Table 3. Taurine S/M ratios in dogfish kidney slices incubated in vitro

Medium	Inhibitor	S/M ratio
EIM*	--	1.77 ± 0.18(9)
EIM (-) Na^+ ‡	--	0.27 ± 0.02(6)+ ‡
EIM (-) K^+ ‡	--	1.59 ± 0.10(6)
EIM (-) Cl^- ‡	--	0.75 ± 0.05(3)+
EIM	Ouabain (0.1mM) no preincubation	1.10 ± 0.08(6)+
EIM	Ouabain (0.1mM) 40 min preincubation	0.67 ± 0.03(3)+
EIM		
EIM	2,4 dinitrophenol (0.1mM)	0.93 ± 0.05(6)+
EIM	Furosemide (0.1mM)	1.56 ± 0.11(3)
EIM	Probenecid (1mM)	2.55 ± 0.36(4)
EIM + Na_2SO_4 (10mM)	--	2.34 ± 0.27(9)+
EIM + $Na_2S_2O_3$ (10mM)	--	1.92 ± 0.19(6)

Values are means ± S.E. Numbers of slices in parentheses.

*EIM = elasmobranch incubation medium.

+Significantly different from value in unmodified EIM.

‡Na^+ replaced by choline, K^+ replaced by Na^+, Cl^- replaced by mixture of $SO_4^=$ and mannitol.

S/M = slice to medium ratio (dpm · g slice^{-1}/dpm · ml medium^{-1}).

Chloride-free incubation medium and preincubation with ouabain both inhibited taurine uptake partially, as indicated by the ratios of 0.75 and 0.67. This is in contrast to the findings in the rat by Awapara and Berg, in "Taurine" ed. R. Huxtable and A. Barbeau, Raven Press, N.Y. (1976) where ouabain did not inhibit taurine accumulation by kidney slices, so a Na-dependence exists. Inhibition of all oxidative phosphorylation by 2,4 dinitrophenol resulted in a tissue/medium ratio of 0.93 indicating that taurine can enter the cell under these conditions but no concentration gradient can be established. The stimulation of taurine transport by sulfate and inhibition by removal of chloride suggest that taurine may be transported into the cell by a low specificity anion transport system. Supported by NSF PCM 75-14322 and NIH HLO4457. Dr. H. Schröck was supported by a stipendium from the Deutsche Forschungsgemeinschaft (West Germany) No. Schr. 215/1.

ACTIVITIES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE, ALDOLASE, LACTATE DEHYDROGENASE, AND ISOCITRATE DEHYDROGENASE IN SCULPIN AND DOGFISH CORNEA AND MUSCLE

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

In mammalian species, the cornea comprises the major refractive component of the eye's optical system. Thus, its transparency is of crucial importance. The limiting layers of the cornea (epithelium and endothelium) maintain this transparency by keeping the hydrophilic stroma at a minimum of physiologic hydration. These layers serve this function by providing 1) barriers to the movement of ions and water, and 2) metabolic pumps which actively maintain

proper stromal hydration. Energy derived from the metabolism of glucose is essential for both the barrier and pump functions of the epithelium and endothelium. ATP generated by glycolysis and the TCA cycle is necessary to fuel both the energy dependent pumps and the synthetic reactions necessary to maintain proper barrier function. Additionally, the NADPH provided by the hexose-monophosphate shunt (HMS) is essential for cellular redox balance and anabolic reactions - both of which are intimately related to corneal transparency.

In aquatic animals, corneal transparency is equally essential for proper ocular function. Little is known, however, about glucose metabolism in corneas of these species, particularly as it relates to the maintenance of corneal transparency. We have previously measured corneal oxygen consumption in marine teleosts (sculpin, flounder, and goosefish) and elasmobranch (dogfish) (Bull. MDIBL 17:7, 1977). Further, we have shown that the HMS is a prominent pathway of glucose oxidation in the sculpin, adult dogfish and dogfish pup. Oxidation of intracellular glutathione by diamide produces a marked stimulation of HMS activity in these corneas. (Bull. MDIBL 18:38, 1978).

In this study, specific activities of representative enzymes of the major pathways of glucose catabolism were determined -- both in the cornea and for comparison in muscle.

Eyes were enucleated and corneas excised from sculpin (Myoxocephalus octodecimspinosus), adult spiny dogfish (Squalus acanthius), and the intrauterine dogfish pup. For enzyme analysis, corneal tissue samples were collected, minced and homogenized at 4°C in 1.5 ml of 0.1M Tris HCL buffer (pH 7.6). The samples were as follows: (1) the epithelia from one pair of adult shark corneas - obtained by scraping, (2) the epithelia from ten intrauterine shark pup corneas - collected similarly - the remaining endothelium and stroma were also retained for analysis, (3) corneas from four sculpin were separated into outer and inner layers, and enzyme activity was assayed in each layer. Muscle samples (0.7 - 1.0 g) were taken from the tails of the adult shark, shark pup and sculpin. Each muscle sample was minced and homogenized at 4°C in 5.0 ml of Tris buffer. All samples were centrifuged for 20 min. at 7500 g (4°C). Aliquots of the supernatants were assayed for glucose-6-phosphate dehydrogenase (G6PDH), aldolase, lactate dehydrogenase (LDH), and isocitrate dehydrogenase (ICDH). The assays were NADH (for aldolase and LDH) or NADPH (for G6PDH and ICDH) coupled. A protein determination (BIO-RAD) was performed on a portion of each supernatant.

Corneal enzyme activities are shown in Table 1, and the corresponding muscle values in Table 2.

Table 1. Corneal Enzyme Activities (mU/mg protein) in Dogfish and Sculpin (MEAN ± SEM)

	G6PDH	ALDOLASE	LDH	ICDH
Dogfish Adult				
Epithelium	51.4 ± 5.6	54.3 ± 2.4	1286 ± 15	18.7 ± 1.9
n	6	6	6	6
Dogfish Pup				
Epithelium	40.2 ± 8.0	82.4 ± 18	1450 ± 171	25.6 ± 18
n	4	4	5	5
Endothelium + Stroma	16.1 ± 5.4	62.8 ± 16	567 ± 111	55.7 ± 12.8
n	4	4	5	5
Sculpin				
Outer Cornea	46.2 ± 6.5	13.1 ± 1.3	20.7 ± 3.1	21.9 ± 1.3
n	6	6	6	6
Inner Cornea	4.9 ± 0.8	6.3 ± 0.9	53.0 ± 3.4	22.4 ± 1.4
n	6	6	6	6

Table 2. Muscle Enzyme activities in Dogfish and Sculpin (MEAN ± SEM)

	G6PDH	ALDOLASE	LDH	ICDH
Dogfish Adult				
White Muscle				
mU/mg protein	0.64 ± 0.07	6972 ± 653	8166 ± 1348	90 ± 19
U/g fresh wt	0.017 ± .002	183 ± 17	215 ± 35	2.4 ± 0.5
n	5	5	8	7
Red Muscle				
mU/mg protein	3.7 ± 0.4	671 ± 151	640 ± 82	719 ± 82
U/g fresh wt	0.12 ± 0.01	22 ± 5	21 ± 2	24 ± 3
n	5	8	8	8
Dogfish Pup (White Muscle)				
mU/mg protein	3.5 ± 0.1	4972 ± 1013	7998 ± 1323	134 ± 26
U/g fresh wt	0.086 ± 0.002	123 ± 25	197 ± 33	3 ± 0.6
n	5	7	7	7
Sculpin (White Muscle)				
mU/mg protein	1.9 ± 0.2	1354 ± 243	3983 ± 423	152 ± 24
U/g fresh wt	0.057 ± 0.006	40 ± 7	119 ± 12	4 ± 0.7
n	4	7	8	8

Values are expressed as milli-international units per milligram tissue protein. In addition, the data for muscle is presented as international units per gram muscle wet weight.

The initial reaction of the hexose-monophosphate shunt is the enzymatic dehydrogenation of glucose-6-phosphate by the enzyme glucose-6-phosphate dehydrogenase. Of the corneal tissues studied, the highest activity of G6PDH was measured in the adult dogfish epithelium (51.4 mU/mg protein), all epithelia and outer corneal layers show similarly high activities. The lowest activity was measured in the sculpin inner cornea, and muscle activities of this enzyme are considerably lower than the corresponding corneal values (Table 2).

Aldolase, an enzyme of glycolysis, is found at the highest activity of corneal tissue in the dogfish pup epithelium (82.4 mU/mg protein) and endothelium + stroma (62.8 mU/mg protein) . The sculpin inner cornea has the lowest aldolase activity. Muscle activities of this enzyme are markedly higher than those of the cornea.

The reversible oxidation of lactate to pyruvate is mediated by lactate dehydrogenase. In corneal tissue, this enzyme is found at relatively high activities in the dogfish epithelium, both of the adult and pup. Considerably lower activities are seen in the corneal layers of the sculpin. White muscle exhibits extraordinarily high activities of this enzyme (8166 mU/mg protein), with red muscle activity being only 640 mU/mg protein.

Finally, the carboxylic acid cycle enzyme isocitrate dehydrogenase mediates the oxidative decarboxylation of isocitrate to form α-ketoglutarate. Corneal activities of this enzyme are similar except for the dogfish pup endothelium and stroma which shows an activity twice that assayed in the other corneal samples. Muscle activities of this enzyme are all considerably higher than corresponding corneal values. Activity in red muscle is especially high.

The transparency of the elasmobranch cornea is ensured by both the impermeability of this tissue and sutural fibers which interconnect stromal collagen lamellae and thus limit stromal swelling (Goldman and Benedek, Invest.

Ophthal. 6: 574, 1967). By comparison, the teleost cornea is also impermeable but lacks the sutural fibers; consequently, this cornea contains more water and does swell when metabolism is inhibited. The potential difference measured in these corneas is probably related to ion transport for deturgescence (Fischer and Zadunaisky, Exp. Eye Res. 25: 149, 1977).

In addition to its protective role, the epithelium of both the elasmobranch and teleost cornea is highly impermeable and functions as a barrier to electrolytes and water. To maintain this function, the epithelium has a high mitotic index which is reflected by this layer's high metabolic activity. Glycolysis, the hexose-monophosphate shunt, and the tricarboxylic acid cycle are well developed in this layer. Glucose oxidation by the HMS and TCA cycle is crucial to the metabolic requirements of this rapidly dividing tissue. Hence, it is not surprising that representative enzyme activities of these pathways are quite similar in both species.

Stromal swelling of the elasmobranch cornea is limited by the passive physical restraint of the stromal sutural fibers. The metabolic requirements of this type of passive mechanism are minimal. It was not possible to measure enzyme levels in the elasmobranch endothelium and stroma. The enzymes are present in extremely low activities which is further manifest by the low metabolic rate of this tissue (Edelhauser and Geroski, Bull. MDIBL, 18: 38, 1978). The endothelium-stroma of the developing dogfish pup cornea, on the other hand, is metabolically quite active. The energy requirements of this developing pup tissue are manifest in the high activity of the TCA enzyme ICDH -- the highest activity measured for any of the corneal tissues studied.

The sculpin cornea, lacking the sutural fibers seen in the elasmobranch, must rely on active transport mechanisms to control stromal hydration. The metabolic requirements of this tissue might be expected to be somewhat greater than that of the corresponding layer (endothelium and stroma) of the adult dogfish, but somewhat less than that of the developing dogfish pup. Enzyme activities of this layer of the sculpin cornea do fall between those of the adult and pup dogfish corneas.

The lower activities of G6PDH measured in muscle suggest that the HMS is less active in this tissue. It is not surprising that the activity of this pathway is greater in actively dividing tissue of the corneal epithelium. Activities of glycolytic and TCA enzymes, by comparison, are much higher in muscle. Of particular importance is the high ICDH to LDH ratio in red muscle compared to white muscle of the shark. The production of ATP would be much greater in red muscle than in white muscle. This data is consistent with the energy requirements anticipated on the basis of the tissue's function.

It thus seems that the measured enzyme activities reflect the particular adaptations made by corneas of marine elasmobranchs and teleosts to extremely different osmotic gradients which enable these tissues to remain transparent.

This work was supported in part by the National Eye Institute grant EY00933.

DIFFERENTIAL INHIBITION OF NaCl ABSORPTION AND SHORT-CIRCUIT CURRENT IN THE URINARY BLADDER OF THE WINTER FLOUNDER, PSEUDO-PLEURONECTES AMERICANUS

David C. Dawson and David Andrew,* Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA and *University of Maine, Orono, Maine.

Ion transport by the isolated urinary bladder of the winter flounder has been previously investigated under conditions of continuous perfusion (Renfro et al, Amer. J. Physiol. 228:52, 1975; Renfro, J. Exp. Zool. 199:383, 1978). These studies showed that the isolated bladder: (1) actively absorbed sodium and chloride at virtually identical rates and (2) was characterized by a small (1-5 mV) mucosa-positive transmural electrical potential difference P.D.). Ouabain abolished both the transmural P.D. and active NaCl absorption.

The purpose of our studies was to investigate the possible interdependence of active ion transport and the electrical properties of the flounder bladder under conditions where the tissue could be mounted as a flat sheet and