Table 2.--Rates of Lactate Oxidation by Perfused Isolated Ocean Pout and Sea Raven Hearts. Data are Expressed as μmoles of Substrate Oxidized·gm dry weight^{-1}·hr^{-1}. All Values Represent The Mean ± S.E. On the Basis of 4-6 Individuals

Lactate concentration	3 - ^{14}C-Lactate	U - ^{14}C-Lactate
	OCEAN POUT	
2 mM	27.6 ± 3.5	26.7 ± 5.2
10 mM	46.8 ± 4.1	150.9 ± 9.7
	SEA RAVEN	
2 mM	2.1 ± 0.4	9.6 ± 2.8
10 mM	8.1 ± 2.0	19.4 ± 3.1
15 mM	5.9 ± 2.1	33.4 ± 5.1

production from 3-^{14}C-lactate is equivalent to that from U-^{14}C-lactate for the ocean pout hearts. In the sea raven hearts though rates of $^{14}CO_2$ production from the latter substrate are 4-5 fold higher than from 3-^{14}C-lactate. As the availability of exogenous lactate increases the ratio of $^{14}CO_2$ production from 3-^{14}C-lactate to $^{14}CO_2$ production from U-^{14}C-lactate decreases for both heart types. The oxidation of 3-^{14}C-lactate leads to the production of $^{14}CO_2$ only by reactions catalyzed by enzymes of the citric acid cycle; whereas, in addition to these sites U-^{14}C-lactate liberates $^{14}CO_2$ in concert with the production of acetyl CoA at the pyruvate dehydrogenase reaction. Thus, at 2 mM lactate essentially all the oxidized substrate is channelled through the citric acid cycle in the ocean pout hearts but only about 1/4 of the oxidized substrate enters the cycle in the sea raven hearts. That is, in the myoglobinrich hearts a higher proportion of the lactate undergoes the first decarboxylation only and is subsequently directed elsewhere probably into lipid synthesis. At all higher concentrations of exogenous lactate the proportion of lactate directed into the citric acid cycle decreases for both ocean pout and sea raven hearts.

In conclusion, at low work rates the ocean pout and sea raven hearts display similar rates of oxygen consumption even though their intracellular content of myoglobin is very different. It is possible that the role of myoglobin in terms of facilitating intracellular oxygen diffusion becomes important only at high rates of metabolism. Under conditions of low mechanical work the ocean pout heart appears particularly well designed to utilize exogenous lactate as a metabolic fuel whereas lactate oxidation in the sea raven heart is less vigorous. The relationship between the presence or absence of myoglobin and lactate metabolism is presently under investigation. This work was supported in part by a grant from the N.B. Heart Foundation. We would like to thank Dr. Barbara Kent for the generous use of the oxygen meter.

MYOGLOBIN CONTENT AND MAXIMAL ACTIVITIES OF ENZYMES OF ENERGY METABOLISM IN FISH WHITE HEART AND SKELETAL MUSCLE

William R. Driedzic, Bruce D. Sidell and John M. Stewart, Biology Department, Mount Allison University, Sackville, N.B., and Department of Zoology, University of Maine, Orono, Maine

It is well recognized that there are significant differences amongst muscle types with respect to patterns of metabolic fuel utilization. White skeletal muscle for instance, is characterized by low myoglobin and mitochondrial content but high levels of glycolytic enzymes. White muscle generates a high proportion of the ATP required to support contractility via the anaerobic conversion of glycogen to lactate. Heart and red skeletal muscle on the other hand are well vascularized and high in myoglobin content which serves to facilitate intracellular oxygen diffusion. The red colouration of tissues of this type is actually due to the presence of myoglobin.

In contrast to most vertebrates the Ocean pout (*Macrozoarces americanus*) possesses a white heart which is apparent after blood wash out. The question arises as to whether the organization of metabolism in this tissue is similar to that of typical red vertebrate hearts or whether the ocean pout heart takes on some of the characteristics of white skeletal muscle. This problem is being approached by measuring the maximal activity of a number of enzymes of energy metabolism and determining the content of a variety of intracellular compounds associated with ATP production mechanisms. For comparative purposes the heart of the sea raven (*Hemitripterus americanus*) which is bright red even after blood washout is being analysed along with the ocean pout tissues.

MATERIALS AND METHODS

Ocean pout and sea ravens were captured by otter trawl in either Passamaquoddy Bay, N.B. or Seal Cove, Maine. The fish were transported to the laboratory and maintained at 10° – 13°C. Animals were sacrificed by severing the spinal cord, the hearts were exposed, excised and with the exception of hearts utilized for myoglobin analysis were placed in cold homogenization media. Hearts utilized for myoglobin analysis were initially perfused with Ringers (see Driedzic et al., this Bulletin) at a rate of 15 ml per min for 2 min to eliminate blood. Thereafter the tissue was homogenized in 10 mM phosphate buffer pH 7.0. Myoglobin content was estimated from the absorption spectra (490–600 nm) of highspeed supernatants (Sidell, Physiol. Zool. **53**:98–107, 1980). Hearts utilized for glycogen analysis were homogenized in cold 30% KOH. Thereafter, glycogen was isolated, hydrolyzed (Walaas and Walaas J. Biol. Chem. **187**:769–776, 1950) and assayed in terms of glucose units by enzymatic procedures. All enzyme assays were conducted at 10°C and were based upon published procedures: hexokinase, phosphofructokinase, and lactate dehydrogenase (Zammit and Newsholme, Biochem. J. **160**:447–462, 1976); pyruvate kinase (Zammit, Beis, and Newsholme, Biochem. J. **174**:989–998, 1978); citrate synthase (Alp, Newsholme and Zammit, Biochem. J. **154**:689–700, 1976); alpha-ketoglutarate dehydrogenase (Read, Crabtree and Smith, Biochem. J. **164**:349–355, 1977) and cytochrome oxidase (Smith and Conrad, Arch. Biochem. Biophys. **63**:403–413, 1956).

RESULTS AND DISCUSSION

The data collected to date are presented in Table 1. The sea raven heart contains about 10 fold higher levels of myoglobin than the ocean pout heart. The content of myoglobin in the sea raven heart is comparable to that found in trout, carp, and catfish (D.J. Chapman, personal communication) whereas the content in the ocean pout heart is similar to that in goldfish white skeletal muscle (Sidell, Physiol. Zool. **53**:98–107, 1980). The activity of hexokinase which is an index of the capacity to utilize blood borne glucose is significantly higher in the hearts of both species than in the skeletal muscle. Similarly, the activities of citrate synthase and alpha-ketoglutarate dehydrogenase, which are indecies of Krebs cycle activity, are higher in the ocean pout heart than in the skeletal muscle. The enzyme data suggest that the ocean pout heart has a greater aerobic capacity than the skeletal muscle despite the similarity in extremely low myoglobin levels. The activities of the glycolytic enzymes phosphofructokinase and pyruvate kinase are significantly higher in skeletal muscle than in ocean pout heart suggesting that the maximum rate of anaerobic metabolism is greater in the former tissue. The significance of high levels of heart glycogen relative to skeletal muscle though is yet to be ascertained. It is possible that elevated heart glycogen relates to physiological aspects other than anaerobic metabolism such as starvation.

Table 1.--Myoglobin and Glycogen Content and Maximal Activity Levels of Enzymes of Energy Metabolism

	Ocean Pout		Sea Raven
	Heart	Skeletal muscle	Heart
Myoglobin (nmoles/g wet weight)	6.34 ± 2.89	--	64.1 ± 4.42
Glycogen (μmoles glucose/g wet weight)	86.11 ± 14.11	18.96 ± 2.69	33.99 ± 5.83
Hexokinase	2.45 ± 0.35	N.D.	2.52 ± 0.70
Phosphofructokinase	1.17 ± 0.15	2.51 ± 0.23	1.31 ± 0.11
Pyruvate kinase	36.34 ± 0.42	67.55 ± 5.9	--
Lactate dehydrogenase (.33 mM pyr)	41.34 ± 3.69	68.94 ± 2.99	83.68 ± 25.50
(10 mM pyr)	127.79 ± 4.16	177.4 ± 55	189.59 ± 36.91
Citrate synthase	12.78 ± 1.17	0.23 ± 0.01	--
α Ketoglutarate dehydrogenase	1.80 ± 0.13	N.D.	--
Cytochrome oxidase	34.64 ± 12.32	--	39.88 ± 8.97

Enzyme data are expressed as μmoles/gm wet weight·min at 10 C.

N.D. indicates not detected. The symbol - indicates that the assay has not been attempted. All values are expressed as mean ± S.E. on the basis of 3 - 8 individuals.

In conclusion, it is clear that the organization of energy metabolism in the ocean pout white heart is very different than that which occurs in white skeletal muscle. In keeping with the general findings from other systems the ocean pout heart appears to have a greater aerobic capability than the skeletal muscle; however, maximum rates of anaerobic glycolysis are higher in the skeletal muscle. The question still remains as to whether any quantitative differences exist between the red and white hearts. This problem is currently under investigation.

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EFFECTS OF ETHACRYNIC ACID AND RELATED COMPOUNDS ON ACTIVE Cl SECRETION BY THE DOGFISH RECTAL GLAND

H. Clive Palfrey, Patricio Silva and Franklin H. Epstein, Department of Pharmacology, Yale University School of Medicine, New Haven, Ct., and Department of Medicine, Beth Israel Hospital, and Harvard Medical School, Boston, Ma.

"Loop" diuretics of the 5-sulfamoylbenzoic acid class, such as furosemide and bumetanide, inhibit cAMP-stimulated rectal gland secretion possibly by interacting with the putative NaCl cotransport system in the basolateral membrane (Silva et al., Amer. J. Physiol., 233: F298, 1977; Palfrey et al., Bull. MDIBL., 19: 58, 1979). The specificity of a series of related drugs of this type appears to be similar to that found for inhibition of Na/K/Cl cotransport in avian erythrocytes, and for diuretic activity *in vivo* in dogs (Palfrey et al., *op cit*.; Palfrey et al.,