Arch. Soc. amer. oftal. optom. (1959) - II - 173

ENDOTHELIAL STUDIES OF CORNEAS PRESERVED IN VARIOUS MEDIA

BY

H. M. KATZIN, M. D., M. MARTINEZ, M. D.

New York, U. S. A.

The refinement of surgical methods for keratoplasty in conjunction with the better understanding of its physiopathology has made corneal transplantation more popular in recent years, with a resulting increase in the need for fresh donor material. The supply of this material fluctuates and corneal preservation is one method by which we have tried to compensate for the discrepancy between the increasing demand and the inconstant supply.

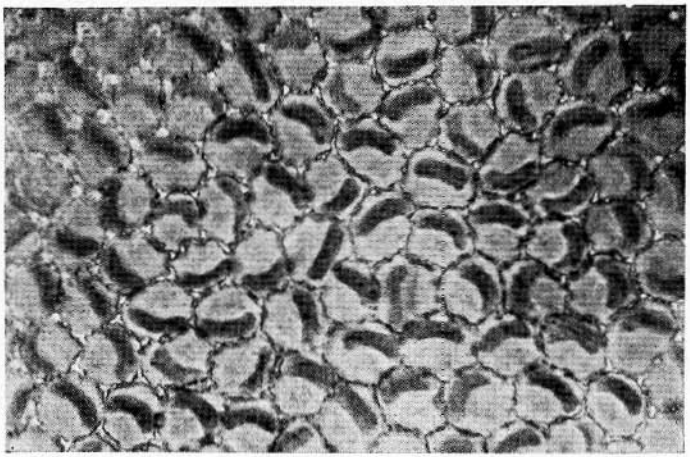

Fig. 1. Normal cat endothelium, alizarin red stain, phase contrast microscope, 320 X.

* From the laboratory of The Eye-Bank for Sight Restoration, Inc., Manhattan Eye, Ear and Throat Hospital, aided by a grant from The Knights Templar Eye Foundation.

Several methods for corneal preservation have been developed, studied and tested: dehydration, preservation in tissue culture media, cold temperatures, mineral oil, water soluble plastics, glycerine, glycerine-dehydration, sugars, homologous serum or whole blood, etc. Stocker and his co-workers [1] and researchers

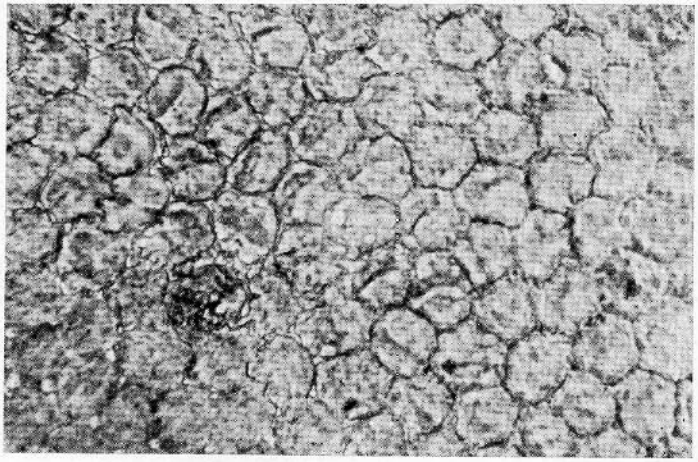

Fig. 2. Typical picture of (*) endothelial damage. Moderate cell shrinkage, mild contraction of the cytoplasm. Alizarin read stain, phase contrast microscope, 320 X.

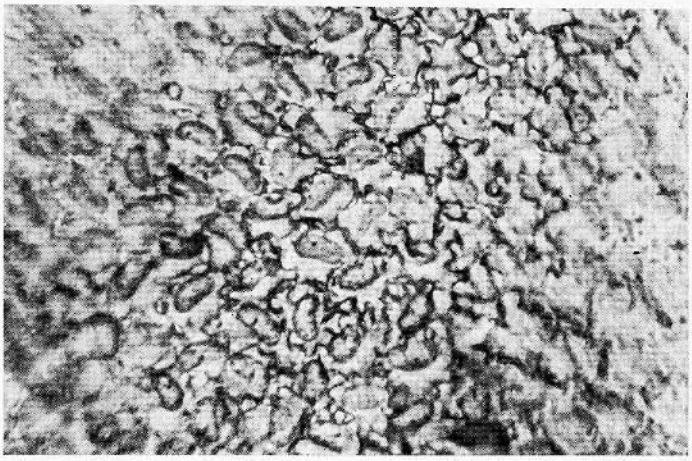

Fig. 3. Typical (**) endothelial changes. Cornea inmersed for one hour in dextrose-saline at room temperature. Marked cell distortion due to dehydration and moderate increase in size of nuclei. Alizarin red stain, phase contrast microscope, 320 X.

at the University of Toronto[2] have tested a number of these techniques of preservation by trying to grow the preserved corneal tissues by tissue culture methods.

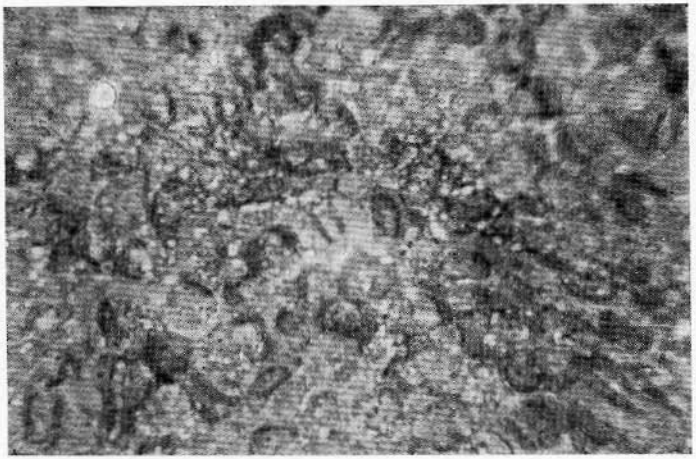

Fig. 4. Typical (***) endothelial changes. Cornea preserved three days in 5% glycerine-saline at 4ºC. Moderate increase in size of nuclei, marked formation of vacuoli, rupture of the membranes in several cells. Alizarin red stain, phase contrast microscope, 320 X.

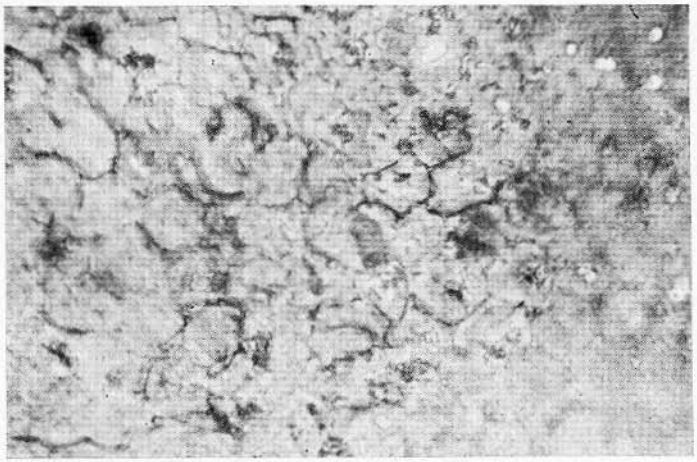

Fig. 5. Typical (****) endothelial changes. Cornea preserved four days in glycerine 5% at -10ºC. Almost complete disorganization of cell structure. Alizarin red stain, phase contrast microscope, 320 X.

Other investigators have evaluated the preservation methods clinically in experimental animals. Whatever means is used to preserve the tissues, it is our impression that corneas preserved for more than several days may be satisfactory for partial thickness grafts only.

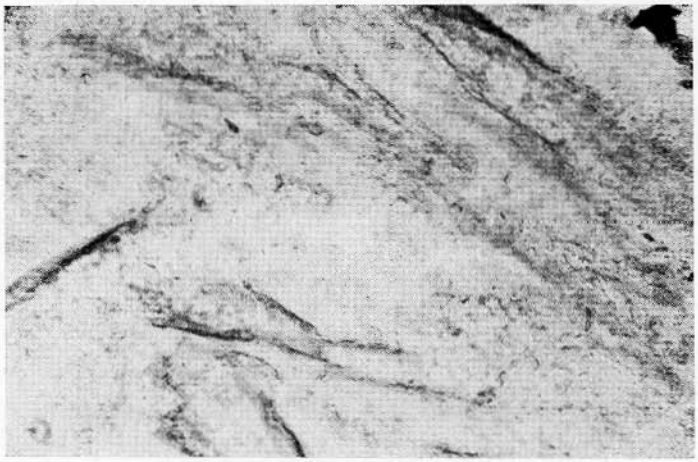

Fig. 6. Typical (*****) changes. Cornea preserved in 15% glycerine thee days at 4°C. Complete cellullar disorganization, endothelial detachment in several places. Alizarin red stain, phase contrast microscope, 80 X.

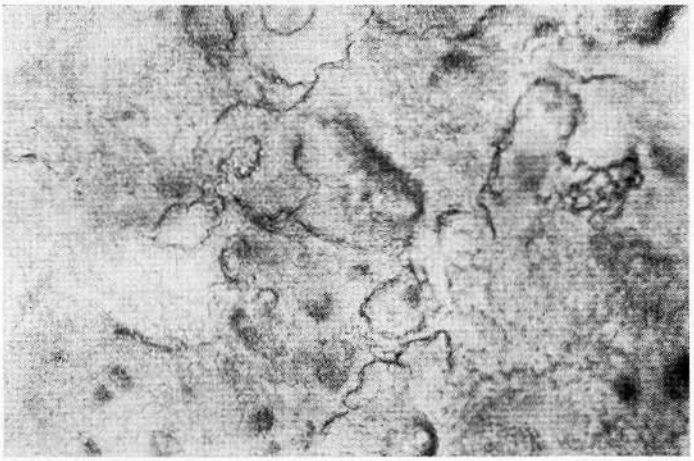

Fig. 7. Endothelial changes after inmmerision in 95% glycerine at —10°C. Complete cell destruction. Alizarin red stain, phase contrast microscope, 80 X.

The success of clinical tests in the case of lamellar transplants has raised the question as to whether full viability of all layers of the cornea is really necessary. Some experience seems to indicate that it is not.

In addition to tissue culture and actual transplantation of preserved corneas, oxygen consumption, vital staining, lipogenesis, and the uptake of isotopes are among the more popular testing techniques, and while none of them give us the information needed to predict accurately the clinical result, they do give us some idea of the comparative chance of success.

The problem of full-thikness transplantation with preserved material, however, remains an important one and is at present unresolved. Most attempts at fullthickness grafts with corneas preserved for a prolonged time resulty, sooner or later, in the opacification of the tissue and failure to restore useful vision. The reason for this failure are complex and not well understood, but the viability and functional integrity of the endothelium and the role of Descemet's membrane apepar to be of primary importance.

We have conducted a study on the morphological changes taking place in the endothelium durig the preservation process.

METHODS

Our endothelial studies have been made on cat corneas obtianed from the American Society for the Prevention of Cruclty to Animals, where the cats are sacrificed by sudden decompression and vacuum in a hermetic chamber. After that the

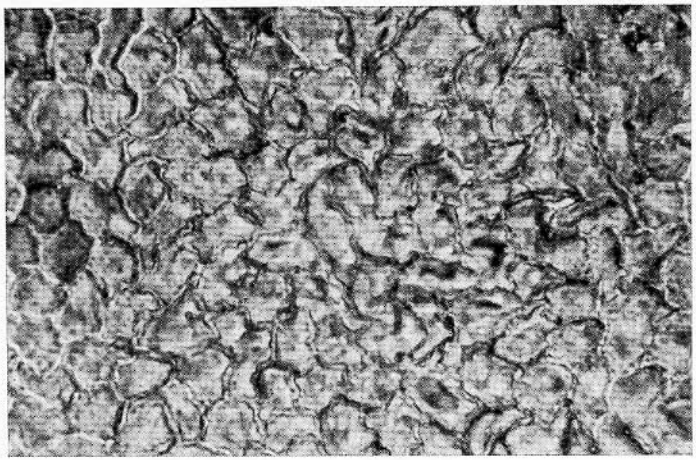

Fig. 8. Endothelial changes after three days preservation in mineral oil at -10ºC. Mild cell distortion. Mild cytoplasmic changes. Alizarin red stain phase contrast microscope, 320 X.

animal is decapitated and the head is brought immediately to our research laboratory. The eyes are nucleated and the whole cornea is removed, cutting 1 mm. behind the limbus with razor blade and curved scissors, taking extreme care to avoid mechanical trauma to the endothelium. Immediately after removal the cornea is placed in the preservative medium or solution. The preserved corneas are removed at varying intervals for study, and rehydrated, when necessary, in normal salin or Hank's solution at pH 7.2.

The preserved corneas are evaluated as to transparency, pliability, rehydration properties and the condition of the endothelium. This report will concerns itself chiefly with studies of the endothelium using flat preparation, vital staining, and phase contrast microscopy. This technique reveals the intercellular cement substance and shows up the structure of the endothelium exceptionally well. Extreme care is taken during the microscopic examination to avoid pressure on the tissue or drying of the preparation since this would induce profound changes in the delicate cells.

Table I shows the degree of change found in the endothelium of corneas examined after various intervals of time.

Corneas were stored in 5, 15, 50 and 95% glycerine at room temperature, 4° C and —10° C, for varying lengths of time. We also used solutions of the same concentrations of dextrose, fructose and sorbitol at the same temperatures.

The results of our experiments indicate that the endothelium suffers profound morphological changes with storage in glycerin-saline in all of the various concentrations, temperatures or time intervals are increased. They range from simple cellular shrinkage due to dehydration to the complete rupture and disorganization of the cellular elements.

Of the various conditions of concentrations and temperatures, the low concentrations of dehydrating substances at room temperature produce the most rapid deterioration of the endothelium. With higher concentrations, up to 50%, the changes are delayed somewhat but they take place nevertheless, and they are not reversible upon rehydration. Similary, if the lower concentrations are used in conjunction with a temperature of 4° C the changes are delayed, but by the tenth day there is complete disorganization of the cellular elements.

At temperatures of —10° C parallel results were noted. Rapid changes occur in low concentrations and slower changes with higher concentrations, but here there is the added factor of trauma produced by ice crystal formation. In short, every possible combination of temperature and dehydration will, by the tenth day, produce irreversible morphological changes in the endothelium.

We believe that neither sub-zero temperatures nor partial dehydration will prevent autolysis and pyknosis. Corneas preserved at low temperatures in concentrations of 15% glycerine invariably show detachment of the endothelium by the thir day. The fact that the cellular elements in these tissues stain deeply with 1% alizarin red, while fresh tissues take the stain lightly or not at all, would seem to

EXTENT OF ENDOTHELIAL CHANGE IN PRESERVED CORNEAS

%	GLYCERINE +20°C	GLYCERINE +4°C	GLYCERINE -10°C	DEXTROSE +20°C	DEXTROSE +4°C	DEXTROSE -10°C	FRUCTOSE +20°C	FRUCTOSE +4°C	FRUCTOSE -10°C	SORBITOL +20°C	SORBITOL +4°C	SORBITOL -10°C
15 MIN.												
5	+		+	+	+	+	+	+	+	+	+	+
15	+		++	+	+	++	++	++	++	+	+	++
50	+++		+++			+++			+++			++++
95	+++		++++			↓			+++			↓
1 HOUR												
5	+			++	+		+++	++	+	++	++	+
15	++			++	++		+++	++	++	+++	++	++
50	+++				+++		++++	+++	+++	++++	+++	+++
95					↓		↓	↓	↓	↓	↓	↓
3 DAYS												
5	+++	+++	+++	+++			++++	+++	++	++++	+++	++
15	+++	++++	++	++++			++++	++++	+++	++++	+++	+++
50	+++++	↓	++++	++++			+++++	↓	↓	↓	↓	↓
95	+++++	↓	↓	↓			↓	↓	↓	↓	↓	↓
10 DAYS												
5			++++	++++	++++	+++	++++	++++	+++	+++++	++++	+++
15			++++	↓	↓	↓	↓	↓	↓	↓	↓	++++
50			+++++	↓	↓	↓	↓	↓	↓	↓	↓	↓
95			↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
15 DAYS												
5			++++									
15			+++++									
50			↓									
95			↓									

\+ MILD CELL SHRINKAGE.

++ CELL SHRINKAGE, CYTOPLASMIC DISTORTION, MILD INCREASE SIZE OF NUCLEI.

+++ MARKED CELL DISTORTION, INCREASE SIZE OF NUCLEI.

++++ CELL MEMBRANE RUPTURE, PYKNOSIS OF CELL ELEMENTS.

+++++ COMPLETE DISORGANIZATION OF CELL STRUCTURE.

indicate that some degenerative change has taken place. We believe that the mobilization, diffusion and adsorption of cellular enzymes play an important part in this problem, causing partial digestion of the corneal structures. This process is, of course, irreversible.

We undertook a study of corneas preserved in various sugars in the belief that sugars might be less toxis than glycerine, but subsequent chemical and histological studies did not bear this out, and experimental transplants in cats were unsuccessful.

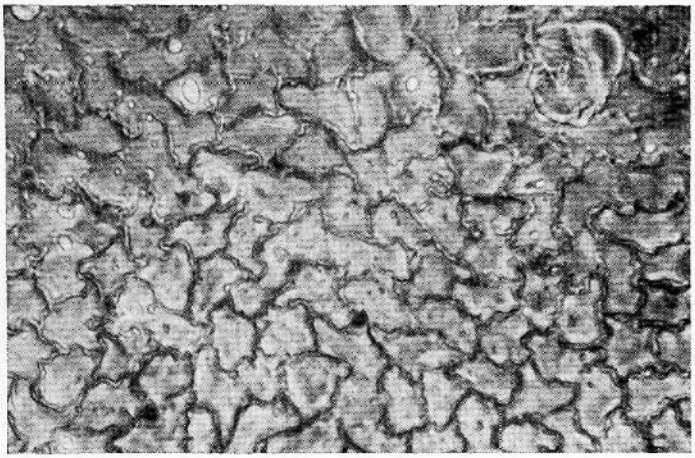

Fig. 9. Endotelial changes after 10 days of preservation in mineral oil at 40° C. Mild distortion of cellular elements. Alizarin red stain, phase contrast microscope, 320 X.

SUMMARY

Studies were made of the endothelium of corneas preserved by various methods for varying lengths of time.

According to our criterion based on the morphological appearance of the endothelium when stained with alizarin red and examined under the phase contrast microscope, all of the corneas preserved for more than three days exhibited degenerative changes such as disorganization of the cellular elements and detachment of the endothelium.

The results of our studies lead us to believe that none of the methods we used satisfactorily arrests the action of autolytic processes that cause irreversible changes in the endothelium.

210 East 64th Street

REFERENCES

1. STOCKER, F. W., EIRING, A., GEORGIADE, R., and GEORGIADE, N., Evaluation of viability of preserved rabbit corneas by tissue culture procedures. Am. J. Ophth. 47: 772, 1959.
2. COCKERAM, A. M., BASU, P. K., and ORMSBY, H. L., The effect of glycerine, dehydration, merthiolate, and zephiran on the viability of cornea. Am .J. Ophth. 47: 308, 1959.