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IN VIVO FREEZING OF THE CORNEA: AN EXPERIMENTAL STUDY

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An important problem in ophthalmology is the maintenance of corneal transparency. Corneal opacities account for more blindness than any other single condition in most of the world. It is estimated that in underdeveloped countries about one-sixth of those that are blind have scarred corneas (1). We still have many gaps in our knowledge concerning factors that govern metabolism, transparency, degeneration and vascularization of corneal tissue.

Many agents, pharmaceuticals and techniques have been used in treating corneal disease with varying degrees of succes. In the last few years cryocautery has been introduced in ophthalmology, after having been used with a measure of success in several other fields of medicine and surgery. To date, cryo-techniques in ophthalmology have been almost exclusively confined to cataract extractions and retinal detachment surgery.

This study was undertaken to determine the effect of freezing, in vivo on the normal rabbit cornea, both from a clinical and a pathological viewpoint and to ascertain whether this technique has any potential therapeutic possibilities.

Methods

Mixed rabbits of 1 to 3.5 kg. were used as subjects. The animals were anesthetized with intravenous sodium pentobarbital and topical ophthaine instilled in the eyes. A freezing lesion of the cornea was produced by holding the tip of the Cooper Linde (2) cryogenic probe (4 mm. in diameter) in contact with the

cornea for a period of 5 to 10 seconds (Fig. 1), until a gross white ice ball was seen (Fig. 2). The animals were examined daily for the first week both with loupe and slit lamp, and at least three times weekly thereafter. Photographs taken at appropriate intervals.

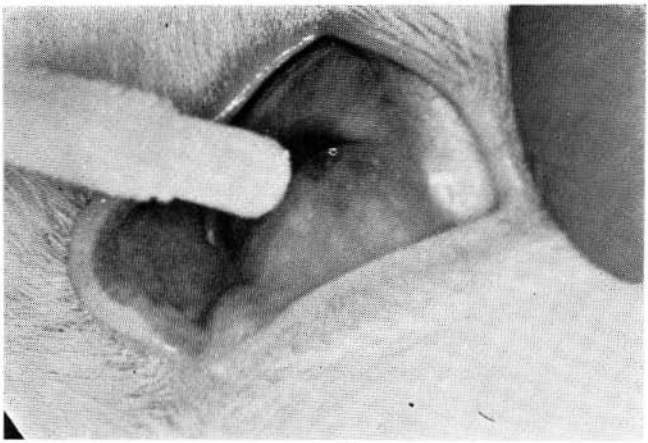

Fig. 1 The freezing probe applied to the center of the rabbit cornea.

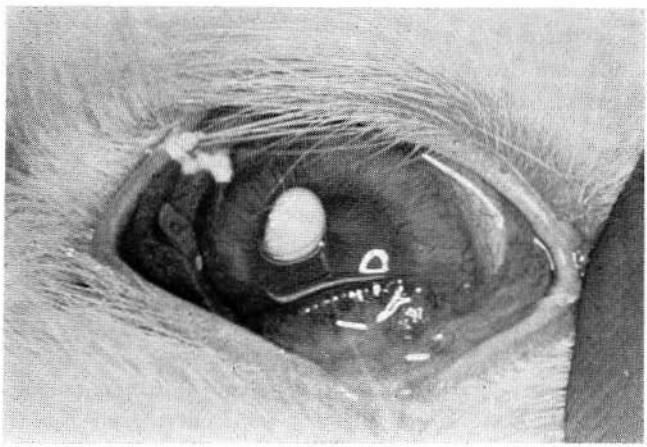

Fig. 2 Ice ball seen immediately after removal of the freezing probe from the cornea.

Animals were sacrificed at intervals varying from 0 minutes to 2 months from the time of production of the lesions. The eyes were removed with great care and were fixed 10% neutral formalin dehydrated and embedded in paraffin in the

usual manner. Semi-serial sections were made through the area of the lesions. The tissues were then stained with hematoxylin and eosin.

Three arbitrary temperatures were selected -192° C., -50° C., and -10° C. Lesions produced in the right cornea were always central and in the left cornea, superior and peripheral.

In the -192° C. group, rabbits were sacrificed at 0 hours, 8 hours, 24 hours, 48 hours, 4 days, 1 week, 2 weeks, 3 weeks, 1 month, and 2 months.

In the -50° C. group, rabbits were sacrificed at 8 hours, 30 hours, 48 hours, 5 days, 1 week, 2 weeks, 1 month, 6 weeks and 2 months.

In the -10° C. group, rabbits were sacrificed at 8 hours, 24 hours, 3 days, 5 days, 1 week, 16 days, 1 month, and 2 months.

The experiments were terminated two months after the production of the lesions.

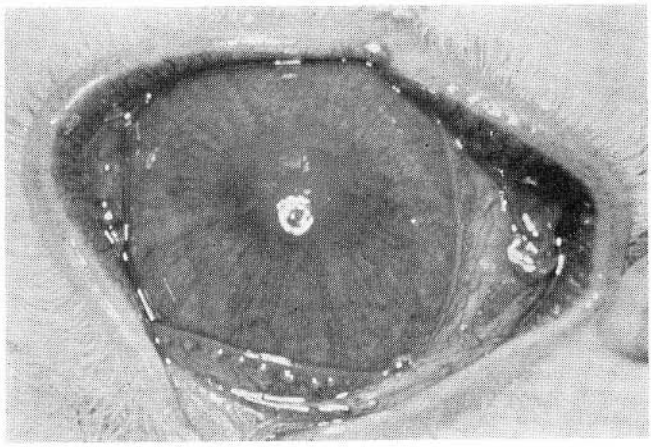

Fig. 3 Central lesion seen 6 hours post-injury at -192° C. Note the zone of central cloudiness and the increased vascularity of the iris.

Results

A latent period of approximately 2 hours existed between the time the cornea was touched with the freezing probe and the appearance of corneal cloudiness due to edema which was localized to the corneal stroma (Fig. 3). In the interim, the cornea remained perfectly clear. The epithelium, denuded after initial contact with the probe covered over the site of the lesion (i. e., the lesion did not stain with fluorescein) in all instances after 24 to 36 hours. The endothelium regenerated between 24 and 48 hours post-injury in the -10° C. series and -50°

C. series and between 48 and 96 hours in the -192º C. series. In all instances the epithelium and endothelium recovered more rapidly than did the stromal cells.

Peripheral lesions healed slightly faster and exhibited a greater inflammatory response than central lesions. With some of the peripheral lesions, vascularization ocurred into the peripheral cornea at the site of the lesion, but for distances not exceeding 1 or 2 mms. This did not interfere with the clarity or deturges-

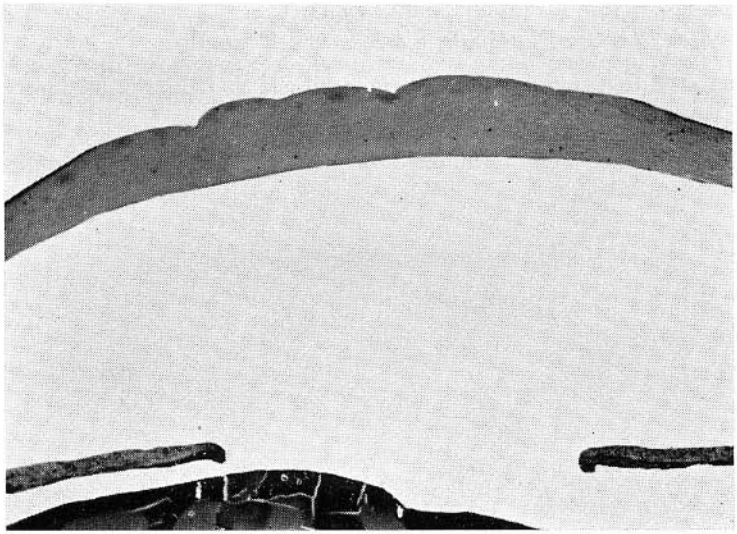

Fig. 4 Central lesion - 8 hours post-injury at -192º C. There is an absence of epithelium and endothelium with a decreased number of stromal cells. There is an increased curvature of the anterior surface with moderate stromal edema. The epithelial cells are starting to spread out over the denuded area (X 25).

cence mechanisms of the cornea. In pigmented eyes with peripheral corneal lesions, a localized depigmented sector of the anterior surface of the iris in proximity with the corneal lesion was noted 4 to 5 days after the lesion was produced and at all three temperatures (Figs. 10 and 11).

All cells in a full thickness section of the cornea (epithelium, stroma, and endothelium) could be destroyed by freezing (Figs 4 and 5). The stromal cells died by nuclear fragmentation. Central corneal lesions were repaired primarily by undamaged stromal cells (Fig. 6), peripheral corneal lesions were repaired by undamaged stromal cells and wandering macrophages. In the -192º C. series, reparative activity was strongest between 4 and 7 days, with complete healing in

all instances after 2 weews. In the -50° C., series, reparative activity was strongest between 2 and 7 days, with complete healing mostly after 1 week. In the -10° C. series, reparative activity was strongest between 1 and 5 days, with complete healing in all instances after 1 week. Most corneas regained their normal clinical and histologic appearance (Fig. 7).

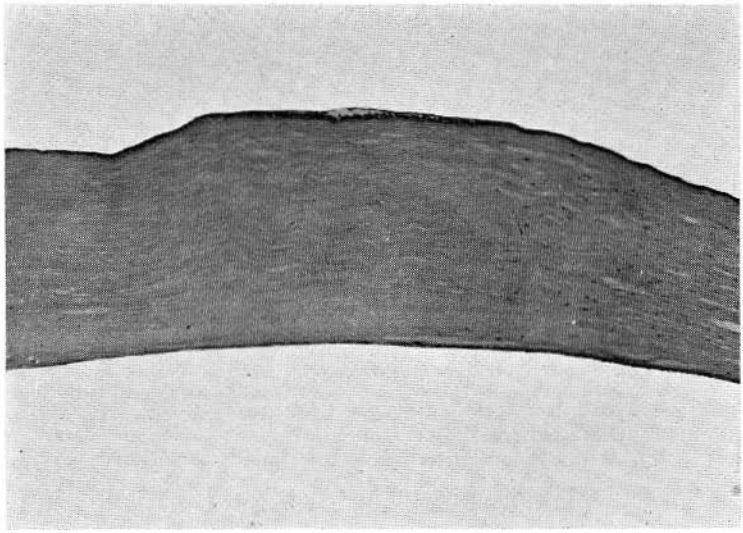

Fig. 5 Central lesion - 24 hours post-injury at -192° C. The epithelium is one cell layer in thickness. The stroma is totally acellular and markedly edematous. The endothelium is still absent. Descemet's mebrane shows patchy staining (X40).

Infection was not found with any of the freezing lesions.

In a preliminary study, one eye of each rabbit was used as a control. None of these control eyes showed any demonstrable change as the result of the freezing of the opposite eye.

Discussion

Several earlier investigators (3, 4, 5), who did not use temperatures lower than -78° C. produced freezing lesions on normal healthy rabbit corneas without leaving any residual corneal opacity or significant change in any other portion of the internal or external eye. This study is the first to use such low temperatures (-192° C.) and to note similarities and differences between the central and peripheral corneal lesions at the same and at different temperatures.

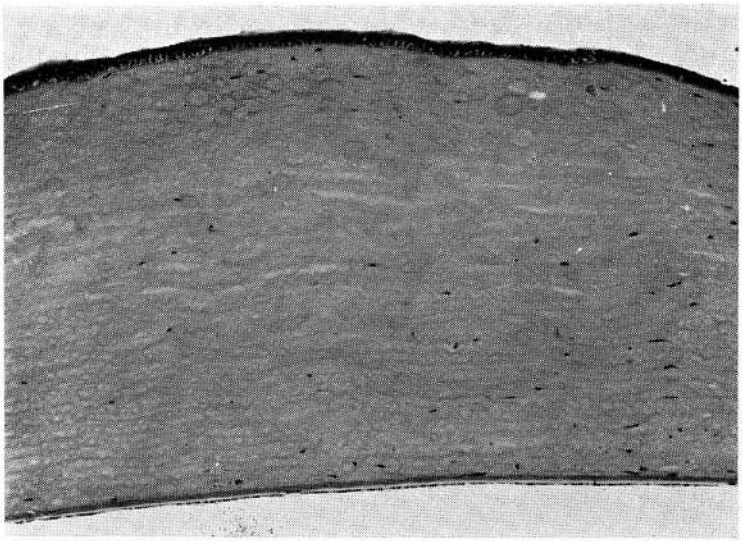

Fig. 6 Central lesion - 4 days post-injury at -192º C. The epithelium is 3 to 4 cell layers in thickness. Some stromal cells from the normal peripheral cornea have migrated into the injured central area. The endothelium appears normal. There is moderate edema of the stroma but no distortion of the lamellar architecture (X75).

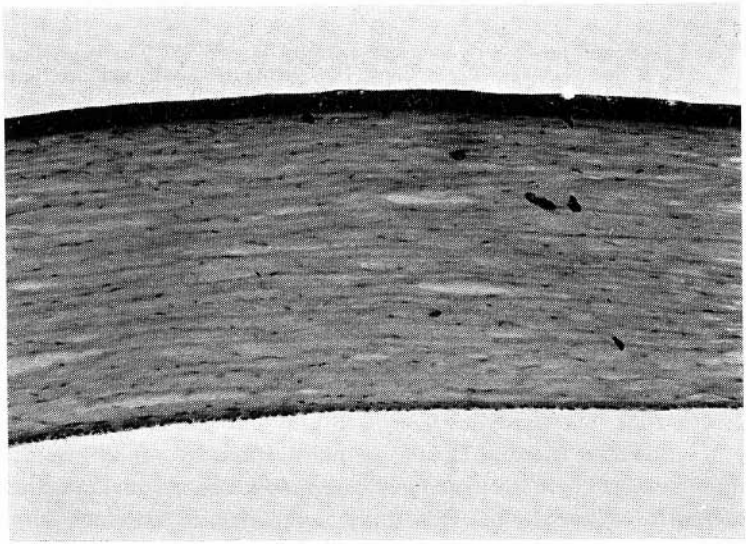

Fig. 7 Central lesion - 2 weeks post-injury at -192º C. The cornea is completely normal. Some artefacts present. (X100).

In the -192ºC., series, reparative activity was strongest between 4 and 7 days, with complete healing mostly by 1 week (with 3 exceptions - all occurring in central lesions). One eye at 1 week showed posterior stromal cloudiness or slit lamp examination, and on histologic examination this cloudiness was interpreted as due either to endothelial reduplication or organization of inflammatory exudate on the posterior surface of the cornea. (Fig. 8). Another eye showed midstromal swelling at 1 week and was perfectly normal at 2 weeks. Another eye showed midstromal edema at the end of 6 weeks. We do not have a satisfactory explanation as to why these changes occurred. In the -10º C series, reparative activity was strongest between 1 and 5 days, with completely normal corneas by 7 days.

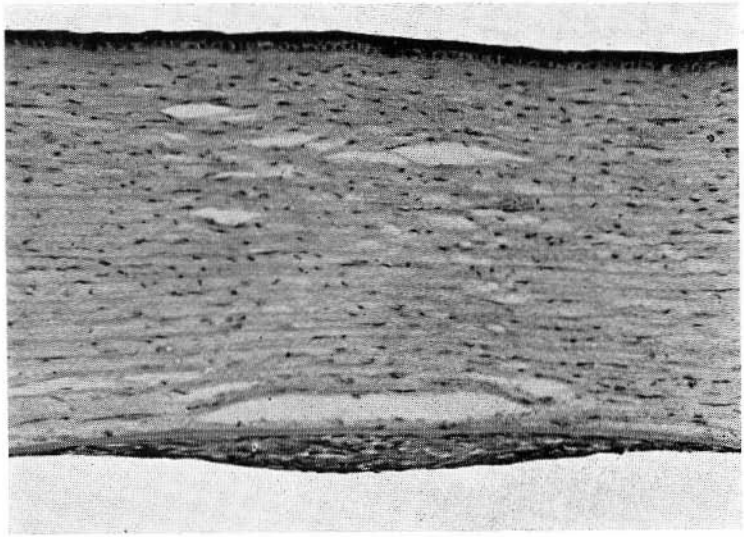

Fig. 8 Central lesion - 1 week post-injury at -50º C. Note the posterior corneal surface. This may be organized inflammatory exudate or endothelial hyperplasia. (X100).

In all instances regardless of temperature or the site of the lesion, epithelium covered over the site of the lesion after 24 to 36 hours. The endothelium regenerated between 24 and 48 hours post-injury in the -10 C. series and -50º C. series and between 48 and 96 hours in the -192º C. series. Healing in all the cellular layers generally was a little faster with peripheral than with the central lesions. This factor was a function of proximity to the perilimbal vessels; the closer the lesion was to the blood supply, the quicker the healing; the farther from the blood supply, the longer the healing.

With the central lesions, healing took place withthout any vascularization. Two weeks after the lesions were produced (with one exception - midstromal

edema at 6 weeks), all the corneas were clear and glistening and the rest of the eye was normal and unchanged. For the first 48 to 72 hours there were minimal inflammatory changes in the anterior and posterior chamber as evidenced by the slit lamp changes and the microscopic changes in the iris, ciliary body and ciliary processes. The central corneal lesions were most distant from a blood supply and this could explain the minimal inflammatory changes despite the severity of the corneal insult.

With the peripheral lesions, healing was a little faster than with the corresponding central lesions. In all instances the peripheral lesions healed, leaving a clear cornea. In few eyes, minimal vascularization occurred into the peripheral

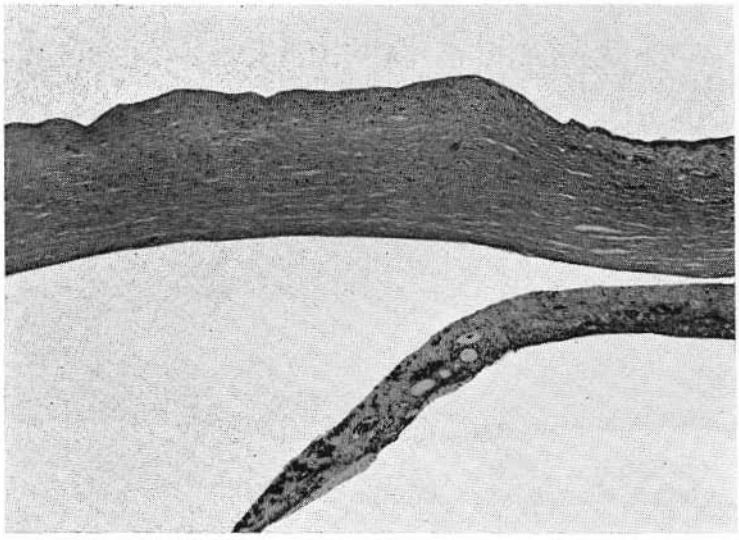

Fig. 9 Peripheral lesion - 8 hours post-injury at -192º C. There is a heavy cellular infiltrate (polys) in the injured stroma. The epithelium and endothelium are absent. Note the vascular changes in the iris. (X35).

cornea at the site of the lesion, but for distances, not exceeding 1 or 2 mms. from the limbal margin. This minimal degree of vascularization did not interfere with the clarity or deturgescence of the cornea. There was a greater inflammatory response with the peripheral (Fig. 9) than with the central lesions. This was evidenced by increased perilimbal vascular and cellular responses, considerable anterior chamber exudate, segmental iris hyperemia and subepithelial edema of the ciliary processes - most marked in an area closest to the corneal lesion, but still diffuse throughout. An interesting finding with pigmented eyes was

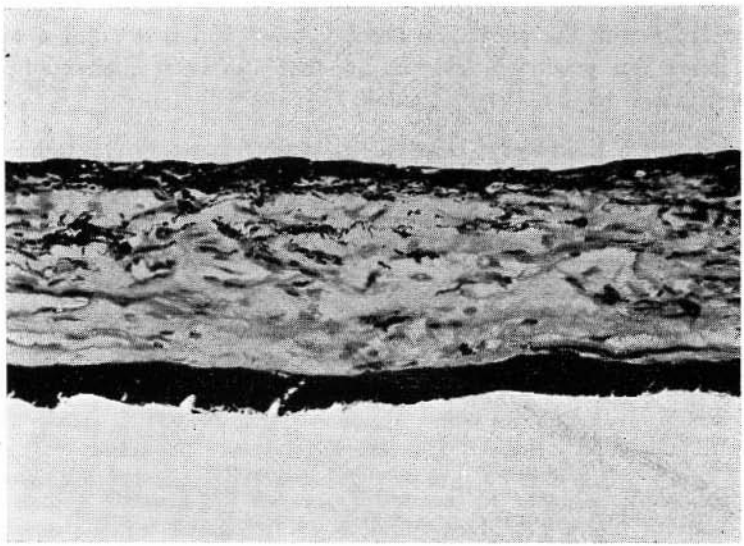

Fig. 10 Normal iris of pigmented rabbit. (X200).

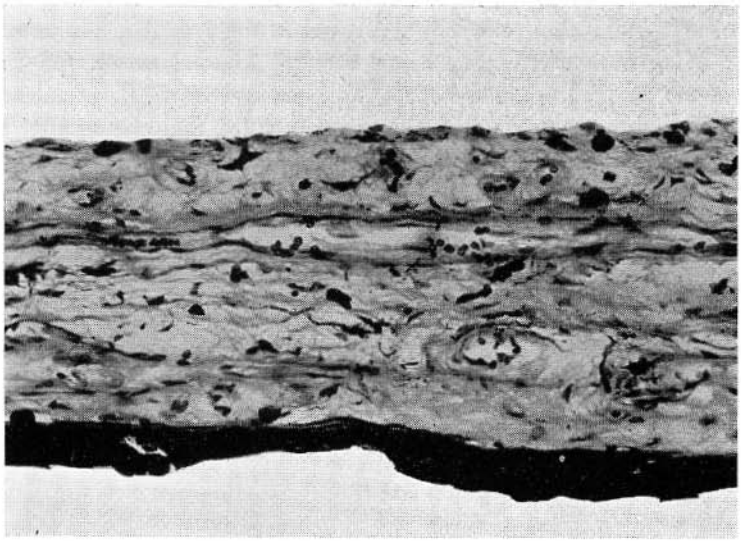

Fig. 11 Note depigmentation of anterior surface of iris closest to peripheral corneal lesions. This was noted 3 to 5 days after injury (X200).

the presence of a localized depigmented sector of the anterior surface of the iris in proximity with the peripheral corneal lesion and first noted 4 to 5 days after the lesion was produced. This finding has also been observed by several other investigators (6, 7) (Figs. 10 and 11).

Although there has been a recent upsurge of interest in cryotechniques because of the availability of more refined freezing instruments, it is to early to predict the practical application of in vivo freezing of the cornea.

Summary

Central and peripheral freezing corneal lesions were produced using a Cooper Linde (2) cryogenic probe 4 mm. in diameter. Three groups of rabbits were used, producing lesions at 3 different temperatures –192ºC., - 50ºC., - 10ºC. The experiments were concluded 2 months after the lesions were produced.

Application of the probe to the rabbit's cornea for 5 to 10 seconds resulted in a localized ice ball which disappeared 5 to 10 seconds after the probe was removed. The cornea, initially clear, gradually became cloudy 1½ to 2 hours later, as the stroma became edematous. When the edema subsided, the cornea resumed its normal clarity.

On histologic examination, all of the cells of the cornea, including the epithelial, stromal and endothelial cells were destroyed in the area that had been frozen; the degree of cellular destruction being directy proportional to the degree of reduction of the temperature. The corneal lamellae and intercellular substance did not appear to be permanently damaged by this procedure. Eight hours after freezing, the corneas at all temperatures were edematous, and by 24 hours remnants of the stromal cells had disappeared in the area of the lesion. At -192º C. the stroma was completely acellular, at -50º C. an occasional scattered stromal cell was seen, and at -10º C., some stromal cells were seen in the posterior 1/3 of the stroma. Healing varied with the temperature at which the lesion was produced. At -192º C., reparative activity was strongest between 1 and 5 days with complete healing after 1 week. Central lesions were repaired by wandering macrophages and undamaged stromal cells.

REFERENCES

1—BISLEY, G. G.: Quoted by Trevor-Roper, P. D.: Editorial: Deep Frozen Full-Thickness Corneal Grafts. A. M. A. Archives of Ophthalmology. 72: 741, 1964.

2—COOPER, I. S., GRISSMAN, F., and JOHNSTON, R.: A. Complete System for Cryogenic Surgery. St. Barnabas Hospital Medical Bulletin. Volume I, Nº 3, Spring, 1962.

3—DUNNINGTON, J. H. and SMELSER, G. K.: Incorporation of S^{35} in Healing Wounds in Normal and Devitalized Corneas. A. M. A. Archives of Ophthalmology. 60: 116, 1958.

4—MAUMENEE, A. E. and KORNBLUETH, W.: Regeneration of the Corneal Stromal Cells. I. Technique for Destruction of Corneal Corpuscles by Application of Solidified (Frozen) Carbon Dioxide. American Journal of Ophthalmology. 31: 699. 1948.

5—MAUMENEE, A. E. and KORNBLUETH, W.: Regeneration of the Corneal Stromal Cells. II. Review of Literature and Histologic Study. American Journal of Ophthalmology. 32: 1051, 1949.

6—SUDARSKY, R. S., HULQUIST. R. and CHI, H. H.: Cryogenically Induced Lesions. American Journal of Ophthalmology 60: 217, 1965.

7—TOST, M., FÜHRMEISTER, H., and SCHULZE, F.: Experimenteller Beitrag Zur Kälterinwirkung am Auge. Klin. Mbl. Augenheilk. 146: 1, 1965.

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