CHAPTER 3

EFFECT OF HYPOPHYSECTOMY ON LACTATE DEHYDROGENASE (LDH) ACTIVITY IN THE REGENERATE AND LIVER DURING TAIL REGENERATION IN THE HOUSE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Considerable amount of attention has been paid to biochemical and histochemical localization and distribution of enzymes during regeneration of vertebrate appendages (Schmidt, 1968; Shah and Hiradhar, 1978). Of these, dehydrogenases have received greater attention in this context. Relevant literature can be found in the studies of Geczik and Wolsky (1959), Wolfe and Cohen (1963), Schmidt and Weidman (1964), Johnson and Singer (1964), Hessler and Bromley (1969) and Balek and Chan (1969) on amphibian regeneration; Shah and Ramachandran (1970), Magon (1970) and Shah *et al*. (1979a) on regeneration in reptiles. The effect of hormones on the synthesis of lactate dehydrogenase (LDH) has been investigated (Degroot and Cohen, 1962; Goodfriend and Kaplan, 1964; Giri and Singh, 1978). No such studies during regeneration have been reported in reptiles so far. Hence, the present study on LDH in the liver and regenerating tail of hypophysectomized and sham operated house lizard, Hemidactylus flaviviridis was undertaken with a view to understand the influence of pituitary hormones on LDH activity and its relation to the observed retardation in the rate of growth of the regenerate.

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 to get them acclimatized to the laboratory conditions. Adult lizards (weighing 10-12 gms) were selected for hypophysectomy and sham operation, and were operated as described in Chapter 1. Tail autotomy was induced 10 days after operation. Fifty lizards in each case viz., hypophysectomized and sham operated were used for the present experiments. At specific intervals in accordance with the various phases of tail regeneration, 6 to 8 animals per ~~each~~ phase were sacrificed.

Quantitative estimation of LDH:

Regenerates and liver pieces from the hypophysectomized as well as sham operated lizards were quickly excised and blotted free of blood and tissue fluid, homogenized in cold distilled water and were used for estimations of lactate dehydrogenase (LDH), spectrophotometrically, employing the method of Farrar and Bush (1969), using sodium lactate as the substrate and nicotinamide adenine dinucleotide (NAD) as a cofactor. LDH activity was expressed as m μmoles $NADH_2$ formed per mg protein per minute. Protein concentration in the homogenate was estimated by Biuret method (Layne, 1957).

RESULTS

The data on quantitative values of LDH in the regenerating tail and liver are presented in Tables 1 & 2 and Figs. 1 & 2.

LDH activity in the tail, 10 days after operation was found to be 53.639 ± 5.119 and 49.877 ± 2.341 in hypophysectomized and sham operated lizards respectively. After the tail autotomy there was a fall in the activity of the enzyme from the preautotomy level, which was found to be the lowest during the wound healing phase in the hypophysectomized (6.642 ± 0.469) and sham operated (7.234 ± 0.482) lizards. Once the wound healed, the enzyme activity gradually increased during subsequent phases of tail regeneration. The pattern of the levels

Table 1

Lactate dehydrogenase activity in the tail of hypophysectomized and sham operated lizard, *H. flaviviridis*, during tail regeneration.

Stages	Hypophysectomized (a)	Sham operated (b)	$\frac{a}{b}$ x 100
10 days after operation	53.639 ± 5.119 (NS)	49.877 ± 2.341	107.54
Phases of tail regeneration:			
Wound healing	6.642 ± 0.469 (NS)	7.234 ± 0.482	91.81
Blastema	7.959 ± 1.655 ($P < .0005$)	14.092 ± 0.994	56.47
Differentiation	8.910 ± 2.013 ($P < .0025$)	15.439 ± 2.455	57.70
Growth (30 days)	11.655 ± 2.784 ($P < .0025$)	16.893 ± 0.173	68.99
Growth (50 days)	18.124 ± 3.154 ($P < .0005$)	36.810 ± 3.750	49.23

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 m μmoles of $NADH_2$ formed/mg protein/min as mean ± S.D.

Table 2

Lactate dehydrogenase activity in the liver of hypophysectomized and sham operated lizard, *H. flaviviridis*, during tail regeneration.

Stages	Hypophysectomized (a)	Sham operated (b)	$\frac{a}{b} \times 100$
10 days after operation	29.986 ± 2.869 $(P < .0005)$	43.246 ± 4.191	69.33
Phases of tail regeneration:			
Wound healing	23.296 ± 5.296 (NS)	26.063 ± 4.693	89.38
Blastema	30.726 ± 2.574 $(P < .0005)$	41.712 ± 0.182	73.66
Differentiation	25.722 ± 1.067 $(P < .0005)$	32.427 ± 2.390	79.32
Growth (30 days)	23.265 ± 3.464 $(P < .005)$	30.020 ± 1.857	77.49
Growth (50 days)	15.933 ± 3.394 $(P < .0005)$	33.335 ± 5.618	47.79

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 m µmoles of $NADH_2$ formed/mg protein/min as mean ± S.D.

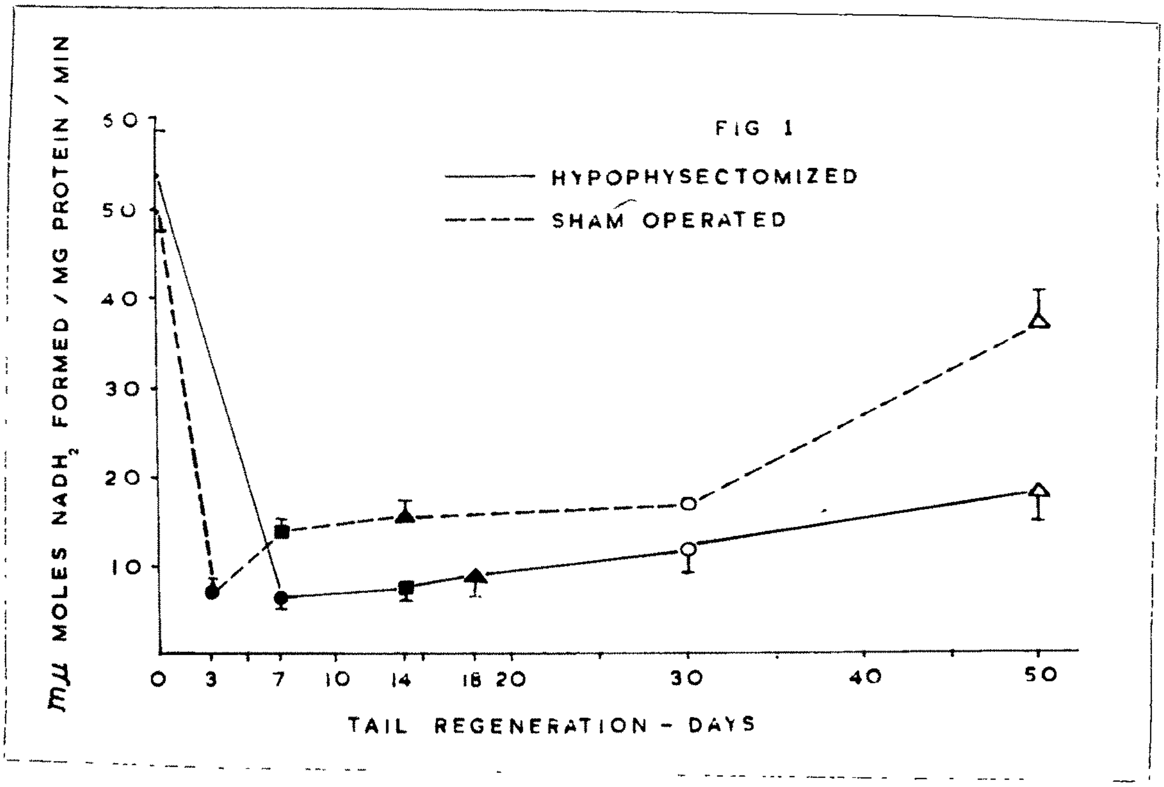

FIG 1

HYPOPHYSECTOMIZED
SHAM OPERATED
mμ MOLES NADH$_2$ FORMED / MG PROTEIN / MIN
50
50
40
30
20
10
0
3
7
10
14
18 20
30
40
50
TAIL REGENERATION - DAYS

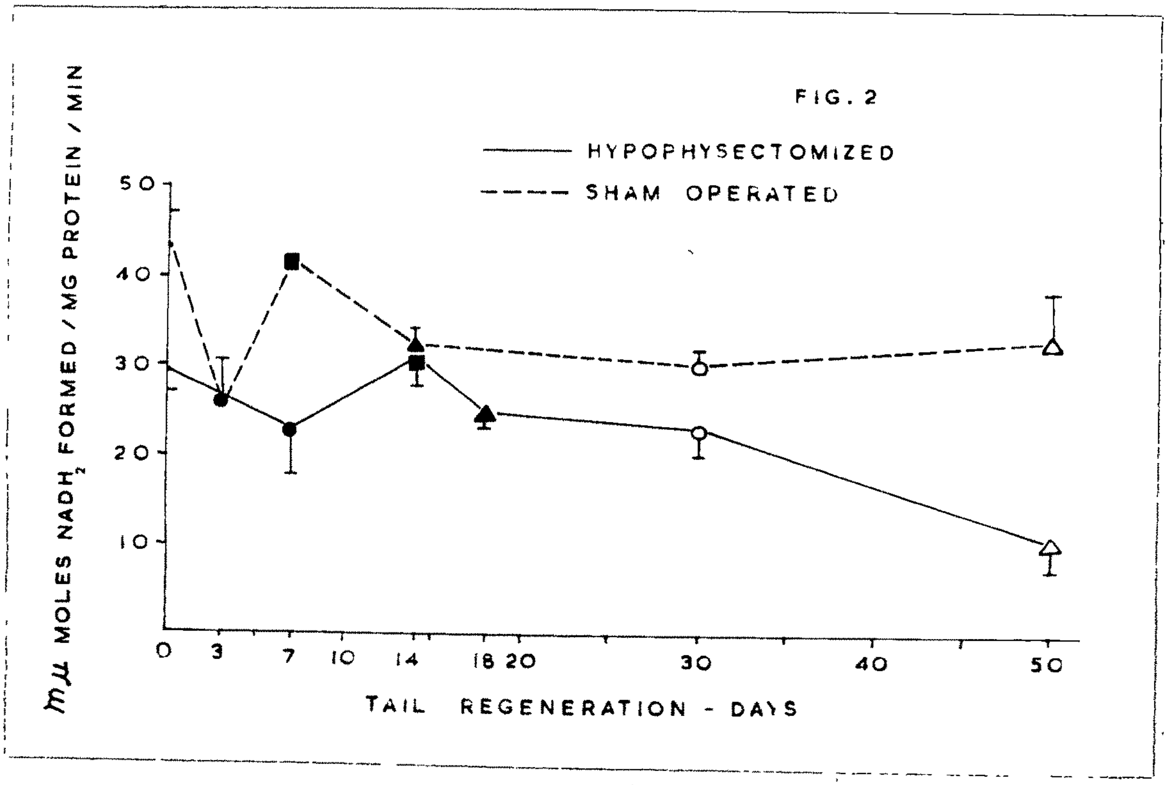

FIG. 2

HYPOPHYSECTOMIZED
SHAM OPERATED
mμ MOLES NADH$_2$ FORMED / MG PROTEIN / MIN
50
40
30
20
10
0
3
7
10
14
18 20
30
40
50
TAIL REGENERATION - DAYS

Fig. I : Graphic representation of levels of lactate dehydrogenase activity in tail regenerate, during different phases of tail regeneration in hypophysectomized and sham operated lizards, _H_. _flaviviridis_.

Fig. 2 : Graphic representation of levels of lactate dehydrogenase activity in liver, during different phases of tail regeneration in hypophysectomized and sham operated lizards, _H_. _flaviviridis_.

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

of the enzyme activity in both the groups viz., hypophysectomized and sham operated lizards were comparable; however, in the former it was nearly 50% lower during the blastema, differentiation and growth phases (50 days) as compared to that in the latter during corresponding phases.

Prior to autotomy, significantly low LDH activity was observed in the liver (Table 2, Fig. 2), of the hypophysectomized lizards (29.986 ± 2.869) as compared to that of the sham operated ones (43.246 ± 4.191). During wound healing a decline in the enzyme activity was noticed in both the groups; however, it rose nearly to the preautotomy level during the blastemal phase but again declined during the differentiation and growth phases. At any stage of the tail regeneration, LDH activity in the liver of the hypophysectomized lizard was lower than that in the sham operated ones.

DISCUSSION

High level of LDH activity in the original tail of the hypophysectomized and sham operated lizards suggests an active glycolysis in the tail tissues. Dependence of the normal tail tissue of the house lizard, *H. flaviviridis* on the anaerobic mode of carbohydrate catabolism for energy procurement has been recognized by previous workers (Chakko, 1968; Magon, 1970), which has been further supported by the studies on respiratory quotient (RQ) of the tail tissue (Shah and Hiradhar, 1974).

The animal which does not have the power of extensive regeneration, wound healing marks an end of the repair process. In such cases where metabolic machinery which gets altered on infliction of injury, returns to the normal physiological state in due course of time as the healing of the wound is completed. But as far as regeneration is concerned, wound healing is just the beginning of a variety of morphological and physiological changes that are to follow. The activity of LDH has been reported to be higher in the epithelium during wound healing, blastema and differentiation phases than in the normal tail skin (Magon, 1970). Besides, the active involvement of LDH with anaerobic glycolysis, the capacity of this enzyme to convert lactate into pyruvate can help in lipogenesis. Lactate is actively utilized by the skin as a precursor to lipogenesis and it can also provide cytosolic $NADH_2$ for glycerogenesis and for the malate cycle (Wheatley, 1974). It is interesting to note, at this point, that high activity of MDH and high level of lipids (Chakko, 1968; Shah and Hiradhar, 1977) in the skin of the regenerating tail indicates the possibility of lipogenesis in the skin. Hence, involvement of LDH in lipogenesis and generation of $NADH_2$ for operation of other pathways can not be ruled out during reptilian tail regeneration.

The activity of the LDH in the blastemal cells of the hypophysectomized lizards was nearly half, when compared to that of the animals of the control group. The blastema is considered as a mass of mesenchymal cells derived from the dedifferentiated cells of the stump tissue which are actively undergoing cell division (Shah and Chakko, 1968b). During cell division, the dividing cells are known to derive energy through anaerobic glycolysis (O'Connor, 1950; Vos et al., 1967; Chakko, 1968; Magon, 1970; Ramachandran, 1972). Hence the involvement of LDH in such a process in the blastema cells is quite understandable. Presently observed low LDH activity in the blastema cells of hypophysectomized lizard suggests lower metabolic rate in the constituent cells. Such a defect in the metabolism could inturn affect the energy demand of the dividing cells. Presently observed delay in the formation of blastema and its retarded growth (Chapter 1) could be due to the low level of enzyme activity prevailing in the hypophysectomized lizards.

Belated cellular proliferation during blastema, when reaches its peak the differentiation and growth of the regenerate ensue and correspondingly gradual increase in the LDH activities occurs. This rise in the LDH activity observed in the tail regenerates of the hypophysectomized lizards during differentiation and growth phases was significantly lower than that observed for the corresponding periods in the control animals. Such a low level of the enzyme activity in the hypophysectomized lizards and consequent poor cell proliferation and differentiation of the regenerate tissues could be due to the absence of pituitary hormone/s. This fact along with the presently observed low levels of LDH activity amply accounts for overall low metabolic activities of the regenerates in the hypophysectomized lizards. During these phases in the normal unoperated lizards increase in glycogen content and phosphorylase activity in the tail regenerates have been observed (Shah and Chakko, 1967b; Radhakrishnan and Shah, 1973; Shah and Hiradhar, 1974). However, the levels of glycogen in the regenerates during these phases in the hypophysectomized lizards were comparatively low (Chapter 2).

Hormones such as testosterone, progesterone, estradiol, thyroxine and cortisone have been shown to influence the activity of LDH (Degroot and Cohen, 1962; Goodfriend and Kaplan, 1964; Giri and Singh, 1978). Hypophysectomized newt showed a reduction in the LDH activity, however, those receiving growth hormone were shown to regulate the isoenzyme synthesis (Hessler and Bromley, 1969) in the regenerating forelimbs. In light of above reports reduction in the LDH activity observed in the tail regenerates in the hypophysectomized lizards could be well understood. Such interference in the synthesis of the LDH could in turn affect the metabolism and structural lay out of the tail regenerate in the hypophysectomized lizards.

Immediately after autotomy of the tail, physiological homeostasis of the lizard body gets altered sufficiently so as to adapt towards the extra energy demands of the injured tissues of the tail stump for their repair and subsequent regeneration (Kinariwala, 1977; Kothari ,1977). Reptiles are known to depend upon anaerobic metabolism (Bennet, 1972b; Bennet and Dawson, 1972; 1973; Bennet and Licht, 1972). Miller and Hale (1968) and Bennet (1972a) have shown that in reptiles glycolytic enzymes have much higher value in their skeletal muscles than in the liver. On the other hand aerobic enzymes in the liver are nearly four times that in the muscles (Bennet, 1972a).

Relatively high activity of LDH in the liver at the preautotomy period in the sham operated lizards suggests a prevalence of anaerobic metabolic activities in this organ. LDH activity in the liver of hypophysectomized lizards during tail regeneration was low as compared to that during corrèsponding stages in the sham operated animals. Such a decrease in the activity of LDH in the liver of rats following hypophysectomy has been reported (Weber et al., 1961; Freedland et al., 1968). Hence the presently observed reduction in the activity of LDH in the liver of the hypophysectomized lizards could be considered due to ablation of the pituitary gland.

Low levels of the enzyme activity in the liver observed during the course of tail regeneration except during the blastema formation in both the groups of experimental lizards are suggestive of shift in the metabolic pattern of the organ. This contention finds support in the studies of Shah et al. (1978a)who have shown that during lizard tail regeneration an increase in the activity of SDH and MDH in the liver occurs, which is considered as an index of the increased TCA cycle operation. Such an alteration in the metabolic pattern is believed to provide the extra energy needed for the tail regeneration. Similar situation would be visualized in the lizards used in the present study. However, the energy yield through such shift in metabolic pattern of the liver could be quite low in absence of hypophysial hormone/s. Hence delay in regenerative process and retarded growth of the regenerate could occur.