RELEASE OF RADIOLABELED GLYCOSAMINOGLYCANS BY THE NONSWELLING CORNEA OF THE DOGFISH SHARK (<u>SQUALUS ACANTHIAS</u>) AND THE SWELLING CORNEA OF THE ALEWIFE (<u>ALOSA PSEUDOHARENGUS</u>) AND SCULPIN (<u>MYOXOCEPHALUS OCTODECIMSPINONSUS</u>)

T.A. Kangas and H.F. Edelhauser, Departments of Physiology and Ophthalmology, Medical College of Wisconsin, Milwaukee, WI 53226

Nearly all vertebrate corneas show a tendency to swell following immersion in aqueous solutions or deionized water. Evidence from our own laboratory, as well as others, suggests that, as stromal edema ensues, glycosaminoglycans (GAGs) are lost from mammalian corneas. The elasmobranch cornea is of interest because it does not swell; edema is minimal in dogfish corneas despite incubation in solutions of various osmolalities. The purpose of these experiments was to determine whether labeled stromal GAGs were lost from the shark cornea during incubation in elasmobranch Ringer's and distilled water and to compare the release of stromal GAGs from shark and teleost corneas.

Corneas were excised from pithed dogfish sharks. Alewives were anesthetized with MS-222, sacrificed, and corneas excised. Sculpins were pithed, and the anterior stroma of the nonfused cornea was gently separated and excised from the posterior stroma.

Dogfish corneas were incubated in elasmobranch Ringer's containing ^{3}H-glucosamine (4 μCi/ml) for 5 hrs at 20°C. Then pair-matched labeled corneas were separated; one cornea was transferred at hourly intervals between vials containing elasmobranch Ringer's, while the mate cornea was similarly transferred between vials of deionized water. Hourly samples were collected for an 8-hr period for each cornea, and a sample was taken every hour thereafter for a total of 40 hrs. During transfer, corneal clarity and thickness were observed. At the end of the experiment, all samples were diluted with Optifluor and the radioactivity was determined by liquid scintillation counting. Corneas were blotted dry, weighed, dissolved with Soluene, diluted with Optifluor, and counted. The above protocol was repeated using dogfish corneas labeled with ^{35}S-sulfuric acid (4 μCi/ml) for 5 hrs. Additionally, this protocol was repeated using teleost Ringer's for: (a) alewife corneas labeled with ^{3}H-glucosamine, (b) alewife corneas labeled with ^{35}-sulfuric acid, (c) sculpin stromas labeled with ^{3}H-glucosamine, and (d) sculpin stromas labeled with ^{35}S-sulfuric acid.

The release of radiolabeled GAGs from shark, alewife, and sculpin corneas into their respective incubation medium is shown in Figures 1, 2, and 3. The data represents the cpms released into the sample vial as a percentage of total corneal cpms. Total corneal cpms include the sum of radioactivity in the 12 incubation vials plus that remaining in the cornea at the end of the experiment.

As evident from Figure 1, there was a marked difference between the ^{35}S labeled corneas; shark cornea incubated in elasmobranch Ringer's released significantly greater amounts of radiolabeled GAGs than the paired cornea incubated in deionized water. However, the loss of ^{3}H-glucosamine from the shark cornea was not different.

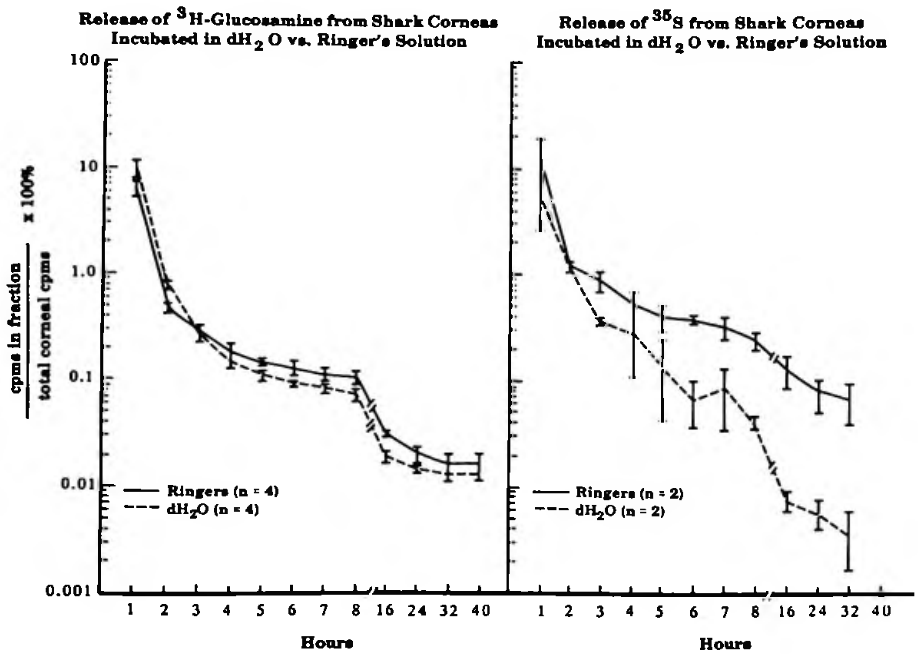

Fig. 1. Loss of shark corneal stromal glycosaminoglycans incubated in deionized water and elasmobranch Ringer's (mean ± SE).

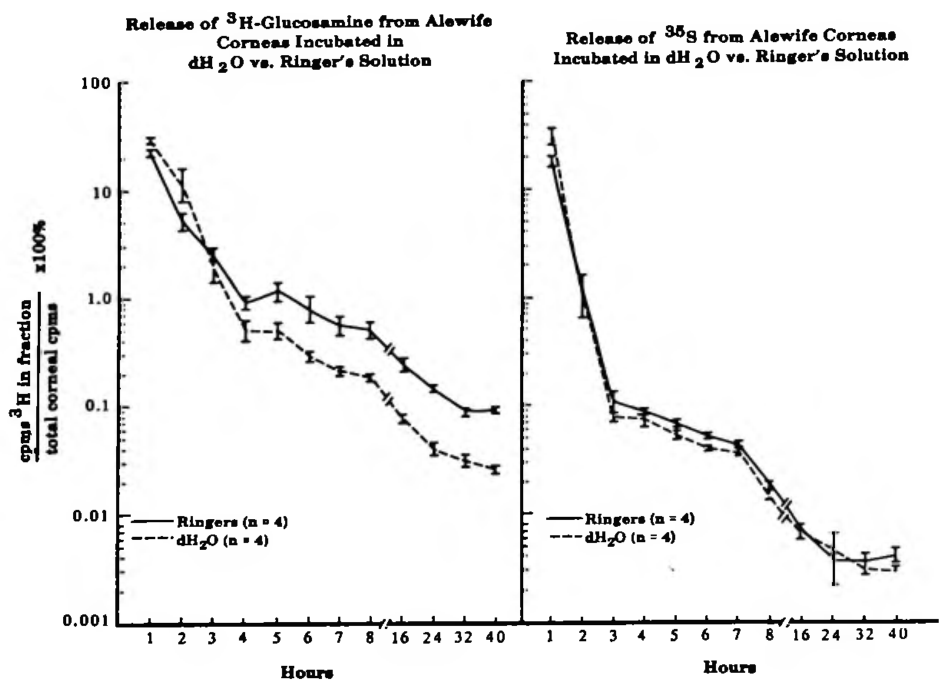

Fig. 2. Loss of alewife corneal stromal glycosaminoglycans incubated in deionized water and teleost Ringer's (mean ± SE).

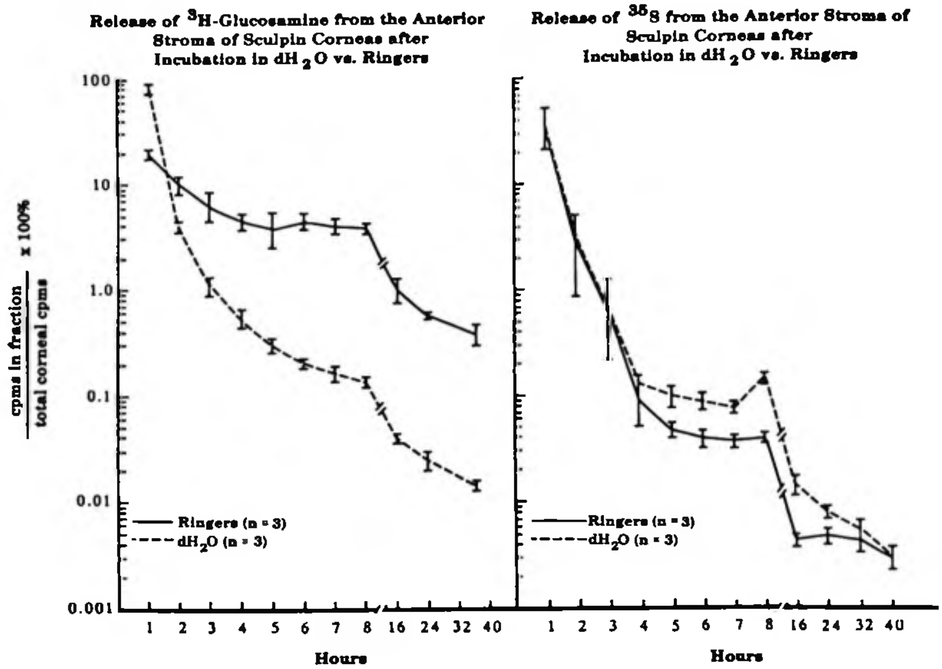

Fig. 3. Loss of sculpin corneal stroma glycosaminoglycans incubated in deionized water and teleost Ringer's (mean ± SE).

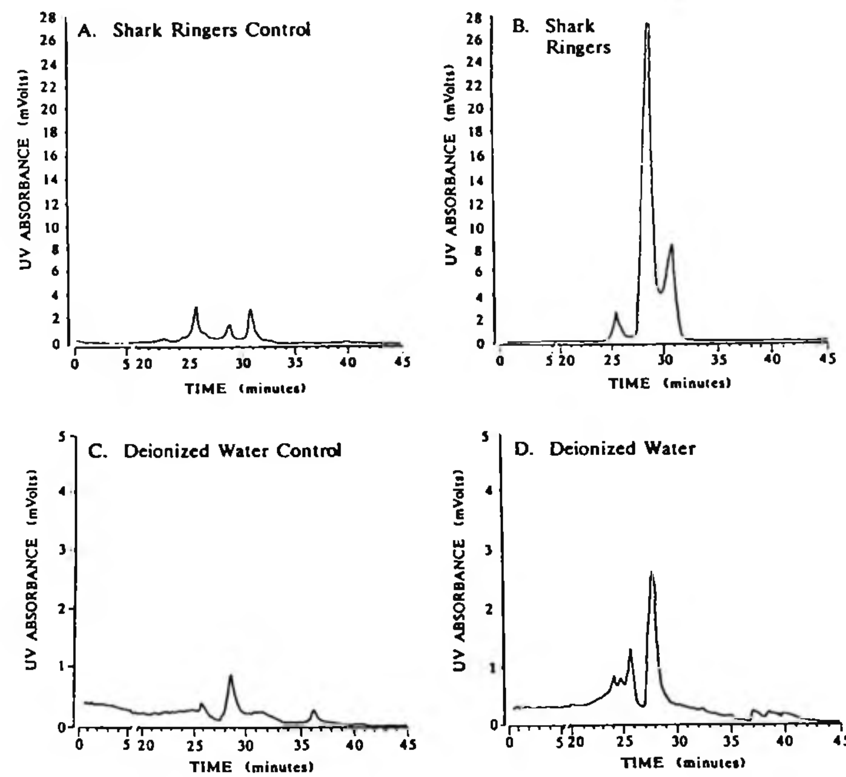

Fig. 4. HPLC analysis of the 7th-hr corneal stromal incubation of shark Ringer's and deionized water (see Fig. 1).

In contrast to the dogfish corneas, both ^{3}H-glucosamine labeled teleost corneas showed a significantly greater release of radiolabeled GAGs into Ringer's than into deionized water. There was little or no difference between the ^{35}S released by teleost corneas into Ringer's compared with deionized water.

Because the release of ^{35}S from shark corneas incubated in elasmobranch Ringer's was significantly greater than that released from their paired corneas in deionized water, it was of interest to further characterize the constituents in the Ringer's and deionized water samples using HPLC. The 7th-hr samples of the elasmobranch Ringer's and deionized water were filtered and injected onto a gel filtration column. The chromatograms are shown in Figure 4. In Figure 4A a control sample of elasmobranch Ringer's is shown. In Figure 4B, the 7th-hr Ringer's sample revealed the presence of three low molecular weight constituents at significantly greater levels than in the control Ringers. Figure 4C shows the control deionized water chromatogram. Figure 4D is the 7th-hr deionized water sample.

It is evident from Figure 1 that dogfish corneas labeled with radioactive GAG precursors did release radioactivity into the media despite the absence of corneal swelling. Although tritium was released from ^{3}H-glucosamine labeled shark corneas, there was little apparent difference between tritium release into Ringer's versus deionized water. However, the radioactivity released from ^{35}S labeled shark corneas significantly exceeded that released into deionized water. When these samples were further characterized on the HPLC, the elasmobranch Ringer's aliquots contain three major low molecular weight constituents that were absent or present in lesser amounts in control and deionized water samples.

The above findings suggest that the constituents released from shark corneas are likely to be either highly sulfated galactosaminoglycans or glycoproteins. Indeed, studies by Praus and Goldman (Invest Ophthalmol Vis Sci 9:131-136, 1970) have shown that dogfish corneas contain little keratan sulfate and considerable highly sulfated chondroitin sulfate in comparison with the corneas of other vertebrates. Likewise, Robert and Schllinger (Arch Ophthalmol, Paris, 27:813-818, 1967) described a remarkably higher content of insoluble structural glycoprotein in dogfish corneas relative to teleost corneas.

In comparison to the dogfish corneas, both the alewife and sculpin corneas showed a significantly greater difference between the ^{3}H-glucosamine released into Ringer's compared with the deionized water, with little difference between the ^{35}S counts released into Ringer's versus deionized water. This suggests that the GAGs released by the teleost corneas are less highly sulfated than those released by the shark corneas. The higher rate of incorporation and release of ^{3}H-glucosamine is consistent with the findings of others which suggest that keratan sulfate is the predominant GAG present in these teleost corneas. Since the teleost corneas manifested corneal edema, it appears that there is a concomitant loss of stromal GAGs associated with corneal swelling. The significant loss of ^{35}S and the release of three low molecular weight substituents from the dogfish cornea in the absence of edema warrants further investigation.