CHAPTER III

LOCAL AND SYSTEMIC ALTERATIONS IN LACTATE AND SUCCINATE DEHYDROGENASES DURING TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA

Dehydrogenase catalized reactions bring about oxido-reduction of metabolic intermediates in a sequential fashion constituting well defined metabolic pathways involving the breakdown and synthesis of proteins, lipids and carbohydrates. Further, by their precisely regulated activities they also bring about controlled interconversions between these metabolites and thus play pivotal roles in bringing about adaptive metabolic alterations and energy transformations as per the spatial and temporal requirements of various tissues and organs of a living system. Activities of specific dehydrogenases apart from controlling the operation of anaerobic or aerobic pathways of metabolism, also generate intermediates useful in the interconversion of various metabolites. In addition, they also control the switching on or off of either anaerobic or aerobic reactions or even the operation of both the pathways simultaneously and perhaps also the switch over from one to the other according to the dictates of the system concerned. Such alterations could be well within the purview of living systems in response to

environmental cues, or even to meet the exigencies of energetics imposed upon the organism either at the tissue, organ or organismal level as an adaptive response to the many physiological events or biological phenomena characteristic of various animals. Regeneration of lost parts of an organism is one such phenomenon which calls for alterations in metabolic features for meeting the changing energy demands and generating the requisite biochemical moieties. This has been well established for both amphibian limb regeneration as well as lacertilian tail regeneration (Johnson and Singer, 1964; Schmidt, 1968; Procaccini et al., 1973; Shah and Ramachandran, 1976; Shah and Hiradhar, 1978). Apart from the reported augmented metabolic activities characteristic of urodelian limb regeneration (Schmidt, 1968), some of the documented evidences on lizard tail regeneration have also highlighted the occurrence of a metabolic shift at the local site from anaerobic to aerobic and back to anaerobic during the course of regeneration (Shah and Ramachandran, 1970;1976; Shah and Hiradhar, 1974a; Chapter IV). Lizards in general are known to rely principally on anaerobic mode of metabolism (Bennet, 1972; Bennet and Dawson, 1972; Bennet and Light, 1972) for meeting their energy requirements. Some of the recent studies from this laboratory have not only implicated the participation of systemic factors as props for the activities at the local site but have also tentatively

indicated the prevalance of an aerobic environment in the body as a whole during the blastemic and differentiation phases of tail regeneration in Mabuya carinata (Shah et al., 1980a, 1981; Chapter IV). Since such a conclusion was reached based mainly on certain circumferential observations like increased oxygen carrying capacity of the blood (Shah et al., 1980 a), depletion of colloidal content of the thyroid follicles (Ramachandran et al., 1981) and shrinkage and loss of lipids from the visceral fat bodies (Shah et al., 1981), a more direct approach was considered a worthwhile exercise. In this wake, a quantitative evaluation of two key dehydrogenases, one which serves as an index of anaerobic metabolism (Lactate; NAD oxido-reductase; EC 1.1.1.27; LDH) and the other which functions as a yardstick of aerobic metabolism (Succinate; INT oxido-reductase; EC 1.3.99.1; SDH) was carried out in liver and skeletal muscle so as to bring out the metabolic alterations that might be occurring in the body during tail regeneration. Since no such quantitative analysis has been done even in the regenerating tail, the assay was also extended to the regenerating system. This study is expected to give a comparative idea about the occurrence of local and systemic metabolic responses to the stress of tail autotomy and its regeneration in Mabuya carinata.

MATERIALS AND METHODS

Healthy Mabuyas of both sexes weighing around 20-24 g and obtained from Hyderabad, India, were maintained on a diet of insects. Autotomy of the tail was done by pinching it off 1.5-2.0 cms distal to the vent. The animals with regenerating tail were sacrificed at fixed intervals of 3, 5, 7, 10, 12, 15, 25, 40 and 60 days post-autotomy along with the normal animals with intact tails. Liver and skeletal muscle (femoral) as well as the tail (regenerating or normal as the case may be) were quickly removed and the tissues weighed and homogenized in ice-cold redistilled water. A 2% homogenate was prepared for liver and skeletal muscle whereas a 4% homogenate was found satisfactory in the case of the tail tissue.

The activity of LDH was assayed following the method of Farrar and Bush (1969) with Sodium lactate (obtained from Sigma Chemicals, U.S.A.) as substrate and NAD (obtained from Sigma Chemicals, U.S.A.) as a cofactor. The homogenate was centrifuged for 15 min at 15,000 X g at 4°C. The supernatant was used for the assay. The specific activity of the enzyme was expressed as mμ of NADH formed/mg protein/min.

Activity of SDH was assayed in the crude homogenate by its potency to reduce p-indonitrophenyl-tetrazolium violet (obtained from Sigma Chemicals, U.S.A.) according to the

method of Pennington (1961). The specific activity was expressed as μ moles of INT reduced/mg protein/min.

The protein concentration was measured according to the method of Lowry et al. (1951).

For each day and each tissue specified a total of five to seven determinations of enzyme activity were made. The mean and standard deviation were obtained and students' 't' test was used to determine statistical significance.

RESULTS

The results of the present study on changes in the activity levels of LDH and SDH in the tail regenerate, liver and skeletal muscle of Mabuya carinata during its tail regeneration are represented graphically in Figs. 1 and 2. The quantitative values of enzyme activity with statistical significance are shown in Table 1. It is evident from the graphs and table that the activity of LDH is highest in skeletal muscle followed by tail and liver in that order, while in the case of SDH, liver showed the highest activity followed by muscle and tail. Subsequent to tail autotomy, the activity of LDH in all the three tissues showed a significant maximal increase by 3rd day in the regenerate, and by 5th day in liver and skeletal muscle. Immediately after this there was a gradual decrement in enzyme activity in all the three tissues

Table 1. Quantitative levels of LDH and SDH in the regenerate liver and skeletal muscle during tail regeneration in M. carinata.

Periods of tail regeneration in days	LDH			SDH		
	Tail	Liver	Muscle	Tail	Liver	Muscle
N	171.53 ±11.25	161.96 ±16.60	267.37 ±14.18	1.63 ±0.29	9.58 ±1.09	2.55 ±0.04
3	244.17* ±19.48@	258.31⁂@ ±13.86@	347.45⁂@ ±11.46@	2.21 ±0.29	10.93 ±1.76	3.82 ±1.02
5	212.79 ±14.81	279.54⁂@ ±17.05@	450.51⁂@ ±12.09@	2.58⁂ ±0.27	11.41 ±1.03	3.91* ±0.66
7	184.23 ±14.58	162.68 ±18.01	416.84⁂@ ±11.04@	3.95⁂@ ±0.51@	14.51⁂@ ±1.05@	4.11⁂@ ±0.48@
10	150.91⁂@ ±14.59@	147.74 ±11.61	404.34⁂@ ±15.77@	2.59* ±0.36	8.69 ±0.61	2.97 ±0.81
12	110.37⁂@ ± 8.14@	144.47 ± 9.29	316.68* ±14.14	3.17* ±0.57	12.82⁂ ±0.49	3.45@ ±0.31
15	110.72⁂@ ±10.31@	131.12 ±15.73	228.83 ±11.81	3.09* ±0.57	13.98@ ±0.14	3.27 ±0.39
25	133.83*@ ±13.22@	187.83 ±16.16	308.24 ±15.57	2.96@ ±0.07	11.11 ±1.35	3.57⁂@ ±0.09@
40	173.78 ±13.26	221.61@ ±11.61	320.98@ ± 9.13	2.54* ±0.25	10.59 ±1.27	3.53* ±0.45
60	207.07 ±11.98	187.86 ±16.66	296.83 ±15.41	2.51 ±0.34	10.31 ±0.32	3.36 ±0.42

Values are expressed as : LDH - mμ Moles of NADH formed/mg protein/min.

SDH - n Moles of INT reduced/mg protein/min.

* $P < 0.01$; ⁂ $P < 0.05$; @ $P < 0.0025$; @@ $P < 0.001$; ⁂@ $P < 0.0005$.

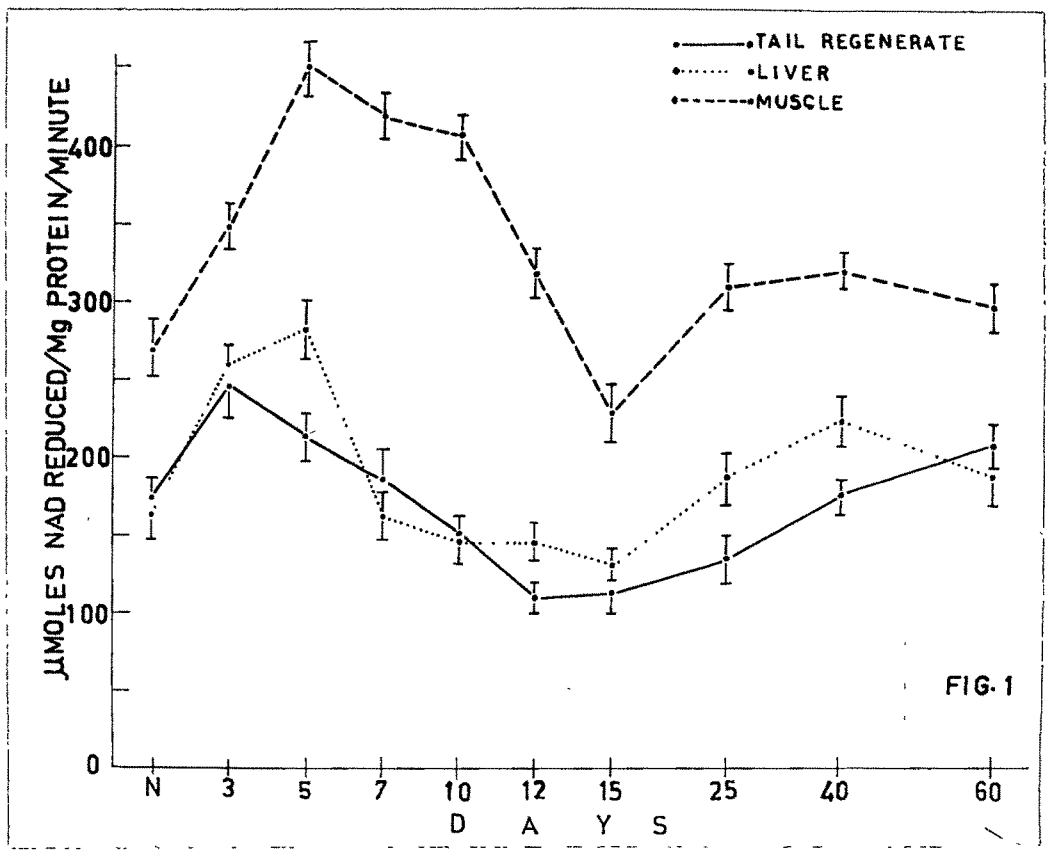

Fig. 1. Changes in LDH activity in the regenerate, liver and muscle during tail regeneration in M. carinata.

ultimately registering the lowest subnormal levels on the 15th day. Whereas in the case of skeletal muscle there was a gradual drop in enzyme activity during 7th and 10th days and sharper drops during 12th and 15th days, liver showed a sharper drop on the 7th day and gradual drop on 10th, 12th and 15th days. At the same time the tail regenerate showed continuous decline through 5th, 7th, 10th and 12th days, with a plateau being maintained between 12th and 15th days. While subnormal levels of LDH activity were recorded by liver and tail regenerate by 10th day itself, the skeletal muscle showed the subnormal level only on the 15th day. Subsequently the enzyme activity depicted an increasing trend on 25th and 40th days in the case of liver and skeletal muscle and 25th, 40th and 60th days in the case of the regenerate. Whereas slightly above normal levels of LDH activity were attained by the liver and skeletal muscle as early as 25th day itself, a near normal level was reached in the regenerate only by the 40th day; and on the 60th day LDH activity tended to remain slightly above normal in the fully regenerated tail. However, between 40th and 60th days, LDH activity in liver and skeletal muscle tended to show slight decrease in order to reach near normal levels.

Succinate dehydrogenase (SDH) depicted a continuous increase in all the three tissues subsequent to tail autotomy to reach maximal levels on the 7th day. A parallel fall in

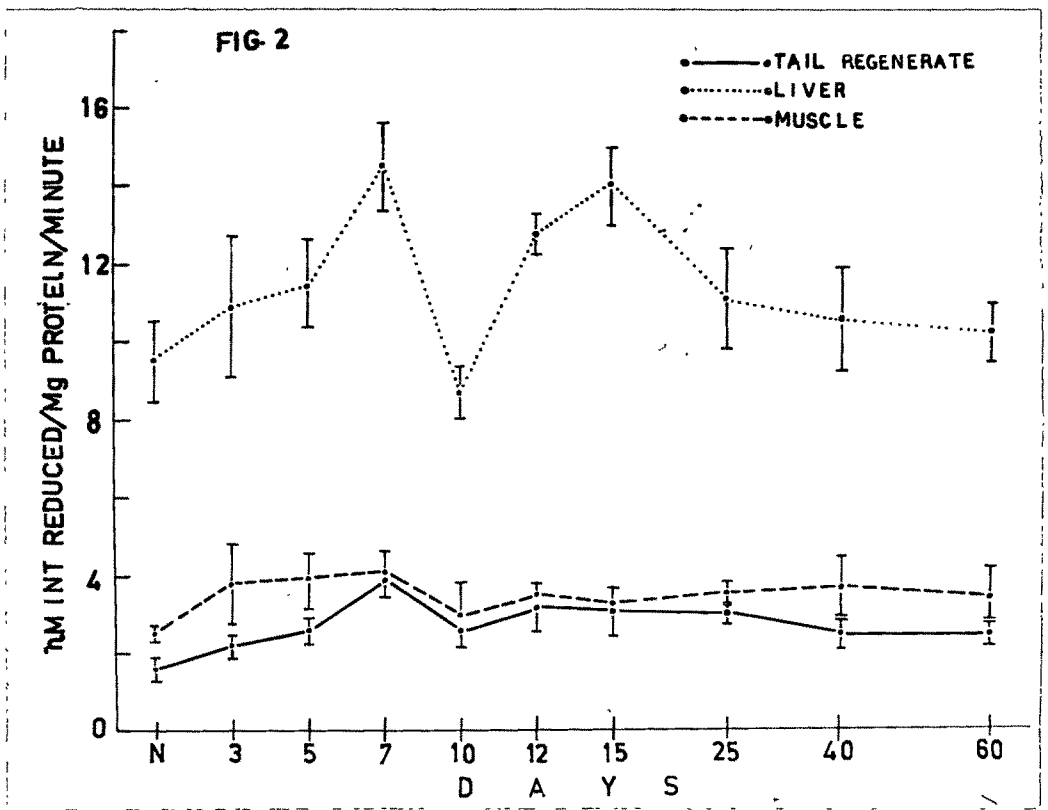

Fig. 2. Changes in succinate : INT reductase (SDH) activity in the regenerate, liver and muscle during tail regeneration in M. carinata.

the enzyme activity from 7th to 10th days was a characteristic feature in all the three tissues. Later, there was again an increase in enzyme activity in all the three tissues by the 12th day. In the case of muscle more or less a steady level of enzyme activity was maintained on the 40th and 60th days of tail regeneration. Concurrently, in the case of regenerate a plateau level was maintained between 15th and 25th days with a fall on 40th day; and this level was maintained so even on 60th day. In contrast, SDH activity in the liver depicted a further increment on 15th day to be followed by a gradual decline on 25th and 40th days which was maintained so more or less on 60th day.

DISCUSSION

The present study, an attempt to elucidate the relative operation of anaerobic and aerobic modes of metabolism at the local site of regeneration as well as in two major visceral organs (liver and skeletal muscle) has proved successful in bringing out phase specific fluctuations in metabolic pattern during tail regeneration. At the very outset it may be pointed out that the total LDH activity assayed in the present study based on the 'forward reaction' of lactate to pyruvate appears to be representative of anaerobic type of LDH. Our observations on quantitative analysis of heart and muscle type LDH subunit concentrations (Chapter IV) seem to be in conformity with this

idea as the changes in total LDH activity obtained in the present study is apparently well paralleled by the recorded changes in the muscle type (A) LDH subunits. Similar conclusions have been drawn by Sjödin (1976) based on his studies on LDH activity in human skeletal muscle. Based on this fact, the presently observed enhanced total LDH activity during the first 5 days of tail regeneration is indicative of increased anaerobiosis occurring in all the three tissues under study in the immediate post-autotomy periods. This is well supported by the noted changes in glycogen content and phosphorylase activity (Chapter II) as well as the reported increased content of muscle type LDH subunits and lowered value of analogue ratio (Chapter IV). However, the fall in LDH activity from 5th day onwards and the attainment of lowest levels by about 15th day in all the three tissues denote the shifting emphasis from the anaerobic mode of metabolism in the post-wound healing period of regeneration corresponding to the blastema and early differentiation phases. Incidentally the increasing trend of SDH activity right from the time of autotomy and maintainence of supranormal levels on the 7th day onwards in general uptill about 25th day signify a definite shift in the metabolic pattern from anaerobic to aerobic in the body as a whole during the blastemic and differentiation phases of tail regeneration in M. carinata. Based on their histochemical studies on enzymes, Shah and Ramachandran (1976)

had already suggested such metabolic alterations to be in operation in the regenerating tail of M. carinata, and they have reviewed the functional significance of these alterations in relation to the changing energetics, macromolecular requirements and other metabolic intricacies associated with the process of cell division and differentiation characteristic of this period of regeneration. Some of the later studies of Shah et al. (1980 a) and Ramachandran et al. (1981) have shown increased red blood cell count and haemoglobin content of blood and depletion of colloidal content from thyroid follicles respectively, during 7th to 12th days post-autotomy. Both these observations when coupled with the presently noted increase in SDH activity in liver, skeletal muscle and the regenerate simultaneously are strongly suggestive of the commissioning of the oxidative pattern of metabolism in the body as a whole in conjunction with the regenerating system. This gets ample confirmation from the reported increase in heart type LDH subunits and the resultant increase in analogue ratio together with the appearance of electrophoretic LDH isozymic bands 1 and 2 during the progressive phases of regeneration (Chapter IV).

Prevalence of above normal levels of LDH and SDH activities during 25th and 40th days of regeneration which correspond to differentiation and growth phases respectively, are reflective of active metabolic events occurring during

these periods to meet the increased energy demands as well as biosynthesis of essential organic molecules associated with the process of differentiation, and laying down of the micro and macro-architecture of differentiated components of the regenerate. Incidentally the increased LDH activity during 25th and 40th days might also indicate a second metabolic shift reverting the pattern of metabolism from the prevailing aerobic to the original anaerobic type. Corresponding increase in the A type subunits with a fall in B type subunits of LDH (Chapter IV), as well as the decreasing oxygen carrying capacity of the blood as denoted by the reported fall in RBC count during these periods of tail regeneration (Shah, et al., 1980 a) are all evidences in favour of this presumption. The increase in the activity of SDH during the first 7 days and a significant fall in its activity during 7th and 10th days followed by a second phase of increased enzyme activity, appear to have relevance with the reported changes in thyroid activity during tail regeneration in M. carinata (Ramachandran et al., 1981), as well as the reported relation of SDH with thyroxine (Thorpe et al., 1967; Wolff and Wolff, 1964). Finally, the decreasing trend in activity of both SDH and LDH from 40th to 60th days of tail regeneration denote the settling down of the metabolic activities towards the original preautotomy condition with the completion of the process of regeneration.

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