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EFFECT OF *IN VIVO* MUSCULAR STIMULATIONS

III. Some Aspects of Carbohydrate Metabolism of Cardiac Tissue

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ABSTRACT

The carbohydrate level of cardiac tissue was drastically decreased under the influence of *in vivo* muscular stimulations. The aminoacid content increased to a large extent. On successive muscular stimulations for 10 days the cardiac tissue appeared to have undergone a shift towards aminoacid oxidations, decreasing the utilization of carbohydrates.

INTRODUCTION

ELECTRICAL stimulation has been widely employed for inducing the muscular exercise[1-2]. Increased levels of oxidative enzymes of all tissues of the body[3] and of myocardium[4], have been reported following prolonged *in vivo* muscular stimulations and conflict stress respectively. Physical exercise also leads to increase in the oxygen demand of myocardium[5], and increases heart rate, regional blood flow and arterial blood pressure[6-7]. The values of these parameters were shown to decrease within 5–7 days of exercise. Since heart is known to involve in rapid activity during exercise, an attempt has been made to understand the possible changes in carbohydrate metabolism of cardiac tissue during muscular stimulations of short duration and prolonged periods.

MATERIAL AND METHODS

Frogs belonging to the species *Rana hexadactyla* (Lesson) were employed in the present investigation. Right gastrocnemius muscles of intact frogs were stimulated with electronic stimulator (INCO/CSIO Research Stimulator—Ambala) as described earlier[3] with a series of impulses (biphasic) of 5 V at a frequency of 120 pulses/min for 30 min per day for one day in one batch of experimental animals and for 10 successive days in another batch. The duration of each impulse was 100 ms, while the delay was 400 ms.

The cardiac tissue was isolated from freshly pithed control as well as experimental frogs and placed in amphibian Ringer to recover from shock effects. The heart was squeezed and washed thoroughly with amphibian Ringer to remove the traces of blood and taken for biochemical assays.

The activity levels of SDH, MDH and LDH were estimated by the method of Nachlas *et al.*[8] and GDH activity by the method of Lee and Lardy,[9] modified as follows. The reaction mixture in a final volume of 2 ml contains 40 μm of substrate (sodium succinate for SDH, sodium malate for MDH, sodium lactate for LDH and sodium glutamate for GDH) 0·1 μm of NAD (for MDH, LDH and GDH only), 100 μm of pH 7·4 phosphate buffer and 2 μm of INT (2-4 Iodophenyl) 3 (4 nitrophenyl) 5 phenyl tetrazoliumchloride). The reaction was initiated by the addition of 0·5 ml of the tissue extract. The incubation was carried out for 30 min at 37 C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight in cold with 5 ml of toluene. The intensity of colour was read at 495 mμ against toluene blank and the activity was expressed as μm of formazan/gm wt/hr. Total

TABLE I

Levels of total carbohydrates, lactic acid, free aminoacids (mg/gm wt) and succinate, malate, lactate, glutamate dehydrogenase activities (μm formazan/gm/hr) in heart of control and experimental animals

The values represent the mean of six observations. Mean ± SD ; + and − indicate per cent increase or decrease over controls

COMPONENT	CONTROL	Experimental	
		1 day	10 days
1. Total Carbohydrates	2·74 ± 0·39	1·14 ± 0·21	1·96 ± 0·24
		− 58·4 P < 0·001	−28·47 P < 0·01
2. Lactic acid	0·938 ± 0·05	1·073 ± 0·052	1·082 ± 0·024
		+ 14·39 P < 0·01	+16·34 P < 0·001
3. Free aminoacids	1·71 ± 0·35	3·78 ± 0·54	1·49 ± 0·14
		+121·05 P < 0·001	−12·87 P < 0·001
4. SDH	28·15 ± 0·707	28·24 ± 1·25	49·18 ± 0·008
		+ 0·32 NS	+74·71 P < 0·001
5. MDH	5·92 ± 1·45	5·87 ± 0·98	6·44 ± 0·9
		− 0·85 NS	+ 8·78 NS
6. LDH	31·06 ± 6·54	31·44 ± 3·47	46·6 ±14·5
		+ 1·22 NS	+50·03 P < 0·05
7. GDH	26·73 ± 1·15	18·86 ± 2·56	43·9 ± 8·9
		− 29·44 P < 0·01	+64·23 P < 0·01

carbohydrates were estimated by the method of Corroll *et al.*[10], free amino acids, by the method of Moore and Stein[11] and lactic acid by the method of Barker and Summerson[12] modified by Huckabee[13].

RESULTS AND DISCUSSIONS

The results presented in Table I Indicate the influence of *in vivo* muscular stimulations on certain components of carbohydrate metabolism of cardiac tissue. The total carbohydrate level of heart decreased drastically following one day of muscular stimulation. As a consequence of carbohydrate degradations, lactic acid level of the tissue has been elevated. While succinate, malate and lactate dehydrogenase activities revealed non-significant changes, glutamate dehydrogenase activity showed considerable decrease, indicating probably the decreased levels of aminoacid oxidations in the tissue. The free aminoacid level was found to be elevated, which might be due to the decreased aminoacid oxidations and increased rate of uptake of aminoacids from blood. The uptake of aminoacids from the blood might be possible in the light of elevated blood aminoacid level after one day muscular stimulations (82% increase per 100 ml blood).

The situation of cardiac metabolism seemed to be different after 10 days of successive muscular stimulations. The tissue was capable of decreasing the extent of depletion of total carbohydrates during the muscular stimulations. The capacity of the tissue to utilize less carbohydrates appeared to have developed by

slight reorientation of the metabolism. Elevated GDH activity and decreased aminoacid content suggested the involvement of aminoacid oxidations. As a consequence of increased GDH activity, continuous addition of α-ketoglutarate could be visualised and this might be responsible for increased SDH activity of the tissue. Besides, NAD dependent LDH activity also showed considerable increase indicating the active addition of lactic acid into the citric acid cycle. In spite of such a large increase in SDH activity, MDH activity showed a non-significant rise indicating the participation of some other cycle which might be actively depleting the citric acid cycle intermediaries at this level. In conclusion it could be said that during prolonged muscular stimulations, cardiac tissue appeared to have decreased the utilization of carbohydrates with a shift towards the oxidations of aminoacids.

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SYMPOSIUM ON " FLORISTIC STUDIES IN PENINSULAR INDIA " AND ANNIVERSARY CELEBRATIONS OF MADRAS HERBARIUM, COIMBATORE

The Herbarium of the Botanical Survey of India at Coimbatore has completed 125 years of its services to the plant-sciences of India. The Botanical Survey of India is planning to celebrate the 125th anniversary and also to organise a symposium "Floristic Studies in Peninsular India" in November 1978 at Coimbatore. Details can be had from Dr. S. K. Jain, Director, Botanical Survey of India, P.O. Botanic Garden, Howrah–711 103 or from Dr. N. C. Nair, Dy. Director, Botanical Survey of India, 29 Dewan Bahadur Road, COIMBATORE–641 002.

INDIAN SOCIETY OF CELL BIOLOGY

The Third Cell Biology Conference organized by Cancer Research Centre will be held at Bombay during January 10–12, 1979. Further information regarding the Conference can be had from Dr. A. N. Baisey, Organizing Secretary, Cell Biology Conference, Cancer Research Institute, Tata Memorial Centre, Parel, Bombay 400 012.