CHAPTER 4

LEVELS OF ACID AND ALKALINE PHOSPHATASES IN THE REGENERATING TAIL IN THE NORMAL AND HYPOPHYSECTOMIZED HOUSE LIZARDS, HEMIDACTYLUS FLAVIVIRIDIS

The loss of an appendage such as limb or tail in amphibians or tail in reptiles initiates a series of local and systemic reactions leading to regeneration and restitution of the lost part. The enzymatic changes associated with regeneration in animals offer a favourable system for evaluation of the biochemical events underlying phenomena like dedifferentiation and differentiation. Phosphatases have received considerable attention in this respect (Ghiretti, 1950; Schmidt and Weary, 1963; Shah and Chakko, 1967a; Miller and Wolfe, 1968; Radhakrishnan, 1972). The lysosomes with their acid hydrolases are known to play important role in the process of intracellular digestion (de Duve and Wattiaux, 1966). Since the loss of certain specialized cellular organelles and structures figure prominantly in the early phases of regeneration, when dedifferentiation of cells occurs. During such phase, changes in the quantitative levels and histochemical

localization of hydrolases in the dedifferentiating cells could prove valuable to understand the remodelling processes associated with the phenomenon of regeneration (Miller and Wolfe, 1968).

Non-specific acid phosphatase, according to the site of its localization and characteristics of the cell, is supposed to be involved in a variety of cellular activities such as cellular phagocytosis (Klockars and Wegelius, 1969), dissolution of tissue components (Weber and Niehus, 1961), protein synthesis (Pearse, 1968) differentiation (Ghiretti, 1950), absorption (Straus, 1964) and phosphorylation (Stetten, 1964).

The alkaline phosphatase is demonstrated to be involved in differentiation, formation of fibrous protein, calcification of bone, formation of mucopolysaccharides forming ground substances (Simkiss, 1964) carbohydrate metabolism (Rosenthal et al., 1960), Phosphate transfer in DNA metabolism (Rogers, 1960) and passage of metabolites across cell membrane (Simkiss, 1964).

Acid and alkaline phosphatases have been well involved in the regenerative process in amphibians (Schmidt, 1968) and in reptiles (Shah and Chakko, 1966

and 1967a; Radhakrishnan, 1972). In the present course of studies it is found that hypophysis removal caused considerable delay in the tail regeneration in Hemidactylus flaviviridis (Chapter 1). Hormonal influence on activities of acid and alkaline phosphatases in rats (Buchanan and Schartz, 1967; Helminen et al., 1972; Yeh and Moog, 1977; Wilfred and Rao, 1977) and in Chick (Manwell and Betz, 1966; McWhinnie and Thommes, 1973) have been studied. It would be fruitful to evaluate effect of hypophysis ablation on the activities of acid and alkaline phosphatases in the regenerating tail of the house lizard, H.flaviviridis. Since no such studies are known to have been done, the present study on quantitative analysis of these two hydrolases in the regenerating tail of hypophysectomized lizards was undertaken.

MATERIALS AND METHODS

The house lizards, H.flaviviridis, were collected from the university campus and maintained in the laboratory on a diet of insects. The animals were kept in the laboratory for a fortnight prior to experimentation, to get them acclimatized to the laboratory conditions. Adult lizards

of both the sexes in more or less the same weight group (10-12 gms) were selected. Hypophysectomy and sham operation were performed and tails were autotomized 10 days after operation (as described in Chapter 1). Autotomy was also performed in a group of normal unoperated animals. Fifty lizards in each case viz., hypophysectomized, sham operated and normal ones were used for the present experiments. At specific intervals in accordance with various phases of tail regeneration 6-8 animals per phase were sacrificed. Tail regenerates were quickly excised and blotted free of blood and tissue fluid. These were homogenized in cold distilled water and were used for quantitative estimations of acid and alkaline phosphatases employing the method described in Sigma Technical Bulletin No.104, using p-nitrophenyl phosphate as substrate. Protein concentrations in the homogenate were estimated employing Biuret method (Layne, 1957). Enzyme activities were expressed as μmoles p-nitrophenol released/mg protein/30 minutes.

RESULTS

The results obtained from the above studies on the hypophysectomized, sham operated and unoperated normal

tail bearing lizards are presented in Tables 1 & 2 and Figs. 1 & 2.

Acid Phosphatase:

When the values of acid phosphatase activities in the normal tail and that in the wound healing phase of regenerating tail were compared, it was noticed that in the experimental animals (Sham operated and hypophysectomized lizards) there was a decline in the enzyme activity during wound healing period. However, in the tail of unoperated lizards the enzyme had higher activity during this phase.

Acid phosphatase activity in the tails of unoperated lizards as well as in that of the sham operated and hypophysectomized ones showed a trend of increase after wound healing and attained its maximum level during differentiation phase. Hereafter, in the first two cases (unoperated and sham operated lizards) the enzyme activity began to decline and reached the value characteristic of the normal tail by about 50th day after autotomy. However, in the case of the hypophysectomized lizards the increase in enzyme activity continued upto early growth phase (30 days

Table 1

Acid phosphatase activity in the tail of hypophysectomized, sham operated and unoperated lizards, H. flaviviridis, during tail regeneration.

Stages	Hypophysectomized* (a)	Sham operated* (b)	Unoperated	$\frac{a}{b}$x100
10 days after operation*	0.04819 ± 0.0171 (P < .01)	0.06985 ± 0.0074	0.04713 ± 0.0053	68.99
Phases of tail regeneration				
Wound healing	0.02004 ± 0.0015 (P < .0005)	0.05065 ± 0.004	0.05262 ± 0.0162	39.56
Blastema	0.05587 ± 0.0181 (NS)	0.06773 ± 0.0152	0.07446 ± 0.0078	82.48
Differentiation	0.06199 ± 0.0192 (P < .0005)	0.13881 ± 0.0122	0.13491 ± 0.0119	44.65
Growth (30 days)	0.10936 ± 0.0131 (NS)	0.11185 ± 0.0026	0.08226 ± 0.0115	97.77
Growth (50 days)	0.04087 ± 0.0292 (P < .01)	0.09630 ± 0.0180	0.06031 ± 0.0277	42.44
Fully regenerated tail (70 days)	-	-	0.04955 ± 0.0039	-

P values in parentheses are obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in μmole p-nitrophenol released/mg protein/30 min as mean ± S.D.

Table 2

Alkaline phosphatase activity in the tail of hypophysectomized, sham operated and unoperated lizards, H. flaviviridis, during tail regeneration.

Stages	Hypophysectomized* (a)	Sham operated* (b)	Unoperated	$\frac{a}{b}$x100
10 days after operation*	0.06124 ± 0.0072 (P < .025)	0.07234 ± 0.0110	0.04166 ± 0.0080	84.65
Phases of tail regeneration				
Wound healing	0.02329 ± 0.0083 (P < .01)	0.00922 ± 0.0037	0.01194 ± 0.0021	252.60
Blastema	0.12335 ± 0.0055 (P < .0005)	0.21264 ± 0.0182	0.20640 ± 0.0165	58.008
Differentiation	0.06879 ± 0.0146 (P < .001)	0.12857 ± 0.0194	0.12197 ± 0.0259	53.50
Growth (30 days)	0.10165 ± 0.0272 (P < .0025)	0.15891 ± 0.0174	0.13444 ± 0.0341	63.96
Growth (50 days)	0.10229 ± 0.0242 (NS)	0.11497 ± 0.0161	0.08040 ± 0.0209	88.97
Fully regenerated tail (70 days)	-	-	0.051706± 0.008	-

P values in parentheses were obtained in comparison with sham operated lizards.

Values are based on 6-8 animals in each stage and expressed in µmole p-nitrophenol released/mg protein/30 min as mean ± S.D.

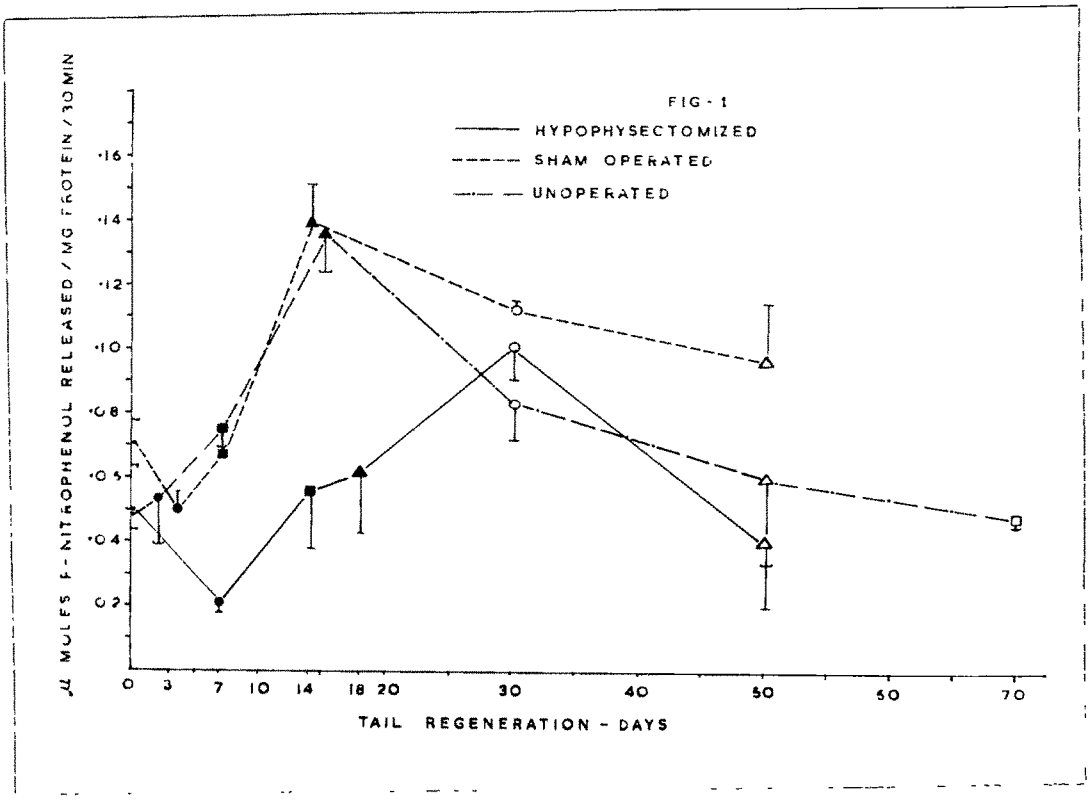
FIG - 1
HYPOPHYSECTOMIZED
SHAM OPERATED
UNOPERATED
μ MOLES P-NITROPHENOL RELEASED / MG PROTEIN / 30 MIN
·16
·14
·12
·10
·08
·06
·04
·02
0 3 7 10 14 18 20 30 40 50 60 70
TAIL REGENERATION - DAYS

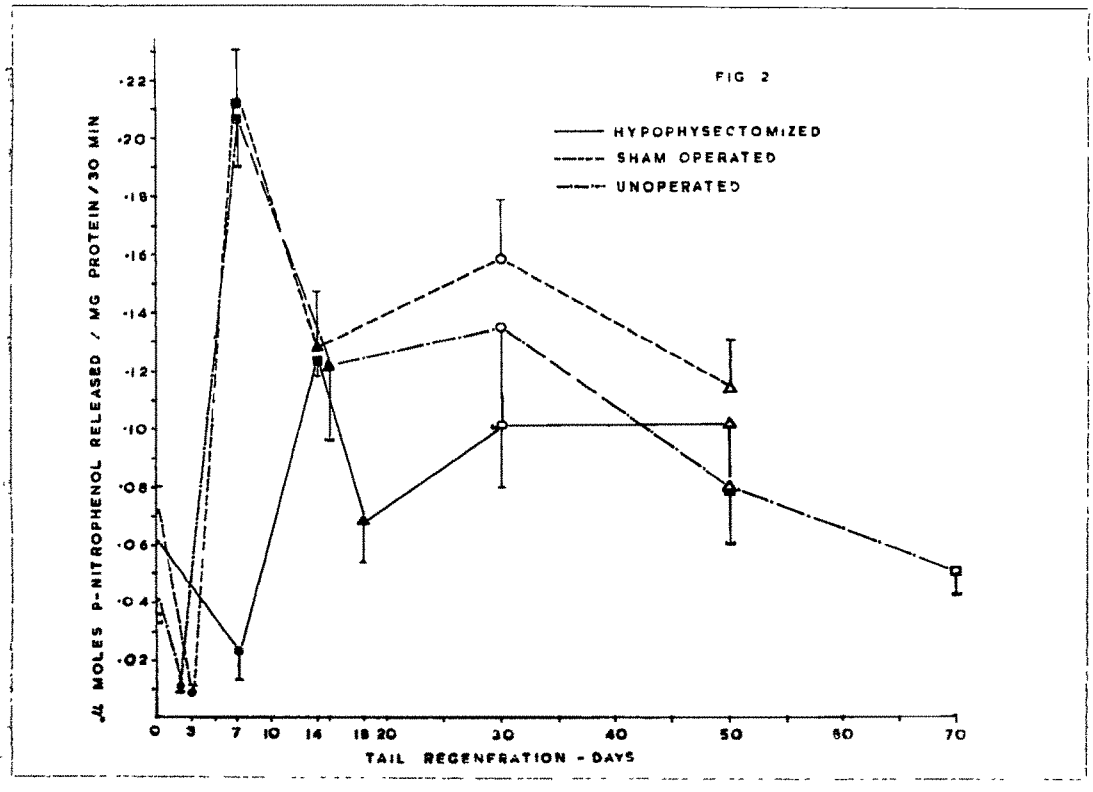
FIG 2
HYPOPHYSECTOMIZED
SHAM OPERATED
UNOPERATED
μ MOLES P-NITROPHENOL RELEASED / MG PROTEIN / 30 MIN
·22
·20
·18
·16
·14
·12
·10
·08
·06
·04
·02
0 3 7 10 14 18 20 30 40 50 60 70
TAIL REGENERATION - DAYS

Fig. I : Graphic representation of levels of acid phosphatase activity in tail regenerate, during different phases of tail regeneration in hypophysectomized, sham operated and unoperated lizards, H. flaviviridis.

Fig. 2 : Graphic representation of levels of alkaline phosphatase activity in tail regenerate, during different phases of tail regeneration in hypophysectomized, sham operated and unoperated lizard, H. flaviviridis.

● Wound healing phase
■ Blastema phase
▲ Differentiation phase
O Growth phase (30 days after autotomy)
△ Growth phase (50 days after autotomy)

after autotomy) and by the 50th day it dropped to more or less the same level that was observed in the hypophysectomized animals prior to autotomy. Throughout the period of tail regeneration the enzyme activity in the regenerate of the hypophysectomized animal was at considerably low level when compared to that in the unoperated and sham operated lizards.

Alkaline Phosphatase:

In all the three groups viz., unoperated, sham operated and hypophysectomized lizards, alkaline phosphatase activity declined from its preautotomy level during the wound healing phase, but increased again to attain highest peak during blastema phase. During differentiation, a decline in the enzyme concentration in the regenerates of lizards belonging to all the three groups was observed, which thereafter again rose during the early growth phase (30 days). However, when the regenerate was 50 days old the enzyme concentration again declined. In the unoperated lizards, when regenerates were fully grown (70 days) the enzyme levels declined further, attaining values similar to those observed at the preautotomy state. The full

grown state of the tail regenerate in the unoperated lizards is reached by about 70 days (Magon, 1970). In the hypophysectomized lizards all the experiments were terminated on 50th day after autotomy since the lizards deteriorated in their health. The sham operated lizards were also considered upto 50 days post-autotomy as they constitute a control group to the hypophysectomized animals.

DISCUSSION

Production of lysosomal enzymes such as acid phosphatase represents one of the first steps in repair processes (Raekallio, 1960). Acid phosphatase is known to be involved in phagocytic activity and mucopolysaccharide synthesis during regeneration of appendages in amphibians and reptiles (Singer and Salpeter, 1961; Shah and Chakko, 1966; Miller and Wolfe, 1968; Radhakrishnan, 1972). Such activities are apparently affected in event of unavailability of an optimum concentration of acid phosphatase. In the present study, the acid phosphatase activity during wound healing phase in the hypophysectomized lizards is less than half (39.56%) as compared to that in the sham operated ones. Such a low

level of the enzyme activity could be due to absence of hypophysial hormones and could be considered as one of the factors for the delayed wound healing observed in the hypophysectomized animals (Chapter 1). On comparison the level of preautotomy acid phosphatase activity in the tail of the three groups of lizards, the sham operated (controls) exhibited highest value amongst the three. This anamoly could be explained in terms of gearing up of the enzymatic machinery involved with the healing of the operational wound prior to autotomy. This apparently leads to prevalence of higher acid phosphatase levels to begin with. In the case of hypophysectomized animals such high levels are not attained probably due to unavailability of the hypophysial hormones.

Regeneration blastema is a mass of undifferentiated mesenchymatous cells that are actively engaged in cellular division and proliferation leading to the growth of the blastema (Shah and Chakko, 1968b). Such activities would certainly demand greater synthesis of macromolecules such as proteins, and acid phosphatase is known to be involved in protein synthesis (Pearse, 1968). Increase in acid phosphatase activity observed during blastema and differentiation phases in unoperated and sham operated lizards

could be correlated with the increase in the damand for protein synthesis. It has been also reported that, increase in concentration of nucleic acids (Shah and Chakko, 1972), total proteins (Shah et al., 1977a) and free amino acids (Chapter 6) occur in the lizard tail blastema and differentiating regenerates. On comparison, acid phosphatase activity levels in the fully formed blastema in all the three groups of animals do not show any significant difference. However, during differentiation phase very low level of enzyme concentration is observed in the regenerate of the hypophysectomized animals only. A low level of the acid phosphatase activity could lead to disturbance in protein and nucleic acid synthesis. Thus a correlation between low acid phosphatase activity and delay in the establishment of the differentiating regenerate in the hypophysectomized lizards is quite apparent. During early growth phase (30 days after autotomy) the regenerate in the hypophysectomized lizard showed an increase in the activity of acid phosphatase compared to that observed during its differentiation phase. This spurt in the enzyme activities is probably an attempt to correct the defect of the earlier phases. Selective augmentation or diminution of acid phosphatase under the influence of thyroid, parathyroid and gonadal hormones

(Helminen et al., 1972; Robinson, 1973; Piechowski and McWhinnie, 1977) has been suggested. From the present study on hypophysectomized lizard it becomes evident that pituitary hormone/s play significant role in controlling the activities of acid phosphatase in the tail regeneration either directly or through other endocrine glands. It is also possible that several acid phosphatase isoenzymes are active during the course of regeneration, synthesis and/or activation of some of these is selectively inhibited, while others are unaffected in the absence of the pituitary. Nevertheless, the increase in the acid phosphatase activity in the tail regenerate of the hypophysectomized animals from blastema till early growth phase could possibly be due to corrective efforts made in control of this enzyme activity by other endocrines viz., thyroid, parathyroid and gonads, under hypophysioprivic conditions.

Alkaline phosphatase showed a decrease in its activity from the preautotomy value during wound healing in the unoperated, sham operated and hypophysectomized lizards. However, it is interesting to note that in the hypophysectomized animals acid phosphatase was at low level while alkaline phosphatase exhibited an elevation

compared to the controls. This may be due to change of the environmental conditions in the cells engaged in wound healing, which probably becomes alkaline, thus creating a condition suitable for alkaline phosphatase activity. This inturn is not conducive for normal progress of wound healing.

During blastemal phase on the other hand, alkaline phosphatase showed a tremendous increase in its activity in all the three groups of animals. Rapidly proliferating cells during embryonic development are also known to exhibit high activity of alkaline phosphatase (Abe et al., 1972; Makawa and Yamana, 1975). Association of this enzyme with protein synthesis and nucleic acid metabolism is also well established (Simkiss, 1964). In limb regeneration of hypophysectomized Triturus alpestris and T. vulgaris (De Coninck et al., 1956) RNA was observed to be at low level as compared to that in unoperated newts. Compared to the controls (unoperated and sham operated lizards) significantly lower level of alkaline phosphatase in the blastema of the hypophysectomized lizards probably affects protein and nucleic acid synthesis.

During differentiation of the tail regenerate in all the three cases, there is a decline in the alkaline

phosphatase activity from that observed during the blastema phase. However, the level is higher than the preautotomy one. Differentiation of the regenerate is characterized by laying down of matrix and synthesis of mucopolysaccharides are considered to be some of the characteristics of differentiating tissues of the tail regenerate (Shah and Hiradhar, 1975) where alkaline phosphatase is known to be involved. Considerably low level of alkaline phosphatase observed in the hypophysectomized lizard would affect laying down of matrix and synthesis of mucopolysaccharidesin the tail regenerate.

Growth of the differentiated regenerate is the next event during tail regeneration. Since alkaline phosphatase has been linked with elaboration of organic matrix and collagen production, high level of the enzyme activity during the growth phase compared to the preautotomy level probably indicates involvement of this enzyme in such activities. It is interesting to note here that, in the hypophysectomized lizard, muscle, connective and adipose tissues of the regenerate were poorly developed and even the weight of the tail regenerate was significantly lower as compared to that

of the control animals (Chapter 1). Hence it is surmised that the low level of alkaline phosphatase activity prevailing in the regenerate of hypophysectomized lizards probably affected the structural lay out of the regenerate.