GENERAL CONSIDERATIONS

The capacity to regenerate certain lost parts of the body in only some animals, allured every one. The study of vertebrate regeneration has evolved gradually from morphological and histological observations and with advent of modern techniques the regeneration research has become the key area in developmental biology. Understanding the process of regeneration is of practical value than mere academic interest. Limb of urodele amphibians has emerged as an excellent model to study the process of epimorphic regeneration, as evidenced by the available literature. Meanwhile, the regeneration of the lost tail in lizards an instance for epimorphic regeneration is relatively neglected and literature available is sparse. Though the regenerated tail is not an exact replica of the lost one, the regenerated spinal cord is a stunning model to study spinal cord injury and repair (Simpson, 1993). Extensive studies have carried out in our laboratory on energetics and certain hormonal aspects of tail regeneration in the gekkonid lizard, *Hemidactylus flaviviridis* (Shah and Hiradhar, 1978; Shah *et al.*, 1979a,b; Shah *et al.*, 1981 a,b; Ndukuba and Ramachandran, 1988; Ramachandran and Ndukuba, 1989). However, the neural contribution during tail regeneration of lizards, is unknown. Hence, the present study has been undertaken to unveil neural and associated hormonal mechanisms of tail regeneration in the lizard, *Hemidactylus flaviviridis* (chapters II - VIII).

In lizards, the crucial stages in regeneration - similar to that in urodele amphibians - are preblastemic(formation of functional wound epithelium) and blastemic stages. Immediately after the formation of wound epithelium dedifferentiation occurs in the stump segment and a blastema forms which consists of undifferentiated cells with high proliferative potential. These cells further redifferentiate to express the cell phenotypes and organise to regain the cytoarchitecture of the lost tail. These progressive events are mediated by both neuronal and hormonal factors, many of which are identified in urodele limb regeneration. However, only few experiments are attempted to ascertain neural and endocrine dependence of these crucial events in tail regeneration of lizards.

The roles of ependyma and spinal nerves have been studied in tail regeneration of lizards(Simpson, 1970). However, the role of sympathetic and parasympathetic nerves are mostly neglected. Recent experimental results on the influence of sympathetic nerves on limb regeneration of the newt, *Notophthalmus viridescens*, suggest a positive role(Taban and Cathieni, 1988). In this perspective, experiments were conducted in tail regeneration of lizards, to explore the influence of adrenergic and cholinergic nerve fibers. Exogenous supplementation of these neurotransmitters and thus augmentation of their level well above the physiological concentration did not evoke any positive

influence on tail regeneration. In contrast, epinephrine administration produced severe growth anomalies. To ascertain the influence of CA on tail regeneration chemical sympathectomy was performed in lizards, using 6-hydroxydopamine and guanethidine. Both of these agents are known to deplete the peripheral CA levels, however, a difference in their mode of action exists. 6-hydroxydopamine depletes the CA levels by destruction of adrenergic nerve terminals while guanethidine destroys both nerve terminals and their cell bodies in pH-dependent manner. Chemical sympathectomy with 6-OHDA surprisingly enhanced the regenerative performance in lizards. The histological features revealed an acceleration of differentiation with a boost in myogenesis. This finding is opposite to that reported in urodele limb regeneration. Though in the present experiments, the hormonal and cyclic nucleotide levels are not measured, based on the available evidences, it can be speculated that, the alteration in hormonal and/or cyclic nucleotide levels might have stimulated myogenesis and enhanced the tail growth.

Guanethidine-induced sympathectomy adversely affected the tail regeneration in pH-dependent manner. The divergence in the action of guanethidine and 6-OHDA can be explained on their mode of action. Guanethidine destroys the adrenergic neurons and their axons at increased pH levels. Several *in vitro* studies have proved this fact (Levi-Montalcini *et al.*, 1954; Johnson and Aloe, 1974). Besides this, guanethidine interferes with the Ca^{2+} flux in mitochondria and inhibits mitochondrial respiration(Malmiquist and Oates, 1968; Juul and Sand, 1973). Several sympathetic ganglia-derived neural peptides are recently identified (Hokfelt *et al.*, 1980) and many of these molecules are postulated as trophic agents in urodele limb regeneration (Globus and Vethamany-Globus, 1985). In newt limb regeneration, guanethidine-induced denervation retards morphogenesis(Sicard and DiNicola, 1974). Thus it is presumable that guanethidine-induced sympathectomy and its adverse effect on tail regeneration of lizards may be a result of deprivation of trophic molecules from sympathetic ganglia and/or metabolic effects due to interference on mitochondrial respiration and/or synthetic process in neurons. Augmentation or depressing the cholinergic actions does not influence any of the stages of tail regeneration. These observations are consistent with that in urodele limb regeneration.

As the adrenergic and cholinergic neurotransmitters act through their specific cellular receptors, inactivation of these receptors with definite blockers can delineate their possible functions. To elucidate the adrenergic and cholinergic functions during tail

regeneration, specific receptor blockers were used alone, together or in combination with agonists in preblastemic and blastemic stages. The findings identified the presence of both α- and ß-receptors within the tail regenerate. α- and ß-receptor blockage alone or together at preblastemic level did not have any influence on the process of dedifferentiation while addition of NE or E with α- or ß-receptors blockers enhanced (nonsignificant) the growth rate. It can be presumed that, in the dedifferentiating stages CA desensitization occurs and the dedifferentiating cells may not have acquired the receptors for catecholamines. The noted effect of addition of NE and E may be direct cellular effect. Further evidence to this notion is obtained from the results of adrenoreceptor blockade at BL stage where,α- and ß-receptor blockers marginally depressed the growth of the regenerate. However, addition of NE or E with one of the receptor blockers evoked marginal increase in tail growth. This leads to the thesis that the CA sensitivity is acquired only during the differentiation stages. The noted growth promoting actions can be related to the effect of CA on general metabolism and cyclic nucleotides.

Among the various hormones, the role of steroids is extensively studied in urodele limb regeneration. It is suggestive that these hormones have inhibitory effects on regeneration(Wallace, 1981). No direct evidence are available for corticosteroid involvement in tail regeneration. Hence, the influence of glucocorticoid in tail regeneration in lizards was studied by chemical adrenalectomy(with metyrapone) and exogenous supplementation of corticosterone. These aspects were also studied in crucial events. In all cases chemical adrenalectomy marginally enhanced the process of regeneration, while exogenous supply of corticosterone suppressed the growth rate. Corticosterone administration at preblastemic level, suppressed the dedifferentiation while supplementation at BL stage had no appreciable effect. The results clearly demonstrate the inhibitory actions of corticosterone during tail regeneration. However, it may be considered that in regenerating animals the glucocorticoids at physiological level may not affect the process of regeneration, in differentiating stages the hormone might be required in a very low threshold to sustain the growth. The hormonal dependence of tail regeneration in lizards is found to be maximum during differentiating stages (Lichet and Howe, 1969; Turner and Tipton, 1971). It has been reported that a low level of glucocorticoids favours cell proliferation through increase in cyclic GMP levels(Vesely, 1980). Suppressing the glucocorticoid level might be creating a similar situation during dedifferentiating stages, in differentiating stages the glucocorticoid may be exerting permissive influence.

In the preceding experiment(chapter-III), chemical sympathectomy depressed the cell dedifferentiation, but no effect was observed in differentiation. As 6-OHDA-induced sympathectomy alters the glyceamic and hormonal levels of corticosteroids and growth hormone(Rintamaki, 1986), it is assumed that the inhibitory effect may be due to excess corticosterone release. Supporting evidence to this notion obtained from the results of another set of experiments(chapter - VI), where chemical adrenalectomy increased while CORT administration depressed the regeneration. To test this assumption another set of experiments were conducted(chapter-VIII). Chemical adrenalectomy was performed prior to 6-OHDA administration and the regenerative performance was assessed at critical events. This procedure confirmed that no corticosterone elevation occurs after sympathectomy. Resperine treatment was also given to ensure the total CA depletion. Measurement of glycaemic levels revealed that chemical adrenalectomy counteracts the 6-OHDA-induced corticosterone elevation as indexed by reduction in glycaemic levels. However, the growth inhibition observed at WE stage could not be reserved by this combined chemical adrenalectomy and sympathectomy. Based on these experiments, it can be assumed that the noted inhibitory effect is due to the mechanisms other than glucocorticoid release. Catecholamines suppressed the process of dedifferentiation. The overall findings suggest that glucocorticoids and CA exert inhibitory effect on tail regeneration in lizards. The CORT may be exerting regulatory influence on differentiating stages of tail regeneration at a very low threshold. At physiological level these hormones and neurotransmitters may not exert any inhibitory effect on the regeneration.

The importance of calcium in epimorphic regeneration has recently been recognised(Globus *et al.*, 1983). However, the importance of calcium in mediating the tail regeneration remains unknown. Hence experiments were conducted at preblastemic and blastemic phases of tail regeneration using calcium entry blockers, calmodulin inhibitors and calcium flux modifiers. That calcium entry blockers inhibited the cell proliferation and differentiation, implies an active calcium flux during regeneration. Calmodulin inhibition totally suppressed the progressive phases of regeneration suggesting an intimate involvement of calcium - calmodulin complex in initiating cell cycle and other calcium regulated intracellular enzymes. Calmodulin inhibition suppresses the mitosis in the newt limb regeneration (Globus *et al.*, 1987). Though the calmodulin content was not elevated in the limb regenerate it is suggested that the increase in the number of active Ca^{2+}-CaM complexes in turn increased the mitosis. In the present experiment, trifluoperazine which specifically inhibits calmodulin activation, suppressed the dedifferentiation, proliferation and differentiation processes, emphasising

the pivotal role of calmodulin in regulation of regenerative events.

The presumption of calcium-calmodulin involvement during tail regeneration has been strengthened by the finding that intracellular calcium depletion by calcium efflux mediators inhibited the process of regeneration. It appears that maintenance of intracellular calcium at critical level is a prime requisite in promoting the cell proliferation during dedifferentiation and differentiation. Calcium ionophore A23187 acted as a mitogen in early events of tail regeneration. Ionophore-induced Ca^{2+} influx promoted the cell proliferation notably in myoblast cell line and precaucious differentiation of epidermis. The mitogenic properties of ionophore has been suggested by Luckasen(1974). In several oocytes, ionophore-induced calcium influx initiated DNA synthesis and oocyte development(Steinhardt *et al.*, 1974; Steinhardt and Epel, 1974). In newt limb regenerates, addition of ionophore A23187, both *in vivo* and *in vitro*, produced two fold increase in mitotic index(Globus *et al.*, 1983). The selective increase in the proportion of mesenchymal cells observed in the tail regeneration is notable. The response of epidermal cells to increased calcium levels is well-known. In cultured epidermal cells increase in calcium levels resulted in terminal differentiation of keratinocytes(Hennings *et al.*, 1980). It appears that increased Ca^{++} level can reprogramme the regenerating cells channelising into another pathway.

The behaviour of apical epidermal cap(AEC) of the tail regenerate, in decreased calcium level needs particular attention. It has been noted that in decreased calcium levels, the distal epidermal areas show increased cell proliferation resulting in hyperplasia. A low calcium milieu in favour of epidermal cell proliferation is established(Hennings, 1980). But the fact that only the AEC responded to the decreased calcium level, raises several questions. The role of AEC in governing the limb regeneration is hypothesised(Tassava and Olsen, 1982; Stocum, 1985; Wolsky, 1988). However, further experiments are required to establish the role of AEC in tail regeneration of lizards.

On the whole, the present experiments unveiled certain positive and negative aspects of neural and endocrine regulation of tail regeneration in lizards. Though the depletion or supplementation of various agents enhanced or suppressed the blastema formation or its subsequent differentiation, none of this modification could totally inhibit the process of dedifferentiation. This fact presumes that the stump cells of the tail possess 'innate ability' to dedifferentiate and produce a mass of blastemal cells, aided and abetted by neural and endocrine factors.