INTRODUCTION

The ability to shed or readily loose appendages as a method of escape from enemies is known as autotomy (self-amputation) and is quite widespread throughout the animal Kingdom. Among invertebrates, it is found in certain molluscs, crustaceans and echinoderms (McVean, 1975). Among the vertebrates, the tail appears to be the only organ that can be lost in this way. The capacity for caudal autotomy is developed to varying degrees in certain salamanders (Arnold, 1984), in the tuatara (Sphenodon) and many lizards (Guibe, 1970), in some amphisbaenians (Gans, 1978), in a few snakes and in certain rodents (McVean, 1975). It is, however, interesting to note that autotomy seems to be unknown in fishes, despite the diversity of their structure and habits. In the context of autotomy, regeneration may be defined as the ability to reproduce relatively complicated organs or structures after they have been lost through trauma or other causes. Although organs that can be autotomized usually have some power of regeneration, the two phenomena are not necessarily linked, and regeneration is much more widespread than autotomy. Examples of renewable organs among the vertebrates are the fin rays of certain teleostean fishes, the limbs and tails of larval urodele amphibians, the mammalian liver and the antlers of deer (Cervidae) which are physiologically shed and replaced each year (Goss, 1969). Among the reptiles, only the tail (in Sphenodon and most lizards) and, probably, the shell in Testudines have extensive powers of regeneration. It is obviously difficult to define the border between regeneration and reparative processes, such as wound healing and the renewal of skin after trauma (Maderson *et al.*, 1978) as well as the normal processes, such as skin-shedding and tooth replacement (Lillywhite and Maderson, 1982).

Regeneration is customarily distinguished into two types *viz.*, physiological regeneration and reparative regeneration. Physiological regeneration is the restoration of the lost elements of a living organism during its normal day to day activity (*eg.*epithelial and red blood corpuscles regeneration). Physiological regeneration is a universal phenomenon characteristic of tissues and cells of animals, plants and micro-organisms; and it is not lost during phylogenesis and ontogenesis. On the contrary, reparative regeneration is restoration of lost parts caused by trauma or damage, and is restricted to lower animals, while in higher animals it is limited to such an extent that in man even minor wounds often leave scars after they are healed. Wound healing in higher vertebrates, especially man, is far from ideal, and regenerative capacity is highly restricted. Studies now underway in different laboratories on this aspect, attempt to minimize or even eliminate scarring, expedite the healing process and possibly activate regeneration of lost appendages. Maderson and Salthe (1969) have suggested that poorly controlled wound healing is a good factor for regeneration. Regeneration, like other biological processes, is susceptible to analysis at various levels of organization. It occurs in varying degrees in plants and animals. Goss (1969) in his treatise on "Principles of Regeneration" has opined that if there were no regeneration there could be no life, if everything regenerated there could be no death. And the process of regeneration of an organ can be considered as an extension on a grander scale of the process of wound healing (Bullough and Laurence, 1966). According to Morgan (1901) regeneration is the resumption of temporarily arrested growth. However, the numerous studies that followed showed that regeneration is something more than mere growth; it includes transformation, differentiation and quantitative and qualitative changes in metabolic profile to restore the lost parts of an organism. Therefore, it appears that the capacity to regenerate a lost appendage in a vertebrate would depend on both the local potentiality as well as the ability to evoke specific, synergistic, supportive and permissive systemic responses (Valsamma, 1982).

The influence of external factors like temperature, humidity, light and electric current on appendage regeneration has been well documented (see Borgens *et al.*, 1979), and the influence of light on tail regeneration in the lizard, *Anolis carolinensis*, has been reported (Turner and Tipton, 1972).

However, there is no report on the influence of photoperiodism on tail regeneration in a tropical saurian. Hence, the present study attempts to investigate the influence of increasing or decreasing photoperiodim on tail regeneration in the Gekkonid lizard, <u>Hemidactylus</u> <u>flaviviridis</u> (chapter 1).

A feature of every vertebrate pineal is its capacity to synthesize different indoleamines including serotonin (5-hydroxytryptamine) and melatonin (5-methoxy-N-acetyltryptamine). The daily rhythmic secretion of the pineal indoleamine, melatonin, is able to interpret seasonal and photoperiodic changes (for references see, Tamarkin <u>et</u> <u>al</u>., 1985; Skene <u>et</u> <u>al</u>., 1987). Light and temperature are two important stimuli that can affect pineal melatonin levels in vertebrates, and their variations alter the levels of nocturnal melatonin secretion in some nonmammalian vertebrates, such as the quail (Underwood and Siopes, 1985), the rainbow trout (Diston and Bromage, 1987), the laying chicken (Liou <u>et</u> <u>al</u>., 1987), the box turtle (Vivien-Roels <u>et</u> <u>al</u>., 1988) and the anole lizard (Underwood, 1985). It is also known, at least in homeotherms, including man, that light is the main environmental stimulus affecting pineal melatonin levels whereas both light and temperature can control pineal melatonin levels in poikilotherms (Birau and Schlooi, 1981; Underwood, 1985). The pineal complex of lizards, the most extensively studied group of reptiles, is photosensory (Wurtman et al., 1968; Gundy and Wurst, 1976). And since it has been shown (Turner and Tipton, 1972) that long-length photoperiods stimulate tail regeneration in *A. carolinensis*, it was thought pertinent to study the possible roles of the photoreceptor organs (lateral eyes and pineal organ) on photoperiodic photoreception in *H. flaviviridis* during its tail regeneration (chapters 2 and 3) and across the seasons (chapters 4 and 5). The results may indicate a positive influence of increasing photoperiodism and a negative influence of decreasing photoperiodism on tail regeneration in *H. flaviviridis* (chapter 1), ~~and~~ Furthermore, the lateral eyes, or retinae, do not participate in this photoperiodic influence as blinded lizards regenerate their lost (autotomized) tails like their sighted counterparts exposed to similar experimental photoperiodic conditions (chapter 2). Moreover, it has been shown that the pineal organ is the principal site of extraretinal photoreception in Hemidactylus since both pinealectomy as well as light deprivation to the pineal organ abolished the stimulatory influence of increasing lengths of light and significantly retarded the regeneration process (chapter 3). It has also been demonstrated that seasonal (temperature) variations have pronounced influences on lacertilian tail regeneration; the best regenerative performance was observed during the summer season (30°C) and the worst performance in the winter months (17°C) with the regenerative performance during the monsoon season (26°C) in between (chapter 4).

The fine thermosensitivity function of the pineal organ is clearly revealed at temperature ranges above the optimal (monsoon temperature - 26°C) as pinealectomized lizards exhibited minimal variations at temperature ranges above the optimal (chapter 5).

During the last few decades, studies have been carried out to reveal the factors that either suppress or entirely inhibit repair and regenerative processes among vertebrates. Such inhibition has been accomplished by a wide array of physico-chemical means, including application of X-rays, ultraviolet irradiation, antimetabolites and other toxic agents as well as induced lack or excess of endocrine hormones. There is now considerable evidence to indicate that the pineal organs of all vertebrate classes are capable of synthesizing and secreting indoleamines, the most widely studied of which is melatonin, a putative hormone secreted principally although not exclusively by the pineal organ (Ralph, 1981;,Gern and Greenhouse, 1988; Vivien-Roels et al., 1988). Prolactin (PRL), another putative vertebrate hormone, is known to be involved in the regulation of a wide variety of biological processes, including osmoregulation, growth and development, and reproduction (Nicoll et al., 1980). In fishes, PRL is predominantly involved in osmoregulation prompting Nicoll (1981) to suggest that this action may have been the original vertebrate PRL regulatory function. In Amphibia, PRL is known to be implicated in the developmental, osmoregulatory and reproductive processes (Delidow et al.,1988) and exogenous PRL is actively involved in transcutaneous Na^+ transport in the Japanese newt, *Cynops pyrrhogaster* (Takada and Komazaki, 1988). Other studies indicate that PRL promotes growth of target organs in rats and pigeons by both direct and indirect actions. The direct effect involves stimulation of the liver to secrete a PRL - synergizing factor, synlactin (Nicoll *et al.*, 1985; Mick and Nicoll, 1985). Subsequent studies have shown that synlactin is actively secreted by the liver of a number of species representing all major classes of vertebrates (Delidow *et al.*, 1986). In rats (Nicoll *et al.*, 1985), bull frog tadpoles, and one teleost fish (Delidow *et al.*, 1986), hepatic secretion of synlactin was correlated with physiological states in which PRL is known to be active. There are reports on the influence of exogenous melatonin in reptilian gonadal development (Levey, 1973, Haldar, 1977; Thapliyal and Haldar, 1979; Underwood, 1981) and of exogenous PRL in appendage regeneration of Amphibia (see Maier and Singer, 1981). No comparative studies have addressed the problem of tail regeneration in lizards in general and Hemidactylus in particular. Hence, the present study was intended to elucidate the possible roles of these two putative hormones on lacertilian tail regeneration. The results are the first which demonstrate that the time of administration of the indoleamine, melatonin, determines its proregenerative or antiregenerative effect in a lizard and that the~~ir~~re is an intricate inter-relationship between photoperiodism, pineal and PRL. The administration of exogenous melatonin to intact Hemidactylus at dawn produced an antiregenerative effect and a proregenerative effect at dusk but has no effect on pinealectomized animals, leading to the suggestion that the diurnal rhythm in sensitivity to melatonin in the intact lizards results from some aspect of pineal function (chapter 6). Exogenous PRL stimulated tail regeneration in intact, but not pinealectomized, animals maintained in continuous darkness, indicating that the pineal organ is somehow linked to the favourable influence of PRL on tail regeneration in lacertilians (chapter 7).

Neuroendocrine studies using neuropharmacological agents have amply demonstrated that dopamine has an inhibitory role in the control of PRL release (see Clemens, 1976). It is now well documented that dopamine is the main inhibitory regulator of pituitary PRL secretion and that it exerts its effects directly at the level of the lactotroph (for details and references see Fernandez-Ruiz et al., 1981). The dopamine agonist, bromocriptine is known to depress the circulating level of PRL in several species of mammals (see Knight et al., 1986). The indoleamine serotonin (5-HT) has often been implicated as a neurotransmitter which influences anterior pituitary function by way of the hypothalamic release of release-inhibiting factors. 5-HT neurons are known to terminate diffusely in the rat hypothalamus, with particularly strong concentrations in the suprachiasmatic nucleus (SCN), the median eminence (ME), and in the area of the paraventricular nucleus (PVN) (Hutchison, 1978). Similarly, 5-HT-containing neurons have been observed in the paraventricular organ of pigeons, quail, and ducks (Calas and Bolster, 1980; Oksche and Hartwig, 1980), in the preoptic and supraoptic nuclei of quail (Oksche and Hartwig, 1980), and in the ME of ducks and quail (Calas and Bolster, 1980). Thus, both the avian and mammalian hypothalamus are well innervated by serotonergic neurons, providing anatomical evidence that this monoamine could modulate hypothalamic neurosecretory function (see Hargis and Burke, 1984). Parachlorophenylalanine (p-CPA) has been shown to selectively decrease the concentration of 5-HT in the brain without altering the concentration of either noradrenaline or dopamine. This selective action is probably affected by inhibition of the enzyme tryptophan hydroxylase (Koe and Weisman, 1966; Walker, 1982). Blockage of 5-HT synthesis by p-CPA completely inhibits the rise in PRL that is normally associated with the return of broody turkeys from cages to the nest (ElHalawani *et al.*, 1980). Quipazine maleate, a 5-HT agonist, has been shown to increase basal PRL levels when administered systemically to male chickens (Rabii *et al.*, 1981), as well as to young male turkeys (Fehrer *et al.*, 1982). Thus, limited, indirect evidence indicates that 5-HT has a stimulatory role in the regulation of PRL secretion in birds. In mammals, p-CPA has been reported to block the surge of PRL as well as luteinizing hormone (LH) and affects the secretion of oestradiol-17β (Horn and Fink, 1985). Studies with catecholamine synthesis inhibitors have unequivocally demonstrated that a catecholamine is involved in the inhibitory control of PRL release (see Clemens, 1976). The catecholamine antagonists block the action of the catecholamine on its receptor. The antipsychotic drugs were a good source of catecholamine receptor blockers, because among most antipsychotic drugs, a positive correlation between dopamine receptor blocking ability and antipsychotic potency exists. Out of the group of antipsychotic drugs, only pimozide is known to be a specific blocker of dopamine receptors over a limited dose range. While most of the drugs appear to implicate some monoamine as being involved in the inhibitory control of PRL secretion, only the studies with pimozide clearly focus on dopamine in inhibiting PRL release (Clemens *et al.*, 1974).

The present study is the first to investigate the mechanism of PRL release during lacertilian tail regeneration, using pharmacological agents. Daily intraperitoneal injection of bromocriptine to intact lizards exposed to either continuous light or continuous darkness did not alter the regeneration process (chapter 9), while daily intraperitoneal administration of high dose of p-CPA, the serotonin synthesis inhibitor and an agent employed for chemical pinealectomy, significantly retarded the regeneration process in lizards exposed to continuous illumination, indicating that lizards with physically intact pineals but deprived of their ability to synthesize

5-HT do not exhibit the favourable influence of light on tail regeneration (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 may suggest that serotonergic and not dopaminergic mechanism of PRL release is operative under this regimen. It may be tentatively surmised that bromocriptine failed to retard the regeneration process in lizards kept in continuous darkness because the dopamine receptors at the lactotrophs are fully saturated with dopamine, thereby leaving no available sites for its agonist to bind. This contention is substantiated by the observation that daily intraperitoneal injection of pimozide, a potent dopamine receptor antagonist, significantly enhanced the regeneration process in animals maintained in continuous darkness (chapter 10). Thus, with the results enumerated above, it may be conclusively stated that both serotonergic and dopaminergic mechanisms of PRL release are operative in H. flaviviridis during its tail regeneration. Evidence now exists (chapter 11) that the two systems may be operative on par, at the intermediate photoperiodic regimen of 12 hours of light and 12 hours of darkness (LD 12:12), since tail regeneration in lizards exposed to this regime was significantly retarded by bromocriptine treatment. Further experimental evidence suggests that melatonin codes for day length in regenerating lacertilians exposed to LD 12 : 12 since exogenous melatonin produced a dual effect in lizards exposed to this schedule but had no effect in animals exposed to photoperiodic extremes (LD 24 : 0 and 0:24) (chapter 12).