GENERAL CONSIDERATIONS

The study carried out for this thesis has been divided into two main sections. In the first section, an attempt was made to investigate the effects of some external factors, such as photoperiod and seasonal (temperature) variations as well as the possible roles of the photoreceptor organs (lateral eyes and pineal organ) on the morphology of regenerating lacertilian tail. The second section was primarily concerned with neuroendocrine studies, using neuropharmacological agents, and their modulatory influences on tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis.

"That there is a small gland in the brain, the pineal, in which the soul exercises its functions more particularly than in any other part". This quotation, rendered by Rene Decartes in the 17th century, is familiar to nearly every endocrinologist and has spawned many legends concerning the alleged functions of the pineal gland. The organ characteristically has been associated with an aura of mysticism and has frequently been a topic of discussion for prophetic soothsayers. Reversing the well-ingrained tenet that the pineal is a lingering relic has

proved to be an ardous task. The decline of pristine concepts of the pineal "function" primarily was chronicled by discoveries within the last few decades. During this time, investigators have rejected speculations of their predecessors and have based their judgements on irrevocable experimental evidence. Contributing significantly to the downfall of primitive epiphyseal doctrines was the chemical isolation of a possible hormonal material from bovine pineal (Lerner et al., 1958). Probably equally as noteworthy as the identification of potentially hormonal substances was the demonstration of the exclusiveness of some of the pineal enzymatic systems (Axelrod et al., 1961). Moreover, the proposal that the pineal's biochemical activity is partially governed by radiant energy (Wurtman et al., 1963) altered appreciably the design of subsequent investigations of the organ physiologically, which hitherto had provided equivocal information (Kitay and Altschule, 1954; cf. Reiter, 1970).

The most remarkable feature of pineal organs is their exceptional morphological diversity. When considered across all the vertebrate groups, pineal architecture is surely the most varied among all organs (Oksche, 1965). Such phylogenetic lability invites curiosity about the functions of the organ and encourages much speculation. It also suggests the possibility of a lack of common physiological role, a situation that has led to considerable frustration in precisely defining the essential actions these enigmatic

structures are performing. Since there is no pan vertebrate function for the pineal gland, a brief review of some of its known functions in different vertebrate groups is probably appropriate here. The pineal gland has been implicated as a photoperiod transducer in a number of mammalian species (see Ralph et al., 1979 for reviews). Pinealectomy prevents short-day induced gonadal regression in Syrian hamsters (Hoffmann and Reiter, 1965). In long-day hamsters, implants of melatonin (a pineal gland indoleamine) mimic the effects of a short-day photoperiod (Turek et al., 1976) although this effect has not been observed in some studies (Reiter, 1980). Similar evidence regarding the role of the pineal gland in mediation of photoperiodic adjustments associated with temperature regulation has also been reported in the white-footed mouse, Peromyscus leucopus (Lynch et al., 1980). It is generally held that the pineal organ of many nonmammalian vertebrates (fishes, amphibians, and some reptiles) is directly photosensory while in other vertebrates (some reptiles, birds, and mammals) it is supplied with photoreceptor information via sympathetic pathways from the lateral eyes (Kappers, 1971).

In its most diverse form, the reptilian pineal complex is composed of a superficially situated parietal eye (parapineal) and a deeper pineal organ on the dorsal aspect of the diencephalon (see Firth et al., 1988). Some lizards

and the rhynchocephalian, Sphenodon, possess both structures, while other groups, such as turtles and snakes, possess only the pineal organ; in crocodilians, both parapineal and pineal components are apparently absent (Quay, 1979). The reptilian pineal complex transmits photic and perhaps other environmental information to the brain (Oksche, 1984). The complex is involved in numerous functions, including reproduction (De Vlaming and Olcese, 1981), circadian activity rhythms (Underwood, 1984) and thermoregulation (Firth et al., 1988). The pineal organ of lizards, the most extensively studied group of reptiles, is photosensory

. In dealing with photoperiod-sensitive ectotherms, it is often difficult to determine which are the important factors that control a physiological process. As Hoffman (1970) has pointed out, spontaneous activity, feeding, and, metabolic activity, all of which are modifiable by light and temperature, may themselves have much to do with reproductive development or regression. Indeed, Licht (1967) has shown that optimal temperatures must coincide with the longer photoperiods to demonstrate photic stimulation of reproductive condition in Anolis carolinensis. The pineal complex may exert its effects on the reproductive state in lizards indirectly by influencing one or more of the above - mentioned factors. Pinealectomy of Sceloporus occidentalis led to equivocal results regarding exposure on

the surface of the ground and locomotion, but the mean body temperature of pinealectomized animals was 1.1°C below that of sham-operated controls (Stebbins, 1960). Pinealectomy as well as light deprivation to the pineal organ of H. flaviviridis produced unequivocal results as demonstrated by the retardation in the regeneration process in lizards exposed to continuous illumination (Ramachandran and Ndukuba, 1989a, Chapter 3). The administration of exogenous melatonin, a pineal indoleamine, produced an antiregenerative effect at dawn and a proregenerative effect at dusk in intact lizards but had no effect in pinealectomized animals (Ramachandran and Ndukuba, 1989d, Chapter 6), and exogenous PRL stimulated tail regeneration in lizards exposed to continuous darkness but had no effect in pinealectomized animals (Ndukuba and Ramachandran, 1989a, Chapter 7). These findings may suggest a diurnal rhythm in sensitivity to melatonin in intact Hemidactylus as well as an intricate inter-relationship between photoperiod, pineal and PRL in lacertilians. Further, earlier studies revealed that light and temperature modulate tail regeneration in H. flaviviridis (Ndukuba and Ramachandran, 1989c, d; Chapter 1 & 5; Ramachandran and Ndukuba, 1989c, Chapter 4) and this when coupled with the finding that blinded lizards regenerated their lost (autotomized) tails like their sighted counterparts exposed to similar experimental photoregimes (Ndukuba and Ramachandran, 1988b, Chapter 2), conclusively demonstrate that the photoreceptive pineal organ, and not the lateral eyes, is

principally responsible for the transduction and translation of photic information into hormonal and/or physiological responses favouring tail elongation in Hemidactylus. Structural, biochemical and functional analogies between retinal photoreceptors and pineal transducers as well as homologies between different types of pineal transducers have been established (Collin et al., 1986; Van Veen et al., 1986). For instance, several types of proteins involved in phototransduction, enzymes of indole metabolism, melatonin, taurine are common to both retina and pineal. Several examples could prove that data obtained from retinal photoreceptors have increased our knowledge of the pineal transducers and vice versa (Collin et al., 1986; O'brien and Klein, 1986; Roman et al., 1988).

Serotonin (5-hydroxytryptamine; 5-HT) concentrations in the pineal gland are higher than in any other tissue of the body (Quay and Halevy, 1962). Furthermore, pineal concentrations of 5-HT fluctuate more than 50% each day reaching peak levels slightly after the mid-point of the light period and falling nearly 20% befofe the onset of darkness (Quay and Meyers, 1978). Secretion may account for at least part of these daily changes. The first report to imply that 5-HT is secreted by the pineal gland was by Shein and Wurtman (1971). Secretion of pineal 5-HT is also suggested by its intracellular localization within cytoplasmic vesicles resembling secretory structures (Juillard and

Collin, 1980). It has been proposed that a mechanism for regulating intracellular concentrations of 5-HT should exist in the pineal gland, if it secretes 5-HT (Ducis and DiStefano, 1980a,b). This proposition was supported by the discovery and characterization of a high-affinity uptake system for 5-HT in bovine pinealocytes (Ducis and DiStefano, 1980a,b). More recently, pineal 5-HT was identified in primate cerebrospinal fluid (Taylor et al., 1982; Garrick et al., 1983), suggesting that some 5-HT reaching the brain originates from the pineal gland. Preliminary studies by Walker and Aloyo (1985) and Walker et al., (1986) provided direct evidence for 5-HT release from rat pineals in vitro. The involvement of 5-HT in the regulation of prolactin (PRL) release is well accepted. This neurotransmitter generally has stimulatory effects on pituitary PRL secretion (Wilson, 1979). Thus the administration of 5-HT or its precursors and agonists increases serum concentrations of PRL (Ruszas et al., 1982). Moreover; inhibitors of 5-HT synthesis, 5-HT neurotoxins or 5-HT receptor blockers inhibit PRL release at pro-oestrus or PRL release induced by oestrogens or suckling (Horn and Fink, 1985; Jahn and Deis, 1987).

In some teleost species studied to date, PRL cells appear to be primarily under an inhibitory control since their activity is increased in autotransplanted pituitaries compared with in situ glands (Ball et al., 1972). In Gillichthys mirabilis (Nagahama et al., 1974) and in Poecilia

latipinna (Batten and Ball, 1977) hypothalamic type B fibres make direct contact with the PRL cells and James and Wigham (1984) suggest that these fibres form a pathway for inhibitory control of PRL secretion by the hypothalamus, based on their in vivo and in vitro studies with the trout, Salmo gairdneri. Dopaminergic fibres have been identified in the brain and pituitary gland of Mugil platanus (Zambrano, 1975), however, not in the rostral pars distalis of G. mirabilis (Swanson et al., 1975), and the results of pharmacological treatments have provided further evidence that the PRL inhibitory factor (PIF) involved in the regulation of PRL in some teleosts is dopamine (Batten and Ball, 1976). Studies with catecholamine synthesis inhibitors have unequivocally demonstrated that a catecholamine is involved in the inhibitory control of PRL release. The dopamine agonist, bromocriptine is known to depress the circulating level of PRL in several species of mammals, including the ewe (Land et al., 1980), rabbit (McNeilly and Friesen, 1978), cow (Karg et al., 1981) and man (Besser et al., 1972). There are also some indications that a stimulatory system is involved in the control of PRL secretion in teleosts. The results of the treatment of Anguilla anguilla (Olivereau and Olivereau, 1979) and Carassius auratus (Olcese et al., 1979) with precursors and other agents which alter brain 5-HT levels, have provided evidence that 5-HT may be involved in this stimulation.

Furthermore, serotonergic fibres have been identified in the brain and pituitary of A. anguilla (Fremberg et al., 1977). In addition, Wigham et al., (1977) investigated the in vitro effects of a number of other putative regulatory factors, including GABA, cortisol, somatostatin and TRH, on PRL secretion in Sarotherodon mossambicus and concluded that some may have a role in the regulation of PRL cells in this species. The in vivo and in vitro studies by James and Wigham (1984) provided direct evidence for the presence of both dopaminergic and serotonergic regulation of PRL cell activity in S. gairdneri. Daily intraperitoneal injection of bromocriptine to intact H. flaviviridis exposed to either continuous light or continuous darkness did not affect the regeneration process (Ramachandran and Ndukuba, 1989c, Chapter 9), while daily injection of a high dose of p-CPA, an agent used for chemical pinealectomy, significantly retarded tail regeneration in lizards exposed to continuous illumination (Ramachandran and Ndukuba, 1989b, Chapter 8). The failure of bromocriptine to retard tail regeneration in animals exposed to continuous light, coupled with the observation of a 50% retardation effect with p-CPA, suggest that serotonergic and not dopaminergic mechanism of PRL release is operative under this schedule. Further experimentation with serotonin receptor antagonists, such as cyproheptadine, methysergide, or SQ10,631 is necessary to conclusively support this contention. It may be tentatively surmised that bromocriptine failed to retard tail regeneration in lizards maintained in

Continuous darkness because the dopamine receptors at the pituitary lactotrophs are fully saturated with dopamine, thereby leaving no available sites for its agonist to bind. However, the injection of the antipsychotic drug, pimozide, a potent dopamine receptor blocker (see Clemens, 1976), enhanced the regenerative performance of lizards kept in continuous darkness (Ndukuba and Ramachandran, 1989b, Chapter 10). Experimental evidence now exists, from recent studies in our laboratory, that bromocriptine retards tail regeneration in lizards exposed to 12L : 12D, indicating the existence of the dopaminergic mechanism in this regime (Ndukuba and Ramachandran, 1989e, Chapter 11). Further evidence suggests that exogenous melatonin produced a dual effect in regenerating lacertilians exposed to LD 12 : 12; an antiregenerative effect at dawn and a proregenerative effect at dusk but did not affect lizards exposed to constant photoperiods (LD 24:0 and 0:24). Moreover, a low dose of melatonin ($2mg.kg^{-1}$) did not alter the regenerative performance of PX lizards exposed to LD 12:12 but a high dose of $10mg.kg^{-1}$ restored the regenerative ability of PX lizards to near the NL level (Ramachandran and Ndukuba, 1989a, Chapter 6; Ndukuba and Ramachandran 1989f, Chapter 12).

These results may suggest that in lizards, as in teleosts (James and Wigham, 1984), both dopaminergic and serotonergic mechanisms of PRL release are operative. In lacertilians, it is presumed that both the systems are operative on par at the intermediate photoperiodic regimen of 12 hours of light and 12

hours of darkness. With increasing photoperiodism, there is a direct antagonism by 5-HT of the dopaminergic system that inhibits PRL release. The antagonistic effect of 5-HT on the dopaminergic system probably reaches the peak in continuous light with the serotonergic neurons fully activated. With decreasing photoperiodism, the dopaminergic mechanism becomes activated and a direct antagonism by dopamine of the serotonergic system that stimulates PRL lrease occurs. This may attain its peak in continuous darkness where the dopaminergic neurons are fully activated, (for detailed discussions of the serotonergic and dopaminergic mechanisms of PRL release during lacertilian tail regeneration, see chapters 9-12).

It is proposed that melatonin codes for day length in lizards by integrating/synchronizing the daily LD cycle, and the pineal and its putative hormone, melatonin activity participate in the neuroendocrine mechanism that leads to sustained tail elongation in regenerating lacertilians.

Waddington (1935) has suggested that "the fundamental fact about cancerous tissue is that it has escpaed from the normal growth controlling agents of the body". These "growth controlling" agents, called individuation fields, are highly potent in animals that are capable of regeneration. During regeneration, the primitive cells initially multiply rapidly with a certain abandon resembling that of a cancerous growth. But unlike a cancer, which would continue to grow in a disorderly

fashion, the regenerating tissue eventually grows at a slower rate and gradually takes the form of a functional organ. The present thesis, although far from forming a bridge between regeneration and cancer, has certainly done its bit in opening a new frontier for students of vertebrate regeneration. And since regeneration and the multiplication of cancer cells are both governed by same principles of differentiation, high mitotic activity and cell proliferation, the work reported here may, with further extension, contribute to a better understanding and possible control of one of man's most dangerous afflictions-cancer.