CHAPTER

6

Thyroid Hormone and Tail Regeneration : Differential *in loco* and Systemic Effects and Seasonal Variation in Responsiveness

Thyroid hormones exert control over oxidative metabolism and metabolic activities of various organs of lacertilians like in mammals (John-Alder, 1980; Jacob and Oommen, 1990). Apart from metabolic activity, of late, thyroid hormones are also implicated in many other functions. (Allain and McGregor, 1993; McNabb, 1995; Studer and Derwahl,1995). In anuran tadpoles, regeneration of their limbs and tail is restricted to only the pre-metamorphic stages, and with metamorphosis, this capacity remains totally unrepresented in the adult condition. The thyroid hormone is considered to be inhibitory to regeneration in this group of amphibians. However, paradoxically, the thryoid hormone is reported to be an active evocator of regeneration in reptiles. Studies on newt limb regeneration have yielded no unified concept regarding the role of thyroid gland. Richardson (1940), Schotte and Washburn (1954) and Liversage and Brandes (1977) demonstrated impeded regeneration and abnormal development of skeletal elements following concomitant thyroidectomy and amputation in the adult newt, *Notophthalmus viridescens*. Regeneration was greatly enhanced if thyroidectomized adult newts were given daily injections of thyroxine beginning 10 days post-amputation. However, Connelly *et al.* (1968) observed that, hypophysectomised adult newts regenerated their limbs poorly when thyroxine was added to the medium. But Connelly *et al.* (1968) and Tassava (1969a,b) observed that hypophysectomized adult newts when treated with both thyroxine and prolactin regenerated their limbs more effectively. A later study showed that thyroxine alone

does not support regeneration in organ cultured adult newt tail blastema while, thyroxine in combination with insulin and, more particularly, when combined with insulin, growth hormone and hydrocortisone, advanced growth and cartilage differentiation (Vethamany-Globus and Liversage, 1973). The above observations suggested a multiple hormonal background or "*hormonal milieu*" for appendage regeneration in adult newts (Liversage and Scadding, 1969; Vethamany-Globus and Liversage, 1973; Liversage and Fisher, 1974). Subsequently, Korneluk and Liversage (1978) noted greater localization of labelled thyroid hormone in the regenerating forelimbs. However, Liversage and Korneluk (1978) did not observe any significant difference in circulating levels of T_4 and T_3 between regenerating and control newts. Ironically, none of the above studies had focused on the possible seasonal variations or even specified, the exact season of study. This is very pertinent as, poikilotherms are known to show significant variations in circulating levels of hormones and metabolic activities (See Ceusters *et al.,* 1978) and as such, Liversage and Korneluk (1978) had broached this aspect as a possible explanation for their observation on thyroid hormone levels.

Though hormonal dependence of amphibian appendage regeneration had received greater attention, there have been only very few studies on this aspect in Saurians. Two early studies suggested the importance of the pituitary gland on tail regeneration in *Anolis carolinensis*. (Licht and Jones, 1967; Licht and Howe, 1969).

Subsequently, Turner and Tipton (1971) evaluated the role of the lizard thyroid gland in tail regeneration and concluded that hypothyroidism inhibits tail regeneration by retarding the formation of the ependymal vesicle, which evokates the formation of a regeneration blastema (Kamrin and Singer, 1955; Simpson, 1964, 1965, 1970). In the same experiment they observed an early emergence of blastema by hyperthyroidism. Turner (1972) reported normal growth and differentiation in young hypophysioprivic regenerates by thyroxine treatment. It was again concluded from this study that thyroxine plays some role in regulating ependymal growth. Based on previous observations of altered haemopoietic changes and systemic metabolic profile in relation to thyroid histology during tail regeneration in *Mabuya carinata*, a tropical lizard, (Kinariwala *et al.*, 1978; Ramchandran *et al.* 1979, 1981b, 1985; Shah *et al.*, 1980a, 1982a) and, a later observation of inhibition of the above responses in 6-propyl-thiouracil (PTU) induced hypothyroidic animals, it was contended that thyroxine exerts its regulating influence on regeneration, even indirectly, by altering the adaptive modulations of systemic responses in the initial periods (Shah *et al.*, 1982b, Ramachandran *et al.* 1984, 1996). Observations detailed in Chapter 1 have provided compelling evidences for photothermal influences on regeneration and a seasonal variation thereat. Poikilotherms in general, and lacertilians in particular, are known to show variations in thyroid activity and metabolic feature on a seasonal basis (Lynn, 1970; Bennett and Dawson, 1976). Since both these factors are implicated in

tail regeneration in lizards, it is pertinent to evaluate the influence of thyroid hormone deficiency or excess on tail regeneration in lizards on a seasonal basis. The effects if any, on regeneration, by such manipulations of thyroid funtions could provide a rational explanation for the earlier observed seasonal difference in regenerative growth in terms of circulating titres of thyroid hormones and sensitivity. The present study in this context, deals with the effects of thyroid hormone deprivation or thyroid hormone excess on the course of tail regeneration in *H. flaviviridis* in the summer, monsoon and winter months.

MATERIAL AND METHODS

Adult *Hemidactylus flaviviridis* of 10 ± 2 grams body weight and a snout-vent length of 80 ± 5 mm were used for the present experiment. The procurement, acclimation and maintenance of the lizards are as described in Chapters 1 and 2. The experiments were conducted in the months of summer (April-May), monsoon (July-Sept.) and winter (Dec-Feb.) and are designated as Experimental schedule I, II and III respectively.

Experimental Schedule I (Summer)

This consisted of three set-ups involving nine groups of ten lizards each.

Set up 1 : (Hyperthyroidism)

This consisted of 3 groups of 10 lizards each. The lizards of group I received daily intraperitoneal (*ip*) injections of thyroxine at a concentration of 0.09 µg/lizard in 0.1ml of saline for 30 days at 09.00 hrs starting from the day of autotomy. These lizards served as the experimentals receiving systemic administration of thyroxine. Lizards of group II were injected locally (the tail) with 0.09 µg of thyroxine in 0.1ml saline for 30 days starting from the day of caudal autotomy and these served as experimentals receiving *in loco* thyroxine. The third group of lizards served as control and five of them received same amount of vehicle systemically while, the other five received locally.

Set up 2 : (Hyperthyrodism)

This consisted of 3 groups of lizards. Two groups of experimentals received ip. or *in loco* thyroxine daily for 15 days from the day of autotomy followed by injections every alternate day for the remaining 15 days at 09.00 hrs. The third group served as control and 5 of them received same amount of vehicle intraperitoneally while other five received the vehicle *in loco* as per the schedule of the experimentals.

Set up 3 : (Hypothyroidism)

These also consisted of three groups, of which , one served as control and the others as experimentals. The two experimental groups received 20 µg, or 50 µg of

methimazole (MMI), in 0.1 ml saline/lizard intraperitoneally at 1700 hrs starting 5 days prior to autotomy and continued for 30 days after autotomy. The control group received 0.1 ml of vehicle at the same time.

Experimental Schedule II (Monsoon)

This consisted of 8 groups of 10 lizards each and were done under 3 set ups.

Set up 1 : (Hyperthyroidic)

It consisted of two groups, one control and one experimental. The experimental group received 0.09 μg thyroxine in 0.1ml saline/lizard intraperitoneally daily for 30 day, at 09.00 hrs starting from the day of autotomy. The control group received same amount of vehicle for the same period at the same time.

Set up 2 : (Hyperthyroidic)

This consisted of three groups of lizards of which two were experimentals and one control. The two experimental groups received 0.09 μg thyroxine in 0.1ml saline either ip or *in loco* daily for 15 days from the day of autotomy and followed by every alternate day for the remaining 15 days at 09.00 hrs. In the control group, 5 of the lizards received the vehicle ip and the other 5 *in loco* as per the experimental schedules.

Set up 3 : (Hypothyroidic)

This again consisted of three groups, two of which were experimentals and one control. The two experimental groups received 50 μg MMI in 0.1ml saline per lizard ip. starting 5 days prior to autotomy at 17.00 hrs. Following autotomy, one group continued to receive MMI injections every day, while the other group received injections every alternate day. The control group received equal amount of saline, with 5 of them receiving the vehicle as per the schedule of one experimental group, and the other 5 as per the schedule of the other experimental group.

Experimental Schedule III (Winter)

This again consisted of three groups of lizards and the experiments were carried out under one set up.

Set up 1 : (Hyper and Hypothyroidism)

This consisted of four groups, two experimentals and two controls. One experimental group received 0.09 μg thyroxine *in loco* daily at 09.00 hrs for 15 days from the day of autotomy and thereafter every alternate day for the next 15 days. The control group received same amount of vehicle as per the same schedule. The other experimental group received 50 μg MMI in 0.1 ml saline per lizard (*ip*) daily at 17.00 hrs starting 5 days prior to autotomy and continued for 30 days thereafter. The control group received vehicle as per this schedule.

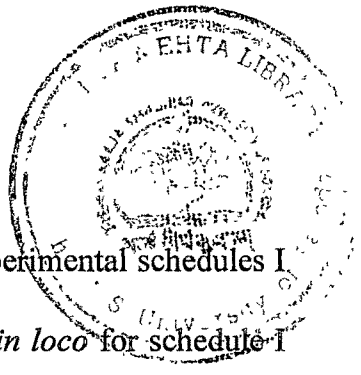
Preparation of Solutions

Thyroxine, commercially available as thyroxine sodium tablets (Glaxo India Ltd.) and each uncoated tablet containing thyroxine sodium IP 0.1 mg (equivalent to 0.091 mg of anhydrous thyroxine Sodium) synthetic thyroid hormone was used. Each tablet was dissolved in 0.6% saline and then diluted to obtain the final concentration of 0.091mg in 0.1 ml. Methimazole (Sigma Chemical Co, St. Louis, U.S.A) was prepared freshly daily before injection. Methimazole was dissolved in few drops of ethanol and then diluted appropriately with 0.6% saline to obtain a final concentration of either 20 μg /0.1 ml or 50 μg /0.1 ml.

Experimental Protocol

The cages housing the animals measured 18" x 15" x 10" with one side made of transparent glass and ventilated on three sides. Each cage housed a total of 10 lizards and they were balanced for size and sex. The studies were carried out during three seasons, viz., summer, monsoon, and winter and were maintained under natural photoperiodic conditions and temperature ranges.

RESULTS



Since the experimental groups under set up 2 in both Experimental schedules I & II produced similar results, the data of only one group (i.e. *in loco* for schedule I and systemic for II) are presented.

Experimental Schedule I (Tables 1-3& figs. 1-3)

All the experimental groups receiving thyroxine either systemic or *in loco* showed early formation of blastema and initiation of growth by two days, compared to the controls. However, the total length of tail replaced at the end of 30 days and the total percentage replacement were significantly lower in the experimental groups receiving thyroxine daily. Both the experimental groups showed identical tail replacement. Though there was an increased growth rate during the initial periods, it remained significantly low after 20 days.

The experimental groups receiving thyroxine every alternate day after 15 days showed however, a significant increment in total tail regeneration and total percentage replacement. Not only was there early growth initiation and increased growth rate, but even after 20 days, the growth rates appeared to remain steady as compared to the control. The hypothyroid lizards receiving MMI, formed a regeneration blastema and initiated growth at the same time as the controls. With both the dosages of MMI,

LEGEND TO FIGURE

Plate - 6

Photographs of (c) control (a) thyroxine administered (locally) and (b) thyroxine administered (systemically) lizards with thin regenerated tails at the end of 15 days post-autotomy. Note the improved growth due to systemic administration and more noticeable, due to local administration compared to control. (Lizards were maintained under LD 12:12 in the summer months).

PLATE- 6

TABLE - 1

Showing the number of days taken, the total length of tail regenerated and the percentage replacement at the end of 30 days in control and hyperthyroidic and hypothyroidic lizards.

Manipulations	Total length	Percentage replacement	Number of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	25.50 ± 4.20	40.47 ± 2.64	7.5	9	11	12
THYROXINE(S) DAILY	20.33[b] ± 3.57	32.26[b] ± 2.71	7	8	9	10
THYROXINE (L) DAILY	20.55[b] ± 3.44	32.61[b] ± 2.22	6	7.5	8.5	9.5

b - $P < 0.005$ compared to control.

Set up II (Hyperthyroidism)

CONTROL	23.00 ± 3.02	35.93 ± 3.08	8	10	11	12
THYROXINE (S) Alternate after 15 Days	28.00[b] ± 3.28	43.75[b] ± 3.43	6	7	8	9

b - $P < 0.005$ compared to control.

Set up III (Hypothyroidism)

CONTROL	29.00 ± 3.82	47.85 ± 3.66	5	6	7	8
MMI (20 µg)	21.4[b] ± 2.96	35.66[b] ± 3.47	6	7	8	9
MMI (50 µg)	11.00[b] ± 1.49	18.33[c] ± 1.52	6	7	8	9

WH - wound healing; PB - Preblastema; B - Blastema; IG - Initiation of Growth; s - Systemic l- Local; MMI - Methimazole, b - $P < 0.001$, c - $P < 0.001$.

TABLE - 2

Length of tail regenerated at different time periods post autotomy in control and experimental lizards.

Manipulations	DAYS				
	10	15	20	25	30
CONTROL	-	5.00 ± 5.20	12.80 ± 1.26	19.15 ± 1.89	25.40 ± 2.22
THYROXINE(S) DAILY	1.6 ± 3.57	5.90 ± 0.32	12.90 ± 1.00	16.56 ± 1.08	20.33[b] ± 2.12
THYROXINE (L) DAILY	1.8 ± 0.20	6.80 ± 0.71	13.80[a] ± 1.24	17.63[a] ± 1.86	20.55[b] ± 2.32

a - $P < 0.01$, b - $P < 0.005$.

Set up II (Hyperthyroidism)

CONTROL	-	4.65 ± 0.52	12.95 ± 0.76	18.60 ± 1.43	23.25 ± 2.68
THYROXINE (S) DAILY	1.12 ± 0.08	7.97[b] ± 0.88	16.22[b] ± 1.28	22.97[b] ± 2.44	27.97[b] ± 2.43

b - $P < 0.005$

Set up III (Hypothyroidism)

CONTROL	2.37 ± 0.44	8.22 ± 1.20	16.52 ± 1.53	24.30 ± 2.21	29.30 ± 2.58
MMI(20 μg)	1.5b ± 0.08	7.25b ± 1.12	12.23b ± 1.48	18.08b ± 2.52	21.60[b] ± 2.68
MMI (50 μg)	1.15[b] ± 0.12	4.41[c] ± 1.50	6.70[c] ± 1.73	8.49[c] ± 1.82	10.89[c] ± 1.90

S - Systemic L - Local; MMI - Methimazole, b - $P < 0.005$, c - $P < 0.001$ compared to control.

TABLE - 3
Per day rate of growth in control and experimental lizards in blocks of 5 days.

Manipulations	PER DAY RATE OF GROWTH				
	5-10	10-15	15-20	20-25	25-30
CONTROL	-	1.00	1.56	1.27	1.25
THYROXINE (S) DAILY	0.32	0.86	1.40	0.73	0.75
THYROXINE (L) DAILY	0.36	1.00	1.40	0.76	0.58

Set up II (Hyperthyroidism)

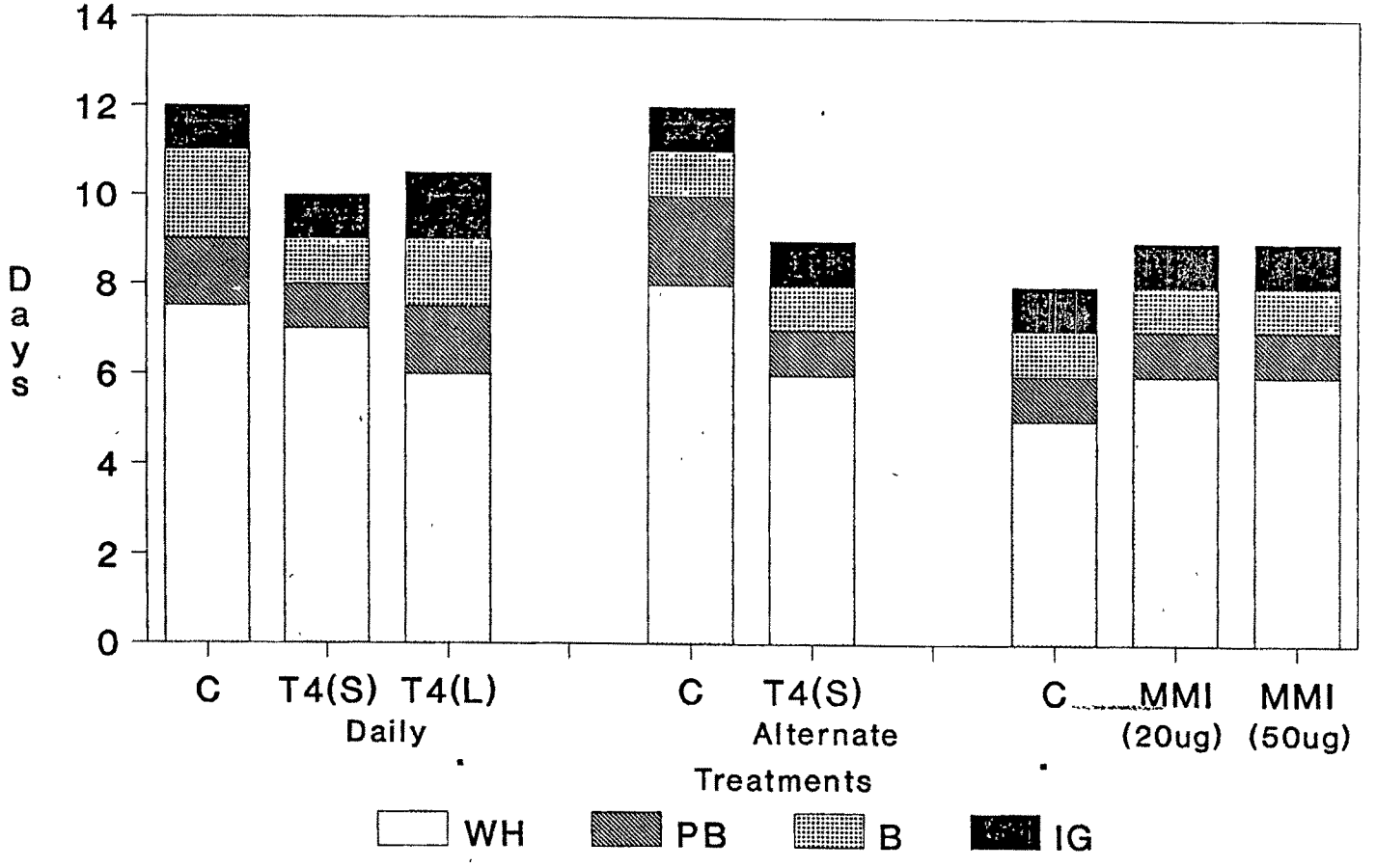
CONTROL	-	0.93	1.66	1.13	0.93
THYROXINE (L) Alternate after 15 days	0.22	1.37	1.65	1.35	1.00

Set up III (Hypothyroidism)

CONTROL	0.47	1.16	1.66	1.55	1.00
MMI(20 μg)	0.30	1.15	0.99	1.17	0.70
MMI (50 μg)	0.23	0.65	0.45	0.35	0.48

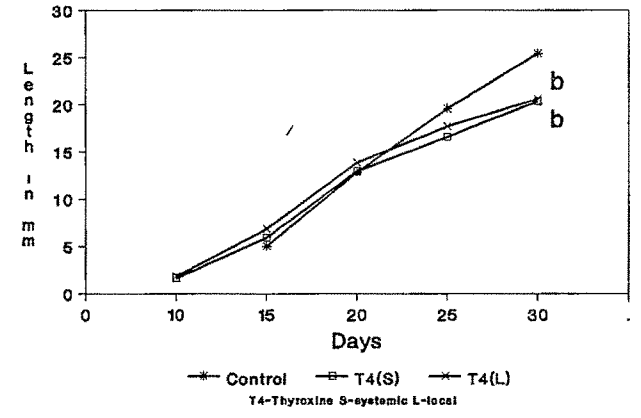
S - Systemic L - Local; MMI - Methimazole.

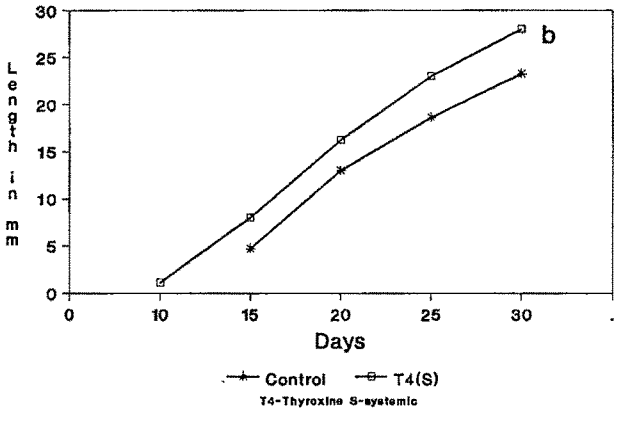
Fig.1 Number of days taken to attain the arbitrary stages in control (C) and experimental lizards.

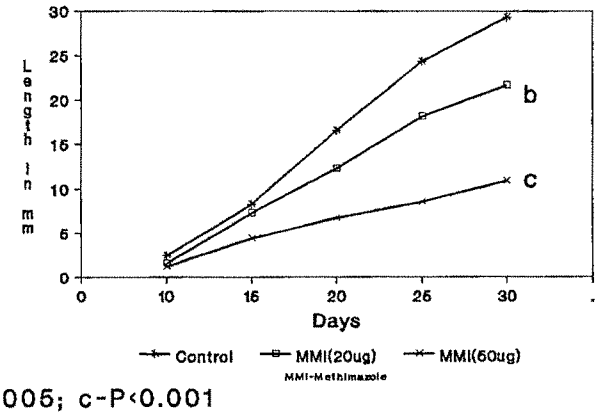


T4(S) - thyroxine(systemic) T4(L) - thyroxine(local) MMI-methimazole

Fig.2 The length of tail regenerated in control(C) and experimental lizards.

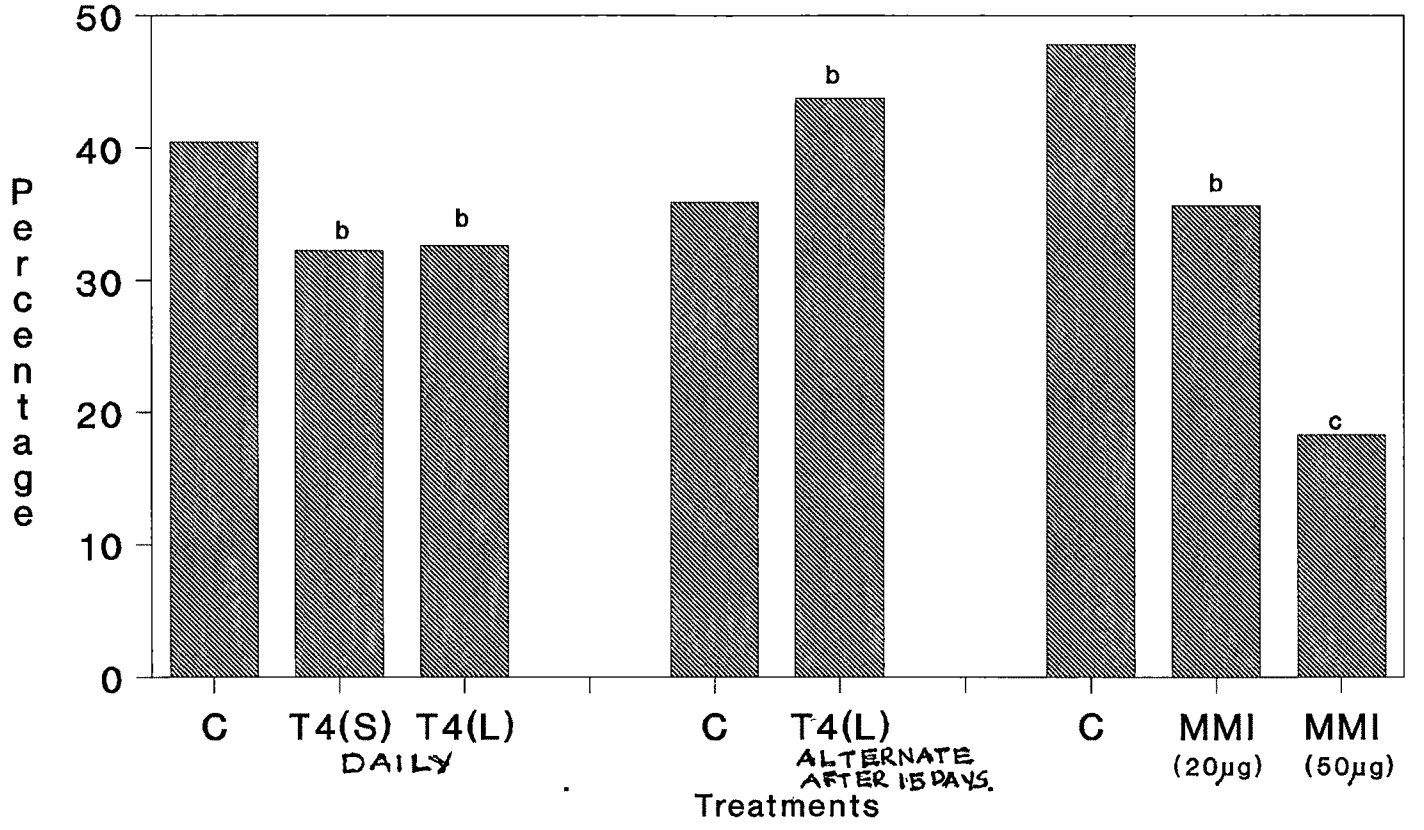






b-P<0.005; c-P<0.001

Fig.3 The percentage of tail replaced in control(C),hyper- and hypothyroid lizards at the end of 30 days in summer.



T4-Thyroxine (S)-systemic (L)-local MMI-Methimazole

a- P<0.01, b-P<0.005, c-P<0.001

there was significant retardation in the length of tail regenerated and, the total percentage of tail replaced at the end of 30 days. However, this retardatory influence was highly pronounced with the higher dosage of MMI. The overall growth rate in MMI treated lizards was significantly lesser than controls at all the time periods, this was more prominent in the 50 μg group.

Experimental Schedule II (Tables 4-6; figs. 4-6)

All the experimental groups receiving either thyroxine (hyperthyroidic) or MMI (hypothyroidic) showed a delay in the formation of blastema and initiation of regenerative growth by 2-3 days. The total length of tail replaced at the end of 30 days and the total percentage of tail replaced were also significantly lower in all the experimental groups. The retardation by hypothyroidism was more pronounced with, either the daily schedule or the alternate schedule showing no ultimate difference. The growth rate remained significantly low compared to control at all the time periods. The MMI treated lizards not only showed very poor growth rates, but also showed a fast tapering off of the growth rate after 20 days.

Experimental Schedule III (Tables 7- 9., fig.7)

There was significant delay in the control animals in the formation of regeneration blastema (24 days) and, initiation of growth (25 days). Both the experimental groups receiving, either MMI or thyroxine, showed a further delay by

TABLE - 4

Showing the number of days taken, the total length of tail regenerated and the percentage replacement at the end of 30 days in control and hyperthroidic and hypothyroidic lizards.

Schedule - II (Monsoon)

Setup I (Hyperthyroidism)

Manipulations	Total length	Percentage replacement	Number of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	16.44 ± 2.08	26.76 ± 3.12	8	9	11	12
THYROXINE DAILY (S)	9.87[c] ± 0.96	16.09[c] ± 1.63	8	10.5	12	13.5

Set up II (Hyperthyroidism)

CONTROL	15.85 ± 1.28	25.49 ± 2.58	5	6	7	8
THYROXINE DAILY (S) Alternate after 15 days	11.50[c] ± 0.86	18.85 ± 2.61	7	8	9	10

Set up III (Hypothyroidism)

CONTROL	15.85 ± 2.28	25.49 ± 2.12	5	6	7	8
MMI (50 µg) DAILY	7.5[c] ± 0.73	13.33 ± 1.58	10	11	12	13
MMI (50 µg) Alternate	7.8[c] ± 1.49	12.78[c] ± 1.66	7	8	9	10

WH - wound healing; PB - Preblastema; B-Blastema; IG - Initiation of Growth; S - Systemic L- Local; MMI - Methimazole, c - $P < 0.001$ compared to control.

TABLE - 5

Length of tail regenerated at different time periods post autotomy in control and experimental lizards.

Manipulations	DAYS				
	10	15	20	25	30
CONTROL	-	2.10 ± 0.12	5.75 ± 0.46	10.75 ± 0.88	16.35 ± 1.23
THYROXINE (S) DAILY	-	1.00[a] ± 0.08	3.00[b] ± 0.32	4.88[c] ± 0.28	9.83[c] ± 0.92

Set up II (Hyperthyroidism)

CONTROL	2.00 ± 0.08	5.57 ± 0.14	10.14 ± 0.54	13.59 ± 1.12	15.84 ± 1.22
THYROXINE (S) Alternate after 15 days	0.55[b] ± 0.03	2.60[c] ± 0.28	6.30[c] ± 0.43	8.97[c] ± 0.96	11.47[c] ± 1.08

Set up III (Hypothyroidism)

CONTROL	2.00 ± 0.06	5.57 ± 0.21	10.14 ± 0.56	13.59 ± 1.12	15.84 ± 1.34
MMI(50 µg) DAILY	-	2.00[c] ± 0.12	5.15[c] ± 0.62	6.65[c] ± 0.70	7.50[c] ± 0.63
MMI (50 µg) Alternate	1.16[a] ± 0.28	3.16[c] ± 0.32	5.66[c] ± 0.65	7.39[c] ± 0.99	7.86[c] ± 0.92

MMI - methimazol, S - systematic.

TABLE - 6
Per day rate of growth in control and experimental lizards in blocks of 5 days.

Schedule-II (Monsoon)

Set up I (Hyperthyroidism)

Manipulations	PER DAY RATE OF GROWTH				
	DAYS				
	5-10	10-15	15-20	20-25	25-30
CONTROL	-	0.43	0.73	1.00	1.12
THYROXINE (S) DAILY	-	0.20	0.40	0.37	0.99

Set up II (Hyperthyroidism)

CONTROL	0.40	0.71	0.91	0.69	0.45
THYROXINE (L) Alternate after 15 days	0.11	0.41	0.74	0.53	0.50

Set up III (Hypothyroidism)

CONTROL	0.40	0.71	0.91	0.69	0.45
MMI (50 µg) DAILY	-	0.40	0.63	0.30	0.17
MMI (50 µg) Alternate	0.23	0.40	0.50	0.34	0.09

MMI - methimazole, S - Systematic, L - Local.

Fig.4 Number of days taken to attain the arbitrary stages in control (C) and experimental lizards.

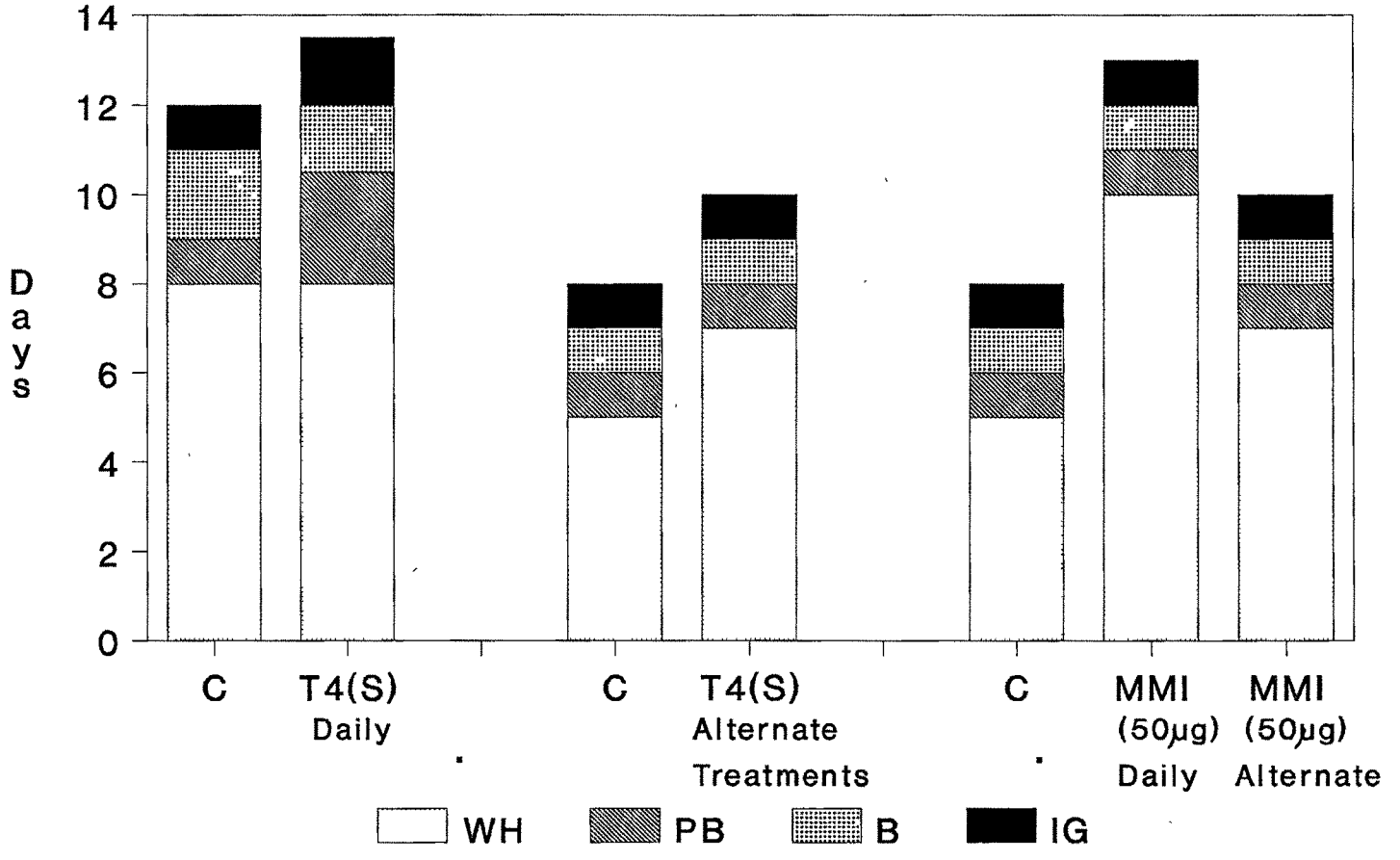
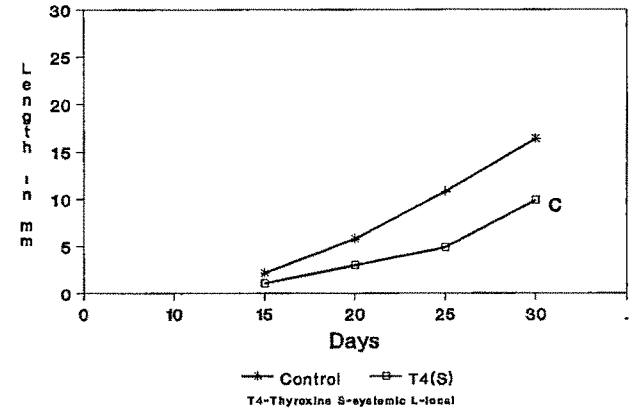
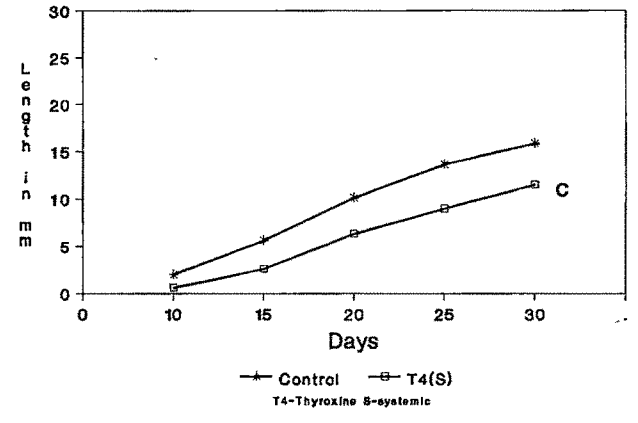
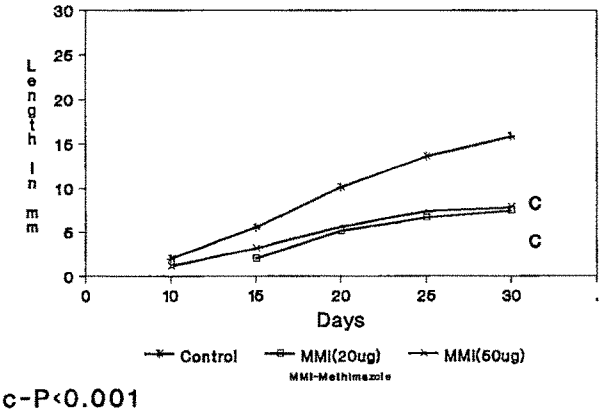


Fig.5 The length of tail regenerated in control(C) and experimental lizards.

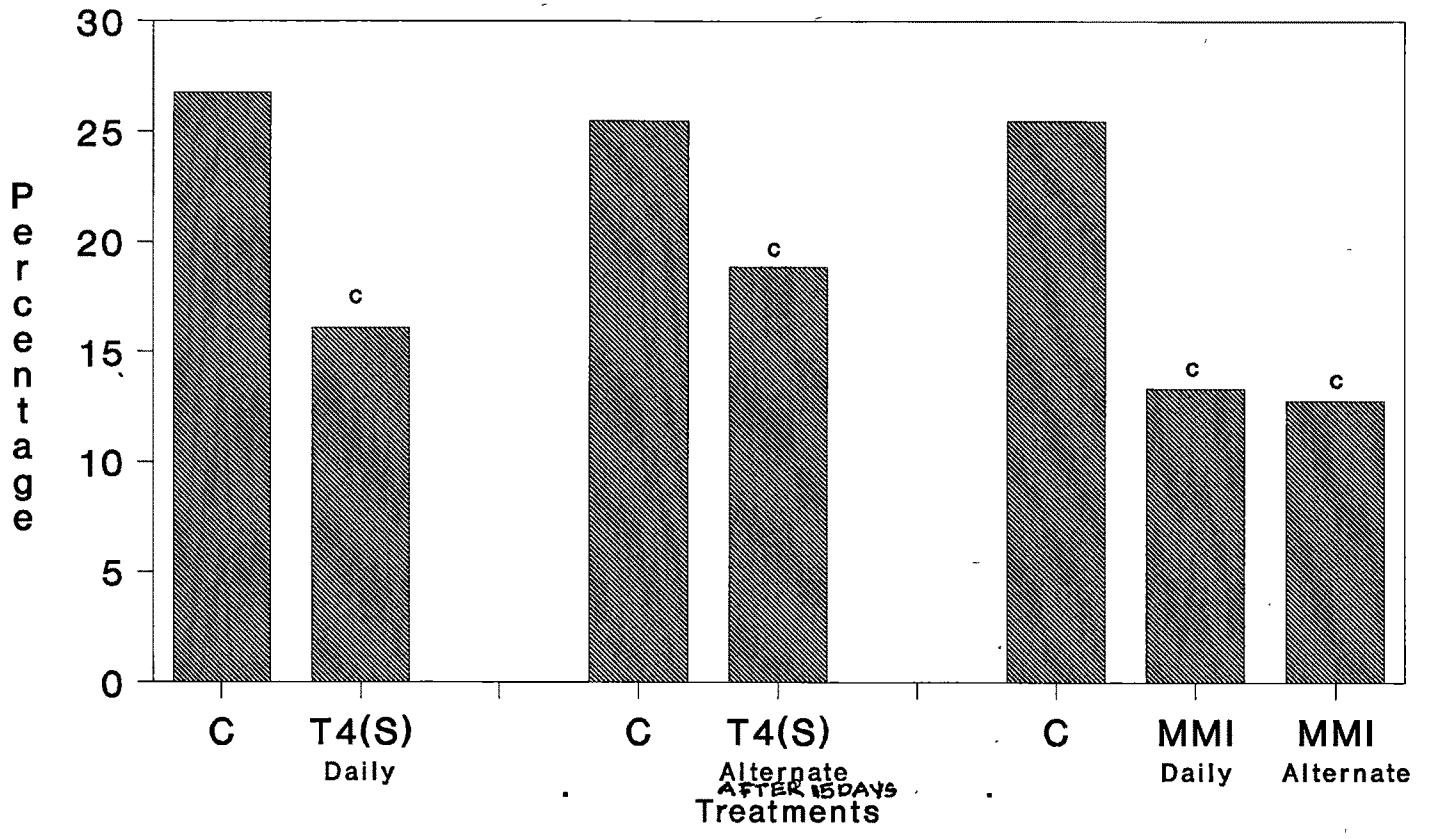






c-P<0.001

Fig.6 The percentage of tail replaced in control(C), hyper- and hypothyroid lizards at the end of 30 days in monsoon.



T4-Thyroxine (S)-systemic MMI-Methimazole

a- P<0.01, b-P<0.005, c-P<0.001

TABLE - 7

Showing the number of days taken, the total length of tail regenerated and the percentage replacement at the end of 30 days in control and hyperthroidic and hypothyroidic lizards.

Manipulations	Total length	Percentage replacement	Number of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	3.00 ± 0.12	4.90 ± 0.28	22	23	24	25
THYROXINE (L) Alternate after 15 days	3.07 ± 0.32	4.50 ± 0.15	23	24	25	26
MMI (50 µg)	2.8 ± 0.09	5.03 ± 0.61	23	24	25	26

TABLE - 8

Length of tail regenerated at different time periods post autotomy in control and experimental lizards.

Manipulations	DAYS				
	10	15	20	25	30
CONTROL	-	-	-	-	3.00 ± 0.28
THYROXINE (L) alternate after 15 days	-	-	-	-	3.07 ± 0.18
MMI (50 µg)	-	-	-	-	2.80 ± 0.12

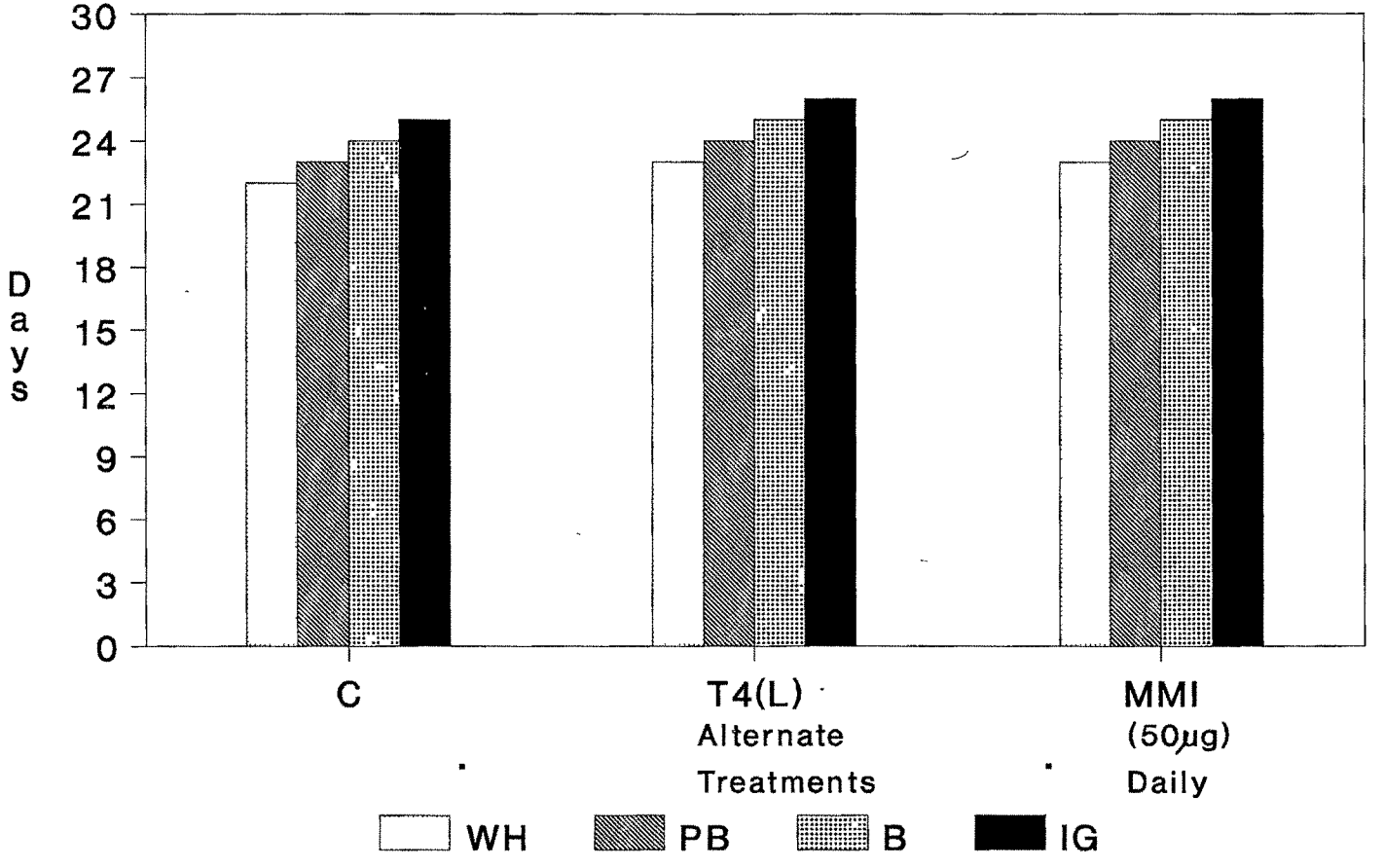
S - Systemic; L - Local; MMI - Methimazole.

TABLE - 9
Per day rate of growth in control and experimental lizards in blocks of 5 days.

Manipulations	PER DAY RATE OF GROWTH				
	DAYS				
	5-10	10-15	15-20	20-25	25-30
CONTROL	-	-	-	-	0.60
THYROXINE (L) Alternate after 15 days	-	-	-	-	0.16
MMI (50 µg)	-	-	-	-	0.56

L - Local; MMI - Methimazole

Fig.7 Number of days taken to attain the arbitrary stages in control (C) and experimental lizards.



T4(L) - thyroxine(local) MMI - methimazole

one day. The total length of tail regenerated at the end of 30 days and the total percentage replaced, were very poor with no significant difference between the experimentals and controls. Similarly, the growth rate was also identical in the experimentals and the controls. Though different controls have been used corresponding to the different treatment regimes under the three experimental schedules, data of only one control is presented as there was no apparent difference.

DISCUSSION

The present results have revealed interesting season specific (temperature dependent) and phase specific differential effects of thyroid hormone deficiency or excess on tail regeneration in *H. flaviviridis*. Two previous studies, one on the Scincid lizard, *Mabuya carinata* and the other, on the Gekkonid lizard, *H. flaviviridis*, had both demonstrated retarded tail regeneration under induced hypothyroidism (Ramachandran *et al.,* 1984; Ramachandran and Abraham, 1990). The study on *M. carinata* had also shown the reversal of hypothyroidism induced retardation by T4 replacement. Neither of these studies were on a seasonal basis, though, the study on *M. carinata* had a seasonal angle in terms of breeding activity. Nevertheless, the authors had documented no significant difference in the ambient temperature.

The retardatory influence of hypothyroidism has been related to a direct action of thyroxine at the local site to induce the outgrowth of the ependyma (a *priori* for the initiation of regeneration), as well as an indirect one by preventing the adaptive systemic responses (Turner and Tipton 1971; Turner 1972; Shah *et al.,* 1977, 1980a; Ramachandran *et al.,* 1979, 1981a,b, 1984). In the present study on hypothyroidism, maximum retardation was induced by higher dose of MMI and the retardation with this dosage was about 50% in the monsoon months and 60% in the summer months. Apparently, there is a similar retardation by hypothyroidism at both the summer and monsoon temperature ranges. However, an interesting observation, was a delay in the formation of regeneration blastema by hypothyroidism during the monsoon months, which was not evident in the summer months. Another observation that merits to be viewed together is the proportionately decreasing regenerative performance from the high summer temperatures to the lower winter temperatures through the intermediary temperature ranges in the monsoon, as also recorded previously (Chapter 1). A rational idea that emerges from these observations is, decreasing thyroid activity (resultant decrease in circulating thyroid hormone levels) and thyroid hormone sensitivity with decrease in temperature. In this respect, the above observed discrepancy in the number of days taken to form a regeneration blastema in hypothyroid lizards during the summer and monsoon months can be explained as due to a greater sensitivity to the thyroid hormones, despite their lower levels, in the

summer months. Obviously, formation of a blastema triggered by the ependymal outgrowth locally, as well as by adaptive modulations systemically, can occur even under subnormal thyroid hormone levels when the thyroid hormone sensitivity or responsiveness is higher. It can be hypothesized from the present observations that, temperature has dual but independent effects on thyroid activity and thyroid hormone sensitivity; higher temperatures increase thyroid activity and thyroid hormone sensitivity while, lower temperatures decrease both. However, the decrease in thyroid hormone levels induced experimentally at higher temperatures, does not affect the prevailing sensitivity. Concievably, the formation of the regeneration blastema is dependent on optimum sensitivity towards thyroid hormones rather than on the absolute levels of the thyroid hormones. There are evidences to show that the thyroid activity and thyroid hormone levels are dependent on temperature with, higher temperatures increasing and lower temperatures decreasing them (Wilhoft, 1958, 1964; Lynn, 1970; Thapliyal and Chandola, 1973). Our seasonal observations on thyroid histology in *H. flaviviridis* also affirm the same.

The growth rates and the ultimate total length of tail regenerated and, the total percentage replacement were, all significantly lower in the hypothyriod animals during both summer and monsoon months. On a comparative basis, the retardation in linear growth was more pronounced in the summer, despite an early formation of blastema and initiation of growth. This suggests that the proportionate increase in

regenerative growth occurring at higher temperatures is dependent on absolute levels of thyroid hormones along with sensitivity or responsiveness. At both the temperature ranges, the growth rate was proportionately decreased in response to the decreased thyroid hormone levels under the prevailing hormone sensitivity status. In the winter months, under the prevailing low temperatures, the formation of regeneration blastema as well as regenerative growth were significantly retarded. There is a protracted delay in the formation of blastema (25 days from the day of autotomy) in the control animals and, even MMI treatment showed same degree of delay (26 days). Even the regenerative growth was the same in the control and hypothyroid lizards. Apparently, at lower temperatures (winter months) the thyroid activity and thyroid hormone levels are so negligibly low that, further suppression by methimazole is of no consequence.

The corollary experiments involving hyperthyriodism, induced either by systemic or local administration of thyroxine, had yielded more variable observations. Continuous daily administration of thyroxine for 30 days from the day of autotomy, either systemically or *in loco*, produced an overall smaller tail regenerate and decreased percentage tail replacement. These were reflected in the comparatively reduced growth rates after 20 days despite an early initiation of growth and formation of regeneration blastema, in the hyperthyroid lizards. Clearly, exogenous thyroid hormone during the first 15 days not only hastens the formation of regeneration blastema but also provides an early growth spurt. However, continued administration

of thyroxine thereafter affects progressive tail elongation which would suggest an inhibitory effect of hyperthyroidism. There is obviously a favourable influence of supranormal thyroid hormone levels during the initial phases of regeneration while, such a level exerts an inhibitory influence in the later phases. Credence for this influence is provided not only by other observations from the present study but, also by some previous observations. The herein observed better regenerative performance in lizards administered with exogenous thyroxine daily for the first 15 days and only every alternate day thereafter, is one in this context. The observed increase in biphasic thyroid activity and serum thyroid hormone levels subsequent to caudal autotony in *M. carinata* and *H. flaviviridis* respectively, once during the first 10 days and the other after 25 days (Ramachandran *et al.*, 1981b, 1993) as well as the observation of the tapering of the growth rate in hyperthyroidic *A. carolinensis* (Turner and Tipton 1971) are others to this end. The early formation of regeneration blastema by either systemic or *in loco* thyroxine administration, is clearly a compounding effect of higher thyroid hormone concentration in the prevailing high sensitivity. This is in accordance with the earlier discussed ineffectiveness of hypothyroidism to alter the time course of blastema formation in the summer months. It can only be speculated as to how continuous supranormal levels of thyroid hormones retard tail regeneration in the later phases. Apart from the possibility of local effect on progressive histodifferentiation

leading to maturation of the regenerating tissues, a metabolic burnout, both *in loco* and systemic, can be the purported reasons.

In contrast to the observed effects during the summer months, thyroxine administration, either systemically or *in loco*, daily for 30 days or, daily for 15 days and every alternate day thereafter in the monsoon months, resulted in significantly decreased regenerative performance, though relatively more pronounced with continuous administration. Apart from the retardative influence on tail elongation, thyroxine administration also induced a temporal delay in blastema formation, unlike in the summer months. The poor regenerative performance and the reduced percentage replacement are reflected in the overall reduced growth rates throughout. Inferably, at the mean temperature ranges prevailing in the monsoon months (25°-28°C), supranormal thyroid hormone levels, exert a negative influence in every phase of regeneration.

A rational explanation for this is not forthcoming from the current status of understanding and, moreover, neither has any study brought out such an enigmatic revealation nor, has any study been conducted on a seasonal basis in relation to regeneration. However, considering the previous observaions on photothermal influences and inferred alterations in the features of melatonin rhythm due to photothermal effects (Chapter- 1), as well as the observed effects of melatonin on

regenerative process (Chapter-2), it is possible to seek some speculative explanations. It was previously reported that, while temperature increases the amplitude of the nocturnal melatonin signal, increased darkness produces a long duration melatonin signal. Further, it is also known that a greater fluctuation in the daily maximum-minimum temperatures maintains a robust melatonin rhythm (Chapter-1). The monsoon months not only show a much reduced fluctuation in the daily maximum-minimum temperatures but, also the variation in the duration of light-dark phases is minimal due to the approaching equinox. It is likely that, during these months and, at the prevailing features of temperature ranges and photoperiodism, there could be an overall elevated melatonin levels, (photophase + scotophase), with an optimum amplitude and duration of the nocturnal melatonin signal as inferred previously (Chapters 1 and 4). A longer duration melatonin signal and an overall increase in melatonin levels together, could, not only dampen prolactin release (needed for linear tail elongation) but, also possibly minimize thyroid hormone sensitivity. It is also likely that, the growth inhibitory influence of melatonin could be potentianted under hypothyroidism. This speculative reasoning needs to be subjected to appropriate experimental scrutiny before they can gain credence. In the absence of validity to the above contentions, other explanations may have to be sought to rationalize the present unique observations.

At the lower temperatures, thyroxine administration, either systemically or *in loco*, had no influence whatsoever on the course of tail regeneration. Both hypo and hyperthyroidism were inconsequential and produced same length of tail regenerates like the controls. Apparently, at lower temperature ranges, the thyroid activity, the thyroid harmone levels and, thyroid hormone responsiveness are all very low as discussed earlier and hence, either methimazole treatment or exogenous thyroxine cannot induce any alterations.

Overall, the present observations suggest

1) Changing thyroid activity and thyroid hormone responsiveness on a seasonal basis.

2) At higher temperatures, increased hormone sensitivity could compensate for reduced hormone levels.

3) At higher temperatures in summer months, there are differential effects of supranormal thyroid hormone levels.

4) In the monsoon months, supranormal thyroid hormone levels have an overall inhibitory influence.

5) At the lower temperatures in winter months, thyroid activity and thyroid hormone levels, both being low, experimental manipulations resulting in excess and/or deficiency of thyroid hormones have no effects.

SUMMARY

Thyroid hormones have been implicated in the control of vertebrate appendage regeneration. In lizards, thyroid hormones have been reported to induce ependymal outgrowth and also exercise control over adaptive systemic metabolic activities. However, there have been no attempts to correlate the seasonal differences in regenerative performance with thyroid activity. The present study has evaluted the effect of induced thyroid hormone excess or deficiency (by T_4 administration or methimazole treatment respectively) on tail regeneration in *H. flaviviridis* on a seasonal basis in summer, monsoon and winter months. The experiments revealed a retardative influence of hypothyroidism in tail regeneration in both summer and monsoon months; however blastema formation occurred in the normal time course in the summer months Hyperthyroidism induced by daily T_4 administration either systemically or *in loco* hastened the formation of blastema and provided an early growth spurt but, ultimaltely retarded regenerative growth in the summer months. However, T4 administration daily and every other day thereafter, favoured a better regenerative growth. In contrast, during the monsoon months, both daily administration or administration every alternate day, either systemically or *in loco,* delayed blastema formation as well as retarded linear growth. Neither hypothyroidism nor hyperthyroidism exerted any influence on the sluggish regenerative performance

charteristic of winter months. It is concluded from the present observations, that thyroid activity and thyroid hormone responsiveness vary on a seasonal basis with maximum activity and responsiveness at higher temperatures and minimal at lower temperatures. It is also concluded that there is differential sensitivity to thyroid hormone during summer and monsoon seasons.