(Forster, R.P., J.A. Hannafin and J.S. Shiffrin, this issue). This led to the following experiments in which we tested the direct action of catecholamines, especially the β-adrenergic agonist isoproterenol, on excitation and contraction of skate atrial and ventricular myocardial fibers.

The preparation used to measure action potential and contraction of isolated heart muscle fibers was similar essentially to that used by Morad, M. and Y. Goldman (Bull. MDIBL 13:82-84, 1973) for their excitation-contraction coupling studies recorded from ventricular strips of the dogfish, *Squalus acanthias*. Muscle fibers approximately 100-200 μ in diameter and 0.5-1 cm long were drawn through small holes in 2 latex membranes which separated the preparation into 3 compartments. Isosmotic sucrose solution in the central compartment electrically isolated the extracellular spaces of the fiber in the 2 outer compartments. The left compartment contained a balanced isotonic elasmobranch medium of the following mM composition: NaCl 280, KCl 6, $CaCl_2$ 5, $MgCl_2$ 3, Na_2SO_4 0.5, NaH_2PO 1, urea 350, glucose 5 and $NaHCO_3$ 8. In the left compartment glass microelectrodes measured action potential and association of the fiber was made here with an isometric tension transducer. The right compartment contained a depolarizing solution of isotonic KCl.

Figures 1-4 show that the myocardial fibers of both atrium and ventricle respond positively to direct catecholamine administration despite a total absence of sympathetic adrenergic innervation to the skate heart. The positive inotropic response to various agents is indicated directly by the recordings of isometric contraction, and indirectly by contour changes in the action potential recordings. Details are provided in the legends under each figure.

These experiments demonstrate a direct positive inotropic action of β-agonists (isoproterenol) and other catecholamines on isolated skate myocardial fibers where there is no anatomical or physiological evidence of a sympathetic adrenergic innervation, but actually there may not be the difference between rat and skate hearts we originally sought in designing our experiment testing the relationship between β-adrenergic stimulation and taurine fluxes. It is reasonable to believe that extrinsic control of the heart in elasmobranchs may represent an intermediate situation in the evolution of that in higher vertebrates. There is evidence of intrinsic chronotropic responses of the fish myocardium to increased perfusion pressure, and there exists also a fully developed extrinsic inhibitory cholinergic "parasympathetic" innervation. The absence of an adrenergic sympathetic innervation to complete the autonomic dual control of the elasmobranch heart may be compensated for by a unique strategically situated pair of whitish 'axillary bodies' lying in the posterior cardinal sinus just behind the heart (Young, op. cit.). They contain sympathetic ganglia and a mass of chromaffin tissue shown to be rich in norepinephrine and other catecholamines (Shepherd, D.M. et al. Nature (Lond.) 112:509, 1953). When released into the blood stream these would quickly pass to the heart, in effect functioning thereby as neurotransmitters would in stimulating β-receptors within the elasmobranch myocardium. This work was supported by NIH grant HL 04457-20.

FURTHER STUDIES ON THE PHYLOGENY OF VERTEBRATE CARBONIC ANHYDRASE IN RED CELLS AND SECRETORY ORGANS

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We are trying to learn the pattern of the development of carbonic anhydrase in vertebrates. Last year (Maren and Rittmaster, Bull. MDIBL 17:35-39, 1977) we showed that the enzyme in elasmobranch red cells is of low specific activity, and that there is a striking increase in teleosts. Indeed, the teleost red cell carbonic anhydrase has a turnover number $\frac{V_{max}}{E}$ K_{cat} as high as primate carbonic anhydrase C (also called II), the greatest of any known enzyme.

TABLE 1

PROPERTIES OF RED CELL CARBONIC ANHYDRASES IN SEVERAL ORDERS OF FISH

	Enzyme units per ml [a]	E/e.u. [b] M x 10^8	k_{cat} [c] sec.$^{-1}$ x 10^{-4}	K_m [d] mM
Myxine				
M. glutinosa (hagfish)	1,000	2	1.3	-
Elasmobranchii				
S. acanthias (dogfish)	2,400	1	2	5
Teleostei				
P. americanus (flounder)	13,000	0.1	25	-
M. americanus (goosefish)	25,000	0.05	50	10
S. gairdneri (trout)	24,000	0.03	70	-

a. In method described in Bull. M.D.I.B.L. 17, p. 35, 1977.

b. From titration with benzolamide or ethoxzolamide, J. Pharm. Exp. Ther. 130, page 389 (1960).

c. $\frac{V_{max}}{E}$ As described in text or from Lineweaver-Burk Analysis.

d. From Lineweaver-Burk Analysis.

TABLE 2

CILIARY FOLDS, RECTAL GLAND, AND LENS CARBONIC ANHYDRASE OF CERTAIN FISH

	Enzyme units per gram	E/e.u. M x 10^8	k_{cat} sec.$^{-1}$ x 10^{-4}	K_m mM
S. acanthias				
Ciliary folds	600	0.25	8	14
Rectal gland	3,200	0.3	12	14
Lens: Corneal endothelium	0	-	-	-
P. americanus[+] and M. scorpius[*]				
Ciliary folds[+]	344	0.3	8	-
Ciliary folds[*]	538	-	-	-
Lens: corneal epithelium[+*]	0	-	-	-
S. gairdneri				
Ciliary folds	1,800	0.2	13	-
Lens	45	-	-	-
Corneal endothelium	176	-	-	-

The methods were as previously described (vide supra). For estimation of k_{cat}, it was not necessary to run a Lineweaver-Burk analysis in each case. This is due to the fact that in the system described, 1 enzyme unit doubles the uncatalyzed rate of hydration of CO_2. Since the latter is constant for given conditions, V_{max} for 1 enzyme unit is known = 0.25 mM per second. The titration of the particular enzyme with a powerful sulfonamide yields the molar concentration of enzyme (E) per enzyme unit. Thus we may calculate for any enzyme unitage, $\frac{V_{max}}{E} = k_{cat}$. This method cannot yield an accurate K_m; where this is sought the usual relation between varying substrate (CO_2) and rate of H^+ formation was measured.

Table 1 shows that there is a notable increase in the specific activity of red cell carbonic anhydrase between representatives of the so-called "primitive classes" Agnatha (suborder Myxinoidea) and Chondrichthyes (order Elasmobranchii), and the bony fish (order Telostei). Data include our previous findings (vide supra) and are of further interest in showing that Myxine and Elasmobranchii appear similar, and that fresh and salt water teleosts are similar. Some chemical properties including the amino acid composition of shark red cell carbonic anhydrase are known (Maynard and Coleman, J. Biol. Chem. 246: 44-55, 1971). Similar studies are now planned for the teleost, in view of the present data showing large differences from shark, and kinetic kinship with mammalian carbonic anhydrases.

Turning to the secretory tissues, Table 2 shows that carbonic anhydrase in the ciliary body and rectal gland is of the high activity type. This is of particular interest in *S. acanthias*, whose red cells are clearly of low activity (Table 1). The lens and corneal endothelium in sea-going fish (flounder and sculpin) contain no carbonic anhydrase; the fresh water trout has the enzyme in both these tissues. This is similar to the situation in the kidney of these fish (Maren, Physiol. Rev. 47:595-781, 1967). It should be added however, that the concentrations of enzyme in trout lens is low, and inhibition by ethoxzolamide or benzolamide required some 100 times greater drug than for other tissues tested (see below). More work is required on this subject.

TABLE 3

INHIBITION OF FISH RED CELL AND SECRETORY CARBONIC ANHYDRASE BY SULFONAMIDES AND ANIONS

	Red Cells	Ciliary Body $K_I \times 10^9$ M	Rectal Gland (Gill)
Benzolamide			
Dogfish	10	20	10 (7)
Flounder	0.4	4	-
Trout	0.4	5	-
Ethoxzolamide			
Dogfish	20	10	-
Anions, Dogfish		$K_I \times 10^4$ M	
Cl^-	700	1160	16200
I^-	90	40	3900
ClO_4^-	2	1	1
CNO^-	0.3	0.3	0.3
F^-	- no inhibition -		

Of further interest is the susceptibility of the fish carbonic anhydrases to sulfonamides and anions. Table 3 shows the data. The teleost red cell enzyme is much more sensitive (25 X) to the sulfonamides than the dogfish red cell enzyme. Ciliary body of teleosts is also more sensitive than that of dogfish. The dogfish ciliary body and rectal gland carbonic anhydrase are about like red cells, 50% inhibited at 10^{-8} M benzolamide or ethoxzolamide. This is a fairly high degree of activity, although an order of magnitude less than found for these drugs against the human red cell C or secretory enzyme (Maren and Rittmaster, vide supra). The ciliary bodies of the teleosts are some 5 X more sensitive to the sulfonamides than this tissue in the dogfish.

Turning to the anions, we find a similar pattern of inhibition among the three tissues, with the interesting and important exception that the rectal gland is resistant to inhibition by the halides. Since this gland pumps a fluid of 500 mM chloride its carbonic anhydrase should not function if it were inhibited at the level of red cells or ciliary body. But since the rectal gland K_I for Cl^- (or I^-) is at least 16 X greater than for these other tissues, there is essentially no physiological inhibition. This finding implies strongly that carbonic anhydrase does have a function in the rectal gland, although this function has eluded definition because sulfonamides have had no demonstrable effect on the secretory rate *in vivo* or *in vitro* (Rawls, Bull. MDIBL 4:58, 1962; Siegel et al. Comp. Biochem. Physiol. 51A:593, 1975; P. Silva and F.H. Epstein, personal communication).

The anion inhibitory pattern in red cells (or tissues) of shark corresponds only qualitatively to those of either human isozyme B or C; this is not surprising since the enzyme in *S. acanthias* is chemically (Maynard and Coleman, vide supra) and kinetically (Maren and Rittmaster, vide supra) quite distinct from any of the mammalian carbonic anhydrases. It should be noted that F^- had no inhibitory activity; that found last year (Maren and Rittmaster, vide supra) appears to be an error. F^- does not inhibit other carbonic anhydrases.

The general conclusions from our 1977 and 1978 work and the relevant literature on this subject follow:

1. Red cell carbonic anhydrase in Elasmobranchii and Myxine is very different from human isozymes B or C. It is of low activity type, moderately sensitive to sulfonamides and anions, and relatively labile.

2. Red cell carbonic anhydrase in teleosts is of high activity type and very susceptible to sulfonamides and (limited data in goosefish) anions. The teleost red cell enzyme thus appears to be an important phylogenetic development.

3. Secretory tissues in *S. acanthias* have enzyme(s) with much higher turnover number than that of red cells. Susceptibility to sulfonamides and anions agrees with that for red cell enzyme, except for rectal gland, which is very resistant to halide inhibition. These findings suggest at least three separate loci for carbonic anhydrase in this species.

4. The ciliary folds in the three teleosts and the dogfish have a high activity enzyme, susceptible to sulfonamide and anion inhibition. Thus far there is nothing to distinguish these enzymes from the carbonic anhydrase in ciliary processes of mammals, including primates. Our earlier physiological work shows that the chemistry of aqueous humor formation in fish is similar to that of mammals (Maren, Wistrand, Swenson and Talalay, Invest. Ophthal. 14:662, 1975).

5. We could not detect carbonic anhydrase in lens or corneal endothelia in the sea-going fish of any of the species tested. In these structures the enzyme appears in low concentration in fresh water fish. It is hoped that these differences will be useful in revealing the function of lens carbonic anhydrase, which is presently unknown (Friedland and Maren, this Bulletin). Supported by NIH Grant HL 22258.

PILOT STUDIES ON ION MOVEMENT IN LENS

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Although lens was one of the first tissues to be recognized for its high carbonic anhydrase content through most of the vertebrate phyla, this structure remains one of the few in which the function of carbonic anhydrase is entirely unknown. Nearly twenty years ago, it was found that elasmobranch lens lacked carbonic anhydrase (Maren, Comp. Biochem. Physiol. 5:201, 1962). This summer (this Bulletin) we found carbonic anhydrase also was absent from lens of saltwater teleosts.

We surmise that these differences may be a useful investigative key in the search for the role of carbonic anhydrase in lens. We report here the beginning of work in which we shall try to discover how movements of anion and cation are linked in vertebrate lens and the possible dependence of this on carbonic anhydrase. Both mammalian and amphibian lens have high K^+ (120 mM) and low Na^+ (17 mM), and efflux of Na^+ is an active process. Cl^- and HCO_3^- are present at about 20 mM, and Cl^- efflux appears to be passive. Thus there is a very large anion gap; presumably negative charges on large molecules and/or PO_4 or other anions. Relatively little attention has been given to HCO_3^- efflux (Duncan, G. in The Eye, vol. 5, ed. by H. Davson and L.T. Graham, Academic Press, New York, 1974; Kinsey, V.E., Documenta Ophthalmologica, Proceeding Series, vol. 8, p. 310, 1976).

We describe pilot experiments on the efflux of labeled Cl^- and HCO_3^- from dogfish lens. The procedure was to soak the freshly dissected lens in shark-Ringers solution containing $Na^{36}Cl$ or $NaH^{14}CO_3$ for one to two hours, then place it in a chamber arranged so that it is supported on a plate of perforated plastic immersed in 17 ml fresh shark-Ringers solution at 12°C. We measured the rate of appearance of isotope in the external fluid at early periods, well before equilibrium was reached. As controls for the free diffusion of ions in this situation, dialysis bags of about the same volume as lens were filled with shark-Ringers solutions containing the isotopes and placed in the chamber. We define a rate constant, k_{out}, for this specific system as

$$\frac{\text{total counts appearing in medium per min}}{\text{total counts in lens or bag}}$$

For efflux of each isotope, four lenses and four bags were studied. In a few cases the use of paired lenses enabled a rough decay rate to be calculated from differences in isotope concentrations in lens at several time intervals, also yielding k_{out}.

The chloride efflux from dogfish lens was rapid and the same from both lens and bag, $k_{out} \cong 0.12\ min^{-1}$. These rates are faster than efflux from mammalian and amphibian lens. Data indicate that Cl^- movement is passive as in mammalian and amphibian lens (Duncan, vide supra).