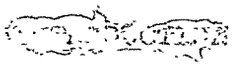
CHAPTER IX

FAILURE OF THE DOPAMINE AGONIST, BROMOCRIPTINE, TO RETARD TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS* EXPOSED TO CONTINUOUS LIGHT AND CONTINUOUS DARKNESS.



Previous experimental studies have shown that amphibian regeneration, especially the early stages, is highly dependent on hormonal participation (Rose, 1964). Prolactin (PRL) has been established as a growth promoter in developing organisms (Crim, 1975) and in regenerating systems of Amphibia (see Maier and Singer 1981) and has been shown to stimulate, protein synthesis in developing tadpoles (Yamaguchi and Yasumasu, 1977). Niewelinski (1958) and Waterman (1965) demonstrated that PRL enhanced regeneration in the non-hypophysectomized newt, in fact, PRL was more effective in this respect than growth hormone. There is strong experimental evidence that limb regeneration can proceed in hypophysectomized newts if they are kept on a prolactin-thyroxine regime (Connelly *et al.*, 1969). In the iguanid lizard, *Anolis carolinensis*, PRL is shown to influence several growth-promoting processes, including appetite, oxidative metabolism, and metabolic patterns involving protein synthesis and lipid storage (Light and Jones, 1967). Evidence exists that tail regeneration can be restored

to near normal levels in hypophysectomized lizards if they are treated with a combination of growth hormone, prolactin, gonadotropin and thyrotropin (Light and Howe, 1969). And numerous hormones have been shown to synergize with PRL in other vertebrates, producing pronounced physiological responses (Meier and Farner 1964).

It is now well documented that dopamine is the main inhibitor 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., 1987). Secretion of PRL from the anterior pituitary gland is primarily under negative control by the hypothalamus (Meites and Clemens, 1972) and dopamine appears to be the hypothalamic inhibitory factor (Macleod, 1976). The evidence is that dopaminergic agonists inhibit PRL secretion in intact animals (Takahara, et al., 1974). In addition, the inhibitory activity found in hypothalamic extract has properties of catecholamines (Shaar and Clemens, 1974). To date, no study has attempted to investigate the problem of the mechanism of PRL release during saurian tail regeneration. Most investigations on the inhibitory role of dopamine and its agonists have focussed on mammalian species as models. Bromocriptine (2-bromo-α-ergocryptine), a potent dopamine agonist has been shown to reduce PRL levels to normal in patients with functional hyperprolactinemia as well as those with pituitary tumors (Thorner et al., 1980a). Furthermore, there is now increasing evidence that the drug may lead to a reduction in tumor size of PRL- and

growth hormone secreting adenomas in upto 40% of cases (Thorner et al.,1980b). Bromocriptine inhibits colony formation by rat pituitary turmor cells (Melmed, 1961) and has been shown to effectively inhibit pituitary tumor growth and PRL secretion in rats (Vrontakis, et al.,1987). Bromocriptine has been widely used in man for the treatment of prolactinomas (Archer et al., 1982).

Recent studies in our laboratory have shown that continuous light stimulates tail regeneration while continuous darkness depresses the same in Hemidactylus flaviviridis. (Ndukuba and Ramachandran 1989 Chapter 1). It was also shown that exogenous PRL can overcome the depressive effect of continuous darkness, thereby confirming our earlier suggestion that the stimulatory effect of continuous light is mediated by increased PRL release (Ndukuba and Ramachandran, 1989a Chapter 7). The present investigation was designed to test whether bromociptine, a dopamine agonist, can reduce the purported light stimulated PRL release and thereby retard tail regeneration in lizards exposed to continuous light.

MATERIALS AND METHODS

A total of 60 lizards was used for this investigation and they were balanced for size and sex in order to eliminate any error in the final statistical analysis due to size and sex differences. They were then divided into six groups of 10

lizards each and exposed to the two photoperiodic extremes. Food and waterware provided ad libitum throughout the period of the experiment.

Group 1. Bromocriptine treated (1mg kg^{-1} body mass).

The first group of 10 lizards, exposed to LD 24 : 0 schedule, received once daily intraperitoneal (ip) injection of 1mg kg^{-1} bromocriptine, 5 days prior to and 50 days after tail autotomy.

Group 2. Bromocriptine treated (1mg kg^{-1} body mass)

A second group of 10 lizards, maintained in the LD 0 : 24 condition, received once daily ip injection of 1mg kg^{-1} bromocriptine, 5 days prior to tail autotomy and 50 days thereafter.

Group 3. Bromocriptine treated (2mg kg^{-1} body mass)

The third group of 10 animals, exposed to LD 24 : 0 received once daily injection of 2mg kg^{-1} bromocriptine, 5 days prior to and 50 days after tail autotomy.

Groups 4-6. Saline treated controls (0.6%).

The fourth, fifth and sixth groups of 10 lizards each received once daily ip injection of 0.6% saline, 5 days prior to tail autotomy and 50 days thereafter. These served as controls for groups 1, 2 and 3 experimental animals.

Preparation of bromocriptine (1mg and 2mg kg^{-1}).

Bromocriptine (2-bromo-α-ergocryptine, as proctinal by

Biddle Sawyer Pvt. Ltd., Bombay, India) was prepared and stored in 4°C in a refrigerator for the daily injections. The drug was weighed and dissolved in few drops of ethanol. Warmed (40°C) Saline (0.6%) was then added to give the required concentration. For the high dose of 2mg/kg^{-1}, twice the weight of bromocriptine used for the low dose (1mg) preparation was used. 0.1ml of the prepared solutions was injected daily giving an approximate daily dose of 10µg/animal(Low) and 20µg/animal (high)

Preparation of saline (0.6%).

0.6gm of reagent grade sodium chloride (NaCl) was dissolved in 100ml re-distilled water with few drops of ethanol and stored in a refrigerator for daily use.

Experimental design.

Two photoperiodic conditions were investigated: continuous light (LL :LD 24 : 0) of 2500 lx intensity and continuous darkness (DD : LD 0 : 24). The detailed description of the light schedules and the dimensions of the cages that housed the animals have been well documented on pages 12 and 13.

Statistical analysis

The length of new growth (regenerate) in mm, was measured with a graduated meter rule and recorded at fixed time intervals of 5, 10, 20, 30, 40 and 50 days after tail autotomy. The

measurements were later used for morphometric calculations. This investigation was conducted during the monsoon months of September and October and the average daily cage temperature at the level of the animals in the lighted and dark chambers was 27°C and 25°C, respectively. The data on the length of tail regenerated and total percentage replacement were subjected to student's t test and further to Duncan's multiple range test (Duncan, 1955) for statistical significance. Values which were different at the $P < 0.05$ level were considered to be statistically significant.

RESULTS

Growth rate, total length of tail regenerated and total percentage replacement.

The regeneration blastema appeared in bromocriptine (both 1mg and 2 mg/kg^{-1}) and saline treated lizards exposed to the LD 24 : 0 schedule by day 7 and in those kept in LD 0 :24 by day 14 post-caudal autotomy. The total length of tail regenerated by the 50th day in control lizards exposed to LD 24 : 0 and injected once daily with 0.6% saline was 39.3mm and 37.8mm, and in their counterparts injected with 1mg and 2mg/kg^{-1} bromocriptine, 38.4 mm and 37.2 mm respectively, representing a replacement of 74.1% and 68.7% in the controls, and 72.4% and 67.6% in the experimentals respectively. In animals exposed to LD 0:24, the same was 26.3 mm and 26.1mm respectively, which was a replacement of 49.5% and 49.2% respectively

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN BROMOCRIPTINE TREATED AND CONTROL LIZARDS H.FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT AND CONTINUOUS DARKNESS.

EXPERIMENTAL ANIMALS AND PHOTOREGIMES	WOUND HEALING	BLASTEMA	EARLY DIFFEREN-TIATION	MID DIFFER-ENTIATION	LATE DIFFEREN-TIATION	GROWTH
LD 24 : 0						
Bromocriptine (1mg/Kg)	3	5 - 7	7 - 9	10	20	30*
Bromocriptine (2mg/Kg)	3	5 - 7	7 - 9	10	20	30
Saline control (0.6%)	3	5 - 7	7 - 9	10	20	30
LD 0 : 24						
Bromocriptine (1mg/Kg)	8	12 - 14	16 - 18	25	40	45
Saline control (0.6%)	8	12 - 14	16 - 18	25	40	45

LD 24 : 0 - CONTINUOUS LIGHT (24 HOURS OF LIGHT AND 0 HOURS OF DARKNESS)
LD 0 : 24- CONTINUOUS DARKNESS(0 HOURS OF LIGHT AND 24 HOURS OF DARKNESS)
* - DAYS POST - CAUDAL AUTOTOMY

TABLE 2. LENGTH OF TAIL REGENERATED AND TOTAL PERCENTAGE REPLACEMENT IN BROMOCRIPTINE TREATED AND CONTROL H. FLAVIVIRIDIS EXPOSED TO PHOTOPERIODIC EXTREMES DURING THE MONSOON SEASON. (±SD).

EXPERIMENTAL ANIMALS AND PHOTOREGIMES	DAY 10	DAY 20	DAY 30	DAY 40	DAY 50	TOTAL % TAIL REPLACE-MENT
24L : 0D						
BROMOCRIPTINE TREATED	12.9 ± 2.07	17.9 ± 2.38	28.7 ± 3.46	35.4 ± 2.45	38.4* ± 1.95	72.5%
SALINE INJECTED (CONTROL)	16.4 ± 2.05	20.1 ± 2.34	29.5 ± 3.32	37.1 ± 2.42	39.3 ± 2.41	74.1%
0L : 24D						
BROMOCRIPTINE INJECTED	- -	5.7 ± 1.61	13.7 ± 1.18	21.3 ± 1.55	26.1 ± 1.81	49.2%
SALINE TREATED (CONTROL)	- -	5.1 ± 1.57	13.8 ± 1.93	21.8 ± 2.31	26.3 ± 2.00	49.6%

24L : 0D - 24 HOURS LIGHT AND 0 HOURS DARKNESS (CONTINUOUS LIGHT)
0L : 24D - 0 HOURS LIGHT AND 24 HOURS DARKNESS (CONTINUOUS DARKNESS)
* - TOTAL LENGTH OF REGENERATED IN mm.

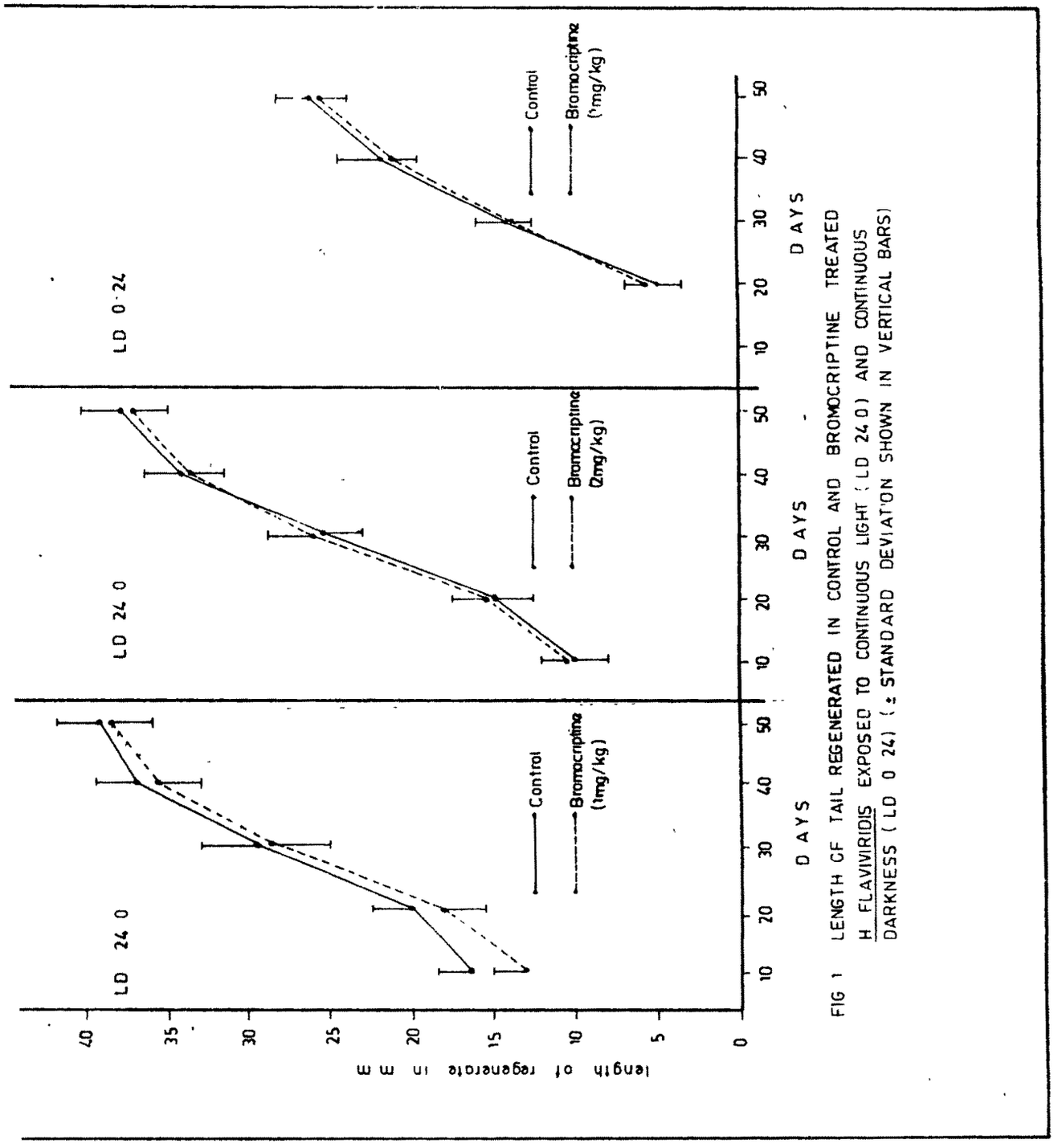


FIG 1 LENGTH OF TAIL REGENERATED IN CONTROL AND BROMOCRIPTINE TREATED H. FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT (LD 24 0) AND CONTINUOUS DARKNESS (LD 0 24) (± STANDARD DEVIATION SHOWN IN VERTICAL BARS)

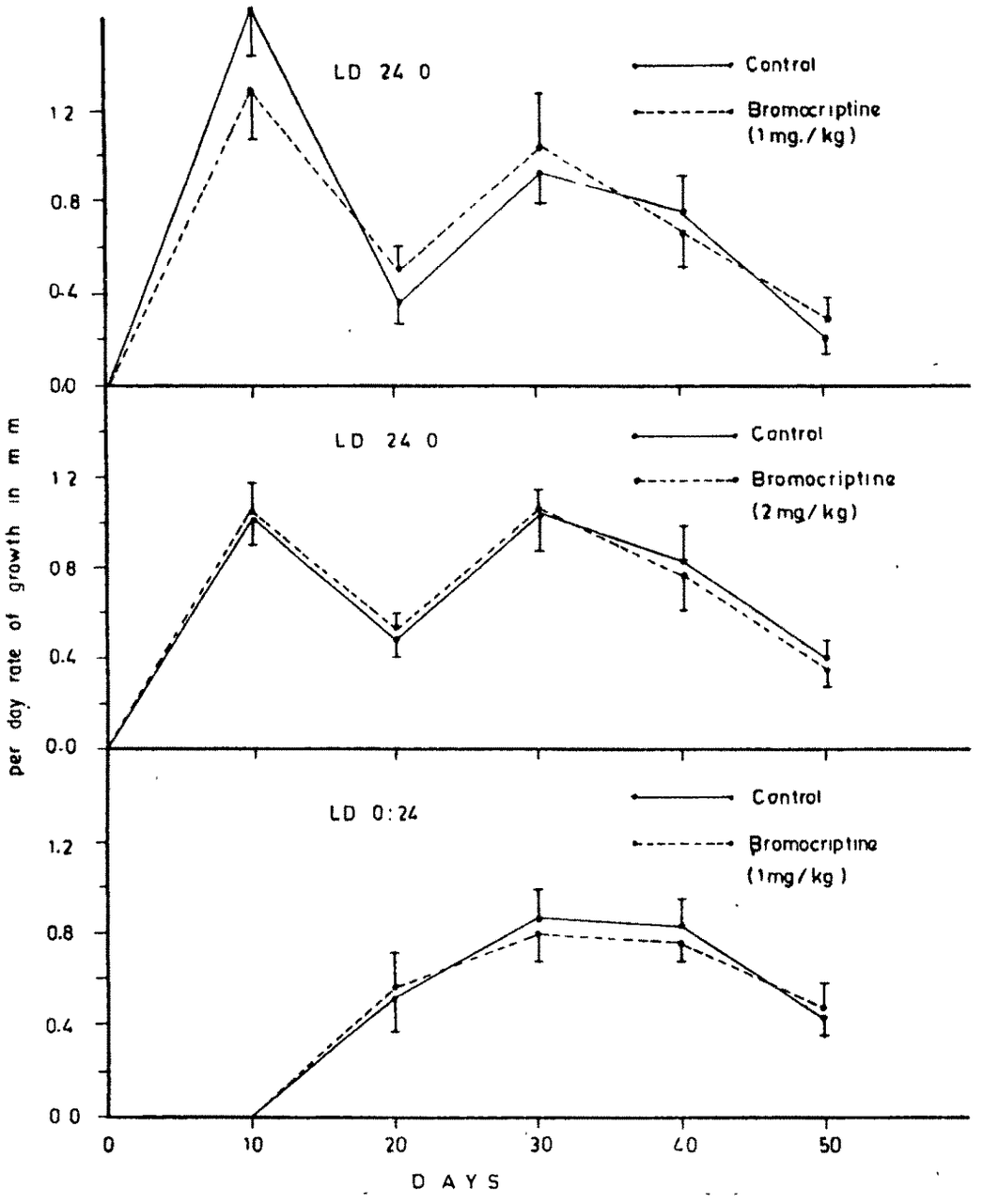


FIG 2 PER DAY RATE OF GROWTH OF TAIL REGENERATE IN CONTROL AND BROMOCRIPTINE TREATED H FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT (LD 24 0) AND CONTINUOUS DARKNESS (LD 0 24) (± STANDARD DEVIATION, SHOWN BY VERTICAL BARS)

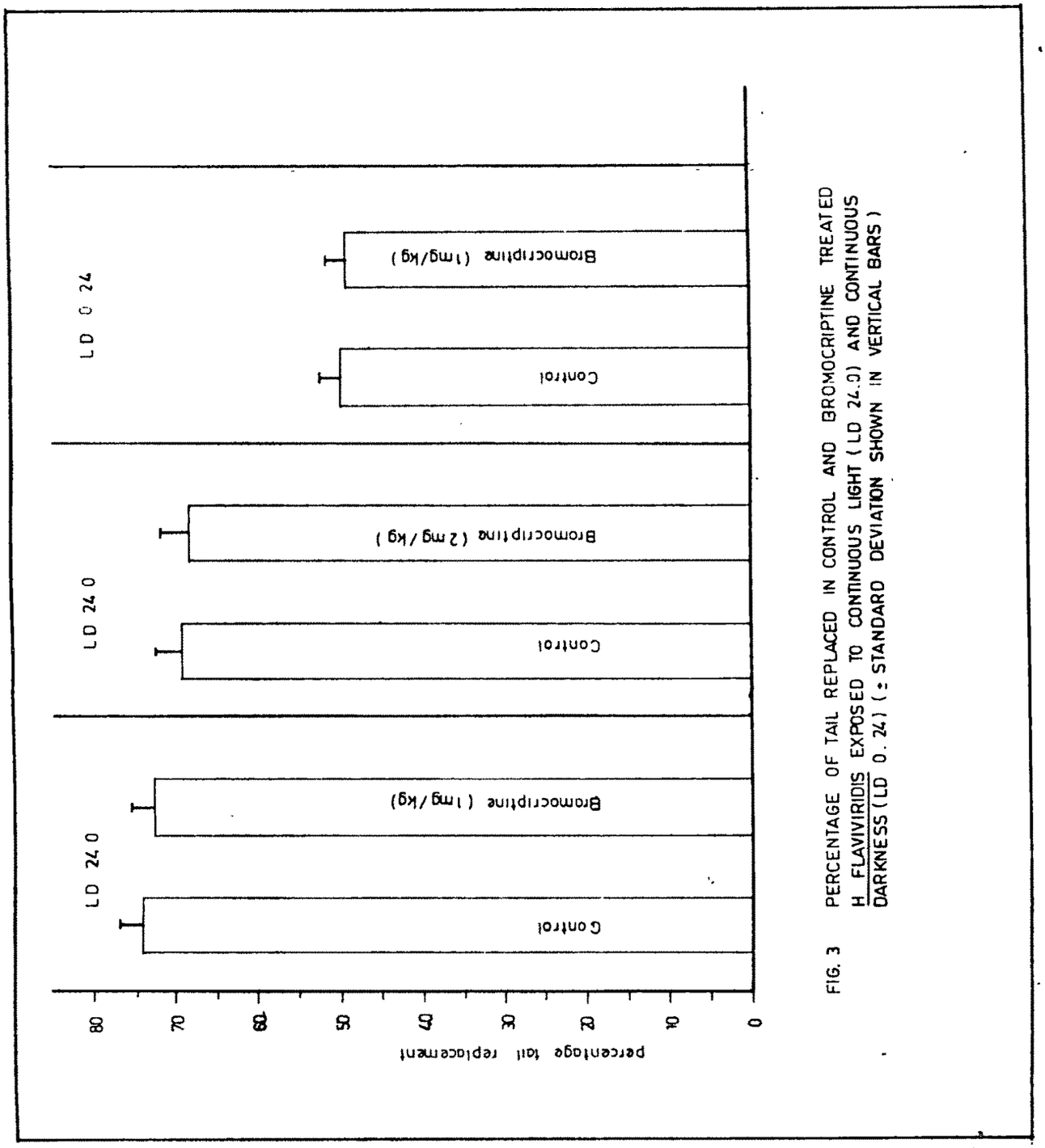


FIG. 3 PERCENTAGE OF TAIL REPLACED IN CONTROL AND BROMOCRIPTINE TREATED H. FLAVIVIRIDIS EXPOSED TO CONTINUOUS LIGHT (LD 24.0) AND CONTINUOUS DARKNESS (LD 0.24) (± STANDARD DEVIATION SHOWN IN VERTICAL BARS)

(Figs. 1 and 3). The pattern of growth rate (Fig.2) indicates a biphasic growth rate curve in the LD 24 : 0 exposed animals (one during the first 10 days and the second between 20-30 days) and a linear increase peaking at 20-30 days in the lizards maintained in the LD 0:24 regime. All possible comparisons between saline- and bromocriptine-treated lizards in LD 24:0 on one hand and saline - and bromocriptine-treated animals in LD 0:24 on the other (Student's 't' test) showed no statistically significant difference. However, comparisons of the differences between the groups of animals in LD 24 : 0 and 0 : 24 were statistically significant at the 5% level (Duncan, 1955).

DISCUSSION

The results show that once daily intraperitoneal injection of two different doses of bromocriptine (1 mg and 2mg kg^{-1}) to lizards exposed to LD 24 : 0 and 1mg kg^{-1} to those kept in LD 0 : 24 did not affect the regeneration process when compared with their counterparts injected with 0.6% saline. The initiation of regeneration, the daily growth rate, the total length of new growth (regenerate) produced by day 50 and the total percentage replacement of the lost (autotomized) tails at the end of 50 days did not show any statistically significant difference in either LD 24 : 0 or 0 : 24 exposed animals when compared with their respective saline-treated controls.

It was previously reported that continuous light stimulates

tail regeneration in the lizard, Hemidactylus flaviviridis while continuous darkness depresses it (Ndukuba and Ramachandran, 1989c). It has also been shown that the pineal organ is the principal site of extraretinal photoreception in H. flaviviridis, since, both pinealectomy as well as light deprivation to the pineal abolished the stimulatory influence of continuous illumination and significantly retarded the regeneration process (Ramachandran and Ndukuba, 1989a). Chapter 3 Evidence now exists that parachlorophenylalanine (p-CPA), an inhibitor of serotonin (5-HT) synthesis at the level of the enzyme tryptophan hydroxylase (Koe & Weissman 1966; Walker 1983) and an agent used for chemical pinealectomy, produced similar retardation effect as did complete pineal ablation, indicating that lizards with physically intact pineals but deprived of their ability to synthesize 5-HT failed to exhibit the favourable influences of light on tail regeneration in lacertilians (Ramachandran and Ndukuba, 1989b). Chapter 6 And exogenous PRL stimulated the regeneration process in normal (NL), but not pinealectomized (PX) lizards kept in continuous darkness, prompting the authors (Ndukuba and Ramachandran, 1989a) Chapter 7 to suggest that the pineal organ is somehow linked to the favourable influence of PRL on tail regeneration in lizards.

Neuropharmacological studies have amply demonstrated that dopamine has an inhibitory role in the control of PRL release (Fernandes-Ruiz, et al., 1987). Chronic hyperprolactinaemia increases dopamine turnover in the tuberoinfundi-

bular dopaminergic neurons in the median eminence (Demarest, et al., 1984), consistent with a positive feedback, allowing plasma PRL to control its own secretion. Evidence abounds that inhibition of anterior pituitary PRL secretion is regulated mainly by dopamine released from the median eminence terminals (see Gallardo, et al., 1985, for references). PRL secretion is inhibited by ergot alkaloids or their derivatives such as bromocriptine (Knight, et al., 1986). Bromocriptine treatment results in normalization of the circulating PRL levels, and return of ovarian function in lactating mice (Knight, et al., 1986).

The present study is the first investigation aimed at characterizing the mechanism of PRL release during tail regeneration in saurians, using neuropharmacological agent(s). Once daily intraperitoneal injection of 1 mg or 2mg. kg^{-1} bromocriptine failed to retard tail regeneration in the LD 24:0 exposed animals, and coupled with our earlier observation of a 50% retardation effect with p-CPA (Chapter-8), suggest that serotonergic and not dopaminergic mechanism of PRL release is operative under this regime. The failure of bromocriptine to affect the regeneration process in lizards kept in the LD 0:24 condition deserves a tentative explanation. Our previous reports that regenerating lacertilians maintain basal PRL levels as was deduced from 50% regenerative ability in PX lizards (Ramachandran and Ndukuba, 1989a, Chapter 3) and in the LD 0:24 exposed NL animals

Ndukuba and Ramachandran, 1989a) may be relevant in this context. We have recently demonstrated that exogenous PRL stimulated tail regeneration in lizards exposed to LD 0:24 implicating PRL as a growth promoter in lizards (Ndukuba and Ramachandran 1989a). It is presumed that the dopaminergic mechanism of PRL release may be the one operative in this experimental photoperiodic schedule. Apparently, bromocriptine did not affect tail regeneration in lizards maintained in LD 0: 24 because the dopamine receptors on pituitary lactotrophs are fully saturated with dopamine, thereby leaving no available sites for its agonist to bind.

Having, thus, enumerated several literatures supporting an inhibitory role for dopamine and its agonists in PRL secretion in mammals, coupled with the demonstration of PRL as a growth promoter in regenerating systems (Maier and Singer, 1981; Ndukuba and Ramachandran, 1989a), it is difficult to give a sound interpretative explanation for the observed failure of bromocriptine, a potent dopamine agonist, to retard tail regeneration in H. flaviviridis. However, based on present observation and past findings, it is surmised that the positive influence of continuous light on tail regeneration in Hemidactylus is probably brought about by increased PRL release mediated through the stimulatory serotonergic mechanism rather than the inhibitory dopaminergic mechanism. Further, it is presumed that under LD 0:24, there is suppressed PRL release

resulting in minimal regenerative growth. This dark effect may be mediated through the activation of the inhibitory dopaminergic mechanism and the presently observed inability of bromocriptine to alter the regenerative performance in lizards exposed to LD 0:24 may indicate a state of full saturation of dopamine receptors on lactotrophs. Recent experimental evidence from our laboratory (Ndukuba and Ramachandran, 1989b, Chapter 10) of improved regenerative performance in lizards maintained in LD 0:24 condition by the use of the dopamine receptor antagonist, pimozide, tend to validate this concept. Our concept is that, during the process of tail regeneration in lizards, both serotonergic and dopaminergic systems of PRL release are operative on par at the intermediate photoperiodic regimen of 12 hours of light and 12 hours of darkness (LD 12:12). With increasing photoperiodism, there is a direct antagonism by serotonin of the dopaminergic system that inhibits PRL release. This antagonism probably reaches the peak in LD 24:0 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 release occurs. This may attain its peak in LD 0:24 where the dopaminergic neurons are fully activated. Since bromocriptine failed to block PRL release in regenerating lacertilians exposed to photoperiodic extremes and, thus, could not alter the regeneration process in either LD 24:0

or 0: 24 exposed animals (this report), the authors thought it pertinent to investigate with bromocriptine in lizards exposed to LD 12: 12 where it has been proposed (also this report) that both the serotonergic and dopaminergic mechanisms of PRL release are operating on par.