PART - I

PHYSIOLOGY
OF
TAIL REGENERATION

CHAPTER I

TAIL REGENERATION IN *MABUYA CARINATA* RELATED WITH BREEDING SEASONS AND THYROID ACTIVITY

In lizards the regenerated tail is almost a true replica of the original tail which was lost with respect to the anatomical architecture and size except for the replacement of the caudal vertebrae by a cartilagenous canal and ependymal tube in place of spinal cord with segmental spinal nerves. The total length of time taken by an animal for its regenerate to attain the original morphological dimentions exhibits both species as well as individual variations. The variations in rate of growth of the regenerate usually reflect the vagaries of multiple influencing factors such as the amount of tail autotomized, environmental factors like temperature, humidity etc., and also to a greater extent seasonal and hormonal factors. Attempts made to evaluate the role of these factors as isolated components, have yielded confusing and at times contradictory results even within the same species due to the cris-cross pattern of interactions of these factors having both synergestic as well as antagonistic effects. Added to these the tropical and temperate differences in metabolic adaptations, reproductive cycles, photoperiodism etc., have all precluded the development of crystalized ideas on extrinsic and intrinsic control on regeneration and the present concepts available on these lines are mostly enigmatic. Literature on environmental and hormonal control on regeneration either separately or in combination are comparatively more with respect to amphibians than reptiles. A few studies carried out on reptiles have not yielded any conclusive inferences and moreover, they are all pertaining to temperate species (Licht, 1967; Licht and Jones, 1967; Madersen and Licht, 1968; Licht and Howe, 1969; Turner and Tipton, 1971; Turner, 1972).

Since scant attention has been paid to these aspects in reptiles in general and in fact none in the case of tropical saurians, effect of seasonal factors in correlation with breeding cycle on one hand and thyroid hormone involvement on the other hand on tail regeneration in the Scincid lizard, Mabuya carinata, has been attempted presently. The rate of growth of regenerating tail has been studied during late non-breeding (June - August) and early breeding period (Sept. - Dec.) to evaluate the change in regenerative capacity, if any, in the changing hormonal 'milieu internae'. Further, effect of chemical thyroidectomy rendered by 6-propyl, 2-thiouracil (PTU) feeding as well as replacement with Thyroxine (T4) on lizard tail regeneration has also been evaluated during the early breeding period and compared with that of the euthyroidic controls of the same period.

MATERIALS AND METHODS

Adult Mabuyas of both sexes in the weight group of 20-24 gms, obtained from Hyderabad, India were kept on a diet of insects and were allowed to get acclimated to the laboratory conditions for about 10 days. Autotomy was performed by pinching off the tails at about 1.5 to 2.0 cms from the vent. The length of new growth in mm, was recorded at fixed time intervals i.e. 5th, 7th, 10th, 12th, 15th, 25th, 40th and 60th days post-autotomy.

Seasonal variations :

A total of 60 animals of both sexes served the experimental purpose. Thirty animals were autotomized during the late non-breeding period (June - Aug.) and their rate of tail elongation measured at the time intervals mentioned above. Another group of thirty animals were autotomized during the early breeding period (Sept. - Dec.) and the growth in the length of the regenerate measured at the specified intervals and compared with those of the earlier period.

Chemical thyroidectomy :

A total of 150 animals were divided into three groups each containing 50 animals comprising of both sexes.

Group A : This group of animals served as the euthyroidic controls.

Group B : The animals in this group were thyroidectomized chemically by force feeding them with 0.1 ml solution (pH 8.0-8.2) of 0.1% 6-propyl,2-thiouracil (PTU) (obtained from Fluka Chemicals, Switzerland), every alternate day starting 30 days prior to autotomy. PTU treatment was continued even after tail autotomy till the end of the experimentation.

Group C : These animals were chemically thyroidectomized as that of group B animals; however, they received 1 µg/gm body weight of Thyroxine (obtained from Sigma Chemicals, USA) i.p., 20 days after the commencement of PTU treatment and 10 days before tail autotomy (dissolved in 0.1 ml solution, pH 8.2-8.4). The PTU treatment as well as thyroxine replacement were continued till the end of experimentation.

The length of growing regenerate of all the three groups were measured at the time intervals specified and compared.

For each day specified a total of five to seven observations were made. The mean and standard deviations were obtained and students' 't' test was used to determine statistical significance.

OBSERVATIONS

Seasonal Growth pattern :

The comparative account of the new growth in mm., shows slightly faster growth rate in late non-breeding period (see Tables 1 and 2; Fig. 1). However, the growth rate appears to be accelerated in the group of animals in the early breeding period specifically after 25 days of regeneration as a result of which total new growth by 60 days attained an almost identical figure. The percentage reduction in new growth was about 20% upto 12 days and maximum of about 28%-29% between 25th to 40 days, after which it fell to 11% and 0.7% on 40 and 60 days, respectively. The percentage reduction in terms of rate of growth was minimal during 5th and 10th days and maximal during 7th, 12th and 15th days, while during 25th through 60th days the percentage growth rate increased positively above the pre-breeding levels (Tables 1 and 2; Fig. 1). As is clear from the tables 1 and 2 and Fig. 1, though there was a slight difference in the growth rate of the regenerate during different periods, there was not, however, any significant difference in the attainment of various morphological stages of regeneration such as wound healing, blastema, differentiation and growth.

PTU treatment and T4 replacement :

The mortality rate in groups B and C animals was about

Table 1. Length of tail regenerate during two seasons under study of *M. carinata*.

Periods of tail regeneration in days	Length regenerated in mm	
	Late non-breeding season	Early breeding season
0	-	-
3	-	-
5	1.5 ± 0.5	-
7	2.5 ± 0.5	2.0 ± 0.41
10	5.25 ± 0.65	4.13 ± 0.63
12	8.75 ± 0.29	7.01 ± 0.91
15	16.25 ± 1.11	11.63 ±1.65
25	36.0 ± 3.91	25.38 ± 1.11
40	51.75 ± 1.47	45.75 ± 4.35
60	87.35 ± 7.5	86.75 ± 5.38

Table 2. Rate of growth per day of tail regenerate during two seasons under study of *M. carinata*

Blocks of periods in days	Rate of growth per day in mm	
	Late non-breeding season	Early breeding season
0 - 3	-	-
3 - 5	0.3	-
5 - 7	0.5	0.3
7 - 10	0.9	0.62
10 - 12	1.75	1.5
12 - 15	2.5	1.5
15 - 25	2.0	1.4
25 - 40	1.03	1.3
40 - 60	1.79	2.0

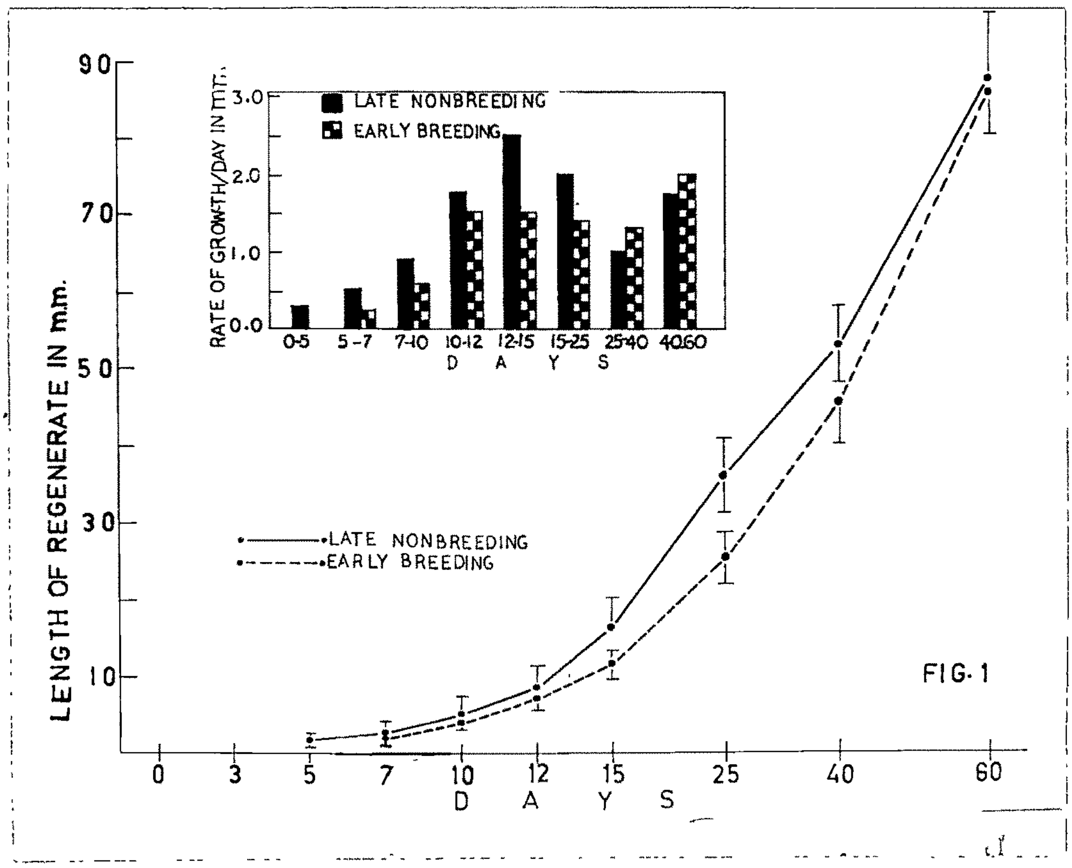

Fig. 1. Length of tail regenerated during late non-breeding and early breeding seasons in *M. carinata*. Inset - Per day rate of growth during different time intervals of the tail regenerate during the same seasons.

5% during the first 20-40 days of experimentation. Maximal mortality rate of about 33% occurred in the PTU fed animals between 70 to 90 days of experimentation; with no mortality occurring in group C animals after 40 days. In general, all the surviving animals appeared to be in good health, though the group B animals tended to show a general loss of body weight and inanition during the later part of the studies.

From Tables 3 and 4 and Fig. 2 it becomes apparent that PTU induced hypothyroidism does show marked retardation in regeneration and animals which have been given T4 replacement show near normal regeneration though the total new growth attained by 60 days post-autotomy was slightly lesser as compared to the euthyroidic controls. Though the hypothyroid animals did show some growth in terms of length, the girth and mass values of new growth was markedly very poor and hence the regenerate appeared highly shrunken and stunted. The percentage difference in the total new growth at various phases as well as the percentage difference in the per day rate of growth between the 3 groups of animals are represented in Tables 3 and 4; Fig. 2. In the T4 administered group of animals the percentage reduction in growth came down from about 54.5% on day 10 to about 7.4% by 25th day. Thereafter, however, the percentage growth rate increased to 19.4% and 25% respectively on 40th and 60th days. Similarly the difference in per day rate of growth fell gradually on first

Table 3. Length of tail regenerated under euthyroidic, hypothyroidic and T4 replaced conditions in *M. carinata*.

Periods of tail regeneration in days	Length Regenerated in mm		
	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)
0	-	-	-
3	-	-	-
5	-	-	-
7	2.0 ±0.41	-	-
10	4.13 ±0.63	-	±1.88 ±0.48
12	7.01 ±0.91	1.00 ±0.08	4.13 ±0.63
15	11.63 ±1.65	2.63 ±1.11	9.13 ±1.18
25	25.38 ±1.11	6.51 ±1.47	23.5 ±2.26
40	45.75 ±4.35	13.13 ±1.44	36.88 ±2.06
60	86.75 ±5.38	26.01 ±3.37	65.01 ±9.13

Table 4. Rate of growth per day of the tail regenerate under euthyroidic, hypothyroidic and T4 replaced conditions in *M. carinata*.

Blocks of periods in days	Rate of growth per day in mm		
	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)
0 - 3	-	-	-
3 - 5	-	-	-
5 - 7	0.5	-	-
7 - 10	0.62	-	0.62
10 - 12	1.5	0.3	1.25
12 - 15	1.5	0.5	1.65
15 - 25	1.4	0.4	1.45
25 - 40	1.3	0.45	0.9
40 - 60	2.0	0.62	1.4

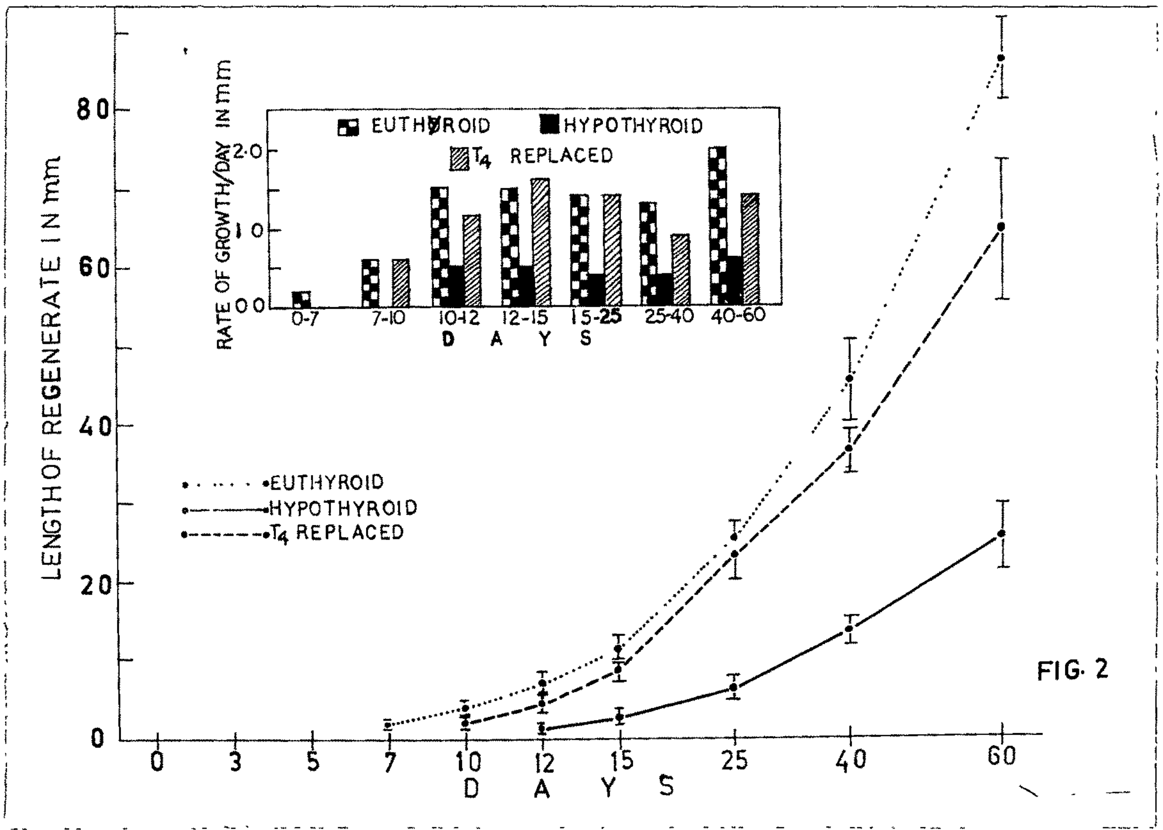

FIG. 2

Fig. 2. Length of tail regenerated in control euthyroidic, PTU induced hypothyroidic and T4 replaced Mabuyas. Inset - Per day rate of growth during different time intervals of the tail regenerate under above conditions.

Table 5. Average maximum temperature and relative humidity recorded in Baroda, India, during June-Aug. 1979 and Sept.-Dec., 1980.

Months of study	Maximum temperature in °C	Relative humidity as %
1979 June *	39.1	72.1
July *	33.8	85.3
Aug. *	36.1	89.8
Average of three months	36.3	82.4
1980 Sept. @	34.4	82.5
Oct. @	32.7	71.9
Nov. @	35.2	61.4
Dec. @	32.5	72.7
Average of four months	33.7	72.1

* Late non-breeding period of *M. carinata*.

@ Early breeding period of *M. carinata*.

Table 6. Percentage difference of length of the regenerate on different days in early breeding season compared with that of late non-breeding season.

Periods of tail regeneration in days	In animals in early breeding	Percentage difference
0	–	—
3	—	—
5	–	—
7	-0.5	-20%
10	-1.12	-21%
12	-1.75	-20%
15	-4.62	-28%
25	-10.62	-30%
40	-8.75	-11%
60	-0.60	- 1%

Table 7. Percentage difference in per day rate of growth of the regenerate during different blocks of periods of tail regeneration in early breeding season compared with that of late non-breeding season.

Blocks of periods in days	In animals in early breeding	Percentage difference
0 - 3	—	—
3 - 5	—	—
5 - 7	—	—
7 - 10	-0.06	-17%
10 - 12	-0.25	-14%
12 - 15	-1.0	-40%
15 - 25	-0.6	-30%
25 - 40	+0.27	+26%
40 - 60	+0.21	+12%

Table 8. Percentage difference of the length of the regenerate on different days in hypothyroid and T4 replaced groups of animals compared with euthyroid controls.

Periods of tail regeneration in days	In hypothyroid group	Percentage difference	In T4 replaced group	Percentage difference
0	—	—	—	—
3	—	—	—	—
5	—	—	—	—
7	—	—	—	—
10	—	—	-2.25mm	-54%
12	- 6.0 mm	-86%	-2.87mm	-41%
15	- 9.0 mm.	-77%	-2.50mm	-22%
25	-18.88 mm.	-75%	-1.88mm	- 7%
40	-32.62 mm.	-71%	-8.87mm	-19%
60	-60.75 mm.	-70%	-21.75mm	-25%

Table 9. Percentage difference in per day rate of growth of the regenerate during different blocks of periods of tail regeneration in hypothyroid and T4 replaced groups of animals compared with euthyroid controls.

Blocks of periods in days	In hypo-thyroid group	Percen-tage differ-ence	In T4 replaced group	Percen-tage differ-ence
0 - 3	–	–	–	–
3 - 5	–	–	–	–
5 - 7	–	–	–	–
7 - 10	–	–	-2.25	-54%
10 - 12	-0.5mm	-86%	-2.87	-41%
12 - 15	-1.0mm	-67%	-2.56	-22%
15 - 25	-1.0mm	-71%	-1.88	- 7%
25 - 40	-0.85mm	-65%	-8.87	-19%
40 - 60	-1.38mm	-69%	-21.75	-25%

12 days to attain an identical value as that of the normal between 12 and 15 days, and, in fact the percentage rate of growth rose above the euthyroidic levels by about 18% between 15 and 25 days of regeneration. Thereafter, however, it fell below the control levels for the remainder of the period of regeneration. The PTU fed animals showed an average retarded growth to 74% whereas growth rate was reduced to the tune of about 70%. The plateauing effect of the percentage retardation in growth and increase in percentage retardation in growth rate are indicative of a gradual inhibition or cessation of growth.

DISCUSSION

The present study which facilitates evaluation of a few parameters of tail regeneration in a tropical lizard, has shown no apparent sex difference in tail elongation nor much difference in growth characteristics during late non-breeding and early breeding periods. Whatever difference is depicted does not seem to be dependent on temperature changes as the variation in average day time temperature during the two periods is hardly significant. From the data recorded herein it becomes apparent that though the final length of the new growth attained towards the end of the regenerating period was more or less the same, there are phase specific variations in the rate of growth between the two groups. The rate of growth remains faster in the late non-breeding group from the time of autotomy till about the late differentiation phase, with the maximum delay of about 30-40% being during the period of early differentiation in the early breeding season. During the late differentiation and growth phases of regeneration this trend was reversed with the result these phases of tail regeneration exhibited an increment in growth rate of about 26% and 12% respectively during the early breeding season. The observed differences in the growth rate during the two periods may have to be explained on the basis of shifting hormonal balance as temperature as a factor is invalidated by the recorded nearness of the temperature range. Moreover, the ambient temperature range of 33°C to 36°C (see Table 5) in the two periods of study appears to be an optimal thermal condition, as Licht (1967) has inferred 32°C to represent an ideal temperature for lizards in general as they obtained maximal regenerative tail elongation of 1.0 to 1.2 mm per day in Anolis carolinenses. A significant report in this respect is that of Schauble (1972) wherein he has shown distinct seasonal variation in the rate and amount of regenerative forelimb growth under a constant experimental temperature regime of 20°C. in newt, Notophthalmus viridescens. Since the summer temperature is quite high in the 'Deccan belt' of India, Mabuyas in this region probably undergo aestivation and have an active breeding phase between Nov. - Jan. Hence during the current experimental period of Sept. - Dec., the lizards could be expected to show high metabolic rate associated with increased physical activity and thereby maximal thyroid activity (Wilhof, 1964; Licht, 1967). Further, elevations in endogenous gonadotrophins associated with gonadal recrudescence and prolactin associated with reproductive activity can also be surmised. Under such a hormonal setup a highly geared up metabolic activity and favourable energy equilibrium can be considered to exist during this period to meet the demands of breeding activities. During such a predisposed condition, stress of caudal autotomy and regeneration might entail the necessity of diverting part of the energy resources for meeting the regenerative requirements. This sharing of resources and partial channelization of the metabolic potentialities may be one of the reasons for the observed comparatively reduced rate and amount of regenerative growth upto the late differentiation phase during the early breeding season. The reported action of prolactin in decreasing metabolic activity and inhibiting the peripheral action of thyroxine (Licht and Jones, 1967; Derby and Etkin, 1968; Gona, 1968) is also worth keeping in mind in the present context. Licht (1967) observed a slight reduction in total tail regenerative growth in his experimental group of lizards treated with exogenous prolactin and gonadotrophins as compared to the control animals during the non-breeding period. In this context the better rate of growth observed during the non-breeding periods of July-Sept., might be due to the autotomy induced output of hormonal complements (Ramachandran et al., 1980a, 1981 a) and the prevailing hypersensitivities of the tissues leading to swift and effective response of the organs in the form of increased metabolic machinisations and total channelization of all available metabolic and energy potentials to the regenerating system. However, the better regenerative ability during late differentiation and growth phases in early breeding period might probably indicate the favourable disposition of the prevailing hormonal equilibrium towards late differentiative activities and growth of differentiated structures in the regenerate.

The second experimental setup aimed at the understanding of the involvement of thyroid gland in tail regeneration of Mabuya carinata has revealed the dependence of lizard tail regeneration either directly or indirectly on thyroxine. Though there is a sizable literature on thyroid and regeneration in amphibians which are all confusing and contradictory (see Schmidt, 1968; Liversage and Brandes, 1977; Liversage and Korneluk, 1978), there are only two studies of this type with respect to lizard tail regeneration and both these studies have demonstrated unequivocally the dependence of lacertelian tail regeneration on thyroxine (Turner and

Tipton, 1971; Turner, 1972). The present results are in perfect conformity with those of the above two reports and depict a significant initial delay and ultimate inhibition of tail regeneration in the PTU fed (Group B) animals. A previous study from this laboratory on the effect of surgical thyroidectomy on tail regeneration in *Hemidactylus flaviviridis* has however, shown only a retardation in regeneration but no inhibition (Kothari *et al*., 1979). This discrepancy might be due to the availability of some thyroxine from the extrathyroidal tissues which are known to be present in vertebrates (Schmidt, 1958; Chaven and Bouwmann, 1965; Evans *et al*, 1966) as surgical thyroidectomy cannot anhilate these sources. Comparison of the tail regeneration process of B and C groups of animals with that of group A animals reveals the following : (1) The regenerative growth in group B animals was quantitatively and qualitatively poor with a shrunken and stunted lean linear growth. (2) This poor lean linear growth occurred at a constant subnormal rate of 0.5 mm per day between 12 and 60 days post-autotomy. A thin blastema like protuberance occurred between 13th and 14th days and some sort of histodifferentiation by about 18 days. (3) From all tables and graphs depicting the growth pattern in Group B animals it becomes evident that the new growth that occurred was at an average only 30% of the control (A Group) animals. Further from the observations made on the 75th day it becomes clear that the growth remained as that of 60th day. (4) In the T4 replaced (C Group) animals from an initial delay of 3 days in blastema formation the regenerative process progressed faster and the delay was totally abolished by the late differentiation phase. In fact the difference in percentage rate of growth got completely nullified by early differentiation phase (12 to 15 days) and even showed an increased rate of growth of about 18% over the controls during the period of active differentiation (15-25 days). (5) Since then the rate of growth remained below the control levels by about 30% for the rest of the periods of regeneration.

A similar retarded growth of tail regenerate observed in thiourea fed hypothyroidic Anolis carolinenses was attributed to an inhibition in out growth of the ependyma. However, based on our previous observations of altered haemopoietic changes and systemic metabolic profile in relation to thyroid histology during tail regeneration in M. carinata (Shah et al., 1977b; 1980a; Ramachandran et al., 1979; 1980 b; 1981) and the currently observed inhibition of the above responses in PTU induced hypothyroidic animals (Chapters VIII-XII), it is our contention that thyroxine exerts its regulatory influence on regeneration in an indirect fashion by adaptive modulations of the systemic responses of the animal at least in the initial periods of regeneration. The regenerative out growth, however poor, that is observable in the hypothyroidic group of animals appears to corroborate this contention. However, a direct dependence of the regenerate on thyroxine can also be expected, especially associated with the relationally interlinked events of cell proliferation and differentiation as evidenced by the better growth rates during these periods in the T4 replaced group of animals. Pertinent to quote in this context are the reports of favourable influence of thyroid on cell differentiation (Siegel and Tabias, 1966 and Greenberg et al., 1974) and the reported increase of hyaluronic acid concentration and decrease of chondroitin sulfuric acid synthesis in states of thyroid hormone deficiency (Schiller et al., 1962). Another observation which merits some consideration is the reduced percentage rate of growth in the (Group C) animals administered with T4 during the late differentiation and growth periods. This might be suggestive of the retarding influence of continued presence of thyroxine on post-differentiative growth processes. Some validity comes in this context from the observation of a reversal of thyroid morphology to a near normal state after the 25th day of tail regeneration in M. carinata (Ramachandran et al., 1981). In the light of these facts it may be safely concluded that the thyroid gland exerts a bimodal phase specific influence on tail regeneration in lizards.

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