Transscleral freezing of the retina: An experimental study

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Transscleral freezing of the dog’s retina for 2 seconds was done experimentally and demonstrated ophthalmoscopically and histologically. The resulting lesion was studied from the instant of freezing to the stage of final healing. Attempts were made to alter the lesion by methods known to affect the freezing of live tissue. The Cooper-Linde cryoprobe was used to produce the lesions. Retinal freezing occurred when the temperature of the probe tip was between -30 and -40° C. Adhesive chorioretinitis involving sclera was observed, first evident in 4 days, and healed in 7 days. The lesion was resistant to retinal separation and without hemorrhage. The conjunctiva appeared unaffected. Retinal destruction increased with increased duration of freezing up to 20 seconds, with little further damage thereafter. Decreasing the temperature of the probe tip to -60° C, for 2 seconds caused a marked increase in retinal destruction, but little change with longer durations at that temperature. Refreezing increased retinal destruction slightly and choroidal reaction markedly. Rapid thawing and subconjunctival injection of glycerol had no effect.

Recent development of a cryoprobe that allows accurate control of tissue freezing in the live organism has given ophthalmic surgeons an entirely new and promising therapeutic tool. It has theoretical application in almost all ophthalmic surgical procedures. Not the least of these is the production of an adhesive chorioretinitis for treatment of retinal detachments.

The purpose of this paper is to present the ophthalmoscopic and histologic features of the lesion produced by transscleral freezing of the retina, and to report the results of attempts to change the lesion by methods known to affect the freezing of living tissue.

Methods and materials

Dogs weighing 10 to 20 kilograms were used. Low temperatures were obtained with the Cooper-Linde cryogenic probe. This instrument consists of a probe made of two concentric tubes; the inner one brings liquid nitrogen to the tip, the other carries liberated gas away. The tubes are insulated with a jacket containing a vacuum. A thermocouple in the tip permits a constant record of the temperature to be obtained. The flow of liquid nitrogen is controlled by a dial, allowing the operator to adjust the temperature of the tip, measuring 2 by 2 mm., to any desired level down to -196° C. within 5 to 7 seconds, as well as to stop the freezing instantaneously.

To obtain a fundus photograph of the chorioretinal ice crystallization and subsequent lesion, a surgical approach was used that allowed the placing of the cryoprobe tip on the posterior part of the globe. After shaving and preparation of the skin in the usual manner, a longitudinal incision

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Fig. 1. A trocar is placed retrobulbarly through the orbital ligament. A cryoprobe is inserted into the cannula.

was made superotemporally beginning one fingerbreadth behind the orbital rim.

The orbital ligament that runs from the supraorbital process to the zygomatic arch was exposed (in the dog the bony wall of the orbit is absent in this area). This thick, tough tissue was incised posterior to the position of the globe. A hemostat introduced into the incision was then spread. A trocar, 4 mm., in diameter, was placed in the incision and subsequently pushed retrobulbarly. It was then directed toward the posterior part of the globe. After the operator was satisfied that the trocar tip was resting against the globe near the disc, the obturator was withdrawn and the cryoprobe placed in the cannula (Fig. 1). The fundus camera was aligned with the indentation made by the probe, the temperature decreased, and pictures of the ice crystallization made (temperature held for 2 seconds at -40° C.). The globe and cannula were withdrawn and fascia and skin were sutured with 2-0 catgut and 3-0 silk, respectively. This dog was anesthetized every day and pictures of the lesions taken up to 2 weeks and again 1 month after the lesions had been made.

Lesions for histologic study were produced in the medial and lateral peripheral parts of the retina.

The operator, equipped with an indirect ophthalmoscope, was positioned to one side of the animal. Traction was then applied to four traction sutures previously placed at the limbus so that maximal prolapse of the eye was obtained with the least amount of force. The cryoprobe tip was placed on the globe on the side opposite the operator, posterior to the traction suture, and below the inferior margin of the rectus muscle. The globe was indented until the ora serrata came into view. This was easily accomplished by controlling the position of the eye with the traction sutures. The probe tip was then moved posteriorly until a good, clear view of the peripheral retina was obtained. An assistant turned the dial on the apparatus to decrease the temperature of the probe tip at the fastest rate possible. When the operator saw ice crystals, which appeared suddenly as an easily identified, bright white spot, form in the retina and choroid, he signaled the assistant to stop decreasing the temperature. The ice crystallization was held for 2 seconds, whereupon the apparatus was turned off.

Eyes were enucleated 1 hour and in 1, 2, 4, 7, 10, 14, and 30 days after lesions were made. They were preserved 24 hours in Formalin and then cut anteroposteriorly through the lesions. Specimens were embedded in paraffin and sectioned with a microtome. Sections were mounted on slides and stained. All lesions had representative sections stained with Harris' hematoxylin and eosin and with Lawson's elastic tissue stain and Van Gieson's connective tissue stain.

The conjunctiva was observed grossly but not preserved for histologic study.

An attempt was made to change the retinal lesion in five ways:

1. By varying the duration of freezing. This consisted of making a series of lesions as previously described in which the duration of freezing was 0, 2, 5, 10, 20, 30, 45, and 60 seconds, respectively.

2. By dropping the temperature of the probe tip to much below that needed simply to freeze the retina. This consisted of producing a series of lesions exactly as just mentioned but with the probe tip at -60° C.

3. By refreezing. This consisted of producing a similar series as follows: After the retina had been frozen for the desired length of time, the freezing apparatus was turned off and the probe tip allowed to reach a temperature of 1° C. The retina was then refrozen at the previous temperature and duration.

4. Rapid thawing. Another series of the same duration of freezing increments was produced to demonstrate the effects of fast thawing. To obtain fast thawing, a globe separatory funnel was secured at a high level above the operating field. A polyethylene tube ¼ inch (1.3 cm.) in diameter ran from the funnel to the probe. The tube was taped to the probe so that the end of the tube was approximately 1 cm. from the tip. The funnel and tube were filled with saline at 44° C. A large hemostat clamped the tube in such a manner that it could be reached easily with the hand not holding the probe. When the retina was frozen for the desired length of time, the hemostat was re-
leased. The operative field was immediately flooded with a continuous stream of the saline.

5. By subconjunctival injection of 0.5 ml. of 15 per cent glycerol in saline 30 minutes before freezing. The injection was made directly over the site of operation. The same durations of freezing increments were used.

The interval from the instant of turning off the freezing apparatus to the instant the probe tip reached 0° C. (thawing time) was recorded for all lesions produced. All eyes in these five series were enucleated in 2 weeks, preserved, sectioned, and stained as previously described.

Results

Gross changes. The conjunctiva showed no permanent changes. Hyperemia was noted immediately after freezing and there was an occasional small capillary hemorrhage. The hyperemia lasted a few hours. The hemorrhage absorbed in a few days. No scarring or adhesions were noted when the eyes were enucleated.

Ophthalmoscopically the first thing observed on freezing was the sudden appearance of white ice crystals in the choroid. This was followed almost immediately by the appearance of a white circular area of frozen retina involving the entire thickness of the retina (Fig. 2). At this time the signal was given to stop reducing temperature. This produced a frozen area invariably less than the diameter of the probe tip (2 mm.). Retinal freezing occurred between the temperatures of -30 and -40° C.

When the freezing component of the instrument was turned off, the frozen area decreased in size at a somewhat slower rate than that at which it formed. After complete thawing, an area of retinal edema remained that corresponded to the frozen area, but was surrounded by a concentric ring (Fig. 3). This ring proved to be a buckle in the retina, which disappeared after 24 hours. The borders of the edematous area were quite sharp. At no time, during freezing or thawing, were any of the blood vessels affected in any way, and no hemorrhage was noted.

The appearance of the lesion did not change in the first 6 hours. In 24 hours the edematous area was of the same size but appeared whiter and sharper in outline than at 6 hours. In 48 hours the edema had subsided so that it could be seen only in the center of the lesion. The area between the edematous part and the margin showed a loss of pigment and a granular, clumped arrangement. In 4 days the lesions were free of edema. At the end of 7 days the lesion had well-demarcated boundaries and the granular, clumped-pigment appearance was quite clear. There was pigment
migration to the larger retinal blood vessels (Fig. 4).

The appearance of the lesion changed very little from the seventh day on. The pigment-free areas became somewhat whiter between the second and fourth week. The blood vessels remained unaffected and no hemorrhage was noted.

The lesion produced by freezing was quite evident ophthalmoscopically even when thawing was begun immediately after the retina was seen to freeze.

Increasing the duration of freezing up to 10 seconds caused the frozen area to increase slightly in size; for longer durations the frozen area remained of the same size.

At probe tip temperature of -60° C., the frozen area of the retina appeared as described above at the instant of retinal freezing (-30° C.), but, as the temperature decreased further, the frozen area not only increased in diameter in the chorioretinal plane but also extended into the vitreous. The area frozen was larger than the probe tip. The same edematous area resulted after thawing as after the previous procedure, only it was more prominent. In the lesion held at -60° C. for more than 45 seconds, two small punctate hemorrhages were seen in one specimen. These hemorrhages were confined, and at no time were large blood vessels affected, nor was there any hemorrhage into the vitreous. No gross effects were noted by increasing the duration of the -60° temperature except that the lesions of longer duration were slightly larger and a few specimens exhibited some adhesion of vitreous to the lesion.

Upon refreezing, a frozen area was produced in the same amount of time and at the same temperature and was of the same size as that observed with the previous freezing. The resulting retinal edema appeared more prominent than did the edema resulting from single freezing procedures.

Rapid thawing and subconjunctival injection of glycerol produced no visible changes in the lesions.

Microscopic changes. At the end of one hour the retina was diffusely edematous and the ganglion cell and nerve fiber layers were separated. The outer nuclear layer was partially intact and the pigment epithelium appeared normal. The choroid was edematous throughout, with spreading of the choroidal pigment cells. The collagen fibers were thickened and stained poorly. No change was seen in the sclera.

At the end of 24 hours the inner layers had been replaced by edematous shredded debris. The outer nuclear layer remained partially intact; only a few inner nuclear cells remained. The pigment-cell layer was still intact. The choroid remained diffusely thickened by edema, thus spreading out the pigment cells. Pigment granules were seen extracellularly. No scleral change was observed.

At the end of 48 hours all that remained of the inner layers was necrotic edematous material, with the outer nuclear layer showing disruption and some cell death. The pigment-cell layer was still intact but had a few areas of fragmentation and loss of continuity. The choroid and sclera were unchanged from the foregoing description.

In 4 days much of the debris had been cleared away. The retina was three or four cells thick, composed of small cells with...
large, prominent, pigment-filled macrophages. The pigment-cell layer was disrupted in many places. The choroid was still thickened with many extracellular pigment granules. Some melanophages had infiltrated into the inner layer of the sclera. Increased connective tissue was evident in the choroid for the first time.

In 7 days the retina consisted entirely of pigment-filled macrophages and glial cells. The pigment-cell layer was gone. There were increased infiltration of the sclera with melanophages and obvious connective tissue infiltration into the choroid.

In 10 days the macrophages had migrated to the inner layers of the retina. The choroidal pigment cells were farther apart and clumped.

In 14 days tissues were well organized and all debris was gone. The retina was thinned to 2 to 4 cells in thickness. The macrophages were fewer and were situated more toward the inner cell layers. The choroidal pigment cells were fewer and

Fig. 5. Transscleral freezing of the retina for 2 seconds (2 weeks postoperative). (x185.)

Fig. 6. One month after retinal freezing, the lesion is well organized and resistant to detachment. The normal retina became detached in fixing process. (x54.)
those present were clumped in areas associated with connective tissue infiltration. Melanophages were evident in the inner layers of the sclera (Fig. 5).

In one month the only changes were large depigmented areas in the choroid between clumps of pigment cells accumulated in connective tissue (Fig. 6).

The general effect of transscleral freezing of the retina for 2 seconds was a thinning of the retina, thickening of the choroid, and melanophage infiltration into the inner part of the sclera (Fig. 5). The retina was 3 or 4 cells thick with pigment-filled macrophages in the inner layers. The pigment epithelium was gone. The choroid was thickened with connective tissue, and pigment cells were clumped around blood vessels and at the borders of the lesion. Other than the presence of melanophages, the sclera was unchanged. Blood vessels were unaffected in all specimens, and no hemorrhage was seen in any of the sections.

Increasing the duration of freezing affected primarily the retina. Two seconds of freezing reduced the retina to a thickness of 3 or 4 cells, 20 seconds reduced it to a single layer of pyknotic cells with overlying macrophages, and 1 minute completely destroyed it, producing a layer of pigment-laden macrophages with a few glial cells. The choroid and sclera showed no change over that previously described.

A probe tip temperature of -60° C. for 2 seconds produced almost complete retinal destruction. The choroidal and scleral changes were similar to those produced at higher temperatures. The retinal blood vessels were not affected. Little change in the lesion was noted with increased duration of this temperature.

Refreezing produced somewhat more retinal destruction than did a single freezing procedure, but the greatest difference was in the choroidal reaction. The choroid was thicker and was the site of more connective tissue and melanophage production.

Rapid thawing reduced the thawing time from more than 20 to less than 2 seconds. The resulting lesion appeared identical to those produced with slow thawing.

Lesions produced after glycerol injection were not histologically different from those produced without glycerol injection.

Attempts to produce retinal lesions in the superior part of the globe with a probe tip temperature at -60° C. were unsuccessful.

**Discussion**

The temperature at which freezing of the dog's retina occurred was almost exactly that reported for the human retina (-30 to -40° C.). This reflects the great structural similarity between the dog's eye and the human eye. The sharp borders surrounding the lesions in the retina correspond to the sharp borders of the lesions produced by spot freezing of other tissues. The fact that the large blood vessels were unaffected also was to be expected on the basis of previous studies. From knowledge of the thawing process and the freezing process, we would expect that thawing would take longer than freezing, and this was indeed observed in our studies.

The frozen areas associated with increased duration of freezing were larger because the temperature of the tissue at the edges of the frozen area came into equilibrium with the freezing temperatures. The small hemorrhages observed with extremes of freezing duration were from capillaries and were confined, as was observed in other tissues, indicating that such small hemorrhages would not be a problem clinically.

Although damage is somewhat increased by increased duration of freezing, the tissue is more affected by the lowness of the temperature. In our study, increasing the duration of freezing beyond approximately 20 seconds produced little further damage. When the temperature was decreased to -60° C., damage was greatly increased even with instantaneous thawing, but increased duration of freezing at this temperature produced no significant change. No difference in choroidal or scleral damage was
observed with either increased duration or lower temperature. This indicates the extent to which these tissues generally resist damage by freezing.

Of our attempts to modify the effects of freezing, only refreezing showed any demonstrable change. The increased retinal damage and choroidal reaction evident on refreezing in the shorter durations correspond to the increased scar formation found on refreezing other tissues.12

The failure of fast thawing to modify the effects of freezing indicates that the temperature at which death of the tissue occurred was reached in freezing before thawing was ever begun.10, 11

The fact that subconjunctival injection of glycerol did not cause any change in the lesion probably means only that the glycerol did not penetrate to the inner layers of the eye. The preserving action of glycerol on frozen tissue is well known.14

The failure of a temperature much lower than that necessary to produce a lesion in the retina medially and laterally to cause any change superiorly was due to the increased vascularity in the superior globe of the dog and also to the insulating effect of the two superior muscles. This reflects the extremely low conductivity of tissue and the large modifying effect of blood vessels. It means that any particular area which one wishes to freeze must be observed if one is to be sure freezing has taken place, for the temperature needed varies with thickness and vascularity of the tissue.

Since the retinal cells are highly specialized, one would expect them to be more susceptible than others to freezing.13 The innermost retinal cells were the first to be affected; they are farthest from the warming effect of the choroid.

The fact that choroidal changes were the same despite changes in temperature and duration indicates that the tissue damage occurred at a given temperature in the freezing process and that further damage was limited by individual cell resistance.

Scleral damage does occur as is evidenced by the migration of melanophages into the sclera where they undoubtedly act in either a reparative or a macrophagic role. This is the only change observed, and even it is minimal. The sclera would seem to be little affected by freezing, which is to be expected of connective tissues.8

True adhesive chorioretinitis was first observed at the end of 4 days, although minute changes in the pigment epithelium were seen at the end of 48 hours. The breaking up of pigment epithelium to form macrophages and structural cells is evidence of adhesive chorioretinitis. The proliferation of connective tissue in the choroid at the end of 7 days, as well as melanophage infiltration into the sclera, leads to the conclusion that the healing process involves all three tissues (retina, choroid, and sclera), producing an extensive and firm union. This conclusion is borne out also by the fact that the retinas which became detached in the process of fixation remained adherent at the site of the lesions (Fig. 6).

Conclusions

From the observations made in this investigation, the following conclusions are drawn:

1. Transscleral freezing of the retina produces a well-defined lesion involving the sclera, choroid, and retina.
2. Scleral damage is minimal.
3. The lesion is primarily one of adhesive chorioretinitis, apparently resistant to retinal separation.
4. Ice crystal formation in the retina is all that is necessary to produce a lesion.
5. Large retinal blood vessels are unaffected by freezing.
6. Increasing the duration of freezing increases retinal damage for durations up to approximately 20 seconds.
7. Lowering the probe tip temperature to −60° C. produces increased retinal damage which is then unchanged by increased duration of freezing.
8. Refreezing produces more retinal damage and choroidal reaction than does a single freezing.
9. Rapid thawing produces no demonstrable change in the lesion.
10. Subconjunctival injection of glycerol produces no demonstrable change in the lesion.
11. Adhesion of choroid and retina is observed at the end of 4 days.
12. Temperatures necessary to freeze the dog's retina are comparable to those needed to freeze the human retina.
13. Temperatures necessary to freeze the retina depend on thickness and vascularity of the operative site.
14. Ice crystal formation must be observed to be sure that a retinal lesion is produced in the area to which the cryoprobe is applied.
15. There were no permanent gross changes in the conjunctiva.

REFERENCES

Discussion

Dr. Harvey A. Lincoff, New York, N. Y. I am grateful for the opportunity to comment on this thorough and orderly series of observations on the effects of transscleral freezing.

The observations of the cryosurgery group at Cornell have been similar and would support Dr. Archambeau’s impression that a cold application to the sclera in the range of -20° C. to -50° C. produces an exudative lesion at the chorioretinal level which upon healing results in retinal adhesion, and that the sclera is relatively undamaged.

I was interested in the authors’ discussion of what constitutes the chorioretinal adhesion. What binds the pars optica to the pigment epithelium in a treated area? Is it a fibrous adhesion with fibers interlacing from one layer to the other? One is tempted to think so, but we have never been able to see these fibers in our sections, but only the layers themselves in attenuated form, reoriented, and firmly adjacent to each other.

We observe chorioretinal adhesion in animal sections on the first postoperative day, taking as evidence, as the authors do, a chorioretinal adhesion at the site of freezing where elsewhere the retina shows artifactual detachment as a result of fixation. The clinical evidence suggests that some degree of adhesion occurs in 24 hours. We mobilize our detachment cases treated with cryosurgery in 24 hours and the retinas stay in place. It is too early for fibrous adhesion and suggests that at least early there is a gluing effect of the exudate.
It was our first impression too that the white response seen in the retina at the time of cold application was ice crystal formation. However, where we began treating retinal holes it was noted that the choroid in the hole itself did not turn white, but only the retinas around it. A probable explanation for the transient white response in the retina is a reversible precipitation of protein in a supercooled state. This occurs with minimum and therapeutic applications of cold. If the application gets cold enough, and stays long enough, everything turns white and a mound of ice does form. This is an excessively destructive lesion.

The question of whether the vitreous is affected is of concern to the retinal surgeon. In the intact animal vitreous we found that the vitreous when frozen did develop changes adjacent to the retina and not infrequently chorioretinal adhesion.

The practical questions posed by the clinician are whether the chorioretinal adhesion from cold application is as secure as when initiated by diathermy, and whether the strength of the scleral wall is sufficiently preserved to tolerate reoperation.

In the past 1½ years 135 patients with retinal detachment have been operated upon by a cryosurgical technique at New York Hospital-Cornell Medical Center. After the tenth case, the patients were consecutive and unselected. The clinical follow-up indicates that the retinal adhesion is secure and equivalent to our best diathermy result.

Reoperations in the series gave evidence on the state of the sclera following cold application. Ten patients were operated upon two times, six patients three times, and one a fourth time. In the first postoperative week, tissues separate easily from the scleral wall, and the wall appears grossly normal. I think it is slightly softer but not so soft as not to retain sutures in the operated area. In late reoperations there can be considerable thickening of tenons and adhesion, but when the dissection is carried to the scleral surface the wall is found to be of original firmness and thickness.

I think cryosurgery of retinal detachment is passing from an experimental to a clinical procedure. Dr. Archambeau's careful work contributes significantly to this change.