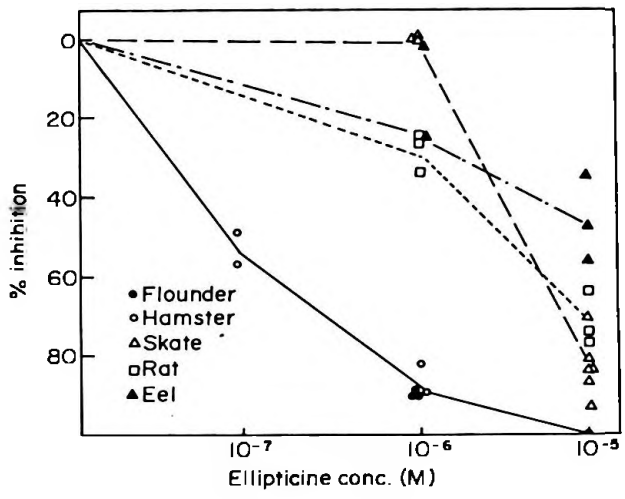

Figure 1

These results indicate that ellipticine may be a valuable probe of the differences in cytochrome P-450 between species and suggest specifically that the cytochrome P-450s of skate and flounder liver may be quite different.

ANOXIC TOLERANCE OF ATLANTIC HAGFISH (MYXINE GLUTINOSA) CARDIAC MUSCLE

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Atlantic hagfish have multiple hearts and a partially open circulatory system with sinuses reminiscent of those found in the invertebrate phyla. The two primary pumps, systemic (branchial) and portal-vein hearts, are composed of true vertebrate cardiac muscle which shows a myogenic origin of rhythmic heartbeat. Unlike most other non-cyclostome vertebrates, however, hagfish completely lack a coronary circulation for delivery of well-oxygenated blood to the cardiac musculature. Both hearts exclusively pump mixed venous blood which, even when fully-oxygenated, has an extremely low oxygen carrying capacity (1 ml O_2/100 ml blood). Functional characteristics of the respiratory pigments from hagfish further suggest chronic hypoxia of the cardiac tissue. Unlike higher vertebrates the hemoglobin of these animals is monomeric (rather than tetrameric) and, accordingly, does not display Bohr or Root effects to aid oxygen dissociation. The P_{50} values of both whole blood and hemoglobin solutions are extremely low, less than 5 mmHg PO_2 (Bauer et al., Nature 256: 66-68, 1975). All factors imply that cardiac muscle of hagfish continuously functions under in vivo conditions that are severely hypoxic by typical vertebrate standards.

The major objectives of this study were to determine: 1. the normal oxygen tensions available to working hagfish cardiac muscle; 2. the mechanical performance of hagfish systemic heart under normoxia and anoxia, and 3. a biochemical index (pyruvate kinase/cytochrome oxidase activity ratio; Gesser and Poupa, Comp. Biochem. Physiol. 48A: 97-103, 1974), which is highly correlated with anaerobic work capacity in hagfish heart compared with that of the obligately aerobic heart of Atlantic cod (Gadus morhua).

METHODS

Atlantic hagfish were captured with baited traps off St. Andrews, Canada, transported to M.D.I.B.L. and held in circulating fresh seawater tanks at ambient temperatures. Atlantic cod were captured with baited lines off Southwest Harbor, M.D.I. Cod hearts were immediately removed and held on ice until enzyme assays were performed that day.

Live hagfish were anesthetized with tricaine methane-sulfonate and subsequently immobilized by injection of 7.5 mg/Kg (body wt.) gallomine triethiodide. A ventro-lateral incision was made through the skin and body wall just posterior of the excurrent gill slits, exposing the systemic heart, portal-vein heart and associated vasculature. Fish were placed in a water jacketed chamber with a tube inserted into the single median dorsal nostril for delivery of controlled-PO_2 ventilatory sea water. Ventilatory sea water and the chamber were temperature controlled at 10°C by an external refrigerated circulating bath. A small open "window" in the chamber allowed access to the hearts and vasculature.

Gross mechanical activity (frequency and amplitude of heart beat) of the systemic heart was recorded utilizing an isotonic force displacement transducer with appropriate amplification and recording equipment. Vascular-PO_2 was measured *in vivo* in the dorsal aorta, left posterior cardinal vein (a major venous input feeding the systemic heart) and supraintestinal vein (a major venous input feeding the portal-vein heart) with oxygen microelectrodes (Transidyne General Corporation). Ventilatory sea water was either air-saturated or 100% nitrogen-saturated.

Cyanide poisoning was through intravenous injection into the left posterior cardinal vein after immobilization of the fish. The bolus of cyanide was diluted with hagfish saline. Final blood concentration was 1 mM cyanide (assuming 17% blood volume to weight ratio).

Maximal tissue activities of pyruvate kinase and cytochrome oxidase were measured at saturating substrate concentrations spectrophotometrically at 15°C by standard techniques.

RESULTS AND DISCUSSION

Vascular PO_2 measured *in vivo* in normoxic hagfish (gills ventilated with air-saturated sea water) clearly demonstrated oxygenation of post-gill arterial blood and utilization by body tissues (Table 1). Lacking a coronary

Table 1.--*In vivo* vascular PO_2 measurement in normoxic Atlantic hagfish.

	PO_2 (mmHg)	
Dorsal Aorta	92.3 ± 5.9	N = 6
Supraintestinal Vein	11.0 ± 1.6	N = 8
Left Posterior Cardinal Vein	12.3 ± 1.7	N = 6

Mean values ± S.E.

circulation, oxygen available to the systemic and portal-vein hearts is limited to the PO_2 of the major venous inputs. The combination of low oxygen carrying capacity of blood and position of the systemic and portal-vein hearts in the circulatory route, i.e., immediately pre-gill, results in extremely low oxygen availability to these tissues. Indeed, *in vivo* measurement of the PO_2 of the major venous inputs to the systemic and portal-view hearts bear this out (Table 1). Thus, even under conditions of high environmental oxygen availability, systemic and portal-vein hearts of hagfish are subject to substantial hypoxia.

Frequency and amplitude of heart beat, although not an accurate measure of the true work performed by the heart, permit an assessment of relative cardiac performance under each experimental treatment. Percent initial (rate x amplitude) was used as an approximate index of work output. Neither anoxia nor intravenous cyanide

poisoning limited the performance of the hagfish systemic heart. Although all preparations showed a decrease in the work output index (13-22%), no differences were found in the mechanical response of the hagfish systemic heart between experimental and control groups (Table 2). In all cases, heart rate decreased with a concomitant increase

Table 2.--Mechanical response of hagfish systemic heart to severe hypoxia and intravenous CN^- poisoning. Mean values ± S.E.

Treatment	Time (min)	Rate (beats min^{-1})	% Initial Amplitude	% Initial (Rate x Amplitude)
Air saturated[1]	0	20 ± 4.3	--	--
n = 4	120	14.8 ± 2.3	112 ± 6.4	82.3 ± 15.5
Nitrogen saturated[1]	0	22 ± 1.1	--	--
n = 9	120	14.7 ± 1.1	114 ± 9.9	78.5 ± 10.3
CN^- injected[2]	0	25 ± 1.8	--	--
n = 5	120	20.5 ± 2.4	108 ± 5.3	87.3 ± 6.8

[1]Ventilatory water perfusing gills of immobilized hagfish. Air saturated PO_2 = 157 ± 4 mmHg; Nitrogen saturated PO_2 < 1 mmHg.

[2]Final blood concentration = 1 mM.

in amplitude with time. At least under conditions of low activity (immobilized animals), anerobic pathways appear to have the capacity to supply ATP at a rate high enough to maintain cardiac performance.

Maximal tissue activities of pyruvate kinase and cytochrome oxidase were determined in hagfish and cod myocardium (Table 3). Cod myocardium has been shown to be relatively intolerant of hypoxia and was used as an

Table 3.--Specific activities of pyruvate kinase and cytochrome oxidase in hagfish and cod myocardium

	Specific Activity[1]		Cod/ Hagfish
	Codfish	Hagfish	
Pyruvate kinase	58.87 ± 1.20 (6)	35.98 ± 2.88 (13)	1.64
Cytochrome oxidase	37.19 ± 1.89 (4)	4.15 ± 0.54 (19)	8.96
PK/CO	1.61 ± 0.61 (4)	9.04 ± 1.35 (13)	

[1]Units (g wet wt.)$^{-1}$. Data shown are means ± S.E.M. Number of fish examines is in parentheses.

example of an obligately aerobic fish heart (O. Poupa and K. Johansen, Am. J. Physiol. 228: 684-688, 1975). The 1.64-fold difference in pyruvate kinase activities between cod and hagfish is small, suggesting a similar maximal glycolytic capacity in both tissues. However, cytochrome oxidase activity was approximately nine-fold greater in cod than hagfish, indicating a substantially greater capacity for aerobic metabolism in cod myocardium. The pyruvate

kinase to cytochrome oxidase activity ratio (PK/CO) was calculated for each species to provide a biochemical index of relative anaerobic to aerobic capacity in the tissue. Gesser and Poupa (1974) have correlated this ratio with the ability of cardiac tissue to produce force under anoxia. The larger the ratio, the greater anaerobic capability. The PK/CO ratio is approximately 5.5 times larger in hagfish than cod.

In summary, low oxygen tensions in the major venous inputs, the similarity of mechanical activity in animals exposed to normoxia, severe hypoxia or intravenous cyanide poisoning, and a biochemical index of relative anaerobic to aerobic capacity points to a substantial ability of hagfish myocardium to maintain performance under anoxia. This research was supported by a grant-in-aid from the American Heart Association, Maine Affiliate, Inc. to B.D.S.

RENAL HANDLING OF PAH IN THE ROCK CRAB (Cancer irroratus) AND THE JONAH CRAB (C. borealis)

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Recent studies from this laboratory have shown that in the rock crab, Cancer irroratus, net urinary secretion of organic anions, e.g., p-aminohippuric acid (PAH), is a result of the action of a potent secretory pump, located in the labyrinth, and a weaker reabsorptive pump in the urinary bladder (Holliday and Miller, Am. J. Physiool. 230: R311, 1980; Miller et al., Renal Physiol. 2: 166, 1980). We present here data showing that the renal systems (antennal glands) of two closely related crabs, the rock crab and the Jonah crab, handle PAH in different ways.

Male, intermoult crabs (250-350 g) of both species were purchased from commercial suppliers on Mt. Desert Island and were kept in Frenchman Bay sea water (30-33°/oo, 14-16°C) in open-circuit or recirculating aquaria at the laboratory. For clearance experiments crabs were injected with 0.5 ml of crab Ringer (CR) solution (Holliday and Miller, op. cit.) containing radiolabeled and unlabeled PAH (^{3}H) and polyethylene glycol (PEG-^{14}C). Each crab was placed in 2 l of aerated sea water (15°C), which was changed daily. Urine, serum, and sea water samples were taken over a six-day period and PAH and PEG clearances were calculated. Note that, because crabs filter only about 10% of the vascular fluid (hemolymph) per day and retain large amounts of urine in the paired bladders, PEG clearance values required 48 h to stabilize at 8 - 10 ml hemolymph per day. Both crabs showed overall net secretion of PAH, since PAH/PEG clearance ratios were greater than unity after 48 h (Fig. 1). It is obvious from these data that the clearance ratios for Jonah crabs were well over one order of magnitude greater than those for rock crabs.

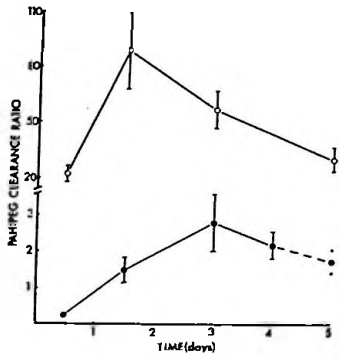

Figure 1.--PAH:PEG clearance ratios for the rock crab (●) and the Jonah crab (○). Initial serum PAH and PEG concentrations were 10 μM and 160 μM, respectively. Both PAH and PEG showed an initial rapid drop during the first 24 h and then declined at constant rates for the next 5 days. Urinary rates averaged = 3% body weight per day. Mean values + SE, n=3 crabs of each species. Note change in ordinate scale.

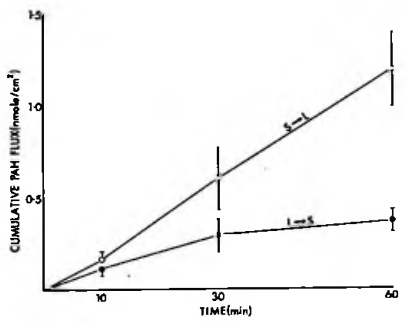

Figure 2.--Unidirectional fluxes of PAH (10 μM added to luminal or serosal bath) across excised Jonah crab urinary bladder. Mean values ± SE, n = 6-10 paired bladders at each point.