CHAPTER

3

Differential Time Dependent Influence of Pineal Indoles on Tail Regeneration

Previous study on tail regeneration in *Hemidactylus flaviviridis* revealed enhancing influence of longer photic schedules and retarding influence of shorter photic schedules on overall growth (Ndukuba and Ramachandran, 1991a). The favourable influence of light on regeneration was shown to be not mediated via the eyes as blinded lizards regenerated their tail as well as the sighted controls (Ndukuba and Ramachandran, 1988). The alluded extraretinal photoreceptor was identified as the pineal, since both pinealectomised (PX) lizards as well as pineal intact lizards exposed to continuous darkness (DD) depicted a 50% deficit in the total length of tail replaced (Ramachandran and Ndukuba, 1989a; Ndukuba and Ramachandran, 1991a). The pineal of all vertebrates is known to produce many methoxyindoles of which three most important known to appear in systemic circulation in order of their importance are melatonin (M), methoxytryptophol (ML), and methoxytryptamine (MT).

Melatonin by its rhythmic secretion has been shown to synchronize seasonal and circadian activity in various vertebrates (Rusak, 1981; Underwood, 1981a, 1989; Bartness and Goldman, 1989; Armstrong, 1989). In addition, both ML and MT have also been reported to influence reproductive functions by their circadian variations (Young and Silman, 1981; Pevet, 1983, 1985; Skene *et al.*, 1986, 1987; Vivien-Roels *et al.*, 1992). Light has been identified as an important stimulus that can affect pineal

indole levels (Skene *et al.*, 1987) and experimental alterations in the duration of light have been shown to alter nocturnal melatonin levels in many non-mammalian vertebrates such as fishes (Duston and Bromage, 1987), reptiles (Firth *et al.*, 1979; Menaker and Wisner, 1983; Underwood, 1985a; Vivien-Roels, 1985) and birds (Underwood and Siopes, 1985; Liou *et al.*, 1987).

In view of the fact that both PX and experimental photic schedules affected tail regeneration in Hemidactylus and, as pineal indoles serve as the essential photo-neuroendocrine transducers, it was pertinent to test the influence of exogenous administration of pineal indoles on tail regeneration. Considering the fact that the effect of indoles administered exogenously on gonadal functions in both mammals and lizards is time dependent (Reiter *et al.*, 1976; Misra and Thapliyal, 1979; Reiter, 1980a,b), in the present study, M, ML, and MT have been administered in the morning or evening to two separate groups of lizards to ascertain their time dependent influence on tail regeneration.

MATERIAL AND METHODS

Adult lizards, *Hemidactylus flaviviridis* of both sexes weighing 10±2g and measuring 80±5mm snout-vent length were procured from a local animal supplier. They were maintained on a diet of cockroaches and water *ad libitum* for a period of 7

days to acclimatize them to the laboratory conditions. A total of 80 lizards was used for this investigation and they were balanced for size and sex in order to eliminate any possible error in the final statistical analysis due to size and sex differences. The lizards were divided into 8 groups of 10 each and treated differently as follows :

Groups I and II (M groups, Mm and Me).

These two groups of lizards received daily intraperitoneal injections of 20 μg M in 0.1 ml saline either at 07.00 hrs. or 17.00 hrs. starting 5 days prior to tail autotomy and 30 days thereafter.

Groups III and IV (ML groups, MLm and MLe)

These groups of lizards received daily intraperitoneal injections of 25 μg ML in 0.1 ml saline either at 07.00 hrs or 17.00 hrs starting 5 days prior to tail autotomy and 30 days thereafter.

Groups V and VI (MT groups, MTm and MTe)

These groups of lizards received daily intraperitoneal injection of 25 μg MT in 0.1 ml saline either at 07.00 hrs or 17.00 hrs starting 5 days prior to tail autotomy and 30 days thereafter.

Groups VII and VIII (Controls)

These two groups of lizards received daily intraperitoneal injection of 0.1 ml saline either at 07.00 hrs or 17.00 hrs starting 5 days prior to tail autotomy and 30 days thereafter.

Preparation of methoxyindoles

The three methoxyindoles (Sigma Chemical Company, St. Louis, U.S.A.) were prepared fresh daily before injection. They were dissolved in a few drops of ethanol before being diluted to the required concentration with 0.6% saline.

Experimental set up

Tail autotomy was performed by pinching off the tail at the third segment from the vent and the animals were exposed to a normal schedule of 12 hrs of light and 12 hrs of dark. The length of tail autotomized was measured for calculating the total percentage replacement at the end of 30 days. The investigation was conducted in the monsoon month of August and the average daily temperature at the level of the animals was 26°C.

Parameters and Statistical Analysis

The number of days taken to reach the various arbitrary stages of regeneration such as wound healing, pre-blastema, blastema and initiation of growth, were recorded. The length of new growth (regenerate) was measured with a pair of compasses and scored against a meter scale. The measurements were recorded every

alternate day and used for recording the tail length at fixed time intervals of 10, 15, 20, 25 and 30 days post-caudal autotomy. The per day rate of growth and total percentage of tail replaced were calculated. The data were subjected to ANOVA and also to Duncan's multiple range test with an alpha level of both 0.05 and 0.01 (Duncan, 1955).

RESULTS

Number of days taken to reach the various arbitrary stages of regeneration (Table 1).

All groups of lizards except Me and MLm completed wound healing by 6 days. The Me and MLm groups of lizards took 5 days. This delay by a day was persistently shown by the other groups of lizards even while attaining the other stages like pre-blastema, blastema and initiation of growth.

Length of tail regenerated (Fig.1)

The total length of tail regenerated at the end of 30 days was similar in control as well as MTm and MTe groups of lizards. In comparison, Mm and MLe groups of lizards regenerated significantly lesser length of tail ($P < 0.01$) and Me and MLm groups of lizards showed significantly greater length ($P < 0.01$).

TABLE - 1

Number of days taken to attain the different arbitrary stages of tail regeneration in control and in methoxyindoles treated *H. flaviviridis* LD 12 : 12.

Group	Wound Healing	Preblastema	Blastema	Initiation of Growth
Control	6.00 ± 1.25	7.00 ± 1.01	8.00 ± 0.97	9.00 ± 1.42
Mm	6.00 ± 0.96	7.00 ± 2.48	8.00 ± 1.21	9.00 ± 1.26
Me	5.00 ± 0.92*	6.00 ± 1.35*	7.00 ± 1.52*	8.00 ± 1.09*
MLm	5.00 ± 0.92*	6.00 ± 1.68*	7.00 ± 1.02*	8.00 ± 1.09*
MLe	6.00 ± 1.04	7.00 ± 0.98	8.00 ± 1.07	9.00 ± 1.12
MTm	6.00 ± 1.08	7.00 ± 0.72	8.00 ± 1.11	9.00 ± 0.98
MTe	6.00 ± 0.86	7.00 ± 0.68	8.00 ± 1.42	9.00 ± 1.06

* - $P < 0.05$

Mm - Morning melatonin, Me - Evening melatonin, MLm - Morning methoxytryptophol, MLe - Evening methoxytryptophol, MTm- Morning methoxytryptamine, MTe - Evening methoxytryptamine, Values are mean ± SD

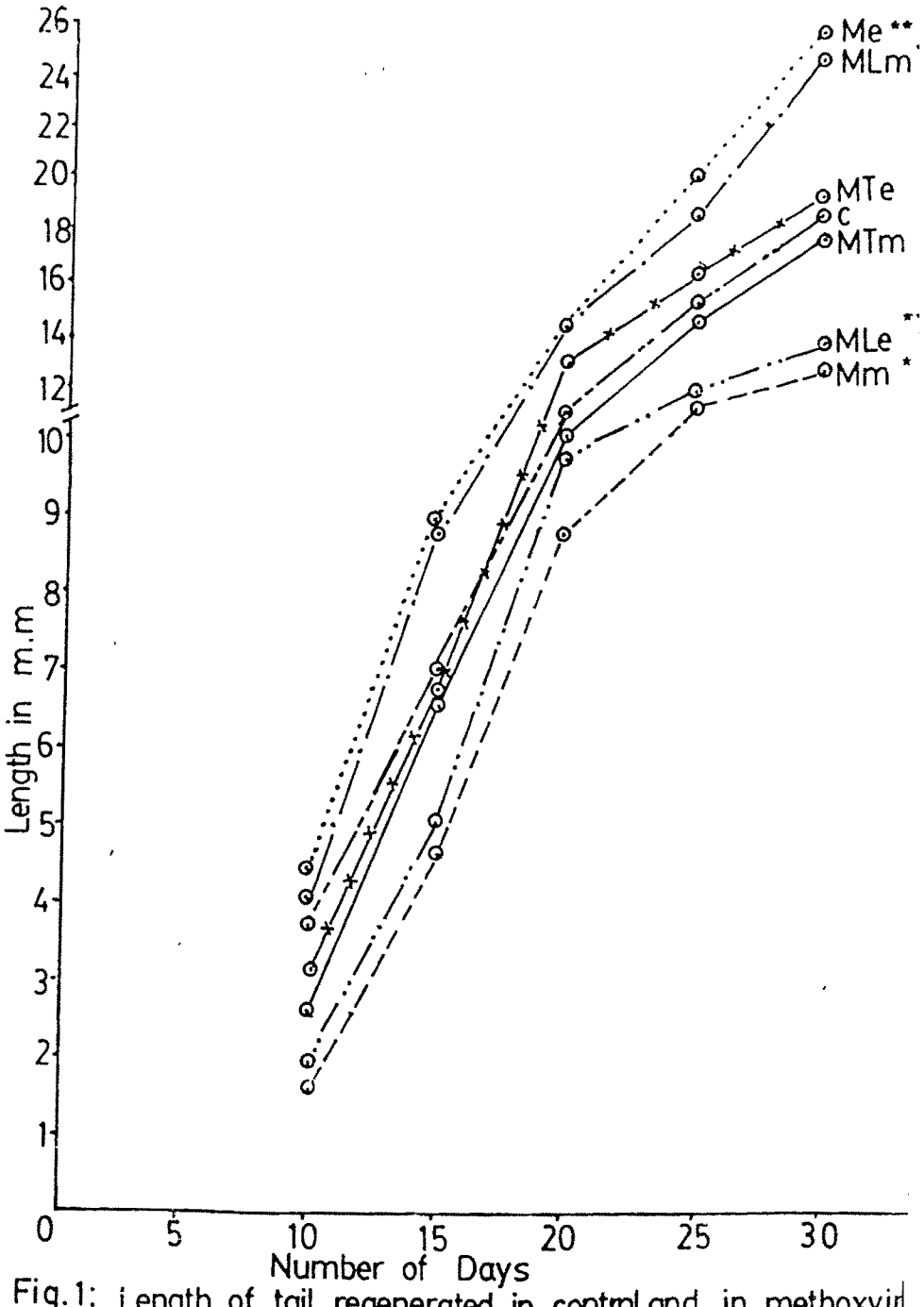

Fig. 1: Length of tail regenerated in control and in methoxyir treated Lizards, H. flaviviridis exposed to L D 12

Rate of growth and total percentage replacement (Table 2 and Fig.2)

The rate of tail elongation calculated for blocks of 5 days shows a continuous increase in control, MTm and MTe groups of lizards to reach a maximum of 0.88 mm between days 20-25 post-autotomy; whereafter the rate declined. The Mm and MLe groups of lizards however showed an initial slow growth rate between 5-10 days and reached maximum growth rates of 0.84 and 0.94 mm respectively between 15 and 20 days. Thereafter, both these groups of lizards showed significantly decreased growth rate. In contrast, Me and MLm, groups of lizards showed significantly greater initial growth rates of 0.88 and 0.76 mm respectively between 5-10 days. Thereafter the growth rates continued to increase gradually till the maximum rates of 1.2 mm and 1.14 mm were reached between 20-25 days respectively. The percentage of tail replaced at the end of 30 days was significantly less in Mm and MLe groups of lizards when compared with those of control, MTm and MTe (25% vs 35%) and significantly more in Me and MLm (50%).

DISCUSSION

The results show that pineal indoles have differential time dependent influence on tail regeneration in *H. flaviviridis*. Of the three indoles tested, M and ML had opposite influence while MT had no effect on the process of tail regeneration.

TABLE - 2

Per day growth rate calculated in blocks of 5 days for 30 days post-caudal autotomy in control and in methoxyindoles injected *H. flaviviridis* LD 12 : 12.

Treatment Groups	BLOCKS OF DAYS					
	0-5	5-10	10-15	15-20	20-25	25-30
Control	-	0.70 ± 0.02	0.60 ± 0.08	0.80 ± 0.06	0.88 ± 0.11	0.68 ± 0.10
Mm	-	0.32 ± 0.08	0.60 ± 0.10	0.84 ± 0.12	0.48 ± 0.09	0.26 ± 0.06
Me	-	0.88 ± 0.15	0.92 ± 0.12	0.96 ± 0.09	1.20 ± 0.02	1.12 ± 0.06
MLm	-	0.76 ± 0.02	1.01 ± 0.08	1.02 ± 0.06	0.92 ± 0.11	1.14 ± 0.10
MLe	-	0.36 ± 0.02	0.64 ± 0.06	0.94 ± 0.06	0.28 ± 0.11	0.48 ± 0.10
MTm	-	0.52 ± 0.10	0.78 ± 0.13	0.70 ± 0.05	0.92 ± 0.05	0.58 ± 0.11
MTe	-	0.62 ± 0.08	0.78 ± 0.10	1.16 ± 0.07	0.68 ± 0.10	0.52 ± 0.12

Mm - Morning melatonin, Me - Evening melatonin, MLm - Morning methoxytryptophol, MLe - Evening methoxytryptophol, MTm- Morning methoxytryptamine, MTe - Evening methoxytryptamine, Values are mean ± SD

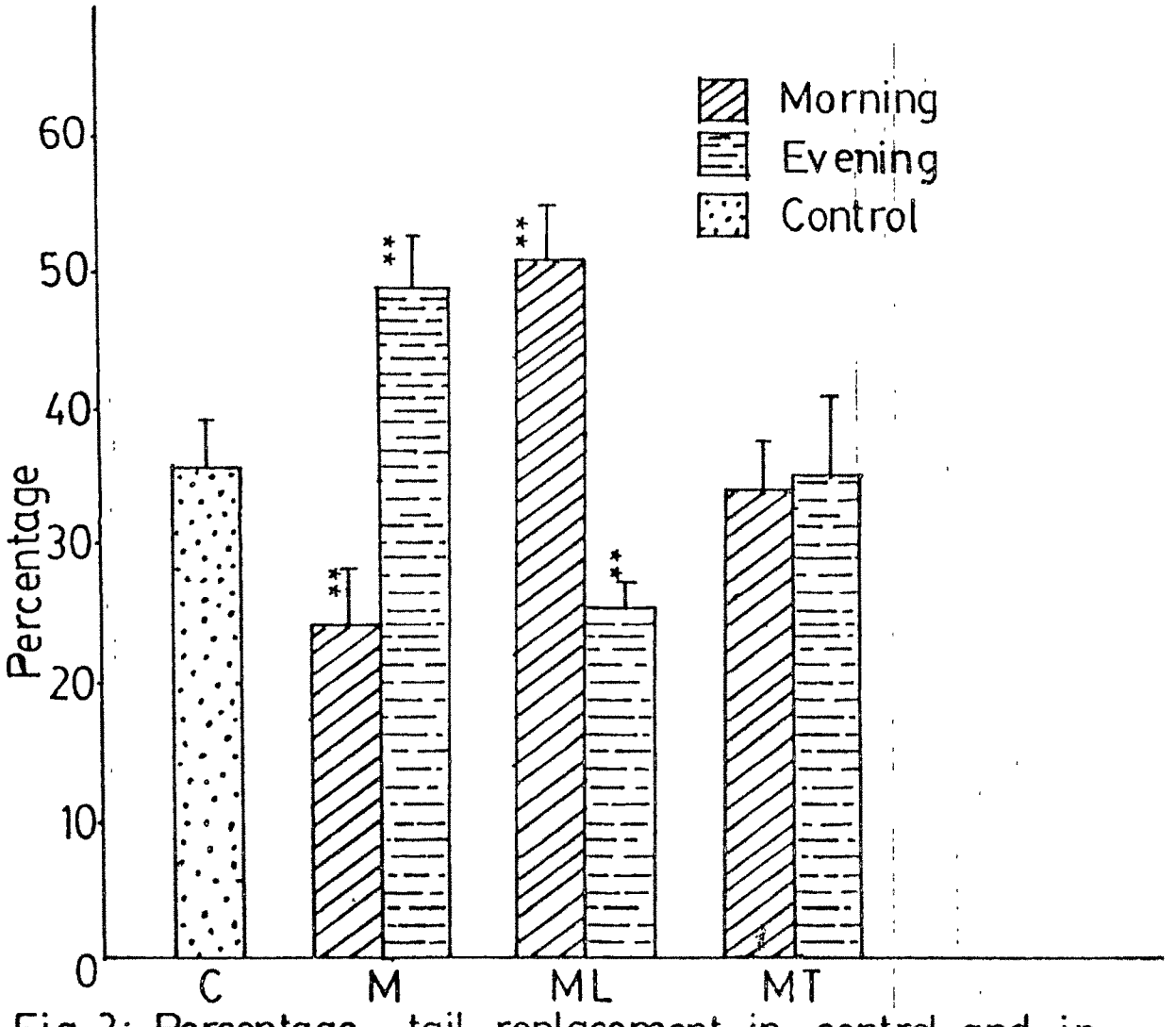

Fig.2: Percentage tail replacement in control and in methoxyindoles treated Lizards. H.flaviviridis exposed to LD 12:12 vertical bars indicates (±SD

** P < 0·0005

Whereas Mm depicted an antiregenerative effect, Me produced a proregenerative effect. In contrast, MLm showed proregenerative effect while MLe had an antiregenerative influence. Time dependent influence of M on gonadal functions of mammals and lizards is well documented (Underwood, 1981b; Carter *et al.*, 1982; Stetson *et al.*, 1986; Ng, 1987). The antiregenerative effect of Mm seems to be essentially due to the morning melatonin injection causing the lizards to read it as an extended dark phase. This is substantiated by the earlier observations of decreasing regenerative tail elongation with decreasing photic schedules (Ndukuba and Ramachandran, 1991a).

In support of the presently observed effect of melatonin in lizards are the studies with ewes in which duration of melatonin level was found to be critical for the induction of photoperiodic effects (Wayne *et al.*, 1988). Based on the present contention that tail regeneration in Hemidactylus is stimulated by the growth promoting influence of PRL and that longer photic schedules lead to increased PRL release and vice versa (Ndukuba and Ramachandran, 1989a; Ramachandran and Ndukuba, 1991), the presently observed antiregenerative effects in Mm lizards could be related with the reduced PRL release occurring, a duration effect of melatonin. Further, the antiregenerative effect of Mm injection was shown to be nullified by PRL administration (Ramachandran and Ndukuba, 1991). Similar nullifying influence of PRL on melatonin effects have been reported in the mink, *Mustela vision* (Murphy *et*

al., 1990). Enough evidence is available to link increasing photic schedule with PRL release (Barrell and Lapwood, 1979; Matthiej and Swarts, 1978; Munro *et al.*, 1980; Brown and Forbes, 1980). There are also documented evidences to show that melatonin can interfere with PRL secretion (Kennaway *et al.*, 1982; Symons *et al.*, 1983; Martinet *et al.*, 1983, 1985; Murphy *et al.*, 1990).

The proregenerative effect of Me seems to be mainly an amplitude effect of melatonin leading to greater PRL release in the early photophase. The high amplitude effect of melatonin can be related with a greater turnover of 5-HT in the hypothalamus, a potent secretogogue of PRL. The ability of 5-HT to induce PRL release is in this context very well documented (Lawson and Gala, 1976, 1978; Clemens *et al.*, 1977; James and Wigham, 1984). Increased serotonergic activity some 16-20 hrs. after melatonin administration have also been reported in the gold fish (Olcese *et al.*, 1981). Further evidence for the amplitude effect of melatonin is provided by our earlier observations of enhanced regenerative tail elongation in LD 12:12 during the summer months as compared to their counterparts in the monsoon and winter months (Ramachandran and Ndukuba, 1989a; Ndukuba and Ramachandran, 1991b). In this context, Vivien-Roels *et al.* (1988) have shown in the box turtle, *Terrapene carolina trianquis* that while photoperiodism is responsible for duration of melatonin in the dark phase, the ambient temperature is responsible for greater amplitude of melatonin. Moreover, the contention that evening melatonin

administration is responsible for greater PRL release caused due to the greater amplitude of melatonin is well supported by the reported elevation of PRL level subsequent to melatonin administration in the evening in rats (Vanage and Sheth, 1991). Additional evidence of 5-HT induced PRL release in Me animals is provided by our finding that both para-chlorophenylalanine, a specific inhibitor of 5-HT synthesis and, 5-HT antagonist, prevented the stimulatory influence of Me on tail regeneration (Ndukuba and Ramachandran, 1989b; Ramachandran and Ndukuba, 1989c; chapter, 5).

The presently observed diametrically opposite influence of ML suggests two possibilities. One, that ML is an active pineal principle in lizards and secondly ML also has a time dependent influence on PRL release. However, the diametrically opposite effect of ML as compared to M is understandable by the reported higher plasma levels of this indole during the night time (Carter *et al.*, 1979; Mefford *et al.*, 1983; Skene *et al.*, 1986). Though the exact mechanisms are not clear, it is presumable that ML administered in the evening dampens PRL release, while in the morning it potentiates PRL release. Some experiments in this context attempting to understand the time dependent influence on PRL release are in progress in our laboratory.

Though MT has been implicated as an active pineal indole with antigonadotrophic properties like M in mammals (Masson-Pevet *et al.*, 1987; Saxena

and Mehrotra, 1992), interestingly this indole was totally ineffective in influencing PRL release in lizards. Overall, the present study gives evidence for both M and ML as potent pineal agents capable of influencing PRL release in a time dependent fashion and thereby influence the process of regenerative tail elongation. Further studies are needed to gauge the relative significance of the two indoles and their actions.

SUMMARY

In order to evaluate the time specific influence of pineal indoles on tail regeneration in lizards, melatonin (M), methoxytryptophol (ML), and methoxytryptamine (MT) were administered intraperitoneally to different groups of lizards in the morning at 07.00 hrs. (m) or in the evening at 17.00 hrs. (e). Attainment of various arbitrary stages of regeneration like wound healing, pre-blastema, blastema and initiation of growth were all delayed in Mm and MLe groups of lizards while they were hastened in Me and MLm groups of lizards. But MTm and MTe treatments did not show any difference compared to the controls. The total length of tail regenerated and percentage replacement at the end of 30 days were significantly less in Mm and MLe groups of lizards. MTm and MTe treatments however did not affect the regeneration process. The results indicate that M and ML have opposite time specific effects on regeneration, while MT has no effect. Apparently, M and ML are linked to photoperiodic and neuro-endocrine transductions related to regenerative growth in lizards.