BARRIER FUNCTION OF THE CORNEAL EPITHELIUM IN FISHES

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The epithelium of the mammalian cornea serves as a barrier to movement of water and ions and prevents uptake of fluid from the tears by the corneal stroma. Therefore, the epithelium plays a role (secondary to the corneal endothelium) in maintaining corneal transparency. Because of this important function, numerous studies have been made of the corneal epithelial healing response after wounding (Ubels et al, J Toxicol--Cut Ocular Toxicol 1:133-145, 1982). The fish corneal epithelium is also a barrier to water and ions and plays the primary role in preventing corneal edema in these animals (Smelser, Invest Ophthalmol 1:11-32, 1962; Edelhauser et al, Am J Physiol 214:389-394, 1968). Freshwater and marine fish corneas are subjected to large osmotic and ionic gradients between the environment and the aqueous humor. Therefore, the barrier function of the epithelium may also help to maintain normal aqueous humor compositon. In contrast, there is essentially no osmotic gradient between tears and aqueous humor in mammals. Because of these structural and functional similarities and differences between mammalian and fish corneas, comparative studies are valuable in understanding ocular function in humans. Most studies of corneal epithelial barrier function have been conducted *in vitro*. The purpose of this study was to investigate the effect of removal of the corneal epithelium on aqueous humor composition *in vivo*. This work has relevance to clinical practice in that the composition of intraocular irrigating solutions used in surgery has profound effects on the function of cornea and lens (Edehauser et al, Arch Ophthalmol 96:516-520, 1978; Haimann et al, Am J Ophthalmol 94:594-605, 1982).

The species used in these studies were alewives (*Alosa pseudoharengus*), sculpins (*Myoxocephalus octodecimspinonsus*), skates (*Raja erinacea*), and dogfish sharks (*Squalus acanthias*). The alewives were taken from the Union River at Ellsworth, Maine, and were kept at the laboratory in freshwater (FW) or adapted to 37% seawater or 100% seawater for at least one week before use. The use of alewife enabled us to study the corneal epithelial barrier function in these varied osmotic environments.

Fish were anesthetized in MS-222, and the entire corneal epithelium was removed by scraping. At 6 hours or 24 hours the aqueous humor was removed from the eye through a 25- or 22-gauge needle attached to a 1-ml syringe and then analyzed for Na^+, K^+, protein, and osmolality.

In alewife adapted to seawater, removal of the corneal epithelium resulted in an increase in aqueous humor Na^+, K^+, and osmolality. In 37% seawater, control aqueous humor Na^+ was reduced compared with seawater adapted animals. Na^+ was increased at 6 hours after corneal wounding, but no changes in K^+ or osmolality were observed. By comparison, in alewive adapted to FW, both the aqueous humor Na^+ and osmolality were decreased. Further decreases in Na^+ and osmolality observed after corneal epithelial removal in FW, although not statistically significant, appeared to be physiologically significant since a cataract formed within 24 hours after removal of the epithelium. Similar cataract formation has been observed after removal of the corneal epithelium of rainbow trout (Ubels and Edelhauser, Cur Eye Res 2:613-619, 1983).

Table 1.--Aqueous Humor Composition Following Removal of the Corneal Epithelium (mean ± SE)

		(N)	Na+	K+	mOsm	Protein
			mEq/l	mEq/l		mg/ml
Alewife Seawater	control	(6)	182 ± 7.8	3.5 ± 0.1	368 ± 6.3	
	6 hr	(6)	196 ± 3.7	3.5 ± 0.1	416 ± 3.2*	
	24 hr	(6)	216 ± 6.0*	4.8 ± 0.2*	483 ± 4.6*	
Alewife 37% Seawater	control	(4)	147 ± 5.2	3.2 ± 0.1	367 ± 2.4	
	6 hr	(6)	169 ± 2.6*	3.5 ± 0.1	377 ± 10.8	
	24 hr	(6)	166 ± 7.2	3.6 ± 0.2	352 ± 6.9	
	plasma		179 ± 3.5	-	342 ± 4.4	
Alewife Freshwater	control	(8)	125 ± 4.9	3.1 ± 0.1	285 ± 8	0.4 ± 0.06
	6 hr	(6)	111 ± 7.7	3.3 ± 0.2	257 ± 8.9*	0.35 ± 0.8
	24 hr	(6)	111 ± 7	3.7 ± 0.2	261 ± 9.1	1.22 ± 0.12
	plasma		149 ± 5.4	3.3 ± 0.5	285 ± 17.1	35.2 ± 3.91
Sculpin	control	(6)	161 ± 3.6	2.9 ± 0.1	345 ± 5.3	0.46 ± 0.17
	24 hr	(6)	180 ± 6*	3.5 ± 0.1*	375*± 9.2	0.53 ± 0.16
	plasma		181 ± 3.8	4.1 ± 0.2	331 ± 5.5	30.5 ± 1.08
Skate	control	(4)	248 ± 22.2	3.5 ± 0.4	918 ± 17.3	
	6 hr	(4)	308 ± 14.9*	5.6 ± 0.5*	903 ± 13.5	
Shark	control	(9)	274 ± 11.6	5.3 ± 0.7	889 ± 16.0	5.56 ± 0.58
	6 hr	(3)	339 ± 1.8*	7.2 ± 0.3*	944 ± 36.6	6.5 ± 0.86
	24 hr	(6)	-	6.9 ± 0.1*	936 ± 10.3*	6.9 ± 1.10
	plasma		253 ± 6.9	3.9 ± 0.5	931 ± 13.0	15.6 ± 1.0
Seawater		(8)	431 ± 9.3	9.7 ± 0.3	898 ± 8.1	
37% Seawater		(2)	161	3.6	346	

* Significantly different from control $p \leq 0.05$

No changes in sculpin aqueous humor were observed 6 hours after wounding; however, at 24 hours Na^+, K^+, and osmolality were increased. These changes were also accompanied by cataract formation. In the skate at 6 hours after wounding, the Na^+ and K^+ in aqueous humor were significantly increased while osmolality was unchanged. In shark aqueous humor Na^+, K^+, and osmolality were also elevated compared to control values 6 hours after wounding. At 24 hours K^+ and osmolality became elevated while urea was reduced to 150 mM from its normal value of 350 mM.

Protein concentration changes in aqueous humor showed no predictable pattern after corneal wounding but tended to be elevated compared with control values. This probably represents a breakdown in the blood aqueous barrier or an inflammatory response due to the removal of the epithelium.

The data obtained in this study illustrate the importance of the corneal epithelium as a barrier to ion and water movement between the environment and the aqueous humor since significant changes in aqueous humor composition occur upon removal of the corneal epithelium. These studies also show the necessity of a fast, adaptational epithelial healing once an epithelial wound occurs (Ubels and Edelhauser, Cur Eye Res 2:613-619, 1983) in order to maintain ocular function. (This research was supported in part by NIH grants EY04069, EY00933, and ES01985.)