

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 Paupa (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. Physiol.* 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°C, 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 ( $^3\text{H}$ ) and polyethylene glycol (PEG- $^{14}\text{C}$ ). 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.

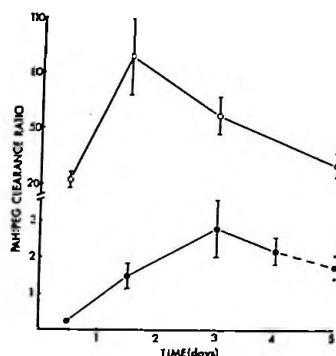


Figure 1.--PAH:PEG clearance ratios for the rock crab (●) and the Jonah crab (O). Initial serum PAH and PEG concentrations were 10  $\mu\text{M}$  and 160  $\mu\text{M}$ , respectively. Both PAH and PEG showed an initial rapid drop during the first 24 h and then declined at constant rates for the next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

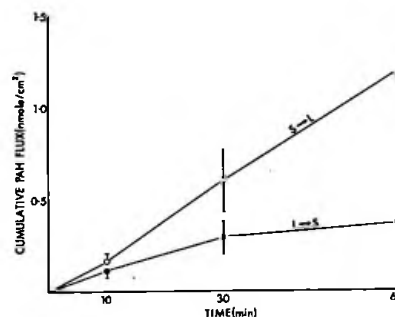


Figure 2.--Unidirectional fluxes of PAH (10  $\mu\text{M}$  added to luminal or serosal bath) across excised Jonah crab urinary bladder. Mean values  $\pm$  SE,  $n=6-10$  paired bladders at each point.

To determine the underlying reasons for the observed differences in the organic anion secretory abilities of the two species, we examined the PAH transport characteristics of two components of the Jonah crab renal system (bladder and labyrinth) and compared the findings to those for rock crab. Unidirectional fluxes of labeled PAH across excised Jonah crab bladder tissues were measured in the same dual flux chamber that had been used for rock crab bladders (Holliday and Miller *op. cit.*). Paired bladder sheets were excised from pithed Jonah crabs and mounted between pairs of cover slips with centered, beveled-edge holes which exposed  $0.283 \text{ cm}^2$  of bladder. Thus mounted, tissues were clamped between the halves of the flux chamber. Separate, 1.0 ml aliquots of CR bathed each side of the two tissues and were aerated and stirred (4 ml/min) by a four-channel peristaltic pump at  $18\text{--}20^\circ\text{C}$ . Labeled and unlabeled PAH ( $10 \mu\text{M}$  final concentration) was added to the CR bathing the luminal side of one tissue and to the serosal side of the other tissue. Unidirectional PAH fluxes were calculated from the rate of appearance of label in the two chambers which were originally isotope-free. Under these conditions Jonah crab bladder showed a significant, net secretory ( $S \rightarrow L$ ) flux of PAH (Fig. 2). Addition of 1 mM bromocresol green (BCG, a competitor organic anion, which, at 1 mM, blocks concentrative PAH uptake in bladder slices) to both the serosal and luminal baths reduced the  $S \rightarrow L$  flux to the same level as the control  $L \rightarrow S$  flux (BCG data not shown). Note, that because of this net secretory flux, the Jonah crab bladder may be a useful model for the vertebrate proximal renal tubule. In contrast, net reabsorption was found in intermoult rock crab bladder (Holliday and Miller, *op. cit.*). Thus, the difference in degree of PAH secretion in these two crabs is related to a difference in the transport function of the bladders. Further, our preliminary micropuncture experiments with Jonah crabs indicate that the labyrinth in this crab secretes PAH less strongly than that in the rock crab (PAH ratios labyrinth fluid: Plasma in lower labyrinths were  $4.2 \pm 0.3$ ,  $n = 3$  in one Jonah crab, and  $14.8 \pm 2.2$ ,  $n = 16$  in four rock crabs).

In conclusion, our results show that PAH is handled differently by the renal systems of two closely related crabs. The rock crab strongly secretes PAH in the labyrinth and partially reabsorbs it in the bladder, resulting in PAH/PEG clearance ratios of 2-3. The Jonah crab shows lower PAH secretion in the labyrinth and strong secretion in the bladder, resulting in PAH/PEG clearance ratios of 30-100. Based on the present findings, one might expect that these two crabs would exhibit greatly different abilities to excrete anionic pollutants, such as the herbicides 2,4-D and 2,4,5-T, i.e., under similar exposure conditions, rock crabs would exhibit a tendency to retain such toxicants for longer times than Jonah crabs. Such retention could result in an increased sensitivity to anionic toxicants for the former species. Supported by N.I.H. grants ES-00920, AM-15973 and RR 05764 and N.S.F. grant PCM-77-26790.

#### FURTHER STUDIES ON CELL VOLUME REGULATION IN SLICES OF DOGFISH (*Squalus acanthias*) RECTAL GLAND

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Our previous studies on cell volume regulation in slices of the rectal gland of the dogfish shark (*Squalus acanthias*) have been extended with the aim of elucidating the role of cell anions and external  $\text{K}^+$  in cellular swelling. Most experimental conditions and analytical procedures were described by Booz, et al., Bulletin, MDIBL 18: 26, 1978; and by Goldstein, et al., Bulletin, MDIBL 19: 3, 1979. The determination of tissue urea and amino acids: 50-100 mg, tissue slices (fresh tissue or after aerobic incubation (air) for 60 min at  $15^\circ\text{C}$ ) were first blotted, weighed, and then extracted with 2 ml ice cold 5% trichloroacetic acid. The deproteinizing agent was removed by extraction with ethyl ether, and the solutes were then determined in an amino acid analyzer.