AMP and calcium ionophore A23187 tended to increase while serotonin decreased the tissue conductance. It is postulated that cyclic AMP primarily inhibits Na and Cl influx from the mucosal surface into the tissue and that the increase in the serosal-to-mucosal Cl movement represents increased permeability in the brush border. Why calcium ionophore and serotonin have different effects on unidirectional fluxes is not known. While it is possible that this is related to different magnitudes in level of change in intracellular calcium content it is also possible that the basic effects on brush border or tight junction permeability of serotonin versus ionophore and/or cyclic AMP may differ. These studies were supported by NIH Grants AM26523, AM20700 (MD); AM27972 (JM); and AM17537 (JT). Dr. Donowitz is a recipient of NIH RCDA 1K04-00588.

CORNEAL ULTRASTRUCTURE AND SWELLING PROPERTIES OF THE SCULPIN AND SKATE CORNEA

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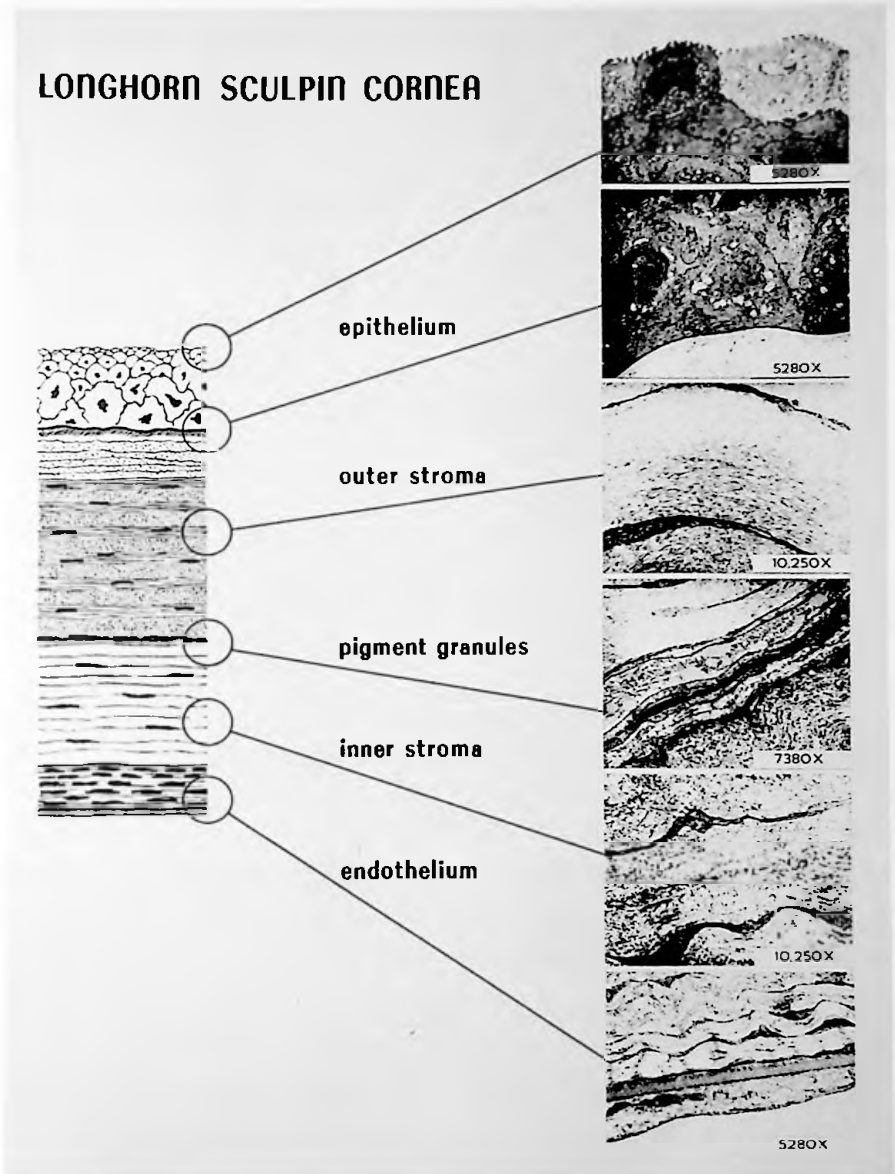
The objective of the current study is to describe the ultrastructure of the Longhorn Sculpin Cornea and to compare the swelling properties of the outer and inner stroma to that of the skate corneal stroma. The stromal swelling was compared in deionized water, teleost and elasmobranch Ringers.

For transmission electron microscopy the sculpin corneas were fixed in 3% glutaraldehyde in phosphate buffer at pH 7.2 and an osmolality of 350 mOsm for at least 8 hrs at 4°C (skate at pH 7.2 at 1000 mOsm). The corneas were then embedded, cut and stained according to our previous studies (Exp. Eye Res. 32:133, 1981). For the swelling studies, the eyes from sculpin (Myoxocephalus octodecimspinosus) and skate (Raja erinacea) were enucleated from euthanized animals, the corneas excised and incubated in 2 cc of either deionized H_2O, marine teleost or elasmobranch Ringers at 15°C in a Dubnoff metabolic shaking incubator. Control corneas and corneas following 30, 60 and 120 minutes of incubation were removed, blotted dry, weighed and dried at 120°C for 24 hrs and reweighed. The percent stromal water was calculated.

The ultrastructure of the sculpin cornea is shown in Figure 1. This cornea reveals a distinct epithelium, outer stroma, inner stroma, Descemet's membrane and an endothelium all of which correspond to the optical reflections of the corneal layers observed in the specular microscope by Fischer and Zadunaisky (Exp. Eye Res. 25:149, 1977). A prominent group of cells that contain pigment granules was also observed between the outer and inner stroma. The pigment granules give rise to the yellow pigmentation in the corneas of many teleosts first reported in 1933 by Walls and Judd (Br. J. Ophthalmol. 17:641, 1933). Other reports by Moreland and Lythgoe (Vis. Res. 8:1377, 1968) and Muntz (Vis. Res. 13:2235, 1973) have shown that marine fish corneas have an absorption maximum of 425, 450 and 480 nm. These authors have postulated that the corneal pigment acts as a filter to improve both visual acuity and contrast discrimination through reduction of chromatic aberration, overhead glare and short-wave scatter. The collagen of the inner stroma adjacent to the endothelium is compact with many cells that contain a highly developed endoplasmic reticulum, which contributes to the irridescent nature of the cornea as reported in many teleost corneas by Lythgoe (Proc. R. Soc. Lond. 188:437, 1975).

The swelling properties of the sculpin and skate corneal stroma following incubation in either deionized water or Ringers is shown in Figures 2 and 3.

In the sculpin cornea the anterior and posterior stroma swell markedly (89-92%) in deionized water. By comparison the skate cornea that contains sutural fibers (Smelser, Invest. Ophthalmol. 1:11, 1962) only shows a minimal increase in water content throughout the 120 min in deionized water (75%).



When the anterior and posterior stromal layers of the sculpin corneas are incubated in teleost Ringers, their water content increases to 88%. However, the skate stroma shows the same increase in water content following 120 min in elasmobranch Ringers (75%) as it did in deionized water. It can be concluded that, although both Ringers solutions are isotonic to the respective stromas, they are not iso-osmotic and stromal swelling ensues. Past studies by Anseth (Exp. Eye Res. 8:297, 1969) and recent studies in our laboratories have shown that as stromal swelling occurs in rabbit corneas there is a progressive loss of stromal proteoglycans and glycoprotein which can lead to collagen fiber aggregation. A similar phenomenon may occur in these corneas and may account for the continuous stromal swelling observed for the stroma in isotonic incubation media.

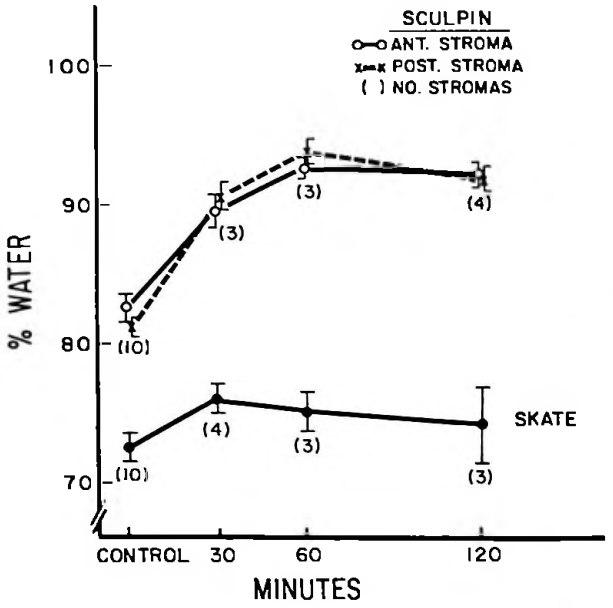


Figure 2.--Left. Corneal stromal swelling (mean+ SEM) of sculpin and skate in deionized water at 15°C.

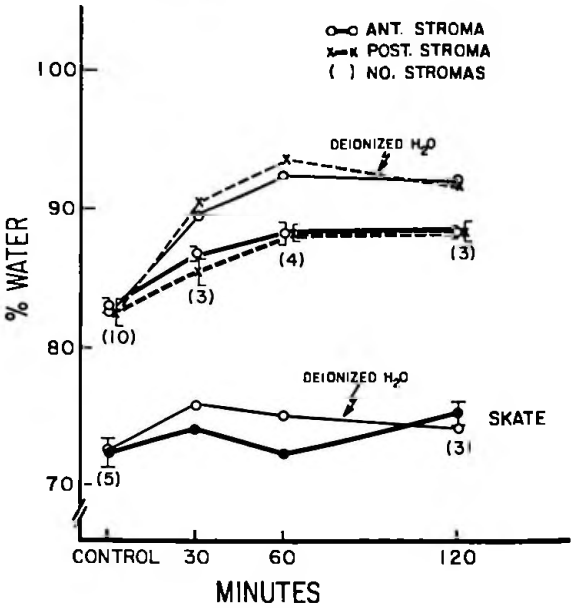


Figure 3.--Right. Corneal stromal swelling (mean ± SEM) of sculpin and skate in teleost Ringers and elasmobranch Ringers at 15°C.

SOLUBLE PROTEINS OF LONGHORN SCULPIN AND SHARK CORNEAS

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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