SUMMARY

Chapter-I

In this chapter the materials and methods employed in the present study are outlined in detail.

Chapter-II

The effects of exogenous supplementation of epinephrine(E), norepinephrine(NE) and cholinergic agent, Acetylcholine(ACh) have been evaluated on the tail regeneration in lizards. Two series of experiments were conducted. In first series, the growth rate of the regenerate and the time required to reach specific stages were recorded. In second series, the mechanism of action of neurotransmitters at WE and BL stages of regeneration was analysed. Supplementation of E retarded the process of regeneration with a delay in wound healing and blastema formation while NE had no effects. The effect of E has been discussed in relation to the diabetogenic action of the drug. The divergence in action of E and NE could be attributed to the difference in density of receptor population. Both at WE and BL stages, E inhibited the process of regeneration. The E-induced growth inhibition may be a direct effect on cell proliferation. The increase in insulin coupled with the cyclic AMP level might have promoted the proliferation and differentiation in NE-supplemented tail regenerate. That increasing the levels during WE stages suppressed the regeneration, could be related to the change in sensitivity of regenerating cells to neurotransmitters. In all experiments, the effect of ACh was nonsignificant. Findings overrule the possibility of any cholinergic influence upon the tail regeneration in lizards.

Chapter-III

6-hydroxydopamine(6-OHDA) depletes catecholamine(CA) content in adrenergic nerve fibres without altering the brain CA levels. In this study, the animals were sympathectomised using 6-OHDA at pre-autotomy level(exp.-1) and preblastemic and blastemic stages(exp.-2) to delineate the stage specific functions of CA during tail regeneration in lizards. Results showed that, sympathectomy at pre-autotomy level highly favoured the regeneration with a boost in tail growth and differentiation. Sympathectomy at WE stage slightly suppressed the tail growth but the differentiation was unaffected. At WE stage the treatment reduced the cell proliferation; instead of forming a blastemal mass, cells channelised into differentiating pathway. Nevertheless, an increased rate of differentiation with significant myogenic potential was revealed histologically, in both the experiments. It seems that a massive depletion of CA

reprogrammes regeneration; possibilities also exist in relation to hormonal variations. Findings suggest that the depletion of CA levels due to sympathectomy and a corresponding decrease in cAMP to cGMP ratio might have favoured the myoblast differentiation during tail regeneration in lizards.

Chapter-IV

Guanethidine, an adrenergic blocking agent which primarily destroys the cell bodies rather than the axons, was used to study the effect of adrenergic denervation on the process of tail regeneration in lizards. Treatments were done at two different pH, as the effect of this drug is pH dependent. The extent of sympathectomy was assessed through the histofluorescence of CA in the cornea of the lizards. Histological and morphological studies were also carried out. The results showed that despite an appreciable CA depletion, the blastema formation and differentiation was unaffected at pH 7.4 - 7.6. However, the drug affected regeneration adversely at pH 10.0 - 10.2; reduction in body weight, ptosis and increased mortality were also observed. This implies that, at higher pH the drug destroys sympathectic ganglia and inhibit body metabolism. These findings suggests that sympathetic ganglia-derived trophic agents might help in promoting the tail regeneration in lizards.

Chapter-V

The neurotransmitters act through specific cellular receptors. Several pharmacological agents are known to block these cellular receptors which in turn block the neurotransmitter functions. Thus neurotransmitter blockers are excellent experimental tool to delineate neurotransmitter functions. In the present experiment, the adrenoreceptor and cholinoreceptor functions during tail regeneration in lizards were assessed through blockage of their specific receptors. These blockers (Benextramine and Propranolol) were used alone, together or in combination with agonists at preblastemic(WE) and blastemic(BL) stages of tail regeneration. Blockage of α- and ß-adrenoreceptors alone or together at WE stage had no inhibitory effects on blastema formation while addition of NE or E with receptor blockers significantly increased the tail growth. Blockage of adrenoreceptors at BL stage suppressed the tail growth. Adrenoreceptor blockers together with α- and ß-adrenoreceptors antagonists marginally increased the tail growth at BL stage. Based on these observations it is presumed that adrenoreceptor sensitivity changes might be occurring during different stages of regeneration. Increasing the cholinergic actions with carbachol had no positive effects on tail growth. However, cholinergic inhibition with atropine at WE stage, suppressed the blastema formation,

which may be due to alterations in cyclic nu c leotide levels. However, further experiments are required to establish the adrenoreceptor mechanisms during tail regeneration in lizards.

Chapter-VI

This chapter deals with the stage-specific influence of Corticosterone (CORT) on the tail regeneration in lizards. Exogenous CORT administration as well as chemical adrenalectomy using Metyrapone- a steroidogenesis inhibitor, were carried out in lizards during the period of pre-autotomy to post-autotomy (30th day) in one set and preblastemic and blastemic stages in another set. Results showed that the regeneration impaired after CORT administration while enhanced by glucocorticoid suppression.

Corticosterone delayed the process of wound healing and progress of differentiation while glucocorticoid suppression accelerated above process as well as the blastema formation that was unaffected by CORT administration. At preblastemic stages, CORT supplementation considerably slowered the dedifferentiation-proliferation processes while hormone suppression had no effects. Glucocorticoid inhibition at blastemic stage accelerated the process of differentiation while CORT administration did not produce significant growth inhibition. Finding reveals that CORT might be required in a low threshold during the process of regeneration in lizards.

Chapter-VII

The inhibitory effect of CA and glucocorticoids on the tail regeneration of lizards have been evidenced by combined chemical adrenalectomy and sympathectomy. Different types of experiments such as chemical sympathectomy with 6-OHDA(1) or in combination with reserpine (2) or reserpine alone (3), chemical adrenalectomy with metyrapone(4) or in combination with sympathectomy(MET+6-OHDA+RES)(5) and CORT supplementation (6) were conducted at preblastemic and blastemic stages. The results have been discussed after determining the growth rate and plasma glucose levels. The glycaemic levels were notably changed in different treatment groups; chemical sympathectomy elevated while those were given a combined treatment showed a marked hypoglycaemia. Chemical adrenalectomy prevented the 6-OHDA-induced hyperglycaemia, suggesting that glucocorticoid-induced hyperglycaemia can be controlled by inhibiting adrenal steroidogenesis. However, glucocorticoid depletion could not reverse the growth suppression observed after 6-OHDA treatment in preblastemic stages. The combined treatment at BL stages produced a maximum enhancement in tail growth rate pointing to the fact that both CA and CORT exert inhibitory effect on tail regeneration.

Chapter-VIII

The cellular functions of Ca^{2+} in promoting the process of tail regeneration in lizards have been investigated by experimentally altering their level at preblastemic and blastemic stages. Calcium entry blockage with verapamil(VRL) and diltiazem(DTM) inhibited the cell proliferation at WE stage. However, VRL had no effect in blastema cell proliferation and differentiation. Diltiazem inhibited all progressive phases of regeneration. The observed difference of these drugs could be related to their concentration and mode of action. Calmodulin antagonists Chlorpromazine(CPZ) and trifluoperazine(TFP) were used to study the calmodulin dependent functions. Trifluoperazine a specific calmodulin antagonist inhibited all regenerative events which implicates the importance of this protein in Ca^{2+} functions through formation of Ca^{2+}-CaM complex. Calcium efflux with papaverine (intracellular calcium depletor) totally suppressed the proliferation-differentiation stages of tail regeneration, emphasizing the importance of intracellular calcium. That the influx of Ca^{2+} and increasing the intracellular Ca^{2+} levels, increased the cell prolilferation notably in myogenic cell line. This can be primarily attributed to the changes in cyclic GMP levels; ionophore is known to elevate cGMP levels, which in turn favours cell proliferation. In decreased calcium levels the apical epidermal cap(AEC) of the regenerate showed increased cell proliferation resulting in hyperplasia, the cause of which remains unknown. The findings suggest that intracellular calcium is an important modulator of tail regeneration in lizards.