INTRODUCTION

"If there were no regeneration, there could be no life, if everything regenerated there would be no death", thus observes Goss (1969) in his treatise on "Principles of Regeneration". All organisms exist between these two extremes. In an antagonistic and competitive world all living creatures are constantly beset, through injury and disease, with the problem of replacing the affected areas or succumbing to the results of disaster. Fortunately for living organisms, ability to repair injured or lost tissues and parts has been innate. Realization of the regenerative phenomena is by no means recent. According to Greek mythology Prometheus who was punished by Zeus for the theft of fire from Olympian Gods and its deliverence to man, chained to rock in the Caucasus mountains, he was beset daily by vulture who ate away at his liver. At night Prometheus' fever subsided and his liver regenerated (Fitzgerald, 1970). Though this statement appears to be a fable, nevertheless it does suggest that even at that ancient time it was believed that human liver possesses power of regeneration.

More than two centuries ago an Italian investigator Spallanzani (1768) undertook first systematic studies utilizing larval and adult amphibians. His observations have been re-evaluated and strengthened by several investigators since the turn of this century.

Several definitions have been put forth to explain the phenomenon of regeneration. However, in the final analysis, rebirth or restoration of lost or damaged organs and parts thereof, appears to be a fairly workable definition.

Two main forms of regeneration, viz., physiological and reparative regeneration, are customarily distinguished from each other. Physiological regeneration is the restoration of the lost elements of a living organism during its normal day to day activity (e.g. epithelial and RBC regeneration). Physiological regeneration is a universal phenomenon characteristic of tissues and cells of animals, plants and microorganisms. Physiological regeneration is not lost during phylogenesis and ontogenesis. On the other hand, reparative regeneration is restoration of lost part caused by trauma or damage, and is restricted to lower animals while in higher animals it is rather limited to such an extent that in man even minor wounds often leave scars.

For a long time many biologists and physicians considered regeneration as a phenomenon of growth, assuming that it is nothing else but accumulation of cells and growth of tissue as a result of damage to the organ. In one of the first extensive reviews on problems of regeneration, Morgan (1901) wrote : "Regeneration is nothing else but resumption of temporarily arrested growth". However, the numerous studies that followed in subsequent few decades showed that regeneration is something more than mere growth; it includes transformation, differentiation and quantitative as well as qualitative changes to restore the lost parts of an organism.

The power of regeneration extends right from the most primitive coelenterates to highly evolved mammals. However, the regenerative powers are feeble in the higher vertebrates when compared to the lower ones. The regenerative potentials appear to be limited due to the complexity of organisms, which are higher up in the evolutionary tree.

The larvae of Petromyzon (ammocoetes) are said to regenerate their tail (Niazi, 1964). Number of fishes can regenerate their fins, optic nerves and barbels (Nicholas, 1955; Goss, 1956; Haas, 1962; Raghuvanshi and Swarup, 1978). Among the amphibians, newts, salamanders and anuran tadpoles have regenerative potentials. Salamanders seem to have the most remarkable regenerative ability compared to all the other vertebrates. Amongst reptiles, some species of lizards have retained considerable capacity to regenerate their tail, which is however, described as 'imperfect' regeneration by Simpson (1964). It is interesting to note that lizard embryos do not have the ability to regenerate their tail (Moffat and Bellairs, 1964). In birds and mammals, physiological regeneration is well exemplified. Epidermal appendages such as feather, nails and hair either grow continually or are replaced by periodic molts. Regeneration of functional axon in the central nervous system is better developed in birds than in mammals, and perhaps is better in embryo than in adults (Windle, 1955). Ability for regeneration in mammals is restricted to addition of cells of the same basic type e.g. epithelia, blood cells and liver cells than in the production of a complex new and wholly functional limb.

Appendage regeneration in vertebrates involves a series of morphogenetic events. After amputation, the cut end of the stump tissue is covered with wound epithelium. Maderson and Salthe (1969) have suggested that "poorly controlled" wound healing is a good factor for regeneration.

Goss and Grimes (1972) have made a clear distinction between wound healing and regeneration. In the regenerating system a number of cell types readily revert to more embryonic state under conditions of trauma and start dividing. Dedifferentiation is absolutely necessary prior to proliferation of cells to form a blastema in a regenerating system (Hay, 1968). Regeneration fails to occur in the absence of a blastema, hence number of studies related to the origin of blastemal cell (Trampusch and Horrebomee, 1965; Hay, 1966; Schmidt, 1968; Cox, 1969); potentiality of blastema (De Both, 1970; Michael and Faber, 1971); autonomous self regulating and organizing capacity of blastema (Faber, 1965; Stocum, 1968a,b) have been carried out during recent years.

During last few decades studies have been carried out to reveal the factors that either suppress or entirely inhibit repair and regenerative processes among vertebrates. Such inhibition has been accomplished by a wide array of physico-chemical means, including application of X-rays, ultraviolet irradiation, antimetabolites and other toxic agents as well as induced lack or excess of endocrine hormones (Schmidt, 1968).

The influence of external factors like temperature, humidity, light and electric current on appendage regeneration has been well documented (Becker, 1961a,b; Bodemer, 1964; Maderson and Licht, 1968; Magon, 1970; Schauble,1972; Smith, 1974; Schauble and Nentwig, 1974; Borgens et al., 1977; 1979). The tail of Anolis carolinensis regenerates more rapidly when exposed to a long day photoperiod (Turner and Tipton, 1972). Similarly in Triturus (Notophthalmus viridescens), rate of limb regeneration was enhanced by photoperiod length and depressed by total darkness; the early phase of regeneration (mitosis) being influenced by light (Maier and Singer, 1977).

Considerable amount of attention has been paid in recent years to the neurotrophic influence on the process of regeneration. Maden (1978) has reported that so called neurotrophic factor regulates the cell cycle in a regenerating system. Denervation remarkably affected the mesenchymal DNA synthesis than that in the epidermis (Geraudie and Singer, 1978). A depressive effect of denervation was observed on RNA synthesis (Morzlock and Stocum, 1972; Singer and Caston, 1972; Dearlove and Stocum, 1974; Bantle and Tassava, 1974); on glycosaminoglycan synthesis (Smith et al., 1975) and on enzyme activity involved with nucleotide synthesis (Manson *et al*., 1976). Other important contributions to this field are the "protein" isolated from the brain of newt, which has the power to restore the DNA and protein synthesis in denervated blastema (Singer, *et al*., 1976; Jabaily and Singer, 1977). Kamrin and Singer (1955) in the lizard, *A. carolinensis*, and Simpson (1964) in *Lygosoma laterale* have suggested the influence of neurotrophic factor/s in lacertilian tail regeneration.

The morphologic changes occurring during various phases of regeneration are undoubtedly reflected in the metabolic manifestations of the tissues involved. All the information available in this respect is gathered mainly by Geczik and Wolsky (1959); Wolfe and Cohen (1963); Schmidt and Weidman (1964) in amphibian limb regeneration and Shah and Chakko (1967b; 1969); Magon (1970); Shah and Ramachandran (1970; 1972; 1973); Radhakrishnan and Shah (1973); Shah and Hiradhar (1974; 1977) in reptilian tail regeneration. These studies are associated with repair and regeneration at the site of the damage and local metabolic adaptations in the regenerate. But there are fewer studies suggestive of systemic metabolic responses during amphibian and reptilian regeneration (Procaccini *et al.*, 1971; 1973; Connelly *et al.*, 1974; Shah *et al.*, 1977a,b; 1979a).

Importance of hormones in the regeneration has also received considerable attention, especially in amphibians and fishes (Liversage, 1963; 1967; 1973; Schmidt, 1968; Liversage and Scadding, 1969; Liversage and Liivamagi, 1971; Liversage *et al.*, 1971). However, studies regarding hormonal influence on reptilian regeneration are sporadic (Licht and Jones, 1967; Shah and Chakko, 1968a; Licht and Howe, 1969; Turner and Tipton, 1971; Turner, 1972; Shah *et al.*, 1979b; Kothari *et al.*, 1979).

Schotte and Hall (1952) have reported that hypophysectomy inhibits urodelan forelimb regeneration, by severely affecting wound healing. They proposed that, the role of pituitary in urodelan regeneration is possibly in conjunction with adrenal. Pituitary adrenal synergism in amphibian regeneration has been investigated by number of workers (Schotte and Lindberg, 1954; Schotte and Chamberlain, 1955; Schotte and Bierman, 1956; Liversage and Price, 1973; Bromley, 1977).

Richardson (1945) has studied the effect of interrelationship of thyroid and hypophysis upon limb regeneration in amphibians. The hypophysectomy in a thyroidectomized animal has a greater inhibitory effect on regeneration as compared to those with intact thyroid and operated only for hypophysectomy. Administration of thyroxine in hypophysectomized amphibians did not prove effective in stimulating regeneration of their limb (Thornton, 1968; Tassava, 1969). Influence of thyroid gland during reptilian tail regeneration has been investigated in *Anolis* (Turner and Tipton, 1971; Turner, 1972) and in *Hemidactylus flaviviridis* (Shah and Chakko, 1968a; Shah *et al*., 1979b; Kothari *et al*., 1979).

The promotive influence of androgens on gonopodium regeneration in certain teleostian fishes (Hopper,1949a,b) and ear hole regeneration in rabbits (Joseph and Dyson, 1966; Dyson and Joseph, 1968) has been reported. Androgens which are of considerable physiological importance in reptiles through their effects on oxidative metabolism (Chandola *et al*., 1974a,b) are considered to influence tail regeneration in lizards (Shah *et al*., 1979b; Kothari *et al*., 1979).

In addition, Vethamany-Globus and Liversage (1973a,b,c) have proposed a multihormonal (insulin, growth hormone, hydrocortisone and thyroxine) control of limb regeneration in amphibians. Similarly, the studies of Licht and Howe (1969) have shown that in hypophysectomized *Anolis*, administration of growth hormone, prolactin, gonadotropin and thyrotropin can restore the tail regeneration to near normal levels.

Tail regeneration in lizards is an unique example of regeneration, where the regenerated tail though functionally restored, is considered far from perfect structurally (Woodland, 1921; Barber, 1944; Kamrin and Singer, 1955; Simpson, 1965; Cox, 1969). A vertebrate body is known to respond to injuries at the site as well as systemically wherein circulating metabolites and the distant organs get involved. Considerable amount of work on morphological, histological, histochemical, biochemical and certain experimental aspects of lacertilian tail regeneration has been done (Shah and Hiradhar, 1978). A regenerating system, such as lizard's tail provides a unique opportunity to evaluate hormonal influences in an adult organism in which metabolic shifts and extensive developmental events can be studied simultaneously. Data on circulating and stored metabolites in some tissues in lizards during tail regeneration are available from our laboratory. The current study has been designed to examine these in the light of endocrine involvement and to comprehend the extent of influence of hormone/s on the lizard tail regeneration. Changes occurring in the levels of free aminoacids in the lacertilian tail regenerate which has not been done in the past have been also presented herein. A gekkonid lizard, *H. flaviviridis* is utilized for the purpose.

In the recent years a number of developmental biologists have become aware of the fact that hormones play important role in the process of regeneration. Hormonal dependence of tail regeneration has been suggested in the lizard, *Anolis* and *H. flaviviridis* (Shah and Chakko, 1968a; Licht and Howe, 1969; Turner, 1972; Shah *et al*., 1979b; Kothari *et al*., 1979). To evaluate the involvement of hypophysial hormones in regeneration, hypophysectomy was performed in the lizard. Effect of hypophysis ablation on the rate of growth, time taken to attain various stages of regeneration, relative weight of the regenerate to that of the body and the histomorphology of the tail regenerate have been examined (Chapter 1).

Importance of glycogen as a major energy yielder in animal tissue has been well recognized. In the tissues of the normal and regenerating tail of the house lizard *H. flaviviridis*, utilization of glycogen either through

Embden Meyerhof pathway (EMP) or Hexose monophosphate Shunt (HMP) has been implicated (Shah and Hiradhar,1978). Bearing the importance of glycogen as the principal energy yielder of animal tissues, in mind, the relative levels of glycogen in the regenerate, liver and thigh muscles and the corresponding levels of glucose in blood have been determined during different phases of tail regeneration in the hypophysectomized and sham operated house lizards (Chapter 2).

Dehydrogenases are significantly involved in metabolic activities concerned with energetics. Lactate dehydrogenase (LDH) features prominently in anaerobic metabolism. Hence a study of LDH activity could give us an insight in the metabolic mechanics of the regenerate. With this view, quantitative levels of lactate dehydrogenase (LDH) were estimated in tail regenerate and liver during different phases of tail regeneration following hypophysectomy (Chapter 3).

Involvement of non-specific phosphatases such as acid and alkaline phosphatases has been widely recognized in metabolic interactions and structural lay out of vertebrate tissues. Histochemical distribution of these enzymes during tail regeneration in H. flaviviridis (Shah and Chakko, 1966;

1967a) has indicated significant correlation of these hydrolases with normal progress of regeneration. Quantitative estimations of these hydrolases were carried out to obtain strengthening evidences during normal course of tail regeneration and also the influence of hypophysial hormones, in general, on these enzymes during tail regeneration (Chapter 4).

Role of ascorbic acid (AA) in repair, connective tissue formation, lipid and carbohydrate metabolism has been well accounted. The importance of this vitamin in physiological processes underlaying regenerative phenomenon has not been ignored. Taking into account the high content of AA at wound site (Shah _et al_., 1971) and mobilization of this vitamin from the distant organs (Shah _et al_., 1976) during tail regeneration; it was thought desirable to study the effect of hypophysectomy on the levels of this vitamin with regards to tail regeneration in the lizard. Keeping this fact in mind, quantitative estimations of AA have been undertaken in liver (storage organ), kidney (organ of synthesis) and regenerating tail of hypophysectomized and sham operated house lizards (Chapter 5).

Development of an organ demands greater synthesis of macromolecules such as proteins, carbohydratesand lipids.

Synthesis of protein is particularly important to a regenerating system during histodifferentiation of tissues forming the new regenerate. Since amino acids are the building blocks of proteins, involvement of amino acids could be anticipated in any regenerating organ or system. To evaluate utilization of free amino acids in a normal tail and in regenerates during different phases of regeneration, quantitative estimations were carried out (Chapter 6).

CHAPTER 1

EFFECT OF HYPOPHYSECTOMY ON THE RATE OF GROWTH OF THE REGENERATING TAIL OF THE HOUSE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Tail regeneration in lizards entails long and complex series of morphogenetic events. Factors controlling initiation and continuance of regeneration have invariably sparked off imagination of many developmental biologists who have visualized and executed extensive experimental protocols in order to comprehend these elusive avenues of morphogenesis. Involvement of hormones has received considerable attention in this respect. Rose (1964) has pointed out significant hormonal dependence of early stages in amphibian regeneration.

Involvement of endocrine glands, such as thyroid, pancreas, adrenal, in amphibian regeneration, has been investigated by a number of workers (Schmidt, 1968; Vethamany-Globus and Liversage, 1973a,b,c; Liversage and Brandes, 1977; Liversage and Korneluk, 1978). Very little attention has been paid to this aspect in reptilian regeneration. Hormonal dependence of tail regeneration has been suggested in the lizard Anolis (Licht and Jones, 1967; Licht and Howe, 1969; Turner and Tipton, 1971; Turner, 1972). A histological evaluation of thyroid gland during various phases of tail regeneration in the house lizard, Hemidactylus flaviviridis has been reported by Shah and Chakko (1968a), Magon (1970). A thyroid-gonad axis has been proposed by Kothari et al. (1979) and Shah et al. (1979b) during lizard tail regeneration.

Pituitary hormones are known to be required for normal regeneration in adult vertebrates (amphibian and reptiles) but their exact mode of operation is unknown (Licht and Howe, 1969; Tassava, 1969). Pituitary is not a prerequisite for larval forelimb regeneration in an amphibian nor for its survival (Liversage, 1967) but it plays a major role in regeneration in the adults (Richardson, 1945; Hall and Schotte, 1951; Schotte and Hall, 1952; Niwelinski, 1958; Wilkerson, 1963; Waterman, 1965; Tassava, 1969; Vethamany-Globus and Liversage, 1973b; Liversage and Fisher, 1974). Although there are many discrete endocrine tissues scattered throughout a vertebrate body, a large part of the system is controlled by the pituitary.

Studies encompassing hypophysial involvement in lacertilian tail regeneration have been lacking. Current study is an attempt in this direction by determining rate of growth and histological profile of the tail regenerates through entire period of regeneration in the lizard, H.flaviviridis, following hypophysectomy.

MATERIALS AND METHODS

The lizards, H. flaviviridis, were collected from the University campus and were maintained in the laboratory on a diet of insects. The animals were kept in the laboratory for a fortnight, so that they could get acclimatized to the laboratory conditions. Hypophysectomy was performed through the pharyngeal approach. A small transverse incision was made at the level of the junction of sphenoid and parasphenoid bones. Pituitary gland was exposed after gently lifting the bones and picked out with fine forceps. The operated area was cleaned with 0.9N saline and the fluid was sucked up with the help of a syringe. Bone was let back in its position and covered with a small amount of plaster of Paris. For Sham operation entire procedure as stated above was followed except for the removal of the gland. Completion of hypophysectomy was confirmed by examining the brain under binocular microscope and in the histological preparations at the end of the experiments. In all hypophysectomized animals natural pigmentation gets dull and the animals become pale and remain so throughout the experimental period. Such a change in colouration has been considered as an indicator of successful removal of the hypophysis. Tail autotomy was induced in the lizards, 10 days after hypophysectomy, (by this time operation wound is healed and the animal has resumed feeding) by pinching off the tail leaving two to three segments intact after the vent.

Twenty-five hypophysectomized and same number of sham operated adult lizards (irrespective of sex), weighing approximately 10 to 12 gms and measuring from snout to vent 8 to 10 cm, were used for the present experiments. The growth of the regenerates was measured at 10 day intervals starting from the 10th day after autotomy till the 50th day. The time taken (expressed in days) for attainment of each stage viz., wound healing, blastema, differentiation, and growth (Shah and Chakko, 1968b) was recorded.

For histological studies the regenerates at different phases from hypophysectomized as well as sham operated lizards were removed and placed on a chuck of a cryostat microtome maintained at -20°C. Longitudinal sections of 9 μ thickness were cut and spread on previously chilled albuminized slides. The sections were fixed in Bouin's fluid at room temperature for 24 hrs. These were thoroughly washed in distilled water and were stained using Hematoxylin-eosin and Mallory's triple stains.

RESULTS

Mortality in the groups of hypophysectomized lizards was about 15-20%, however, all the sham operated lizards survived the entire experimental period. By and large, the post-operative condition of the surviving hypophysectomized lizards was good. Data regarding the rate of growth and the time taken (in days) for attainment of each of the stages of tail regeneration are presented in Tables 1 & 2 and Figs. 1 & 2.

Hypophysectomy showed very obvious effect on the time required for wound healing and subsequent achievement of various stages of the tail regeneration. The hypophysectomized animals took about 7 days to heal their autotomy wound as compared to sham operated ones where the event took only 3 days. Similarly there was a considerable delay in the blastema formation in hypophysectomized animals, where it

Table 1

Number of days taken by regenerate to reach different stages of tail regeneration after hypophysectomy and sham operation in the lizard, H.*flaviviridis*.

Stages of regeneration	Hypophysectomized	Sham operated
Wound healing	7	3
Blastema formation	14	7
Commencement of differentiation	18	14
Commencement of growth	22	18

Time gap

Trophic regulation ?

FIG.1

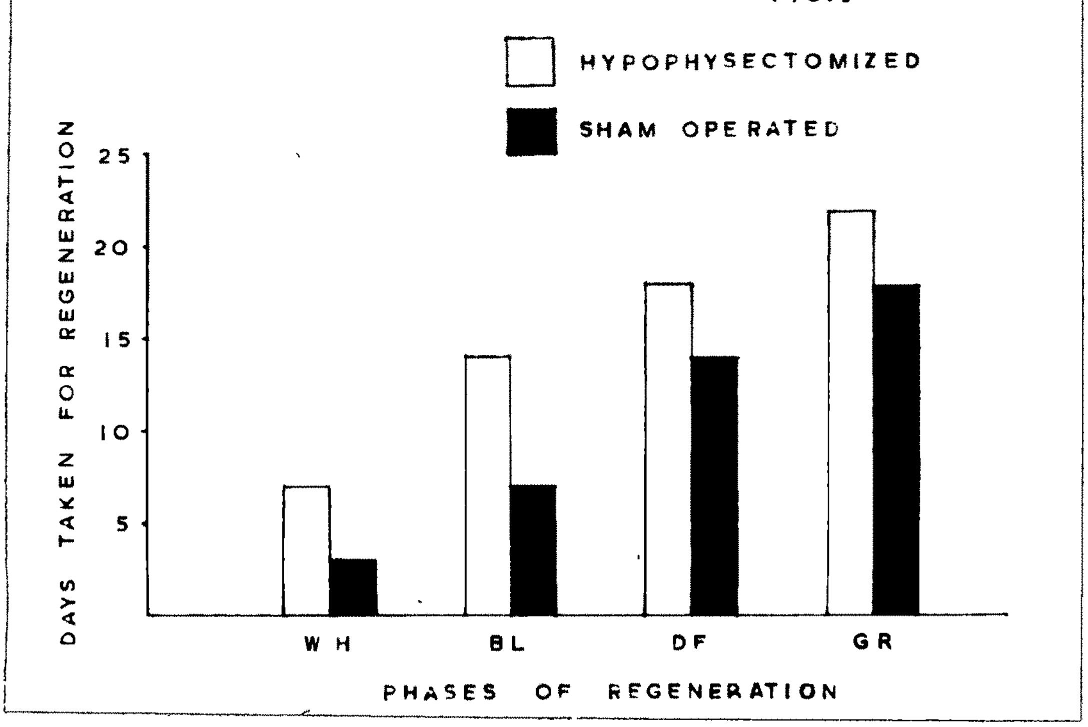

Fig. 1 : Graphic representation of time taken (in days) for attainment of various stages of tail regeneration after autotomy in hypophysectomized and sham operated lizards, *H. flaviviridis*.

WH - Wound healing phase

BL - Blastemaphase

DF - Commencement of differentiation

GR - Commencement of growth phase

Table 2

Rate of growth in mm of the regenerating tail of hypophysectomized and sham operated lizards, <u>H.flaviviridis</u>.

Days after autotomy	Hypophysectomized (a)	Sham operated (b)	$\frac{a}{b}$x100
10 days	2.9 ± 0.76 (P<.0005)	10.2 ± 0.41	28.43
20 days	9.0 ± 0.73 (P<.0005)	25.6 ± 0.46	35.15
30 days	13.5 ± 0.44 (P<.0005)	33.6 ± 0.40	40.17
40 days	26.0 ± 0.86 (P<.0005)	39.4 ± 0.82	60.98
50 days	32.6 ± 0.50 (P<.0005)	41.4 ± 0.47	78.74

rapid in [illegible]

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

Values are based on 25 animals and expressed in mm as mean ± S.D.

↑ 10-15 % mortality

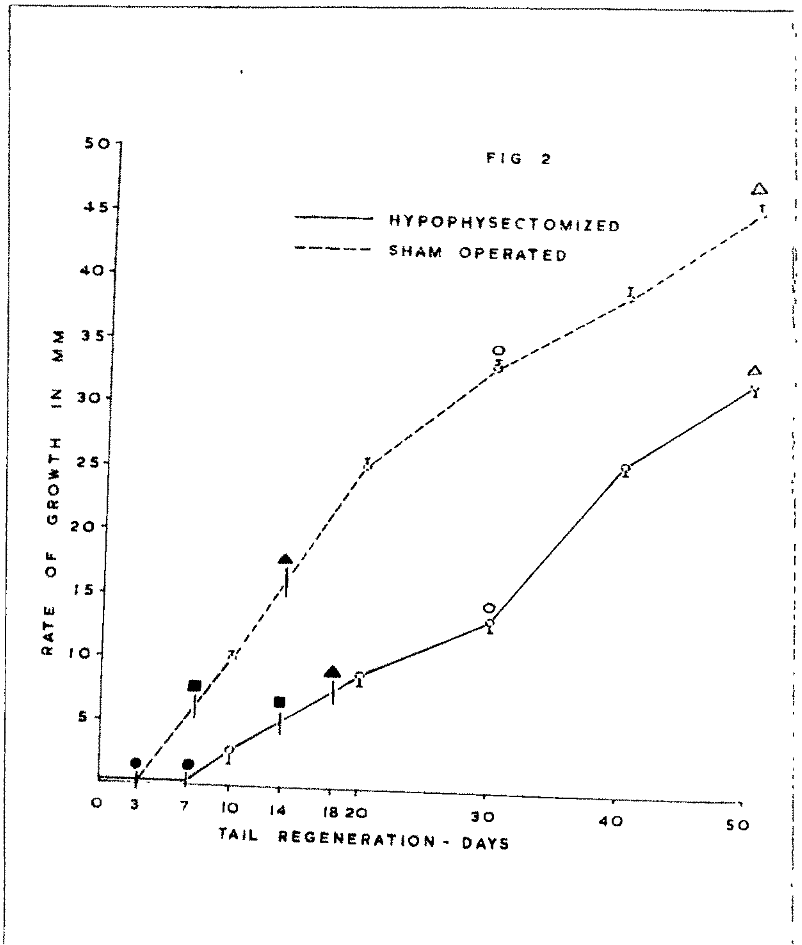

Fig. 2 : Graphic representation of the rate of growth of the regenerating tail of hypophysectomized and sham operated lizards, *H. flaviviridis*.

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

Table 3

Relative weight of regenerate per 100 gm body weight during different phases of tail regeneration in the lizard, H.*flaviviridis*

Stages of regeneration	Weight of regenerate x 100 / Body weight Hypophy-sectomized	 Sham operated
Blastema	0.1359	0.1468
Differentiation	0.3388	0.4451
Growth (30 days)	0.5285	1.4580
Growth (50 days)	0.8405	5.5075

original body wt. normal

was formed 14 days after autotomy as against 7 days in the sham operated lizards. As the regeneration progressed, differentiation phase of the regenerate in the case of hypophysectomized animals was noticed by 18th day while in the sham operated ones it was seen by about 14th day.

On the 10th day the extent of regenerate growth in the hypophysectomized lizards was one-third (2.90 ± 0.76 mm) of that noticed in the sham operated ones (10.2 ± 0.41 mm) at that specific time. As the tail regeneration progressed further, the extent of difference in the growth rate between the hypophysectomized and sham operated lizards was not as great as it was observed in the earlier phases (Table 2 and Fig. 2).

Data regarding relative body weight to tail regenerate weight for the hypophysectomized and sham operated lizards are presented in Table 3. This relative weight in the hypophysectomized lizards showed considerable reduction as compared to that observed in the sham operated ones.

In the hypophysectomized lizards no noticeable changes in the gross microscopic structure of the regenerate, during wound healing, blastema, and differentiation were describable as compared to that in either the sham operated or unoperated

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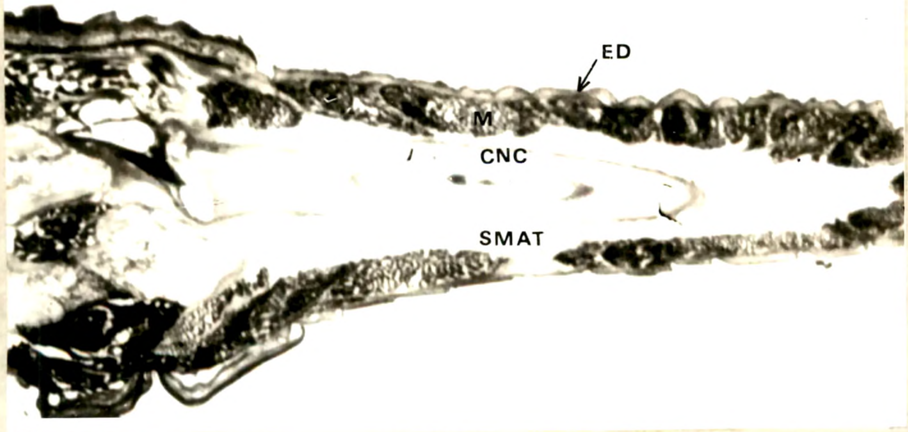

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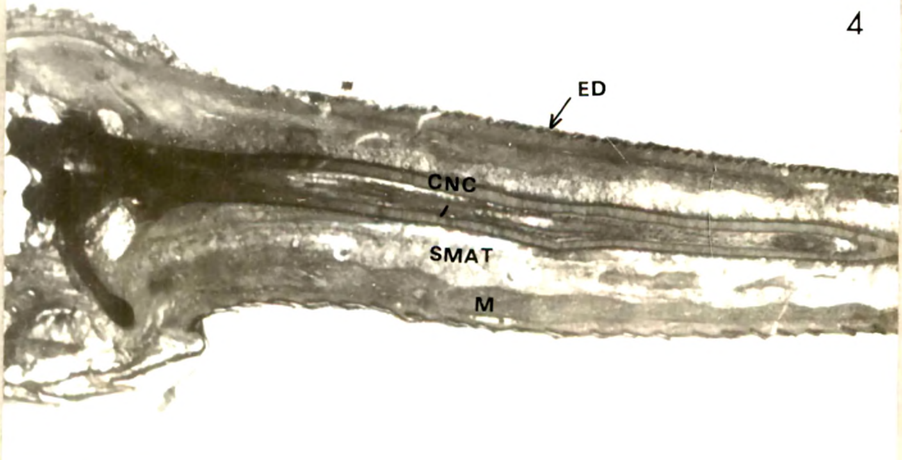

EXPLANATIONS FOR FIGURES

Fig. 3. Photomicrograph of tail regenerate from hypophysectomized lizard. X 16.

Fig. 4. Photomicrograph of tail regenerate from sham operated lizard. X 9.2.

ABBREVIATIONS

CNC	Cartilagenous neural canal
ED	Epidermis
M	Muscles
SMAT	Submuscular adipose tissue

normal lizards. However, the 50 day old regenerate, in the hypophysectomized animals as compared to that in the sham operated and unoperated normal animals showed poorly grown constituent parts *viz.*, muscles, connective tissue, adipose tissue and cartilagenous neural canal (Figs. 3 & 4).

DISCUSSION

Removal of hypophysis prior to autotomy has caused pronounced effect on the development of tail regenerate in *H. flaviviridis* during initial phases of regeneration. Wound healing as well as blastema formation took double the time in the hypophysectomized lizards as compared to the time taken by these events in the sham operated ones. Activity of a hydrolase, acid phosphatase, which is known to be associated with phagocytic function (Klockars and Wegelius, 1969) was remarkably reduced in the hypophysectomized lizards during wound healing phase (Chapter 4). The delay in wound healing could be attributed to a defect in the removal of cellular debris at the wound site. Thus the hypophysectomy in lizard appears to be the cause for reduced enzyme activity correlated with phagocytosis.

The blastemal phase is characterized by active cell division and proliferation. De Coninck _et al_. (1956) have shown a reduction of RNA in the blastema of hypophysectomized newt, and which can be raised by administration of growth hormone. Similar delay was also observed in the formation of blastema in _Anolis_ after hypophysectomy (Turner and Tipton, 1972). A multihormonal mechanism (growth hormone, thyroxine, insulin, corticosteroid) has also been suggested for an effective growth of the blastemal cells in culture (Vethamany-Globus and Liversage, 1973a).

According to Lebowitz and Singer (1970) only 40% of the protein synthesis in the regenerating amphibian limb is nerve dependent; however, denervation did not alter the rate of transport of amino acids for protein synthesis (Singer and Ilan, 1977). In hypophysectomized lizards enzyme activities in the blastemal cells do get affected (Chapters 3,4). Besides, the hormone dependant transport of amino acids across blastemal cells is assumed to be affected (Tepperman, 1965; Martin, 1976). Such defective metabolic conditions are assumed to be factors in hypophysectomized animals that have caused delay in blastema formation in _H_. _flaviviridis_.

In the hypophysectomized lizards, tissue differentiation in the tail regenerate and its growth were, though, not completely stopped but were qualitatively affected. Ablation of hypophysis did cause significant reduction in the growth of the regenerate. As compared to control animals, in the hypophysectomized lizards the rate of growth of the regenerate during the first 10 days of regeneration was very slow (28.43%) but it gradually increased by the 50th day (78.74%). Such an apparent increase in the rate of growth of the regenerate is taken into account by considering the linear growth pattern only, but when the relative weight of body weight to regenerate weight is taken into account, it showed a significant decrease (Table 3.).

Histological studies confirm the reduction in the weight of tail regenerate which is due to its poorly developed structural elements, such as muscles, connective and adipose tissues, and cartilagenous neural canal. Shah and Hiradhar (1975) have reported synthesis of structural macromolecules like mucopolysaccharides (MPS) during the different phases of tail regeneration in *H. flaviviridis*. Growth hormones are implicated in stimulation of MPS synthesis (Aer *et al*., 1968; Hernneman, 1971). Dorfman and Schiller (1958) have demonstrated decline in turnover and uptake of chondroitin sulphate and hyaluronate in hypophysectomized rats. Presently observed poor development of structural elements of the regenerate in the hypophysectomized lizard *could be* due to derangement in the turnover of MPS, and amino acids in the regenerate tissues. Turner and Tipton (1972) have also reported poorly defined muscle and cartilage tube in the hypophysectomized lizard~~s~~, *Anolis*.

From the present study it becomes evident that early phases of lizard tail regeneration *viz.*, wound healing and blastema formation are very much dependent on hypophysial hormone(s) directly and/or indirectly. However, the later phases *viz.*, differentiation and growth do get gradually emancipated from the dependence on the hormone(s) but achieve only poor growth of the structural elements of the regenerate. This has been believed to be, due to derangement in metabolic machinery in absence of hypophysial hormone(s).