EVALUATION OF VIABILITY OF DONOR TISSUE FOR CORNEAL GRAFTING*

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WITH CORNEAL grafting operations being more widely performed, the demand for donor material has increased. In order to use the available tissue to the best possible effect, a satisfactory method of storing donor material over a prolonged period of time is necessary. The method of keeping donor eyes in a moist chamber at about +4° C., currently used by most eye banks in this country, is far from being ideal. Most surgeons are reluctant to use corneas for grafting which have been stored in this manner for longer than 48 hours. This obviously leads to a considerable waste of precious tissue.

The following methods of preservation have been employed by various investigators in attempts to obtain clear corneal grafts: (*a*) formalin fixation (1); (*b*) drying (2); (*c*) freezing (3); and (*d*) freeze-drying (4, 5). In most cases these methods have not been satisfactory. Three methods have evolved which appear to be more promising. These are (*a*) storage in liquid paraffin (mineral oil) (6); (*b*) vacuum dehydration (7); and (*c*) rapid freezing after glycerol treatment (8). Very satisfactory clinical results with lamellar grafts have been reported both with dehydration and freezing after glycerol treatment. Clear penetrating grafts were also obtained in some cases with the glycerol-freezing method by Eastcott. But the over-all results were not good enough for the author to recommend this procedure for full thickness grafts. On the other hand, excellent results have been reported with perforating grafts which had been stored in mineral oil at +4° C. by Bürki and others. No report on results of perforating grafts is available at this time with regard to the dehydration method.

The ultimate decision on the best method of preserving corneal tissue will have to be based on the clinical experience. Experimental

*From the Duke University School of Medicine Department of Surgery: Plastic Surgery Research Laboratory and Division of Ophthalmology. This work was aided by U.S. Public Health Grant B-1161.

grafts in animals may be used as a preliminary test. Results in animals such as rabbits however are sometimes difficult to evaluate.

It is generally thought that the donor tissue should be viable at the time of the corneal grafting operation, at least where full thickness grafts are concerned, regardless of whether the tissue is subsequently replaced by the recipient. *In vitro* methods which demonstrate the viability of the tissues, therefore, are most helpful. These methods include determination of active tissue metabolism and tissue cultures. The latter would appear to be more reliable in demonstrating the presence of cells which are capable of proliferation as well as metabolic activities. With a positive tissue culture, one can be quite certain that the tissue is viable. A negative culture would not definitely preclude the possibility of obtaining a living graft *in vivo*, but persistent negative results might at least cast severe doubts on the viability of the tissue examined.

McPherson *et al.* (9) and Draheim *et al.* (10) used tissue culture successfully in determining viability of corneal tissue. Fresh rabbit corneas showed excellent migration of epithelial and fibroblastic cells within 48 hours. Corneas which were soaked in dilute glycerol or soaked and then frozen at –79° C., showed a slight lag in migration but were soon indistinguishable in migration and cell appearance from fresh corneas. It was found that storage of glycerol-frozen corneas for longer than one month resulted in changes in the physical appearance and decreased viability. The question, however, of why lamellar grafts were uniformly successful with glycerol-frozen material but perforating grafts were not remained unanswered.

It may not be too improbable to suggest that, while the corneal epithelium and stroma are able to survive the freezing process rather well, the delicate endothelium is more easily damaged and would result in unsuccessful perforating grafts. In order to find out whether this was so, a technique had to be developed by which the three layers of the cornea, particularly the endothelium, could be grown in tissue culture separately.

The details of our experimental technique will be described in another paper to be presented before the Association for Research in Ophthalmology (11). We succeeded in isolating Descemet's membrane with the endothelial layer from the stroma of the rabbit's cornea as previously described (12). Figure 1 demonstrates that except for the endothelial cells no cellular elements are present in such a preparation. Any cells growing from this specimen by necessity will be endothelium.

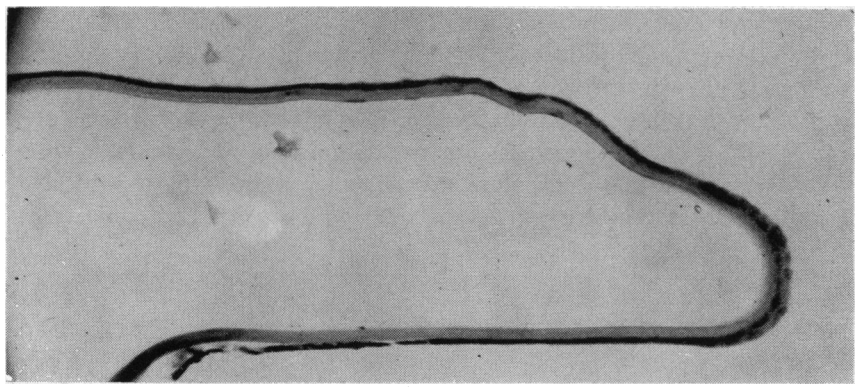

FIGURE 1. DESCEMET'S MEMBRANE TOGETHER WITH THE LAYER OF ENDOTHELIUM SEPARATED FROM THE STROMA OF A RABBIT'S CORNEA

No other cellular elements are present besides endothelia (× 700).

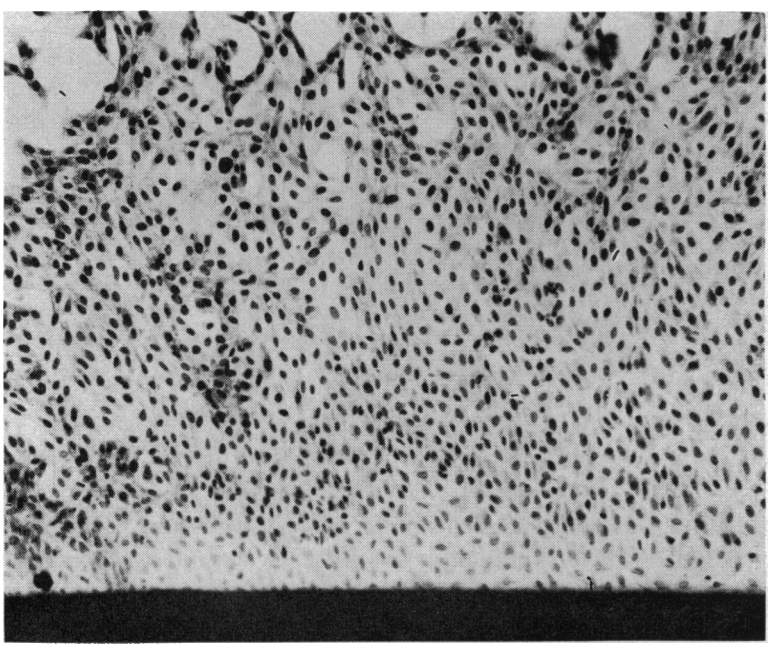

FIGURE 2. ENDOTHELIUM OF RABBIT'S CORNEA AFTER SEVEN DAYS IN TISSUE CULTURE

Definite growth of endothelial cells is observed (× 88).

When Descemet's membrane and endothelium were isolated and tissue cultured, migration of endothelial cells commenced in one to two days and good growth occurred within five days. Figures 2 and 3 show the growth of the culture after seven days. It is interesting to note that the proliferating cells (Figure 4) are very similar in appearance to the *in vivo* regenerating cells after experimental injuries of the corneal endothelium as previously reported (12) (Figure 5). Epithelial cells,

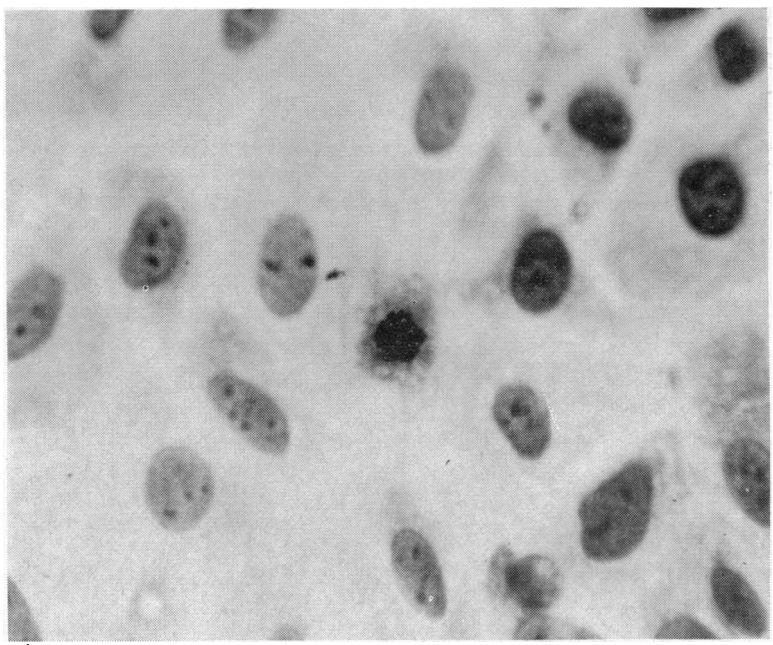

FIGURE 3. ENDOTHELIUM OF RABBIT'S CORNEA AFTER SEVEN DAYS IN TISSUE CULTURE

Same as Figure 2 under higher magnification (× 700). Definite mitosis is seen in center.

in separate cultures, showed similar behavior (Figure 6), whereas stromal cells came out only after five to seven days and needed about two weeks for a good growth to occur (Figure 7).

Once the basic technique of growing the three layers of the cornea separately was established, the influence of various preservatives on each type of tissue could be tested.

The European literature contains several reports on preservation of corneas in mineral oil (liquid paraffin) at +4° C. (6, 13, 14, 15). The

presence of the enzyme cytochromoxydase in corneal tissues after three weeks of storage by this method was considered to be proof of viability by Bürki (13). In contrast, if the tissues were kept in Ringer's solution, the enzymatic activity was reduced after a few days.

Using our technique, we have cultured tissues from rabbit corneas which had been kept in mineral oil at +4° C. for varying intervals of time. After one week of storage, all three layers of the cornea showed

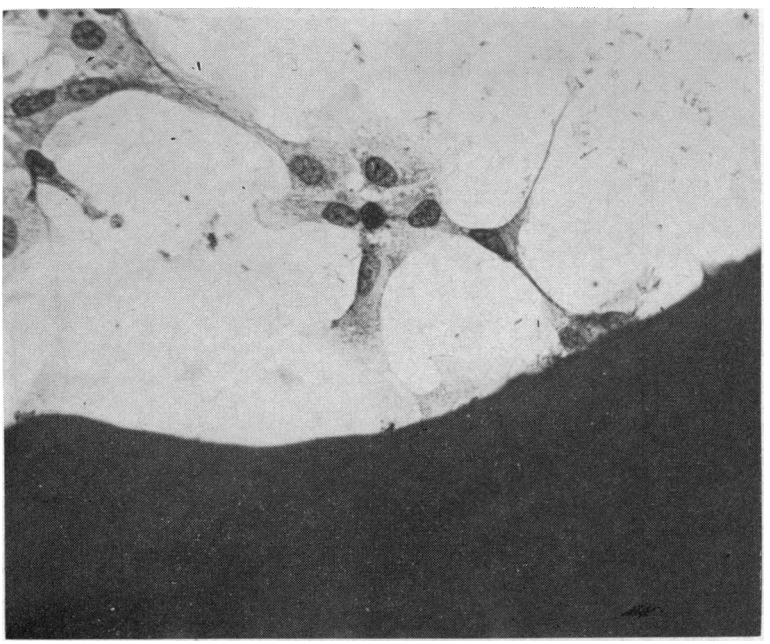

FIGURE 4. ENDOTHELIUM OF RABBIT'S CORNEA AFTER SEVEN DAYS IN TISSUE CULTURE

Same as Figure 2 and 3, (detail) proliferating cells.

excellent growth. The experiments then were extended to two, three, four, five, six, eight, ten, and twelve weeks of storage. Up to five weeks, positive cultures were obtained from epithelium, stroma, and endothelium. Figure 8 shows abundant growth of endothelium after four weeks' storage in mineral oil at +4° C. It is interesting to compare the morphologic appearance of the endothelium of human cornea after storage in moist chamber at +4° C. and in mineral oil at +4° C. Figure 9 shows the endothelium in a flat preparation (technique previously described (12)) and after 48 hours of storage in moist chamber.

In Figure 10 the endothelium is seen as it appears after four weeks' storage in mineral oil. In general, the appearance is comparable, that is, there is a continuous sheet of endothelial cells, somewhat loosened in some areas with vacuolization of the cells. In contrast, after four days of storage in moist chamber, the endothelial layer is completely disrupted with large parts of Descemet's membrane being denuded as shown in Figure 11. After six weeks' storage under mineral oil at

FIGURE 5. FIXED FLAT PREPARATION OF CORNEAL ENDOTHELIUM OF RABBIT 20 HOURS AFTER EXPERIMENTAL INJURY TO THE ENDOTHELIUM OF THE LIVING ANIMAL

There is a striking similarity with the appearance in tissue culture (Figure 4). From Stocker (11).

+4° C., no growth occurred from any of the three layers. From these observations, it appears that all three layers of the cornea may remain viable for roughly one month when kept in mineral oil at +4° C.

Numerous British and European authors have reported excellent clinical results with corneas stored in liquid paraffin (mineral oil) at +4° C. Bürki, who first described this method (6), summarized his experimental studies and clinical experience in a comprehensive

monograph (12). In a comparative series, he reports 10 clear grafts out of 23 (43.5 percent) in a group with conventional methods of preservation (moist chamber). The group of 46 cases for which the mineral oil method was used contained 24 (52.2 percent) clear grafts. In general, he used the donor cornea within a few hours, but he has obtained clear grafts with corneas stored for several days. Among the numerous outstanding foreign ophthalmologists who now use this

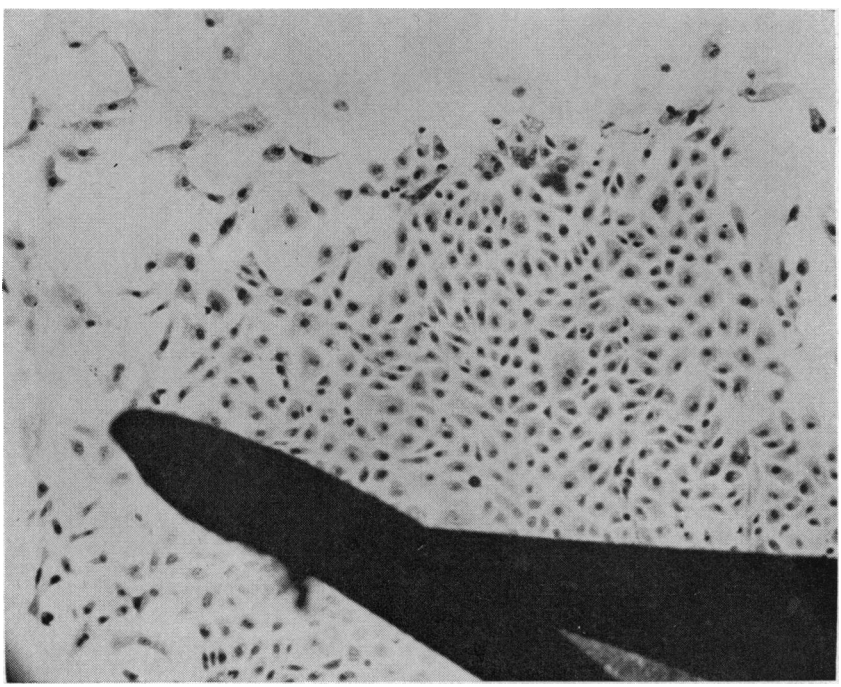

FIGURE 6. EPITHELIAL CELLS FROM RABBIT'S CORNEA GROWING IN TISSUE CULTURE AFTER SEVEN DAYS (× 140)

method routinely are Amsler, Streiff, Sourdille, and Busacca. Rycroft (15) reported successful optical grafts after preservation of the bulbus in mineral oil for as long as three weeks. In a recent publication (16), he advised that it was safe to use material which had been stored in mineral oil at +4° C. for as long as two weeks.

The second method of preservation which seemed promising is that of vacuum dehydration. Through the courtesy of Dr. John Harry King, Jr., we were able to extend our investigation to corneal tissues

which had been dehydrated and stored at room temperature at his laboratory. The four rabbit corneas we received were sealed in glass tubes, two of them in vacuum. They were perfectly clear in that stage. As soon as they were dehydrated they became cloudy and edematous, a change which had already been observed by King himself. The

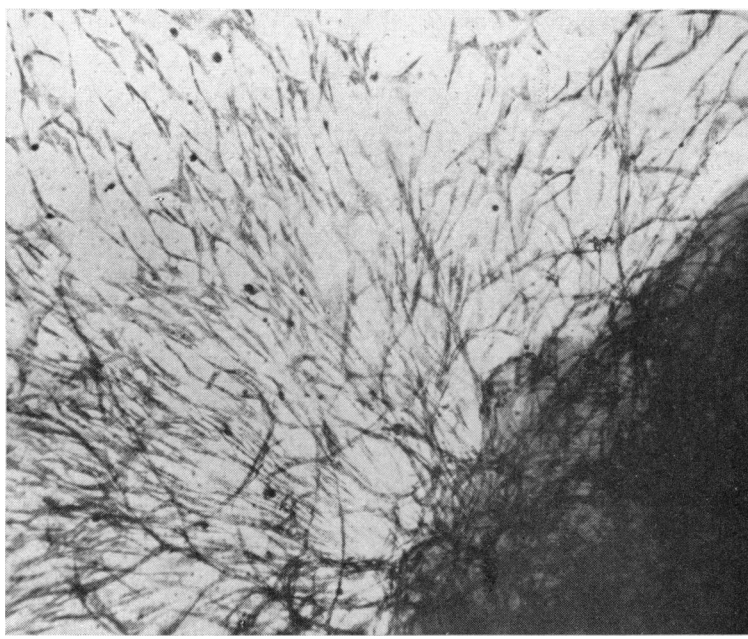

FIGURE 7. STROMAL CELLS FROM RABBIT'S CORNEA GROWING IN TISSUE CULTURE AFTER 10 DAYS (× 80)

tissues were then separated in the same way as in the previous experiments and tissue cultures set up using the same technique. No growth occurred in any of the three corneal layers.

Since King has reported beautiful results with lamellar grafts using dehydrated material, it has to be assumed that, for this type of grafting, it is not necessary for the donor tissue to be viable. Probably proliferation of the recipient cornea readily replaces the graft. After all, even after a simple keratectomy, without graft, a fairly normal cornea may be regenerated.

With perforating grafts the situation seems to be different. If the question of whether the endothelium of the graft will eventually be

replaced by the recipient is put aside, an intact endothelial layer seems to protect the graft from becoming edematous in the early stages. Figure 12 demonstrates that, even in the same graft, the part from which the endothelium had been scraped off is considerably thicker, from absorbing fluid, than the part which was covered by normal

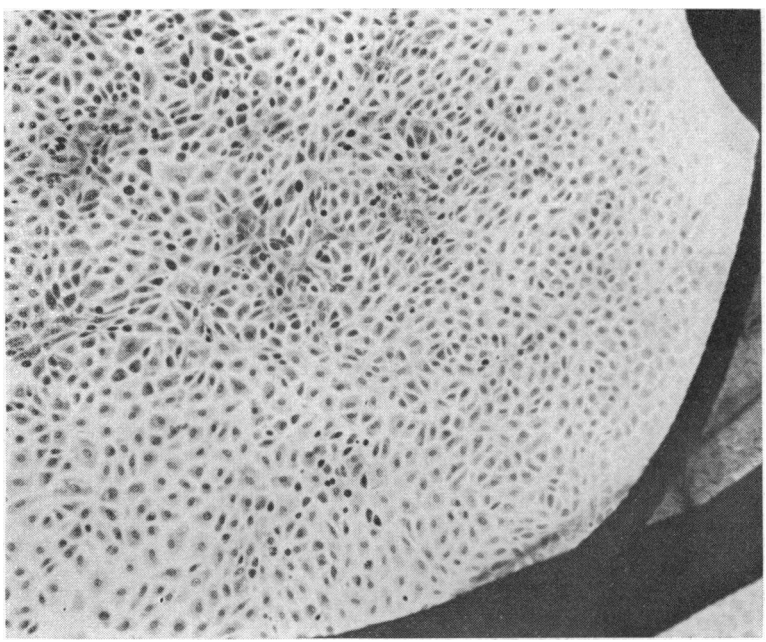

FIGURE 8. ENDOTHELIUM OF RABBIT'S CORNEA

The endothelium which had been stored in mineral oil at +4° C. for 4 weeks shows abundant growth after 10 days in tissue culture (× 80).

endothelium. Clinically early edema causing cloudiness of the graft is a most unwelcome symptom. Although in some cases a clear graft still may result, the prognosis is not as good as when the graft stays clear from the beginning. Thus an intact viable endothelium appears to be of paramount importance for obtaining successful penetrating grafts.

The third method that has been promising is that of glycerol treatment followed by storage at subzero temperatures. Our approach to the problem has been by tissue culture methods in which we utilized

our previously developed methods for separating and growing the three tissues of the cornea separately. By this method we were able to determine the effect of various preservatives and temperatures on the individual tissues and thus determine the optimal conditions for preserving and storing all three tissues in a viable condition.

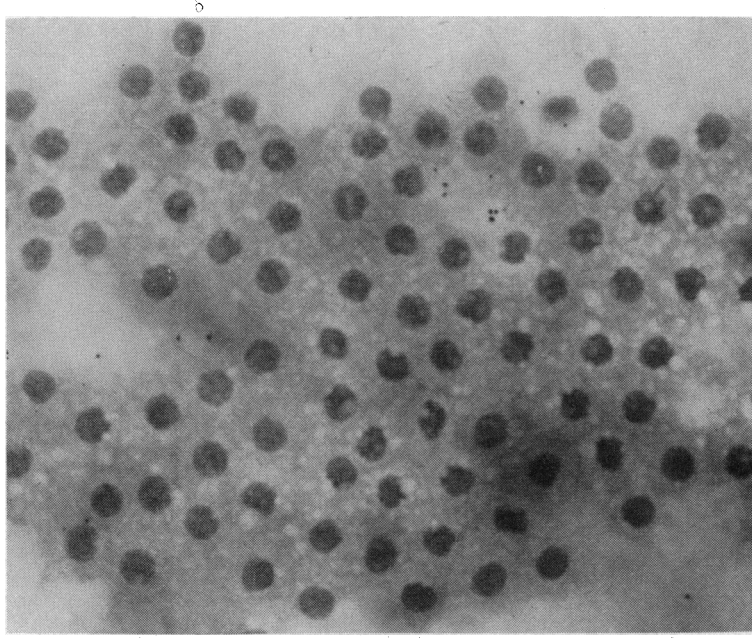

FIGURE 9. FIXED FLAT PREPARATION OF HUMAN CORNEAL ENDOTHELIUM AFTER 48 HOURS OF STORAGE IN MOIST CHAMBER AT +4° C.
The endothelial layer is not continuous any more. Extensive vacuolization of cytoplasm and nuclei is present (× 678). From Stocker (11).

In an initial series of experiments, corneas were exposed to various concentrations of glycerol or ethylene glycol for one hour at room temperature and then tissue cultured. The diluent employed was either Hanks's balanced salt solution without sodium bicarbonate (RBSS) or oxypolygelatin (OPG). The results may be briefly summarized as follows: (*a*) all concentrations (1-100 percent) of ethylene glycol were toxic for all three tissues (epithelium, stroma, and endothelium); (*b*) concentrations of glycerol greater than 50 percent were toxic for all three tissues; and (*c*) RBSS and OPG appeared equally satisfactory as a diluent.

These results were used as the starting point for short term (1 to 48 hours) freezing experiments. The corneas were soaked at room temperature for one hour in the preservative and placed at –45° C., –79° C., or –196° C. No step freezing was employed. With the –45° C. temperature, a comparatively slow rate of freezing was obtained while

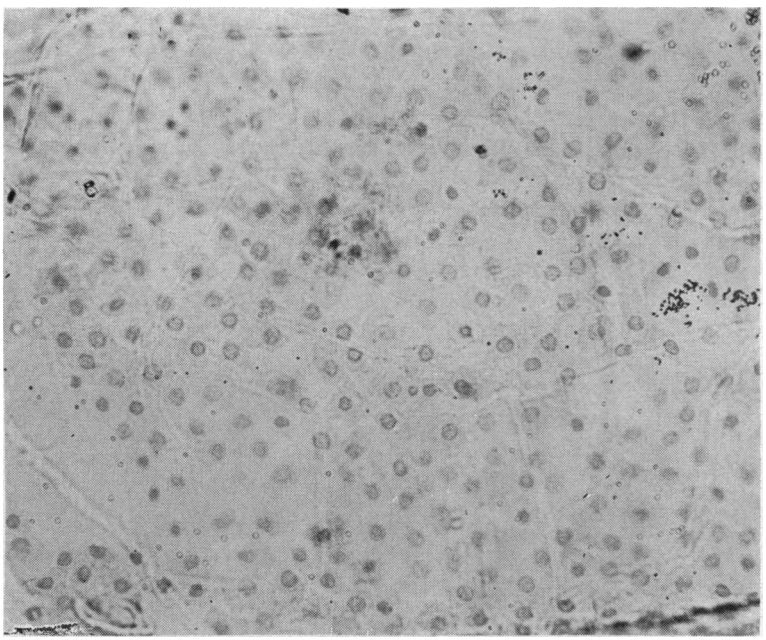

FIGURE 10. FIXED FLAT PREPARATION OF HUMAN CORNEAL ENDOTHELIUM AFTER 4 WEEKS' STORAGE IN MINERAL OIL AT +4° C.
The picture is roughly comparable with that presented in Figure 10 (× 300).

with the liquid nitrogen (–196° C.) a fast rate was achieved. After one hour in liquid nitrogen or one or two days at –45° C. or –79° C., the tube was removed, placed in a +37° C. water bath to thaw, and then tissue cultured. Erratic results were obtained at first with the only consistent finding being that the epithelial tissue survived and grew over a wide range of glycerol concentration and in all three temperatures. The series has been repeated several times and more consistent results have been obtained. The results are summarized in Table 1 and are briefly as follows: (*a*) the epithelial layer is least affected while the endothelium is the most vulnerable, and the stromal tissue is

TABLE 1. TISSUE CULTURE RESULTS OF RABBIT CORNEAS FROZEN AT VARIOUS TEMPERATURES AND IN DIFFERENT CONCENTRATIONS OF GLYCEROL

	STORAGE TEMPERATURE*								
	−45° C.			−79° C.			−196° C.		
DILUENT†	*Ep*‡	*S*	*En*	*Ep*	*S*	*En*	*Ep*	*S*	*En*
OPG	1–20§	10	20	1–20	10	10–20	5–50	¶	—
RBSS	1–50	5–30	20–30	1–30	5–30	20	5–50	20–30	20

*Storage interval is one hour for −196° C.; 24–48 hours for −45° C. and −79° C.

†OPG = Oxypolygelatin; RBSS = Hanks' balanced salt solution without sodium bicarbonate.

‡*Ep* = Epithelial; *S* = Stromal; *En* = Endothelial cultures.

§Numerals indicate glycerol concentration range which resulted in growth.

¶No growth was observed in any glycerol concentration.

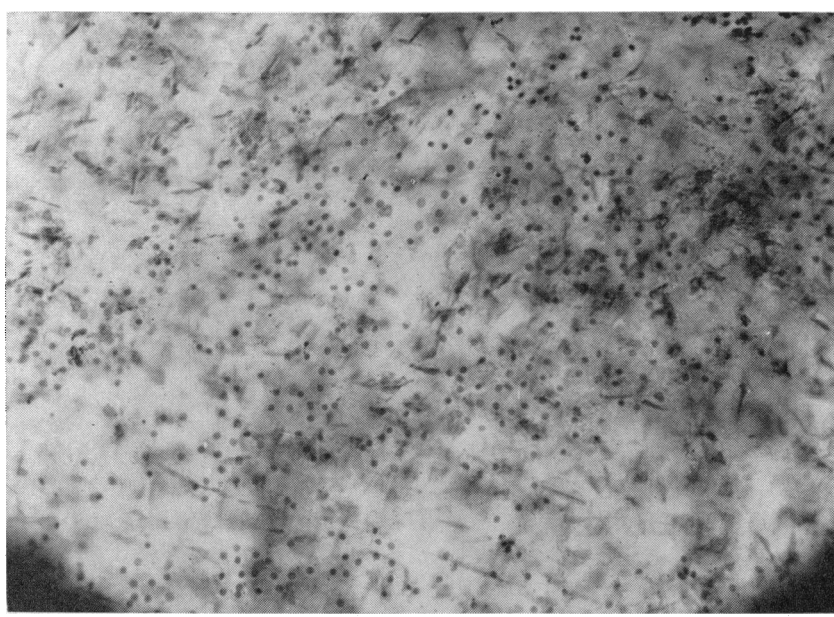

FIGURE 11. FIXED FLAT PREPARATION OF HUMAN CORNEAL ENDOTHELIUM AFTER 4 DAYS' STORAGE IN MOIST CHAMBER AT +4° C.

In large areas the endothelium is absent. The remaining cells are of variable size and stain faintly (× 158). From Stocker (11).

in between; (*b*) growth of all three tissues was obtained over a wider range of glycerol concentration when RBSS was employed as diluent than when OPG was used; (*c*) the –45° C. temperature series resulted in better preservation over a wider range of glycerol concentration than either of the other two temperatures; and (*d*) the optimal conditions from these results are shown to be obtained from a preservative medium composed of 20 percent glycerol with RBSS as diluent and a storage temperature of –45° C.

These results have been used to set up long-term storage experiments at –45° C. and –79° C. utilizing both diluents and a glycerol concentration of 20 percent. The results to date are summarized in Table 2 and

TABLE 2. COMPARISON OF TISSUE CULTURE RESULTS WITH RABBIT CORNEAS PRESERVED AT −45° C. AND −79° C.

Length of storage	−45° C.						−79° C.		
	RBSS*			OPG			RBSS		
	Ep†	*S*	*En*	*Ep*	*S*	*En*	*Ep*	*S*	*En*
2 days	+	+	+	+	+	+	+	+	+
1 week	+	+	+	+	0	+	+	+	0
2 weeks	+	±	±	+	±	0	+	0	0
4 weeks	+	+	+	+	0	0			
6 weeks	+	+	0	+	+	0	+	0	0
8 weeks	+	+	0	+	+	0	0	0	0
12 weeks	+	+	+	+	+	0			

+ = More than 50 percent positive cultures; ± = less than 50 percent positive cultures; 0 = no growth.

* = Preservative media composed of glycerol (20 percent) with either Hanks' balanced salt solution without sodium bicarbonate (RBSS) or oxypolygelatin (OPG) as diluent.

†*Ep* = Epithelial; *S* = Stromal; *En* = Endothelial cultures.

are briefly as follows: (*a*) there is no difference in results for a two-day interval with respect to diluent or temperatures, that is, all three tissues survived and grew under all conditions; (*b*) for longer intervals, the –45° C. temperature has proven superior to –79° C. for protecting the stromal and endothelial layers; (*c*) at –45° C. the RBSS diluent has been better for protecting the endothelial cells, as well as the other two tissues, than the OPG diluent; (*d*) the initial observations on the

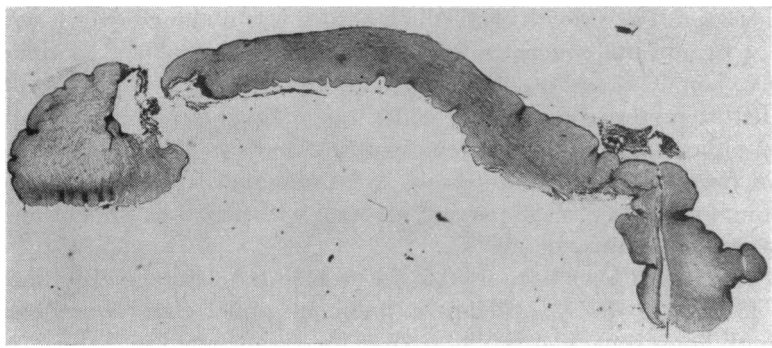

FIGURE 12. RABBIT'S CORNEA FOUR DAYS AFTER A PERFORATING GRAFT HAD BEEN PERFORMED

The part to the right is much thicker (edema) than the part to the left. Prior to grafting, the endothelium had been scraped off the part to the right. From Stocker (11).

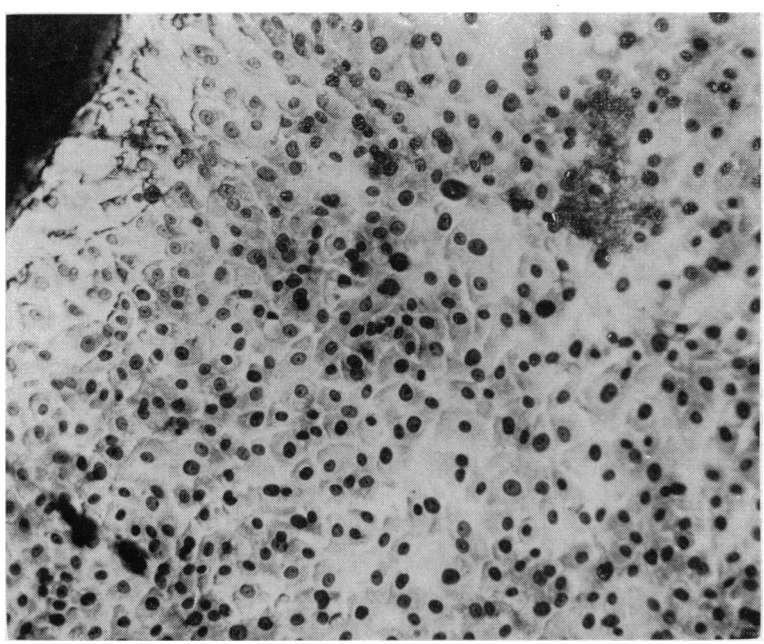

FIGURE 13. EPITHELIUM OF RABBIT'S CORNEA AFTER STORAGE AT —45° C. FOR SIX WEEKS

Abundant growth (× 140).

"hardiness" or vulnerability of the three tissues are demonstrated again, that is, the epithelial cells survive and grow over a wide temperature range and long storage time; the endothelium survives over a much narrower range and time, and the stroma is in between; (*e*) the optimal conditions are a preservative medium composed of glycerol

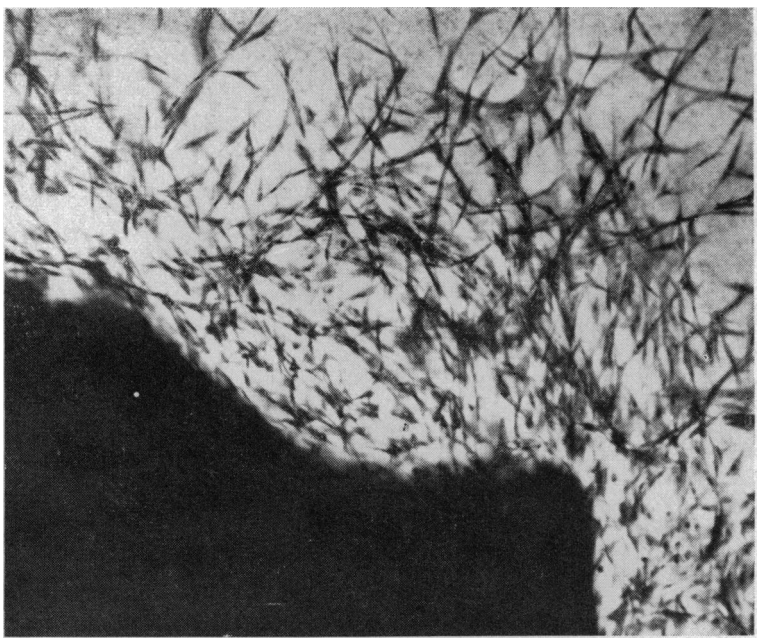

FIGURE 14. STROMA OF RABBIT'S CORNEA AFTER STORAGE AT —45° C. FOR SIX WEEKS

Abundant growth (× 82).

20 percent with RBSS as diluent, and –45° C. as storage temperature; and (*f*) in contrast to the observations of Draheim *et al.*, under the optimal preservation conditions, no changes in the physical appearance of the corneas after prolonged storage were noted. Figure 13 shows growth of epithelium after six weeks' storage at –45° C.; Figure 14 growth of stroma under the same conditions. Figure 15 shows the endothelium of rabbit's cornea after storage at –79° C. for two weeks and left in tissue culture for three weeks. The endothelial layer is fairly intact, but the cells have shrunk, and no sign of migration or

growth is detectable. In contrast, definite growth of endothelium is seen in Figure 16 after storage of 12 weeks at –45° C.

The generally unsatisfactory results by the use of frozen corneas in full thickness grafts has been mentioned. One excellent result reported by Iliff (17) was obtained with a cornea which had been frozen only

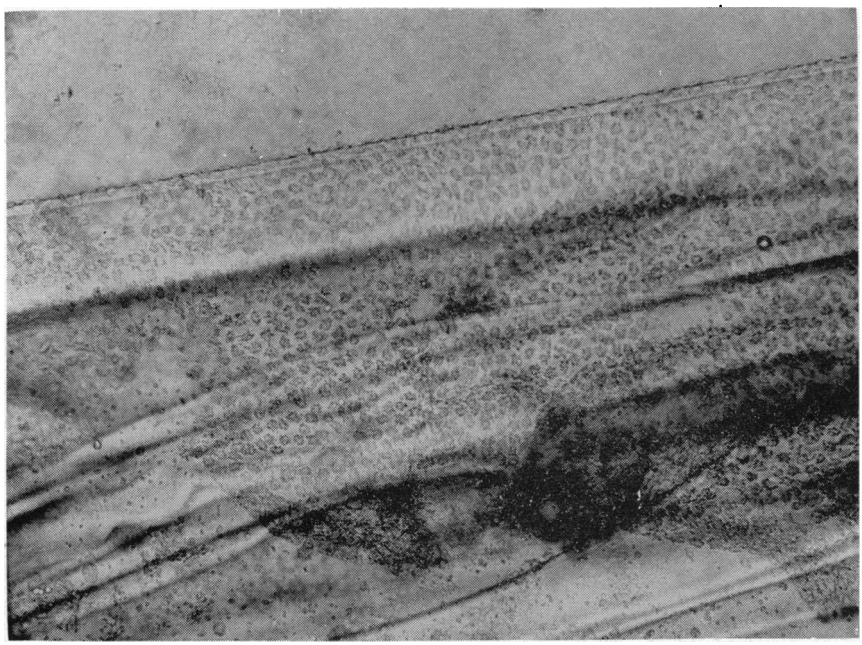

FIGURE 15. ENDOTHELIUM OF RABBIT'S CORNEA AFTER STORAGE AT —79° C. FOR TWO WEEKS AND LEFT IN TISSUE CULTURE FOR THREE WEEKS

The endothelial layer is fairly intact, but the cells have shrunk, and no signs of migration or growth is detectable (× 265).

for one and one-half hours at –79° C. It would appear to be consistent with our experiments that the endothelium would remain viable after having been kept frozen at this temperature for only a short while.

A case reported recently by Rycroft (16) in which a cornea was used which had been frozen and stored at –79° C. for four weeks made an interesting course. The graft at first became more and more opaque until after seven weeks it was completely cloudy. Only after seventeen months did it begin to clear again and after two years it was completely clear. It may be assumed that in this case the endothelium was

severely damaged by the freezing process. Consequently the aqueous humor was able to penetrate into the corneal stroma which led to a cloudiness of the graft. As the endothelium was replaced by the host, clearing of the cornea occurred. While this clearing was fortunate, one would hardly consider such a postoperative course as normal or

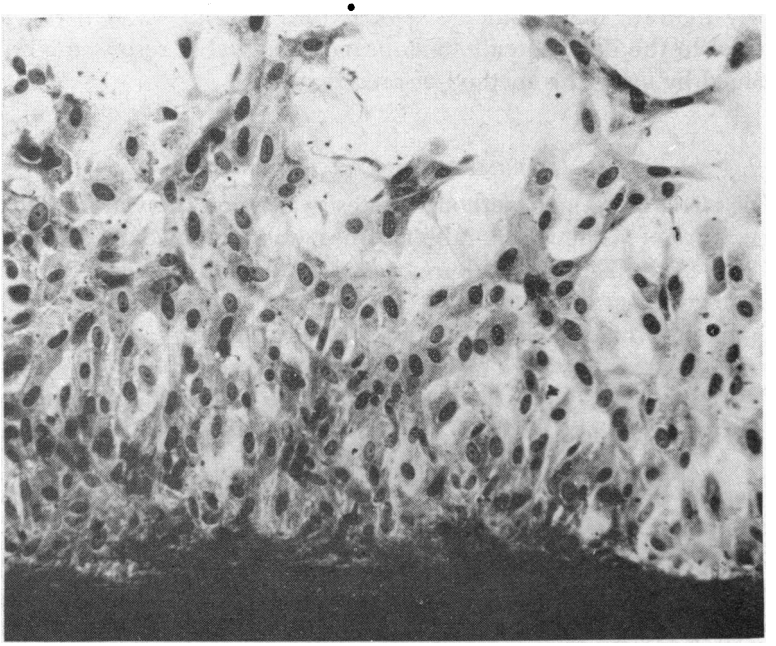

FIGURE 16. ENDOTHELIUM OF RABBIT'S CORNEA AFTER STORAGE AT —45° C. FOR 12 WEEKS

Definite growth. Practically indistinguishable from growth of fresh endothelium (Figures 2 and 3) (× 145).

desirable. Rycroft (16) stated that more recent corneal grafts, with modification of the deep freeze technique, had given very encouraging results, and that this method will become the bank method of preservation for the future. He did, however, not specify what the modifications of the technique were.

When our laboratory findings are correlated with the clinical experience of various authors, it appears that it is important for the corneal endothelium to be intact and viable at the time of the grafting operation if full thickness grafts are concerned. The results with tissue culture indicate that the endothelium is definitely more vulnerable and

reduced in its vitality by freezing than are the epithelium and the stroma. We consider this fact to be the logical explanation for the generally unsatisfactory clinical results which have been reported for the use of frozen donor material for full thickness grafts. It should be remembered, however, that all these reports deal with donor corneas which had been frozen at –79° C. or –196° C. Since our experiments clearly indicate that a storage temperature of –45° C. is much less harmful to the delicate endothelium more favorable results might be obtained by using this method of preservation.

SUMMARY AND CONCLUSIONS

The effect of various methods of preservation on the viability of the three types of tissue of the rabbit cornea was examined by setting up between 850–900 tissue cultures using a method developed in our laboratory for growing the three corneal tissues individually.

In general the epithelium was found to be the least affected by the process of preservation. The endothelium suffered the most.

Positive cultures from all three layers were obtained after preservation of corneas in mineral oil at +4° C. for periods up to five weeks but not longer.

No growth was obtained from corneas dehydrated by the method of King.

Of the freezing techniques, storage in 20 percent glycerol and 80 percent Hanks's balanced salt solution without sodium bicarbonate at –45° C. gave the most satisfactory results. Positive cultures from all three layers were obtained after as long as 12 weeks of storage.

From this study it would appear that storage at –45° C. would be the best method for preserving corneal tissue as far as viability is concerned.

Experiments with human corneas are now under way. These will be followed by the clinical application of the experimentally determined principles.

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DISCUSSION

DR. R. TOWNLEY PATON. The work that Dr. Stocker and his associates have reported on is indeed a valuable contribution to the subject, and I wish to compliment them on their efforts, and the interesting results which they have reported.

Experimentation in this field has certain fundamental difficulties. Successful corneal transplants have been reported with non-viable as well as viable corneal material. Criteria for the efficiency of any method of storage are difficult to evaluate. The clinical result of a series large enough to be statistically significant will always have to be the ultimate proving ground of success. Tissue culture technique is an unquestionable method for demonstrating viability. Viability of the cells is certainly a most desirable platform from which to launch further studies. Dr. Stocker's report on cellular viability of eyes stored under mineral oil for four to five weeks is definitely an important advance in the solution of this problem.

In addition to the methods of preservation noted by Dr. Stocker, whole eyes have been stored in blood, plasma, vegetable oils, various physiologic

solutions, and polyvinyl plastic which was used by the Germans in the last war as a soluble plasma substitute. Excised corneas or corneal discs have been stored in moist chambers, air, mineral or vegetable oils, partially hydrated chambers, and in vacuum following exposure to electronic bombardment.

Experimentation in this field has certain fundamental difficulties. On the one hand, dead tissue has been successfully transplanted, in a clinical sense at least, in some cases. On the other hand, respiratory activity on the part of corneal tissue has been demonstrated to be quantitatively unimpaired (by Duane) in corneas stored for several days beyond the time that they would be considered useful for transplantation purposes. The capacity of the tissues to grow in tissue culture has likewise failed to serve as a useful index for the preservation of corneal tissue for purposes of corneal transplantation. This conclusion may be drawn from the observations of Paul Messier and Ruth Hoffman (Arch. Ophth., 42:148–154) that grafts from corneas cultured *in vitro* fail to give satisfactory clear transplants. The use of experimental animals as a test for transplantability has limited value because of the greater number of surgical complications in animals, and the fact that the "durability" of the corneas (in rabbits particularly) is so much less than in humans.

The criteria of successful preservation (exclusive of clinical trial) which have been used by various authors include the simple examination of the cornea with a slit lamp microscope, histologic examination of donor material, loosening of epithelium, cell migration and mitotic activity, tissue culture, swelling and water uptake, increase in the non-protein nitrogen activity of proteases, respiratory activity, oxidation-reduction potentials, etc.

In the Eye Bank Laboratory, studies have been conducted on several phases of the storage problem, by Dr. Katzin, Dr. Teng, and La Tessa. Frozen dried corneas were used in rabbits. Attempts were made to transplant formalinized grafts. Whole eyes, as well as removed corneas, were stored in conventional moist chambers over solutions with varying concentrations of salt to control the vapor pressure of the atmosphere. Whole eyes were stored after replacement of the aqueous by injected mineral oil, hexadecane, or octadecane. (Hexadecane and octadecane are paraffins which have melting points of 18° and 28° C. and so are fluid at body temperature but solidify rapidly at storage temperature.) Polyvinyl (water soluble) plastic has also been used in 10 percent solution, in the anterior chamber, for whole eye immersion, and for immersion of the cornea alone. The efficiency of storage has been measured by changes in the nitrogen fractions, by histologic examination, by changes in water content, and by transplantation into live rabbits, etc.

Histologically, changes noted after three days in the stroma cells are somewhat less marked in corneas stored in mineral oil than in a moist chamber.

So far as hydration is concerned, less fluid is taken up by corneas stored after replacement of the aqueous by mineral oil, air, or polyvinyl plastic. If the whole eye is immersed in mineral oil or polyvinyl plastic, fluid is taken up to the same degree as when stored in a moist chamber. The best control over hydration has been in cornea suspended over salt solutions of controlled concentration in the vicinity of ½ molar NaCl concentration.

Clinical trials of the various methods of storage lead us to the conclusion that successful grafts may be obtained after prolonged storage with polyvinyl plastic, or any of the methods of controlled hydration of the cornea. Our impression is that this material is not as suitable clinically as is fresh cornea. Since the Eye Bank is constantly operating at a deficit, we have not had the practical problem of storage; there has never been an excess available for that purpose. This is not meant to imply that a suitable method of storage would not be desirable. Some eyes should be kept in reserve for emergencies, especially in areas where Eye Bank eyes are not available.

Dr. Stocker's method of tissue culture of stored eyes is impressive, particularly with regard to the growth of endothelium. Live endothelium in the donor cornea must certainly be an important factor in successful grafting, even if the donor's endothelium regresses after an initial period of growth after transplantation.

DR. JOHN MCLEAN. I believe that we are tremendously indebted both to Dr. Stocker and to Dr. King for their very exciting pioneer work in this field. I agree completely with Dr. King that we must use the utmost conservatism in applying such a radical new method to clinical cases, but if as Dr. Stocker's current studies seem to indicate Dr. King's material is non-viable this may be of very significant basic importance and may be an important clue to clarity and non-clarity of transplants. I am only sorry Dr. King did not see fit to include in his paper a few words about his recent very exciting studies in which he appears to be crossing the species specificity barrier which may be as important in ophthalmology as the sound barrier is in aviation and missile work.

DR. STOCKER. I wish to thank the discussers for their contributions. I have only a few words to add. First, about supposedly successful grafts with non-viable donor material. There has been some talk about Löhlein's (Erfahrungen auf dem Gebiete der Hornhautüberpflanzung, v. Graefe's Arch. für Ophth., 151:1–45, 1950) having reported clear grafts with formalinized corneas. On checking with the original text, I found that what Löhlein really said was that for emergencies formalinized grafts may be used. Some of them will take and some of them will not. Some of them will show a certain amount of translucency, but Löhlein did not report really clear grafts. We have to be careful in the evaluation of such reports. Sometimes the meaning is not accurately transmitted in translation. As to the

method of experimentally evaluating the viability of donor tissue by animal grafts, I agree with Dr. Paton that this is a difficult field. The animals react differently from humans, and the rabbit especially has a tremendous regenerating power. I have seen corneal transplants in rabbits which had become porcelain white after two or three weeks. We left the rabbit alone, and in six months we looked at it again. To my astonishment, we found a clear cornea. Such a thing would hardly occur in humans. Also I would like to mention just briefly another experience to illustrate what a rabbit can do. Some time ago, I was interested in the possibility of dissolving the lens by proteolytic enzymes. I did that by injecting such enzymes into the lens of rabbits, and I could see, to my satisfaction, the melting away of the lens tissue until only a bag of capsule remained. I then left the rabbits alone, but after several months they had a lens again, and I did not know what to think about this unexpected change. Sometime later I happened to run across a publication in which it was mentioned that if a rabbit has a few cells of lens capsule epithelium left it can regenerate the whole lens.