CHAPTER III

PRELIMINARY EVIDENCE FOR PINEAL MEDIATED EXTRARETINAL PHOTORECEPTION IN RELATION TO TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS.

It is well established that among fishes, amphibians and reptiles the pineal organ, a small structure embedded in the top of the brain, and such associated structures as the parietal "eye" are sensitive to light. The pineal system (pineal organ and parietal eye) is light sensitive on the basic of neurophysiological and cytological evidence (Wurtman et al., 1968). Ultrastructural and neurophysiological studies have convincingly shown that the lizard's parietal eye is a functional photoreceptor (Eakin, 1973). The parietal eye often contains a well defined cornea, a lens, and a retina; the retina contains photosensory cells similar in appearance to those found in lateral eyes (Hamasaki and Eder, 1977). These photosensory cells synapse with ganglion cells which send axons to the rest of the brain. In some studies, removal or shielding of the parietal eyes of lizards has affected photoperiodic responses and activity of individuals exposed to field conditions or to photothermal gradients in the laboratory (Stebbins, 1963,1970;

Stebbins and Eakin, 1958). The obvious photoreceptive capabilities of lizard parietal eyes have prompted studies on the role this organ may play in mediating such light-dependent processes as activity, reproduction, metabolism and thermoregulation (Eakin, 1973; Ralph et al., 1979). These studies suggest that the parietal eye has an inhibitory role since removal or shielding the parietal eye in some cases, causes increased exposure of the lizards to photothermal stimuli. In such cases, however, it is not clear whether the parietal eye is directly involved as a photoreceptor organ or indirectly involved via the role it plays in thermoregulation (Ralph et al., 1979).

The pineal body is present in all vertebrates and generally appears to be glandular in nature. In lower forms, cells are present which are not unlike rod and cone cells in the retinae of normal eyes but are not organized as such. It is possible, however, that in the course of evolution there has been a change from primitive photoreceptive type of organ which can translate photic stimuli into physiological controls of different types, to a sensory structure which can carry out similar functions in response to stimuli affecting normal optic pathways. Many species of fishes, reptiles and amphibians monitor the light-dark cycle by way of a third eye (partietal or parapineal eye) or via the pineal itself (Eakin, 1973; Adler, 1976). Photosensory cells, ependymal (supportive) cells

and certain other types of cells have been recognized and described in the pineal organs of lacertilians (Steyn,1960; Eakin et al., 1961; Collin, 1967; Wartenberg and Baumgarten, 1968; Hamasaki and Dodt, 1969) and chelonians (Vivien-Roels, 1969). The consensus of opinion seemes to be that the peculiar cells having outer segments containing laminated cells are photosensory.

The perception of light provides important information for the organism about its environment. For this purpose, most animals possess well-developed photoreceptors and neuronal networks in the retinae of their lateral eyes. Interestingly, even in species with highly organized ocular photoreceptors, additional photoreceptive structures - extraocular photoreceptors-are utilized in the transmission of photic information about the day-night schedule and seasonal photoperiod changes. Considerable evidence supports the view that the pineal organ is the principal site of extraocular photoreception in lower vertebrates (cf. Meissl and Dodt, 1981). Recent radio-immunoassay studies have revealed the presence of circadian oscillators in the isolated pineal organs of the lizard, Anolis carolinensis (Menaker and Wisner, 1983).

Previous studies with lizards demonstrated that extraretinal photoreceptors are involved in the photoperiodic response in A. carolinensis (Underwood, 1975). Accordingly, long-stimulatory photoperiods have been shown to induce testicular recrudescence and maturation in blinded Anoles.

Similar studies have been condcuted on a variety of vertebrate species such as fishes and birds where testicular growth could be induced in blinded animals by exposure to stimulatory photoperiods (Underwood, 1979). According to Maier and Singer (1977) and Turner and Tipton (1972), long-length photoperiod can speed up the rate of forelimb regeneration in the newt and tail regeneration in lizards, respectively. This effect is not mediated by the optic system as blinded newts kept in continuous light regenerated their forelimbs more rapidly than their sighted counterparts kept in total darkess (Maier and Singer, 1977).

The response of homeothermic animals to light is known to be influenced by pineal activity (reviews by Kappers, 1971; Sorrentino and Benson, 1970; Relkin, 1975; Oksche, 1976). In reptiles, the effects of pinealectomy have been studied minaly with regard to gonadal development, and behavioural activity (Stebbins, 1970; Levey, 1973; Haldar and Thapliyal, 1977; Thapliyal and Haldar, 1979; Underwood, 1981). To date, no investigation has yet been carried out on pineal mediated extraretinal photoreception in relation to tail regeneration in lizards in general and Hemidactylus in particular. Hence, the present preliminary investigation was designed to elucidate the role of the pineal organ in photoperiodic photoreception during the process of tail regeneration. One group of

Hemidactylus flaviviridis had their heads painted with a mixture of Indian ink and Nile Blue Sulphate (II-NBS) in order to prevent light from penetrating to the pineal organ, another group had their organs surgically removed (pinealectomy) and the regenerative potentials were compared to the normal (unoperated and non-painted) counterparts exposed to similar experimental photoperiodic schedules.

MATERIALS AND METHODS

A total of 760 lizards was used in this investigation and they were divided into four groups and exposed to the eight experimental photoperiodic schedules as described on pages 12 and 13.

Group 1 - Experimental (PX) :

The first group of 320 lizards, which served as the experimentals, had their pineal organs surgically removed (PX). A small cut was made with a sharp scissors on the skin above the skull, thereby exposing the skull. A semi-circular incision was then made on the skull with a sterilized surgical blade in such a way that a flap of the skull was lifted up to expose the brain and the attached pineal organ. The pineal was removed with a sterilized forceps. Bleeding occurred

and the blood was wiped off with a piece of clean cotton wool. The flap of skull was replaced in its original position and then covered with the skin. Neomycin skin ointment was applied over the operated region to prevent possible infection due to surgery. Pinealectomy in each animal took about three minutes and PX lizards were allowed 5 days recovery period in order to eliminate any traumatic side effect due to surgery. These were then divided into eight batches of forty lizards each and exposed to the eight lighting schedules as described on pages 12 and 13. Mortality in PX animals was negligible. At the end of the experiment, PX lizards were sacrificed and microscopic examination as well as histological study showed that the pineal was removed completely and no damage was done to the brain. Food and water were provided ad libitum.

Group 2 - Experimental (NL -HP) :

The second group of 80 lizards had the brain region of the head painted with a mixture of equal parts of Indian ink and 10^{-3} Nile Blue Sulphate, in order to prevent light from penetrating to the pineal organ (NL - HP). Since H. flaviviridis sheds its skin epithelium periodically, it was necessary to reapply the painting mixture every alternate day. The mixture was applied, using a thin brush, to an area extending rostrocaudally from the snout to the base of the skull and laterally to an area between the ears.

Two batches of forty lizards each from the group were exposed to continuous light and 12 hours of light regimes.

Group 3 - Controls (NL) :

The third group of 320 lizards had intact pineals without any head paint. Forty lizards each were then exposed to the eight photoregimes described on pages 12 and 13.

Group 4 - Controls :

To be certain that the results observed contained no toxicity artefact from the application of Indian ink-Nile Blue Sulphate mixture, a group of forty lizards had their dorsal pelvic region painted with the mixture. Twenty lizards each from this group were then exposed to continuous light and 12 hours light schedules.

Tail autotomy was performed by pinching off the tail at the third segment from the vent. The length of new growth (regenerate) in mm, was measured with a graduated meter rule and

recorded at fixed time intervals of 5,10,20,30,40,50 and 60 days post-caudal autotomy. At the end of the experimentation, the pinealectomized animals were sacrified and microscopical examination of the head region as well as histological study of the brain were made to ensure complete removal of the pineal without any damage to the brain. This investigation was conducted during the post-breeding monsoon months (August-October) and the recorded average monthly ambient, room and cage temperatures are given in table 2. The average daily temperature at the level of the animals in the lighted and dark chambers did not differ by more than 2°C. The data on the length of tail regenerated and the percentage replacement were subjected to an analysis of variance and further to Duncan's multiple range test with an alpha level of both 0.05 and 0.01 (Duncan, 1955).

RESULTS

The results are depicted in table 1 and figures 1-3. The blastemic stage appeared in LL and LD 18 : 6 exposed animals by day 5 to day 7 and in DD and LD 6 : 18 exposed animals by day 12 to day 14 post-caudal autotomy. In PX lizards, the same occurred by day 8 to day 10 and day 18 to day 20, respectively. In the intermediate photoperiod of (NLD; LD 12 : 12) and LD 16 : 8; the regeneration process started by day 8 to day 10 in NL and by day 15 in PX, while in the LD 8 : 16 exposed animals it occurred by day 10 to day 12 in NL and day 16 to day

18 in PX (Table 1). This temporal difference in regenerative outgrowth persisted and got amplified till the early differentiation phase after which it got minimized during the late differentiation and growth phase (Table 1). The arbitrary stages of regeneration shown in table 1 are described in Chapter 1.

Growth rate and total length regenerated :

A measurable growth occurred in LL and LD 18 : 6 groups of animals by day 5 in the case of NL and by day 8 in the case of PX while in NLD, LD 16 : 8 and LD 8 : 16 groups of animals it occurred between day 8 and 12 and days 15-18, respectively. However, in lizards exposed to DD and LD 6 : 18, a measurable growth occurred only between days 12 and 14 in the case of NL and days 18-20 in the case of PX (Table 1). The regeneration process was completed in LL (H), LL (L) and LD 18 : 6 photoperiodic schedules by the 50th day in both groups of animals at which time the total length of tail regenerated was 41.7 mm, 33.3 mm and 38.7 mm respectively in NL, and 28.3 mm, 27.8 mm and 27.1 mm, respectively in PX (Figure 1 and table 1). In the other groups of lizards, the regenerative growth ceased by day 60 and the least length regenerated (28.2mm - NL, 27.2 mm-PX, 27.4 mm - NL, 26.7 mm-PX) were in the LD 6 : 18 and DD groups. The total length of tail regenerated in the remaining groups of animals were nearly similar and were 33.6 mm (NL) and 27.9mm (PX) in LD 16:8, 33.0 mm (NL) and 27.5 mm (PX) in LD 12:12 and 31.0 mm (NL) and 26.6 mm (PX) in LD 8:16 (Figure 1 and table 1).

From figures 1 and 3, it is obvious that in NL, both the total length of tail regenerated and the percentage replacement are maximal under LL (H) and minimal under DD. Though the values with regard to these two parameters were quite similar in LL (L), NLD and LD 16 : 8 on one hand and LD 6 : 18 and DD on the other, a definite linear correlation between the length of photoillumination and the ultimate length of tail regenerated and total percentage replacement can be inferred. This fact is confirmed by the observed values under LD 18 : 6 which were significantly more than all the groups except LL (H) (Figures 1 and 3). In PX animals, the stimulatory influence of long-length photoperiods is abolished as can be deduced from the significant retardation in their regenerative potential when compared to their NL counterparts (Figures 1 and 3). Secondly, there is no significant alteration either in the initiation and onset of regeneration, the daily growth rate, the final length of tail replaced at the end of regeneration or the percentage replacement of the autotomized tail in PX lizards exposed to the eight experimental photoperiodic regimens under investigation (Figures 1-3 and table 1).

The pattern of growth rate depicted in figure 2 indicates a linear increase peaking at 30-40 days in NL lizards exposed to all photoperiodic schedules from DD to LD 16 : 8. However, LD 18 : 6 and LL photoregimes induced a very significant initial growth spurt which rendered the growth rate curve a biphasic one with increasing lengths of light beyond 16 hours having a

definite stimulatory influence on this initial spurt. The stimulatory influence of long-day photoperiods was further revealed by the gradually decreasing peak growth rate from LD 16:8 to DD (Figure 2). A biphasic growth pattern, though quantitatively attenuated, was also discernible in PX and NL (HP) lizards under LD 18:6 and LL photoperiodic schedules, while the animals exposed to the other lighting regimens showed a linear increase peaking at 30-40 days post-caudal atutotomy (Figure 2).

Total percentage replacement :

Percentage replacement in NL lizards, calculated in terms of total length of tail regenerated and total length of tail autotomized, revealed a minimum of 50.5 in DD exposed lizards and a maximum of 75.3 in LL (H) exposed animals followed by 70.4 in animals exposed to LD 18:6 photoperiodic schedule (Figure 3). Lizards exposed to LL (L), NLD and LD 16:8 recorded nearly similar replacement of 62.5%, 61.7% and 62.7% respectively. Lizards exposed to 8 hours of light showed slightly reduced percentage replacement of 57.8 while those exposed to 6 hours of light produced a replacement of 52.7%, more like the DD exposed animals (Figure 3). Pinealectomy as well as head paint in general, nullified the stimulatory effects of light and produced a near similar replacement of 49.51% (Figure 3).

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN NORMAL AND PINEALECTOMIZED H. FLAVIVIRIDIS.

PHOTOREGIMES	WOUND HEALING		BLASTEMA		EARLY DIFFEREN-TIATION		DIFFEREN-TIATION		LATE DIFFEREN-TIATION		GROWTH		FULLY REGEN-ERATED TAIL	
	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px	NL/BL	Px
LL (H)	3	5	5-7	8-10	7-9	10-12	10	15	20	25	30	32	50	50
LL (L)	3	5	5-7	8-10	7-9	10-12	10	15	30	35	40	42	50	50
LD 18 : 6	3	5	5-7	8-10	7-9	10-12	10	15	25	30	35	40	50	50
LD 16 : 8	5	7	8-10	15-17	12-14	18-20	20	25	30	35	40	42	60	60
LD 12 : 12	5	7	8-10	15-17	12-14	18-20	20	25	30	35	40	42	60	60
LD 8 : 16	5	7	10-12	16-18	14-16	20-22	22	27	32	37	42	44	60	60
LD 6 : 18	8	10	12-14	18-20	16-18	22-24	25	36	35	40	45	46	60	60
LD 0 : 24	8	10	12-14	18-20	16-18	24-26	25	38	40	42	45	47	60	60
LL (HP)	8	10	12-14	18-20	16-18	24-26	25	38	40	42	45	47	60	60
NLD (HP)	8	10	12-14	18-20	16-18	24-26	35	38	40	42	45	47	60	60

NL-NORMAL LIZARDS
BL-BLINDED LIZARDS
Px-PINEALECTOMIZED LIZARDS

LL(H) - CONTINUOUS LIGHT (HIGH INTENSITY), LL(L) - CONTINUOUS LIGHT(LOW INTENSIT
LD 18:6 - 18 HOURS OF LIGHT (HIGH INTENSITY) AND 6 HOURS OF DARKNESS
LD 16:8 - 16 HOURS OF LIGHT (HIGH INTENSITY) AND 8 HOURS OF DARKNESS
LD 12:12 - 12 HOURS OF LIGHT (HIGH INTENSITY) AND 12 HOURS OF DARKNESS
LD 8:16 - 8 HOURS OF LIGHT (HIGH INTENSITY) AND 16 HOURS OF DARKNESS
LD 6:18 - 6 HOURS OF LIGHT (HIGH INTENSITY) AND 18 HOURS OF DARKNESS
LD 0:24 - CONTINUOUS (TOTAL) DARKNESS
LL (HP) - HEAD PAINTED LIZARDS EXPOSED TO CONTINUOUS LIGHT(HIGH INTENSITY)
NLD(HP) - HEAD PAINTED LIZARDS KEPT IN NORMAL LIGHT AND DARKNESS (LD 12:12)

TABLE 2. AVERAGE AMBIENT, ROOM AND CAGE TEMPERATURES DURING THE PERIOD OF STUDY.

MONTHS*	TEMPERATURE MEASUREMENT IN °C						HUMIDITY	
	AMBIENT		ROOM		CAGE			
	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.
AUGUST 1986	29.0	23.9	28.0	21.0	28.0	22.0	99%	42%
SEPTEMBER 1986	35.4	24.6	32.0	22.0	23.0	21.0	94%	40%
OCTOBER 1986	37.7	22.0	34.0	20.0	35.0	20.0	93%	13%

AVERAGE DAILY TEMPERATURES

LIGHTED CHAMBER = 27°C

DARK CHAMBER = 25°C

* POST-BREEDING MONSOON SEASON.

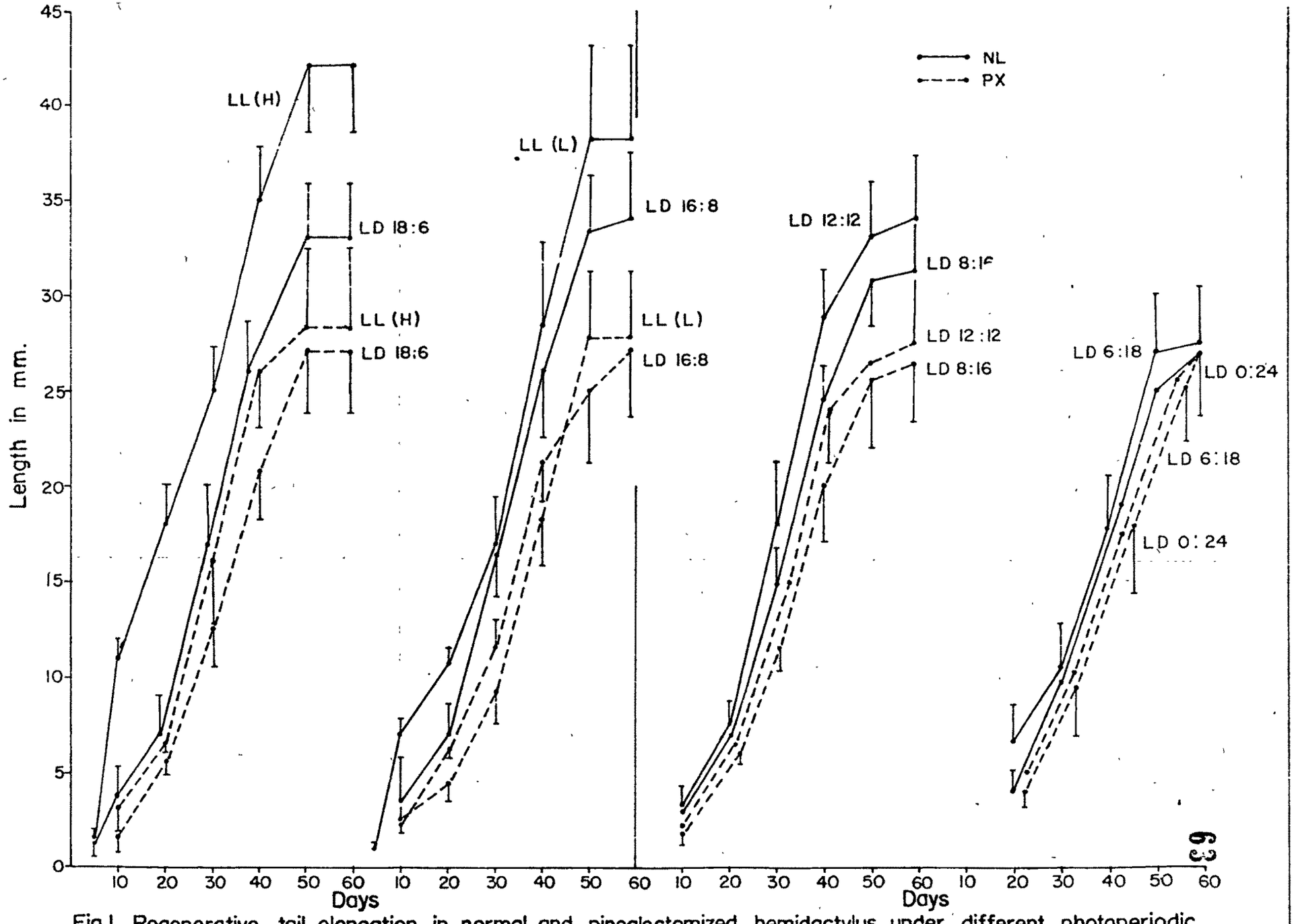

Fig.1. Regenerative tail elongation in normal and pinealectomized hemidactylus under different photoperiodic regimens.

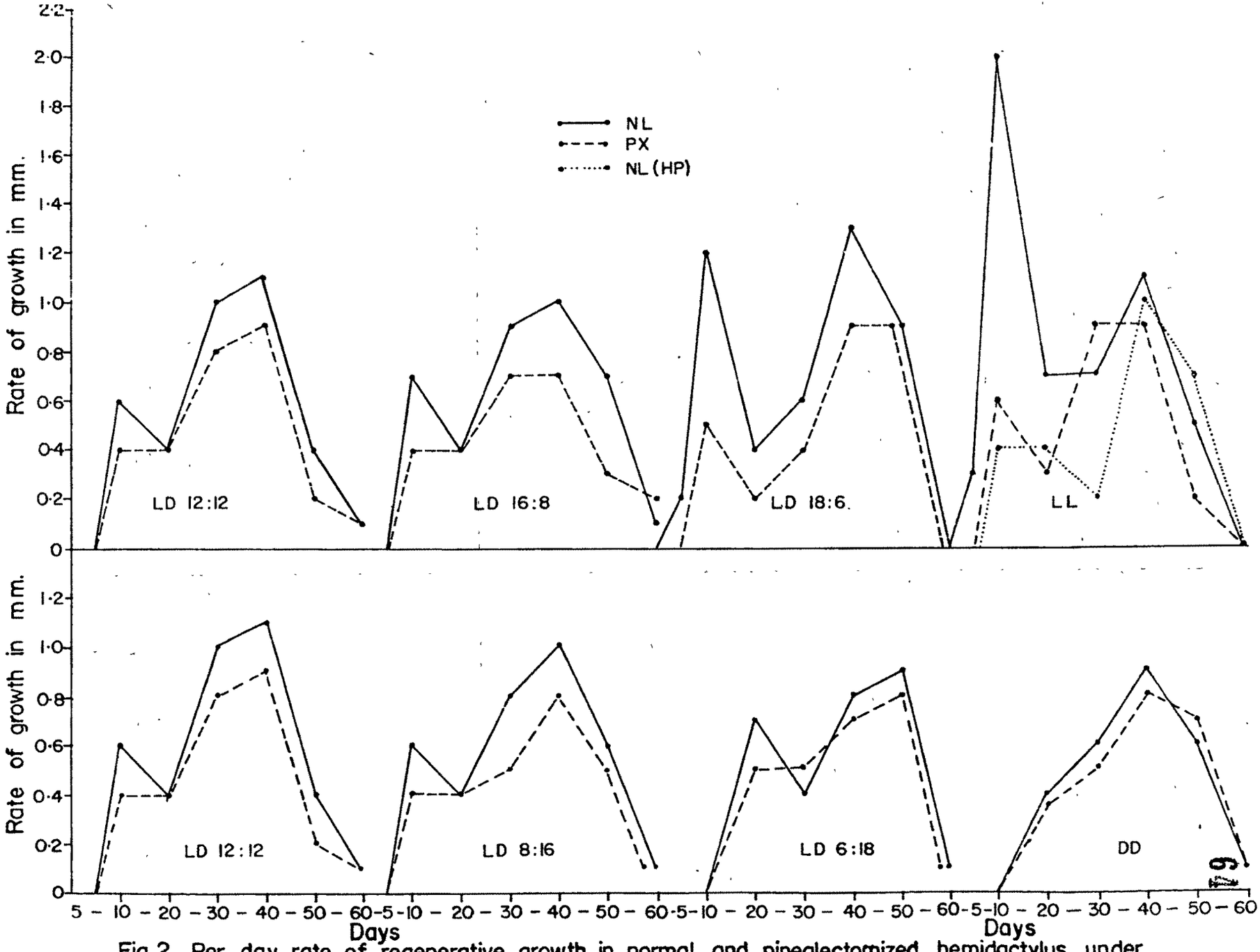

Fig.2. Per day rate of regenerative growth in normal and pinealectomized hemidactylus under different photoperiodic regimens.

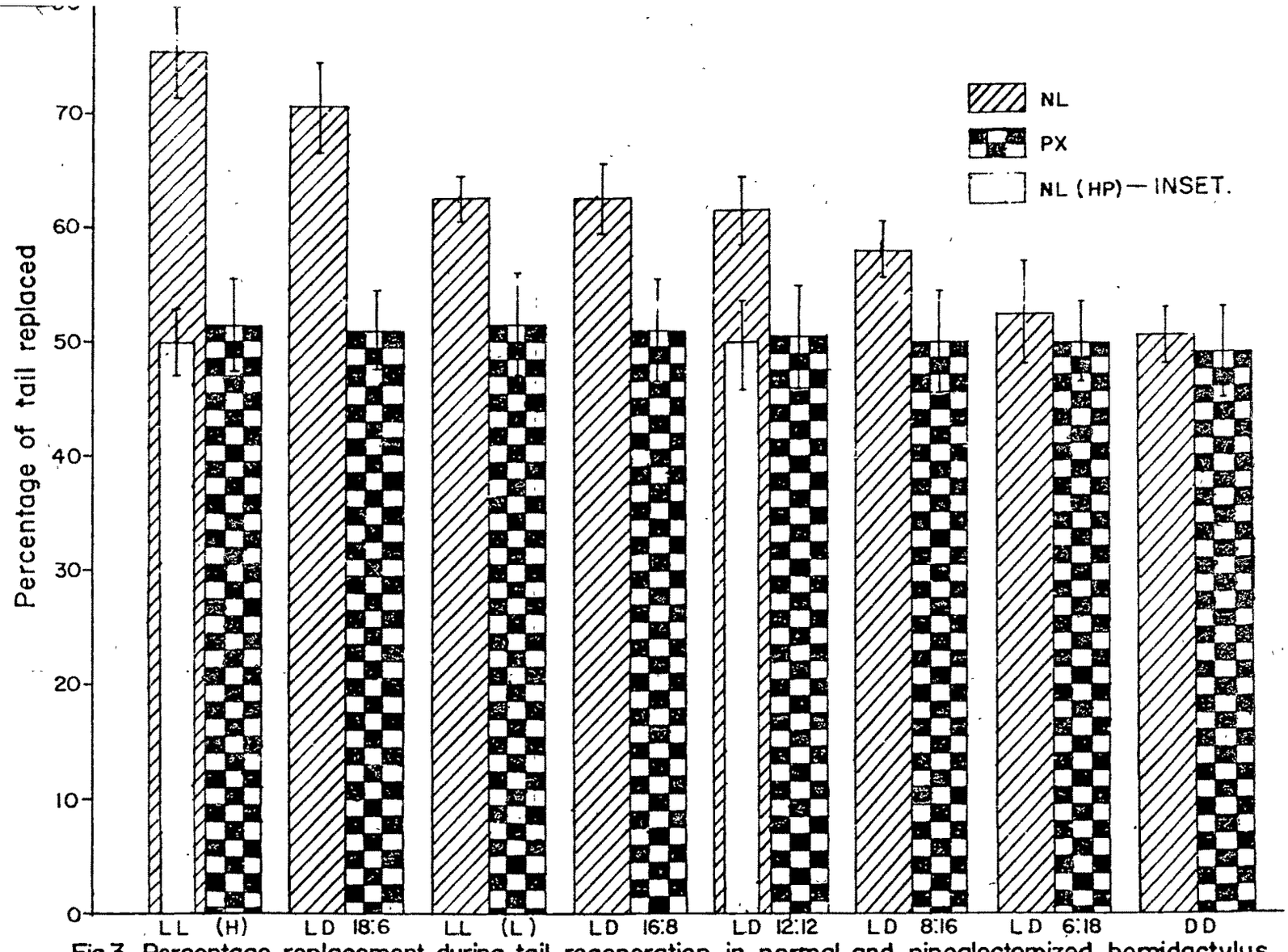

Fig.3. Percentage replacement during tail regeneration in normal and pinealectomized hemidactylus under different photoperiodic regimens.

LL(H)-continuous light (high intensity); LL(L)-continuous light (low intensity); DD-continuous (total) darkness; NLD-normal light and darkness; LD 18:6-18 hours light and 6 hours darkness; LD 6:18-6 hours light and 18 hours darkness; LD16:8-16 hours light and 8 hours darkness; LD 8:16-8 hours light and 16 hours darkness; NL-Normal lizards; PX-pinealectomized lizards; NL (HP)-normal lizards with head painted

EXPLANATION OF PHOTOGRAPH

EXTRARETINAL PHOTORECEPTION IN LACERTILIAN TAIL REGENERATION

BLINDING DID NOT AFFECT TAIL REGENERATION BUT PINEALECTOMY SIGNIFICANTLY RETARDED IT IN LIZARDS EXPOSED TO LL(H) PHOTOREGIME.

PX - PINEALECTOMIZED LIZARD.

BL - BLINDED LIZARD.

NL - NORMAL (UNOPERATED) CONTROL LIZARD.

LL(H)- CONTINUOUS LIGHT (2,500 LX INTENSITY).

PX BL NL

All possible comparisons between the eight experimental set ups in NL, NL (HP) and PX were made with reference to Duncan's multiple range test (Duncan, 1955). No statistical significance was found between NLD, LL (L) and LD 16:8 amongst the NL groups and between all PX and NL (HP) groups of animals. However, all other comparisons other than these amongst NL as well as between NL, PX and NL (HP) groups were statistically significant at both 5% and 1% levels.

DISCUSSION

Previous studies in our laboratory have demonstrated that the duration of photic input as well as its intensity have a definite stimulatory influence on lacertilian tail regeneration (Ndukuba, and Ramachandran, 1988 Chapter 1). Furthermore, it has also been shown that the lateral eyes, or retinae, do not participate in photoperiodically significant photoreception in the Gekkonid lizard, Hemidactylus flaviviridis since blinded lizards regenerated their lost (autotomized) tails similar to their sighted counterparts exposed to similar experimental photoperiodic regimes (Ndukuba and Ramachandran, 1988 Chapter 2). This study, aimed at evaluating the possible role of the pineal organ in mediating the photic influence on tail regeneration in lacertilians, has revealed that in H. flaviviridis, the pineal is the principal photoreceptor organ since both pinealectomy as well as light deprivation to the pineal, abolished the

stimulatory influence of long-length photoperiods on tail regeneration. A comparative assessment of the new growth (regenerate) shows that the initiation of regeneration, the daily growth rate, the final length of tail replaced at the end of regeneration and the percentage replacement of the autotomized tail are all significantly retarded in PX Hemidactylus and also in NL (HP) lizards as compared to their NL counterparts exposed to similar experimental photoregimes.

Most investigations on a potential role for retinal receptors have been conducted with birds. A long series of investigation by Benoit on the domestic duck demonstrated the participation of extraretinal receptors in the photoperiodic response of ducks (Beniot, 1935). Many other different combinations of experiments led Benoit to conclude that both retinal and extraretinal photoreceptors are involved(Benoit, 1970). However, a careful reconsideration of the published work of Benoit by McMillan et al. (1975) led them to conclude that a retinal participation in photoperiodism in ducks has not been conclusively demonstrated. The participation of extraretinal receptors in testicular responses in a second avian species, the house sparrow, Passer domesticus, was shown by Menaker and Keatts (1968). A series of experiments with house sparrows, utilizing several different experimental approaches,demonstrated that the eyes are not involved in photoperiodic photoreception, extraretinal receptors located in the brain are fully capable of

mediating this response (Underwood and Menaker, 1970; McMillan et al., 1975). Previous studies with lizards demonstrated that extraretinal photoreceptors are involved in photoperiodic response in Anolis carolinensis (Underwood, 1975). Accordingly, long stimulatory photoperiods have been shown to induce testicular recrudescence and maturation in blinded Anoles. Similar studies have been coducted on a variety of vertebrate species such as fishes and birds where testicular growth could be induced in blinded animals by exposure to stimulatory photoperiods (Underwood, 1979).

In our study of pineal mediated extraretinal photoreception in H.flaviviridis during the process of tail regeneration, NL lizards replaced a minimum of 50.5% in LD 0:24 and a maximum of 75.3% in LL (H). In contrast, PX lizards replaced about 50% of the autotomized tail in all photoperiodic schedules investigated. Obviously, a 50% replacement can be considered as basal or innate level of regenerative ability which is independent of any photoperiodic influence and can occur irrespective of the presence or absence of light. However, replacements beyond 50% are positively correlatable with increasing photoperiodism which could be nullified by pinealectomy as well as light deprivation to the pineal organ. Presumably, an intact pineal is the essential receptor cum synchronizer of the photostimulatory response in Hemidactylus during its tail regeneration. However, a biphasic growth pattern, though quantitatively attenuated, was discrenible in

both PX and NL (HP) lizards under LD 18:6 and LL photoregimes. It is difficult to give a sound interpretative explanation to this observation. Nevertheless, it could be speculated that in the absence of the pineal, the principal photoreceptor organ in lacertilians (Ramachandran and Ndukuba, 1989a), the lateral eyes, or retinae, may, indeed, be able to absorb and transmit some amount of photic information under long stimulatory photoperiods of higher light intensities which could be responsible for the expression of the biphasic growth pattern in PX lizards. It is known that the hypothalamus controls and integrates many of the neuroendrocrine functions in vertebrates and that the suprachiasmatic nucleus serves as a circadian pace maker (Rusak and Zucker, 1979). In this context, it may be presumed that under long photoperiods of higher light intensities, small amount of retinally transmitted light impinges upon the suprachiasmatic nucleus to produce the biphasic growth spurt observed in PX lizards.

On the basis of current knowledge, the most likely photoreceptor in lower vertebrates is the pineal organ. Over the years, a body of information, based largely upon indirect morphological evidence has accumulated to the effect that the epiphyseal complex of the lower vertebrates is responsive to light and darkness. A few electrophysiological studies now lend direct evidence of such activity in fishes (Dodt, 1963; Morita, 1966; Hangri et al., 1969; Falcon and Meissl, 1981) and reptiles (Miller and wolbarsht, 1962). The pineal complex of lizards, the most extensively studied group of reptiles,

is photosensory. Many lizard species have as part of the pineal complex a superficial parietal or "third" eye (Gundy and Wurst, 1976). The eye, an excellent wavelength discriminator, is more highly organized than the homologous frontal organ of amphibians (Dodt and Scherer, 1968). In Cyotaphytus collaris, the parietal eye and the intracranial pineal organ have a feedback relationship, wherein, the parietal eye sends afferent impulses to the pineal body and the pineal body sends efferent signals to the parietal eye (Engbretson and Lent, 1976). A parietal nerve in Lacerta viridis projects into the habenular region and a nerve from the pineal body reaches the subcommisural organ with some fibers traversing the posterior commisure (Kappers,1987). Recent radioimmunoassay studies have revealed the presence of circadian oscillators in the isolated pineal organ of the lizard, Anolis carolinensis (Menaker and Wisner,1983). Circadian rhythms are characterized by three major properties: they oscillate under constant conditions (free-run), they can be synchronized by environmental light-dark (LD) cycles (entrainment), and their periods vary only slightly with changes in ambient temperature (temperature-compensation). The pineal organ of A. carolinensis contains one or more temperature-compensated circadian oscillators coupled with photoreceptors on the input side and to melatonin synthetic pathways on the output side. In A. carolinensis, some of the photoreceptors are coupled with the circaidan oscillators that regulate the synthesis of melatonin, since the rhythm in isolated Anolis pineals can be entrained by LD cycles (Menaker and Wisner, 1983).

How the pineal may respond to affect the rate of tail regeneration in H. flaviviridis can only be speculated upon. Melatonin is produced by the pineal gland and is a mitotic inhibitor (Banerjee and Margulis, 1974). Melatonin can be suppressed by extended exposure to light (Brownstein, 1975). Litwiller (1940) demonstrated that the mitotic rate of blastemal cells peaks during the light phase of the diurnal cycle. Results of the present study demonstrate that it is during the pre-blastemic, blastemic and early differentiation stages of regeneration, characterized by high mitotic potential, that the stimulatory influence of increasing lengths of light is essentially exerted in NL animals. Apparently, photic input is being transduced and translated into hormonal and,or,physiological responses favouring growth potential, though the exact action at the cellular level remains speculative. It may be that the increased mitotic rate during the daylight hours and its subsequent decline during the dark phase bears a causal relation to the melatonin cycle. Alternatively, increased or decreased lengths of light may affect the production of prolactin which is a known growth promoter (Crim, 1975). Bourne and Tucker (1975) had, in fact, demonstrated the positive influence of increasing lengths of light on the level of serum prolactin. Serotonin could, in this respect, mediate the light effect since it is enhanced by light (Brownstein, 1975). Moreover, serotonin and its precursors have been shown to elevate serum prolactin levels (Lu and Meites,1973) and, therefore, could operate as a mitotic stimulator by way of its ability to induce prolactin release. These modulatory effects

of light in NL animals are abolished by pinealectomy as well as light deprivation to the pineal, since the regeneration process in PX and NL (HP) groups of lizards are not affected by either increased or decreased lengths of exposure to light.