Table 1

	Inulin Space (%)	Intracellular Electrolytes (mEq/1) (Na)	(K)	(Cl)
Mean	29.00	42.1	161.6	116
SD	1.69	8.4	19.1	23.7

the viability of the preparation.

The presence of a patent lumen (even in glands unstimulated by theophylline or dibutryl cyclic AMP) containing fluid of very high Na and Cl content was thought to complicate the interpretation of intracellular electrolyte measurements made on whole tissue. The following preparation permitted the free entry of labeled inulin into the lumen, and correction for this extracellular compartment.

Freshly harvested rectal glands from 5 dogfish were sectioned by means of a Stadie-Riggs microtome so that the individual glandular tubules were cut in cross section (Fig. 2). Sections approximately 200 in thickness and 4-5 mm in diameter were then observed under a dissecting microscope to ensure that the direction of sectioning was correct; lumina of the sectioned tubules appeared to be patent, giving a "doughnut-like" appearance. The sections were then transferred to conical flasks where they were incubated at 15°C, with air bubbling, in 4 ml of dogfish Ringer's to which ^{14}C inulin (50 ci/ml) was added. Three sections from each gland were incubated for 15 minutes and another section for 30 minutes to ensure complete labeling of the extracellular space. For the determination of total extracellular space, sections were removed from the flasks, blotted dry on filter paper, weighed, placed in counting vials and extracted in 0.4 ml NCS tissue solubilizer (amersham/Searle Corp., Ill.) for 24 hours. The tissue was then reweighed. For Na and K determinations the tissue was digested in 100 l of concentrated nitric acid for 24 hours, and Na and K concentrations measured using a flame photometer. For chloride determination, the dried tissue was boiled in 100 l of deionized water in sealed tubes for 2 hours and chloride concentration measured using a Cotlove chloridometer.

The results of the above measurements are depicted in Table 1. The measured inulin space of 29±1.69% of wet tissue weight is slightly, but significantly greater than the value of 26.7±1.2 obtained in vivo by Silva et al. (*Bull. MDIBL, 14: 116, 1974*). There was no difference between the inulin space determined at 15 and 30 minutes incubation and only the former values are listed. intracellular electrolyte concentrations agree reasonably closely with the previously reported in vivo measurements of Silva et al; however, both intracellular Na and Cl concentration are higher in the present studies.

These studies demonstrate that in vitro studies on isolated tubules of the dogfish rectal gland are possible and that further useful information may be derived using the technique of in vitro perfusion of single tubule segments.

Supported by NIH Grants AM 03853 and HL 05928.

17 • 1975

The Electrical And Hydrophilic Properties Of Fish Cornea

Frank H. Fischer and Jose A. Zadunaisky, New Yo
University Medical Center, New York, N.Y.

The transparency of the cornea is linked to th optical properties of its connective tissue layer, called th stroma. The optical properties depend on the degree o hydration of the stroma, and the hydration is contro by ionic pumps located in the epithelium and endotheliu of the cornea. There is ample evidence for the propert of the cornea of mammalians and amphibians, howeve studies extending to the lower vertebrates and inve tebrates have been less frequently reported.

We have examined the electrical properties, thic ness and hydration characteristics of the corneas of a elasmobranch, the spiny dogfish, *Squalus acanthias* a some teleosts, the sculpins' *Myoxocephalus octodece spinosus* and *Myoxocephalus scorpius*, and one specim of the cod fish, *Gadus callarias*. The background to the studies is that sharks' corneas are known not to swel become opaque. On the other hand, the cornea o teleosts is known to swell and in this sense they a similar to amphibians and mammalians. (Smelser, Inves Ophthal, 1:1, 1962). However a detailed study of th thickness and electrical properties of these fish cornea does not exist. In the case of the shark cornea, previou attempts to determine the existence of any electric potential difference across it, when placed as membrane in a lucite chamber showed that the resistan was extremely low. In fact, we have found that this is du to a lesion of the corneal epithelium produced durin collection of the specimens, because of rubbing of th corneal epithelium against the sand paper like skins of th companion dogfish in the catch. Appropriate protectio was provided, by harnessing the recently caught dogfis with a protection made of metal wires and rubber tha prevented lesions to the cornea. The protective harness shown in Figure 1. The lesion of the epithelium is eas observed if a few drops of fluorescein are instilled in th eyes of the fish. This classical test shows the geographi borders of the lesion of the epithelium where fluoresce penetrates. Figure 2 shows the lesions produced i unprotected animals. These lesions do not occur in th protected eyes. The values for potential differences an resistances observed in protected corneas, unprotecte and others handled specially are shown in Table I. Th corneas were disected out and mounted in special lucit chambers for corneal research of an aperture of 1 cm.^{2} i is clear that the results that approximate more to th

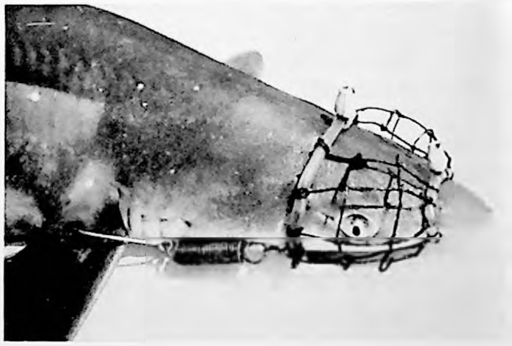

Figure 1

ormal conditions are those in protected eyes. The esistance reached an average value of more than 1000 hms. cm.^{2}, in contrast with the unprotected eyes where e resistance was only 134 ohms cm.^{2}. An intermediate alue of 378, still extremely low, was found when the ead of the dogfish was sectioned immediately after apture, and kept on ice until disection. The potential ifference was virtually nihl in the corneas when rotected, with the high resistances. The average otential difference was +0.2 mV which resulted from alues that ranged between -0.7 and +0.9 mV; the + or sign indicates the polarity of the epithelial side. On the ther hand the electrical potential was of the same small agnitude in the corneas with the low resistance. It is our onclusion that the epithelium f the dogfish cornea does not roduce a sizable potential dif-erence. These results in the artk contrast, from the elec-cal point of view, with the ndings in the teleosts corneas Table 1). The resistances of e sculpin corneas were al-ays in a higher range, most of em above 1000 ohms. cm.^{2} d, most importantly, they owed a potential difference ways oriented epithelial side egative of an average value of 5 mV. In this sense, they are, om the electrical point of view, so similar to amphibians and ammalians. The corneas of ese teleosts were short cir-ited and the average value tained was 1.8 uAm/cm.^{2} e have not yet completed the aluation of the best *"in vitro"* perimental conditions for the olated teleost corneas, therefore these potential differences may well be in the lower range of those values the teleost cor-eal epithelium is capable of roducing.

Figure 2

Table 1

Electrical Properties of Protected and Unprotected Corneas of the Spiny Dogfish, *Squalus acanthias*

	Protected Eyes		Unprotected Eyes		Special Handling*	
	Potential Difference mV	Electrical Resistance ohms.cm^2	Potential Difference mV	Electrical Resistance ohms.cm^2	Potential Difference mV	Electrical Resistance ohms.cm^2
MEANS	+0.2**	1010	+0.2	134	-0.3	378
S.E.M.	±0.1	+186	±0.1	±55	±0.4	±101
	17	17	4	6	4	4

* No harness was used on these fish. The heads were severed immediately after capture, and kept on ice until dissection at the laboratories.

** The average represents the algebraic sum of values ranging from — .07 to +0.9 mV will 11 cases epithelial side + and six cases epithelial side — . It is interpreted that the P.D. oscillates around 0, in spite of the high values of R.

The differences between the values of R for the three groups were statistically significant.

Table II

Electrical Properties of Isolated Corneas of Some Teleost Fish

	Potential Difference mV	Electrical Resistance ohms.cm^2	Short Circuit Current uAmp/cm^2
Longhorn sculpin (*Myoxcephalus octodecimspinous*)	1.7±0.2* (13)	1540±256 (11)	1.4±0.3 (6)
Shorthorn Sculpin (*Myoxcephalus scorpius)*	1.5±0.2 (3)	1744±525 (3)	1.5±0.0 (2)
Codfish (*~~Gadus callarias~~*))	1.3 (1)	743 (1)	1.8 (1)

* Means ± SEM. The potential difference was always oriented epithelial side negative.

The thickness of the cor-eas was determined with the pecular microscope. This instrument permits the determi-ation of corneal thickness *in ivo* or *in vitro* with an accuracy of 1%. The corneal thickness was measured 1) in an intact nucleated eye, the corneal epi-helium bathed with the appropriate Ringer solution or 2) he excised cornea was mounted in an apparatus and an rtificial anterior chamber perfused with Ringer by means of a pump. The thickness of the corneal layers is measured as the distance in microns between images reflected y the different layers.

Thickness for whole cornea, epithelium and depth of anterior chamber for *Squalus acanthias* before and after exposure to low temperature are shown in Table 2. The values found coincide with previous quanitative evaluation on the basis of histological sections. The most interesting fact is that practically no change in thickness, therefore in hydration, occurs after exposure to 2°C for more than 12 hours.

Table III

Thickness of the Cornea Measured with the Specular Microscope for an Elasmobranch and a Teleost and the Effect of Low Temperature

Spiny Dogfish (*Squalus acanthias*)	Control				Low Temperature*		
	Total Thickness		Epithelium	Depth of Anterior Chamber	Total Thickness		Epithelium
	Central	Periferal			Central	Periferal	
Means	161	184	66	342	159	155	67
±SEM	9	17	6	102	11	13	3
N	14	9	6	6	7	7	4

Longhorn Sculpin (*Myoxcephalus Octodecimspinous*)	Epithelium	Intermediate Layer of Stroma	Descemet-Enoothelium	Total Thickness of Cornea		Depth of Anterior Chamber
				Control	Cold *	
Means	14	124	213	241	820	725
±SEM	3	7	16	12	90	129
N	4	7	4	7	7	6

* Eye kept overnight a 2° C covered with Ringer.

In the teleosts the images obtained were 5. They correspond to the layers of relexion of the images in the complex cornea of these fish. The first two images corresponded to the anterior and posterior portion of the epithelium, the third to the anterior or dermal portion of the stroma, and the fourth and fifth to the outer and inner limits of the Descemetendothelial zone. The actual thickness of the regions and the effect of low temperature is shown in Table 3. It is evident that the cornea of teleosts swells and increases its thickness in the cold.

In summary, the ancient cornea of the shark, possesses the ability to remain at normal hydration and thickness in adverse metabolic conditions, and at the same time, no significant electrical potential difference is found arising from the epithelial layers. On the other hand, in the more recent teleosts, the cornea swells in the cold and shows he consistent presence of an electrical potential difference arising from the epithelium, similar to amphibians and mammalians. Specular microscope measurements of corneal thickness *in vivo* and in mounted corneas of sharks and sculpins is reported.

This work was supported by research grant #01349 of the National Eye Institute, National Institutes of Health, and a grant from the Seeing Eye Foundation, Inc.

18 • 1975

Plasma And Urina Proteins In The Aglomerular Teleost, *Lophius americanus*

R. Galaske, State University of N.Y. at Buffalo and Medizinische Hochschule, Hannover

Bieter, using the less sensitive methods of his time, foud no protein in the urine of *Lophius* (*J. Pharm. and Exper. Therap. 43:406, 1931*). His finding that injected hemoglovin appeared in the urine of glomerular but not of aglomerular fish led to the conclusion that most urinary protein was of glomerular origin.

A more valid examination of the renal handling of plasma proteins in aglomerular fish has been made possible by the recent develoment of micro techniques of polyacrylamide gel electrophoresis. The appearance of any plasma protein in the urine of an aglomerular fish is of interest with respect to its mode of access, secretory or otherwise. Moreover, since filtration and tubular reabsorption of low molecular weight proteins (LMW) are important processes in their catabolism in a filtering kidney we may question if accumulation in plasma or alternate pathways of disposal for LMW proteins have evolved with the loss of glomerular function.

In this report data are presentd for the renal clearance of plasma proteins by *Lophius* and for the clearance of an inert macromolecule (PVP) injected intravenously.

Clearance experiments were performed in male fish weithing from 2000 to 3000 g. Fish were placed ventral side up with head and gills submerged. Both ureters were catheterized with PE tubing through a ventral abdominal incision. Blood samples were taken from the caudal vein. Total protein concentration in plasma and urine was measured by the Lowry method. Micro-polyacrylamide electrophoresis with a continuous gradient from 2 to 40% was performed on diluted plasma and unconcentrated urine samples. Some urine samples were concentrated by ultrafiltration in dialysis tubing (Union Carbide) and Amicon filters(UM 2). Concentrated urine or unconcentrated plasma was separated by gel filtration on Sephadex G 100, G 75 and G 50 columns. Reconcentrated eluted fractions were again separated by gradient gel electrophoresis. One fish was injected with PVP (MW 40,000) and renal clearance and plasma disappearance curve of macromolecules were determined.