ACKNOWLEDGEMENT

I am thankful to Dr. R. P. Sharma, Geneticist, N.R.L., I.A.R.I., New Delhi, for technical help and constructive suggestions.

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EFFECT OF *IN VIVO* MUSCULAR STIMULATIONS
VI. Inhibition of Cardiac Proteolysis by the Electrical Stimulations of Leg Muscle

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ABSTRACT

The cardiac proteolysis was inhibited by the short term and prolonged *in vivo* muscular stimulations with an increase in the levels of soluble and structural protein fractions. The cardiac tissue appeared to have diverted the free amino acids towards the synthesis of both proteins and carbohydrates.

INTRODUCTION

PHYSICAL exercise is known to alter the metabolism of cardiac tissue[1–3]. Prolonged *in vivo* muscular stimulations[2–3] and conflict stress[4] increased the levels of oxidative enzymes of the tissues of the body and of myocardium respectively. Increased oxygen demand of myocardium[5], heart rate, arterial blood pressure and regional blood flow[6] are reported during physical exercise. Induced proteolysis is reported in various tissues of the body during heavy exercise[7] and repeated electrical stimulations[8]. Attempts to demonstrate changes in the protein levels of cardiac tissue during exercise led to contradictory results[4,9–10]. In the light of these conflicting statements an attempt is being made to understand the effect of short term and prolonged *in vivo* muscular electrical stimulations on the protein metabolism of cardiac tissue.

MATERIAL AND METHODS

Frogs, *Rana hexadactyla* (Lesson), were employed for the present investigation. Right gastrocnemius muscles of intact frogs were stimulated with electronic stimulator (INCO/CSIO Research Stimulator—Ambala) as described earlier[2], with a series of impulses (biphasic) of 5 V at a frequency of 2 pulses/sec for 30 minutes per day for one day in one batch of experimental animals and for ten successive days in another batch. The duration of each impulse was 100 ms, while the delay was 400 ms.

The cardiac tissue was isolated as usual for biochemical studies. Protein contents in the supernatant (water soluble) and the residue (water insoluble —structural) as obtained by the centrifugation of tissue homogenate at 3000 rpm for 30 min are estimated by the method of Lowry *et al.*[11]. Protease acti-

vity and free amino acids (Moore and Stein[12]), free ammonia (Beigmeyer[13]) and the activities of alanine amino transferase (AlAT—EC 2.6.1.2) and aspartate amino transferase (AAT EC. 2.6.1.1) by the method of Reitman and Frankel[14] are determined.

TABLE I

Levels of soluble and structural proteins (mg/gm wt), protease activity (μM Tyrosine/mg protein/hr), free amino acids (μM Tyrosine/gm wt), AlAT and AAT (μM Pyruvate/mg protein/hr) and ammonia (mg/gm wt) in the cardiac tissues of control and experimental animals

Values are mean of six observations. Mean ± S.D. + and − indicate percentage increase and decrease over control. 'P' indicates level of significance

Sl. No.	Component	Control	Experimental	
			1 day	10 days
1.	Soluble proteins (water soluble)	94·42 ± 8·48	116·04 ± 13·11 + 22·9 P < 0·01	125·11 ± 4·12 + 32·5 P < 0·001
2.	Structural proteins (water insoluble)	56·74 ± 9·08	73·49 ± 5·74 + 29·52 P < 0·001	59·07 ± 9·89 + 4·1 NS
3.	Protease activity	0·09 ± 0·001	0·08 ± 0·004 − 11·11 P < 0·001	0·069 ± 0·003 − 23·33 P < 0·001
4.	Free amino acids	9·44 ± 1·93	20·86 ± 2·98 +120·97 P < 0·001	8·22 ± 0·77 − 12·92 P < 0·05
5.	AlAT	0·137 ± 0·01	0·143 ± 0·006 4·38 NS	0·172 ± 0·04 + 28·55 P < 0·05
6.	AAT	0·166 ± 0·028	0·155 ± 0·002 − 6·63 NS	0·161 ± 0·03 − 3·01 NS
7.	Ammonia	0·029 ± 0·004	0·027 ± 0·008 − 6·9 NS	0·031 ± 0·001 + 6·89 NS

RESULTS AND DISCUSSION

The data presented in Table I reveal the extent of changes in cardiac tissue protein metabolism in response to the *in vivo* muscular stimulations. The protease activity of the heart decreased significantly on one day muscular stimulation with an elevation in both soluble and structural proteins. The increased protein levels might be due to decreased proteolysis and increased protein synthesis. The elevation in structural proteins in one day stimulation might suggest the possibility of alterations in solubility properties of the proteins. This observation is in consonance with the earlier reports where increase in cardiac protein content[5,10] and cardiac hypertrophy[5] have been demonstrated during exercise. In spite of significant decrease in protease activity, free amino acid level showed an increase, indicating the possibility of active uptake of amino acids by the tissue from some other source. The blood amino acid level was reported to have been increased to a large extent on one day muscular stimulation[15] and this

might be responsible for the increase in free amino acid level of the cardiac tissue The activities of aminotransferases, *viz.*, AAT and AlAT, and the level of free ammonia showed non-significant changes, suggesting less involvement of amino acids in the metabolism of the tissue during short term muscular electrical stimulation.

Protease activity recorded further decrease on 10 days of muscular stimulations. Soluble protein fraction increased significantly, while structural protein showed non-significant change suggesting the possibility of slight change in the solubility of proteins. The level of free amino acids decreased indicating their utilization in the tissue towards protein biosynthesis. Increase in GDH activity as reported earlier during prolonged muscular stimulations[3] is notable. Besides, AlAT activity increased suggesting the possible diversion of the amino acids towards glyconeogenesis[15]. Since both asparate aminotransferase activity and free ammonia level showed non-significant change, it can be suggested that to a large extent, free amino acids might have been utilized for protein biosynthesis and glyconeogenesis. Hence on 10 days of muscular stimulations, the cardiac tissue seems to inhibit proteolysis with a switch over towards the biosynthesis of proteins and carbohydrates.

ACKNOWLEDGEMENTS

One of the authors (PR) is grateful to the Council of Scientific and Industrial Research, New Delhi, for the award of Research Fellowship, during the tenure of which this work was carried out.

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WORKSHOP ON STRATIGRAPHY AND CORRELATION OF THE LESSER HIMALAYAN FORMATIONS

A four-day Workshop on the Stratigraphy and Correlation of the Lesser Himalayan Formations, from April 6 to 9, 1979, is organised by the Department of Geology, Kumaun University, Nainital. The objective of the Workshop is to make a serious effort to resolve the stratigraphic tangle, establish an agreed stratigraphic standard, formulate a common nomenclature and work out a mutual correlation of different lithological units in order to prepare the basis for the construction of the geological history of the Lesser Himalaya. For details please contact Prof. K. S. Valdiya, Head, Department of Geology, Kumaun University, Nainital, Uttar Pradesh

I.C.A.R. GRANT TO *CURRENT SCIENCE*

The Current Science Association acknowledges with thanks the receipt of the publication grant of Rs. 20,000 towards the publication of its Journal CURRENT SCIENCE during the year 1978–79 from the Indian Council of Agricultural Research, New Delhi.