26 μmoles/g·min and deaminating activity was 2.3 μmoles/g·min. Activity levels of gluconeogenic enzymes were 1.44 μmoles/g·hr for PEP carboxykinase, 33.6 μmoles/g·hr for fructose-1,6-diphosphatase, and 1.68 μmoles/g·hr for glucose-6-phosphatase. Transaminase activity could not be detected by spectrophotometric or by radiometric methods using a number of amino acids (asp, met, ala, ser, gly, leu, orn, arg, cys) as the amino donor. Other sources of renal ammonia including the glutaminase reaction and purine nucleotide cycle were not assayed.

The identification of glutamate dehydrogenase and the gluconeogenic enzymes in dogfish kidney extracts indicates the potential for renal ammoniagenesis and support the results of *in vivo* experiments showing the ability of the dogfish kidney to increase renal ammonia excretion in response to an acid load. The inability to demonstrate transaminase activity leaves the source of renal ammonia in question. Glutaminase and purine nucleotide cycle enzyme activities need to be investigated. This work was supported by NSF grant PCM 75-14322.

ION TRANSPORT IN ELASMOBRANCH AND MARINE TELEOST LENSES WITH PARTICULAR RESPECT TO HCO_3^- MOVEMENT.

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Elasmobranch and marine teleost lenses, unlike those of "higher" vertebrates, lack carbonic anhydrase (Maren, T.H. and B.R. Friedland, Bull. MDIBL., 18:79, 1978). Ion transport studies in these species may elucidate the relationship between carbonic anhydrase and the movement of bicarbonate and other ions in this tissue. Last year, we reported preliminary initial efflux rate constants for chloride and bicarbonate (Friedland and Maren, Bull. MDIBL 18:82, 1978). This study reports steady state entry and efflux rates for labelled Na^+, $K(Rb)^+$, Cl^-, and HCO_3^- using *in vitro* lens incubation techniques. *Squalus acanthias* lenses were used in most cases; data on ^{86}Rb uptake in the flounder lens is included. Additionally, we report intracellular ion concentrations, electrical potential, and Na-K ATPase activity of the elasmobranch lens.

Table 1 gives the electrolyte composition of freshly dissected lenses of *S. acanthias*. There are several unusual

Table 1. Comparison of lens (intracellular) and aqueous (extracellular) ionic composition in *S. acanthias* with nernst equilibrium potentials. Values are ±s.e.m., number of samples (n)

	Aqueous* (mM)	Lens[@] (meg/kg lens H_2O)	Lens Intracellular[+] (mM)	Calculated Lens Nernst Potential (mV)
Na^+	279±6 (9)	54±1.6 (15)	40±1.6	+50±3
K^+	5±1 (10)	61±1.1 (18)	61±1	-64±0.4
Cl^-	253±5 (9)	77±2 (18)	63±2	-36±1
total CO_2	8.5±0.3	33±0.6	32±0.6	+34± .5
Urea	350	340±26	323±23	

*from Maren, T.H., Comp. Biochem. Physiol., 5:193, 1962.

[@]Lens water content was measured to be 54±2%.

[+][Intracellular] calculated assuming a 5% extracellular space and extracellular concentrations equal to aqueous.

features. It appears hypotonic, like the cephalopod lens (Duncan, G., in The Eye, Davson, H. and L.T., Graham, ed., Academic Press, 5:357, 1974). However, unmeasured amino acids (i.e. taurine) and trimethylamine oxide may account for the deficit. Secondly, the K/Na ratio is considerably lower than that generally seen in tissues, including mammalian and frog lenses (Duncan, vide supra). Finally, total CO_2 (or HCO_3^-) is very high compared to aqueous humor. Little other data on this point are available; Kinsey (Doc. Ophthalmolgica, Proceedings Series, 8:310, 1976) found values 30% less in lens than aqueous in the rabbit, and we currently find HCO_3^- in rat lens to be about the same as in aqueous.

Membrane potential was measured with a glass microelectrode (tip diameter 0.1 micron) filled with 3M KCl. The lens bathing solution was dogfish Ringers with $[HCO_3^-]$ of 8.5 mM. The measured potential across the lens was -61 mV, agreeing closely with the calculated potential for potassium distribution (Table 1).

Potassium. The influx of ^{86}Rb (as a K^+ tracer) was studied using the method of Becker (Invest. Ophthalmol. 1:502, 1962). The appropriate fish Ringers solution was used, gassed with 1% CO_2 in air. $[HCO_3^-]$ was 8.5 mM. Lenses weighing 50-800 mg were incubated in 5 ml, with tubes continuously and gently shaken at 15°C. Figure 1 shows the data. The substantial difference between adult Squalus and pup Squalus lens uptake can be related to the ~5 fold difference in surface area. The influx rate in pup lens is given by the rate constant (0.0016 min^{-1}) x K^+_{out} (5mM) = 8 μM/min (see also Table 3). The high rate in flounder (even compared to rabbit) is unexplained. Size (50-100 mg)

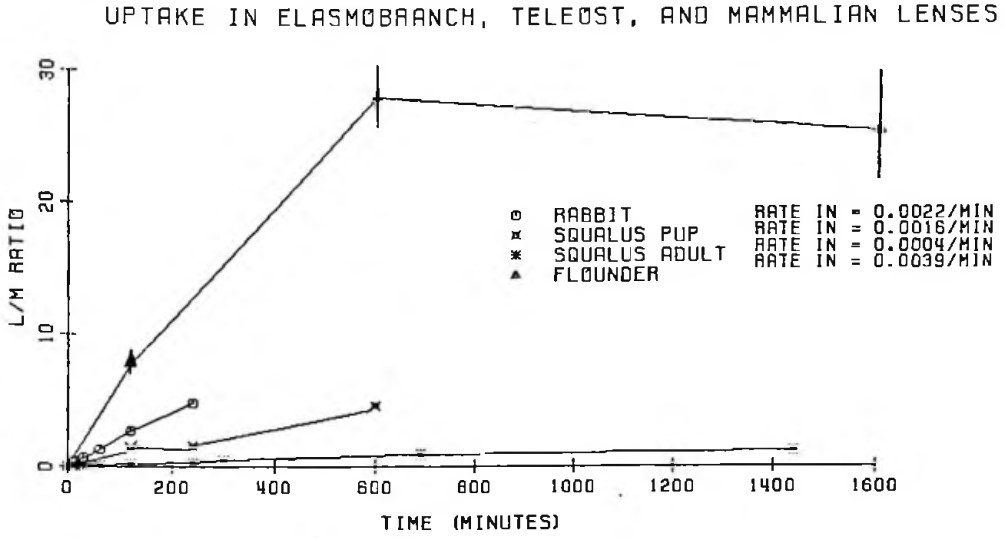

Figure 1. ^{86}Rb uptake in elasmobranch, teleost and rabbit lenses. Lenses were incubated with 2-3 μCi ^{86}Rb per lens. The symbols are the size of 1 standard error. $n \geq 4$ for each point; n = 6 for each point in rabbit and flounder experiments.

is comparable to dogfish pup and both lack carbonic anhydrase. The Lens/Medium isotope ratio at equilibrium agrees well with the K^+ ratio found in the cod, Gadus morhua (Duncan, vide supra).

Figure 2 shows the inhibitory effect of Ouabain (2×10^{-5}M) on ^{86}Rb influx in both Squalus and flounder lenses. Ouabain lowered k_{in} from 0.0016 min^{-1} to 0.0006 min^{-1} in Squalus and from 0.0024 min^{-1} to 0.0007 min^{-1} in flounder. In connection with this experiment, we found Na^+-K^+-ATPase activity in lens epithelium and capsule (n=4) of 0.4 μmoles Pi/mg protein/hr. (147 mmoles Pi/kg wet weight/hr); this agrees well with data of Bonting (invest. Ophthalmol. 4:723, 1965) for mammalian lenses. Earlier data (Jampol et al Bull. MDIBL 11:47, 1971) suggesting little or no Na-K-ATPase activity in Squalus lens was probably due to dilution of epithelial activity by the remainder of the lens.

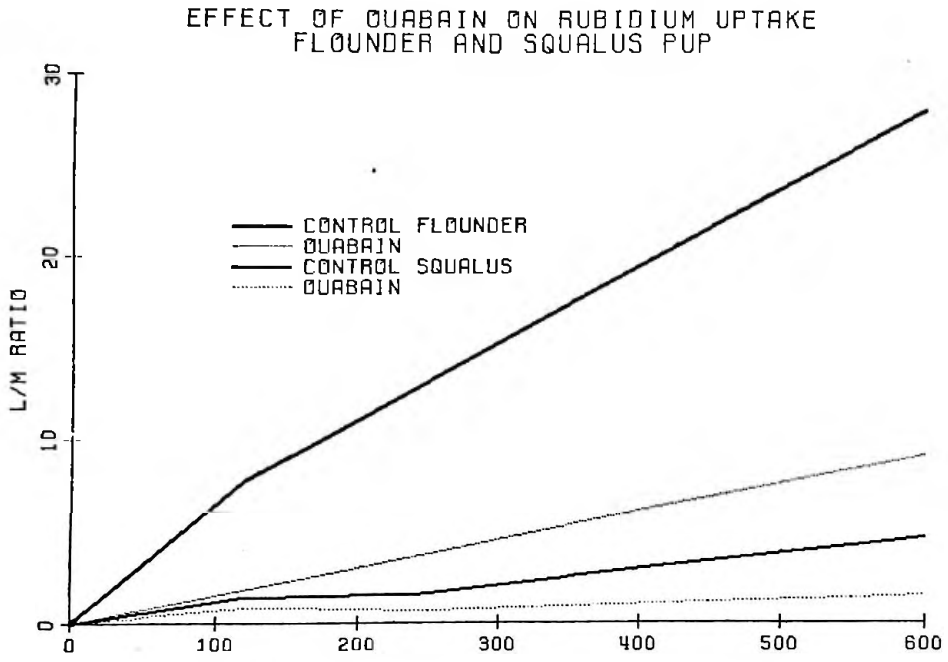

Figure 2. Effect of 2×10^{-5}M Ouabain on ^{86}Rb uptake in lens. n = 4 for each point, shark pups; n = 6 for each point, flounders.

Table 2. Efflux rate constants from lens of Squalus pup. $min^{-1} \pm$ s.e.m. number of experiments, (n)

	Na^+	Cl^-	HCO_3^-
k_{out}	.021 ± .0008 (12)	.010 ± .0005 (6)	.012 ± .0008 (14)
k_{out}, ion free	.007 ± .0004 (6)	.0053 ± .0006 (6)	.011 ± .0004 (6)
k_{out}, ouabain (10^{-4}M)	.010 ± .0007 (8)	.011 ± .0008 (6)	.010 ± .0009 (6)

The rate constants for exit were calculated as

$$k_{out} = \frac{\left(\frac{\text{counts appearing in medium during interval}}{\text{size of interval in minutes}}\right)}{\text{counts in lens at beginning of interval}}$$

the denominator of this expression is obtained by adding the counts in lens at the end of the experiment to the sum of counts which appeared in the medium from the specified interval to the end of the experiment.

These values were calculated after a steady state rate of counts appearing in the medium was achieved, usually after 30-40 minutes.

^{22}Sodium. Influx was studied as described for ^{86}Rb. The pup lens yields an approximate steady state rate constant for sodium influx (k_{in}) of 0.01 min^{-1} whence from the concentration of Na_{out} = 279 mM, the influx is 2.8 mM/min (Table 3). Efflux was studied using the continuous perfusion method of Patterson (expt. Eye Res. 10:331, 1970). Two hours of loading with isotope yielded equilibrium ratios of lens to medium counts. The external solution simulated aqueous humor (Table 1) which is very similar to plasma. Additionally we used a Cl^- - free medium with 280 mM Na acetate and a Na^+ free medium with 280 mM choline chloride. HCO_3^- free medium had a pH of 7.3 after 1% CO_2 gassing.

Table 3. Comparison of rates for various ions in the dogfish pup (mM/min) number of experiments, (n)

	Rb^+	Na^+	Cl^-	HCO_3^-
Influx	.008	2.8	2.5	.05
	(16)	(26)	(18)	(26)
Efflux	--	0.84	0.6	0.32
		(12)	(6)	(14)

Values for rates are calculated using k_{in} · aqueous concentration (influx rate) and k_{out} · lens concentration (efflux rate).

Efflux is given by k_{out} x lens concentration (from Tables 1 and 2 = 0.84 mM/min (Table 3). The 3-fold discrepancy with influx is not surprising, in view of the large number of variables, including multiphasic flux curves, and some technical differences between influx and efflux experiments. Ouabain inhibited 53% of efflux, and a sodium free solution inhibited 67%. Ethoxzolamide, 10^{-4}M, had no effect on Na^+ efflux (4 experiments). The data are consistent with active efflux of sodium, as with lenses of mammals and toad (Duncan, vide supra).

^{36}Chloride. Influx yielded an approximate rate constant of 0.01 min^{-1}, which gives 2.5 mM/min for entry. Efflux as calculated from data of Tables 1 and 2, is 0.6 mM/min; again a discrepancy with influx rate (Table 3). Ouabain did not alter efflux, but it was reduced about 50% in external Cl^- free solution.

$H^{14}CO_3^-$. These experiments were done in sealed tubes after the medium was gassed. The k_{in} was 0.006 min^{-1} which gives 0.05 mM/min for entry (Table 3). Table 2 shows k_{out}; the high intracellular total CO_2 (Table 1) or HCO_3^- then generates an outflux rate of 0.32 mM/min (Table 3). Thus we find a marked asymmetry, both with respect to concentration and flux. This deserves special comment in view of our interest in CO_2 metabolism. On the whole there is reasonable balance between the summed anion efflux and Na^+ efflux (Table 3).

Co_2 is a product of lens metabolism, predominantly through the pentose phosphate pathway (Kinoshita and Wachtel, J. Biol. Chem. 233:5, 1958). We propose (Figure 3) that this CO_2 drives the production of HCO_3^- in the lens with or without carbonic anhydrase (path 1) and that the steady state concentration of HCO_3^- is relatively high in dogfish lens since carbonic anhydrase is lacking to speed its dissipation (path 2). The mammalian lens and aqueous (HCO_3^-) are about the same; in the presence of carbonic anhydrase only a very small gradient is necessary for its dehydration (path 3). In the absence of enzyme the HCO_3^- gradient becomes critical. In this view, HCO_3^- does not have primarily an ionic role in lens; rather it functions, as in red cells and plants, as a carrier and reservoir for CO_2. This idea is consistent with the marked excess intotal CO_2 outflux over influx (Table 3) since outflux can be considered a chemical change and dissipation of HCO_3^-, rather than transport. It also agrees with the fact that carbonic anhydrase inhibition in the mammalian lens does not affect K^+ influx (Becker, vide supra) or Na^+ efflux (work in progress). ^{86}Rb uptake is even faster in flounder than in rabbit lens (Figure 1), despite absence of carbonic anhydrase in the fish lens.

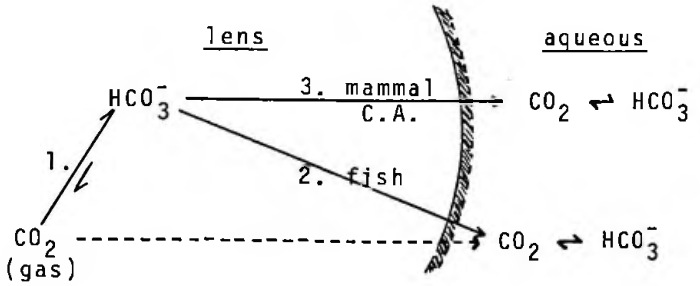

Figure 3. Schematic diagram of CO_2/HCO_3^- relationships in lens.

In summary, this comparative approach has been of great value in showing that fish and mammalian (or amphibian) lens have similar Na^+-K^+ transport systems and rates of ion transport) although the fish tissue lacks carbonic anhydrase. The data suggest that the role of lens enzyme is in dissipation of metabolic CO_2.

This work was supported by NIH grants EY-02227 and NRSA EY-05378. We thank the following colleagues for making these measurements: Martin Morad (lens potential); Larry Reinking (urea); Jill Eveloff and David Miller (ATPase); Ness Pessah and Richard Solomon (electrolytes); Robert Rosenthal (computer and graphics).

EXCITATION-CONTRACTION COUPLING IN VENTRICULAR MUSCLE OF DOGFISH (SQUALUS ACANTHIAS)

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Comparative ultrastructural and electrophysiological studies in amphibian and mammalian ventricular muscle suggest that the development of t-tubules and the sarcoplasmic reticulum (SR) parallels the development of internal and releasable Ca^{2+} stores. Thus in the absence of T-system and a rudimentary SR the frog heart contrasts with that of mammalian heart by showing no phasic tension, no post-extrasystolic potentiation, and no post-clamp potentiation. In the experiments to be described below we compare and contrast the structure function characteristics of the dogfish heart (Squalus acanthias). The results provide further support for the hypothesis that t-tubular system and the adjoining SR is required for the development of inotropic characteristics of the heart associated with internal release and re-circulation of the activator Ca^{2+}.

Ultrastructural studies. Exposed trabeculae on the ventricular wall of dogfish heart were fixed in 3% glutaraldehyde buffered with 0.2 M cacodylate with 0.2 mM $CaCl_2$. After 2-3 hours the specimens were rinsed in buffer, and post-fixed for 1 hour in 1% osmium tetroxide and 1.5% potassium ferrocyanide in cacodylate buffer (Karnovsky, Abstracts of Papers of 11th Ann. Mtg. for Cell Biol., 284:146, 1971), then dehydrated in ethanol and propylene oxide and embedded in Epon. Thin sections were stained with uranyl acetate and lead salts and examined in Jeol 100B and 100S electron microscopes.

The ventricular fibers of shark myocardium are fusiform in shape with an average diameter of 4-6 μm at their greatest width (Fig. 1, panels A & B). The cells are tightly packed and connected to each other at their tapering ends by intercalated discs. The extracellular space between fibers appears as a continuous electrondense matrix often crossed by prominent bundles of collagen fibrils. The contractile material is located at the periphery of the cells whereas the nucleus and mitochondria occupy the central core of the fibers. The sarcolemma frequently invaginates into the cytoplasm as small flask shaped inpocketings (caveolae) of uniform size; they all appear to c communicate with the extracellular space through a narrow ostium.

Sarcolemmal invaginations of t-tubules were absent but internal membrane systems resembling SR were frequently observed (Fig. 1, panels B & C). These membraneous tubules seem to be qualitatively less organized than in the mammalian heart. Specialized enlargements of the SR into flattened cisternae were not directly observed but saccules adjacent to the sarcolemma resembling SR cisternae were often observed (panels B & D). These couplings were always present at the level of the Z-lines or at A-I junctions (panel D).. Regularly spaced densities spanning the gap between the cisterns and plasma membrane were not observed. In general the lateral couplings seem to be less specialized as compared to those found in the mammalian ventricle. Free running trabecula 0.3 to 0.6 mm in diameter were isolated from the ventricular wall and mounted in a single sucrose gap setup. The shark Ringers had the following composition in mM: NaCl 280, KCl 6, $CaCl_2$ 5, $MgCl_2$ 3, Na_2So_4 0.5, NaH_2PO_4 1, urea 350, 99% 0_2.