CHAPTER III

LOCAL AND SYSTEMIC ALTERATIONS IN PHOSPHODIESTERASE ACTIVITY DURING TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Cyclic nucleotides have gained wide recognition as essential links in the molecular chain of events that constitute specific cellular responses to various stimuli that impinge upon the cells *in vivo*. Fluctuations in the intracellular level of cAMP have been reported to mediate many crucial cellular events, ranging from cell division to cell differentiation (Thomas *et al*., 1973; Berridge, 1975; Friedman, 1976; Miller, 1977; Taban and Cathieni, 1978; Carlone and Foret, 1979; Kosher and Savage, 1980), macromolecular synthesis (Sharma and Talwar, 1970; MacManus *et al*., 1972, 1973; Babich and Foret, 1973; Dokas, 1973; Foret and Babich, 1973; Short *et al*., 1975), and metabolic alterations (Rall *et al*., 1957; Drummond *et al*., 1969; Beriz *et al*., 1977; Ishibashi and Catton, 1978).

As a process which includes a conglomerate of all the above events, epimorphic regeneration is ideal for evaluating functionally correlatable changes in cyclic AMP level. Some attempts have been made in the past to understand the

involvement of cyclic nucleotides in regeneration (Jabaily et al., 1975; Liversage et al., 1977). It is known that the activity levels of phosphodiesterase (PDE) can give an indirect estimate of the prevailing concentration of cAMP in any tissue (Butcher and Sutherland, 1962). Due to this fact, and owing to our practical limitations in the direct assay of cAMP, quantitative evaluation of alterations in phosphodiesterase activity in loco was thought pertinent during different phases of tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis. Since the involvement of systemic factors has been established, liver and skeletal muscle PDE levels were also assayed along with the tail regenerate in the present study.

MATERIALS AND METHODS

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

A total of sixty animals were used for the study. The animals with regenerating tail were sacrificed at fixed intervals of 3, 5, 7, 10, 15, 25, 40 and 60 days post-autotomy along with lizards with intact tail prior to autotomy. The visceral organs (liver and skeletal muscle) 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 the enzyme assay and the activity of cAMP phosphodiesterase (PDE) in the crude homogenate was estimated according to the method described by Butcher and Sutherland (1962). The inorganic phosphate released was estimated by the method of Fiske and Subbarow (1925).

The amount of protein was estimated in the same homogenate by the method described by Lowry et al. (1951).

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 the statistical significance.

TABLE-1 : Alterations in tissue phosphodiesterase activity (µg phosphate released/mg protein) during tail regeneration in H. flaviviridis. (± SE).

Periods of regeneration in days	0	3	5	7	10	15	25	40	60
Liver	0.23 ±0.04	0.34 ±0.04	0.20 ±0.05	0.11$^{@}$ ±0.02	0.21 ±0.03	0.08* ±0.01	0.26 ±0.03	0.20 ±0.004	0.24 ±0.04
Muscle	0.46 ±0.02	0.41 ±0.02	0.36 ±0.06	0.38 ±0.05	0.18** ±0.03	0.35$^{@}$ ±0.04	0.38* ±0.01	0.42 ±0.04	0.39 ±0.03
Tail	0.94 ±0.02	0.41** ±0.04	0.73$^{@}$ ±0.08	0.14** ±0.02	0.31** ±0.03	0.41** ±0.05	0.25** ±0.07	0.25** ±0.01	0.87 ±0.04

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

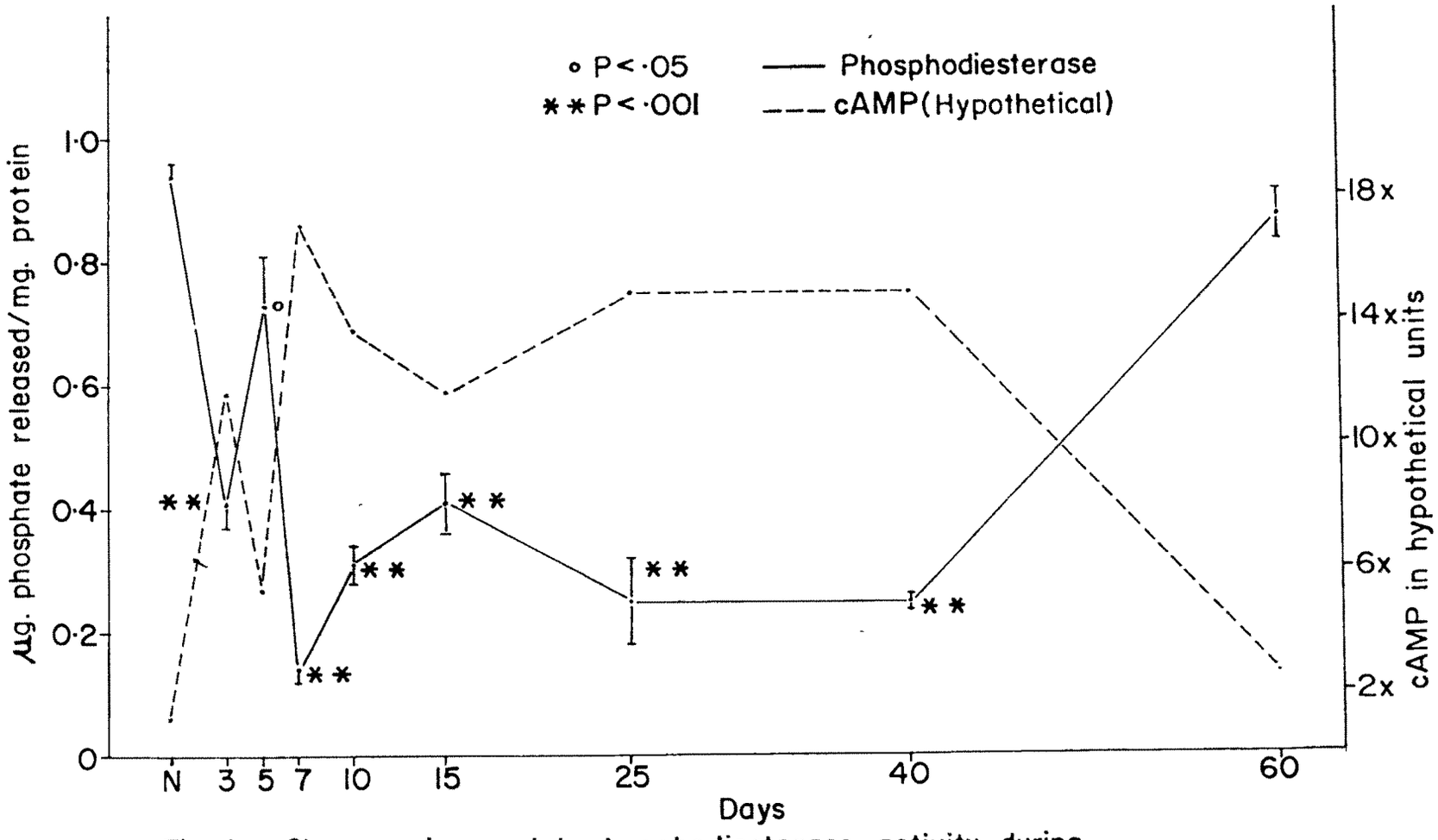

Fig. 1 . Changes in caudal phosphodiesterase activity during tail regeneration in H. flaviviridis.

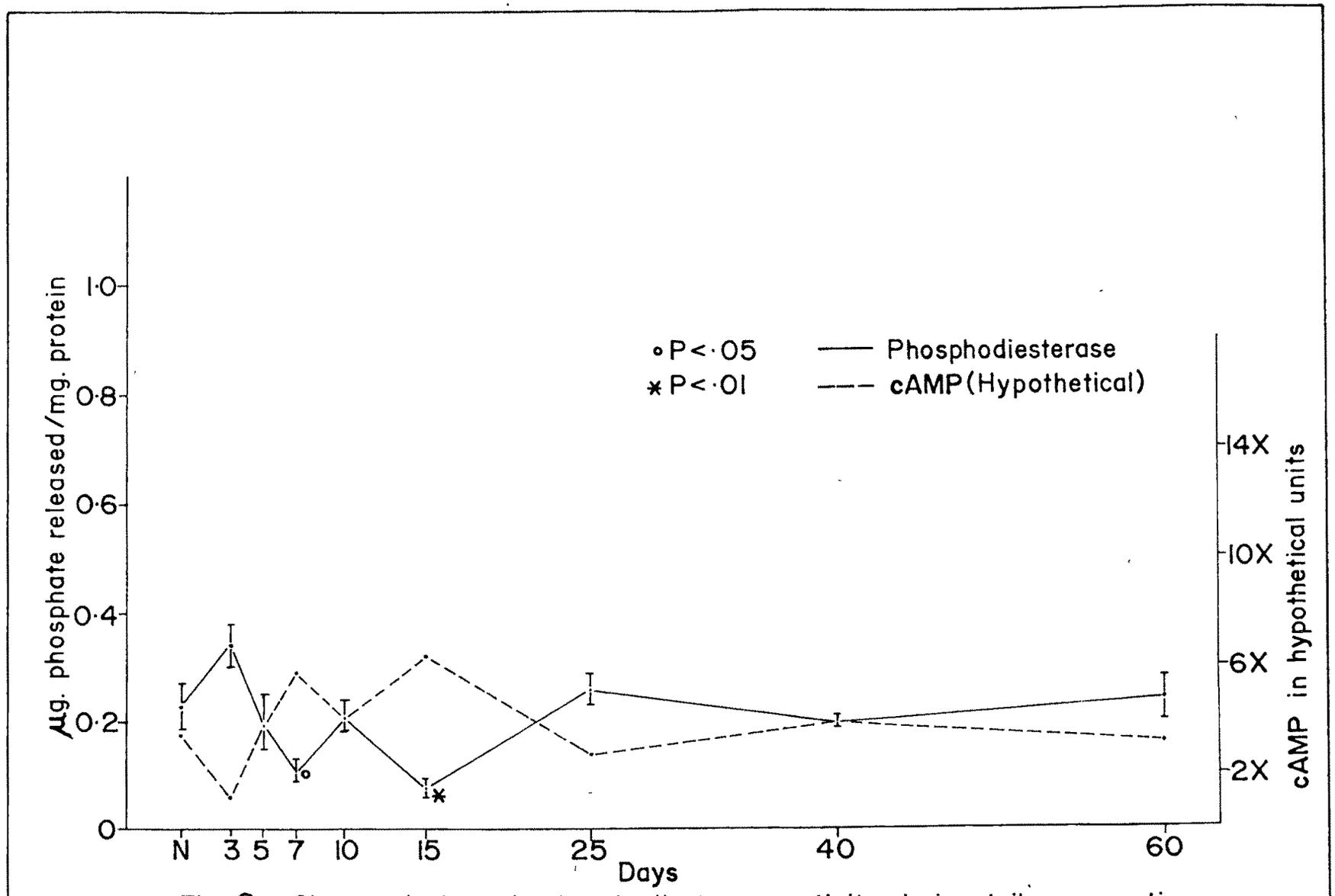

Fig. 2. Changes in hepatic phosphodiesterase activity during tail regeneration in H. flaviviridis.

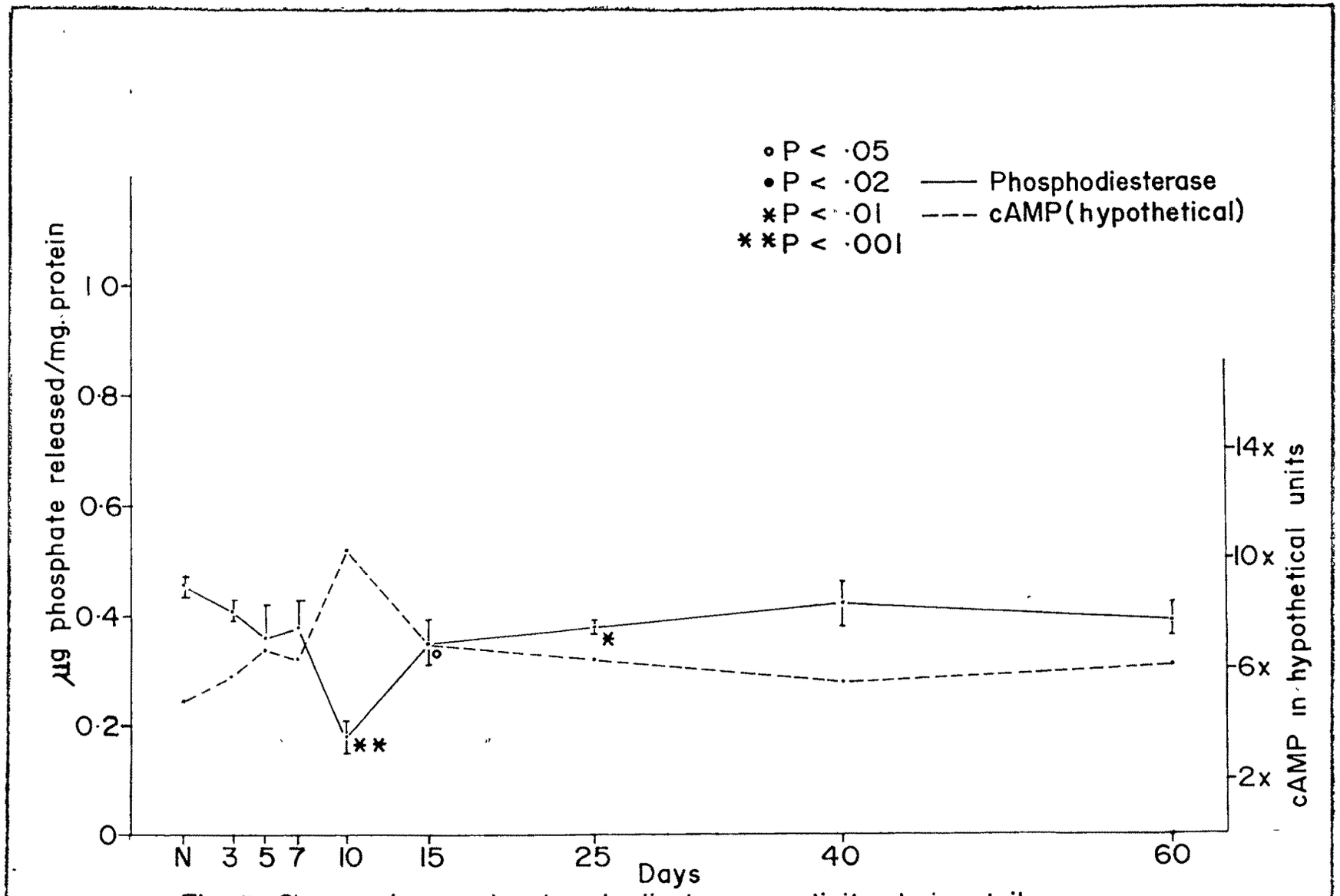

Fig. 3. Changes in muscle phosphodiesterase activity during tail regeneration in H. flaviviridis

DISCUSSION

Though the intracellular level of cAMP is essentially controlled by the synthetic enzyme, adenylate cyclase, the degradatory enzyme PDE can also play an important regulatory role. Variations in the prevailing level of PDE activity could denote either a cause or effect relationship with the cAMP content. In this context, whereas an increase in PDE activity could be taken as a primary response in prelude to a lowering of cAMP level, or as a secondary inductive response following an elevation in cAMP level, a decrease in PDE activity could essentially suggest a regulatory mechanism for increasing the cAMP content or prolonging its action. The present study has not revealed any increase in PDE activity during tail regeneration either *in loco* or systemically, which might suggest no net increase in total cAMP turnover during the regenerative mechanics. However, significant decrease in PDE activity has been the feature during regeneration both in the tail regenerate as well as in muscle and liver. This is suggestive of an increase in the active pool of cAMP essentially modulated by PDE activity without actually bringing about an increased generation of cAMP. Viewed in this context, increased cAMP activity at the local site of regeneration could be presumed to occur on the 3rd, 7th and 25-40th days post-autotomy. The period between 3rd

to 7th days represents dedifferentiative to blastemic phases and the herein informed increased cAMP content during this period could indicate the involvement of this cyclic nucleotide with a number of preparative and prerequisitive events associated with cell proliferation such as phosphorylation of histones (Greengard and Kuo, 1970; Langan, 1970; Bradbury et al., 1974), induction of ornithine decarboxylase and synthesis of polyamines (Theoharides and Cannelakis, 1975; Russel and Stambrook, 1975) and generating precursors for, and regulating DNA synthesis (MacManus et al., 1972, 1973; Foret, 1973; Foret and Babich, 1973; Short et al., 1975). Based on a previous study on PDE activity during tail regeneration in the Scincid lizard, Mabuya carinata, Ramachandran et al. (1983) had also suggested similar cAMP mediated activities during dedifferentiation and early blastemic phases. Pertinently, direct evaluation of cAMP during newt limb regeneration by Jabaily et al. (1975) had also reported increased content in the initial phase of regeneration. The report of Shappard (1972) of the attainment of certain levels of intracellular cAMP as a prerequisite for mitosis also appears significant in this context. Another phase of increased cAMP content visualised during the progressive phases of differentiation i.e., 25-40 days, as also reported by Ramachandran et al. (1983) in Mabuya carinata, denotes the involvement of this cyclic nucleotide in differentiation and morphogenesis (Robinson, 1973; Miller, 1977; Kosher and Savage, 1980).

The systemic alterations in PDE activity appear less significant and the decreased enzyme activity in skeletal muscle from 10th to 40th days post-autotomy may be linked with the glycogen depletion noted to occur during this period (Chapter 1). In contrast, the liver depicted an increased PDE activity on the 3rd day which corresponds to the period of hepatic glycogen depletion post-autotomy and could be viewed as an aftermath of increased cAMP content induced by adenylate cyclase activity. Whereas the increased cAMP content that may be purported to occur on the 15th day is correlatable with the second phase of hepatic glycogen depletion (Chapter 1), that on the 7th day defies any possible correlation at this juncture. It is quite likely that the increased cAMP in the liver on the 7th day may be related to the many biochemical and metabolic adjustments that have been noted to occur in liver during tail regeneration (Ramachandran et al., 1979, 1982, 1985; Kinariwala et al.,1981; Shah et al., 1982a,b). On a comparative basis, though the temporal in loco changes in PDE activity levels noted to occur in the present study during tail regeneration in Hemidactylus flaviviridis are in general similar to the picture obtained in Mabuya carinata (Ramachandran et al.,1983), the changes in the hepatic and skeletal muscle PDE activity are however of a differential nature in the two lizards. This tends to emphasize the fact that the systemic, metabolic

and biochemical adjustments in relation to regeneration though essentially existent in both cases are nevertheless different in keeping with their metabolic adaptations related to their habit and habitat.

SUMMARY

A quantitative evaluation of cAMP phosphodiesterase, the only known hydrolytic enzyme of cAMP, was undertaken in liver, muscle and tail during various periods of tail regeneration in Hemidactylus flaviviridis. Though changes have been observed in all the three tissues, the regenerating tail depicted maximum variation. In general, the changes in phosphodiesterase activity tend to suggest elevated cyclic AMP levels during the 3rd, 7th, 25th and 40th days of regeneration which correspond to early wound healing, preblastema and differentiation phases. In contrast, the systemic levels of PDE activity as evaluated in liver and femoral muscle showed less pronounced changes. The in loco and systemic alterations in PDE activity are correlated with changing levels of cAMP and its role in molecular mechanisms underlying regeneration and in the associated systemic, metabolic and biochemical modulations.