Introduction

Cuvier (1836) described the study of regeneration as "*Une belle recherche physiologique*". Though fascinating in manifestation, regeneration is an intricate developmental phenomenon of great implications. It represents the capacity of animals to generate lost parts. Regeneration being a complex phenomenon, the *modus operandi* and its biological significance merit the utmost interest and curiosity. Many workers have noted that, the ability to regenerate is pristine in both the phylogenetic and the ontogenetic sense.

Regeneration is nature's way of making good a deficit; transplantation is man's solution to the problem. Regenerative powers are inconveniently deficient in higher vertebrates. Worse still, our immunological defence ensures that the foreign tissue grafts shall be efficiently rejected. With the odds stacked heavily against us, the problem of how to repair the depreciation of the body's organ is as formidable as it is intriguing. And even if we cannot do much about the situation yet, perhaps some consolation is to be found in understanding why such a state of affairs should have evolved in the first place.

Regeneration is a negative phenomenon in the sense that it presupposes a vulnerability on the part of all organisms. The fact that the phenomenon exists at all testifies to the prevalence of this susceptibility to the hazard of life. Nature has invented no end of ingenious ways to provide for the replacement of or compensation for lost parts. Nevertheless, the absence of regeneration in certain forms may sometimes be taken to indicate that the problem of vulnerability may have been solved by preventive measures rather than remedial ones. In other words, regeneration is a good thing when an organism has failed to insure its own security from injury. But it is far better not to be injured in the first place, thus making regeneration superfluous. Hence the phylogenetic reduction in regenerative potential signifies not so much a loss of developmental versatility as the successful evolution of self-protective adaptations. This may be regarded as an encouraging trend. Of one thing there can be no doubt, that regeneration was originally universal and synonymous with development and reproduction. From the beginning, the potential to regenerate has been sacrificed as other advantages have evolved. Yet, regenerative capacities have not been abandoned lightly. Indeed, the remarkable thing is how cleverly organisms have contrived to retain the ability to regenerate while evolving ever more complex strategies to avoid extinction. Only as a last resort has regeneration been forfeited in order to secure the advantages of attributes with which regeneration proved to be incompatible. Admittedly, nature can achieve almost anything if the stakes are high enough, provided the laws of chemistry and physics are not violated. Thus, in some instances regeneration manifests itself in extraordinarily unexpected places. In other cases, its unaccountable absence might never have been predicted.

In the course of evolution, the pros and cons have been weighed by each group of animals. Some have found regeneration to be indispensable, and have devised all manner of mechanisms to insure its regeneration. Others have opted to get along without it, and have derived the benefits of investing their energies in other directions. Most animals have struck a compromise by keeping the ability to regenerate some parts of the body, but not others.

It is generally accepted, with some important reservations, that regenerative ability has tended to decline during the course of evolution. Yet this notion must be qualified with reference to the level of organization at which structures are replaceable. At the molecular and ultrastructural levels, regeneration is equally efficient in all organisms throughout the phylogenetic scale. It is at higher levels of organization, where histologically complex body parts are involved, that the ability to grow replacement has been curtailed in the course of evolution. Thus, higher animals are less capable of regenerating heads, tails or appendages than lower ones, but their molecular and cellular turnover is just as good as it ever was. Therefore, it is not regeneration *per se* which has been selected against during phylogeny, but the level of organization at which it occurs.

There is, in addition, more than one way to regenerate. The most direct, of course, is to grow back what was lost in exactly the same form and location as the original. The new, thus becomes a replica of the old, indistinguishable in every detail from the structure it replaces. Yet, sometimes a given body part is not so individually important that its loss cannot just as well be made good in ways other than by reproducing a faithful copy of itself. Homologous structures, for example, may be increased in size or number to compensate for missing parts, and thereby achieve the ultimate goal of regeneration, viz., the recovery of function through replacement of structure. To expect organisms always regenerate precisely what was lost is to deny evolution of the most efficient way of achieving an end. Evolution is not that rigid. It has no loyalty to one mode of development over another. All that counts is whether or not the method which evolves is the one that works best.

Man is in no position to dispute the dogma that what evolves is by definition that which is the fittest of our progenitors, and hopefully of ourselves. This is despite the inability of most of us to regenerate parts of ourselves we might often wish to replace. Yet, we have eluded extinction despite such shortcomings. Nature may indeed be capable of achieving virtually anything so long as it is within the rules of the game, but in the final reckoning the advantages of such achievements must be weighed against the costs. He who spends money on luxuries at the expense of necessities is not a very good financial risk. Thus, if we lack certain attributes that other animals possess, let us not lament the unfairness of things, for what we do not have would probably have cost too much anyway (Goss, 1969).

Many queries are unanswered and hence the phenomenon of regeneration remains shrouded in mystery and repeated attempts by scientists to unmask this mystery have remained unsuccessful. Thus this phenomenon offers the most challenging opportunities to the modern biologist. Apart from the theoretical and experimental interests for the biologists to evaluate and comprehend many developmental events such as wound healing, dedifferentiation, cell proliferation, tissue interactions, differentiation, and growth, all of which occur as a continuum in a precisely synchronized sequential order during epimorphic regeneration, it also holds other practical interests. One such interest, is the fond thought that some day we may be able to regrow our extremities lost due to surgical reasons or by accidents. Another one, of more immediate interest is the possibility of finding some clues for the causative mechanisms of cancer as, the early phases of regeneration marked by dedifferentiation and proliferation of cells bear close resemblance to tumerogenesis. Moreover, the understanding of mechanisms which commit cells to orderly differentiation during regeneration may also provide us with ways and means of controlling neoplastic growth. The importance of studying the process in lizards in the above context needs no elaboration when we consider the fact that reptiles represent the closest ancestral stock from which the homeotherms have evolved.

The adaptive value of autotomy as an antipredator device can well be perceived by the act of lizards snapping off their tail and leaving it with the predator and making good the escape. Its value is apparent from the high incidence of broken and regenerated tails in wild populations. The violent writhing of an autotomized tail, perhaps a reflex triggered off when the spinal cord is ruptured, on the one hand distracts the aggressor initially and on the other hand offers him an easily obtained meal. Autotomy has so obvious a survival value that it is valid to inquire why such an ability has been lost, reduced or never gained in such groups as agamids, some iguanids, chameleons, a few geckos, and skinks and all platynotids. A common view is that autotomy is not manifested by those lizards in which the tail performs any specialized and more or less indispensable function (Woodland, 1920, Pratt, 1946; Boring *et al.,* 1948). It was in this perspective that Vitt *et al.* (1977) classified the tails of lizards broadly into "actively functional and passively functional" categories, the latter being useful mainly by virtue of their power of autotomy.

The prehensile tails of chameleons, essential for their arboreal life-style, the powerful, heavy tail of monitor lizards (varanus) which may be employed both for swimming and for defence, the long smooth tails of Anolis and other forms, which are used as adjunct for climbing or those of gliding lizards, which are used as rudders, clearly fit into the actively functional category. Predictably, their tail lack fracture planes. Fracture planes tend to be absent, reduced or prone to undergo ontogenic obliteration in such lizards. Additionally, the tail may function in certain types of defensive and sexual displays and may also serve as a status symbol. In the hierarchical communities of the chuckwalla (*Sauromalus obesus*) many 'tyrannt males' lose their dominant position after injury to the tail. "A male that loses 30 to 50% of its tail appears to manifest psychological as well as physical damage leading to loss of social status."

An important feature of most autotomous lizards is that they have an abundant reserve store of energy in the form of subcutaneous and submuscular caudal adipose tissues (Pond, 1978, 1981). Such fat stores are very nominal or even lacking in majority of non-autotomous forms. It appears that fat storage in the tail performs an important adaptive function and the tails of such lizards can be regarded as actively functional (Vitt *et al.*, 1977). Apparently, the tails of most lizards have various roles, of which autotomy on the one hand and locomotion and nutrition on the other are the most significant (See Minton, 1983). The relative importance of these partly incompatible roles show all gradations among different lizards. In some forms such as the gecko, *Lygodactylus Klugei*, they appear to be of almost equal weight, moreover, a strong trend in favour of autotomy could be balanced by a high regenerative capacity (Vitt and Ballinger, 1982). This to some extend, invalidates the distinction between actively and passively functional tails. (Vitt *et al.*, 1977; see Vitt and Ballinger, 1982), although it remains a useful concept. In a large number of species, the integrity of the tail is probably not essential under normal circumstances; the tail is indispensable only in non-autotomous lizards. The relative costs and benefits may vary among different species (see Arnold, 1984) perhaps even among different members (e.g. sexes) of the same species and are related to such factors as the extend of fat storage and the energy required for regeneration and other purposes. Autotomy can be regarded as a compromise adaptation, often involving sacrifice, which a lizard cannot easily afford but which is preferable to the alternative of certain death. Autotomy seldom occurs more than twice in a single life span, although a few individuals show as many as four breaks. Whereas the study of animal regeneration as a whole is a fashionable and rapidly advancing field, only a fairly small population has been carried out on reptiles.

The regenerate that forms after autonomy is of special note because of both its structure and its method of origin. Morphologically it is a quite imperfect tail. It lacks vertebrae and, in their place, has an unsegemented cartilaginous tube. This tube contains the regenerated spinal cord, which consists of an extension of the ependymal lining of the central canal of the cord and descending fiber tracts from the old cord. No new neurites are differentiated in the regenerated tail.

The origin of a regenerate is from a mass of mesenchymal cells accumulated below the wound epithelium (Plates I Aa, Ba; IIA). This epithelium gets gradually multilayered and together with the accumulated mesenchymal cells from a dome shaped projection referred to as the regeneration blastema (Plate I Ac, Bc; III). The multilayered apical epithelium is designated as the blastemic epithelium, while the

LEGENDS TO FIGURES

Plate - I

A & B : Photographs of lizards showing arbitrary stages following caudal autotomy.

Aa & Ba : Lizards showing wound healing stage. The wound surface is covered by a wound epithelium.

Ab & Bb : Lizards showing preblastema stage with initiation of dedifferentiation and accumulation of cells beneath the wound epithelium, giving a turgid appearance to the wound surface.

Ac & Bc : Lizards showing the blastema stage. The wound surface gets projected in the form of a dome shaped structure by the accumulation of dedifferentiated mesenchymal cells below the wound epithelium.

PLATE-I

LEGENDS TO FIGURES

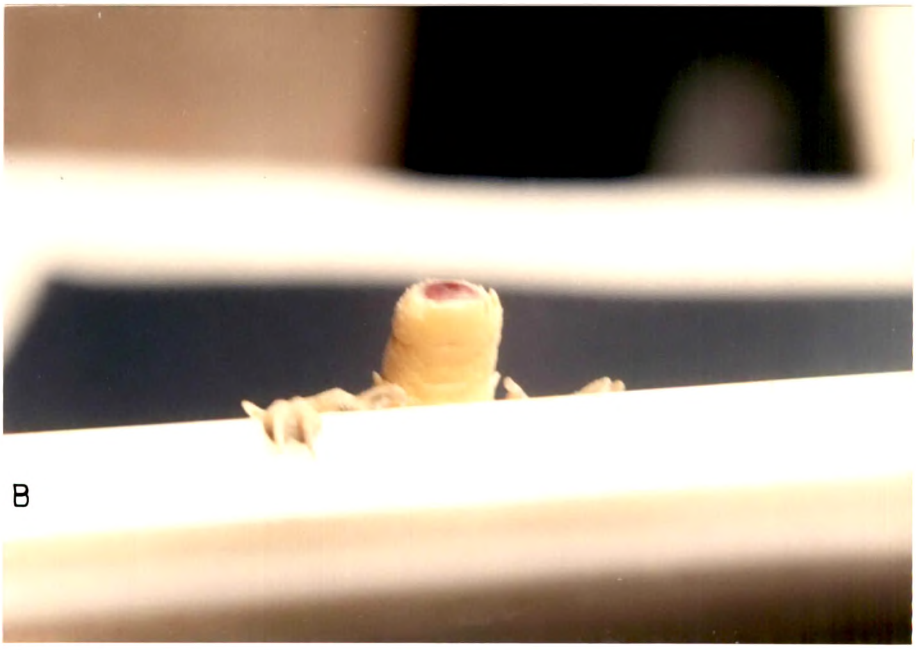
Plate - II

A & B : Photographs of close-up view of the autotomized tail stump showing wound healing and pre-blatema tages.

A : Tail depicting wound surface covered by a wound epithelium.

B : Preblastema stage showing trugid appearance of the wound surface due to accumulation of dedifferentiated cells.

PLATE - II

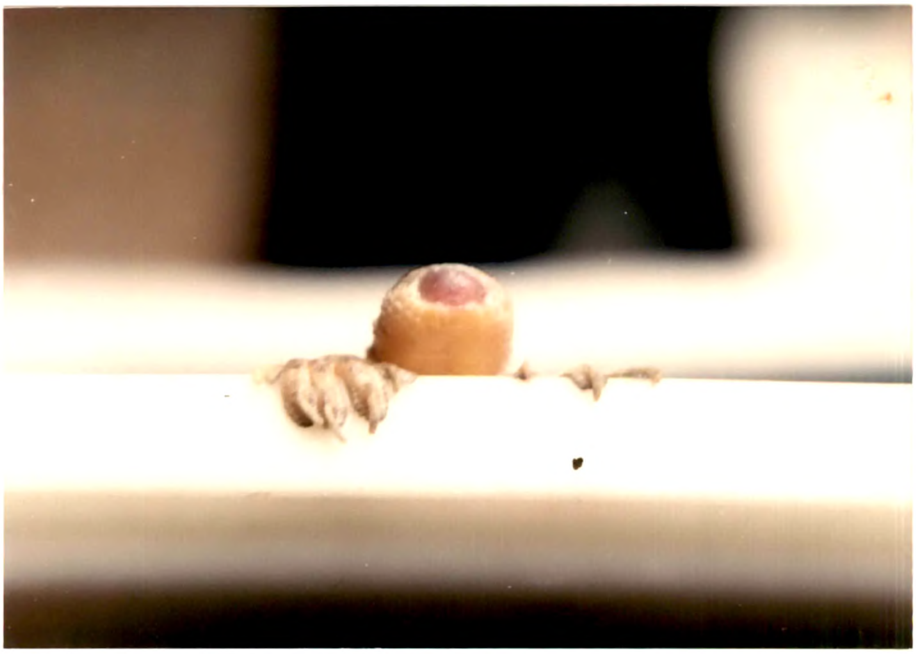


LEGEND TO FIGURE

Plate III

Photograph showing a close-up view of blastema stage marked by a dome shaped protuberance from the wound surface due to the accumulation of dedifferentiated mesenchymal cells.

PLATE-III



underlying undifferentiated mesenchymal cells are termed blastemal cells. The source of blastemal cells is the dedifferentiating mesodermal components of the tail primarily the various connective tissues comprising muscle septae, dermis, adipose tissue, periosteum and perhaps osteocytes of the vertebrae (Simpson, 1965; Cox 1969 a, b). However, in the tropical Indian Lizards, *Hemidactylus flaviviridis* and *Mabuya carinata* some degree of dissolution of caudal muscle from the cut end of the tail has been observed (Shah and Chakko, 1968; Radhakrishnan and Shah, 1986). This attests to the possibility that even muscles could contribute in the formation of dedifferentiated blastemal cells. Further, even nomadic cells in the form of migrating lymphocytes have also been shown to populate the formative regeneration blastema (Shah *et al.*, 1980a; 1982a; Ramachandran *et al.*, 1985). The accumulated blastemal cells then proliferate below the blastemic epithelium and then redifferentiate into cartilage, muscles and other tissues in a proximo-distal direction. Within the newly forming cartilaginous tube, the ependyma proliferates and gradually and continuously extends distally.

If the spinal cord is removed a distance anterior to the autotomy plane, regeneration of the tail fails to occur (Kamrin and Singer, 1955). This inability was first thought to be due to failure of nerves to reach the cut end. Subsequently, however, it was shown that the presence of nerve is not a requisite for tail regeneration; rather, the ependyma is the essential ingredient. Destruction of the greater part of the nerve cord at the amputation plane does not inhibit regeneration as long as ependyma is present. Very importantly, small bits of regenerating ependyma within the cartilage tube grafted into the dorsal muscle mass of the tail will induce supernumerary regenerates whereas implants of cartilage tube alone will not (Simpson, 1964, 1970). The transport of presumed neurotropic substances by nerve is thus not required for tail regeneration as the ependyma produces no neurites in the regeneration situation.

When the limb of a lizard is amputated, it does not regenerate. Singer (1961) and Simpson (1961) both showed, however, that after amputation of one hindlimb, a limited regrowth occurs with low frequency if the sciatic nerve of the opposite limb is deviated surgically to enter the amputated limb. The regenerates in these cases are merely short extensions from the stump. Bryant and Wozny (1974) found, however, that an amputated lizard limb will regenerate in a majority of cases and to a greater length if supplied with cartilage and ependyma from a regenerating tail.

It is rather clear, as Goss (1969) pointed out, that a warm-blooded animal requires frequent nutrition, in order to maintain its heightened metabolic rate and elevated body temperature. Should a bird or mammal lose a limb, it might very well starve, freeze or be preyed upon before it could regenerate a functional replacement. The ability to regenerate limbs was apparently lost in the course of evolution in exchange for other advantages of the warm-blooded condition. At present there are no indications that regeneration of major limb parts in mammals will be accomplished in near future. There is now considerable evidence, however, that amputation through the terminal phalanges can be followed by complete regeneration of the fingertip. Its full length may be restored and the nail and fingerprint whorls appear normal. There is seldom loss of sensitivity and mobility.

However, it is intriguing that amphibians have greater regenerative potential in the larval stages, which is lost in the adult stage as in the case of anurans while, reptiles have regenerative potential in the adult stage not expressed whatsoever in the larval stage. This discrepancy questions the logistic thought that embryonic forms must express greater regenerative potential than adult forms when viewed from the phylogenetic evolutionary scale. On the other hand, the progressive restriction in regenerative potential envisaged in terms of evolutionary hierarchy is clearly exemplified by the observed ability of amphibians to regenerate both their limbs and tail while in the reptiles it is restricted to only the tail in few lizards.

Though initiation of the regenerative outgrowth is an innate process, it is however responsive to modulations of various endogenous and exogenous factors. Hormones such as thyroxine (T4) and prolactin (PRL) have the greatest influence on lizard tail regeneration. Amongst the environmental factors, though temperature variations on a seasonal basis do have influence, light or photoperiodism is also a major factor in the regulation of various endogenous rhythms capable of modulating the regenerative potential. It was the importance of light and temperature *per se* on regeneration and, existing controversies, that led to investigations on these aspects in a detailed manner in this laboratory.

Studies on these lines conducted previously have revealed definite influence of photic schedules on the course of regeneration (Ndukuba and Ramachandran, 1991a). Moreover, a seasonal influence was also demonstrated (Ramachandran and Ndukuba, 1989a) However, photothermal interactions in terms of photic extremes and different light intensities in relation to altered temperature need to be ascertained. This has been attended to and, forms the subject matter of the first chapter, wherein, photothermal manipulations have been carried out to assess the rate and course of regenerative growth.

The alterations in regeneration performance recorded under different photic schedules have been correlated with PRL release (Ndukuba and Ramachandran, 1991 a,b; Ramachandran and Ndukuba, 1989a). Previous investigations from this laboratory had hypothesized the involvement of high serotonergic activity for elevated PRL release occurring under long photic schedules (Ndukaba and Ramachandran, 1991 a, b; Ramachandran and Ndukaba, 1989a). This hypothesis has been tested in the present study by the use of cyproheptidine, a known pharmacological agent which functions as 5-HT receptor antagonist. The effect of administration of this agent either in the morning or evening under normal light-dark schedule and under extremes of photoperiod has been assessed so as to draw relevant and logical conclusions. A distinct array of studies on developing embryos have not only localised neurotransmitter substances in the pre-nervous stages but, also implicated them in various aspects of developmental process independent of their neurotransmitter functions. (Buznikov, 1973,1979). Relevantly, a previous study from this laboratory had shown involvement of acetyl cholinesterase in the early stages of the regenerative process, even before the establishment of any cholinergic innervation (Ramachandran *et al.*, 1981a; Abraham and Ramachandran, 1988). These reports ignited the curiosity to see the possible involvement of 5-HT in the developmental events associated with the regeneration process. To this end, cyproheptidine, a 5-HT receptor blocker, was also administered locally either in the morning or evening to see its influence on the course of regenerative growth.

Pineal as the extra-ocular photoreceptive organ mediating the influence of light on regeneration in lizards was established previously (Ramachandran and Ndukuba, 1989b). The above studies revealed that both pinealectomy as well as light deprivation to pineal could block the favourable influence of light on regeneration. Since melatonin is the principal indoleamine hormone of pineal involved in photo-neuroendocrine transductions, a preliminary evaluation to test the effect of exogenous administration of melatonin either in the morning or in the evening was conducted. This study revealed a proregenerative effect when melatonin was administered in the evening and an antiregenerative effect when administered in the morning. (Ramachandran and Ndukuba, 1993; Kurup *et al.*, 1995). Since it is documented that there are differential effects of morning or evening administration of melatonin on the reproductive functions of seasonally breeding mammals and, that both morning and evening administration nullifies the antigonadal effects of evening administration (Reiter, *et al.*, 1976. Reiter, 1980a,b, 1991), it was imperative that similar experimental paradigms be tested on the regenerative process as well. In this context, the influence of administration of melatonin either in the morning or evening or, both morning and noon, or morning and evening, noon and evening or even, morning, noon and evening on regenerative performance has been evaluated in the present study.

Since the discovery of melatonin as the pineal principle, even other potent indoleamines have been shown to be elaborated and secreted by the pineal gland with obvious physiological effects. Two such pineal indoles recorded to exhibit circadian rhythms in secretion and exerting potent effects are, methoxytryptophol and methoxytryptamine. These two principles have been documented to have effects on seasonal reproduction in mammals like that of melatonin (Pevet, 1983, Raynaud *et al.*, 1989). In this scenario, the influence of both these pineal principles has been evaluated on the regenerative process in *H. flaviviridis*.

The fact that the attendant effects of timed exogenous administration of melatonin is by way of induced alterations in PRL release, needed verification. Hence in the present study, the credibility of this contention has been arraigned by the use of pimozide, a dopamine receptor antagonist and, cyproheptidine, a 5-HT receptor antagonist. Pimozide was co-administered along with melatonin in the morning. The effects of these agents can confirm the operation of inhibitory dopaminergic and stimulatory serotonergic regulation of PRL release in lacertilians.

Amphibian appendage regeneration and reptilian tail regeneration, apart from other differences, also differ in terms of the impetus for the initiation of regeneration. Whereas in the case of amphibians, it is the presence of an optimum level of nervous innervation, it is the spinal cord that is implicated in the case of reptiles. It is well established that the ependymal outgrowth from the spinal cord from the cut end of the tail is indispensible for the organization of a regeneration blastema. Further, it has been deciphered that the outgrowth of ependyma is dependent on thyroid hormones. (Turner and Tipton, 1971; Turner, 1972). It is also well neigh recognized that there is seasonal variation both in the initiation of regeneration as well as in the subsequent linear tail elongation. Though these variations are related to the variations in photoperiod and temperature, their possible relation with thyroid hormone is not elucidated. In this behest, in order to detail the possible variations or differences in thyroid hormone status and/or sensitivity on a seasonal basis, experiments involving manipulations of thyroid status have been undertaken. One set of experiments involved studying the effect of either thyroidectomy or treatment with the antithyroid drug Methimazole (MMI) or even both, on the process of tail regeneration. In another set of experiments, systemic or local administration of thyroxine was carried out. The consequent effects on the formation of regeneration blastema and initiation of tail regeneration are likely to provide some clues regarding the possibilities of season dependent altered status of thyroid as well as sensitivity towards thyroid hormones in relation to photoperiod/temperature changes.

Another hormone that has been related with the regenerative process is prolactin. Prolactin has been identified as the essential growth promoter during regenerative tail elongation in lizards (Ndukuba and Ramachandran, 1989a). Obviously both thyroxine and prolactin are regeneration promoting hormones in lizards. However, the relative roles and sequential importance of these two hormones need to be clearly identified. To this end, a series of experimental manipulations in the form of thyroidectomy combined with pimozide treatment, systemic and local administration of thyroxine together with bromocriptine or even replacement with thyroxine in thyroidectomized and methimazole and bromocriptine treated lizards or, treatment with pimozide instead of thyroxine have been carried out. The results from these experimental manipulations are likely to provide evidence for the relative importance and temporal involvement of these two hormones in the control of tail regeneration in lizards.

Involvement of growth factors during development as well as in the control of cell cycle and even in the synthesis of extracellular matrix components has been increasingly and clearly brought out (See, Pfeilschifter, 1990). Some studies have shown the favourable influence of FGF in amphibian appendage regeneration (Cuny *et al.*, 1986 Gospodarowicz *et al.*, 1987, Gospodarawicz and Mescher 1981). Similarly a previous study from the laboratory highlighted the importance of NGF in lizard tail regeneration and its ability too nullify the retardative influence of hypothyroidism (Kumar, 1990). Since it is likely that, many growth factors might be involved in temporally orchestrated sequential fashion during tail regeneration, attempts have been made in the present investigation to evaluate the effects of exogenous *in loco* treatment with EGF or TGF-β during the post-autotomy phases on the course of tail regeneration. The observations are likely to provide some idea about the relative roles of these two growth factors and their functions during regeneration.

The detailing of observations/results of the above series of investigations and a discussion on them in available background and/or related literature and the conclusions drawn are embodied in the ensuing chapters.