INTRODUCTION

The existence of regenerative capacity in animals was curiously noted by Aristotle in fourth century B.C. Since then, the interest and fascination in regenerative phenomena increased overwhelmingly. Today, the approach to the regeneration studies varies from invertebrates to vertebrates at the molecular level, raising new questions and opening up new avenues of research. The ability to regenerate the lost structures is present in all animals to some extent. The invertebrates have amazing power of regeneration. In vertebrates, the regenerative power exists in all classes. The fins and gill filaments of teleost fishes readily regenerate after amputation. The most extensive power of regeneration is vested with the amphibians. Urodele amphibians are capable of regenerating their limbs, tail, jaws and snout. In anurans the regenerative ability is restricted to hind limbs and tail in larval life. Among the reptiles, only the tail of lizards and probably the shell of testudians have extensive regenerative capabilities. Higher vertebrates have very limited power to regenerate lost appendages. However, certain epidermal structures such as feathers, hairs and claws can be regenerated. The only mammalian appendage possessing the faculty to regenerate are the antlers of deer.

The process of caudal autonomy in saurians and the subsequent regeneration of the lost tail has been noted as early in the seventeenth century. Cuvier(1836) gave a detailed account of the process of tail regeneration in lizards. The histology of the tail during the process of regeneration in lizards have been studied in 1960's and 1970's by various investigators in different species of lizards (Ali, 1941; Huges and New, 1959; Werner, 1967; Shah and Chacko, 1968; Simpson 1965,1970; review, Bellairs and Bryant, 1985). Though many outstanding works have been carried out in animal regeneration, the investigations done on regenerative capabilities of reptiles are scanty (Bellairs and Bryant, 1985).

The process of tail regeneration in lizards is comparable to that seen in urodele amphibians(Iten and Bryant, 1976). The structural changes that occur in lizards during tail regeneration is similar in almost every species studied. An extensive critical review of these works has recently been described by Bellairs and Bryant(1985). Shah and Chacko(1968) described the histology of the process of tail regeneration in the lizard, *Hemidactylus flaviviridis*. The principal phases of tail regeneration in lizard are wound healing, blastema formation, differentiation and growth phase. The differentiating stages can be further arbitrarily classified as early, mid- and late-differentiating stages. As in the case of urodele limb regeneration, the 'critical stages' of tail regeneration in lizards are the formation of functional wound epithelium and the appearance of blastema. The functional wound epithelium appears after the vertebral ablation and shedding of the

scab. The process of dedifferentiation takes place beneath the wound epithelium and eventually a conical elevation appears from the stump comprising dedifferentiated cells- embryonic and fibroblast like cells - that accumulate beneath epithelium forms the blastema. Subsequently, a rapid elongation in the tail occurs, during which the blastema cells differentiate to express the phenotypes and form the new tissues. This process lasts for few weeks and the late stages are marked by the growth phase in which only the growth of tail takes place.

Histological changes after the blastema formation include the formation of myoblasts and their fusion to form multinucleate myotubes that get finally arranged segmentally. The procartilage cells around the ependymal tube also differentiate to form the cartilage tube which constitutes the skeleton of the regenerate. New nerve fibers originate from the severed end of the spinal cord and grow backwards into the new regenerate. The epidermal invagination and formation of scale ridges begin after two weeks of autotomy. The contribution of stump tissues to the new tail regenerate has been studied in several species of lizards. The epidermis of the regenerate, ependymal tube and nerve fibers grow out directly from the stump tissues (Cox, 1969). These cells do not dedifferentiate or contribute to the blastema. The source of blastemal cells is believed to be derived mainly from original dermis, the connective tissue autotomy septum and fat layers which all incorporate tritiated thymydine (Simpson, 1965). The cell contribution from original stump muscle cells is recorded in *Hemidactylus flaviviridis* (Shah and Chacko, 1968). Satellite cells from the severed muscle fibers also found to be released and contribute to myoblast proliferation in *H. flaviviridis* (personal observation). These observations are in accordance with the reports from other species of lizards studied (Simpson, 1970; Mufti and Iqbal, 1975; Goss, 1978).

Numerous *in vitro* and *in vivo* studies have demonstrated the requisite of neural and hormonal factors in the process of limb regeneration in the newt, *Notophthalmus viridescens*(reviewed by Wallace, 1981; Brockes, 1984; Tassava and Olsen, 1985; Carlone and Mescher, 1985; Globus and Vethamany-Globus, 1985; Liversage *et al.*, 1985; Boilly and Albert, 1988; Taban and Cathieni, 1988; Cathieni and Taban, 1992). The neural and hormonal dependence of tail regeneration in lizards were studied by a few investigators (Kamrin and Singer, 1955; Cox, 1969; Simpson 1965,1970; Lichet and Howe, 1969; Turner and Tipton, 1971; Kothari *et al.*,1979; Shah *et al.*, 1981b). Hypophysectomy retards the process of differentiation in *Anolis carolinensis* and *Hemidactylus flaviviridis* (Licht and Howe, 1969; Shah *et al.*, 1981b). Thyroid hor-

mones, gonadal steroids (Kothari *et al.*, 1979; Shah *et al.*, 1979) and prolactin (Ndukuba and Ramachandaran, 1988) are found to influence the process of tail regeneration in the lizard, *Hemidactylus flaviviridis*. However, the literature available on the neural influence of tail regeneration in lizards is scanty. In particular, the importance of neural and hormonal factors in crucial events (preblastemic and blastemic satges) are not well attempted. Hence, in the present study an approach has been made to investigate certain neural, hormonal and ionic intracellular regulation of calcium in the crucial stages of tail regeneration in lizards. The experimental protocol has been designed to investigate the neuroendocrine dependence at preblastemic and blastemic phases of tail regeneration. This approach enables to study two processes, (i) hormonal and/or neural dependence on dedifferentiation and (ii) the neural and hormonal influence in promoting the proliferation-differentiation phases. Besides, attempt has also been made to study certain neuroendocrine aspects in the overall process of tail regeneration in lizards.

The neural contributions in tail regeneration of lizards have been studied with emphasis on spinal cord and ependyma (Kamrin and Singer, 1955; Cox, 1969; Simpson, 1965). On the basis of these experimental evidences it is hypothesised that the ependyma might be exerting profound trophic influence on tail regeneration in lizards (Simpson, 1970). However, the role of sympathetic nerves which also extend into the caudal region remains unexplored. In lizards, the sympathetic trunk also regenerates on either side of caudal artery along with the spinal cord (Terni, 1922). The adrenergic influence and catecholamines are well studied in urodele limb regeneration. Wallace(1981) opined that both adrenergic and cholinergic influences are negligible during limb regeneration in newts. However, Sicard (1983) hypothesised that catecholamines may play a pivotal role in proliferation-differentiation stages of amphibian limb regeneration. Recently, it has been shown that both chemical sympathectomy and catecholamine biosynthesis inhibition retards the process of limb regeneration in the newt, *Notophthalmus viridiscens* (Taban and Cathieni, 1988). On the other hand, the cholinergic influence on limb regeneration in urodele amphibians is found to be negligible (Singer, 1965; Drachman and Singer, 1971).

In the light of above findings, it has been decided to investigate the adrenergic and cholinergic influence on tail regeneration in lizards. The adrenergic and cholinergic regulation of tail regeneration in the lizard, *Hemidactylus flaviviridis* has been studied by exogenous supply of neurotransmitters (chapter-II) and preventing their action by specific blockers (chapter-III and IV). Adrenergic denervation is achieved through

chemical sympathectomy with 6-Hydroxydopamine and Guanethidine. Both these agents deplete the peripheral catecholamine levels. 6-Hydroxydopamine destroys the adrenergic nerve terminals leaving the cell bodies intact (Thoenen and Trazer, 1973) while guanethidine destroys the nerves and their cell bodies (Burnstock *et al.*, 1971). This approach also helps to evaluate the specificity and varying actions of different drugs.

The neurotransmitters are modulators of vast number of metabolic and even endocrine functions. Many of these changes induced by catecholamines are as a consequence of their binding to α- and ß-adrenoreceptors. Several of the developing and regenerating systems are known to largely depend upon both adrenergic and cholinergic neurotransmitters (Buznikov *et al.*, 1964, 1971; review MacMohan, 1974; Rathbone *et al.*, 1980; Taban and Cathieni, 1988). Catecholamines have been found in association with specific cells during embryogenesis and implicated in the control of morphogenesis. Buznikov and Co-workers (1968, 1970, 1972) extensively investigated the role of catecholamines during sea urchin embryogenesis and implicated them in the control of morphogenesis. Subsequently, presence of catecholamines has been detected in rat ova-zygotes and 2-4 cell embryos (Burden and Lawrence, 1972). In chick embryonic development the acetylcholine content has been detected in early stages and suggested in neural tube closure (Ignarro and Shide, 1968; Lawrence and Burden, 1973).

In urodele limb regeneration, the role of catecholamines and acetylcholine have been studied (Singer, 1960; Drachman and Singer, 1971; Taban *et al.*, 1977, 1978). Norepinephrine has been found to act through ß-adrenoreceptors and influence cyclic nucleotide metabolism in cultured newt blastemas (Taban *et al.*, 1977,1978; Rathbone *et al.*, 1980). However, the receptor-mediated functions of catecholamines and acetylcholine during tail regeneration in lizards remain largely unknown. Therefore, a series of experiments has been conducted in the crucial stages of tail regeneration in lizards, employing adrenoreceptor and cholinoreceptor agonists and antagonists(chapter-IV).

Langley and Ehrlich in 19th century proposed the hypothesis that drugs have their effects by interacting with specific 'receptive' substances. Years later, Ahliquist (1948) found that catecholamines act through specific receptors. Later on his concepts were confirmed by the development of specific receptor antagonists. Similarly, the parasympathetic nerves act through the release of neurotransmitter acetylcholine at their nerve endings. Several adrenoreceptor and cholinoreceptor agonists and antagonists are available today for studying the receptor-mediated functions of neurotransmitters.

Among the ß-receptor antagonists, propranolol non-specifically blocks both $ß_1$- and $ß_2$-adrenoreceptors. Benextramine, a recent introduction into the family of α-receptor antagonists, irreversibly blocks the α-adrenoreceptors. Among the cholinomimetic agents carbachol is a potent stimulator of cholinorecptors, while the alkaloid atropine inhibits cholinergic muscarinic receptors.

The endocrine system appears to play a role in all facets of regeneration except pattern formation (Sicard, 1985). It is now suggestive that hormones may be exerting a permissive influence on regeneration, providing adequate metabolic background (Sicard, 1985; Liversage *et al.*, 1985). The current concepts suggest a 'hormonal milieu' in governing the process of limb regeneration in urodele amphibians (Liversage *et al.*, 1985; Liversage, 1987). Among the various hormones, the role of glucocorticoids in control of regeneration has been studied by several investigators (Schotte, 1953; Schotte and Christiansen, 1957; Tassava, 1969).

Several conflicting reports are available on the role of adrenal steroids in urodele limb regeneration. None of the several experiments conducted provide a convincible evidence for a positive role of adrenal steroids in regeneration. However, these hormones may have a temporary or trivial influence on regeneration (Wallace, 1981). In the lizard, *Hemidactylus flaviviridis*, adrenocortical influence on tail regeneration has been studied through hypophysectomy (Shah *et al.*, 1981b) and adrenocorticoid suppression with synthetic glucocorticoid, Dexamethasone (Ramachandran and Abraham, 1990). In both these experiments, the process of tail regeneration found to be retarded. The hypophysectomy-induced tail growth retardation may not be only due to the depletion of ACTH and corticosteroids, but can also be related to insufficiency of other pituitary hormones. Likewise, the supplementation of dexamethasone and thus indirectly suppressing ACTH level, may not demonstrate corticosteroid insufficiency. Dexamethasone itself is more potent than the corticosterone. Hence, chemical adrenalectomy was performed in lizards to evaluate the role of glucocorticoids in tail regeneration (chapter-VI). Metyrapone (metopirone), a drug which inhibits the 11-ß-hydroxylase enzyme of the steroid synthesis pathway, has been employed to achieve chemical adrenalectomy. Exogenous corticosterone was also administered to study the effect of hormone supplementation in tail regeneration of lizards(chapter-VI).

Several *in vivo* and *in vitro* investigations are reported on the involvement of various endocrine secretions in urodele limb regeneration (review, Liversage *et al.*,

1985). Based on these findings a concept evolved was that the hormonal systems interact interdependently in the promotion of growth and differentiation of the blastema (Liversage, 1987). Data obtained in the present experiments (chapters-II,III and IV) helped to draw certain assumptions on the influence of catecholamines and glucocorticoids, that might be exerting regulatory/inhibitory effects on the process of tail regeneration in lizards. It is speculative that a change in sensitivity to neurotransmitters and/or hormones occur with specific stages of regeneration (Sicard, 1983). On the basis of these assumptions and to gain more insight into the regulatory mechanism involved in the process of tail regeneration in lizards, further experiment has been carried out in the crucial stages (preblastemic and blastemic) of tail regeneration (chapter-VII). In this experiment, chemical adrenalectomy and chemical sympathectomy were performed together to understand the catecholamine regulation of hormone secretion, if any, and its net effects on the process of tail regeneration. Adrenergic dysfunciton could lead to tremendous metabolic changes. In birds, chemical sympathectomy produced several metabolic and hormonal alterations including elevation of glycemia, increase in corticosterone and growth hormone levels (Harvey *et al.*, 1984; Rintamaki, 1986; Oommen, 1992).

The divalent calcium ion has been implicated in a multitude of regulatory functions. Calcium ions directly regulate nucleotide metabolism, biosynthesis of proteins, secretion of neurotransmitters and hormones. The most fascinating aspect of Ca ion is their involvement in cell proliferation and differentiation. Several *in vivo* and *in vitro* studies provided indirect evidences for the Ca^{2+} participation in cell proliferation (Whitefield *et al.*, 1976; Hazelton *et al.*, 1979; Fairly *et al.*, 1986; Al-Ani *et al.*, 1988; Means and Rasmussen, 1988; Kunert-Radek *et al.*, 1990). Lymphocyte proliferation was stimulated during hypercalcaemia and depressed when serum calcium level was below normal (Hanagan, 1982). An increase in the intracellular Ca^{2+} is proposed to be an intracellular signal for division in many cells(Berridge, 1975; Rasmussen, 1975; Whitefield *et al.*, 1979; Nagle and Egrie, 1981). In sea urchin embryos artificial increase in intracellular Ca^{2+} by Ionophore A23187 activated the unfertilized eggs to commence cell division parthenogenetically (Epel, 1980; Uehera and Yanagimachi, 1977). Activation of medaka eggs at fertilization results in a large increase in free Ca^{2+} in the cytoplasm (Ridgway *et al.*, 1982).

'Calmodulin' the ubiquitous high affinity calcium binding protien acts as an intracellular intermediary in calcium regulated functions. This unique protein is structur-

ally conserved and functionally preserved throughout the animal and plant kingdom (Means and Dedman, 1980). Calmodulin is also a dynamic component of the mitotic apparatus which regulates cell motility, and microtube and microfilament assembly (Marcum *et al.*, 1978). Calmodulin levels are elevated two fold at late G1 and/or early S phase and are maintained throughout the cell cycle until cytokinesis (Chafouleus *et al.*, 1982).

The Ca^{2+} ion requirement in repair and regeneration is well recognised. In regenerating rat liver, hepatocyte proliferation is preceded by an obligatory transient requirement of Ca^{2+} in the early stages of DNA synthesis (Whitefield *et al.*, 1976). In the blastema of regenerating newt limbs, the requirement of Ca^{2+} for cell proliferation has recently been reported (Globus *et al.*, 1983). A very low Ca^{2+} has been found in regenerating feather follicles of hypothyroidic pigeons, while euthyroidic animals maintain a higher Ca^{2+} levels which might be associated with the active cell proliferation (Paul, 1991).

It is now accepted that Ca^{2+} flow occurs through specific 'calcium channels', which are transmembrane proteins that in the open confirmation allow the passive flux of Ca ions across the plasma membrane down the electrochemical gradient (Carafoli, 1987; Hess, 1990). These voltage sensitive Ca^{2+} channels are found in most cell types studied (Bean, 1989). The calcium channels can be blocked by several agents. The organic calcium channel blockers, diphenylalkylamine-verapamil and benzothiazipine-diltiazem block the calcium channel from inside after having entered in the open state, thus their effects are voltage and usage dependent (Heschel *et al.*, 1982; Lee and Tsien, 1983; review, Godfraind *et al.*, 1986). The calcium channel antagonists have been shown to inhibit the cell proliferation in a variety of systems both *in vivo* and *in vitro*. Verapamil inhibited the mitogenic response induced by Con A and phytohaemmagglutinin(PHA) in mouse spleen cells (Blitstein-Willinger and Diamantstein, 1978) and human blood lymphocytes (Kennes *et al.*, 1981). Mitogen and co-mitogen induced stimulation of lymphocytes has been inhibited by calcium channel antagonists, verapamil, nicardipine and TMB-8. (Grier and Mastro, 1985). Verapamil inhibited the oral regeneration in the ciliate, *Stentor coeruleus*(Maloney *et al.*, 1991). Many of the calcium channel antagonists vary in their mode of actions and exhibit other properties like interacting with Na^+ transport. Diltiazem, a recent introduction among the calcium channel blockers is known to have intracellular actions (Nayler and Dillion, 1986) which largely remains unknown. Diltiazem has also been shown to slow down Na^+:Ca^{2+} exchange across the mitochondrial membrane (Matlib *et al.*, 1983).

Phenothiazine antipsychotics (Chlorpromazine, Trifluoperazine) and aromatic compounds (W_7) are known to bind to calmodulin and thereby inhibit a wide range of calmodulin-dependent processes. The calmodulin antagonists W_7 suppresses the proliferation of CHO-K cells in culture, by specifically inhibiting the G_1-S transition (Hidaka and Sasaki, 1985). In star fish oocytes, trifluoperazine inhibited the oocyte maturation by inhibiting the formation of Ca^{2+}-calmodulin complex (Meijer and Guerrier, 1982, 1983). Trifluoperazine inhibited the oral disc regeneration in the ciliate, *Stentor coeruleus* (Maloney *et al.*, 1991). In chick embryonic myoblast cells trifluoperazine, chlorpromazine and W_7 inhibited the degree of myoblast fusion and phosphorylation of 100kDa protein (Sun *et al.*, 1992). In newt limb blastemata, chlorpromazine suppressed the mitosis (Globus *et al.*, 1987).

The effects of calcium influx and efflux have been investigated in several developing systems. Calcium influx in the cells by ionophore A23187 activated the unfertilized eggs in the bat star, *Patria miniata*, the toad, *Xenopus laevis* and the hamster *Mesocricetus auratus* (Steinhardt *et al.*, 1974). Also, during neurulation in *Ambystoma maculatum* embryos, ionophore A23187 induced Ca^{2+} influx and promoted neural tube closure while papaverine an agent which depletes the intracellular Ca^{2+}, inhibited these processes (Moran and Rice, 1976). In regenerating limbs of *Notophthalmus viridescens* calcium influx promoted mitosis while efflux suppressed it (Globus *et al.*, 1987).

Calcium-calmodulin regulated functions in epimorphic regeneration have recently been recognized (Vethamany-Globus *et al.*, 1978; Globus *et al.*, 1983, 1987). The calcium-calmodulin regulation of tail regeneration in lizards are so far not attempted. Preliminary observations from this laboratory (Sharma, 1989) reports a moderately high level of Ca^{2+} within the blastema of the lizard, *Hemidactylus flaviviridis*. A fairly high level of Ca^{2+} has been found throughout the regenerating stages of tail regeneration in lizards (personal observations). Thus it is imperative to know the influence of Ca^{2+} and calmodulin in regulation of tail regeneration in lizards. Therefore, an attempt has been made to ascertain the calcium-calmodulin involvement in tail regeneration in lizards through calcium entry blockade, calmodulin inhibition and modification of calcium flux (chapter-VIII).

The inferences of the preceding experiments are highlighted and discussed in 'general consideration' to create a perspective of the interrelationships of certain neuroendocrine factors in the process of tail regeneration in lizards.

CHAPTER - I

MATERIALS AND METHODS

SECTION -I

(i) Source of Animals and Maintenance

Adult wall lizards, *Hemidactylus flaviviridis* of both sexes with intact tail and weighing 10 ± 2 gms were procured from animal dealer and maintained in cages in the animal house facility with 12:12 light-dark cycles. Water was available to the animals all the time and cockroaches were fed 2-3 times a week. In all experiments the animals were acclimatized for a period of 7 days. The experiments were conducted during the months of March-July or September-November. Animals were maintained in a temperature controlled room at 30 ± 2°C as this temperature is necessary to produce optimum tail growth in lizards. The tail autotomy was performed in lizards by pinching off the tail by exerting mild thumb pressure leaving three segments intact from the vent.

(ii) Experimental Protocol

Throughout the study, two series of experiments were conducted. In first series, the animals were treated with the drug 3 to 5 days prior to autotomy and continued the treatment after autotomy till 30 days to study the process of regeneration. In second series of experiment, the treatments were given at two crucial events of tail regeneration; preblastemic (wound epithelium stage) and blastemic stages to study the stage-specific effects. The formation of wound epithelium occurs during the preblastemic phase, immediately after the process of wound healing and shedding of the scab. The wound epithelium appears as a smooth shining surface. The formation of wound epithelium initiates the process of dedifferentiation. Blastemic stage is characterized by a conical elevation (average growth from the stump is 2 mm) which consists of accumulated dedifferentiated cells from the stump. The balstema cells further differentiate to express the cell phenotypes.

SECTION - II

Drugs and Dosage

1. **Norepinephrine Bitartrate** . 500 µg/kg body wt prepared in 0.6 % saline immediately before use.

2.**Epinephrine Bitartrate** . 500 µg/kg body wt prepared in 0.6 % saline, fresh before use.

3.**Acetylcholine Chloride** . 5 mg/kg body wt dissolved in 0.6% saline, fresh before use.

4.**6-Hydroxydopamine Hydrobromide** (6-OHDA.Hbr., a selective catecholamine neurotoxin). A total of 700 mg/kg body wt at different intervals in experiment-1 and 300 mg/kg body wt in experiment-2 in a single dose.
The drug was prepared in 0.6% saline containing 1% ascorbic acid as stabilizer, immediately before use.

5.**Guanethidine sulphate** (an anti-adrenergic agent). 50 mg/kg body wt prepared in 0.6% saline. The drug was injected in two different pH, i.e., pH 10.0-10.2 (pH of guanethidine) and pH 7.4-7.6 (adjusted with 0.1 N HCl).

6.**DL-Propranolol** (ß-adrenoreceptor antagonist). 5 mg/kg body wt. The drug was prepared fresh by dissolving in one drop of ethanol and then diluting with 0.6% saline.

7.**Benextramine Tetrahydrochloride** (α-adrenoreceptor antagonist). 5mg/kg body wt prepared in 0.6% saline, fresh before use.

8.**Carbachol** (Carbamylcholine chloride, cholinomimetic agent). 500 µg/kg body wt prepared in 0.6% saline, fresh before use.

9.**Atropine sulphate** (Cholinergic muscarinic antagonist). 2 mg/kg body wt dissolved in 0.6% saline prepared immediately before use.

10.**Metyrapone** (metopirone, adrenal steroidogenesis inhibitor). 50 mg/kg body wt in experiment - 1 and 200 mg/ kg body wt in a single dose in experiment-2. The drug was prepared by dissolving in one drop of ethanol and diluting in distilled water.

11.**Corticosterone** . 5 mg/kg body wt prepared as slow release form in 0.3%

Carboxyl Methyl Cellulose (CMC) in distilled water, stirred thoroughly to make a homogeneous suspension.

12. **Reserpine** (catecholamine storage depletor). 5 mg/kg body wt prepared by dissolving in one drop of ethanol and diluting with 0.6% saline.

13. **Verapamil Hydrochloride** (calcium channel antagonist). 5 mg/kg body wt made by dissolving in one drop of ethanol and diluting with 0.6% saline.

14. **Diltiazem Hydrochloride** (calcium channel antagonist). 100 mg/kg body wt dissolved in 0.6% saline, fresh before use.

15. **Chlorpromazine Hydrochloride** (neural antagonist; calmodulin inhibitor). 100 mg/kg body wt prepared in 0.6% saline, fresh before use.

16. **Trifluoperazine Dihydrochloride** (specific calmodulin inhibitor) . 100 mg/kg body wt prepared in 0.6% saline, fresh before use.

17. **Papaverine Hydrochloride** (intracellular calcium depletor). 100 mg/kg body wt prepared fresh in 0.6% saline.

18. **Calcium Ionophore A23187** (a mitogen, elevates the intracellular calcium level). 5×10^{-7} M prepared in 0.5% dimethyl sulfoxide (DMSO). Ionophore A23187 was dissolved in full strength distilled DMSO and stored at -20 °C and dilutions were made from this stock solution.

Atropine and carbachol were obtained from National Chemicals, Baroda. Acetylcholine chloride was supplied by CSIR research chemicals, New Delhi. All other chemicals were purchased from Sigma Chemicals Co., St.Louis, MO, U.S.A.

SECTION-III

(i) Morphological measurements of tail growth

The growth in length of the tail regenerate was measured using millimeter scale at fixed intervals. The time taken to reach various arbitrary stages of tail regeneration, such as wound healing (WH), blastema (BL), early differentiation (ED), mid-differentiation (MD), late differentiation (LD) and growth phase (GR) was recorded.

(ii) Histological Studies

For histological studies, the regenerating tissue with one stump segment was cut off, blotted dry and immediately transferred into Bouin's fluid and fixed for 48 hrs. The fixed tissue was then washed thoroughly in running tap water and transferred to decalcification fluid (10% E.D.T.A. disodium salt, pH-6.0), and kept for 15-20 days with frequent changes. Finally the decalcified tissues were cleared in tertiary butanol, embedded in paraffin and serially sectioned at 10 μm. The sections were stained with Haematoxylin-Eosin or Masson's trichrome stain. The low power photographs were taken on a Zeiss Tessovar photographic zoom system and desired high magnifications on Carl-Zeiss or Zeiss photomicroscope.

(iii) Histofluorescence Studies

(a) Localization of catecholamines in the cornea of the lizards: The extent of sympathetic denervation in 6-OHDA and Guanethidine treated lizards was assessed by the fluorescence intensity of catecholamines in cornea of lizards. The cornea from lizards were dissected out immediately after decapitation and immersed in ice-cold 0.1 M phosphate buffer (pH-7.3) containing 2% glyoxalic acid. After 5 min of immersion, the corneas were taken, blotted dry, stretched on a microscopic slide and dried under warm air. The completely dried tissues were exposed to glyoxalic acid vapours for 5 min at 100°C and mounted in Entellen (Terro, 1977). The slides were observed under Carl-Zeiss Fluorescence microscope equipped with epiillumination and filter settings B 224 (440

nm) excitation filter and G 247 (510 nm) barrier filter. The desired areas were captured on 100 ASA/21 DIN colour negative film.

(b) *Fluorescence localization of nucleic acids*: For the nucleic acid (DNA and RNA) localization, the metachromatic fluorochrome Acridine Orange was used. The tail regenerate was removed immediately after decapitatin, blotted dry, and transferred to a cryostat microtome maintained at -20 ^{0} C. Tissues were embedded in OCT compound (Tissue Tek-II) and sectioned at 12 μm in longitudinal plane. The sections were exposed to 0.1% acridine orange in 0.1 M phosphate buffer (pH-6.0) and observed under fluorescence microscope with 410 excitation filter and 510 emission cut off filter. The acridine orange induces specific yellow emission for DNA and flame red emission for RNA at the ultra violet range (Culling, 1974).

(iv) Estimation of Blood Glucose:

Blood was obtained from different experimental and control group of lizards by cardiac puncture under hypothermic anesthesia. The deproteinised plasma was used to estimate the glucose by enzymatic method (glucose oxidase-peroxidase) using assay kit supplied by Stangen Immunodiagnostics, Hyderabad, India.

SECTION - IV

Statistical Analysis

The significance level of the experimental and control group was evaluated by Student's 't' test. For multiple group comparison and difference between the groups the data were subjected to One Way Analysis of Variance followed by Duncan's Multiple Range Test (Duncan, 1955) using SPSS-PC Statistical Analysis Package. A P value of 0.05 or less was accepted as being statistically significant.