7.6, the reaction in epithelial cells was almost absent, but at pH 8.0 to 9.0, the cells demonstrated strong reaction. On the other hand, the optimal pH value of the peroxidase in the exorbital lacrimal gland is 6.5. These peroxidases may differ in their function.

I thank Mrs. H. Ueno for her help in preparing this paper.

From the Departments of Bacteriology and Cytochemistry, Chest Disease Research Institute, and Department of Ophthalmology, Faculty of Medicine, Kyoto University, Kyoto, 606, Japan. Submitted for publication Nov. 7, 1975. Reprint requests: Dr. Takeshi Iwata.

Key words: peroxidase, epithelium, conjunctiva, cornea, goblet cell, cytochemistry.

REFERENCES

Distribution of hexosamines in bovine cornea. Frederick A. Bettelheim and Dennis Goetz.

Excised bovine cornea were sectioned from epithelium to endothelium into 6 to 8 fractions. The glucosamine/galactosamine ratio steadily increases from epithelium to endothelium in all bovine corneas investigated. The glucosamine/galactosamine ratio reflects the keratan sulfate/chondroitin-4-sulfate ratio in the cornea. The significance of this topographic distribution is discussed in terms of the different hydration properties of proteoglycans containing predominantly keratan sulfate or chondroitin-4-sulfate chains.

About 60 per cent of the corneal glycosaminoglycans is keratan sulfate and the remaining 40 per cent is mainly chondroitin-4-sulfate. Small amounts of dermatan sulfate and chondroitin-6-sulfate2 have also been reported. It has been alleged3 that although the glycosaminoglycans are largely responsible for the hydration of the cornea, the keratan sulfate plays a different role than the chondroitin-4-sulfate in the hydration process. For one, the corneal wound healing shows reduced keratan sulfate and increased chondroitin-4-sulfate synthesis and injury to the Descemet membrane and endothelium transforms keratoocytes to dermal sulfate-producing cells. Further, non-swelling shark cornea contains little keratan sulfate and considerable amount of chondroitin-4-sulfate.4 The water vapor absorption properties of these two glycosaminoglycans are also quite different. While keratan sulfate absorbs large amounts of water, it retains very little in the dehydration process5; the reverse is true for chondroitin-4-sulfate.7

However, the glycosaminoglycans in the cornea are in the form of proteoglycans. These are heterogenous macromolecules carrying keratan sulfate and chondroitin-4-sulfate in different proportion. In our laboratory we isolated from cornea one proteoglycan with predominantly keratan sulfate and one with predominantly chondroitin-4-sulfate side chains.8

In characterizing the proteoglycans we also found that the water absorption and retention power of these proteoglycans were different, reflecting the behavior of the side chains. If the different proteoglycans differ so much in their hydration properties, it is possible that there is some specific distribution in the cornea reflecting these roles. Anseth1 reported that there was no difference in the distribution of glycosaminoglycans in the central or peripheral parts of the cornea. The glucosamine/galactosamine ratio "seemed to be higher in the anterior than in the posterior part. This difference, however, was not
significant." On the other hand, Borcherding and co-workers\textsuperscript{9} found that in human cornea the keratan sulfate content decreases steadily proceeding from the center to the periphery. They attribute this specific distribution to the collagen fiber organizing ability of acidic glycosaminoglycans. In view of the data on the role of proteoglycans in the hydration of the cornea,\textsuperscript{8} it was deemed necessary to analyze the topographic distribution of proteoglycans from epithelium (anterior) to endothelium (posterior).

It was decided to use the techniques of Antonopoulos\textsuperscript{10} to determine quantitatively glucosamine and galactosamine on a microgram scale. Bovine eyes (two years old) were obtained from a slaughterhouse less than 12 hours postmortem. Corneal epithelium and endothelium were removed by scraping with a scalpel. Two incisions were made in the cornea so that the whole cornea could be flattened on a planchet and sectioned parallel to the endothelium. The cornea was sectioned in a frozen state by microtome and the wet weight of each section was determined. The sections were hydrolyzed for eight hours in sealed Pyrex tubes in 6 N HCl. After hydrolysis the samples were evaporated to dryness by opening the tubes and placing them in a desiccator over NaOH pellets. The residues were taken up with
Figs. 2, B, C, and D. For legend, see opposite page.
0.1 ml. H₂O and chromatographed on a Dowex 50 by 8 250 mesh column under air pressure of 100 torr. The material was eluted by 0.3 N HCl and the effluent was collected in 0.25 ml. fractions in a fraction collector. The amino sugars were analyzed after acetylation with acetylacetone and using p-dimethylaminobenzaldehyde. The chromophore absorbance was read at 535 nm. Fig. 1 shows the separation of glucosamine and galactosamine with this method. The glucosamine/galactosamine ratios are calculated from such graphs. These ratios are presented in a histogram form in Fig. 2 for four corneas.

All corneas investigated showed the same trend, namely, there was a gradual increase in the glucosamine/galactosamine ratio when one proceeds from the epithelium to the endothelium of the cornea. No particular trend was observed regarding the total hexosamine content.

The glucosamine/galactosamine ratio reflects the keratan sulfate/chondroitin-4-sulfate ratio in the cornea thus implying that relatively more chondroitin-4-sulfate chains bearing proteoglycans are in the anterior part of the cornea than in the posterior part. If proteoglycans play a vital role in the transparency of cornea by maintaining the proper hydration then such a distribution makes sense. We have shown that proteoglycans bearing predominantly keratan sulfate side chains absorb two to three times as much water as proteoglycans with moderate or high chondroitin-4-sulfate content. Proteoglycans with predominantly keratan sulfate side chains make up about 25 per cent of the total proteoglycans in the cornea. According to the topographic distribution presented in Fig. 2, these keratan sulfate proteoglycans are concentrated near the posterior of the cornea near the endothelium. The proteoglycans not only absorb water to a great extent but also transfer them with ease since little is retained by them in the dehydration process.

Thus, their positioning near the endothelium enhances the proper hydration by preventing water loss through dehydration.

We thank Adriel Bettelheim for his help in the preliminary part of this study and Dr. Howard Grob for the use of the microtone.

From the Chemistry Department, Adelphi University, Garden City, N.Y. 11530. This research was supported by a research grant of the National Eye Institute EY 00501-07 United States Public Health Service. Submitted for publication Nov. 1, 1975.

Key words: bovine cornea, galactosamine, glucosamine, hydration, proteoglycans.

REFERENCES


Corneal membrane water permeability as a function of temperature. KEITH GREEN AND SUSAN J. DOWNS.

Osmotically driven water flow across the corneal epithelium and endothelium has been measured as a function of temperature. For both membranes deviations from a single straight-line relationship are found in a logarithmic plot of hydraulic conductivity against 1/T. Both mem-