CHAPTER XIII

LOCAL AND SYSTEMIC ALTERATIONS IN CHOLINESTERASE ACTIVITY DURING TAIL REGENERATION IN NORMAL AND ADRENAL SUPPRESSED GEKKONID LIZARDS, *HEMIDACTYLUS FLAVIVIRIDIS*

Acetylcholinesterase (AChE) has long been recognised to be involved in neuronal functions by its ability to hydrolyse acetylcholine. It occurs in both nervous and non-nervous tissues and is generally regarded as a membrane-bound enzyme (Silver, 1974; Wheeler *et al.*, 1972). In recent years, ample literature has accumulated on the presence of cholinesterases in tissues and sites where neurotransmitter mechanisms are inoperative (Silver, 1974). These have given impetus to notions regarding the non-transmission functions of these enzymes. Singer (1952) and Thornton (1970) have demonstrated the dependence of newt limb regeneration on the presence of an adequate nerve supply at the site of amputation. Though the precise mechanism by which the neuronal contribution affects regeneration is not known, neurotrophic influence on increase in volume and mitotic activity of the early regenerate (Dresden, 1969; Lebowitz and Singer, 1970;

Singer and Caston, 1972; Morzlock and Stocum, 1972; Bantle and Tassava, 1974) have been demonstrated. Successful restoration of protein synthesis to the level of non-denervated regenerate by infusing nerve and brain homogenates into the denervated regenerates, was achieved by Lebowitz and Singer (1970) and Singer et al. (1976). Unlike the case of Urodelean limb regeneration, the lizard tail regeneration is considered to be mostly influenced by the spinal cord or the ependyma, rather than nerves. Though a specific influence of peripheral nerves is not well established as yet, the above studies have suggested some sort of neurotrophic influence on early events associated with lizard tail regeneration. Major studies on these lines have been restricted to amphibian limb regeneration. A previous study by Ramachandran et al. (1981b) in the Scincid lizard, Mabuya carinata have shown the involvement of both [illegible] acetylcholinesterase as well as non-specific cholinesterase in the regenerative mechanics of Mabuya. In the present investigation, AChE activity has been quantitatively assayed in relation to tail regeneration in H. flaviviridis under induced adrenocortical insufficiency. Quantitative alterations in enzyme activity in the regenerate and in liver and skeletal muscle have been recorded in order to understand the involvement of AChE both in loco as well as systemically.

MATERIALS AND METHODS

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

A total of 120 lizards were used for the experimental purpose. They were divided into two groups of 60 each. One group served as the control and the other group was chemically adrenalectomised using the synthetic corticosteroid dexamethasone (DXM) administered intraperitoneally (15 µg/0.1 ml/day/animal) in the evening at 17.00 hrs. starting 10 days prior to tail autotomy and continued post-autotomy every alternate day till the end of experimentation. Controls received an equal amount of distilled water intraperitoneally. Lizards from both the groups were sacrificed at regular time 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 tissues were excised and homogenised with cold 0.9% NaCl. The homogenates were centrifuged in cold at 3000 rpm for

10 mins. and the supernatant was used for assaying the activity of AChE according to the method of Guenther and Klaus (1970). AChE activity was measured by using acetylthiocholine iodide (obtained from Sigma Chemicals, USA) as the substrate and the readings were obtained without and with prostigmine (an inhibitor of AChE). The difference between the readings was then taken as that for AChE. The specific activity of the enzyme was expressed as μ moles of ACh hydrolysed/mg protein/10 minutes.

The protein content was measured by the method described by Lowry et al. (1951). For each day and each tissue specified a total of 5-7 determinations were made. The mean and standard error were calculated and Student's 't' test was used to determine statistical significance.

RESULTS

During tail regeneration in intact controls the AChE activity was noted to increase to a maximum level in all the three tissues through 7th to the 10th days. However, the tail recorded a significant increase on the 3rd day post-autotomy followed by a fall to the normal range by the 5th day itself. Subsequent to the increase on the

TABLE-1 : Alterations in tissue AChE activity during tail regeneration in normal H. flaviviridis. Values are expressed as μ mole ACh hydrolysed/mg protein/10 min. ± SE.

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.19 ±0.03	0.23 ±0.03	0.20 ±0.02	0.28 ±0.03	0.44** ±0.01	0.37* ±0.02	0.27 ±0.02	0.23 ±0.02	0.15 ±0.02
Muscle	0.19 ±0.003	0.16* ±0.005	0.16* ±0.01	0.26* ±0.05	0.37** ±0.02	0.32** ±0.02	0.23* ±0.01	0.24@ ±0.02	0.14* ±0.01
Tail	0.25 ±0.02	0.43* ±0.02	0.17@ ±0.01	0.41* ±0.01	0.72** ±0.04	0.41* ±0.01	0.53** ±0.03	0.50* ±0.04	0.38@ ±0.02

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

TABLE-2 : Alterations in tissue AChE activity during tail regeneration in adrenal suppressed *H. flaviviridis*. Values are expressed as μ mole ACh hydrolysed/mg protein/10 min.±SE.

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.33 ±0.02	0.22* ±0.005	0.21* ±0.01	0.15** ±0.01	0.27 ±0.04	0.11** ±0.01	0.14* ±0.04	0.15** ±0.01	0.13** ±0.02
Muscle	0.26 ±0.02	0.15* ±0.02	0.16* ±0.01	0.13** ±0.005	0.11* ±0.003	0.11* ±0.02	0.14* ±0.02	0.22 ±0.01	0.17@ ±0.03
Tail	0.45 ±0.07	0.29 ±0.02	0.22@@ ±0.02	0.19* ±0.006	0.28 ±0.07	0.35 ±0.03	0.51 ±0.04	0.59 ±0.03	0.31 ±0.04

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

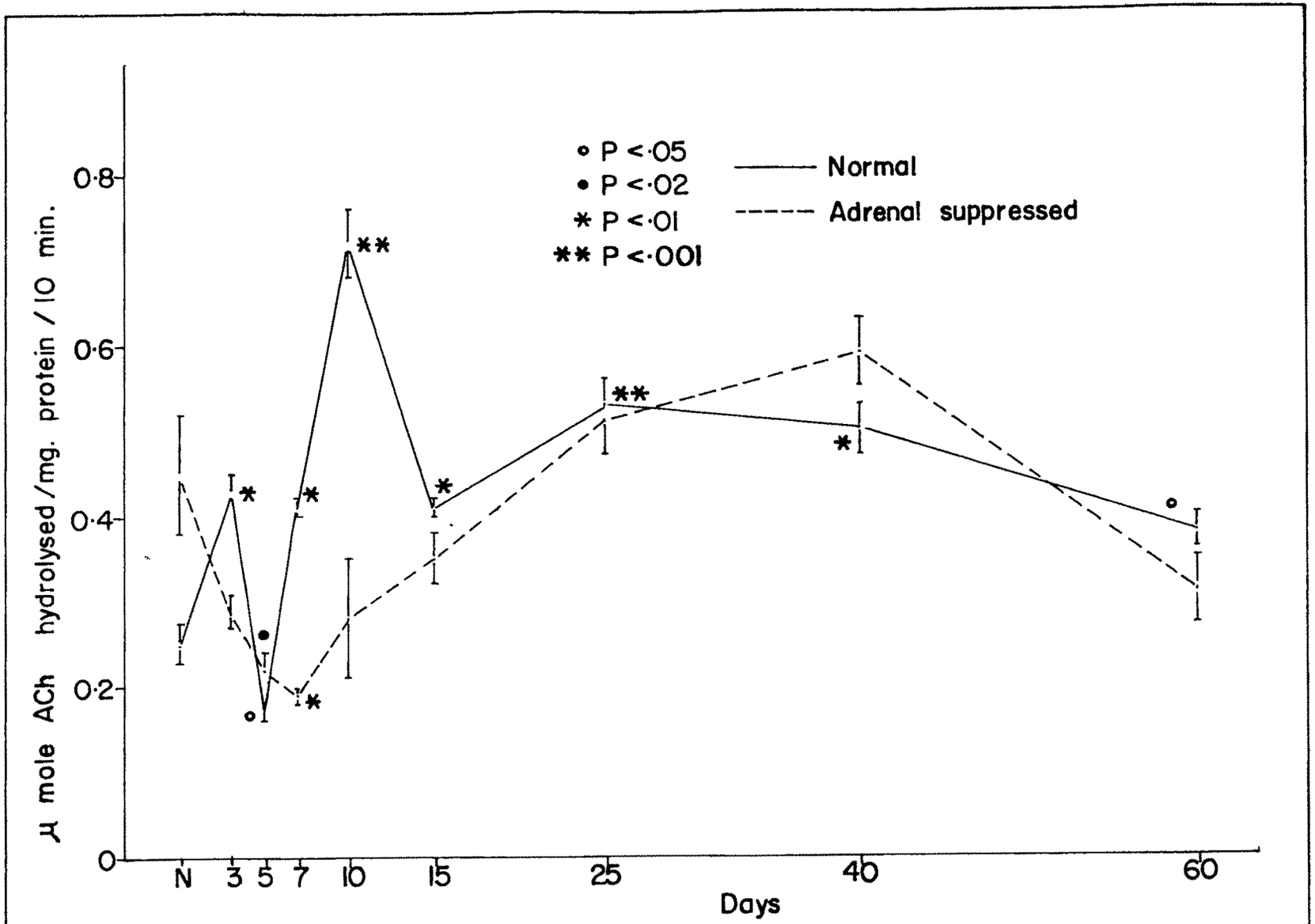

Fig. I. Changes in caudal AChE activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

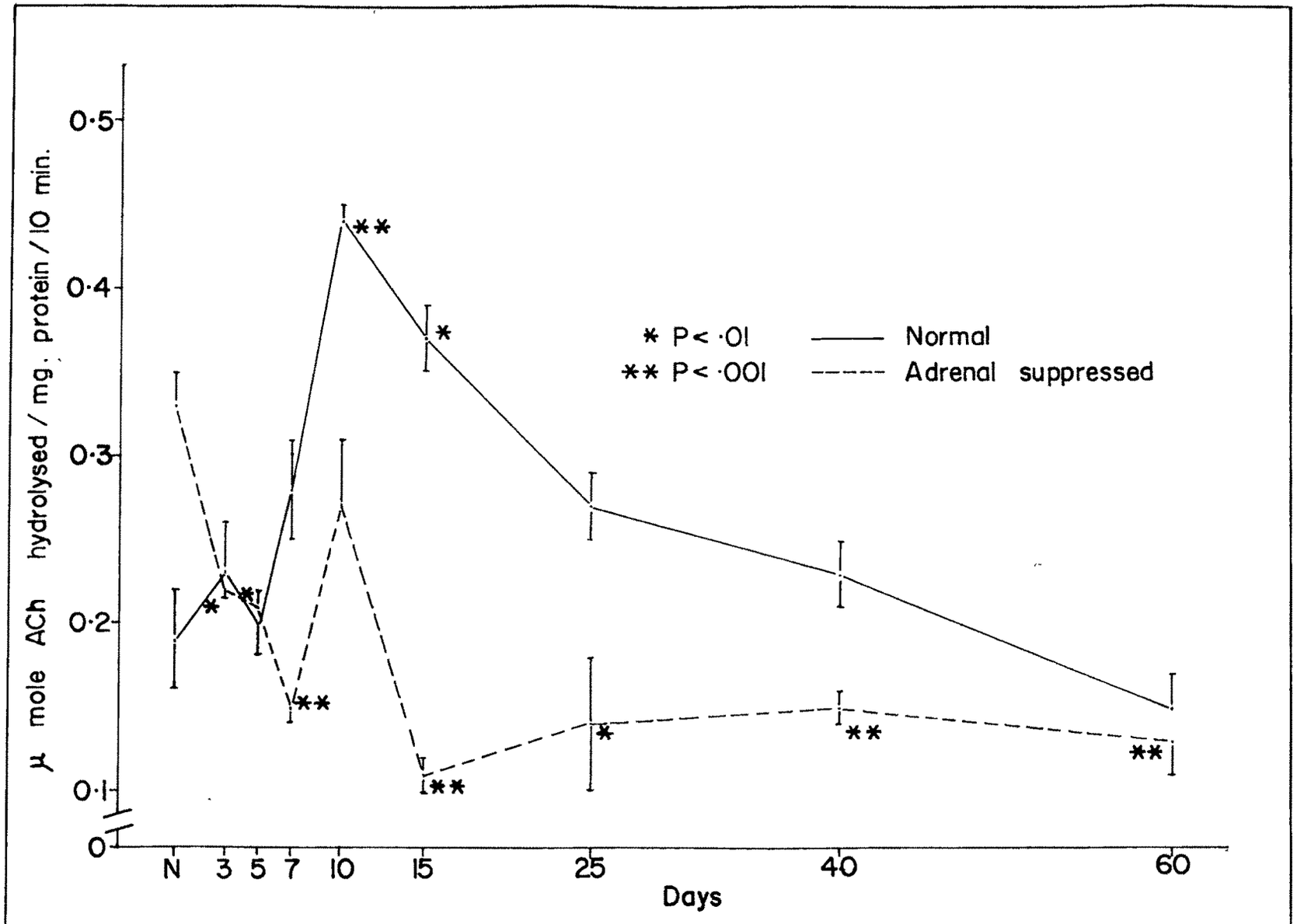

Fig. 2. Changes in hepatic AChE activity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

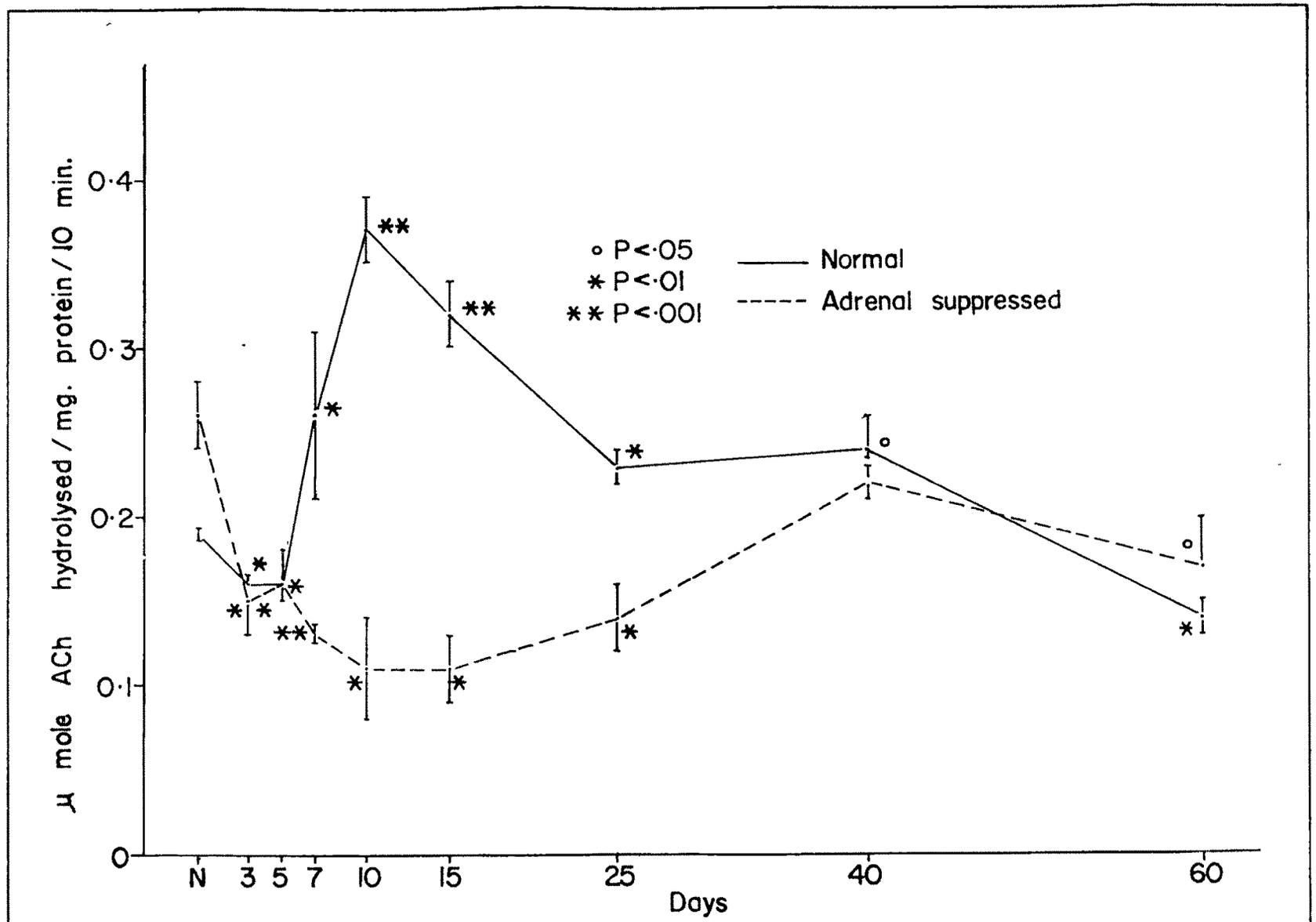

Fig. 3. Changes in muscle AChE activitity during tail regeneration in normal and adrenal suppressed H. flaviviridis.

10th day whereas the enzyme activity tended to settle gradually to the normal pre-autotomy level in the case of liver and muscle, it tended to remain higher in the regenerate till the 40th day. In the DXM treated lizards the enzyme activity was increased significantly in all the three tissues prior to autotomy. By the 3rd day itself subsequent to autotomy, the AChE activity in all three tissues decreased to the pre-autotomy levels characteristic of control lizards. This level of enzyme activity in liver and muscle was maintained all throughout tail regeneration except for a slight increase in muscle AChE activity on the 40th day. However, the tail regenerate recorded an increase in enzyme activity by the 15th day which gradually rose to a maximum level from 25th to the 40th day. By the 60th day the enzyme activity decreased to the levels characteristic of the control lizards.

DISCUSSION

The most characteristic change in AChE activity observable during regeneration *in loco* and systemically is an increase between the 7th and 40th days post-autotomy with a maximum level on the 10th day.
In addition, the skeletal muscle has depicted a decreased enzyme activity during the first five days post-autotomy

while the tail regenerate showed such a decrement only on the 5th day following a significant increase on the 3rd day. Such a decrement in enzyme activity *in loco* and systemically was also noted by Ramachandran *et al.*, (1981b) during the initial periods of tail regeneration in *Mabuya carinata*. Decreased AChE activity leading to increased ACh content in the early periods of regeneration seems to be the feature as similar observations have been made by Singer *et al.* (1960) during limb regeneration in *Triturus viridescens*. Such an increased ACh content by inducing alterations in membrane permeability and increasing cAMP content by inhibiting PDE activity (Rasmussen, 1975) has been suggested to aid in dedifferentiation and early blastemic phase of regeneration (Ramachandran *et al.*, 1981b). In this context the functional correlation between ACh and cAMP seems applicable in the case of *H. flaviviridis* as well as increased PDE activity has been noted to occur in the regenerate during the first five days post-autotomy (Chapter 3). However, the increased AChE activity noted herein by the 3rd day post-autotomy eludes explanation. The increased AChE activity in all the three tissues from the 7th to 40th days of regeneration corresponding to late blastemic and differentiation phases of regeneration appears a common feature associated with lizard tail regeneration as an identical

observation has been made in Mabuya carinata (Ramachandran et al., 1981b). The contention of the above workers regarding the role of elevated AChE activity during periods of regeneration (whence neurotransmission activities are more or less nonexistent), in membrane permeability, ionic transport, metabolic activities and protein synthesis related to differentiation and development appears tenable in the present case too as many metabolic and biochemical alterations have been noted to occur during tail regeneration in Hemidactylus flaviviridis (Shah et al., 1979c, 1987; Chapters 9-12). Adrenal suppression significantly affected the pattern of AChE activity both prior to and after caudal autotomy. Though there are no reports regarding hormonal regulation of AChE activity, the present study has indicated increased tissue AChE activity due to adrenocortical insufficiency. This could either indicate a negative regulatory influence of corticosterone on AChE, or, a short term response of adrenocortical insufficiency. However the fall in AChE activity in all the three tissues to the pre-autotomy control levels immediately subsequent to caudal autotomy (3rd day), and the maintenance of low level of activity in both liver and muscle for the rest of the periods of regeneration probably represent the setting in of a regeneration specific mileu internae which nullifies

the adrenocortical insufficiency induced elevation in AChE activity. However the involvement of adrenocortical hormones in modulating systemic AChE activity during regeneration is denoted by the absence of regeneration specific increase in hepatic and muscle AChE activity between the 7th and 40th days in DXM treated lizards. But the *in loco* modulations in enzyme activity appears to be relatively independent of corticosterone as both the initial short-living decrease as well as the later prolonged increase in enzyme activity occurred, though slightly delayed on the time scale post-autotomy in relation to the control animals. Accordingly, the initial decrement in AChE activity occurred on the 7th day instead of 5th, and the prolonged increment from 15th to 40th instead of 7th to 40th days, and as such may be relevant in the context of the delay in blastema formation and retardation in tail regeneration observed in DXM treated lizards (Chapter 4). Apparently, the present study tends to suggest a relative independence of the *in loco* modulations in AChE activity from corticosterone and the dependence of the systemic modulations in AChE activity on corticosterone.

SUMMARY

A quantitative evaluation of acetylcholinesterase (AChE) in the tail regenerate, liver and skeletal muscle

was undertaken during various periods of tail regeneration in normal and adrenal suppressed H. flaviviridis. During tail regeneration in the controls, the AChE activity was noted to increase gradually to a maximum level in all three tissues by the 10th day. Thereafter, whereas the enzyme activity tended to settle to the normal (unautotomized) range in the case of liver and muscle, it tended to remain higher in the regenerate till the 40th day. DXM suppressed animals depicted an increased AChE activity in all the three tissues prior to autotomy. However, post-autotomy stages were marked by reduced levels of AChE activity in both liver and muscle all throughout while in the case of the regenerate the enzyme activity did depict the elevation characteristic of differentiation phase. These changes indicate an altered modulation in systemic AChE activity due to adrenal suppression, and the relative indifference of caudal AChE to the functional status of adrenal cortex during regeneration.