Demonstration of acid mucopolysaccharides in the trabecular meshwork of the Rhesus monkey

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The ability to demonstrate AMPS in the trabecular region in the normal eye of the Rhesus monkey was shown to be critically dependent upon technical variation. Staining the fixed specimen prior to dehydration and embedding permits the uniform demonstration of AMPS in the trabecular region of the Rhesus monkey and shows it to be hyaluronidase-sensitive. Electron microscopy using the modified technique shows the reaction products to be present within the trabecular band, the intertrabecular spaces, and the canal of Schlemm. More impressive distribution was seen in the basement membrane of trabecular endothelium intimately related to the cell wall and in the ground substance and basement membrane of the endothelium of the inner wall of the canal of Schlemm. The technique is also successful in the human eye and suggests a greater abundance of trabecular AMPS in open-angle glaucoma.

Key words: trabecular meshwork, trabecular endothelium, Rhesus monkey, canal of Schlemm, acid mucopolysaccharides, colloidal iron stain, basement membrane, endothelial vacuoles.

A remarkable increase in aqueous outflow facility was shown to result from the intracameral administration of hyaluronidase in vitro in several animal species. This was followed shortly by the demonstration of hyaluronidase-sensitive acid mucopolysaccharides (AMPS) in the trabecular meshwork by histochemical staining methods in man and in the experimental animal. While the potential significance of this discovery to our understanding of the control and regulation of aqueous outflow facility in the normal eye and in glaucoma was obvious, histologic and physiologic studies have been complicated by lack of uniformity of results. Some histochemical studies demonstrated AMPS in the trabecular region while others failed to confirm this finding or describe their exact localization. Variation in technical details and in tissue preparation were thought to be significant causes of variation in results. Using the colloidal iron technique, AMPS was demonstrated in the normal human eye with the electron microscope.

From several standpoints, primates have been highlighted as the appropriate experi-
mental animal for physiologic and pharmacologic investigations in the field of glaucoma research. The presence of AMPS in the trabecular region of the Rhesus monkey has not been satisfactorily resolved. We therefore undertook to investigate this question and to look for an effective technique. The results, which are the subject of this presentation, affirm the presence of AMPS in the trabecular region of the normal eye of the Rhesus monkey by light and by electron microscopy and describe their distribution. They highlight the importance of technique variation in this regard and have led to the development of one technique capable of providing uniform results.

Materials and method

The clinically normal eye of the adult Rhesus monkey was used in all experiments.

For light microscopy, the following procedure was used: following enucleation under Nembutal anesthesia small blocks of tissue, including the trabecular region, were carefully excised using the dissecting microscope and placed in fixatives overnight. Two fixatives were used independently: (1) 4 per cent glutaraldehyde, and (2) 4 per cent glutaraldehyde + 0.2 per cent cetyl pyridinium chloride. Fixatives were in 0.15M phosphate buffer solution at pH 7.2.

Following fixation, specimens were divided into two groups: in group one specimens were dehydrated and embedded in paraffin and cut in eight micron sections. Alternating sections were incubated in purified testicular hyaluronidase (800 IU per milliliter for one hour. All sections were then carried through the following staining techniques: colloidal iron, alcian blue, and toluidine blue.11, 12 Those with colloidal iron stain were counterstained with Van Gieson stain. In group two, following fixation, the specimens were rinsed with distilled water then carried through two routes: in one, the specimen was incubated in physiologic saline at 37° C, whereas in the second they were incubated in physiologic saline containing 800 to 1,000 IU per milliliter of purified testicular hyaluronidase at 37° C. Following an incubation period of 15 minutes to three hours, all specimens were rinsed in distilled water and incubated in saline solution containing 800 to 1,000 IU of purified testicular hyaluronidase. Those incubated in saline came from the control or the saline-injected eye and those incubated in hyaluronidase came from the hyaluronidase-injected eye. Incubation period varied from 15 minutes to 22 hours at 37° C. Following incubation, specimens were rinsed with distilled water and the entire specimen was carried through the steps of the colloidal iron stain technique. Following staining, the specimens were again rinsed in distilled water, dehydrated, and embedded in Epon 812. One micron sections were then cut using Blum-2 ultramicrotome and stained with 1 per cent toluidine blue for orientation. Appropriate thin sections (500 A) were cut with a diamond knife and stained with uranyl acetate and lead citrate and examined with AEI 6B electron microscope.

Results

Light microscopy. In group one where conventional processing methods for paraffin sections were used, there was uniform failure to demonstrate unequivocally the presence of AMPS in the trabecular region of the Rhesus monkey with all three stains and two methods of fixation. In contrast, all specimens from group two incubated in saline and in which the entire specimen was carried through the steps of colloidal iron stain prior to dehydration and embedding uniformly demonstrated AMPS in the trabecular region and in the iris stroma (Figs. 1 and 2). There was no significant difference in the results between the two methods of fixation. Furthermore, in group two, specimens incubated in testicular hy-
Fig. 1. Light microscopy showing the positive reaction of colloidal iron stain for acid mucopolysaccharide in the trabecular meshwork of normal Rhesus monkey eye using the technique described in the text. (×100.)

Fig. 2. Higher magnification (×250) of the positive reaction for the colloidal iron stain (using the technique described in the text) in the trabecular bands of normal Rhesus monkey eye. (×250.)

Fig. 3. Same staining as in Fig. 2, but the tissues were pretreated with testicular hyaluronidase; greater reaction in positive reaction to colloidal iron stain demonstrates that AMPS in the trabecular meshwork of the normal Rhesus monkey eye is hyaluronidase-sensitive. (×100.)
aluronidase prior to staining failed uniformly to demonstrate AMPS in the trabecular meshwork indicating the sensitivity of this AMPS to hyaluronidase digestion (Fig. 3). There was no difference in the results between the short incubation period of 15 minutes and the long incubation period of three hours. AMPS in the trabecular region were diffusely distributed and seen in relation to trabecular bands as well as in the intertrabecular spaces and canal of Schlemm. The AMPS were more intense and abundant in the posterior portion of the trabecular region close to the tip of the ciliary muscle.

Alcian blue stain in both methods showed equally weak and inconclusive staining reaction in the trabecular meshwork.

**Electron microscopy.** Positive reaction products of colloidal iron were seen in the following locations in the control and in the saline-treated eyes: within the core of the trabecular band, in the basement membrane of the trabecular band endothelium closely related to the cell wall, on the sur-
Fig. 5. Electron micrograph of the trabecular band of normal Rhesus monkey eye. En—endothelial cell; C—collagen, positive reaction product of iron particles (arrows) are aggregated along the endothelial cell wall and among the collagen core of the trabecular band. (×37,500.)

In all these locations, positive reaction products were either completely absent or drastically diminished in the hyaluronidase-treated eye. All specimens (control and hyaluronidase-treated) that were postfixed with osmium tetroxide failed to demonstrate any positive reaction products for colloidal iron in the trabecular region.

Comments and interpretation

The results point out the importance of technique for the success or failure to demonstrate AMPS in the trabecular region.
In light microscopy, the variation in fixative used did not prove to be important in this regard. The most important step for a successful demonstration seems to be the application of the stain to the entire specimen prior to dehydration and embedding. This suggests that the process of dehydration and embedding may alter the physiochemical properties of AMPS or lead to their elimination from their trabecular sites. Similarly, for electron microscopic studies, postfixation with osmium tetroxide seems to exert a similar influence, possibly due to competitive binding to AMPS. While the source of AMPS in trabecular spaces and the canal of Schlemm has been presumed to be aqueous humor, the intimate association between the reaction product and the
basement membrane and cell wall of the trabecular endothelium is most provocative. These sites are not in ready contact with aqueous humor and the density of the reaction product suggests the possibility that AMPS are actually elaborated by the endothelium itself. Such a role has been already proposed for vascular endothelium. The demonstration of AMPS in the ground substance, basement membrane, and vacuoles of the endothelium of the inner wall of the canal of Schlemm encourages speculation as to a possible role of AMPS in regulating resistance to aqueous outflow in this particular region. With our present understanding of trabecular resistance, AMPS may exert their influence not only by mechanical obstruction or reduction of caliber of existing openings but, in addition, may play a role in modulating the activity and properties of the endothelial cells themselves which in turn influence the resistance to outflow. It would be of interest to study the physiologic and pharma-
Fig. 8. Electron micrograph of the outer wall of Schlemm's canal (SC); acid mucopolysaccharides (arrows) are also found along the endothelial lining of the outer wall. (×50,000.)

Physiologic properties of the trabecular region under conditions of normal and greatly reduced AMPS presence. Of obvious interest is the study of AMPS distribution with this method in normal and glaucomatous human eyes. Preliminary results using this technique showed successful demonstration of AMPS in the trabecular region in both groups of human eyes with greater abundance in eyes with open-angle glaucoma. A search for quantitative description of trabecular AMPS is currently being pursued.

REFERENCES