Original Paper

THYROID HORMONE CONTROL OF TAIL REGENERATION: DIFFERENTIAL *IN LOCO* AND SYSTEMIC EFFECTS AND SEASONAL VARIATION

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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 evaluated the effect of induced thyroid hormone excess or deficiency (by T4 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 winter months; however blastema formation occurred in the normal time course in the summer months. Hyperthyroidism induced by daily T4 administration, either systemically or *in loco*, hastened the formation of blastema and provided an early growth spurt but, ultimately, retarded regenerative growth in the summer months. However, T4 administration daily for the first 15 days and every other day thereafter favored a better regenerative growth. In contrast, during the monsoon months, both daily administration and 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 performance characteristic of winter months. It is concluded that thyroid activity and thyroid hormone responsiveness vary on a seasonal basis with maximum activity at higher temperatures and minimal at lower temperatures. It can also be concluded that there is differential sensitivity to thyroid hormone during summer and monsoon seasons.

Key words: Regeneration, tail, lizard, thyroid, season

INTRODUCTION

Thyroid hormones exert control over oxidative metabolism and metabolic activities of various organs of lacertilians (1, 2). Apart from metabolic activity, of late, thyroid hormones are also implicated in many other functions (3-5). Thyroid hormones are reported to be active evocators of regeneration in reptiles.

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 pituitary gland on tail regeneration in *Anolis carolinensis* (6, 7). Subsequently, Turner and Tipton (8) 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 evocated the formation of a regeneration blastema (9-12). In the same experiment, the authors observed an early emergence of blastema by hyperthyroidism. Turner (13) reported normal growth and differentiation in 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 hemetopoietic changes and systemic metabolic profile and thyroid histology during tail regeneration in *Mabuya carinata*, a tropical lizard (14-19), 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 regulatory influence on regeneration, even indirectly, by altering the adaptive modulation of systemic responses in the initial periods (20-22). Earlier studies have provided compelling evidences for photothermal influence on regeneration and a seasonal variation there at (23).

Poikilotherms in general, and lacertilians in particular, are known to show variations in thyroid acitivity and metabolism on a seasonal basis (24, 25). Since both these factors are implicated in tail regeneration in lizards,

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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 manipulation of thyroid functions could provide a rational explanation for the earlier observed seasonal difference on regenerative growth in terms of circulating titers of thyroid hormones and sensitivity. The present study, in this context, deals with the effect of thyroid hormone deprivation or thyroid hormone excess on the course of regeneration in *Hemidactylus flaviviridis* in the summer, monsoon and winter months.

MATERIAL AND METHODS

Adult *Hemidactylus flaviviridis* of 10 ± 2 g body weight and 80 ± 5 mm snout-vent length were used in the experiment. The experiments were conducted in the summer (Apr-May), monsoon (Jul-Sep) and winter (Dec-Feb) months and are designated as experimental schedules 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 three groups of 10 lizards each. The lizards in group 1 received daily *intra-peritoneal* (*ip*) injections of thyroxine at 09.00 hr at a concentration of 0.09µg/ lizard in 0.1 ml of saline for 30 days starting from the day of autotomy and these lizards served as the experimentals receiving systemic administration of thyroxine. Lizards in group 2 were injected locally (the tail) with 0.09µg 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 (Hyperthyroidism):

This consisted of three 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 hr.The third group served as control and five of them received the same amount of vehicle *intraperitoneally* while the other 5 received the vehicle *in loco* as per the schedule for the experimental lizards.

Set-up 3 (Hypothyroidism):

This also consisted of three groups, of which one served as control and the others as experimentals. The two experimental groups received 20 mg or 50µg of methimazole (MMI), in 0.1ml saline per litre *intra-peritoneally* at 17.00 hr starting 5 days prior to autotomy and continued for 30 days after autotomy. The control group received 0.1ml of the vehicle at the same time.

Experimental schedule II (Monsoon):

This consisted of eight groups of 10 lizards each and divided into 3 set-ups.

Set-up 1 (Hyperthyroidism):

It consisted of two groups, one control and one experimental. The experimental group received 0.09 µg thyroxine in 0.1 ml saline per lizard *intra-peritoneally* daily for 30 days at 09.00 hr starting from the day of autotomy. The control group received the same amount of vehicle for the same period at the same time.

Set-up 2 (Hyperthyroidism):

This consisted of three groups of lizards of 10 each, of which two were experimental and one control. The two experimental groups received 0.09 µg thyroxine in 0.1 ml saline either *ip* or *in loco* daily for 15 days from the day of autotomy, followed by every alternate day for the remaining 15 days at 09.00 hr. In the control group, five lizards received the vehicle *ip* and the other 5 *in loco* as per the experimental schedules.

Set-up 3 (Hypothyroidism):

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

Experimental schedule III (Winter)

This consisted of three groups of lizards and experiments were carried out in one set-up.

Set-up 1 (Hyper- and hypothyroidism):

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

Preparation of solution

Thyroxine, commercially available as thyroxine sodium tablets (Glaxo India Ltd.), each uncoated tablet containing thyroxine sodium *ip* 0'.1 µg (equivalent to 0.091 µg 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 a few drops of ethanol and then diluted appropriately with 0.6% saline to obtain a final concentration of either 20 µg /0.1ml or 50 µg /0.1 ml.

Experimental protocol

The cages housing the animals measured 18" x15" x 10" with one side made of transparent glass, and ventilated on three sides. Each cage housed a total of 10 lizards 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 1 and 2 produced similar results, the data of one group (*i.e., in loco* for schedule 1 and systemic for 2) are presented.

Experimental schedule I (Tables 1-3)

Lizards in all 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 the 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 increased growth rate during the initial periods, it remained significantly low after 20 days.

The experimental group 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 rate 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, 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 retarding influence was highly pronounced with the highest dose of MMI. The overall growth rate in MMI- treated lizards was significantly lesser than controls at all the time periods, being more pronounced in the 50µg group.

Table 1. 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

Schedule I

Set-up-1 (Hyperthyroidism)

Manipulation	Total length	Percentage replacement	No. of days taken to attain various arbitrary stages			
			WH	PB	BL	IG
Control	25.50± 4.20	40.47± 2.64	7.5	9	11	12
Thyroxine (S) Daily	20.33± 3.57[b]	32.26± 2.71[b]±	7	8	8	10
Thyroxine (L) Daily	20.55± 3.44[b]	32.61± 2.22[b]	6	7.5	8.5	9.5

b-P<0.005 compared to control

Set-up-2 (Hyperthyroidism)

Manipulation	Total length	Percentage replacement	No. of days taken to attain various arbitrary stages			
			WH	PB	BL	IG
Control	23.00± 3.20	35.93± 3.08	8	10	11	12
Thyroxine (S) Alternate, after 15 days	28.00± 3.28[b]	43.75± 3.43[b]±	6	7	8	9

b-P<0.005 compared to control

Set-up-3 (Hyperthyroidism)

Manipulation	Total length	Percentage replacement	No. of days taken to attain various arbitrary stages			
			WH	PB	BL	IG
Control	29.00± 3.82	47.85± 3.66	5	6	7	8
MMI (20mg)	21.4± 2.96[b]	35.66± 3.47[b]	6	7	8	9
MMI (50mg)	11.00± 1.49[b]	18.33± 1.52[c]	6	7	8	9

b-P<0.001 c- P<0.005

WH-wound healing; PB-preblastema; B-blastema; IG-initiation of growth; S-syatemic; L-local; MMI-methimazole.

Table 2. Length of tail regenerated at different time periods post-autotomy in control and experimental lizards

Set-up 1 (Hyperthyroidism)

Manipulation	10	15	20	25	30
Control	-----	5.00± 5.20	42.80± 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± 2.12[b]
Thyroxine (L) Daily	1.8± .20	6.80± 0.71	13.80± 1.24[a]	17.63± 1.86[a]	20.55± 2.32[b]

a- P < 0.01, b-P <0.005

Set-up 2 (Hyperthyroidism)

Control	-----	4.65± 52	12.95± 76	18.60± 1.43	23.25± 2.68
Thyroxine (S) Daily	1.12± 0.08	7.97± 0.88[b]	16.22± 1.28[b]	22.97± 2.44[b]	27.97± 2.43[b]

b- P < 0.005

Set-up 3 (Hyperthyroidism)

Control	2.37± 0.44	8.22± 1.20	16.52± 1.53	24.30± 2.21	29.30± 2.58
MMI (20m)	1.5± 0.08	7.25± 1.12	12.23± 1.48[b]	18.08± 2.52[b]	21.60± 2.68[b]
MMI (50m)	1.15± 0.12[b]	4.41± 1.50[c]	6.70± 1.73[c]	849± 1.82[c]	10.89± 1.90[c]

b- P< 0.005, c- P < 0.001 compared to control .
S-systemic L-local ; MMI- methimazole,

Table 3: Per day rate of growth in control and experimental lizards in blocks of 5 days

Set-up 1 (Hyperthyroidism)

Manipulation	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 2 (Hyperthyroidism)

Manipulation	Per day rate of growth				
	5-10	10-15	15-20	20-25	25-30
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 3 (Hyperthyroidism)

Manipulation	Per day rate of growth				
	5-10	10-15	15-20	20-25	25-30
Control	0.47	1.16	1.66	1.55	1.00
MMI(20mg)	0.30	1.15	0.99	1.17	0.70
MMI(50mg)	0.23	0.65	0.45	0.35	0.48

Experimental schedule II (Tables 4-6)

Lizards in 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 the tail replaced at the end of 30 days and the total percentage replacement were also significantly lower in all the experimental groups. The retardation by hyperthyroidism 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 time periods. The MMI-treated lizards not only showed very poor growth rate, but also showed a fast tapering off of the growth rate after 20 days.

Table 4: The number of days taken, the total regenerated and the percentage replacement at the end of 30 days in control, hyperthyroidic and hypothyroidic lizards

Schedule 2

Set-up-1 (Hyperthyroidism)

Manipulation	Total Length	Percentage replacement	No. of days taken to attain various arbitrary stages			
			WH	PB	BL	IG
Control	16.44± 2.08	26.76± 3.12	8	9	10	11
Thyroxine (S) Daily	9.87± 0.96	16.09± 1.63	8	10.5	12	13.5

Set-up-2 (Hyperthyroidism)

Manipulation	Total length	Percentage replacement	No. of days taken to attain various arbitrary stages			
			WH	PB	BL	IG
Control	15.85± 1.28	25.49± 2.58	5	6	7	8
Thyroxine (S) Daily	11.50± 0.86	18.85± 2.61	7	8	9	10

Set-up-3 (Hyperthyroidism)

Manipulation	Total length	Percentage replacement	No. of days taken to attain various arbitrary stages			
			WH	PB	BL	IG
Control	15.85± 2.28	25.49± 2.12	5	6	7	8
MM(50 mg)daily	7.5± 0.73	13.33± 1.58	10	11	12	13
MM(50 mg)Alternate	7.8± 1.49	12.78± 1.66	7	8	9	10

Table 5: Length of tail regenerated at different time periods post-autotomy in control and experimental lizards

Set-up-1(Hyperthyroidism)

Manipulation	Days				
	10	15	20	25	30
Control	-	2.10± 0.12	5.75± .46	10.75± 0.88	16.35± 1.23
Thyroxine, Daily	-	1.00± 0.08	3.00± 0.32	4.88± 0.28	9.83± 0.92

Set-up-2 (Hyperthyroidism)

Manipulation	Days				
	10	15	20	25	30
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± 0.03	2.60± 0.28	6.30± 0.43	8.97± 0.96	11.47± 1.08

Set-up-3 (Hyperthyroidism)

Manipulation	Days				
	10	15	20	25	30
Control	2.00± 0.06	5.57± 0.21	10.14± 0.56	13.59± 1.12	15.84± 1.34
MMI (50mg) Daily	---	2.00± 0.12	5.15± 0.62	6.65± 0.70	7.50± 0.63
MMI (50mg) Alternate	1.16± 0.28	3.16± 0.32	5.660± 0.65	7.39± 0.99	7.86± 0.92

MMI- Methimazole, S- Systemic

Table 6: Per day rate of growth in control and experimental lizards in blocks of 5 days.

Set-up-1 (Hyperthyroidism)

Manipulation	Per day rate of growth				
	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-2 (Hyperthyroidism)

Manipulation	Per day rate of growth				
	5-10	10-15	15-20	20-25	25-30
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-3 (Hyperthyroidism)

Manipulation	Per day rate of growth				
	5-10	10-15	15-20	20-25	25-30
Control	0.40	0.71	0.91	0.69	0.45
MMI (50mg) Daily	---	0.40	0.63	0.30	0.17
MMI (50mg) Alternate	0.23	0.40	0.50	0.34	0.09

MMI - methimazole, S-systemic, L - local

Experimental schedule III (Tables 7-9)

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

Table 7: The number of days taken, the total length of tail regenerated and the percentage replacement at the end of 30 days in control and hyper- and hypothyroid lizards

Table 8: Length of tail regenerated at different time periods post-autotomy in control and experimental lizards

Manipulation	Days				
	10	15	20	25	30
Control	-	-	-	-	3.00±0.28
Thyroxine(L) Alternate, after 15 days	-	-	-	-	3.07±0.18
MMI (50mg) 2.80±0.12		-	-	-	-

L- local; S- systemic; MMI- methimazole

Table 9: Per day rate of growth in control and experimental lizards in blocks of 5 days

Manipulation	Per day rate of growth				
	5-10	10-15	15-20	20-25	25-30
Control	-	-	-	-	0.60
Thyroxine(L) Alternate, after 15 days	-	-	-	-	0.16
MMI (50mg)	-	-	-	-	0.56

DISCUSSION

The present results have revealed interesting season (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 demonstrated retarded tail regeneneration under induced hypothyroidism (21, 26). The study in *M. carinata* had shown the reversal of hypothyroidism-induced retardation by T4 replacement. Neither of these studies had bearing on the seasons, 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 ratardatory influence of hypothyroidism has been related to 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 indirect systemic responses (8, 13, 15, 16, 18, 21, 27). In the present study on hypothyroidism, maximum retardation was induced by the higher dose of MMI and the retardation with this dose was about 50% in the monsoon months and 60% in 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 due to hypothyroidism during the monsoon months which was not evident in summer months.

Another observation that merits to be viewed together is the proportionately decreasing regenerative performance from the high summer temperature to the lower winter temperature through the intermediary temperature ranges in the monsoon. A rational idea that emerges from these observations is decreasing thyroid activity (and the resultant decrease in circulating thyroid hormone level) and thyroid hormone sensitivity with decrease in temperature. In this respect, the above observed discrepancy in the number of days taken to form regeneration blastama in hypothyroid lizard during the summer and monsoon months can be explained as due to greater sensitivity to the thyroid hormones despite their lower levels in the summer months. Obviously, formation of blastema triggered by the ependymal outgrowth locally as well as by adaptive modulations systematically 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. Conceivably, the formation of regeneration blastema is dependent on optimum sensitivity towards thyroid hormone rather than on the absolute level of thyroid hormone. There are evidences to show that the thyroid activity and thyroid hormone level are dependent on temperature, with higher temperature increasing and lower temperature decreasing them (24, 29-31). Our seasonal observations on thyroid histology in *H. flaviviridis* also affirm the same.

The growth rates, the ultimate total length of the tail regenerated and the total percentage replacement were all significantly lower in the hypothyroid animals during summer as well as monsoon months. On a comparative basis, the retardation in the linear growth was more pronounced in the summer, despite an early formation of the 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 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 autonomy) in the control animals and even MMI treatment caused the same degree of delay (26 days). Even the regenerative growth was the same in the control and hypothyroid lizards. Apparently, at lower temperature (winter months) the thyroid activity and the thyroid hormone levels are so negligibly low that further suppression by methimazole is of no consequence.

The corollary experiments involving hypothyroidism, induced either by systemic or local administration of thyroxine, yielded more variable observations. Continuous daily administration of thyroxine for 30 days from the day of autotomy, systemically or *in loco*, produced smaller tail regenerate and decreased percentage tail replacement. These were reflected in the comparatively reduced growth rate after 20 days despite an early initiation of growth and formation of regeneration blastema in the hypothyroid 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 favorable influence of supranormal thyroid hormone levels during the initial phases of regeneneration 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 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 autotomy in *M.carinata* and *H. flaviviridis*, respectively, once during the first 10 days and the other after 25 days (28, 32) as well as the observation of the tapering off of the growth rate in the hyperthyroidic *A. carolinensis* (8) 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 ineffectiveness of hypothyroidism to alter the time course of blastema formation in the summer months, already discussed. 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 regenerating tissues, a metabolic burn-out, both *in loco* and in 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 degrees 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 revelation nor has any study been conducted on seasonal basis in relation to regeneration. However, considering the previous observations on photothermal influences and inferred alterations in the features of melatonin rhythm due to photothermal effects (23), as well as the observed effects of melatonin on regenerative process (23), it is possible to seek some speculations. It was previously reported that while temperature increases the amplitude of the nocturnal melatonin signal while increased darkness produces a long duration melatonin signal. Further, it is known that a greater fluctuation in the daily maximum - minimum temperatures maintains a robust melatonin rhythm (23). The monsoon months not only had much reduced fluctuation in the daily

maximum - minimum temperatures but, also the variation in the duration of light - dark phases is minimal due to approaching equinox. It is likely that during these months, and at the prevailing features of temperature ranges and photoperoidism, there could be an overall elevated melatonin levels (photophase + scotophase), with an optimum amplitude and duration of the nocturnal melatonin signal as inferred previously (23, 33). 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 likely that the growth inhibitory influence of melatonin could be potentiated under hypothyroidism. This speculation needs to be subjected to appropriate experimental scrutiny before it can gain credence. In the absence of validity to the above contention, 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 in the controls. Apparently, at the lower temperature ranges, the thyroid activity, the thyroid hormone 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 the following:

1) Thyroid activity and thyroid hormone responsiveness differ 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.

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