Experimental retinal detachment with a sulfated polysaccharide

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Research in the pathophysiology of retinal detachments would be aided if an experimental model of such a lesion could be achieved. In an attempt to produce such a model, it was felt essential to cause vitreal as well as retinal alterations. This role of vitreoretinal adhesions and liquefaction of the vitreous as the two main anatomic factors in the etiology of the idiopathic type of retinal detachments has been stressed by Teng and Chi. Balazs noted that in the rabbit the hyaluronic acid has its highest concentration in the boundary layer next to the retina and possibly acts as a barrier to diffusion. Harris noted that hyaluronic acid sulfate caused vitreous liquefaction. We, therefore, injected hyaluronic acid sulfate into the vitreous of a living rabbit and noted that this caused many vitreous alterations leading to retinal detachments comparable to the idiopathic type of retinal detachment found in humans.

C rodin first reported on experimental retinal detachments in 1875. Since then, various authors have attempted to produce a model for experimental retinal detachments in animals which would closely resemble idiopathic retinal detachments in humans. Until the present time, such a model for the production of retinal detachments has not been achieved. Since spontaneous reattachment of the retina occurs in the normal eye of a rabbit following a physically induced retinal tear, it appears that the retinal tear is not the only factor in maintaining a detached retina, but that some other abnormality of the vitreous, retina, or choroid is necessary. Teng and Chi have stressed the role of vitreoretinal adhesions and liquefaction of the vitreous as the two main anatomic factors in the etiology of the idiopathic type of retinal detachment. The presence of vitreous shrinkage has also been noted by many authors. Balazs has described the vitreous as a meshwork of collagen fibers interlaced with coiled hyaluronic acid molecules. He also noted that in the rabbit the hyaluronic acid has its highest concentration in the boundary layer next to the retina and possibly acts as a barrier to diffusion. A sulfated form of hyaluronic acid which does not occur normally in nature was noted by Harris to produce vitreous liquefaction. From the foregoing it would seem clear that if one were to produce a retinal detachment which is at all comparable to that in humans, a method should be used which would produce vitreous liquefaction with as little trauma as possible to the retina and underlying choroid.

With this aim, it was the purpose of our study to inject hyaluronic acid sulfate into...
the posterior vitreous of a living rabbit and to observe alterations in the vitreous and the occurrence of retinal detachment. Periodically, the animals were to be killed for gross and histologic examinations.

Materials and methods

Rabbits of approximately equal weights (about 2.5 kilograms) and of either sex were used. All the rabbits but two were pigmented because fundus details are seen more clearly in pigmented rabbits. All intraocular injections were performed under aseptic conditions and under Nembutal and Thorazine anesthesia. The pupils were maximally dilated with 10 per cent Neo-Synephrine and 2 per cent homatropine. The lids were retracted by a Williams eye speculum. Solutions of 2.5 per cent, 3.15 per cent, and 5 per cent were freshly prepared by dissolving 100 mg. of sterile, purified hyaluronic acid sulfate with pyrogen-free, sterile distilled water.16 A tuberculin syringe was utilized to measure accurately the solutions of 2.5 per cent, 3.15 per cent, and 5 per cent into 0.1 c.c. and 0.2 c.c. volumes for the injection of hyaluronic acid sulfate in measured amounts of 2.5 mg., 5 mg., 7.5 mg., and 10 mg. A one-half inch No. 30 gauge needle on the tuberculin syringe was then placed temporally to the superior rectus muscle and approximately 4 mm. posterior to the limbus. The needle was then inserted through the sclera at the edge of the pars plana ciliaris. Because of the fact that the pars plana ciliaris in the rabbit is a very narrow structure, it was realized that, although it was planned to insert the needle in this area, occasionally it might enter the periphery of the retina, and on several occasions a perforation of the periphery of the retina was noted. These perforation sites in the periphery of the retina were observed to heal quickly without the retina becoming detached at this site.

Through the dilated pupil the operator continuously monitored the course of the needle with the binocular indirect ophthalmoscope. The point of the needle was directed toward the visual streak and when it reached the posterior vitreous, the material was injected. The fellow eye was used as a control by injecting an equal volume of sterile water in a like manner into the posterior vitreous. Immediately following the intraocular injections, the momentary increase in intraocular pressure caused the cornea to become slightly steamy, but this cleared in seconds.

A standard Schiotz tonometer was used to obtain some estimate of the intraocular pressure at various intervals. Measurements were taken prior to injection, one hour after injection, and three weeks following injection.

Stereo fundus photographs were taken in Kodachrome and color Polaroid with the Zeiss fundus camera.10 The rabbits were sacrificed at periodic intervals and the eyes were removed for gross and microscopic examination. All results are based on the examination of such enucleated eyes.

Results

1. Alterations in the vitreous.

A. Vitreous liquefaction. In 100 per cent of the eyes injected with hyaluronic acid sulfate, liquefaction of the vitreous occurred in varying amounts. In all injected eyes, liquefaction of the vitreous was apparent when such eyes were examined on both gross and microscopic section. Such alteration was characterized by the presence of large intravitreal, fluid-filled cavities surrounded by condensed vitreal bands and membranes. The vitreous maintained some normal attachments to the retina, but some pathologic vitreoretinal adhesions were present. Liquefaction of the vitreous took place as early as two days after injection and was quite evident in the eyes examined three months or more following injection. No attempt to measure the amount of liquefaction was made because of the heterogenous nature of the vitreous,17 but on gross section this change could easily be observed. In the control group vitreous liquefaction was not seen and the vitreous remained in its gel form (Figs. 1 to 4).

No vitreal or retinal changes were observed clinically in the enucleated control eyes, other than at the site of the injection. This rapidly healed without resulting in a retinal detachment by either ophthalmoscopic or histologic examination.

B. Vitreous contraction. In addition to the vitreous liquefaction as noted by Harris,3 we also noted in the majority of eyes other vitreous alterations including a definite contraction of the vitreous. This change was fairly characteristic; the vitreous was contracted into a funnel-shaped, somewhat opaque mass with its apex at the optic nerve and with occasional vitreoretinal adhesions to the posterior pole (Figs. 1, 3, and 4). The base of this contracted vitre-
ous appeared to be firmly attached to the posterior lens capsule with many of its attachments to the pars plana ciliaris still intact. In vivo, even with the biomicroscope, this contracted vitreous frequently was difficult to distinguish from a central posterior lens opacity. However, in most instances one could visualize the fundus with the indirect ophthalmoscope through the periphery of the lens, where the contracted vitreous was quite thin.

C. Vitreous strands. In several eyes vitreous strands were noted to extend from the central mass of contracted vitreous to the retina at a point which apparently contained a vitreoretinal adhesion (Figs. 1, 3, and 4).

2. Retina. An even more interesting al-

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**Fig. 1.** Eighteen days after injection. There is vitreous liquefaction and contraction with an early retinal detachment. S, sclera; Rd, retinal detachment; VI, vitreous liquefaction; a, vitreo-retinal adhesion; Vc, vitreous contraction; v, visual streak; L, lens; Ir, iris. (×9.)

**Fig. 2.** Control fellow eye of Fig. 1. The vitreous is clear and intact with no liquefaction or contraction. A small artifactitious retinal detachment occurred during photography. S, sclera; R, retina; V, vitreous; L, lens; Ir, iris; C, cornea. (×9.)
Retinal detachment with sulfated polysaccharide

Fig. 3. Five days after injection. There is vitreous liquefaction and contraction. S, sclera; R, retina; VI, vitreous liquefaction; a, vitreoretinal adhesion; Vc, vitreous contraction; L, lens; Ir, iris. (×7.)

Fig. 4. One month after injection. There is vitreous liquefaction and massive contraction. Note a thin vitreous strand (see arrow) and its shadow below on the visual streak. Ir, iris; L, lens; Vc, vitreous contraction, Vs, vitreous strand; v, visual streak; R, retina; S, sclera. (×10.)

teration was the development of a retinal detachment which resembled in many ways the serous retinal detachment of humans. This detachment was noted in 13 of 21 eyes injected.

A series of fundus photographs was taken to record the development of one of the retinal detachments in the eye of a living rabbit. This was followed with gross and microscopic studies of the same eye. The following sequence of events occurred in this rabbit: Approximately two and one-half days following the injection of 5 mg. of a 2.5 per cent solution of hyaluronic acid sulfate into the posterior vitreous, a slight haze was noted in the vitreous with the appearance of a white patch on the retina below (Fig. 5).

On the eighth day following injection, the misty haze in the vitreous, which was not unlike the typical vitreous contraction, was still present. This haze extended to the patch of pale retina below. This locale appeared to be migrating anteriorly as though...
Fig. 5. Two days after injection. Fundus photo of retina below visual streak. There is a slight retinal haze with a white patch on the retina below. R, retina; Rp, pale retina. (x40.)

Fig. 6. Eight days after injection. There is now a retinal detachment forming. A misty haze in the vitreous extends down to the pale area of retina. The pale retina appears to be migrating anteriorly as though being pulled by a vitreous strand. R, retina; Rd, detached retina; Vh, vitreous haze; Rp, pale retina. (x30.)

Fig. 7. Three weeks after injection. There is now a hole in the retina, and about 30 per cent of the retina is now detached. Rd, retinal detachment; Rh, retinal hole. (x30.)

It was now noted that the retina surrounding the spot of pale retina was becoming detached. Gradually, the locale of white retina was becoming separated from its neighboring detached retina. In Fig. 7 a retinal hole is present in the area previously occupied by the pale retina, three weeks following injection.

The area of detached retina surrounding the hole gradually enlarged and stereo fundus photographs were taken of the retinal detachment. The animal was killed on the thirty-first day after injection and the area of retinal detachment examined microscopically. Fig. 8 illustrates the retinal hole on a mounted section with vitreous strands extending from the edge of the hole toward the pars plana ciliaris.

In Fig. 9 is illustrated a large strand of contracted vitreous, Vs, with attached retinal tissue, O, from the area of the retinal hole; such retinal tissue has the same staining characteristics as the detached retina.

The use of the Rhinehart, Abul-Haj stain demonstrated the presence of acid mucopolysaccharide in the vitreous cavity of both injected and control eyes and also in the rod and cone layer of the retina. Treating mounted sections with hyaluronidase for one hour indicated the vitreal acid mucopolysaccharide to be hyaluronidase sensitive while that of the rod and cone layer was hyaluronidase resistant.

Since we also found that hyaluronic acid sulfate stains as an acid mucopoly-
saccharide with the Rhinehart, Abul-Haj stain and is also hyaluronidase sensitive, the presence of injected material could not be demonstrated in the vitreous body by this technique. The staining intensity of the injected vitreous was slightly greater than the counterpart controls, but this may have been caused by vitreous contraction.

The gross specimen in Fig. 10 illustrates the retinal hole and surrounding retinal detachment one month after injection. In Fig. 11 is illustrated the retinal hole with the typical rolled edges and a cellular vitreous strand attached to one edge of the retinal break and partially covering it. In Fig. 12 a higher magnification illustrates the firm attachment of the cellular vitreous strand to the rolled edge of the retinal hole.

The intraocular pressure as measured by the Schötz tonometer was utilized only for a gross estimation of the intraocular pressure. There was no significant difference in intraocular pressure between the pre-
injection pressure and the pressure three weeks following intraocular injection. As noted previously by Harris, an occasional corneal and/or lens opacity developed. In certain cases there was a spontaneous dislocation of the lens with fragmentation of the zonular fibers being noted on histologic examination. With all these changes, there was little or no inflammatory response. An occasional eye showed a slight beam but no cells were noted in the aqueous. The eye was usually white but occasionally circumcorneal injection was noted.

With the use of special staining techniques for acid mucopolysaccharides, the relationship between the vitreal and retinal elements was more easily determined.

Discussion

Previous experimental models of retinal detachment have not duplicated successfully the vitreal contraction, liquefaction, and formation of vitreoretinal adhesions seen so frequently in humans. By the injection of hyaluronic acid sulfate into the posterior vitreous, liquefaction and contraction of the vitreous produced vitreous strands extending to the sites of vitreoretinal adhesions. At these sites of vitreal traction, a retinal hole and subsequent detachment were observed in 13 of 21 injected eyes. The detachments in all 13 eyes were progressive with two becoming total, one as early as one month and one as late as three months following injection. No spontaneous reattachment was noted for the three-month period of observation. More extensive studies are presently in progress.

The exact mechanism by which the injected hyaluronic acid sulfate causes changes in the ocular structures is, of course, unknown. Such changes may well be due to a fundamental alteration and possibly a replacement of the polysaccharide of the ground substance of ocular
It is also possible that there was a direct toxic effect on the retina and vitreous at the site where the solution was injected. The effect of other sulfated and nonsulfated mucopolysaccharides on the retina and vitreous will have to be determined by further research. However, the most striking result of this experimental procedure was the production of vitreous liquefaction, contraction, vitreoretinal adhesions, and a progressive retinal detachment. Assessment of the role of hyaluronic acid sulfate in the pathogenesis of human retinal detachments will have to await further study as no etiologic significance can be drawn from these results. However, the experimental models so created should be useful for further research in this field.

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REFERENCES