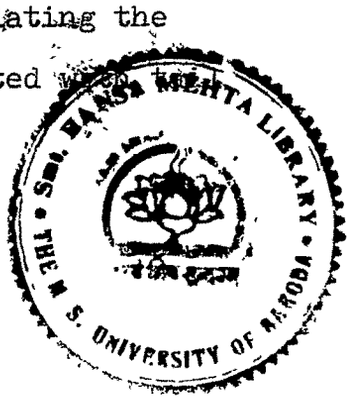
CHAPTER XI

QUANTITATIVE IN LOCO AND SYSTEMIC ALTERATIONS IN LDH ACTIVITY DURING TAIL REGENERATION IN NORMAL AND ADRENAL SUPPRESSED GEKKONID LIZARDS, HEMIDACTYLUS FLAVIVIRIDIS

Developmental processes like regeneration wherein large scale restoration of lost tissues occurs, would predictably raise the energy demands of the organisms. Apart from the reported augmented metabolic activities characteristic of urodelian limb regeneration (Schmidt, 1968), some of the gathered evidences on the 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; Shah and Hiradhar, 1974; Shah and Ramachandran, 1976; Shah et al., 1982d; Swamy et al., 1982b). It is generally realised that tissues exhibiting high mitotic index derive energy mainly through anaerobic glycolysis, and high LDH activity is an indication of anaerobic energy production through glycolytic pathway. The effect of hormones on lactate dehydrogenase (LDH) activity has been reported by a few workers (Degroot and Cohen, 1962; Goodfriend and Kaplan, 1964; Giri and Singh, 1978). Thomas (1980) and Swamy et al. (1982b) have also studied LDH activity in

relation to regeneration under hypophysectomised and hypothyroidic conditions respectively. In this wake it was deemed fit to study LDH activity in relation to regeneration in the regenerate as well as liver and muscle of normal and adrenal suppressed lizards, so as to understand the involvement of the adrenal gland in regulating the adaptive modulations in LDH activity associated with regeneration in H. flaviviridis.



MATERIALS AND METHODS

The lizards, H. flaviviridis procured from the local animal dealer were maintained in the laboratory on a diet of cockroaches. The animals were allowed a fortnight's acclimatisation to the laboratory conditions prior to experimentation. Lizards weighing 10-12 gms. and having a snout-vent length of 8-10 cms. were taken for the study and caudal autotomy was done by pinching off the tail two segments distal to the vent.

A total of 120 animals were used for the experimental purpose. They were divided into two groups of 60 each. One group served as the control and the other was chemically adrenalectomised by intraperitoneal injection of the synthetic corticosteroid, dexamethasone (DXM) (15 µg/0.1 ml/day/animal)

every evening at 17.00 hrs. The DXM treatment was started 10 days prior to tail autotomy and was continued for every alternate day even after autotomy till the end of regeneration. Controls were given the same amount of distilled water intraperitoneally. Lizards from both the groups were sacrificed at regular intervals of 3,5,7,10,15,25,40 and 60 days post-autotomy along with the normal animals with intact tail. Liver, muscle and tail were taken immediately and a 2% homogenate was made in cold distilled water.

The activity of LDH was assayed using the method of King (1959) as modified by Varley (1975) with sodium lactate obtained from Sigma Chemicals (USA) as substrate and NAD (obtained from Sigma Chemicals, USA) as the co-factor. The specific activity of the enzyme was expressed as μ moles lactate oxidised/mg protein/15 mins.

For each day and each tissue specified a total of five to seven determinations were made. The mean and standard error were calculated and Student's 't' test was used to determine statistical significance.

RESULTS

The data obtained on the values of LDH activity in the three tissues during the process of regeneration are represented in Tables 1&2 and Figures 1-3.

The pre-autotomy level of LDH activity was elevated in all the three tissues with the liver recording maximum elevation and the tail the minimum. Subsequent to caudal autotomy whereas the control lizards depicted increasing LDH activity in all the three tissues lasting upto 7 days in the case of tail and muscle, and upto 10 days in the case of liver, the adrenal suppressed lizards depicted decreasing enzyme activity lasting upto 7 days in all the three tissues. The blastema to early differentiation phases (10-15th days) were marked by decreased enzyme activity in adrenal sufficient control lizards. The level of enzyme activity at this stage was subnormal in the regenerate and muscle and normal in the liver. There was a significantly increased level of enzyme activity in all the three tissues on the 25th day which was followed by decreasing trend of enzyme activity on the 40th and 60th days with the activity level on the 60th day tending to be subnormal in all the tissues. In contrast, in adrenal insufficient DXM group of lizards, the LDH activity increased on the 10th day from the lowest levels registered on the 7th day. This was followed by a decrease on the 15th day and an increase on the 25th day to the maximum regeneration levels (though still subnormal compared to the pre-autotomy levels). As in the controls, the DXM group also depicted decreasing levels of enzyme activity on the 40th and 60th days to attain subnormal levels on the 60th day.

TABLE-1 : Alterations in tissue LDH activity (μ mole lactate oxidised/mg protein/15 min) during tail regeneration in normal H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	103.55 ±2.38	161.47* ±14.87	118.67* ± 3.11	160.95* ±11.37	168.41* ± 4.13	104.22 ± 2.13	121.8@@ ± 4.91	114.46 ± 6.97	96.01 ±4.41
Muscle	168.05 ± 3.62	179.63 ± 6.88	200.33* ± 6.45	263.7* ±20.35	189.27 ±18.84	153.13@@ ± 3.41	248.87@@ ±23.83	206.1* ±10.61	155.29 ±17.99
Tail	220.32 ±11.90	239.46 ±22.12	249.12 ± 1.51	268.34@ ±13.70	186.14 ±22.69	134.08** ± 6.06	242.36 ±15.34	199.28 ±11.49	180.32 ±11.60

@ P <0.05; @@ P <0.02; * P <0.01; ** P <0.001

TABLE-2 : Alterations in tissue LDH activity (μ mole lactate oxidised/mg protein/15 min.) during tail regeneration in adrenal suppressed H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	147.46 ±11.37	95.25$^{@@}$ ±11.22	76.67** ±3.14	74.13** ±5.07	128.26 ±16.4	101.52* ± 6.36	174.78 ±14.44	93.98* ±5.45	107.11$^{@}$ ± 8.56
Muscle	226.99 ±20.59	130.02* ± 9.76	100.47* ±12.60	92.75* ±9.52	164.47$^{@}$ ± 8.19	138.91$^{@}$ ±21.60	212.9 ± 8.2	168.9$^{@}$ ± 5.28	158.05$^{@@}$ ± 5.42
Tail	236.9 ±15.72	140.08* ± 3.92	124.18** ±3.39	116.64** ± 4.73	210.23 ±13.13	197.68 ±18.92	227.37 ± 5.67	179.95 ± 1.62	153.05$^{@@}$ ±5.84

@ P <0.05; @@ P <0.02; * P <0.01; ** P <0.001

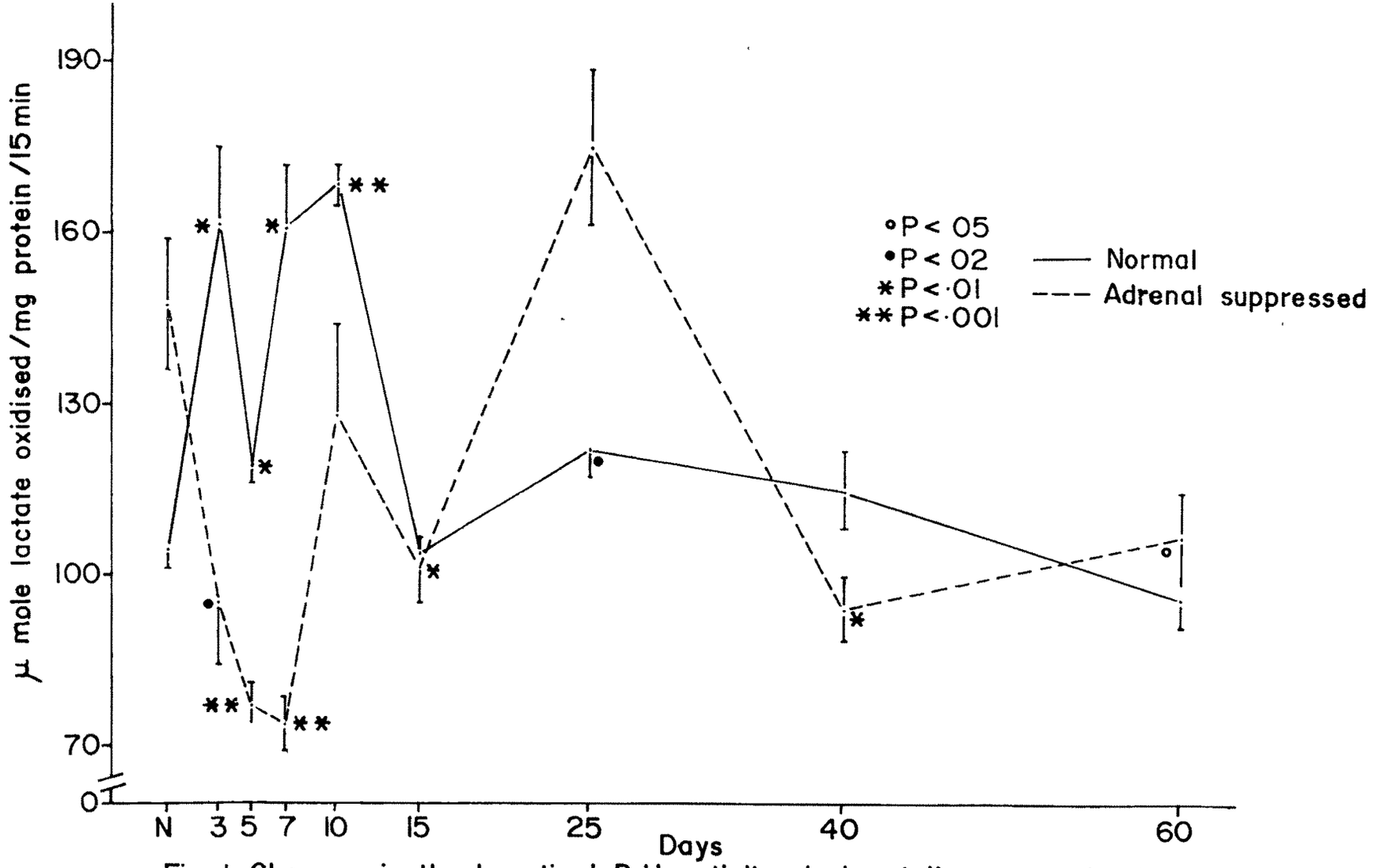


Fig. I Changes in the hepatic L D H activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

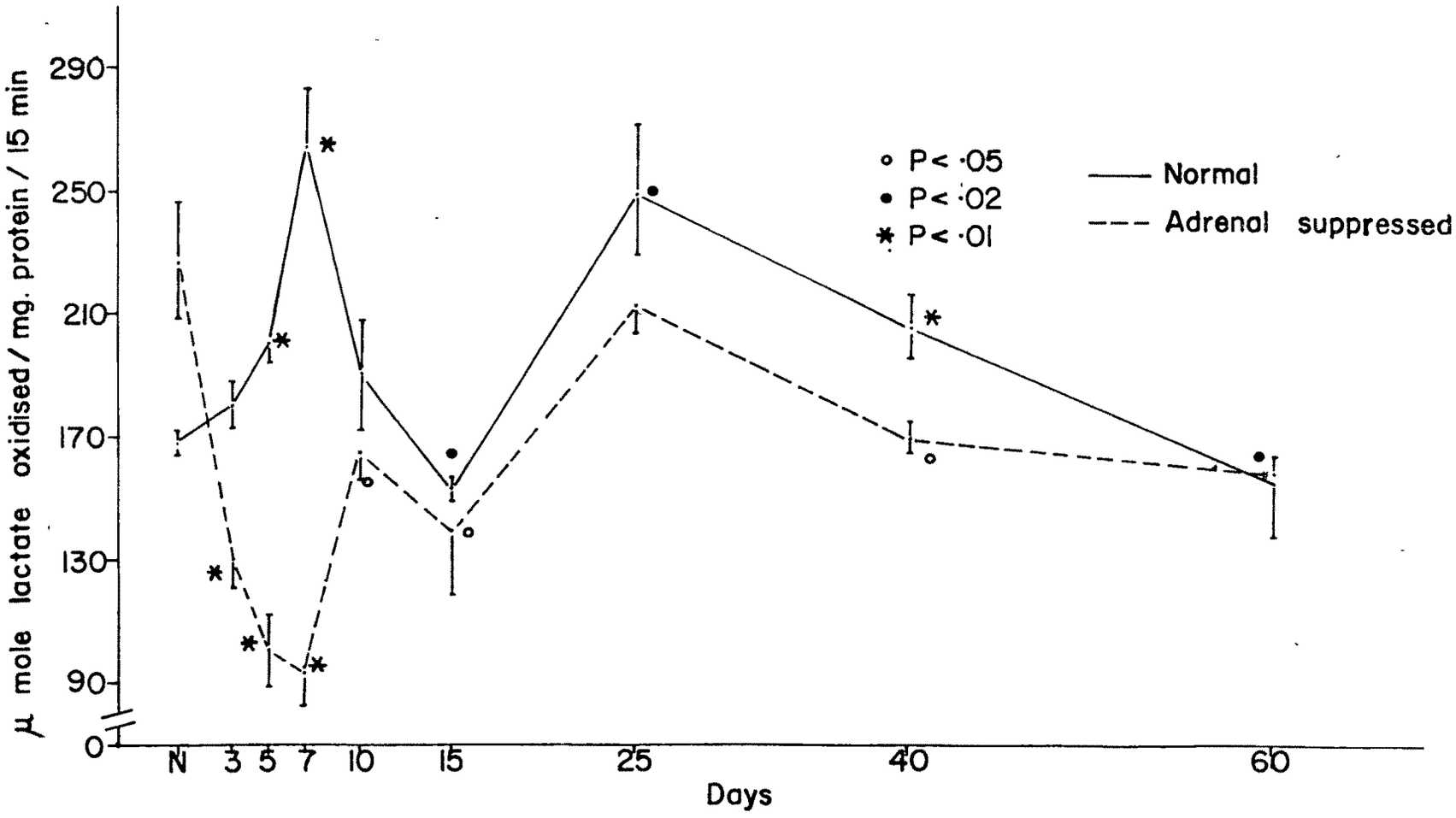


Fig. 2. Changes in muscle LDH activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

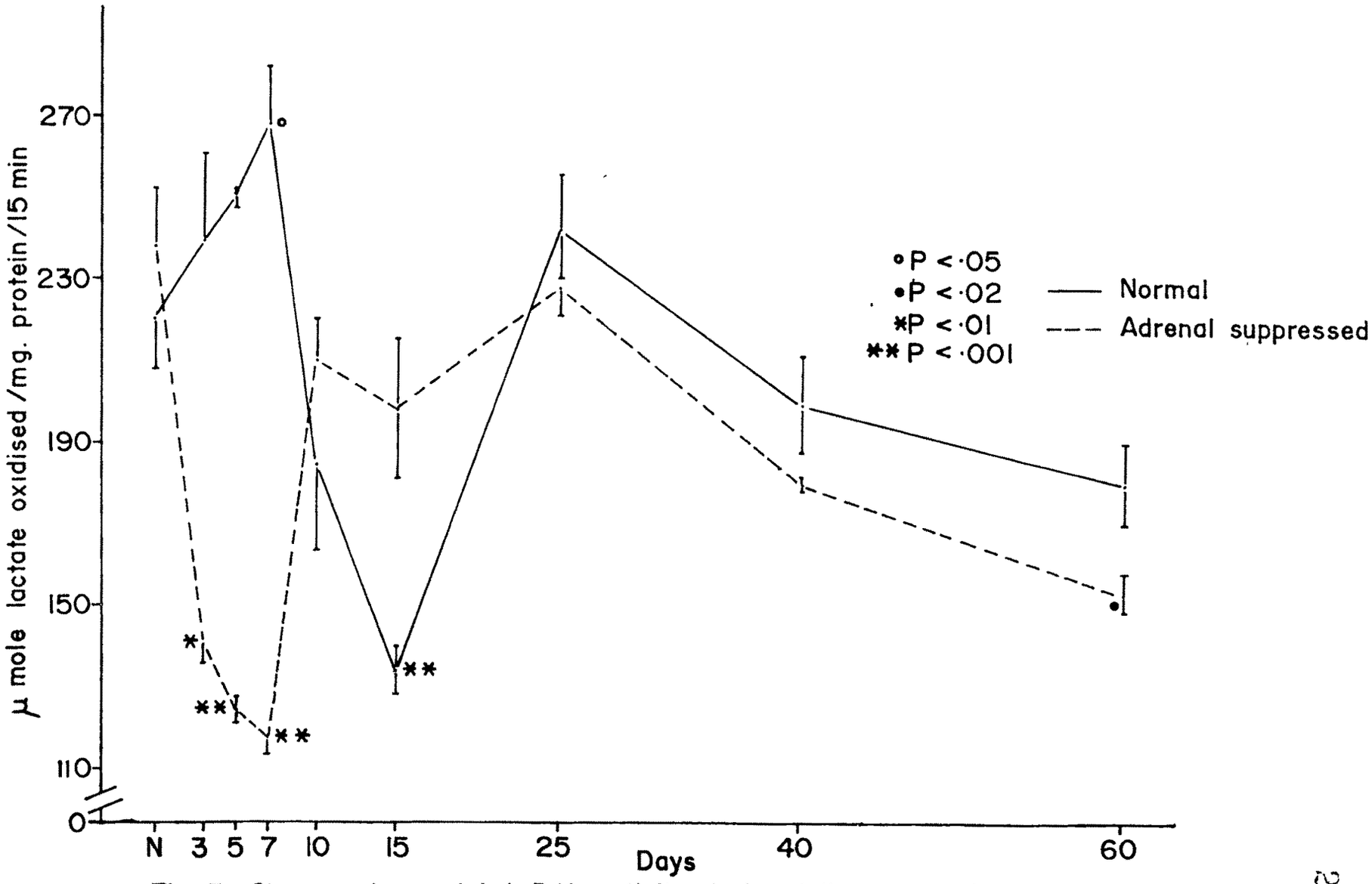


Fig. 3 Changes in caudal L D H activity during tail regeneration in normal and adrenal suppressed H. flaviviridis

DISCUSSION

The results obtained in the current study strongly suggest alterations in the metabolic profile of adrenal insufficient lizards on a temporal scale both prior to autotomy as well as after autotomy. The effect of adrenal suppression in the pre-autotomy condition appears to be increased carbohydrate catabolism, as the LDH activity was increased, and is corroborated by the recorded lowered level of tissue glycogen contents (Chapter 8). The autotomy induced alterations were of a diametrically opposite pattern in the experimental lizards in relation to the controls, upto about 10 days of tail regeneration. The reduced LDH activity in the experimentals could lead to reduced anaerobic glycolysis which has already been established as prime feature associated with the early stages of lizard tail regeneration (Shah et al., 1979c, 1982d; Swamy et al., 1982b). One of the biochemical correlates for the observed significant initial retardation in regenerative growth and the delayed formation of a qualitatively and quantitatively poor blastema in DXM lizards (Chapter 4) may probably be associated with the presently observed fall in LDH activity. The reduced enzyme activity could also denote inhibition in the formation of H type LDH_1 and LDH_2 isozymic forms which are reported to be induced during the post-autotomy phases

of lizard tail regeneration (Shah et al., 1982d). The post-blastemic phases of regeneration were marked by changes in LDH activity in DXM treated lizards which are very much similar to those recorded for the controls. This may have some bearing on the previously noted better regenerative growth in the experimental lizards during the differentiative and growth phases.

On molecular terms, the decreased LDH activity in the initial phases of regeneration in DXM lizards, might represent either decreased induction of LDH subunits or decreased assembly of subunits and/or increased subunit degradation brought about by the autotomy induced regeneration specific milieu internae in the background of prevailing adrenocortical insufficiency. Viewed in this context, the increased enzyme activity observable prior to autotomy could denote either increased subunit assembly or decreased subunit degradation due to DXM induced adrenal insufficiency. The results accrued in the present study tend to suggest the possible involvement of adrenocortical hormones in the in loco and systemic metabolic modulations associated with lizard tail regeneration.

SUMMARY

LDH activity during tail regeneration in H. flaviviridis was quantitatively assayed in liver, muscle and the regenerate under induced adrenocortical insufficiency. An overall reduction in enzyme activity was observable in all the three tissues during the course of regeneration, though the pre-autotomy levels were above normal. There was a biphasic increased LDH activity in the control lizards corresponding to initial periods prior to blastemal formation and peak histo-differentiation. In the experimental lizards the increase in LDH activity, whenever it occurred was of lesser magnitude. The significant delay in regenerative outgrowth in the initial periods in the adrenal insufficient lizards could be correlated with decreased metabolic efficiency characterized by reduced LDH activity.