SUMMARY

CHAPTER I

The tail of the gekkonid lizard Hemidactylus flaviviridis was autotomized at the third segment from the vent and the animals exposed to eight different photoperiodic lengths viz. : Continuous light of high intensity-2500LX (LL(H) : 24L : OD), continuous light of low intensity-638 LX ( LL(L) : 24L : OD), 18hours light and 6 hours dark (18L : 6D), 16 hours light and 8 hours dark (16L : 8D), 12 hours light and 12 hours dark (12L : 12D), 8 hours light and 16 hours dark (8L :16D), 6 hours light and 18 hours dark (6L :18D), and continuous (total) darkness (DD : OL :24D) during the monsoon months of August, September and October. The length of new growth (regenerate) was measured with a ruler graduated in mm. at fixed time intervals of 5, 10, 20, 30, 40, 50 and 60 days after tail autotomy. The measurements were used for morphometric calculations. The results show that there is a positive influence of increasing lengths of light as well as its intensity and a negative influence of decreasing lengths of light from the intermediate photoperiodic reqimen of 12L : 12D as lizards under LL (H) produced the best regenerative performance and those in DD the worst. Further observations revealed that photoreqimes

18L : 6D,LL(L) and LL (H) induced a biphasic growth rate curve (one during the first 10 days and the second between days 30 - 40 postcaudal autotomy). Animals exposed to the other light schedules did not exhibit the first regenerative growth spurt but produced a gradual growth curve peaking between 30 - 40 days after tail autotomy.

## CHAPTER II

Having established in chapter 1 that long-day photoperiods stimulate tail regeneration in Hemidactylus flaviviridis while short-day periods depress it, in chapter 2 an attempt was made to elucidate the role of the lateral eyes, or retinae, in photoperiodic photoreception during the process of tail regeneration in lizards. One group of lizards was blinded by surgical removal of both the lateral eyes (bilateral orbital enucleation) while a second group, which served as the control, remained sighted. Bilateral enucleation was performed as described in chapter II of this thesis. The two groups were exposed to the eight photoregimes described in the summary of chapter 1 and tail regeneration was observed for a peicod of 60 days after autotomy. The results show that there was no statistically significant difference in the regenerative performance of blinded (BL) and sighted lizards in terms of the length of tail regenerated and the total percentage replacement (Duncan's multiple range test Duncan, 1955).

This observation leads to the assumption that the lateral eyes, or retinae, do not participate in photoperiodically significant photoreception in Hemidactylus during its tail regeneration.

## CHAPTER III

Chapter 3 has dealt with the problem of localizing the site (S) of photoreception in regenerating lacertilians since it has been demonstrated (chapter 2) that the eyes, or retinae, are not the principal photoreceptor organs in Hemidactylus flaviviridis. Several lines of evidence suggest that the pineal organ of lower vertebrates is photosensory, conseguently, the pineal organ was selected for the investigation in chapter 3. Lizards were divided into four groups - (1) pinealectomized (PX), (2) normal (NL), (3) normal with head paint (NL - HP) and (4) normal with a small part of the stomach painted. Pinealectomy was performed as described in the text (chapter III). The paint used was a mixture of Indian inK and $10^{-3}$ Nile Blue Sulphate (II - NBS). Groups 1 and 2 animals were exposed to all the eight light conditions, while lizards in groups 3 and 4 were exposed to LL (H) and 12L : 12D regimes. Measurements were made at fixed time intervals of 5, 10,

20, 30, 40, 50 and 60 days after tail autotomy. The regeneration process was significantly retarded by pinealectomy as well as light deprivation to the pineal (50%) suggesting the involvement of the pineal organ of Hemidactylus in photoreception. There was no retardation effect in group 4 animals which served as a control to ensure that the II-NBS mixture had no toxicity side effects. Using Duncan's multiple range test for statistical significance, the differences in the length of new growth (regenerate) and the lost tail in NL groups of animals on the one hand and PX and NL - HP groups on the other were found to be statistically significant at both 1% and 5% levels (Duncan, 1955).

## CHAPTERS IV AND V

The experiments of chapters 4 and 5 were designed with two objectives in mind : (1) to assertain whether the pineal organ of Hemidactylus flaviviridis, in addition to its photoreceptive function, has a thermosensitivity capability and (2) to elucidate the effects of three different seasons (summer-30°C, monsoon- 26°C and winter- 17°C) on lacertilian tail regeneration. NL, BL and PX groups of lizards were autotomized and subjected to the eight photoregimes described in the summary of chapter 1 for 60 days in each of the three

seasons. Measurements of the regenerative tail elongation were recorded at fixed time intervals of 5, 10, 20, 30, 40, 50 and 60 days after tail autotomy. The results show that the regeneration process was enhanced during the summer season (March-May) and retarded during the winter months (November-January) with the regenerative performance during the monsoon season in between (Chapter 4). Further observations revealed that (1) there is a positive correlation between an increase in photothermal input and the total amount of tail regenerated and total percentage of the autotomized tail replaced (Chapter 4), (2) the pineal organ of Hemidactylus may be involved in thermosensitivity at temperature ranges above the optimum (monsoon temperature - 26°C ) just as it has been demonstrated (Chapter III) to be directly involved in photoreception during the process of lacertilian tail regeneration. (3) The stimulatory effect of increasing lengths of light in NL and BL groups of animals was reduced during the winter season and abolished by pinealectomy (Chapter 5). (4) There was an improvement in regenerative performance of NL and BL lizards above the optimum temperature, even when completely deprived of light, which was not discernible in PX lizards indicating a fine thermosensitivity function of the pineal

organ in Hemidactylus at temperature ranges above the optimal (Chapter 5).

## CHAPTER VI

Chapter 6 heralded a new line of studies, using hormones and other neuropharmacological agents (drugs) aimed at demonstrating a possible neuroendocrine participation on tail regeneration in lizards. Melatonin (N acetyl - 5 - methoxytryptamine ) is a putative vertebrate hormone that has been shown to be involved in reptilian gonadal development or regression, but has not been tested in regenerating lacertilians. Hence, melatonin ( 2 mg/kg ) was injected in the morning ( 0700 hrs) and evening (1700 hrs ) in regenerating H.flaviviridis exposed to LD 12 : 12, 5 days prior to tail autotomy and 30 days thereafter. One group of PX lizards received same dose of melatonin at 1700 hrs. Measurements were made at fixed time intervals of 10, 15, 20, 25 and 30 days after tail autotomy. The results show that intraperitoneal (ip) injection of melatonin produced a dual effect, depending on the time of day the hormone was injected. Exogenous melatonin produced an antiregenerative effect at dawn and a proregenerative effect at dusk

in NL lizards, but did not affect the regeneration process in PX lizards at dusk when compared with their respective saline injected controls. It is worth speculating, at this stage, the possibility of exogenous melatonin at dusk sparing or reducing the endogenous conversion of serotonin to melatonin, thereby providing a protracted mitogenic action of PRL release induced by serotonergic mechanism. Similarly, exogenous melatonin in the morning may produce its antiregenerative effect either by its direct antimitotic effect (Banerjee and Margulis, 1973) or indirectly by dampening the increasing release in the light phase as both mitotic potential and PRL level have been reported to peak during the light phase (Litwiller, 1940, Crim, 1975 ). What will be the possible results when exogenous melatonin is injected in regenerating NL Lizards exposed to LL or DD as well as higher doses of the hormone in PX lizards exposed to 12L : 12D were anticipatory guesses at this stage of research (see chapter XII and summary chapter 12, for the final results ).

## CHAPTER VII

In chapter 7, the influence of prolactin ( PRL), another vertebrate putative hormone, was investigated

in regenerating lizards exposed to DD. Six groups of H. flaviviridis were autotomized and exposed to DD schedule. Group 1 - NL (PRL), group 2 - PX (PRL), group 3 - NL (Sal), group 4 - PX (Sal), group 5 - SPX (PRL) and group 6 - SPX (Sal). PRL (5 µg/animal) and saline were injected (ip) into NL, PX and SPX groups of lizards for a period 5 days prior to and 50 days after tail autotomy. Measurements of the new growth ( regenerate ) were made at fixed time intervals of 10, 20, 30, 40, and 50 days post - caudal autotomy. The results show that exogenous PRL enhanced both the length of new growth as well as the total percentage of the lost tail in NL, but not PX, lizards exposed to DD. Comparisons between the six groups of lizards, in terms of the above two parameters, (Duncan's multiple range test ), revealed no statistically significant difference between NL (PRL) and SPX (PRL) lizards on one hand and between PX (PRL), PX (sal), SPX (Sal) and NL (Sal) on the other However, all other comparisons other than these were statistically significant at the 1% level (Duncan, 1955). In conclusion, the growth - promoting influence of PRL was evident in NL, but not PX) groups of lizards, indicating a more intriquing interdependent interaction among photoperiodism, pineal and prolaclin.

## CHAPTER VIII

Parachlorophenyl alanine ( p- CPA ), an inhibitor of serotonin (5 - HT ) synthesis at the level of the enzyme tryptophan hydroxylase (Koe and Weisman, 1966, Walker, 1983 ), was used for chemical pinealectomy in the study of tail regeneration in H. flaviviridis. Three groups of lizards were exposed to LL and given daily ip injection of 200 µg/kg , 400 µg/kg and 0.6% saline, 5 days prior to tail autotomy and 30 days thereafter. The new growth was measured at fixed time intervals of 5, 10, 15, 20, 25 and 30 days after tail autotomy. The regeneration process was significantly polarded in lizards treated with the high dose ( 400 µg/kg) p- CPA (50%) but insignificantly retarded in animals injected with the low dose ( 200 µg/kg ) p- CPA when compared with their respective saline - treated controls (Test for statistical significance was done using Students 't' test ). The results of this study led to the conclusion that : (1) the effect of p- CPA on tail regeneration in Hemidactylus may be dose dependent. (2) lizards with physically intact pineals but deprived of their ability to synthesize 5 - HT do not exhibit the favourable influences of light on tail regeneration. (3) p- CPA has similar retardation effect (50%) on tail regeneration as complete pineal ablation.

## CHAPTER IX

Tail regeneration in the gekkonid lizard, H. flaviviridis exposed to LL and DD was studied with bromocriptine injections. Bromocriptine (2-bromo - α - ergocryptine ) is a potent dopamine (DA) receptor agonist and has been used successfully for lowering PRL levels in patients with functional hyperprolactinemia. Two groups of lizards were exposed to LL and DD along with their saline (control) counterparts for 7 days prior to the experiment for acclimation to the light and dark conditions. 10 lizards in each group were given daily ip injection of 1mg/kg (low dose) and 2mg/kg (high dose) of bromocriptine and the controls received ip injection of 0.6% daily. The regenerative fail elongation in all the groups was measured and recorded at fixed line intervals of 5, 10, 20, 30, 40 and 50 days after tail autotomy and the recorded data were used for morphometric calculations. The results show that bromocriptine had no effect in regenerating lacertilians exposed to either LL or DD as their was no statistically significant difference in terms of the length of tail regenerated and total percentage replacement, in bromocriptine - treated (1mg/kg or 2mg/kg) and their saline - treated controls( Using both students 't' test and Duncan's multiple range test). The failure of bromocriptine, a

potent DA receptor agonist, to retard tail regeneration in LL exposed lizards coupled with a 50% retardation effect by p- CPA suggests that a stimulatory serotonergic rather than an inhibitory dopaminergic mechanism of PRL release may be functioning under this regime. The failure of bromocriptine to retard tail regeneration in lizards exposed to DD was interpreted to indicate a state of full saturation of DA receptors on pituitory lactotrophs, thereby leaving no available sites for its agonist to bind . These assumptions prompted a search for a possible regenerative response to bromocriptine treatment in the intermediate photoperiodic regimen of 12L:12D. This problem has now been solved (see chapter XI and its summary chapter 11 ).

## CHAPTER X

Since bromocriptine failed to retard tail regeneration in lizards exposed to DD, it was thought pertinent to test for the existence of the dopaminergic regulatory system in this regime by the use of the antipsychotic drug pimozide, a potent dopamine (DA) receptor antagonist. Three groups of autotomized H. flaviviridis were exposed to DD. The first group received ip injection of 50 µg/kg pimozide daily. The second group received daily ip injection of 0.6% saline and served as

one control while a third group of animals, which served as the second control, had no saline injection. Measurements at fixed time intervals of 10, 20, 30, 40 and 50 days post-caudal autotomy were used for morphometric calculation. The data on the length of tail regenerated and total percentage replacement were subjected to Student's '*t*' test. Comparisons between the three groups of animals ( Student's '*t*' test) revealed statistically significant difference between pimozide - treated lizards on one hand and saline/nonsaline - treated animals on the other at the 1% level. The marked stimulation of tail regeneration in the DD exposed lizards (this study), confirmed an earlier proposition that the dopaminergic mechanism of PRL release may be operative under this regime ( Chapter IX and its summary Chapter 9). It is interesting to note that the improvement in regenerative performance of pimozide - treated lizards was similar to that observed in earlier study with exogenous ovine PRL (see chapter VII and its summary chapter 7).

## CHAPTER XI

In chapter IX, it was demonstrated that bromocriptine had no effect on tail regeneration in

H. flaviviridis exposed to either LL or DD. The present study ( chapter XI) was a continuation of that investigation and the experimental approach was to test for a possible effect of bromocriptine in lizards obeying the alternating daily light - dark cycle. Two different doses ( 1 mg and 2 mg/kg) of bromocriptine were injected in regenerating lizards exposed to 12L : 12D while the high dose was injected in lizards exposed to 24L : OD, schedule. Both, the 1mg/kg of the drug in 12L : 12D exposed lizards and the 2mg/kg in 24L : OD, produced no effect on tail regenerating lizards. However, the 2mg/kg dose in 12L : 12 D lizards significantly retarded the process of tail regeneration, providing neuropharmacological evidence for the operation of the dopaminergic mechanism of PRL release under the 12L : 12D schedule. These findings lead to the conclusion that : (1) the effect of bromocriptine on lacertilian tail regeneration is dose dependent (2) both serotonergic and dopaminergic regulatory systems of PRL release may be functioning on par in the 12L : 12D photoregime.

## CHAPTER XII

In the present study ( chapter XII) an attempt was made to elucidate the mechanism by which

H. flaviviridis codes for day length during its tail regeneration and to ascertain the possible effect(s) of higher doses of melatonin on tail regeneration in lizards. In an earlier study ( see chapter VI) the injection of melatonin produced a dual effect in the LD 12 : 12 exposed lizards depending on the time of day the hormone was administered. Furthermore, exogenous melatonin ( 2mg/kg) had no effect on PX lizards exposed to LD12:12 condition. The present investigation was an extension of that study. Animals exposed to LL, LD 12 : 12 and DD were given 2mg/kg of melatonin either in the morning or evening while PX lizards exposed to LD12 : 12 received daily ip injection of a high dose (10mg/kg) of melatonin in the evening. The results confirmed the previous finding (chapter VI) that exogenous melatonin produced an antiregenerative effect at ddown but a proregenerative effect at dusk in the LD 12 : 12 exposed lizards. However, melatonin had no effect in regenerating lacertilians exposed to either LL or DD irrespective of the time of day the hormone was injected (the present study).A further observation showed that the high dose of melatonin restored the regenerative performance of PX lizards exposed to LD 12 : 12 to the NL level. These findings

leads to the conclusion that (1) melatonin may code for day length in lizards by integrating/synchronizing the daily LD cycles (2) tail regeneration in LL and DD photoregimes is arrhythmic to exogenous melatonin (3) the pineal organ and its putative hormone, melatonin, actively participate in the neuroendocrine mechanism that leads to sustained tail elongation in regenerating lacertilians.