Effect of freezing on the ciliary body (cyclocryotherapy)

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Freezing of several areas of the ciliary body of normal rabbit eyes was done with a cryocautery. Tonographic tracings were performed before and from 6 to 8 weeks after freezing. It was observed that the average intraocular pressure of these rabbit eyes had decreased from 18 to 14 mm. Hg, the coefficient of outflow facility (C) was reduced from 0.24 to 0.17, and aqueous flow (F) was decreased from 2.26 to 1.06. Histologic observations showed an immediate postfreezing stage of edema, exudates, and hemorrhages, with destruction of cellular elements. The histologic structure of the frozen area regenerated rapidly, acquiring an almost normal appearance one week after freezing. Hyalinization of connective tissue around vessels and in the subepithelial area was observed in sections of eyes 5 to 10 weeks after freezing. It seemed apparent that these changes might be partially responsible for the decreased secretion of aqueous.

Freezing of the ciliary body as an anti-glaucomatous treatment was first described by Bietti in 1950. He reported tonometric and histologic studies on rabbits and also tonometric results in several types of glaucoma in humans. Further reports on this method have not appeared, although it is still used by Bietti in some cases of glaucoma.

The purpose of this investigation was to obtain tonographic information concerning the changes in aqueous production and outflow facility in rabbit eyes following local freezing of the ciliary body (cyclocryotherapy), and to study the histologic changes, especially in the early regeneration period.

Clinical observations have been made by us, and will be reported at a later date.

Methods

Tonography. Adult albino rabbits (2.5 to 3 kilograms) were anesthetized with intravenous sodium pentothal (30 mg. per kilogram) and locally with one or two drops of Ophthaine.* Tonographic tracings were obtained with a Müller electronic tonometer, and recorder in 30 eyes. The 1955 tables of Friedenwald were used to interpret the tonographic results. To obtain a measure of aqueous production, the formula \( F = (P_0 - P_v) \times C \) was used, where \( F \) = rate of aqueous flow (\( \mu l \) per minute), \( P_0 \) = intraocular pressure (mm. Hg), \( C \) = outflow facility (\( \mu l \) per minute per mm. Hg), and \( P_v \) = episcleral venous pressure.

*0.5 per cent proparacaine HCl, E. R. Squibb & Sons.
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(mM Hg). \( P_0 \) was assumed to be 9 throughout these experiments. It is generally recognized that calculating aqueous production from tonographic data is inaccurate, and one cannot obtain reliable data by this method. However, since we were interested only in relative changes in aqueous formation before and after freezing, this method of measuring aqueous humor production seemed adequate for our purposes. Tonography was done in each eye before freezing and then at weekly intervals thereafter.

Freezing of the ciliary body was carried out with a copper cup filled with dry ice and alcohol (-79° C.) with a conical tip 4 mm. in diameter. The cryocautery was applied over the conjunctiva for one minute, 1 mm. from the limbus (Fig. 1). Four, 6, or 8 applications were made. The frozen surface was 5 to 5.5 mm. in diameter, and inside the eye a ball of ice formed gradually, which reached as far as the peripheral vitreous. Since the ciliary processes in the rabbit extend to the posterior surface of the iris, a complete freezing of these structures was probably not always obtained. The eyes were examined daily with penlight and sometimes with slit lamp during the first week after freezing. The tonograms obtained 6 to 8 weeks after freezing were used to evaluate the effects of cryotherapy.

Histology. The eyes of 5 albino and 6 pigmented rabbits (2.5 to 3 kilograms) were frozen in four areas as described above. At periods ranging from 4 hours to 7 days, the animals were killed with intravenous Nembutal. The eyes were removed and fixed in 10 per cent formalin. In addition, the rabbits studied tonographically were killed, and the eyes removed for histologic study at various time intervals ranging from 4 to 10 weeks. Four control eyes were also studied. After fixation, the treated area of the ciliary body was examined macroscopically while being removed from the globe. Tissues were processed in paraffin. Sections were stained with hematoxylin and eosin, Masson’s trichrome stain, and van Giesson’s stain.

Labeling regenerating cells with tritiated thymidine, which is incorporated in the premitotic stage of DNA synthesis, is of interest because one can localize these cells in preparation for mitosis, and obtain a view of their regenerative activity at the time of labeling. For this purpose, in 6 eyes in which cryotherapy had been applied in four areas, 2 \( \mu \)c of tritiated thymidine,* dissolved in 0.10 c.c. of Eagle’s basal media, was injected in the vitreous cavity adjacent to the frozen area 24 hours after freezing. These eyes were removed 24 hours later (48 hours after freezing), fixed in 8 per cent formalin, embedded in paraffin, and cut at 5 \( \mu \). Radioautographs were made with Kodak AR-10 stripping film, and were exposed for 7 days. The slides were developed in Kodak developer D-19, and stained with Harris’ hematoxylin after fixation.

Results

Tonography. After thawing, the treated area showed a moderate amount of hyperemia and occasionally a small conjunctival hemorrhage. Twenty-four hours after freezing there was marked ciliary congestion and dilatation of iris vessels. In one

Table I

<table>
<thead>
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<th>Before freezing</th>
<th>6 to 8 weeks after freezing</th>
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<tr>
<td>( P_0 )</td>
<td>( C )</td>
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<tr>
<td>15</td>
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<td>18</td>
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<tr>
<td>19</td>
<td>0.26</td>
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<tr>
<td>19</td>
<td>0.23</td>
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Average 18.25 0.24 2.26 14.2 0.17 1.06

\( P_0 \) = intraocular pressure.
\( C \) = outflow facility (\( \mu l/\min./\text{mm. Hg} \)).
\( F \) = rate of aqueous flow (\( \mu l/\min. \)).

case, blood was present in the anterior chamber, and for the first 3 or 4 days after freezing all eyes showed a discrete aqueous flare. One week after freezing, the external appearance of all the eyes was normal, and the anterior chambers were clear. Table I represents the tonographic observations in 12 eyes before freezing and 6 to 8 weeks after freezing. Other tonograms were not included because they

Fig. 2. Four hours after freezing, the ciliary processes show edema, some hemorrhages, and loss of the nonpigmented epithelium. In some areas the ciliary epithelium has not been destroyed (arrow). Edema is also present under the pigmented layer of the iris. Fibrinous exudate occupies the posterior chamber. (Masson's trichrome. ×72.)

Fig. 3. Twenty-four hours after freezing, hemorrhages predominate in the stroma of the ciliary processes. Edema has formed cystic deposits between the epithelial layers. The nonpigmented epithelium has been destroyed, and the pigmented epithelium persist for the most part. (Hematoxylin and eosin. ×72.)
were technically inadequate. The table shows that before freezing the average Po was 18, C was 0.24, and F was calculated to be 2.26. Six to 8 weeks after freezing, the tonograms indicated an average Po of 14, a C of 0.17, and a calculated F of 1.06.

**Histology.** Globes of albino rabbits which had been fixed in formalin 4 hours, 1 and 2 days after freezing, on sectioning revealed areas of congestion and hemorrhages in the ciliary body. One week after freezing, these hemorrhages were not seen macroscopically.

Microscopic examination of tissue sections 4 hours after freezing showed swelling of the ciliary processes caused by edema and hemorrhages in the stroma (Fig. 2). Rupture of small capillaries and damage of the endothelium of larger vessels accounted for these alterations. The nonpigmented epithelium was absent over most of the ciliary processes, although in some areas clusters of these cells remained in place. The pigmented epithelium was also destroyed in many ciliary processes; however, in eyes of chinchilla rabbits, where the pigmented epithelium could be better observed, flat pigmented cells remained, lining the injured ciliary body in most places. Vascular congestion and edema were present in the iris stroma, and frequently the pigmented epithelium of the iris was partially detached due to edema (Fig. 2). Coagulated fibrin was present in the anterior and posterior chambers, and irregularly stained strands of vitreous were present around the ciliary body suggesting that this structure was altered by freezing. In the treated area, the sclera showed a pink staining instead of the usual bluish color obtained with the trichrome stain. Subconjunctival edema was present at this level.

Twenty-four hours after freezing, the edema of the injured area had subsided. The appearance of the ciliary processes was otherwise similar to that described. In some sections, hemorrhages predominated (Fig. 3), the basal layer of the ciliary epithelium was ruptured, and red cells were present in the posterior chamber (Fig. 4). Mounds or excrescences of pigmented cells were observed in several

![Fig. 4. Part of a ciliary process 24 hours after freezing, showing stromal hemorrhage and rupture of the basement layer of the ciliary epithelium. (Hematoxylin and eosin, ×144.)](image)

![Fig. 5. Radioautograph of a section of the ciliary body (CB) of an albino rabbit labeled with tritiated thymidine 24 hours after freezing. There is heavy uptake of the isotope by regenerating cells of the ciliary epithelium (E). Arrow points a mitotic figure in the pigmented epithelium. Labeled connective tissue cells are present in the stroma. (Hematoxylin, ×72.)](image)
areas as well as free pigment granules in the surrounding fibrinous exudate. Discrete leukocytic reaction composed of polynuclears and lymphocytes was present in the ciliary body and also in the angle of the anterior chamber. Except in 2 eyes in which no labeling was observed, all radioautographs of the ciliary body labeled 24 hours after freezing and removed 48 hours after freezing showed that cells of both the pigmented and nonpigmented epithelium were heavily labeled, indicating that regeneration of the epithelium was most active at this stage. Figures indicating mitosis were present in the layer of the pigmented epithelium (Fig. 5), as well as clumps of labeled cells which seemed to correspond to pigmented cells. The cells of the pigmented epithelium of the peripheral retina were also labeled in a case where the freezing injury was more posterior (Fig. 6), demonstrating that these cells reacted in a similar way after freezing.

Two to three days after freezing, the edema of the injured area was minimal. A considerable epithelization of the ciliary body was present, and, at one week after freezing, the ciliary processes showed a complete regeneration of the epithelium; except in a few instances where the layer of nonpigmented cells was still absent, the ciliary processes being covered by flat pigmented cells. The appearance of the filtration angle was normal, as well as the staining characteristics of the sclera.

Five to 10 weeks after freezing, the microscopic appearance of the ciliary body and processes in the treated area were indistinguishable from the normal when studied with hematoxylin and eosin, except for a layer of homogeneous hyaline tissue around the blood vessels and in the subepithelial layers (Fig. 7), which was better observed in sections stained with Masson's trichrome stain. Occasionally, areas of hyperplasia of the pigmented epithelium were observed. It also appeared that there was an increase in the amount of connective tissue in the ciliary body when compared to sections of normal eyes (Fig. 8). This thickening of the stroma of the ciliary body was found in eyes 5 weeks or more after freezing.
Fig. 8. Ten weeks after freezing, in most cases there is an increase in the amount of connective tissue of the ciliary body and areas of hyalinization, as shown in the figure. (Hematoxylin and eosin. x72.)

Comments

These experiments indicate that freezing of the ciliary body decreases the intraocular tension in normal rabbit eyes. Tonographic studies confirm the supposition of Bietti that the hypotony was caused by a decrease in the production of aqueous humor. Our studies, although not extensive, indicate that concomitant with the decrease in aqueous flow there is a compensatory decrease in outflow facility, perhaps similar to that observed by Becker and Constant in Diamox-treated eyes. Hemorrhage and edema are the initial lesions in the ciliary body. These alterations are caused by a destruction of the endothelium of capillaries and arterioles and rupture of the wall of small vessels. Destruction of both epithelial layers also occurs, but there is evidence that the pigmented layer persists in several injured ciliary processes. In preliminary experiments we have observed that the intraocular pressure rises somewhat (5 to 10 mm. Hg) shortly after freezing, and remains elevated for 1 or 2 days before hypotony begins. This elevation of the ocular tension is probably caused by the hemorrhages and edema in the treated areas.

The epithelial layers regenerate rapidly, as shown by a high uptake of tritiated thymidine 24 hours after freezing. This regeneration seems more active at the level of the pigmented epithelium, which, as has been noted before, can persist in frozen areas and may show areas of hyperplasia. Cryotherapy did not produce permanent changes in the sclera. This observation is supported by studies proving that freezing of the cornea did not change its collagen structure or transparency.4 Possibly, fibrosis of the connective tissue of the ciliary body results from the hemorrhages caused by freezing and by the stimulus for repair, as may occur in other tissues and as occurs in stomachs frozen in vivo in which hemorrhages and edema were the initial lesions. The changes described in the stroma of the ciliary body might be partially responsible in decreasing the secretion of aqueous humor. Further studies are planned to elucidate this point.
REFERENCES