CONCISE SUMMARY

Though fascinating in manifestation, regeneration is an intricate developmental phenomenon of great implications. It represents a capacity of animals to generate lost parts, often complex, a capacity widely observed. Regeneration being a complex phenomenon, the *modus operandi* and its biological significance merit the utmost interest and curiosity. Many workers have noted that, the capacity to regenerate is primitive 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 ensure 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 retention. 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 ultra-structural 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 apppendages 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, namely, the recovery of function through replacement of structure. To expect organisms always regenerate precisely what was lost is to dey the evolution of the most efficient way of achieving an end. Evolution is not that rigid. It has no loyality 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. Our very existence on earth testifies to the fitness of our progenitors, and hopefully of ourselves. This, 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.

Many queries are unanswered and hence the phenomenon of regeneration remains shrouded in mystery and repeated attempts by scientist to unmask this mystery have remained unsuccesssful. Thus, this phenomenon offers the most challenging opportunities to the modern biologists. Apart 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, growth all of which occur as continum in a precisely synchronized sequential order during epimorphic regeneration, it also holds other practical interest. 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 ones, of more immediate interest is the possibility of finding some clues for the causative mechanisms of cancer at 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.

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 to 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 positive influence of increasing photic schedules and negative influence of decreasing photic schedules (Ndukuba and Ramachandran, 1991a). Moreover, a seasonal influence was also demonsstrated (Ramachandran and Ndukuba, 1989a). As a sequel to the above studies influence of different light schedules and different light intensities in relation to altered temperature has been evaluated in the present study. The various morphometric parameters that have been assessed to evaluate the influence of these experimental schedules as well as for all other experimental paradigms are the approximate number of days taken to reach the various arbitrary stages of regeneration, number of days elapsed post-autotomy for initiation of growth, the daily growth rate, the total length of tail regenerated at the end of the specified time period and the total percentage tail replacement. The photo-thermal manipulations have revealed faster regenerative growth on either side of the normal day-night photoperiodism with the result both continuous light (LL) and continuous darkness (DD) induced faster regenerative growth. Of the two schedules LL was more potent that DD. Increased light intensity upto 600 lux units had no positive influence while a higher intensity of 1500 lux unit produced a significant enhanced response. Temperature increment by 2°C potentiated regeneration under all photic schedules, but relatively greater effect was manifested in lizards experiencing a normal schedule of LD 12:12 (NLD).

Previously, the increased regenerative performance in long photic schedules was ascribed to elevated PRL level mediated by the high serotonergic activity (Leining *et al.,* 1979). This concept has been tested presently by the administration of cyroheptidine, a 5-HT receptor antagonist. This phamacological agent retarded regenerative growth under all the lighting schedules i.e. NLD, LL and DD. Compared to DD retardation in LL was more pronounced. A time dependent influence of cyproheptidine was also seen in the NLD condition with very pronounced retardation occuring when the agent was administered in the morning than in the evening.

Role for pineal was envisaged by the previous observation of retarded tail growth in pinealectomized (PX) lizards (Ramachandran and Ndukuba, 1989a). A preliminary evaluation to decipher the role of pineal was designed in the form of exogenous Melatonin (M) administration in the morning or in the evening. This evaluation showed a proregenerative influence of evening (e) administration and an antiregenerative influence of morning (m) administration (Ramachandran and Ndukuba, 1993). Since it is documented that there are differential responses of morning and evening administration in the reproductive functions of seasonal mammals and that both morning and evening administration nullifies the evening effect (Reiter *et al.,* 1976, 1980; Reiter, 1991). The influence of administration of melatonin either in the morning or evening or both morning and evening or morning and noon, noon and evening or even morning, afternoon and evening on regenerative performance has been assessed currently. The results confirmed the antiregenerative effect of Mm and proregenerative effect of Me and further revealed a more significant retardary influence of morning and evening injections. However, administration of M three times a day produced a retardary effect similar to that of Mm.

Since it is known that pineal elaborates even other indoles like 5-methoxytryptophol (ML) and 5-methoxytryptamine (MT) in addition to M, time specific influence of all the three indoles have been tested on regenerative performance. The finidings suggest that while MT has no influence on regenerative performance, ML has an opposite time dependent influence to that of M. Accordingly, MLm injections had a proregenerative influence while MLe had antiregenerative influence. The antiregenerative effect of Mm and proregenerative effect of Me have been presumed to be due to decreased dopaminergic activity or increased serotonergic activity respectively. (Ramachandran and Ndukuba, 1991; Lawson and Gala, 1976, 1978; Clemens *et al.,* 1977; James and Wigham, 1984).

This concept has been tested presently by the use of pimozide, a dopamine receptor antagonist along with Mm injections and by the use of Bromocriptine, a dopamine agonist or cyproheptidine, a 5-HT receptor antagonist along with Me injections. The above two treatment schedules showed that pimozide can nullify the retardary influence of Mm and that both bromocriptine and cyproheptidine can nullify the positive influence of Me. Of the two, bromocriptine and cyproheptidine, the former was more potent than the latter in nullifying the favourable response of Me. These results have been taken to confirm the operation of inhibitory dopaminergic and stimulatory serotonergic regulation of PRL release and the potentiating time dependent influence of M on the two regulatory mechanisms.

A major point of difference between amphibian appendage regeneration and saurian caudal regeneration is with reference to the control of initiation of regeneration. Whereas, the initial impetus for initiation is essentially provided by nerves in amphibias, the same is provided by the spinal cord in the case of reptiles.

Previous works have shown that it is the ependymal outgrowth from the cut end of the spinal cord that is responsible for the organization of regeneration blastema and that the ependymal outgrowth itself is dependent on thyroid hormones (Turner and Tipton, 1971, Turner, 1972). In order to detail the role of thyroxine in tail regeneration in lizards, two sets of experiments were done on seasonal basis. In one set of experiments lizards were either thyroidectomized (TX) or treated with the antithyroid drug Methimazole (MMI) or even TX and MMI treated. In the second set of experiments lizards were administered thyroxine either systemically or locally. In the first set of experiments MMI treatment resulted in retarded tail regeneration. While complete TX or TX with MMI, both failed to induce the formation of the regeneration blastema even at the end of fifteen days. This suggests the absolute requirement of atleast minimal titers of thyroxine in initiation of regeneration. In the second set of experiments local administration of thyroxine on a daily basis for the first fifteen days post-autotomy had a hastening influence on regeneration in all the seasons, continued daily injections of thyroxine thereafter however had a retardory influence. But if the treatment was continued for alternate days the stimulatory influence was maintained. In contrast systemic administration of thyroxine exerted a retardary negative influence on regeneration right from the beginning in the monsoon and winter seasons. However there was an initial stimulation like in the case of the local administration during the summer season. These results have been taken to develop a hypothesis of seasonal variation in the circulating titer of thyroid hormone and/or altered sensitivity to thyroid hormone. These are been corroborated by seasonal histological observation of thyroid.

It is also established that PRL is a growth promoter during the regenerative tail elongation in lizards (Ndukuba and Ramachandran, 1989b). So it is obvious that both thyroxine and prolactin are regeneration promoting hormones. To ascertain the relative roles and sequential importance of these two hormones a number of experimental manipulations in the form of TX plus pimozide treatments, systemic and local administration of thyroxine in TX plus MMI and bromocriptine treated lizards or treatment with pimozide instead of thyroxine and also sequential replacement with thyroxine followed by pimozide were carried out. These set of experiments have provided convincing evidence for temporal involvement and significance of thyroxine and PRL. Apparently, thyroxine seems to be the initial evocator of regenerative growth while PRL is the essential effector of regenerative tail elongation.