CHAPTER XII

MELATONIN IN LACERTILIAN TAIL REGENERATION: DURATION, PHASE SPECIFICITY AND SYNCHRONIZATION OF THE DAILY LIGHT-DARK CYCLE.

Several lines of evidence suggest that the pineal organ of all vertebrate species, wherever it is present, mediates seasonal and daily photoperiodic cycles (Tamarkin et al., 1985; Firth et al., 1988; Vivien-Roels et al., 1988). Melatonin (N-acetyl-5-methoxytryptamine) is a vertebrate hormone produced principally but not exclusively by the pineal organ (Ralph, 1981; Firth and Kennaway, 1987; Gern and Greenhouse, 1988). Although it has been reported that other sites of melatonin synthesis exist (the eyes, the Harderian gland and the gut), evidence abounds that the pineal organ is the site of the synthesis and secretion, but not the storage, of most of the endogenous melatonin in circulation (Ralph, 1981; Underwood et al., 1984; Firth and Kennaway, 1987). The pineal does synthesize melatonin, and several other indoleamines, through some intrinsic mechanism of its own, modulated by seasonal and daily LD cycle (Underwood, 1986; Skene et al., 1987; Vivien-Roels et al., 1988). Evidence exists that melatonin, in turn, acts as a synchronizer of the seasonal and photoperiodic cycles (Herbert, 1981; Hoffmann, 1987).

Previous studies with different reptilian species have

demonstrated that exogenous melatonin alters thermoregulatory behaviour in Crotaphytus collaris (Cothran and Hutchison, 1979) and in the turtle, Terrapene carolina truinguis (Erskine and Hutchison, 1981). Exogenous melatonin also causes gonadal regression in the lizard Calotes versicolor; the degree of response varies with the time of day at which the hormone is administered (Misra and Thapliyal, 1979). Recent evidence from our laboratory indicates that the time of day at which melatonin is administered determines its proregenerative or antiregenerative effect in NL lizards exposed to LD 12 : 12 photoperiodic condition. Exogenous melatonin produced an antiregenerative effect at dawn but a proregenerative effect at dusk. However, daily injection of a low dose of melatonin (20 µg/animal) to PX lizards at dusk did not affect the regeneration process leading the authors (Ramachandran and Ndukuba, 1989d) to suggest that the diurnal rhythm in sensitivity to melatonin in relation to tail regeneration in intact Hemidactylus results from some aspect of the pineal function.

There are no reports on how melatonin codes for day length in regenerating lacertilians. Experimental evidence from studies with mammals, particularly ewes and hamsters, led several investigators to advance two major hypotheses on how melatonin codes for day length in mammals: duration (Dowell and Lynch, 1987; Wayne, et al., 1988: English et al., 1988) and phase (Stetson and Tay, 1983; Stetson et al., 1986).

Proponents of the duration hypothesis strongly hold that the duration of nocturnal melatonin secretion is the critical parameter in the induction of photoperiodic responses. There are also strong experimental support for proponents of the phase hypothesis whose view is that the effects of melatonin are dependent on the underlying daily rhythm in sensitivity of melatonin receptors to the hormone.

The data on how melatonin codes for day length in Hemidactylus flaviviridis during its tail regeneration are presented here. The experimental approach was to test the influence of exogenous melatonin in lizards exposed to constant photoperiods in comparison with those exposed to the alternating daily LD cycle. Although this experiment was not designed to measure plasma melatonin levels, it has been reported that exposure to continuous light suppresses the nocturnal surge of melatonin in other vertebrates (Binkley, 1981), and the reverse will be the case in animals maintained in continuous darkness. Thus, the level of melatonin in LD 24 : 0 exposed lizards may be kept low, and in those exposed to LD 0 : 24 high, by maintaining them under these regimes.

The effects of pinealectomy and melatonin replacement with regards to gonadal development in the lizards C.versicolor and Anolis carolinensis have been well documented (Misra and Thapliyal, 1979; Underwood, 1981). Our own recent observations show that pinealectomy or light deprivation to the pineal organ retarded tail regeneration in H. flaviviridis

by upto 50% (Ramachandran and Ndukuba, 1989a, Chapter 3), and a low dose of melatonin (2mg kg^{-1}) failed to restore the regenerative potential of PX lizards (Ramachandran and Ndukuba, 1989d, Chapter 6). Further experimental evidence, showing that a high dose of melatonin (10mg kg^{-1}) can restore the regenerative ability of PX lizards to the NL level, is presented here.

MATERIALS AND METHODS

A total of 140 lizards was used for this investigation and they were balanced for size and sex, thereby eliminating any error in the final statistical analysis due to size and sex differences. The animals were then divided into 14 groups as described below:

Groups 1-3. Morning Melatonin injections (MM)

Groups 1-3 consist of 10 lizards each, exposed to LD 24: 0, LD 12 : 12 and LD 0 : 24 photoperiodic schedules and given daily intraperitoneal (ip) injection of 20 µg melatonin per animal in the morning (o700 hrs), 5 days prior to and 30 days after tail autotomy.

Groups 4-6. Evening melatonin injections (EM)

Groups 4-6, composed of 10 lizards each and exposed to LD 24 : 0, LD 12: 12 and LD 0 : 24 conditions, were given daily ip injection of 20 µg melatonin/animal in the evening (1700 hrs), 5 days prior to tail autotomy and 30 days thereafter.

Groups 7-9. Morning saline injections (MS)

Groups 7-9, made up of 10 lizards each and exposed to light — dark schedules as in groups 1-3, were given saline injection in the morning (0700 hrs), 5 days prior to tail autotomy and 30 days thereafter.

Groups 10-12. Evening saline injections (ES)

Groups 10-12, made up of 10 lizards each and exposed to light — dark schedules as in groups 4-6, received daily ip injection of 0.6% saline in the evening (1700 hrs), 5 days prior to and 30 days after tail autotomy.

Groups 13 and 14. (PX + ME and PX + SE) - Evening saline and melatonin (10mg kg^{-1}) injections.

Group 13, made up of 10 PX lizards was injected daily with a high dose of melatonin (10 mg/kg^{-1}) in the evening (PX + ME), 5 days prior to and 30 days after tail autotomy while group 14, composed of 10 lizards was injected daily with 0.6% saline (PX + SE). This group served as the PX control. Groups 13 and 14 were exposed to the LD 12 : 12 schedule. Lizards were pinealectomized by the method described previously (chapter 3). At the end of the experiment, PX lizards were sacrificed under proper anesthesia and microscopic exmination as well as histological studies showed that the pineal organ was completely removed and no damage was done to the brain.

Preparation of melatonin (2mg kg^{-1} and 10mg kg^{-1} body mass)

Melatonin, for the injections, was prepared fresh daily. Crystalline melatonin (Sigma Chemical Company, St. Louis, Mo., U.S.A.) was dissolved in a few drops of ethanol before being diluted to the required concentrations (low dose- 2mg kg^{-1} and high dose - 10mg kg^{-1}) with 0.6% saline. 0.1 ml of the diluted solution of melatonin was injected into each animal, giving an approximate daily dose of 20 µg/animal (groups 1-6) and 100 µg/animal (group 13.)

Preparation of saline (0.6%)

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

Experimental design

Tail autotomy was performed by pinching off the tail at the third segment from the vent and the animals exposed to continuous light (LL : LD 24 : 0) of 2500 lux intensity, 12 hours of light and 12 hours of darkness (LD 12 : 12) and continuous darkness (DD : LD 0:24) throughout the entire period of experimentation. The description of the light schedules and the dimensions of the cage housing the animals have been well documented in chapters 2 and 3. This investigation was conducted during the summer month of April and the average daily temperature at the level of the animals in the

lighted and dark chambers was 31°C and 29°C, respectively.

Statistical analysis

The length of new growth (regenerate) was measured twice, first with a pair of compass and then with a piece of thread, and the measurements were scored against a ruler graduated in mm. The use of a pair of compass and a thread for taking measurements was designed to cross-check one with the other in order to ensure accuracy and not to calculate the average of the two. This is an improvement from the earlier method of directly measuring with a ruler graduated in mm, although the improved method has confirmed the accuracy of the old one. The measurements at fixed time intervals of 5,10,15,20,25 and 30 days after tail autotomy were later used for morphometric calculations. The data on the length of tail regenerated and total percentage replacement were subjected to an analysis of variance and further to Duncan's multiple range test for statistical significance with an alpha level of 0.05 and 0.01 (Duncan, 1955).

RESULTS

The results are clearly shown in figures 1-3.

Growth rate, length of tail regenerated and total percentage replacement

The average length of tail regenerated by the 30th day

in the LD 24 : 0 exposed normal lizards injected with saline in the morning (NL + MS) and evening (NL + ES), normal lizards with morning melatonin injections (NL + MM) and evening injections (NL + EM) was 32.2 mm. 32.6mm, 32.5mm and 32.9mm, respectively which was a replacement of 61.9%, 62.6% 62.5% and 63.2%, respectively. In their counterparts exposed to LD 0: 24, the same was 11.9mm, 12.1 mm, 11.7mm and 12.6mm respectively representing a replacement of 22.8%, 23.2%, 22.5% and 24.2%, respectively. In the LD 12:12 exposed lizards, the same was 19.9mm, 20.4mm, 12.9mm and 26.4mm, respectively which was a replacement of 38.2%, 39.2%, 24.8% and 50.7%, respectively (Figs. 1 and 3). In the pinealectomized (PX) lizards experiment, the average length of tail regenerated in the LD 12 : 12 exposed normal animals injected with saline in the evening (NL + ES), pinealectomized lizards injected with saline in the evening (PX + ES) and those injected with a high dose of melatonin (10mg kg^{-1}) in the evening (PX + EM) was 19.5mm, 10.7mm and 18.6mm, respectively which was a replacement of 39.6%, 20.5% and 37.9%, respectively (Figs. 1 and 3).

The growth rate as represented in fig.2 for animals under constant photoperiods (LD 24 : 0 and 0:24) is a mean of all the groups put together in each case as the length of tail regenerated at various time intervals was identical as can be deduced from fig.1. The pattern of growth rate (Fig.2)indicates a biphasic growth rate curve in animals exposed to LD 24 : 0 (one during the first 10 days and the second between 20-30

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN MELATONIN TREATED NORMAL AND PINEALECTOMIZED H. FLAVIVIRIDIS EXPOSED TO LD 24:0, LD 12:12 AND LD 0:24 PHOTOREGIMES DURING THE SUMMER MONTH

EXPERIMENTAL ANIMALS AND PHOTOREGIMES	WOUND HEALING	BLASTEMA	EARLY DIFFEREN-TIATION	MID DIFFEREN-TIATION	LATE DIFFEREN-TIATION	GROWTH
LD 24 : 0						
NL + SALINE (M)	1	3-5	5-7	8	14	30*
NL + MM	3	5-7	7-9	10	18	30
NL + SALINE (E)	1	3-5	5-7	8	14	30
NL + EM	1	3-5	5-7	8	14	30
PX + SALINE (E)	3	5-7	7-9	10	18	30
PX + EM	1	3-5	5-7	8	14	30
LD 12 : 12						
NL + SALINE (M)	3	5-7	10-12	16	23	30
NL + MM	5	8-10	13-15	20	23	30
NL + SALINE (E)	3	5-7	10-12	16	23	30
NL + EM	3	5-7	10-12	16	23	30
PX + SALINE (E)	5	8-10	13-15	20	23	30
PX + EM	3	5-7	10-12	16	23	30
LD 0 : 24						
NL + SALINE (M)	6	9-11	14-16	20	28	30
NL + MM	8	12-14	17-19	25	28	30
NL + SALINE (E)	6	9-11	14-16	20	28	30
NL + EM	6	9-11	14-16	20	28	30
PX + SALINE (E)	8	12-14	17-19	25	28	30
PX + EM	6	9-10	14-16	20	28	30

LD 24:0 - CONTINUOUS LIGHT, LD 12 : 12 - 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS, LD 0:24 - CONTINUOUS DARKNSS NL + SALINE (M) - NORMAL LIZARDS INJECTED WITH SALINE IN THE MORNING, NL + SALINE (E) - NORMAL LIZARDS INJECTED WITH SALINE IN THE EVENING, NL + MM - MORNING MELATONIN, EM -EVENING MELATONIN, PX - PINEALECTOMIZED LIZARDS
* DAYS AFTER TAIL AUTOTOMY.

TABLE 2. LENGTH OF TAIL REGENERATED AND TOTAL PERCENTAGE REPLACEMENT IN MELATONIN TREATED AND CONTROL H. FLAVIVIRIDIS EXPOSED TO LD 24:0, LD 12:12 AND LD 0:24 PHOTOREGIMES DURING THE SUMMER MONTH OF APRIL

EXPERIMENTAL ANIMALS AND PHOTOREGIMES	DAYS 5	DAY 10	DAY 15	DAY 20	DAY 25	DAY 30	TOTL % TAIL RE-PLACEMENT
LD 24 : 0							
NL + MM	4.1±0.70	14.1±1.13	17.4±1.20	20.7±1.10	29.1±1.70	32.5±1.68*	62.5%
NL + MS	4.0±1.09	14.0±1.48	16.9±1.70	20.5±1.50	28.6±2.24	32.2±1.88	61.9%
NL + EM	4.8±0.97	14.7±1.18	17.9±1.37	20.8±2.18	29.2±1.72	32.9±1.64	63.2%
NL + ES	4.3±1.0	14.4±1.35	20.7±1.55	29.1±1.55	29.1±1.92	32.6±1.85	62.6%
LD 12:12							
NL + MM		2.0±1.1	4.2±0.87	8.8±0.74	11.2±0.87	12.9±1.04	24.8%
NL + MS		3.6±0.66	8.5±1.30	16.0±1.50	18.0±1.50	19.9±1.51	38.2%
NL + EM		5.2±0.87	11.6±1.40	20.0±2.09	24.1±1.50	26.4±1.40	50.7%
NL + ES		3.6±0.66	8.7±1.50	15.7±1.70	18.5±1.40	20.4±1.30	39.2%
PX + EM		5.3±0.45	9.1 ±1.20	15.0±1.60	17.3±1.40	18.6±1.40	37.9%
PX + ES		1.9±0.70	4.0±0.89	7.2±1.10	9.2±1.10	10.7±1.20	20.5%
LD 0:24							
NL + MM		2.2±0.97	4.1±1.7	5.9±2.1	10.8±3.3	11.7±3.1	22.5%
NL + MS		2.5±0.92	4.6±1.6	6.8±2.2	11.2±2.6	11.9±2.5	22.8%
NL + EM		2.4±0.91	5.0±2.0	7.5±2.9	11.7±2.8	12.6±2.9	24.2%
NL + ES		2.3±0.90	4.9±1.5	7.3±2.1	11.9±2.4	12.1±2.8	23.2%

LD 24:0 - 24 HOURS OF LIGHT AND 0HOURS OF DARKNESS (CONTINUOUS LIGHT)
LD 12:12- 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS
LD 0 :24- 0 HOURS OF LIGHT AND 24 HOURS OF DARKNESS (CONTINUOUS DARKNESS)
MM - MORNING MELATONIN INJECTIONS, MS-MORNING SALINE INJECTIONS, EM-EVENING MELATONIN INJECTIONS, ES-EVENING SALINE INJECTIONS, NL-NORMAL LIZARDS, PX-PINEALECTOMIZED LIZARDS
* - LENGTH OF TAIL REGENERATED IN MM BY THE 30TH DAY.

TABLE 3. THE DIURNAL VARIATIONS IN TAIL ELONGATION IN H.FLAVIVIRIDIS EXPOSED TO LD 24:0 AND LD 12:12 PHOTOPERIODIC SCHEDULES.

DAYS AFTER TAIL AUTOTOMY	LD 24 : 0				LD 12 : 12			
	7am - 1pm	1pm-7pm	7pm-1am	1am-7am	7am-1pm	1pm-7pm	7pm-1am	1am-7am
5th day	8.0	5.0	3.0	5.0*	--	--	--	--**
6th day	5.0	6.0	1.0	4.0	--	--	--	--
7th day	5.0	5.0	1.0	5.0	--	--	--	--
8th day	5.0	4.0	3.0	5.0	--	--	--	--
9th day	2.0	2.0	1.0	2.0	--	--	--	--
10th day	4.0	4.0	1.0	1.0	5.0	5.0	5.0	0.0
11th day	3.0	4.0	1.0	2.0	5.0	2.0	3.0	0.0
12th day	1.0	2.0	1.0	6.0	3.0	4.0	1.0	0.0
13th day	2.0	4.0	1.0	6.0	6.0	2.0	1.0	0.0
14th day	3.0	1.0	1.0	6.0	8.0	2.0	2.0	0.0
15th day	1.0	2.0	1.0	2.0	7.0	6.0	2.0	0.0
16th day	1.0	3.0	1.0	0.0	2.0	4.0	0.0	0.0

LD 24 : 0 - CONTINUOUS LIGHT
LD 12 : 12- 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS
* - TAIL GROWTH AT 6 HOURLY INTERVALS (in mm)
** - NO GROWTH AT THIS STAGE

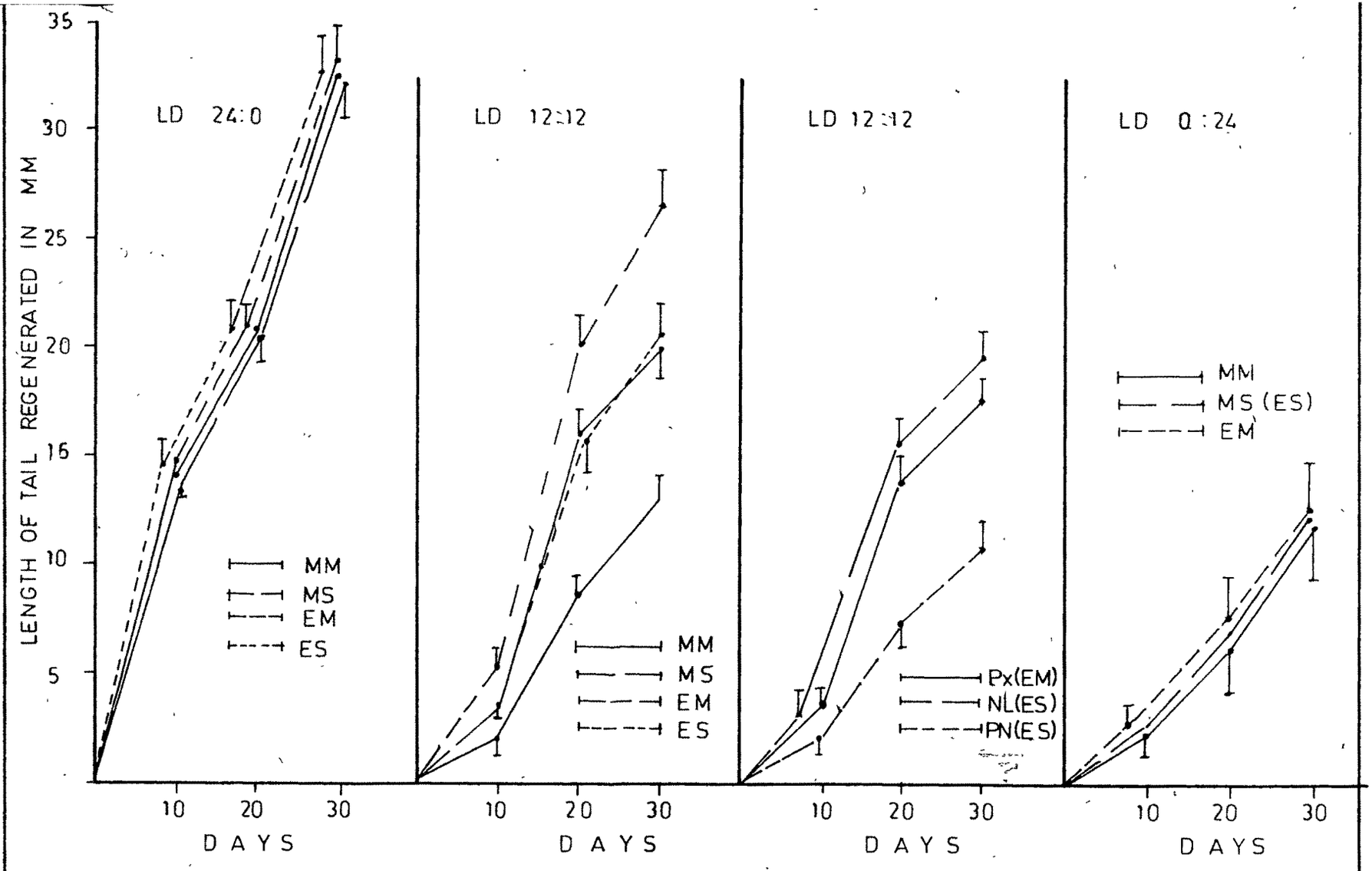


FIG. 1 LENGTH OF TAIL REGENERATED DURING 30 DAYS AFTER AUTOTOMY (± STANDARD DEVIATION SHOWN BY VERTICAL BARS). LD 24:0 – CONTINUOUS LIGHT LD 12:12 – 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS, LD 0:24 – CONTINUOUS DARKNESS, MM – MORNING MELATONIN INJECTIONS, MS – MORNING SALINE INJECTIONS, EM – EVENING MELATONIN INJECTIONS, ES – EVENING SALINE INJECTIONS, NL – NORMAL LIZARDS, Px – PINEALECTOMIZED LIZARDS.

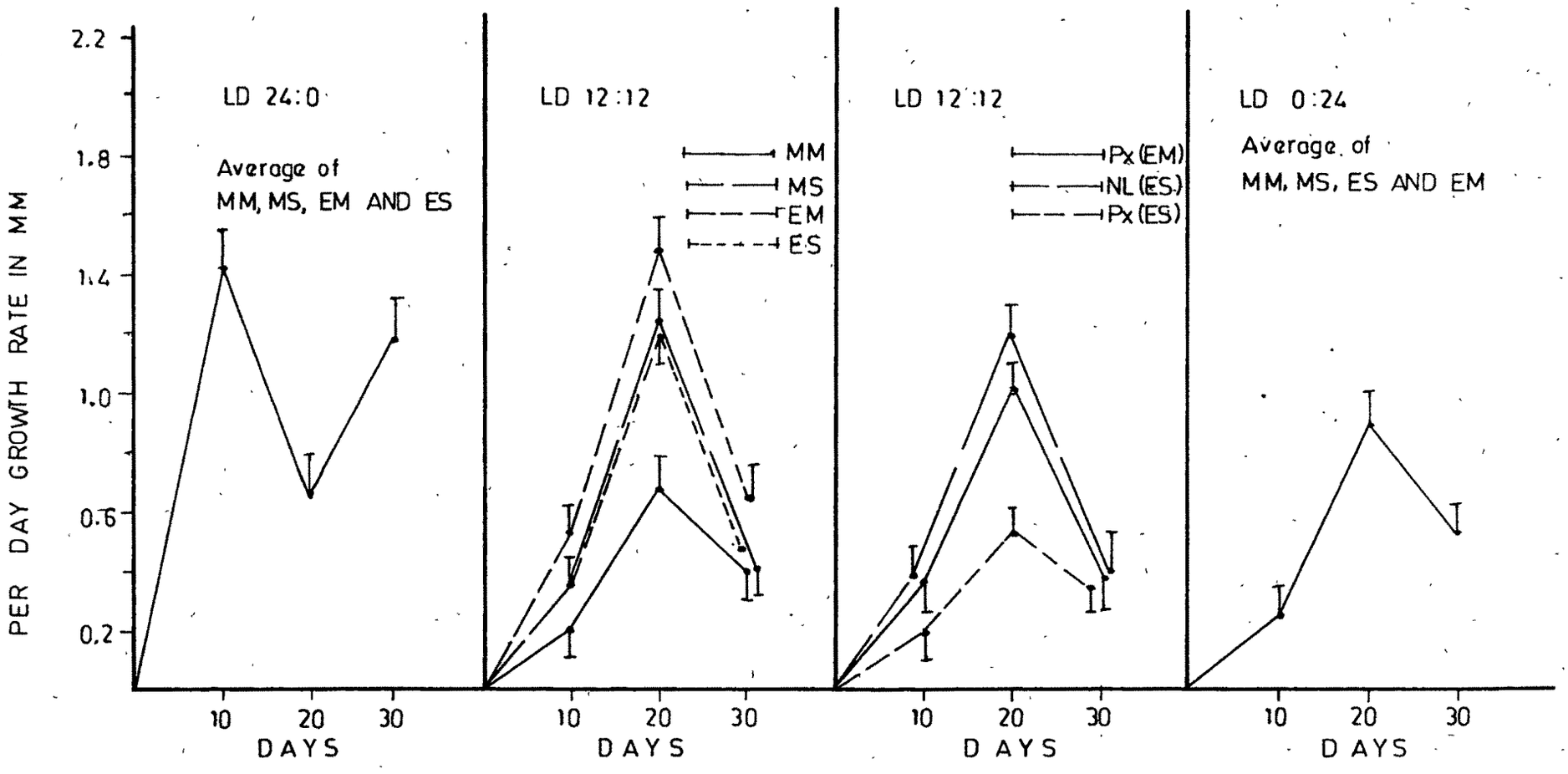


FIG. 2 PER DAY GROWTH RATE DURING INTERVALS OF 10 DAYS AFTER TAIL AUTOTOMY (± SD SHOWN BY VERTICAL BARS) LD 24:0 – CONTINUOUS LIGHT, LD 12:12 – 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS, LD 0:24 – CONTINUOUS DARKNESS, MM – MORNING MELATONIN INJECTIONS, MS – MORNING SALINE INJECTIONS, EM – EVENING MELATONIN INJECTIONS, NL – NORMAL LIZARDS, ES – EVENING SALINE INJECTIONS, PX – PINEALECTOMIZED LIZARDS.

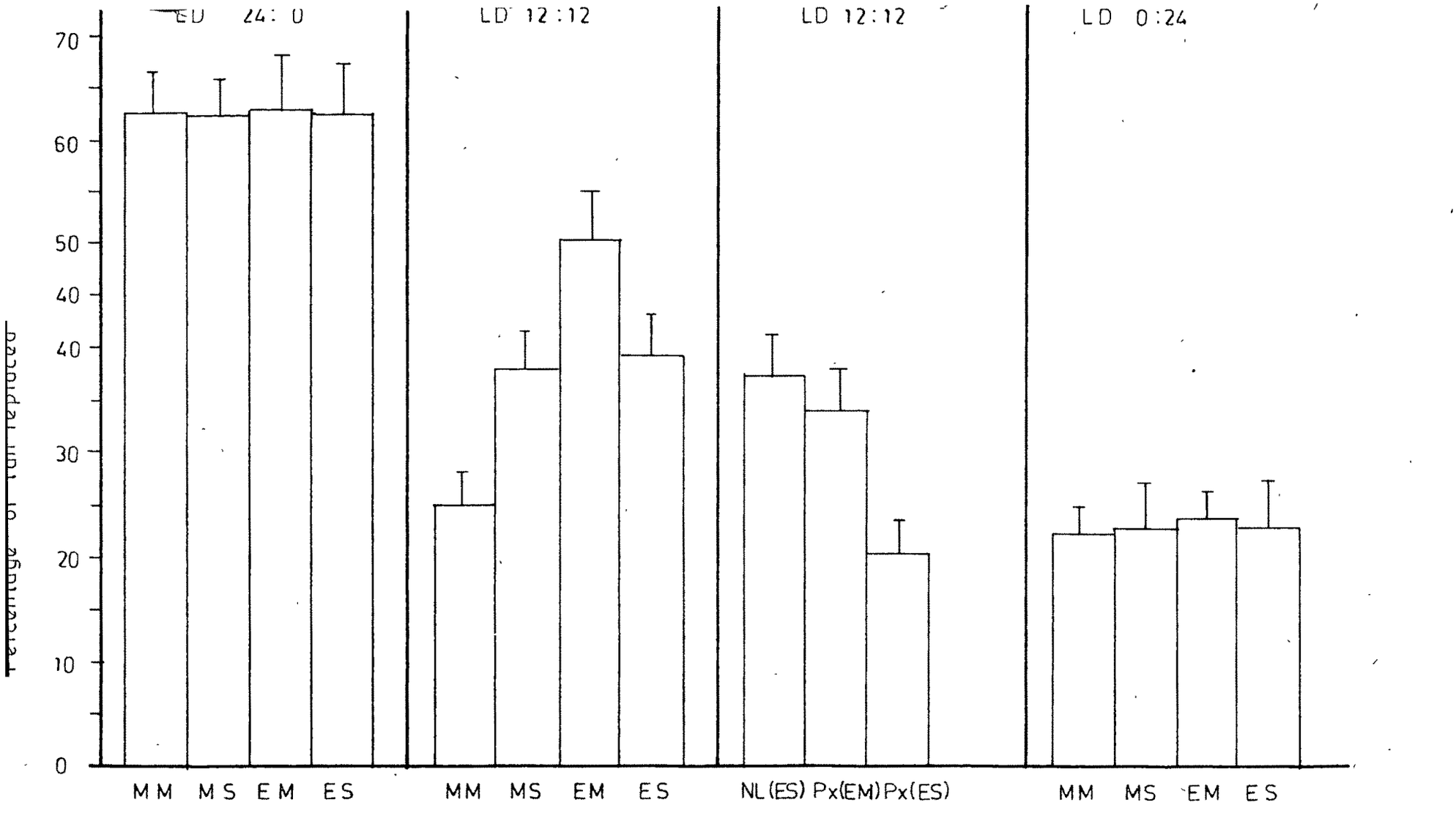


FIG 3 Percentage of tail replaced 30 days after autotomy (±STANDARD DEVIATION SHOWN BY VERTICAL BARS) LD 24:0 = CONTINUOUS LIGHT, LD 12.12 — 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS, LD 0:24 — CONTINUOUS DARKNESS, MM — MORNING MELATONIN INJECTIONS, MS — MORNING SALINE INJECTIONS, EM — EVENING MELATONIN INJECTIONS. ES - EVENING SALINE INJECTIONS, NL — NORMAL LIZARDS, Px — PINEALECTOMIZED LIZARDS.

days) and a linear increase peaking between 20-30 days in all the other groups.

All possible comparisons between the fourteen groups of lizards (Duncan's multiple range test) revealed no statistically significant difference between NL + MS, NL + ES, NL + MM and NL + EM in LD 24 : 0 or LD 0 : 24 on one hand and between NL + MS , NL + ES, and PX + EM in LD 12 : 12 on the other. However, all other comparisons other than these, between lizards in LD 24 : 0, LD 12 : 12 and LD 0:24 and NL + ES and PX + EM in LD 12 : 12 on one hand and PX + ES on the other (Figs. 1 and 3) were statistically significant at both 5% and 1% levels (Duncan, 1955).

DISCUSSION

These results as well as those reported recently (Ramachandran and Ndukuba, 1989d; chapter 6) clearly show that the time of administration of the methoxyindole, melatonin, determines its proregenerative or antiregenerative effect in the gekkonid lizard, Hemidactylus flaviviridis exposed to the alternating daily light-dark (LD) cycle. Melatonin, when administered daily to NL lizards in the early portion of the light phase of the diurnal cycle (0700 hrs) produced an antiregenerative effect, but a proregenerative effect in the early hours of the dark phase (1700 hrs) in the LD 12 : 12 exposed animals. However, melatonin did not affect tail regeneration in lizards

exposed to constant photoperiods (LD 24: 0 or 0: 24). The observations show that the initiation of regeneration, the length of new growth (regenerate) produced by day 30, and the total percentage replacement of the lost (autotomized) tails at the end of 30 days were all significantly enhanced in lizards treated with melatonin at dusk and depressed in animals injected at dawn in the LD 12 : 12 schedule when compared with their saline-injected controls. However, melatonin did not affect the regenerative performance of lizards maintained under constant light or dark condition irrespective of the time of day at which the hormone was administered as there was neither a display of the dual effect observed in lizards under LD 12 : 12 nor a statistically significant difference in the regenerative performance of melatonin treated NL animals and their saline - injected controls under LD 24 : 0 or 0:24 regimes (Figs. 1 and 3), suggesting that melatonin may be the active principle that integrates/synchronizes the LD cycles in regenerating lacertilians.

There is a growing body of evidence that melatonin codes for day length in mammals; however, how the hormone accomplishes this task has remained a subject of continuing controversy. One school of thought holds that the duration of night time melatonin secretion reflects the length of the dark phase; thus, it is opined that duration is the critical paramter in the induction of photoperiodic responses (Dowell and Lynch, 1987;

Wayne et al., 1988; English et al., 1988). The opposing hypothesis supports the view that the effects of melatonin depend on how sensitive the melatonin receptors are to the hormone at each phase of the circadian cycle (Stetson and Tay, 1983; Stetson et al., 1986). The differences of opinion are still unsettled as to which hypothesis best describes the role of melatonin in coding for day length in different mammalian species, particularly ewes and hamsters, although earlier investigators working with the sheep, have interpreted their findings as being consistent with both hypotheses (Phase : Almeida and Lincoln, 1982; duration: Yellon et al., 1985).

There are no comparable studies on how melatonin codes for day length in reptiles. The primary objective in designing the present experiment was to elucidate the mechanism by which melatonin codes for day length in regenerating lizards. One possible approach to solving this problem would be the administration of melatonin, in physiological doses, to lizards exposed to constant photoperiods (LD 24 : 0, where melatonin level may be low and LD 0:24, where melatonin level may be high) and compare the results with those obtained from lizards obeying the daily LD rhythm (LD 12 : 12). Constant photoperiods have unique characteristics with regards to the pineal melatonin-serotonin level; in animals exposed to continuous light, melatonin level is low while serotonin level is high (Brownstein, 1975), whereas in those maintained in continuous darkness melatonin level is high while serotonin

level is low (Underwood, 1986), regardless of the habit (nocturnal or diurnal) of the animals. The rhythm in pineal melatonin content is a true circadian rhythm (ie. driven by an internal biological clock), since the melatonin-serotonin rhythm will persist in constant conditions (Darrow et al., 1986; Chesworth et al., 1987; Mahapatra et al., 1988). Evidence exists that serotonin and its precursors elevate serum prolactin (PRL) levels with increasing lengths of light (Lu and Meites, 1973), and PRL is a known growth promoter in developing organisms (Crim, 1975). In the LD 0:24 exposed animals, regeneration was depressed which could be rectified with exogenous PRL, providing further evidence that PRL is a growth promoter in regenerating systems (Maier and Singer, 1981; Ndukuba and Ramachandran, 1989a, chapter 7). Apparently lizards exposed to LD 24:0, produced the best regenerative performance due to increased PRL release (Pittendrigh, 1974; Goldman et al., 1979) through a stimulatory sertonergic mechanism (Ramachandran and Ndukuba, 1989b, chapter 8) which could not be suppressed by exogenous melatonin. Recent evidence from our laboratory (unpublished data) suggests that in lizards exposed to LD 24 : 0, there was apparent regenerative tail elongation throughout the 24-hrs daily cycle as deduced from our measurements of the tail growth at 6 hourly intervals (Table 3). Concomitantly, in the LD 0:24 exposed animals, where the purportedly melatonin level is high with low serotonin level, worst regenerative performance was observed, probably due to decreased PRL release

through an inhibitory dopaminergic mechanism (Ndukuba and Ramachandran, 1989b, chapter 10). There is a close parallelism between the above findings and recent lines of evidence from our laboratory showing that bromocriptine, a potent dopamine receptor agonist, failed to retard tail regeneration in lizards exposed to either LD 24 :0 or 0:24 (Ramachandran and Ndukuba, 1989a, chapter 9), but did significantly retard those under LD 12 : 12 (Ndukuba and Ramachandran, 1989c, chapter 11) indicating that both the stimulatory serotonergic and the inhibitory dopaminergic systems of PRL release may be functioning on par at the LD 12:12 photoregime.

Since melatonin did not elicit any effect in lizards exposed to constant photoperiods, but did produce a dual effect in those exposed to LD 12 : 12, an antiregenerative effect at dawn and a proregenerative effect at dusk, it may be presumed that its role in integrating/synchronizing the daily LD cycle is a method of coding for day length in lizards. In interpreting the findings from this study and those reported recently (Ramachandran and Ndukuba, 1989d, chapter 6), we suggest that exogenous melatonin produced its antiregenerative effect at dawn in Hemidactylus, as in mammals (English et al., 1988; Wayne et al., 1988), by prolonging the night time melatonin level, thereby inducing the animals to read the dark period of the LD cycle as being extended and, thus, both the dark effect (retardation) and melatonin which is a known

mitotic inhibitor (Banerjee and Margulis, 1973) could be responsible for the observed antiregenerative effect. It is interesting to note (see figs. 1 and 3) that exogenous melatonin in the morning produced similar retardation effects in lizards as did pinealectomy or complete deprivation of light (LD 0:24). This finding supports an earlier proposition (Ramachandran and Ndukuba, 1989a, Chapter 3) that regenerating lacertilians possess basal PRL levels which account for a 50% regenerative ability irrespective of the absence or presence of light. Apparently, exogenous melatonin at dawn could not suppress the basal PRL level but was capable of nullifying the expected rise in PRL level associated with dawn in preparation for the induction of photoperiodic responses, thereby producing an antiregenerative effect. This effect does not support the phase hypothesis since, in our view, if the melatonin receptors are down-regulated after the scotophase, then there should not have been any effect at all when melatonin was injected early in the morning. The observed proregenerative effect in lizards injected with melatonin at dusk may be due to a greater serum PRL surge brought about at dawn, possibly employing the serotonergic mechanism of PRL release. This contention is supported by the report of increased daytime hypothalamic serotonin content and induction of increased serotonergic activity some 16-20 hrs after melatonin administration to intact gold fish (Olcese et al., 1981). The observations

may also be in conformity with the phase hypothesis (Stetson and Tay, 1983; Stetson et al., 1986) by which the rejuvenated/resensitized melatonin receptors during the late photophase could be considered to be responsive to exogenous melatonin thereby initiating the operation of the neuroendocrine mechanism that leads to the release of more serotonin. In interpreting the proregenerative effect of melatonin at dusk, this view is being advanced, because the concept, emerging from the available data, cannot adequately support either the phase hypothesis on its own, or a possible surge in the level of PRL in response to melatonin at dusk. Even if it be considered that there is a surge in the level of PRL at dusk in response to melatonin, it may not lead to effective regenerative growth as unpublished observations from our laboratory indicate no increase in tail length between 1800 hrs and 0600 hrs in the LD 12 : 12 exposed NL lizards (Table 3).

Most of the studies suggest that the pineal organ, through the synthesis and secretion of melatonin, acts as a synchronizer of seasonal and photoperiodic cycles (Herbert, 1981; Hoffmann, 1987). How it achieves this in lizards is a part of the problem of this investigation. The experimental approach was to test the influence of daily administration of a high dose of melatonin (10mg $\cdot$ kg^{-1}) to PX lizards in LD 12 : 12 since a recent study (Ramachandran and Ndukuba, 1989d, Chapter 6) showed that a low dose (2mg $\cdot$ kg^{-1}) could not restore

the regenerative ability of PX lizards to the NL level and, thus, may be a physiologically inadequate dose to compensate for the absence of the "photoneuroendocrine" pineal organ. Several lines of evidence suggest that although melatonin administration alone to PX animals is not sufficient to re-establish prior neuroendocrine conditions, it, however, might be capable of reinstating the natural variation of serotonergic activity when given late, rather than early, in the light phase of the daily photoperiod in the hamster (Tamarkin et al., 1976), the gold fish (Olcese et al., 1981), and the lizard (based on present observations).

The failure of the low dose of melatonin to affect the regeneration process in PX lizards supported the contention that an intact pineal is somehow linked to the favourable influences of light and PRL on tail regeneration in lizards (Ndukuba and Ramachandran, 1989a, Chapter 7). However, results of the present study have revealed that a high dose of melatonin (10mg kg^{-1}) can restore the regenerative potential of PX animals to near NL level, although the observed dual effect in intact lizards exposed to LD 12 : 12 was not discernible. These data provide experimental evidence for the participation of the pineal organ and its putative hormone, melatonin, in the neuroendocrine mechanism that integrates/synchronizes the daily LD cycle that leads to sustained tail elongation in lacertilians.

FLOWCHART - 2

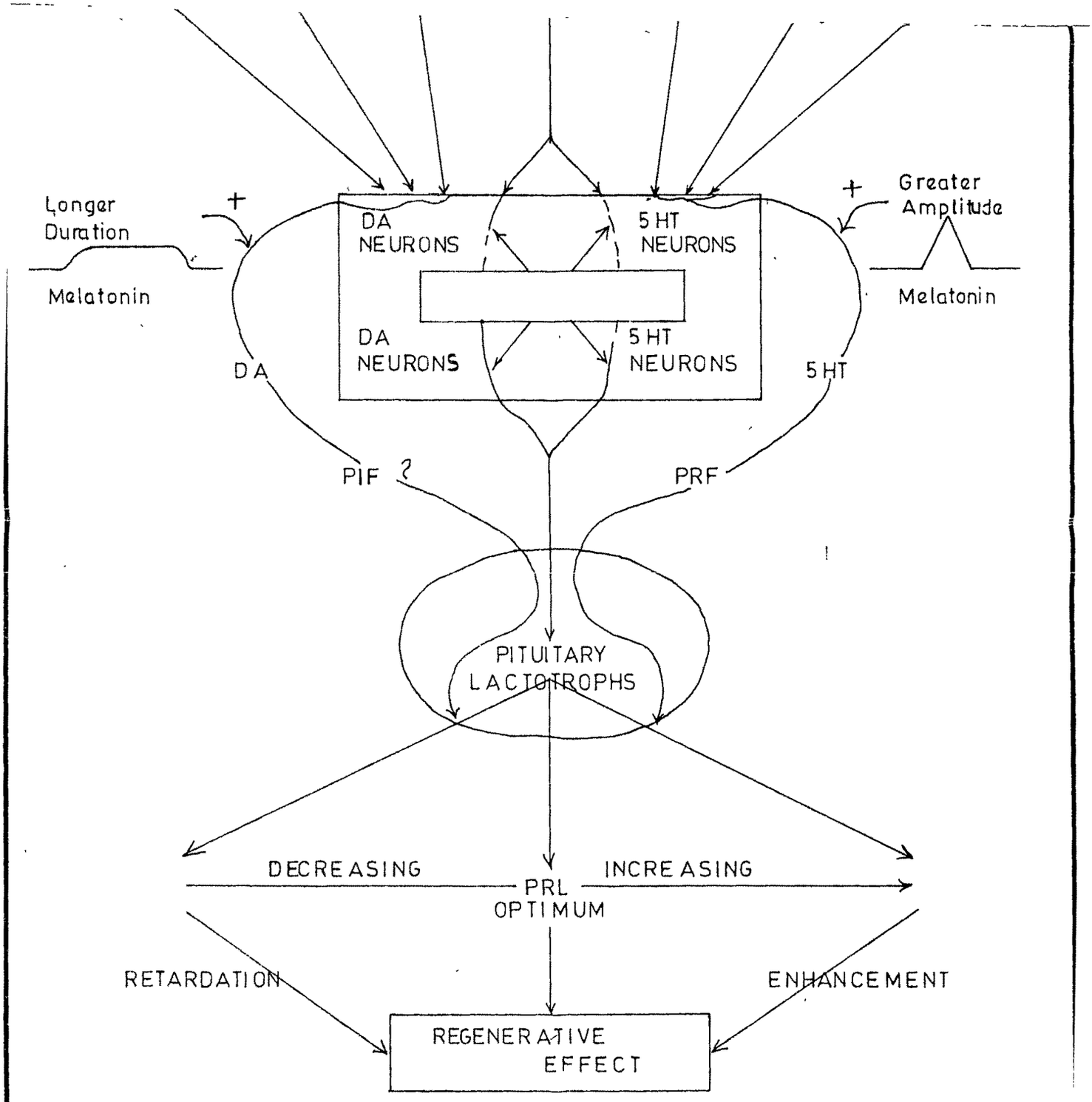
DIAGRAMATIC

REPRESENTATION

OF

SECTION

2.



FLOW CHART - II. NEUROENDOCRINE MECHANISM IN TAIL REGENERATION IN H - FLAVIVIRIDIS

L - LIGHT, D - DARK, DA - DOPAMINE, 5-HT - SEROTONIN, PRF - PROLACTIN RELEASING FACTOR, PIF - PROLACTIN-INHIBITING FACTOR, PRL - PROLACTIN