in one, and wound dehiscence in one. Unsupervised chronic use of topical corticosteroids may have caused or contributed to cataract formation in the remaining two patients.

Wound separation occurred in 9.6% (11) of the cases (Table XI). Of the nine that did not fail, one occurred as a wound leak in a thin vascularized recipient cornea three days postoperatively. In two, the running suture was inadvertently cut when removing interrupted sutures in the first

TABLE XII: ORGAN CULTURED KERATOPLASTY: TYPE OF PROCEDURE

Type	No. (%)*	Successful (%)†	Failed (%)
Aphakic	46 (39.3)	28 (59.1)	18 (40.9)
Pseudophakic	6 (5.3)	6 (100)	0 (0)
Combined	20 (17.9)	18 (90)	2 (10)
Phakic	42 (37.5)	40 (95.2)	2 (4.8)

*Percent of total series.
†Percent of procedure.

postoperative week. Both were resutured and the grafts remained clear. The remainder occurred after suture removal, none of which required resuturing.

Of the seven epithelial defects that did not cause graft failure, all persisted longer than seven days postoperatively, lasting from seven days to three months. All responded to treatment with continuous wear of soft lenses. Five occurred in aphakic bullous keratopathy patients and two in patients with previous herpes simplex keratitis.

As to postoperative infections, there was a 40% recurrence of herpes in the graft postoperatively (Table XI). All were dendritic ulcers that cleared with antiviral therapy and left no scars. Both stitch abscesses were caused by *Staphylococcus aureus* and responded to suture removal and topical antibiotics. The one case of *Torulopsis glabrata* endophthalmitis was discussed in the Background section.

Table XII shows the distribution of the type of procedures performed in this series and their subsequent fate. Aphakic transplants accounted for 39.3% (46) of all the transplants done and for 18 of 22 failed penetrating keratoplasties (81.8%). On the other hand, 35.5% of the transplants were phakic and only two failed (9%). This high failure rate in aphakic patients is a result of the larger number of poor prognosis cases in this group (Tables II and III) and not caused by aphakia per se. Combined procedures were successful because of the good prognosis of Fuchs' dystrophy cases that comprised of most of this group.

DISCUSSION

MICROBIOLOGIC STUDIES

This study demonstrates that donor globe surface decontamination is best accomplished by immersing the donor globe in 3.0% gentamicin for three minutes. This reduces the flora by 78%. Neosporin reduced surface flora only 36%, similar to that seen by Richards and Catzen.[110] Because of the relative ineffectiveness of Neosporin, we changed to gentamicin early in

the study. However, even gentamicin did not sterilize 22% of the donor globes. It was especially ineffective with *Pseudomonas aeruginosa* (Table V). Even though immersion in gentamicin is not 100% effective in sterilizing donor globes, this study agrees with others in demonstrating that immersion in an antibiotic or antiseptic is the most effective way to decontaminate donor eyes.[117,121] This is the first study that shows the superiority of gentamicin over Neosporin when used in this manner.

The incidence and distribution of organisms in this study are similar to those reported by Rollins and Stocker from North Carolina,[111] Boberg et al from Copenhagen,[112] and Pollack et al from New York.[113] As pointed out by others,[129] the kinds of organisms isolated from donor eyes have remained constant from many localities over the past 15 years.

Because none of the decontamination methods are 100% effective, sterile technique, vigorous saline irrigation, and removal of all adnexal tissue and excess conjunctiva are important, especially if the cornea is going to be stored in enriched media.[117,121,122]

Contamination of the media during organ culture comes from three sources: carryover of microbes from donor tissue, contamination (usually bacterial) by personnel, or contamination (usually fungal) from the environment.[130] Contamination of media during organ culture in this study occurred in 11 out of 230 organ cultures over a 1½-year period, for an incidence of 0.55 contaminated cultures per month (Table VI). Contamination was redued by 66% when gentamicin was substituted for streptomycin in medium that also contained penicillin and amphotericin B. This confirms Armstrong's statement that streptomycin is woefully inadequate in trying to control infection in tissue culture.[130] In these 11 instances of contamination, four were probably carried over from contaminated donor globes, demonstrating that antibiotics in the media with this method will not always sterilize donor corneas. This fact has led us to delay using organ cultured corneas clinically until cultured at least one week, and then only with a terminal sterility check of at least an additional seven days (see discussion following). Of the seven other contaminated cultures, two were bacterial and five were fungal species.

A potential advantage of this long-term storage system is the opportunity to check the sterility of the donor cornea. By exploiting the system's vulnerability to microbial contamination, assessment of sterility of the donor tissue is possible. The corneal endothelium maintains its integrity when kept in unchanged media for up to ten days, as long as the media volume is adequate.[83] Seven days was selected to assure adequate time to detect microbial contamination and provide a margin of safety to prevent damage to the corneal endothelium from prolonged storage.

By closing the vial containing the donor cornea at the terminal media change, the risk of contamination during medium change is eliminated. However, placing the donor cornea in M-K medium 18 hours preoperatively breaks this quarantine. To deal with possible contamination that could occur during the transfer of the donor cornea from the closed vial into M-K medium, the M-K medium is kept at 4 C so that if microbes are introduced, replication is prevented. In addition, the antibiotic-antimycotic mixture is added to the M-K medium. Seven days is adequate to detect all but the slowest-growing organisms, especially since antibiotics and mycotics have been removed.[130] Slow-growing fungi, ie, *Histoplasma capsulatum*, may not be detected until four weeks' incubation. However, such organisms are rarely found in tissue culture[130] and are a rare cause of endophthalmitis.[131]

In this sterility check all media, with one exception, became positive at or before seven days. The one exception (No. 7, Table VII) probably represented laboratory contamination and not true media contamination. The CDC group II, K-1 cultured in cases 1 and 2 is a gram-negative rod, a member of the family Pseudomonadaceae, genus *Xanthomonas*.[132] It is a water-borne contaminate, closely related to *Xanthomas campestris*. Since it is a member of the Pseudomonas family, it must be considered a significant pathogen. This contamination occurred during a period when a newly trained technician was involved in changing the media, which shows the importance of well-trained technicians when using this system. No evidence of infection occurred postoperatively in case 1. This is the only case where growth occurred in the media containing the donor cornea. Therefore, 11 of 12 instances of terminal sterility-check medium contamination were false-positive. Because no cases of endophthalmitis have occurred since initiating this new terminal sterility check, no false-negative results have occurred. This indicates the system is extremely sensitive, readily detecting contamination, and emphasizes the importance of a "fail-safe" method of terminal sterility procedures when using 37 C organ culture. Ways are constantly being sought to improve this system. For instance, O'Day et al[133] have reported that cultures from fungal corneal ulcers may require as long as 14 to 19 days before they become positive. This may mean that the seven-day sterility check time should be extended. This is being studied in our laboratory. On the basis of our experience with sterility checks with 108 grafts, the sterility check provides a unique opportunity to assure sterile tissue. I believe it provides us with as safe a donor cornea as that provided by other storage methods. However, microbiologists trained and experienced in sterility check protocol as described in the Appendix are absolutely necessary to perform this function. It is my

opinion that unless one has access to such persons, 37 C organ culture should not be used for donor storage.

Another source of potential contamination in the organ culture system, which we did not study, is that from tissue culture media components such as viruses[134] and bacterial phages in commercial fetal calf serum[135] and endogenous mycoplasma contamination.[136] Multiple exposures with these agents have occurred in human beings for years through the use of vaccinations with vaccine prepared in similar cell cultures, and in none of these instances has human disease been known to occur from such contamination.

CLINICAL STUDIES

This study demonstrates that human corneal endothelial viability is maintained during prolonged storage in organ culture at 37 C. Out of 114 transplants using corneas stored 16.5 days, 92 remain clear after an average of 31 months' follow-up. The higher success rate in this study (81.1%) over that in the preliminary study (65%) is probably due to a larger proportion of poor prognosis cases in the preliminary study.

Preoperative prognosis is extremely important in determining the fate of the graft.[30] In this study, prognosis was favorable in 64% of the successful grafts and in none of the failed grafts (Tables II and III), demonstrating the importance of correlating success to the preoperative prognosis in studies of penetrating keratoplasties. The length of follow-up time is also important.[30] A series of penetrating keratoplasties in patients with good prognosis followed up for a relatively short time would have a much higher success rate than poor prognosis cases followed up over several years.

In this study, as in the preliminary study,[107] and in other studies using 4 C refrigeration storage,[30] donor age and postmortem time were not significant factors in determining graft success. In addition, the duration of organ culture was not significantly different between successful and failed grafts. This finding correlated with the previous laboratory data would indicate that duration of storage could be prolonged beyond 16.5 days without risking graft failure because of loss of endothelial viability.

A measure of the usefulness of 37 C organ culture as a method of donor storage can be gained by comparing it with other storage methods, particularly 4 C refrigeration and M-K medium. However, it is well known that retrospective studies yield data that are difficult to interpret. Mutliple investigators working in different institutions at various times using diverse criteria for patient selection and postoperative evaluation introduce many uncontrolled variables that can cause serious misinterpretations of the

data. These kinds of studies have built-in bias and lack objectivity. A carefully planned prospective randomized clinical trial is the only way to overcome these objections. However, this kind of study is not available, so that retrospective studies are the only source of data that can be used.

The 82.1% success rate in this long-term study of organ culture penetrating keratoplasties favorably compares with the long-term success rates of 83% reported by Saleeby,[20] 78% reported by Stark et al,[137] and 69% reported by Abbott and Forster[30] for 4 C refrigerated tissue. The 59.1% success rate in aphakic grafts (Table XII) is similar to the 66% success rate reported for aphakic grafts using 4 C refrigeration.[138]

There are few long-term series of penetrating keratoplasty using M-K stored tissue in the literature. McCarey et al[58] reported a series of 92 penetrating keratoplasties, 68% of which were clear after an 18-month followup. Approximately 40% of those cases were in a poor prognosis category, and with longer follow-up time more grafts could be expected to fail. The short-term follow-up (90 days) by Aquavella et al[59] of 25 cases, of which 72% were good prognosis cases, had a 92% success rate. Similar short-term results with M-K stored tissue were reported by Bigar et al[60] and Stark et al.[61] Farge[139] reported a group of 330 penetrating keratoplasties selected from 1,026 keratoplasties over a four-year period using both 4 C tissue and M-K tissue. Fifty-nine percent were clear and 41% failed. No breakdown as to which storage methods were used or prognostic category as given in this study.

No cases of primary graft failure were seen in the present study. In the preliminary study, one case of primary graft failure occurred.[107] Primary graft failure has been reported to occur in 7.8% of grafts done with 4 C refrigerated tissue,[137] 11% with cryopreserved tissue[140] and, combining the results of the two reports, 3.5% for M-K medium stored tissue.[60,61] Primary graft failure has become less frequent with improvement in surgical technique, management of postoperative inflammation, and control of intraocular pressure, regardless of which donor storage method has been used. However, the low incidence of primary graft failure with the use of organ cultured corneas suggests this method may improve the quality of donor endothelium during storage in culture, as suggested by previous wound healing experiments[68,69,73] (see Endothelial Wound Healing Studies).

There is no long-term clinical study in the literature of a large number of patients who had penetrating keratoplasty using cryopreserved corneas. Van Horn's recent summary of corneal preservation methods indicates that 350 keratoplasties using cryopreserved tissue have been reported.[141] However, this was a compilation of isolated reports by many authors, with

relatively few grafts followed up beyond one year. Capella et al[142] reported a series of 18 keratoplasties performed on patients with Fuchs' dystrophy and bullous keratopathy with a one-year success rate of 94.4% and a two-year success rate of 88.8%. Kaufman et al[41] reported short-term (three-month) success rates of 75% using cryopreserved tissue in Fuchs' dystrophy and 74% in all cases of bullous keratopathy. Schultz[143] reported 15 cases all clear at 27 months (all but one, with a good prognosis). Mathieu,[144] on the other hand, reported only a 47.5% success rate out of 40 cases followed up at least five months. Twelve of the 21 failures in his group were poor prognosis cases.

Therefore, comparison of organ culture storage with other methods of storage based upon the reported clinical success rates is difficult. However, it would appear that long-term results using organ culture stored tissue are as good as those stored by 4 C refrigeration, M-K medium, or cryopreservation.

To objectively assess graft success, visual acuity results were studied (Table VIII). Seventy-five percent of the patients in this series obtained 20/40 or better visual acuity. Olson et al[145] recently reported 67% of the patients with aphakic bullous keratopathy achieved vision of 20/40 or better using M-K stored tissue. Arentson and Laibson[146] reported that only 41% of combined procedures obtained 20/40 vision or better after surgical treatment using 4 C refrigerated tissue. Visual results from cryopreserved corneal transplants are not available.

Another objective measure of donor corneal success is the rate and magnitude of postoperative thinning (Fig 12). The difference in graft thickness as measured by pachometry in aphakic and phakic eyes seen for the first four weeks postoperatively was not seen in the previous study.[107] Ginsberg et al,[147] using cryopreserved donor tissue, and Irvine et al,[148] using 4 C refrigerated tissue, noted no difference in thickness between aphakic and phakic patients. However, with M-K stored corneas, Bourne[149,150] noted significantly increased graft thickness in aphakia from the early postoperative period to as long as one year postoperative. This delay in postoperative donor corneal thinning in aphakia may represent endothelial dysfunction, perhaps due to a toxic effect of the vitreous on the endothelium. Although this could be a factor in why 20 of the 22 failed grafts were aphakic in the present study, it is more likely that the failures were caused by poor preoperative prognoses (Table III). Whether or not this increased thickness in the early postoperative period is unique to media stored donor buttons is not known. Unlike Bourne's report with M-K tissue,[150] organ cultured grafts are as thin in aphakic as in phakic patients at one year. Therefore, it is unlikely that this increased thickness in

the early postoperative period represents any permanent loss of endothelial cell function.

Specular microscopic examination showed a mean endothelial cell count of 1,598 cells/mm^2 (Table IX). This is almost identical to the mean cell count of 1,548 cells/mm^2 found in the 1976 study of Bourne et al[109] of 14 organ cultured transplants. It compares favorably with the results of other studies of transplanted corneas stored by M-K, cryopreservation, or 4 C refrigeration.[151] Unlike the previous study,[109] no relationship between donor age and mean cell density was seen. However, there was a significant decrease in cell counts with increasing postoperative time unrelated to complications such as glaucoma of immune rejection. This has been shown with studies using M-K[150] and refrigerated donor corneas.[152] This indicates that significant endothelial cell loss occurs following penetrating keratoplasty regardless of the method of interim donor storage.

The four phakic grafts in the present series had cell counts ranging from 905 to 1,852. Although the numbers are too small to test statistical signifiance, the trend is toward lower cell counts in phakic grafts, confirming Bourne and O'Fallen's study that transplants in phakic eyes lose more endothelial cells than transplants in aphakic eyes.[149]

The results of the specular microscopic findings in the present study confirm earlier conclusions that endothelium of organ cultured corneas survives storage and transplantation and maintains endothelial cell density postoperatively as well as corneas preserved by other methods.[109]

Our numbers are too small to comment on the eventual fate of those cases of pseudophakic bullous keratoplasty. This is a new diagnostic category, and patients with the diagnosis will undoubtedly increase in the future. They were arbitrarily placed in an intermittent prognostic group since they all had complications following cataract extraction and intraocular lens implantation leading to corneal decompensation. In all cases, this was associated with intraocular inflammation. All these factors create less than favorable prognostic conditions for the graft. Of the six that have been done, all have remained clear to date.

In this study, immune rejection was the leading cause (36%) of graft failures (Table X). An additional 23 cases of reversed immune rejection occurred (Table XI), for a total immune rejection rate of 28.9%. Pollack[108] has estimated that immune rejection can be expected to occur in 9% to 12% of good prognosis keratoplasties and 30% to 40% in poor prognosis keratoplasties. Based upon these estimates, we would have expected 22 to 29 grafts to develop immune rejection episodes. Since 31 occurred, it appears that organ culture storage as performed in this study failed to modify immune graft rejection.

Perhaps the duration of organ culture storage was too short. In experimental corneal xenografts, no modification occurred until at least three weeks' organ culture.[96] If there are passenger lymphocytes or similar type cells on donor corneas responsible for immune graft rejection, they may not be eliminated under conditions of organ culture as done in this study.

The increased immune rejection rate seen with larger grafts in this study has been noted by others.[153,154] Because larger grafts are closer to the limbus, it is not surprising that they have more rejection episodes because of increased exposure to the host's immune system. Chandler and Kaufman[155] found immune graft reactions in 37.5% of grafts after keratoplasties for keratoconus and believed that the large graft they used for keratoconus (8 to 9 mm) was in part responsible. This probably plays a role in the 45% rejection rate in patients with keratoconus in the present series. Although all reversed, two were severe, requiring large doses of systemic and topical corticosteroids. A cataract developed in one patient.

Multiple variables play a role in immune corneal graft rejection.[154,155] Had smaller grafts been used in this series, the immune graft rejection rate may have been lower and mistakenly attributed to the organ culture storage process, not the size of the graft. On the other hand, if unidentified factors increasing the chances of graft rejection were present in this study, the 28.9% rejection rate may be low, indicating that organ culture may have modified the rejection process. Therefore, on the basis of this study, it cannot be determined whether or not organ culture storage modifies graft rejection. A retrospective analysis as performed in this study cannot answer the question. A well-designed and controlled prospective randomized clinical trial comparing organ culture storage with another method is needed. The most that we can say on the basis of this study is that there is no absolute protection from immune graft rejection.

Glaucoma was responsible for graft failure (Table X) in six (27.3%) and was present at some time in the postoperative period in 34.2% of all grafts in this series (Table XI). Only four phakic graft patients had glaucoma, all of which were controlled medically, whereas 89.7% of all the aphakic patients or patients with combined cataract extraction and keratoplasty had glaucoma. In addition, all the grafts that failed because of the glaucoma were aphakic. Fine[156] reported on the high incidence of postoperative glaucoma in aphakic and combined keratoplasty-cataract patients and believes that all such patients should be empirically treated for glaucoma postoperatively.[156] The high incidence of glaucoma in aphakic and combined cataract-keratoplasty patients in this present series suzstantiates Fine's statement.

Besides graft edema, glaucoma can cause blindness owing to optic atrophy. This was diagnosed in three of the six grafts that failed (diagnosed before graft failure). One patient with a clear graft had optic atrophy caused by unrecognized glaucoma in the postoperative period, resulting in hand motion vision (Table VIII).

Cyclocryotherapy was performed in four of the six patients whose graft failed. In two patients, phthisis resulted, and in two, the pressure was not controlled after two applications. One patient with a clear graft has had good glaucoma control following one cyclocryotherapy treatment. The results of cyclocryotherapy in this study have not been as satisfactory as those reported by West et al.[157] In their series, 12 of 14 patients responded well to cyclocryotherapy. Only two cases resulted in phthisis. The two studies differ in how cyclocryotherapy was applied. West et al[157] applied cyclocryotherapy for 60 seconds around the limbus 360°, whereas in the present study, cyclocryotherapy was applied only 180° for 60 seconds. Although it is possible that the poor response to cyclocryotherapy in this present series could be due to some inherent property of organ cultured donor graft, it is more likely due to the poor preoperative prognosis (Table III).

The remainder of the patients were controlled medically. In some instances treatment was stopped after the sutures were removed. The role that sutures play regarding increased ocular pressure postoperatively in aphakic penetrating keratoplasty has been discussed by Zimmerman et al.[158] Our finding of decreased intraocular pressure when sutures are removed confirms this. Tight sutures may account for the high incidence of glaucoma in the aphakic population of this study. If that is so, it demonstrates that the thicker organ culture donor button does not lead to loose sutures as it thins postkeratoplasty.

Zimmerman et al[159] have reported significant lowering of intraocular pressure when using donor buttons 0.5 mm larger than the recipient bed in aphakic patients. Although most of our aphakic transplants (including combined procedures) were done in this manner in the present series since January 1977, one cannot discern from the data whether or not this lowered intraocular pressure. During the early part of the study, Mackay-Marg tensions were seldom done; therefore, comparisons are meaningless. This technique did not eliminate postoperative glaucoma in the aphakic patient in this series.

The grafts that failed due to persistent epithelial defects were in patients with a preoperative diagnosis of radiation keratitis, a scarred cornea presumably caused by childhood trachoma, and a patient with anterior cleavage syndrome. In each of these cases, the graft gradually

became opacified in the area of the epithelial defect in spite of prolonged treatment with soft contact bandage lenses, topical antibiotics, and corticosteroids. In the case of the anterior cleavage syndrome, the patient also had intraocular pressures ranging between 30 and 50, which may also have accounted for his graft failure. The persistence of the epithelial defect in the patient with radiation keratitis as well as in the patient with old trachoma could be expected because of the basic epithelial disease in these cases. Cavanaugh et al[160] reported 13% epithelial defects using 4 C refrigerated tissue. It would appear that epithelial defects are related to recipient disease and not method of storage.

Wound separation occurred in 9.6% of the grafts in the present study and 11% in the previous study.[107] Binder et al[161] reported 5.7% wound separation in a series of 369 patients. The method of donor storage was not mentioned. Fifty percent of all their wound separations caused graft failure, whereas only 18% of wound separations in this series led to graft failure. As in Binder's series, the vast majority of wound separations in this series were caused by suture removal being performed too early in the postoperative period. Is the wound separation rate higher using organ cultured donor corneas than using donor corneas stored by other methods? Because keratocytes are involved in wound healing and are decreased in numbers during the organ culture process,[71] it is possible that decreased wound healing may be a consequence of organ culture storage. However, only a prospective randomized clinical study comparing results of organ cultured corneal transplants with transplants of corneas stored by other methods will answer this question.

Eight cases of epithelial downgrowth following aphakic keratoplasty have been reported in the literature.[162-165] The cause of the epithelial downgrowth in the present series is unknown. The fistula appeared in the host cornea approximately 2 mm from the graft-host junction as if caused by deep placement of the needle during suturing. If so, this could have allowed epithelium access to the anterior chamber. It is possible the epithelial downgrowth was caused by the previous phacoemulsification procedure. Because overgrowth of epithelium under the endothelial surface during organ culture has been noted after 40 days' storage (Fig 2), this introduces the possibility of epithelium being transplanted into the anterior chamber from the organ cultured corneas. Recall of the residual corneoscleral rim from the donor cornea in both penetrating keratoplasties performed in this patient showed no evidence of epithelium on the endothelial surface. However, these corneas had been frozen, and fine cytologic detail was lost. The donor cornea storage time in both these keratoplasties was less than 15 days. Therefore, it is unlikely that the epithelial

downgrowth came from the donor cornea. Studies are underway investigating the effect of removing all donor epithelium before incubation. Until the issue is resolved, a storage duration of longer than 40 days is not recommended.

Only one case of retinal detachment was in this series. After its repair, the graft failed. Detachments following corneal transplant surgery have been reported in 5.4% of cases using other storage methods.[166] As in the one case in the present series, these detachments were seen only in aphakic eyes where the vitreous was manipulated. Other retinal complications such as cystoid macular edema may have been more common in this series than noted (Table VIII). Had fluorescein angiography been done routinely on all aphakic patients, the incidence may have approached the 33% incidence noted by Forster[167] using 4 C refrigerated tissue. Retinal complications do not appear to be increased with the use of organ cultured stored corneas.

The issue of whether or not the tissue culture medium is cataractogenic is of great concern. Cataract occurred in 11.9% of our phakic transplants. As mentioned in the Results section, all could be explained by postoperative complications or the chronic use of corticosteroids. Wood et al[168] reported that cataract formation after corneal transplantation is related to the use of topical corticosteroids postkeratoplasty. Intraocular surgical intervention is well known to cause or accelerate existing cataracts. Stark et al[137] reported an incidence of 14% cataract formation following penetrating keratoplasty using 4 C refrigerated tissue. No statistics are available for M-K stored cases. It does not appear likely, based upon results in this study, that the incidence of cataract is increased using organ cultured donor corneas.

The 21.1% incidence of synechiae to the graft (Table XI) is similar to the 25% incidence seen using 4 C refrigerated tissue.[169] As in that study, synechia was not a factor in causing graft failure in the present series. Although immune rejection episodes occurred seven times more frequently in grafts with synechiae in the series using 4 C refrigerated tissue,[169] there was no increase in graft rejections with synechiae in this study.

The 40% recurrence of herpes simplex keratitis in the grafts is similar to the 47% recurrence rate reported by Fine and Cignetti[170] using 4 C refrigerated tissue.

The case of endophthalmitis was extensively discussed in the Historical Review and Background section of this thesis and has been reported.[123] This was the major complication of this study. The infection was successfully treated clinically; the retrocorneal mass (Fig 4) disappeared, the inflammation subsided, and the graft remained clear. However, the patient suffered a myocardial infarction and died, probably aggravated by the many

side effects of the systemic antifungal drugs. Since this proved to be a failure of the sterility check procedure used at that time, a moratorium was placed on the use of organ cultured corneas for four months. No other infections occurred clinically and no contaminated donor rims from prior cases were found on recall. The sterility check was modified to one used by the hospital microbiologic laboratories for parenteral solutions and serums. Based upon our experience with this modified terminal sterility check, I believe we are reducing the risk of donor cornea contamination to a minimum.

How valid is the data in this study? The limitations of retrospective analysis and objections of comparisons of such studies have already been discussed. Since the author was the only surgeon involved in this study, uncontrolled variables present when two or more surgeons are involved were eliminated. However, in a study such as this, bias is impossible to avoid. The use of objective data such as visual acuity, specular microscopy, and pachometry, all performed by trained technicians not directly involved in the study, helped to reduce this bias with regard to analysis of successful vs failed grafts. But conclusions based upon causes of graft failure and complications in this study must be qualified owing to the retrospective nature of this study. For instance, is the increased rate of wound separation with organ cultured grafts a consequence of organ culture storage or is it a result of the surgeon removing the sutures too soon and too traumatically? Therefore, even in this study where only one surgeon was involved, there are uncontrolled variables that could lead to misinterpretation of the data.

With these concerns in mind, I believe the results in this series of 114 penetrating keratoplasties using 37 C organ cultured corneas are as good as those reported using 4 C refrigeration, cryopreservation, and M-K medium stored corneas. There did not appear to be any significant difference in causes of graft failure with any of these methods. Except for an increased incidence of wound separation using organ cultured corneas, the postoperative complications were similar in type and amount. There is no apparent modification of the immune response.

How useful is 37 C organ culture as a method of donor storage? How does it fulfill the criteria of the ideal storage method discussed in the Historical Review and Background section of this thesis? It fails to meet the ideal criteria in many ways: It is a complicated, involved technique that is expensive and, like all storage methods used today, does not allow assessment of endothelial viability during storage. It presents a definite biohazard with regard to microbial contamination. It causes the cornea to swell and become opaque during storage. It also has advantages: In organ culture medium the corneas are transportable without special equipment over a

wide range of temperatures. It can be used for both lamellar and penetrating keratoplasty. It may improve the quality of the donor endothelium during storage. The method has the potential for assuring sterile donor tissue. However, its most obvious virtue is the prolonged duration of storage possible with this method. At the author's institution, seven surgeons, three doing retina-vitreous surgery, compete for 40 hours elective time each week in one operating room. Since elective cases are scheduled up to two weeks in advance, the use of organ culture donor tissue has allowed elective scheduling of transplants and prevented the severe disruption that emergency surgery causes to the operating room schedule. Even M-K tissue with its three- to four-day limits would be too short for our needs. Therefore, the advantage of prolonged storage with organ cultured tissue is extremely important in my institution. Because of its complexity and expense, this method would appear to be most useful to a large institution that maintains an eye bank and that has operating room scheduling problems like ours, or to a regional eye bank, in an area where the needs of long-term storage of donor tissue would warrant the expenses involved. Under any circumstances, it is not a method to be used casually without personnel trained in tissue culture and sterile techniques and without access to trained microbiologists to perform the terminal sterility procedure to safeguard against donor cornea contamination.

In conclusion, I believe this clinical study confims that 37 C organ culture storage is a safe (with appropriate personnel and safeguard) and efficacious method of donor corneal storage.

SUMMARY

This thesis presents a study of the efficacy of 37 C organ culture as a method of long-term storage of human donor corneas prior to penetrating keratoplasty. Because bacterial and fungal contamination is a major biohazard using organ culture techniques, microbial studies were performed to assess this risk. Two hundred thirty nonseptic donor eyes had a contamination rate of 76.66% as they were received by the eye bank before any processing of the tissue was performed. Coagulase-negative staphylococcus and diphtheroids comprised 47.4% of all the organisms cultured from these eyes. When the anterior segment of the donor globes was immersed in gentamicin or Neosporin solution for three minutes, gentamicin sterilized 78% of the globes, whereas Neosporin sterilized only 36% of the globes, demonstrating the superiority of gentamicin decontamination of donor globes. During organ culture storage, contamination from the environment or personnel or carryover from contaminated donor eyes occurred at a rate of

0.55 contaminated organ cultures per month in spite of penicillin, gentamicin, and amphotericin B being present in the medium. Therefore, even with careful decontamination procedures of the specimen prior to organ culture, and the addition of antibiotics and antimycotics to the medium, contamination is a constant risk during the organ culture process. To reduce the risk of transplanting contaminated donor corneas, a terminal sterility procedure has been performed on all organ cultured corneas before clinical transplantation to human recipients. The initial terminal sterility check was modified after failing to detect *Torulopsis glabrata* contamination of the organ cultured donor cornea that resulted in endophthalmitis. In 108 transplants since modification, it has functioned satisfactorily. However, this should only be performed by well-trained microbiologists experienced in sterility check procedures.

In the clinical study, 114 penetrating keratoplasties were performed by the author using donor corneas stored in organ culture an average of 16.5 days (range 2 to 35 days) prior to surgery. After a follow-up time from 6 to 63 months (average 31 months), 92 (81.8%) were successful and 22 (18.9%) failed. No cases of primary graft failure have occurred in this series. Immune rejection episodes occurred in 33 (28.9%) of the cases and accounted for the most graft failures (eight). Since this was a retrospective study, definite conclusions regarding modification of the immune graft rejection after organ culture cannot be made. However, based upon the results of this study, it appears unlikely that such modification occurs. The causes of graft failure and their frequency are similar to those reported using other storage methods. The postoperative complications seen using 37 C organ culture were similar to those reported using other storage methods, with the exception of a 9.6% incidence of wound separation using organ cultured donor corneas, as opposed to 5.6% reported using other storage methods.

Technically, 37 C organ culture is a complicated and expensive method of donor cornea storage, requiring a well-trained technician as well as a microbiologic laboratory staff experienced in sterility check procedures. The major advantage of this system is the long-term storage possible with its use. Based upon the results of the investigations presented in this thesis, 37 C organ culture storage is an efficacious method of long-term donor corneal storage prior to penetrating keratoplasty and is safe when used with the appropriate personnel and safeguards.

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