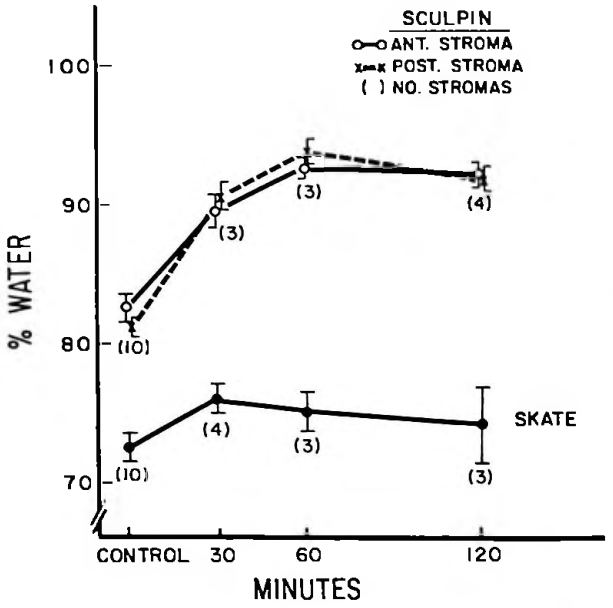


Figure 2.--Left. Corneal stromal swelling (mean+ SEM) of sculpin and skate in deionized water at 15°C.

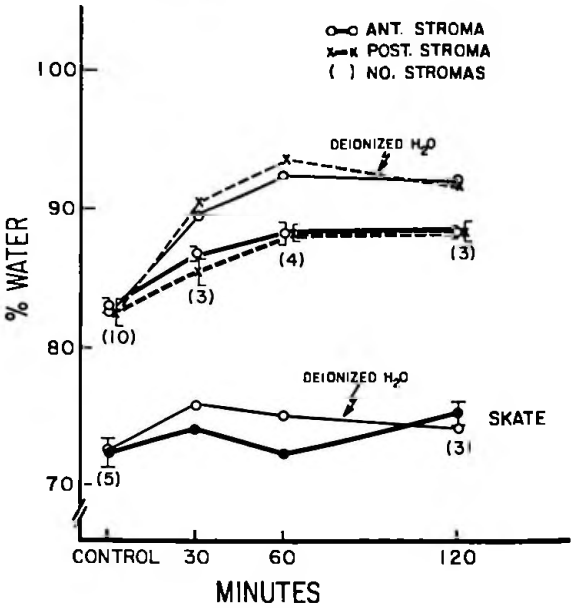


Figure 3.--Right. Corneal stromal swelling (mean ± SEM) of sculpin and skate in teleost Ringers and elasmobranch Ringers at 15°C.

SOLUBLE PROTEINS OF LONGHORN SCULPIN AND SHARK CORNEAS

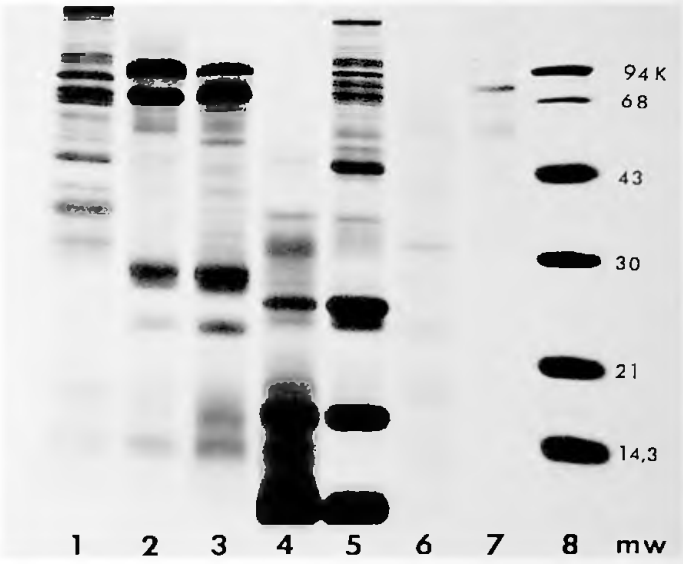
Dayle H. Geroski and Henry F. Edelhauser, Departments of Physiology and Ophthalmology, The Medical College of Wisconsin, Milwaukee, Wisconsin

These experiments were undertaken to characterize the water soluble protein content of a marine elasmobranch (Squalus acanthius) and a marine teleost (Myoxocephalus octodecimspinosus) cornea. Recently the major soluble protein of the bovine cornea has been identified, isolated and characterized (Alexander, Silverman and Henley, Exp. Eye Res. 32:205-216, 1981). This bovine corneal protein has an apparent molecular weight of 54,000 daltons and has thus been termed BCP54. It is also a major soluble protein in human and rabbit corneas and thus seems ideally suited as a macromolecular marker to investigate the biochemistry of corneal phylogeny. A second purpose of these studies was to determine if BCP54 is also a major soluble protein in corneas of marine species.

Freshly obtained corneas of the longhorn sculpin and dogfish shark (adult and pup) were separated into component layers as previously described (MDIBL, Bulletin 19:43-46, 1979). The isolated tissue layers were minced, pooled in 2.0 ml of 0.1M Tris-HCl (pH 7.6, 4°C) containing 0.005M PMSF and 0.015M EDTA, and homogenized. The homogenates were centrifuged at 30,000 x g for 30 min and the supernatants thus obtained were applied to a 12.5% polyacrylamide slab gel (2 mm) containing 0.1% SDS. Gels were electrophoresed for 16 hrs at 8mA, then stained in Coomassie Blue. Protein standards (BioRad) were included in each gel and molecular weights were determined from a plot of relative mobilities vs. log mw for the standard proteins (Weber and Osborn, J. Biol. Chem. 244:4406-4412, 1969).

SDS-PAGE reveals a complex spectrum of soluble proteins in each component layer of the two species studied (Fig. 1). Both striking similarities and differences are apparent in corresponding component layers of the sculpin and dogfish. The epithelium (lanes 1,4,5) is characterized by a particularly complex pattern of soluble proteins. A very prominent group of high molecular weight (67-83K) is present in sculpin and dogfish

pup epithelia but absent in the adult dogfish. Since the sculpin and pup corneas are very active metabolically in comparison to the much less active dogfish adult cornea (MDIBL Bulletin 18:38-40, 1978), it is tempting to postulate that these prominent high molecular weight bands are related to the metabolic machinery of these active corneas. In the dogfish adult most soluble protein is of low molecular weight. All three epithelia share at least two major protein bands (51K and 41.6K). In the dogfish three very striking bands are shared by the adult and pup at 27.5K, 16.5K and 10.7K. These three bands alone account for nearly 30% of the total soluble protein in the dogfish epithelium.



Stromal preparations are shown in lanes 2,3,6 and 7. The sculpin stroma, both inner and outer layers, is characterized by two very prominent bands at 79.4K and 69.9K. These two proteins account for one-third of total soluble protein in sculpin stroma. At least one major protein seems to be present both in sculpin and shark stroma. It has an apparent molecular weight of 25K.

Though none of the samples studied shows a major protein band at 54K, the presence of BCP54 cannot be ruled out in these corneas. The prominent band seen in sculpin and dogfish epithelia at 51K certainly could be the 54K or a closely related protein. Future studies using immunochemical methods will make the final determination.

BASOLATERAL POTASSIUM CONDUCTANCE IN FLOUNDER URINARY BLADDER

David C. Dawson and David Andrew, Department of Physiology, University of Michigan Medical School, Ann Arbor, Mi. and Cornell University, Ithaca, NY

We reported previously that the urinary bladder of the Winter Flounder actively secretes potassium and that net potassium flow can be measured directly as the short circuit current (I_{sc}) produced by the bladder in vitro (Dawson and Andrew, Bull. M.D.I.B.L. 20:89 1980). The results of previous experiments can be conveniently summarized by the simple model shown in Figure 1 in which potassium secretion is envisioned as consisting of two steps: potassium entry across the basolateral membrane via an electrogenic Na/K exchange pump and potassium exit from the cell via a barium-sensitive potassium channel in the apical membrane. Mucosal barium reversibly blocks I_{sc} and net K secretion, presumably by blocking an apical potassium channel. The present experiments were undertaken to investigate the possibility that barium might be used to identify a potassium conductance in the basolateral membrane.