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Some Biochemical Observations on the Ventricular Myocardia of Tuna Fish, *Euthynnus affinis*, Cantor

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Biochemical assay of certain metabolites of the ventricular myocardia of the tuna fish, *Euthynnus affinis*, has been carried out. Significant gradients were exhibited in the distribution pattern of some metabolites, especially glycogen and SDH between the inner and outer myocardial layers.

Key Words: **Inner and outer myocardia, Protein, Fuel reserves, Myoglobin and SDH**

Introduction

Tuna are actively swimming teleosts inhabiting temperate, tropical and even cold waters throughout the world (Menon & Neinan 1975). They exhibit a unique adaptation for maintaining a higher body temperature than the surrounding ambient medium. Considerable attention has been focussed on the ultrastructure, physiology and biochemistry of the cardiac muscles of different vertebrates. Further, biochemical investigations on various chambers of the heart of some mammals have revealed a differential distribution pattern of myoglobin (Alexander 1975). Studies on respiratory activity of mitochondria from the inner and outer layers of ventricular myocardia in juvenile tuna fish have shown a definite variation (Modigh & Tota 1975, Tota 1976).

Generally in fishes, the venitricular myocardium is composed of two distinct muscular compartments—the inner and outer layers. Of these, the inner layer is a spongy core of myocardial fibres supplied by venous blood circulating in the lacunae and interstices from the ventricular luman while the outer compact layer which forms the smooth surface of the ventricle is supplied by the oxygenated blood from the coronary arteries (Satchell 1971, Ostadal & Scheibler 1971). This type of organisation of the ventricular myocardium seems to represent a complete transitory step during the evolution of vertebrates; the spongy myocardium of the most primitive poikilotherms is being replaced by a compact coronary supplied muscle in the higher vertebrates (Poupa et al. 1974). Despite this

interesting example of muscle heterogeneity in 'Pure' myocardial tissue, there has hitherto been a complete lack of information regarding the metabolic and functional characteristics of these two myocardial layers in fishes. Accordingly a biochemical study of these myocardia viz., the inner and outer layers of the ventricle of the tuna fish, *Euthynnus affinis*, has been undertaken.

Materials and Methods

Investigations were carrried out on tuna fishes weighing 2 to 3 kg and having a range at 37–52 cm in total body length. Live fishes were collected directly from the fishing nets at the Sanghumugham Beach at Trivendrum. They were kept in ice, swiftly transferred to the laboratory and the sample analysis was conducted within 30 min of landing. The heart was excised and the inner and outer myocardial layers were carefully separated for biochemical investigations. The moisture content was estimated by dehydrating a known weight of tissue in an air oven as 60°C till attainment of constant weight. The following techniques were employed for the assay of the diverse parameters concerned.

1. Lipid—*Nayeemunissa and Rao* (1972)
2. Glycogen—Anthrone reagent technique—*Seifter et al.* (1950)
3. Protien—*Wong's Microkejeldhal's method* (1923)
4. Myoglobin—*Tappan and Reynafarjee* (1957)
5. SDH—*Kun and Abood* (1947). The optical density of the aliquots were measured in a Bausch and Lomb 'Spectronic 20' photoelectric colorimeter.

Results

The data on biochemical aspects of the inner and outer ventricular layers of tuna fish, *Euthynnus affinis*, are shown in the table.

Comparatively the level of moisture, protein and lipid in the outer and inner layers did not reveal any significant variations viz., Moisture—70.09 and 69.89%, Protein—17.19 and 16.32% and Lipid—13.62 and 14.02%. However the glycogen content revealed an almost two fold increase in the outer layer (101.1472 μg/100 mg) than the inner myocardial layer (64.1288 μg/100 mg). A relatively higher amount of myoglobin was

Region of Heart	No. of fishes used	Moisture % g/wet wt	Protein % g/wet wt	Lipid % g/dry wt	Glycogen μgm/100mg wet tissue	Myoglobin mg/g wet tissue	SDH μg formazan/ min/g
Heart outer layer	10	70.09	17.19	13.62	101.1472	11.9894	75.58
(Epicardia)		(0.2586)	(1.6765)	(0.2520)	(9.1973)	(1.6275)	(16.7842)
Heart Inner layer	10	69.89	16.32	14.02	64.1288	13.0660	85.81
(Endocardia)		(0.2919)	(1.2060)	(0.5482)	(22.4193) $P=<.001$	(2.5808)	(10.4991) $P=<0.05$

Mean values of ten observations from ten fishes with the standard error given in brackets. Statistical analysis for the level of significance is given as $P=<.001$ and $P=<0.05$.

discernible in the inner region (13.07 mg/g) of the tuna fish ventricle than the outer region (11.99 mg/g). The concentration of SDH also exhibited a definite gradient between the outer and inner myocardium viz., 75.58 and 85.81 μg formazan/min/g. in the two layers respectively.

Discussion

Previous investigations on moisture level of the myocardia of representative vertebrates revealed definite variations in accordance with ecological factors and metabolic pattern of the animal concerned. (Mathew Oommen 1980). However, no marked variation was found in moisture levels of the inner and outer ventricular myocardia of tuna fish.

Regarding total proteins, chamber-wise variation have been reported in cardiac muscles in relation to their respective mitochondrial activities (Laguens 1971, Sordabl 1976). In the present study the ventricular myocardium of tuna fish did not reveal any significant variation of the metabolite between the two layers. Lipid constitutes an important source of fuel reserve for muscular contraction; the metabolism of which yield sufficient ATP (West et al. 1956). In tuna, *Euthynnus affinis*, the myocardium was found to have a relatively higher lipid concentration. It may be stated that the red and cardiac muscles of vertebrates have a preference for lipid as a fuel reserve as compared to the skeletal muscles (George & Berger 1966). Further, since the cardiac muscle is unique in as much as it is having continuous sustained activity which warrants a steady supply of energy, for the rhythmic pulsation of the ventricle, it cannot possibly derive its energy from the breakdown of stored glycogen and hence has to derive the same from the aerobic oxidation of fatty acids from the circulating blood (Bing et al. 1953.)

Oommen and Alexander (1980) have reported a regressive variation in the distribution of glycogen in the myocardia of some vertebrates. Regarding the tuna ventricular myocardium, the outer region exhibited a significantly (P=.001) higher level of glycogen than the inner myocardium. Such a higher quanta of glycogen may possibly be due to the fact that outer myocardial fibres are relatively more glycolytic than inner fibres which enable them to produce a maximal amount of mechanical work within a short period. In this respect, this may be compared with the white

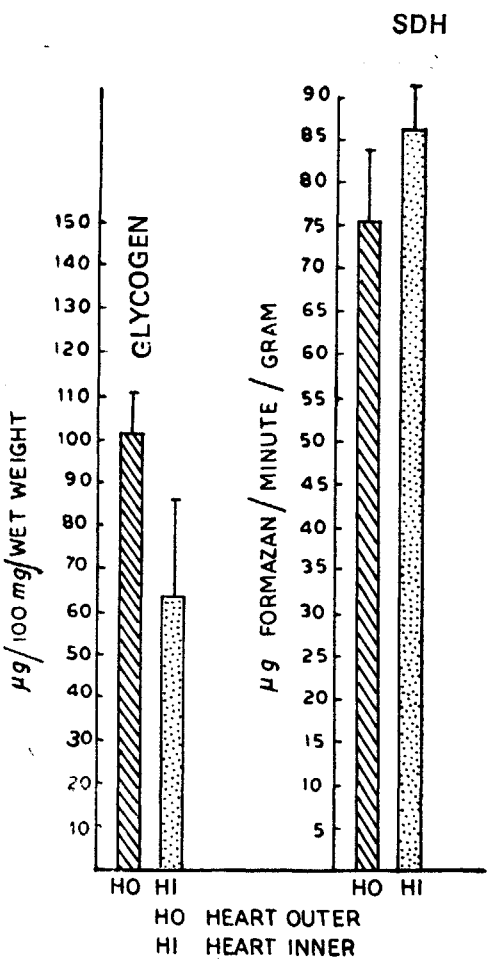

Figure 1 Distribution of Glycogen & SDH

fibres of skeletal muscle in their fuel utilization pattern.

Myoglobin is the muscle respiratory pigment which is concerned with the storage and molecular transfer of oxygen at the cellular level. studies by Tota (1970) in adult tuna, *Thunnus thynnus*, demonstrate that homogenates of the inner myocardial layer maintain a higher level of oxygen consumption than the homogenates of the outer layer of the myocardia. Further, Millikan (1939) had dicserned a pattern in the distribution of myoglobin, whereby myoglobin is generally found in larger quantities in those muscles requiring slow and repetitive activity of considerable force. Myoglobin exists in higher concentration in red muscles of fishes, which are concerned with slow and repetitive activity (Love 1970). In tuna the inner myocardial layer was found to have a higher respiratory activity than outer layer (Basile et al. 1976). In *Euthynnus affinis* the inner myocardium exhibits a slightly higher amount of myoglobin than the outer myocadium which however is not statistically significant.

SDH (succinic dehydrogenase) is the key enzyme of the oxidative cycle—the Kreb's cycle—and is localized at the inner mitochondrial membrane. Since SDH is a mitochondrial enzyme, the level of its activity is an index of mitochondrial metabolism as well as oxidative metabolism of the tissues concerned. In tuna, *Euthynnus affinis*, the inner myocardium exhibited a significantly higher ($P = .05$) quantum of SDH than the outer layer. Studies by Modigh and Tota (1975) on ventricular myocardium of juvenile tuna have revealed that there exists a definite variation in the respiratory activity of the mitochondria from inner and outer layer of the ventricle. Further, Maresca (1976) had reported a variation in the temperature dependence of oxidative phosphorylation in the inner and outer ventricular myocardium of tuna. A similar variation in the distribution pattern of SDH in the inner and outer myocardial layers in the ox (*Bos* sp.) heart has been reported by Eapen and Alexander (1979). In ox myocardia, the concentration of SDH was found to be greater in the inner myocardium than the outer. In fact, the relatively higher SDH level, a general biochemical trait of red muscle of fishes, facilitating a higher functional efficiency, is absolutely essential for a constantly pumping muscular organ like the heart. Moreover in the case of heart, the mitochondria in the inner myocardial layer were found to have a higher cristae density than the outer myocardium. Based on morphometric flow indicator studies, tuna heart ventricle could be regarded as two separate entities as regards structure and vascularization, the outer cortex and the inner spongy layer (Cameron 1975).

In consensus, significant variations are discernible in some of the biochemical parameters especially glycogen and SDH in the inner and outer ventricular myocardium of *Euthynnus affinis* in correlation to the specific functional requirements, such as maintenance of higher body temperature than the ambient medium, adjustment to the varying environments and a suitably adapted vascular supply which facilitate active swimming habits of this teleost.

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References

Alexander K M 1975 Biochemical studies on mammalian cardiac muscles. 1. Distribution pattern of myoglobin in different chambers of the heart of some mammals; *TITJ of Life Sci.* **5** 61–65

Basile C, Golospink G, Modigh M and Tota B 1976 Morphological and biochemical characterisation of the inner and outer ventricular myocardial layers of adult tuna fish (*Thunnus thynnus* L.); *Biochem Physiol.* **B54** 279–283

Bing R J, Siegel A, Vitale A, Balboni F, Sparks E, Taescbler M, Klapper M and Edwards S 1953 Metabolic studies on human heart in vivo. 1. Studies on carbohydrate metabolism of the human heart; *Am. J. Med.* **15** 284–296

Cameron J N 1975 Morphometric flow indicator studies of the teleost heart; *Can. J. Zool.* **53** 691–698

George J C and Berger A G 1966 *Avian Myology* (New York: Academic Press)

George Eapen and Alexander K M 1980 Studies on physiology of mammalian cardiac muscles: 1. A report on differential distribution of some metabolites in myocardia of Ox; *Bos* sp., *Abstr. Proc. 4th All India Symposium on Comparative Physiology and Endocrinology; S.V. Univ., Tirupati, December, 1978*

Kun E and Abood L G 1949 Colorimetric estimation of succinic dehydrogenase by Triphenyl tetrazolium chloride; *Science* **109** 144–146

Laguens R 1971 Morphometric study of myocardial mitochondria in the rat; *J. Cell. Biol.* **48** 673–676

Love R M 1970 *The Chemical Biology of Fishes* (London: Academic Press)

Maresca B, Modigh M; Servillo L and Tota B 1976 Different temperature dependences of oxidative phosphorylation in the inner and outer layers of tuna heart ventricle; *J. Comp. Physiol.* **105** 167–172

Mathew M Oommen 1980 Biochemical studies on the cardiac muscles of some vertebrates; Ph.D. thesis, Kerala University

Menon A G K and Neinan T V 1975 The tuna resources of the Indian ocean; *Sea Food Export Journal.* **7** 25–36

Millikan G A, 1939 Muscle hemoglobin; *Physiol. Rev.* **19** 503–523

Modigh M and Tota B, 1975 Mitochondrial respiration in the ventricular myocardium and in the white and red myotomal muscles of juvenile tuna fish (*Thunus thynnus L*); *Acta Physiol. Scand.* **93** 289–294

Nayeemunnisa and Rao K P 1972 Effects of thermal acclimation in the lipid metabolism in the earth worm; *Lampito mauritii*—A method for lipid estimation; *Comp. Biochem. Physiol* **B42** 167–173.

Ostadal B; and Schiebler T H 1971a Uber die terminale stromahn in Fischherzen; *Z. Anat. Entw Gesch.* **134** 101–110

Poupa O., Gessor H, Jonsson S and Sullivan L 1974 Coronary supplied compact shell of ventricular myocardium in salmonids. Growth and Enzyme pattern; *Comp. Biochem. Physiol.* **A48** 85–95.

Satchell G H 1971 *Circulation in Fishes* (Cambridge; The University Press) 6, 7

Seifter S, Dayton S, Novic B and Muntwyler E 1950 The estimation of glycogen with the Anthrone reagent; *Arch. Biochem.* **25** 191–200

Sordahl L A 1976 Differences in mitochondrial functions from right and left ventricular myocardium of four mammalian species; *Comp. Biochem. Physiol.* **B54** 339–342

Tappan D V and Raynaferjee B 1957 Alteration in enzymes and metabolites resulting from adaptation to low oxygen tension; *Am. J. Physiol.* **190** 93–98.

Tota B 1970 On the respiratory metabolism of the inner and outer layers of tuna fish heart ventricle; *Pubbl. Staz. Zool. Napoli* **38** 223–225

——— 1976 On the metabolic characterisation of the compact and spongious ventricular myocardium of adult tuna fish (*Thunnus thynnus* L.); *Abstract from the Symposium on Fish physiology*, Goteborg, Oct. 1975.

West E S, Todd, W R Manson, H S and Bruggen J T V 1956 *A Textbook of Biochemistry* (New York: The Macmillan Co.)