the possibility of the brain hormone(s) participating in the moulting process, to be of steroidal nature having cholesterol as the precursor.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN DENERVATED AMPHIBIAN GASTROCNEMIUS MUSCLE

PROGRESSIVE decrease of oxidative and glycolytic activity[1–4] and increased proteolytic activity[5,6] are prominent features of hereditary and nutritional muscular dystrophies and atrophy resulting from disuse, denervation, and tenotomy. Succinate[7], malate[3], lactate[9], glutamate[7], isocitrate dehydrogenases[10] of gastrocnemius muscle were found altered in their levels on denervation. Although the relationship between mitochondrial abnormality and the mechanism of muscle atrophy is not immediately evident, the mitochondrial dysfunction may contribute to a loss in muscle structure as a consequence of the decreased amount of energy available for muscular function[11]. Under these circumstances the energy demand for the increased sarcoplasmic or non-contractile protein synthesis may be met by the extramitochondrial oxidations mainly by hexose monophosphate shunt. It was reported that two distinct types of the enzymes having glucose-6-phosphate dehydrogenase activity exist in the tissues of various vertebrates, one located in cytosol relatively specific to NADP and the other located in microsomes which utilizes either NADP or NAD[12–15]. In view of this an attempt was made to study the activity levels of both NAD and NADP-linked glucose-6-phosphate dehydrogenase (G6PDH) on progressive denervation of amphibian gastrocnemius muscle which may provide some insight into the relative importance of co-enzyme specificity in atrophied muscle.

Rana hexadactyla (Lesson) of medium size were denervated by sciatic nerve section about 1 cm from its origin on one side of the leg, while contralateral muscle was considered as control. The frogs were fed *ad libitum* with cockroaches, and water was changed regularly. After 1, 2, 3 and 4 weeks post-operatively, animals were sacrificed and both the denervated and contralateral control gastrocnemius muscles were excised quickly. Homogenates of tissues (10%, wt/vol) were prepared in 0·25 M ice-cold sucrose using Potter-Elvehjem type glass homogenizer and cehtrifuged at 2500 rpm for 15 min. Supernatant (0·5 ml containing 50 mg tissue) was assayed for glucose-6-phosphate dehydrogenase activity by the method of G. D. Lohr and H. D. Waller[16] with the following modifications. The reaction mixture of 2 ml contained 20 μ moles of glucose-6-phosphate, 100 μ moles of trichthanol amine buffer (pH 7·4), 4 μ moles of INT (2-*p*-iodophenyl-3-nitrophenyl tetrazolium chloride), 10 μ moles of $MgCl_2$ and 0·2 μ moles of NAD for NAD linked glucose-6-phosphate dehydrogenase or 0·3 μ moles of NADP for NADP-linked glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of 0·5 ml of supernatant. Controls received 0·5 ml of sucrose in place of enzyme extract. After an incubation of 30 min at 37° C, the reaction was stopped by the addition of 5 ml of glacial acetic acid, and the formazan was extracted into 5 ml of toluene. After overnight stay in cold, the intensity of the colour was measured in UV-spectrophotometer (Hilger and Watts, England) at 495 nm using silica cuvettes of 10 mm path length. Individual zero time controls were maintained for all the samples by the addition of glacial acetic acid to reaction mixture prior to the addition of enzyme. Enzyme activity was expressed as μ moles of formazan/mg protein/hr. Protein levels were determined by the method of Lowry *et al.*[17]. Data were subjected to statistical processing according to standard procedures using students *t* test[18].

Progressive decrease of NAD-linked glucose-6-phosphate dehydrogenase activity level was found in the muscle subjected to denervation, the decrease being 9·81% after 4 weeks (Table I). Contrastingly the NADP-linked glucose-6-phosphate dehydrogenase showed progressive increase, *i.e.*, 26·16% after 4 weeks. Though the NAD-specific G6PDH has shown decreased activity level when compared to normal

TABLE I

Glucose-6-phosphate dehydrogenase activity in the amphibian gastrocnemius muscle

(Values are mean ± S.D. of 10 observations. Activity expressed as μ moles of formazan/mg protein/hr)

	NAD-Specific Activity				NADP-Specific Activity			
Weeks	I	II	III	IV	I	II	III	IV
				Control muscle				
	0·054 ±0·005	0·053 ±0·010	0·058 ±0·009	0·051 ±0·003	0·108 ±0·012	0·109 ±0·010	0·108 ±0·008	0·107 ±0·009
				Denervated muscle				
	0·052 ±0·010	0·049 ±0·007	0·054 ±0·006	0·046 ±0·007	0·115 ±0·042	0·125 ±0·012	0·129 ±0·016	0·135 ±0·010
				% Change on atrophy				
	−3·71	−7·50	−6·90	−9·81	+6·48	+14·68	+19·44	+26·16
				'*t*' values				
	0·667	0·952	1·176	1·923	0·50	3·265	3·62	2·69
				'P' values				
	NS	NS	NS	NS	NS	<0·005	<0·002	<0·02

control muscle, it is statistically not significant showing that denervation does not affect the NAD-specific glucose-6-phosphate dehydrogenase. Further it is evident that this enzyme has shown comparatively less activity than the NADP-specific glucose-6-phosphate dehydrogenase in the control muscle itself.

Progressive denervation atrophy induced an increment in the activity level of NADP-specific G6PDH in the gastrocnemius muscle. Similar increase in NADP-specific G6PDH in atrophic muscle has been reported ealier[19-21]. This increase in shunt metabolism is also related to the production of pentoses to support the substantial rise in the nucleic acids[22] of the atrophic tissue and/or an increased demand for NADPH for synthetic purposes. McCaman[23, 24] suggested that a common feature of dystrophic and denervated muscle is a general elevation in the activities of NADP-dependent dehydrogenases, whereas the activities of NAD-dependent dehydrogenases show a decrease. This is obvious, since one of the major functions of the HMP shunt appears to be the provision of reduced NADP required by the anabolic process outside the mitochondria[25]. Further the increased lipogenesis observed in muscular dystrophy[26] requires NADPH for their synthesis and consequently there is enhanced synthesis of NADP in atrophied muscle, which in its turn may result in elevated levels of NADP-dependent enzymes. It was reported earlier that NADP-specific isocitrate dehydrogenase[19] has shown an increment in its level on denervation and the present finding of enhancement in the level of NADP-dependent glucose-6-phosphate dehydrogenase in denervated frog gastrocnemius muscle is in agreement indicating the presence of synthetic process.

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CIRCADIAN RHYTHMIC ACTIVITY OF ISOCITRATE DEHYDROGENASE IN THE SLUG, *LAEVICAULIS ALTE* (FERRUSAC, 1821)

ALTHOUGH considerable amount of information concerning different types of rhythms in molluscs[1–4] is available, very little work was done on the rhythmic activity of enzymes in these animals. Slugs are shown to be nocturnal animals[5] and hence there could be corresponding variations in the various physiological processes of these organisms. Enzymes like phosphorylase[6], alkaline and acid phosphatase[7], lipase[8] and metabolites like hepatopancreatic glycogen, blood glucose[9] were shown to undergo regular rhythmic changes in the slug. In view of above, it is proposed to assay the activity pattern of isocitrate dehydrogenase a key enzyme in the citric acid cycle, in the hepatopancreas and foot muscle of the slug, as a function of time of the day. The activity pattern of this enzyme during the course of 24h period may reflect the pattern of utilization of energy sources to sustain the energy needs of the animal for various activities.

The details of collection and maintenance of slugs and sampling of tissues were described earlier[6]. Homogenates of tissues (10% w/v) were prepared in 0·25 M ice-cold sucrose and centrifuged at 2500 rpm for 15 min; 0·5 ml of each supernatant (containing 50 mg of the tissue) was assayed for the isocitrate dehydrogenase (EC 1.1.1.41) activity level by the method of Kornberg and Pricer[10] as modified by Mastanaiah *et al.*[11]. The reaction mixture of 2 ml contained 20 μ moles of DL–isocitrate; 100 μ moles of phosphate buffer (pH 7·4); 4 μ moles of INT (2-*p*-iodophenyl-3-nitrophenyl tetrazolium chloride); 10 μ moles of $MgCl_2$, 0·2 μ moles of ADP and 0·2 μ moles of NAD. The reaction was initiated by the addition of 0·5 ml of supernatant. The control reaction mixture received 0·5 ml of sucrose in place of supernatant solution. After an incubation for 30 min at 37° C, the reaction was stopped by the addition of 5 ml of glacial acetic acid and the formazan formed due to reduction of the dye was extracted into 5 ml of toluene (overnight in cold) and the colour was read in UV spectrophotometer at 495 nm using silica cuvette of 10 mm path length. The controls were maintained for all the samples by the addition of glacial acetic acid to the reaction mixture prior to the addition of the enzyme and incubation. The enzyme activity was expressed as μ moles of formazan/mg protein/h. Protein levels were determined by the method of Lowry *et al.*[12]. The data were subjected to statistical processing according to standard procedure (Pillai and Sinha)[13].

The present study shows a typical clock connected rhythm in the ICDH activity, with maximal activity around 00·00 h and minimal activity around 12·00 h of the day in both the tissues. The enzyme ranges from $0·125 \pm 0·015$ to $0·165 \pm 0·011$ μ moles of formazan/mg protein/h in the foot muscle and $0·081 \pm 0·010$ to 0·132–0·012 μ moles of formazan/mg protein/h in hepatopancreas. In both the tissues the difference between the maximal (00·00 h) and minimal (12·00 h) was significant ($P < 0·001$ for muscle; $P < 0·001$ for hepatopancreas). But the pattern of rise and fall in isocitrate dehydrogenase activity in between these intervals were different in