CHAPTER

7

Relative Involvement of Thyroxine and Prolactin During Tail Regeneration

Vertebrate appendage regeneration represents a secondary reactivation of early developmental events at a restricted extremity of the body. Since the regenerating appendage develops in close association with a fully differentiated body, factors of systemic origin from adult organism are likely to exert regulatory/modulatory influences. Embryonic development occurs in a state of complete genomic availability, gradually leading to progressive restriction in availability, and in the post-embryonic adult stage, different regions of the body remain endowed with only restricted or specific genomic expression. In this context, the re-enactment of the developmental phenomenon at an extremity of the body is highly improbable without recreating the genomic open state, even in a restricted sense. Humoral factors in the form of endocrine secretions can be envisaged to have some crucial role in this aspect.

Studies on control of vertebrate appendage regeneration are not quantitatively encouraging. Most of the studies have remained restricted to amphibians. A very interesting case is that of anuran tadpoles which retain their capacity for limb and tail regeneration in the premetamorphic stages, while this capacity is totally lost during metamorphosis. The loss in regenerative capacity with the approaching metamorphosis has been associated with the increasing titres of the metamorphic hormone, thyroxine. The regenerative ability in the premetamorphic stages has been related with the high prolactin levels. Obviously in anurans, prolactin is the regeneration promoting hormone while thyroxine is inhibitory. These and other

observations in lower vertebrates have given rise to a concept of 'prolactin-thyroxine' antagonism (Etkin and Lehrer, 1960; Berman *et al.*, 1964; Grant and Cooper, 1965; Etkin and Gona 1967; Cohen *et al.*, 1972; Brown and Brown, 1973; Crim, 1975; Dodd and Dodd, 1976; Platt, 1976; Clemons and Nicoll, 1977; Lodi *et al.*, 1978; Norris and Duvall, 1981). Most of the evaluations on hormonal regulation of appendage regeneration have been conducted on urodeleans, especially the newts. Dependence of the regenerative process on hormones, especially in early stages has been demonstrated in amphibians (Rose, 1964; Tassava 1968,1969 a, b; Liversage and Scadding, 1969). Based on the studies involving thyroxine, insulin, growth hormone and prolactin, the need for a multiple hormonal background or *"hormonal milieu"* for appendage regeneration in adult newts has been suggested (Liversage and Scadding, 1969; Vethamany-Globus and Liversage, 1973; Liversage and Fisher, 1974). It was shown that in hypophysectomised newts, normal regeneration could be maintained by treatment with a combination of prolactin and thyrotropic hormone (Connelly *et al.*, 1968). The involvement of thyroid hormone in newt limb regeneration has been generally recognized though, there were conflicting reports (See Chapter 6). Prolactin has gained recognition as a versatile hormone in regulating multitude of functions including growth in vertebrates (Newelinski, 1958; Berman *et al.*, 1964; Licht and Jones, 1967; Licht and Hoyer, 1968; Meier, 1969; Schauble, 1972; Schauble and Nentwig, 1974; Crim, 1975; Yamaguchi and Yasumasu, 1977; Maier and Singer,

1981; Nicoll, 1981). Prolactin enhanced regenerative growth was demonstrated in newts (Newelinski, 1958; Waterman, 1965). Schauble and Tyler (1972) showed that prolactin not only enhanced regeneration but also eliminated seasonal variations in the regeneration rate in newts. These studies showed the importance of hormones in the control of regeneration in amphibians. In contrast, there have been only very few studies in this respect in reptiles (Licht and Jones, 1967; Licht and Howe, 1969; Turner and Tipton, 1971; Turner, 1972). All the above studies was on a single species *ie. Anolis carolinensis*, and hence, some studies initiated from this laboratory on tropical lizards like, *Mabuya carinata* and *H. flaviviridis*, had shown a retardatory influence of hypothyroidism, thus implicating thyroid hormones in lizard tail regeneration (Kothari *et al.*, 1979; Ramachandran *et al.*, 1981b, 1984; Ramachandran and Abraham, 1990; Ramachandran *et al.*, 1993). Recent studies on *H. flaviviridis* also demonstrate the growth promoting influence of PRL and its role in photoperiod induced tail regeneration (Ndukuba and Ramachandran, 1989; Ramachandran and Ndukuba, 1991). The variations in regenerative performance noted under photothermal changes (on a seasonal basis) as well as in response to melatonin administration, have been inferred to be due to altered PRL secretion. Further, neuropharmacological evidences also supported the same (previous chapters). In this context, there was a need to understand the relative roles of thyroxine and prolactin as well as the possible interactions either synergistically or sequentially in the control of

tail regeneration in lizards. To this end, a combination of surgical and neuropharmacological manipulations have been carried out to ascertain the roles of these two hormones in lacertilian tail regeneration .

MATERIAL AND METHODS

A total of 280 lizards was used for the present study, consisting of 3 experimental schedules and 28 groups of 10 each.

Experimental Schedule I : (Experiments conducted in April)

A total of 60 lizards was divided into 6 groups of 10 each, 5 of these were experimental groups and one control group. One group of lizards was subjected to surgical thyroidectomy (Tx) as per the method described by Kothari *et al.* (1979). Second group of lizards was subjected to Tx as well as methimazole (MMI) treatment (50 μg/lizard in 0.1ml saline, ip). Third group of lizards was thyroidectomized and treated with MMI and then administered with pimozide (10 μg/lizard) starting from the day of autotomy. Fourth group of lizards was also subjected to Tx and MMI treatment but, was given thyroxine replacement (0.09 μg/lizard in 0.1ml) starting from the day of autotomy. Fifth group of lizards was subjected to Tx and MMI treatment but was given thyroxine replacement as well as pimozide (10 μg/ lizard in 0.1 ml, ip.)

starting from the day of autotomy. Autotomy in all the experimental groups of lizards was performed a week after Tx or MMI treatment.

Control : This group of lizards was subjected to sham Tx and also received 0.1ml saline (ip) from the day of surgery.

Experimental Schedule II : (July to October, Monsoon months)

It consisted of 4 control and 8 experimental groups of lizards.

Set up 1 :

Two experimental groups received (ip) once daily injection of thyroxine (0.09 µg in 0.1ml saline/lizard) at 0900 hrs for 30 days starting from the day of autotomy but, one group received in addition, bromocriptine (20 µg/lizard in 0.1ml saline) at 1700 hrs. One group of control lizards received injection of vehicle alone for 30 days starting from the day of autotomy.

Set up 2 :

This consisted of two experimental groups and one control group. One experimental group was thyroidectomized and treated with MMI and also bromocriptine (20 µg/lizard) seven days prior to autotomy and then received thyroxine (0.09 µg in 0.1ml/lizard) starting from the day of autotomy. Since these lizards were subjected to thyroidectomy as well as MMI treatment, they are considered as functionally athyroidic animals. The second group of lizards was treated with MMI 5

days prior to autotomy and then administered with pimozide from the day of autotomy daily for 30 days. Since these lizards were treated only with MMI which does not ensure complete absence of thyroid hormone, they are considered as hypothyroidic lizards. The control group received injections of vehicle (twice) as per the schedules of the experimentals. Another group of lizards sham Tx and injected with vehicle, was also maintained.

Set up 3 :

This consisted of three experimentals and one control group. One group of experimental lizards received melatonin in the morning at 07.00 hrs (20 μg/lizard in 0.1ml saline) for 30 days starting from the day of autotomy while, the second group received MMI at 17.00 hrs (50 μg/lizard) starting 5 days prior to autotomy and continued for 30 days thereafter. The third group received both melatonin and MMI as per the same schedule. The control group received injections of vehicle alone (0.1ml saline) with one half receiving one, and the other half, two injection.

Set up 4 :

The experimental group in this set up received MMI (50 μg/lizard in 0.1ml saline, ip) starting 5 days prior to autotomy and also pimozide (10 μg/lizard in 0.1ml saline, ip) from the day of autotomy. Both the injections were continued for 30 days after autotomy. The control group received two injections of vehicle as per the experimental schedule.

Experimental Schedule III : (November-February, winter months)

This consisted of three set ups.

Set up 1 :

This consisted of five experimental and one control group. One of the experimental group of lizards was subjected to chemical thyroidectomy by MMI treatment at 1700 hrs starting 5 days prior to autotomy while, a second group was treated with bromocriptine at 1700 hrs (20 μg/lizard in 0.1ml saline) to induce hypoprolactinemia. The third group received thyroxine (0.09 μg/lizard, ip) daily at 09.00 hrs for 30 days from the day of autotomy. The fourth group received pimozide at 17.00 hrs (10 μg/lizard) for the same duration from the time of autotomy. The fifth group of experimental lizards received both thyroxine and pimozide for the same duration. The control group of lizards received injection of saline daily form the day of autotomy.

Set up 2 :

This consisted of four groups of lizards, three experimentals and one control. One group of lizards received daily injection of bromocriptine at 17.00 hrs (20 μg/lizard) daily for 30 days from the day of autotomy. The second group received *in loco* injections of thyroxine at 09.00 hrs (0.09 μg/lizard in 0.1ml saline) daily for 15 days from the day of autotomy and every alternate day thereafter for the remaining 15 days. The third group of lizards received both thyroxine and bromocriptine as per the

above schedule. The control group of lizards received two injections of 0.1 ml saline daily for the first 15 days and one injection every alternate day for the remaining 15 days.

Set up 3 :

This consisted of two experimental groups and one control group. One experimental group of lizards was subjected to chemical Tx by MMI treatment 5 days prior to autotomy and then received pimozide at 17.00 hrs (10 μg/lizard in 0.1 ml saline) daily for 30 days from the day of autotomy along with MMI. The second group of experimental lizards was administered daily with thyroxine and pimozide for 30 days starting from the day of autotomy. The control group of lizards received the vehicle (0.1ml saline ip) starting 5 days prior to autotomy and continued thereafter for 30 days.

RESULTS

Experimental Schedule I : (Plate -7.1)

The control lizards completed wound healing by 6 days and formed blastema by the 9th day and, by 15 days, they produced a new growth of 6.1 mm representing 10% of tail replacement. The Tx plus MMI treated and Tx plus MMI and pimozide treated lizards completed their wound healing by 10 days and showed no signs of formation of blastema till 15 days. The Tx and MMI treated lizards replaced with

LEGEND TO FIGURE

Plate - 7.1

Photographs of thyroidectomized plus Methimazole (Tx + MMI) treated (a) and control (b) lizards at the end of 20 days. Note the pronounced regenerative growth in the control and the absence of regenerative growth in the experimental lizards.

PLATE - 7.1

a. b.

thyroxine completed the formation of various arbitrary stages at exactly the same time periods as in the controls and also produced a new growth of 5.6mm by 15 days but, the Tx and MMI treated lizards given both thyroxine and pimozide also completed the formation of various arbitrary stages at the same time periods and also showed a slightly increased growth by 15 days (7.8mm). These results are depicted in tables 1-2.

Experimental Schedule II :

Set up 1 : (Plate-7.2)

Both the experimental groups of lizards, either administered only thyroxine or, both thyroxine and bromocriptine daily, showed a delay in the formation of blastema by one day. Both these groups also replaced identical length of tail at the end of 30 days with about same percentage of replacement. The growth rates at different time periods remained lower than the controls throughout (tables 3-5 , figures 1-3).

Set up 2 :

The control animals completed wound healing by the 5th day, formed blastema by the 7th day and initiated a new growth by the 8th day. They replaced a length of 24.6mm of tail by the end of 30 days accounting to a replacement of 40.3%. The athyroidic and hypoprolactinemic lizards, receiving thyroxine as well as, the hypothyroidid lizards receiving pimozide, depicted a delay of 1 day in the healing of their wound, formation of blastema and initiation of growth. Whereas athyroidic hypoprolactinemic lizards receiving thyroxine, regenerated as much as 19.8mm of tail,

TABLE - 1

Showing the number of days taken to attain the various arbitrary stages and, total length of tail regenerated and total percentage replacement at the end of 15 days in the control and experimental lizards.

Schedule-I (Summer)

Manipulation	Total length (mm)	Percentage replace-ment	No of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	6.10 ± 0.85	10.00 ± 0.96	6	8	9	10
TX	WH	-	-	-	-	-
TX + MMI	WH	-	-	-	-	-
TX - MMI + Pimozide	WH	-	-	-	-	-
TX + MMI + Thyroxine	5.70 ± 0.96	9.34 ± 1.12	7	8	9	10
TX + MMI + Thyroxine + Pimozide	7.80[a] ± 1.12	12.78[b] ± 1.20	7	8	9	10

WH - wound healing; PB - Preblastema; B -Blastema; IG - Initiation of growth.
TX - Thyroidectomized, MMI - Methimazole,
b - $P < 0.005$,

TABLE - 2

Showing the length of tail regenerated at different time period (days) and per day rate of growth in blocks of 5 days in control and experimental lizards.

Schedule-I

Manipulations	Length of tail regenerated		PER DAY RATE OF GROWTH	
	10	15	5-10	10-15
CONTROL	-	6.10 ± 0.82	-	1.22
TX	-	-	-	-
TX + MMI	-	-	-	-
TX + MMI + Pimozide	-	-	-	-
TX + MMI + Thyroxine	-	5.70 ± 0.90	-	1.14
TX + MMI + Thyroxine + Pimozide	-	7.80[b] ± 1.12	-	1.56

TX - Thyroidectomy; MMI - Methimazole.
b - $P < 0.005$

LEGENDS TO FIGURES

Plate - 7.2

A & B : Photographs of control and thyroxine (systemic) plus bromocriptine treated lizards at the end of 15 days.

A : Photographs of control (a) and thyroxine plus bromocriptine treated lizard (b) Note the reduced regenerative output in the latter.

B : Close-up version of the tail region of control (a) and thyroxine plus bromocriptine treated lizard (b). Note the difference in the regenerative growth.

PLATE - 7.2

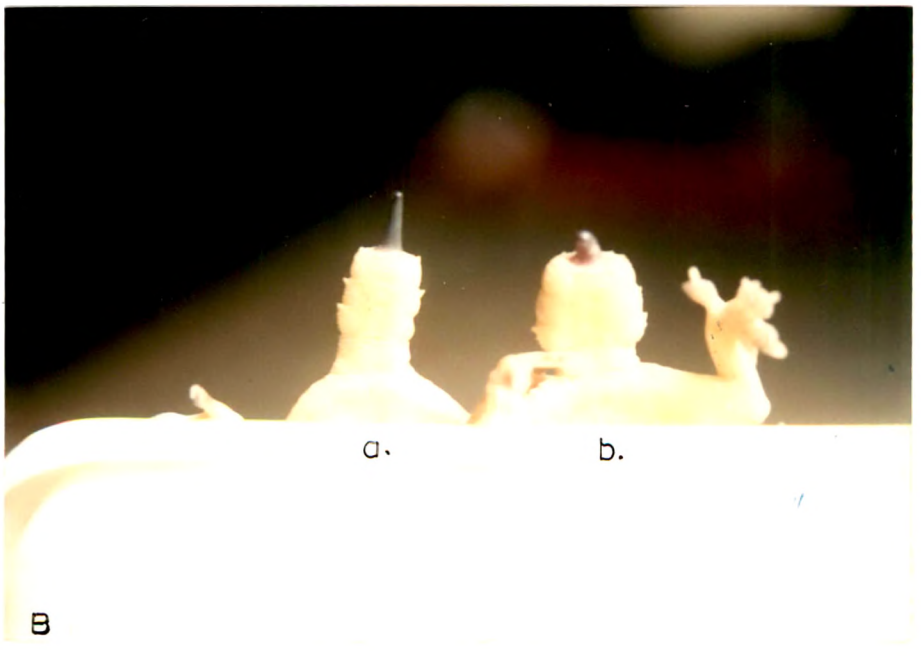


TABLE - 3

Showing the number of days taken for the attainment of various arbitrary stages and total length of tail regenerated and total percentage replacement at the end of 30 days in control and experimental lizards.

Schedule - II (Monsoon)

Setup - I

Manipulation	Total length (mm)	Percentage replace-ment	No of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	16.44 ± 1.28	26.95 ± 2.32	8	9	11	12
Thyroxine(S) DAILY	9.87[c] ± 1.12	16.18[c] ± 1.86	8	10	12	13
Thyroxine(S) + Bromocriptine	9.16[c] ± 1.32	15.02[c] ± 1.38	9	10	12	13

Setup - II

CONTROL	24.60 ± 2.64	40.32 ± 3.53	5	6	7	8
MMI + TX + Bromocriptine + Thyroxine (L)	19.80[b] ± 2.01	32.45[b] ± 3.12	6	7	8	9
MMI + Pimozide	10.80[c] ± 0.98	17.70[c] ± 2.10	6	7	8	9

WH - wound healing; PB - Preblastema; B - Blastema; IG - Initiation of growth; S-Systemic; MMI-Methimazole; TX-Thyroidectomined., L - Local.
b-$P < 0.005$, c - $P < 0.001$.

TABLE - 4
Showing the length of tail regenerated at different time period (days) in control and experimental lizards.

Schedule-II (monsoon)
Set-up-I

Manipulation	DAYS				
	10	15	20	25	30
CONTROL	-	2.15 ± 0.12	5.83 ± 0.88	10.83 ± 1.02	16.44 ± 2.50
Thyroxine (S) DAILY	-	1.00[b] ± 0.08	3.00[b] ± 0.52	4.88[c] ± 0.28	9.83[c] ± 1.12
Thyroxine (S) + Bromocriptine	-	1.25[b] ± 0.05	2.66[b] ± 0.63	5.4[c] ± 0.32	9.3[c] ± 1.28

Set up - II

CONTROL	2.80 ± 0.14	8.80 ± 0.36	14.40 ± 1.58	20.00 ± 2.28	24.60 ± 3.46
MMI + TX + Bromocriptine + Thyroxine (L)	2.50 ± 0.20	8.50 ± 0.40	13.40 ± 1.63	15.05[b] ± 2.12	19.65[b] ± 2.44
MMI + Pimozide	1.50[b] ± 0.08	4.50[b] ± 0.28	6.15[c] ± 0.96	7.15[c] ± 0.96	10.75[c] ± 1.60

MMI-Methimazole; TX-Thyroidectomized; S-Systemic, L - Local.
b-$P < 0.005$, c-$P < 0.001$.

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

Schedule-II (monsoon)
Set-up-I

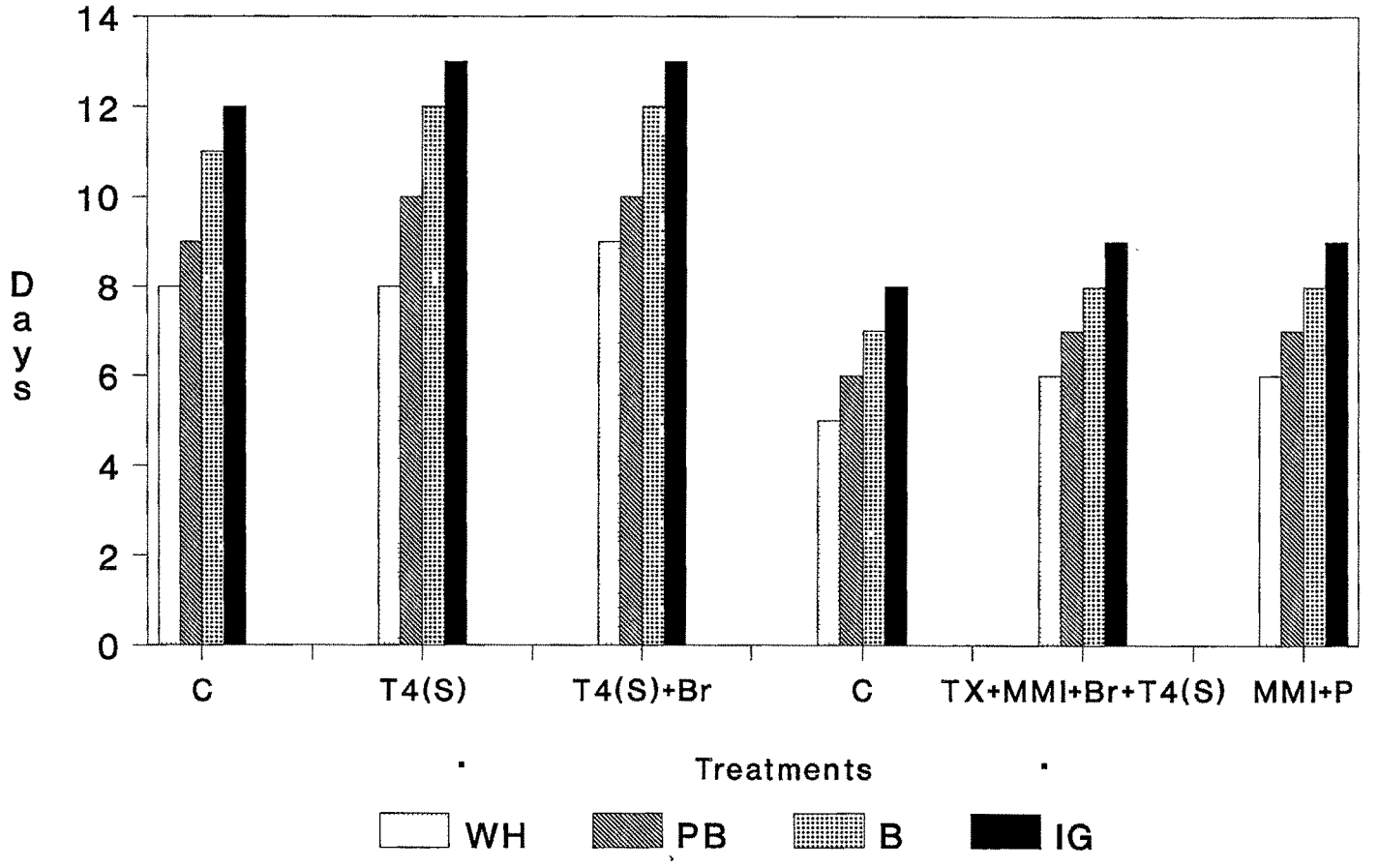
Manipulation	PER DAY RATE OF GROWTH DAYS				
	5-10	10-15	15-20	20-25	25-30
CONTROL	-	0.43	0.73	1.00	1.12
Thyroxine (S) DAILY	-	0.20	0.40	0.37	0.99
Thyroxine (S) + Bromocriptine	-	0.25	0.28	0.56	0.78

Set up - II

CONTROL	0.56	1.20	1.12	1.12	0.92
MMI + TX + Bromocriptine + Thyroxine (L)	0.50	1.20	0.98	0.33	0.92
MMI + Pimozide	0.30	0.60	0.33	0.20	0.72

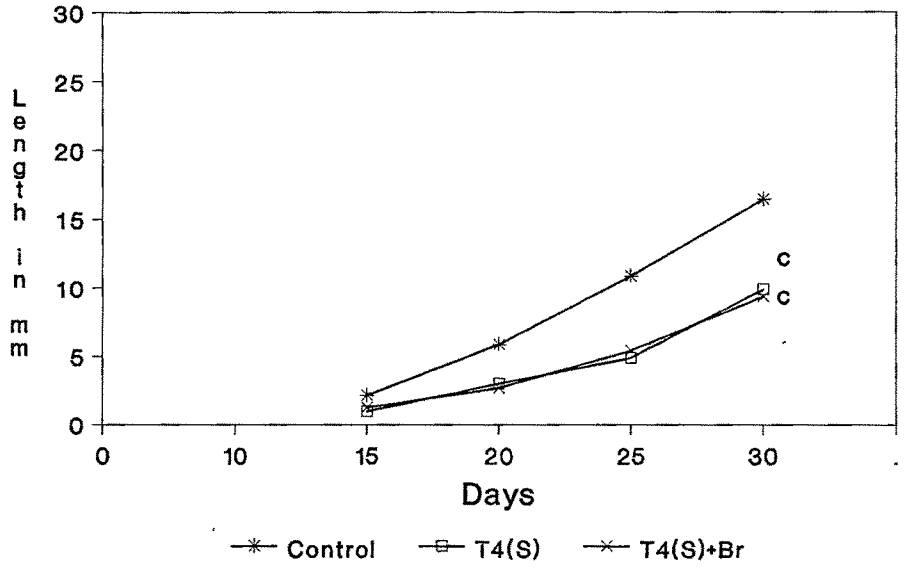
MMI-Methimazole; TX-Thyroidectomized; S - Systemic, L - Local.

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



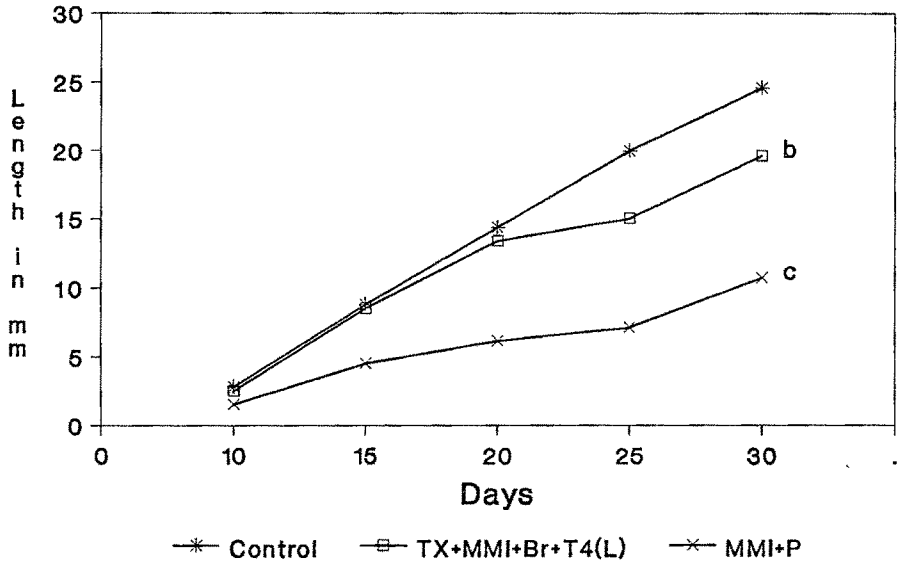
T4(S)-Thyroxine(systemic) Br-Bromocriptine TX-Thyroidectomised MMI-Methimazole P-Pymozide

Figs.2&5 The length of tail regenerated in control(C) and experimental lizards in monsoon.



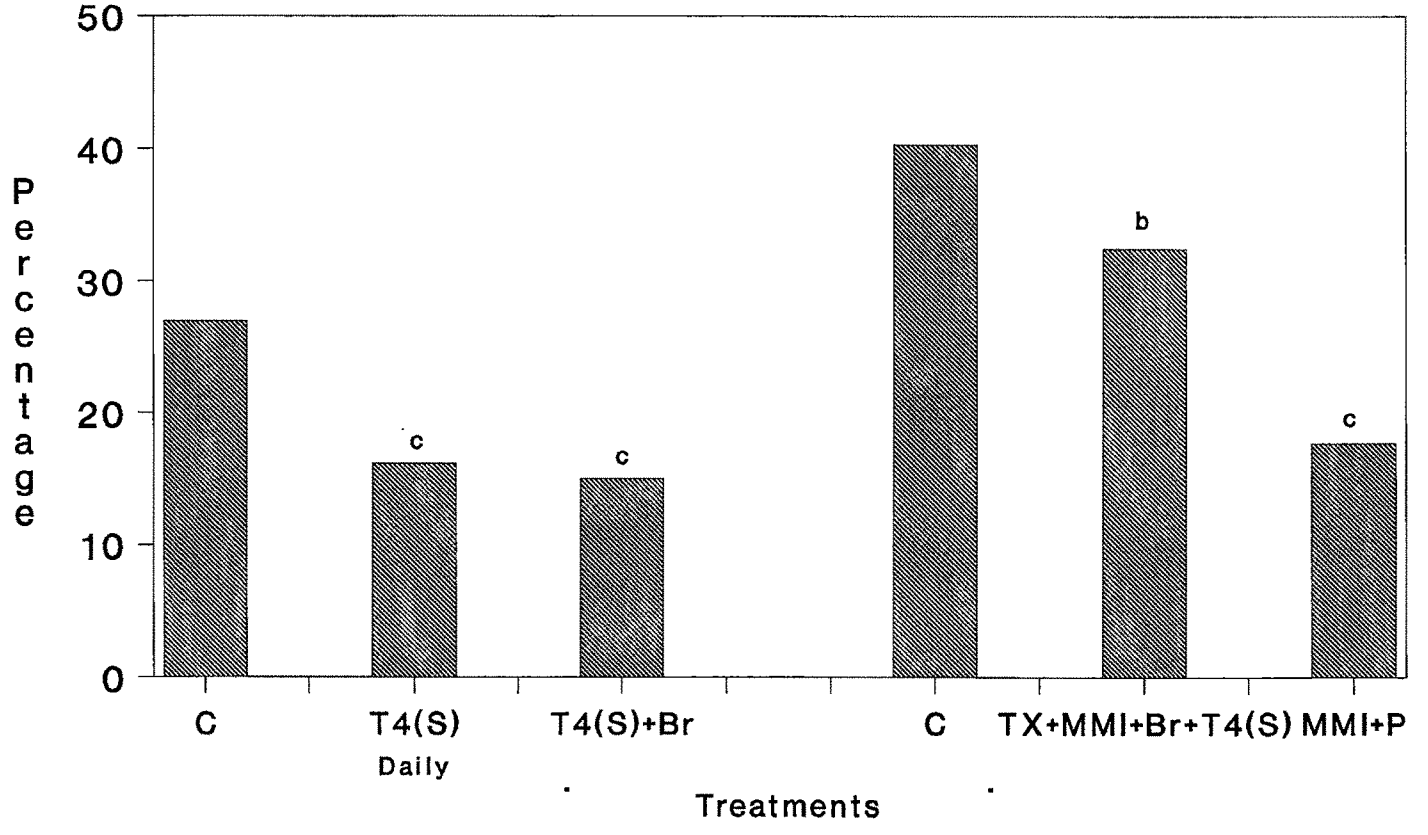
T4-Thyroxine S-systemic,daily Br-Bromocriptine

TX-Thyroidectomised MMI-Methimazole T4-Thyroxine,local P-Pimozide



b-P<0.005; c-P<0.001

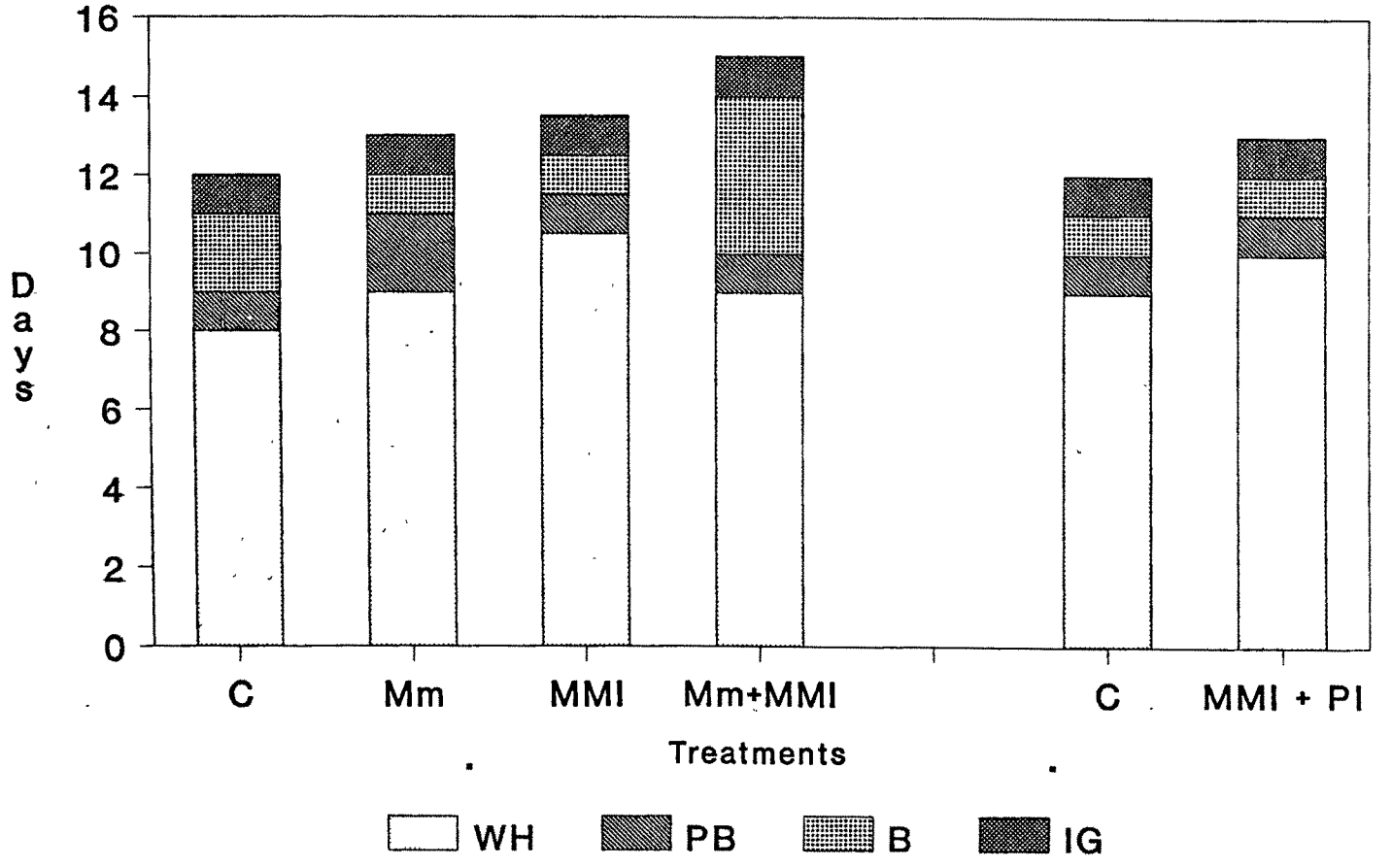
Fig.3&6 The percentage of tail replaced in control(C) and experimental lizards at the end of 30 days in monsoon.



T4-Thyroxine (S)-systemic MMI-Methimazole Br-Bromocriptine TX-Thyroidectomised P-Pimozide

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

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



MMI-methimazole, Mm - morning melatonin, PI - Pimozide

a replacement of 32.4% hypothyroidic lizards receiving pimozide regenerated only 10.8mm long tail representing a replacement of 17.7% (tables 3-5 , Figs. 4-6).

Set up 3 : (Plate. 7·3)

Both the Mm and MMI treated lizards showed a delay of 1 day in the attainment of various arbitrary stages while, lizards treated with Mm and MMI showed a delay of 3 days in the formation of blastema and a delay of 4 days in the initiation of growth. All the 3 experimental groups of lizards regenerated a lesser length of tail at the end of 30 days with the least in Mm plus MMI lizards and, the maximum in MMI lizards. The data of this experiment is depicted in tables 6-8 and Figs. 7-9.

Set up 4 :

compared to the control lizards, the hypothyroidic lizards receiving pimozide showed a delay of one day in the attainment of various arbitrary stages and showed only 17.2% of tail replacement as against 25.9% as in the controls at the end of 30 days (tables. 6-8).

Experimental Schedule III

Set up 1 :

The experimental groups receiving MMI, bromocriptine, pimozide or thyroxine depicted no difference relative to the controls in terms of the number of days taken to attain the arbitrary stages or, the growth rate and the total length of tail replaced.

LEGENDS TO FIGURES

Plate - 7.3

A & B : Photographs of control (b) and Methimazole plus Melatonin morning (MMI + Mm) treated lizard (a).

A : Close-up version of the tail stump of the lizard at the end of 15 days. Note the noticeable regenerative growth in control lizard (b) and early stages of initiation of regenerative growth in the experimental lizard (a).

B : Same lizards at the end of 30 days. Note the clear cut difference in the regenerative growth (a) experimental (b) control.

PLATE - 7.3

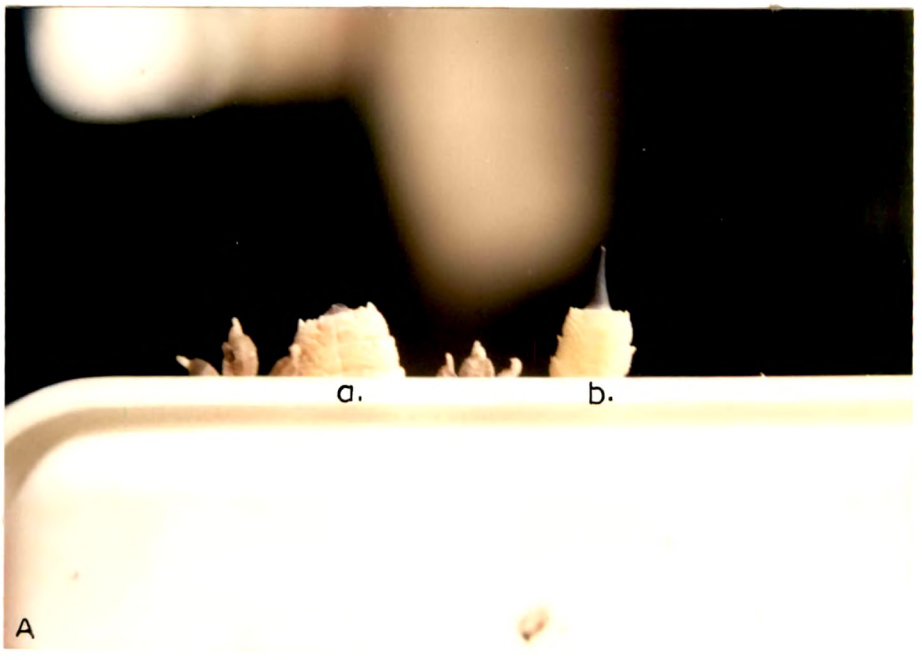


TABLE - 6

Showing the number of days taken to attain the various arbitrary stages and the length of tail regenerated and the total percentage replacement in control and experimental lizards.

Schedule-II (Monsoon)
Setup-III

Manipulations	Total length	Percentage replacement	Number of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	16.44 ± 2.51	26.95 ± 2.48	8	9	11	12
Mm	11.10[b] ± 1.32	18.19[b] ± 2.31	9	11	12	13
MMI	7.50[c] ± 0.98	13.30[c] ± 1.42	10.5	11.5	12.5	13.5
Mm + MMI	3.83[c] ± 0.12	6.28[c] ± 0.86	9	10	14	15

Set up IV

CONTROL	15.80 ± 1.99	25.90 ± 2.40	9	10	11	12
MMI + Pimozide	10.50[b] ± 1.26	17.21[b] ± 2.12	10	11	12	13

Mm - Morning melatonin, MMI - Methimazole, WH - Wound healing; PB - Preblastema; B - Blastema; IG - Initiation of Growth.
b - $P < 0.001$, c - $P < 0.001$.

TABLE - 7
Length of tail regenerated at different time periods post autotomy in control and experimental lizards.

Schedule-II (Monsoon)

Setup - III

Manipulations	DAYS				
	10	15	20	25	30
CONTROL	-	2.15 ± 0.12	5.83 ± 0.15	10.83 ± 0.28	16.44 ± 1.48
Mm	-	1.65	5.05	8.95	11.10
MMI	-	2.00 ± 0.06	5.15 ± 0.12	6.55^{b} ± 0.22	7.55^{c} ± 0.86
Mm + MMI	-	1.12^{b} ± 0.04	1.52^{c} ± 0.06	2.37^{c} ± 0.09	3.77^{c} ± 0.32

Set up IV

CONTROL	-	2.83 ± 0.08	6.33 ± 0.18	10.18 ± 0.44	15.68 ± 1.66
MMI + Pimozide	-	2.07 ± 0.12	5.39^{a} ± 0.22	8.49^{b} ± 0.52	11.79^{c} ± 1.00

Mm - Melatonin morning; MMI - Methimazole
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$.

TABLE - 8
Showing the per day rate of growth in control and experimental lizards.

Schedule - II (Monsoon)

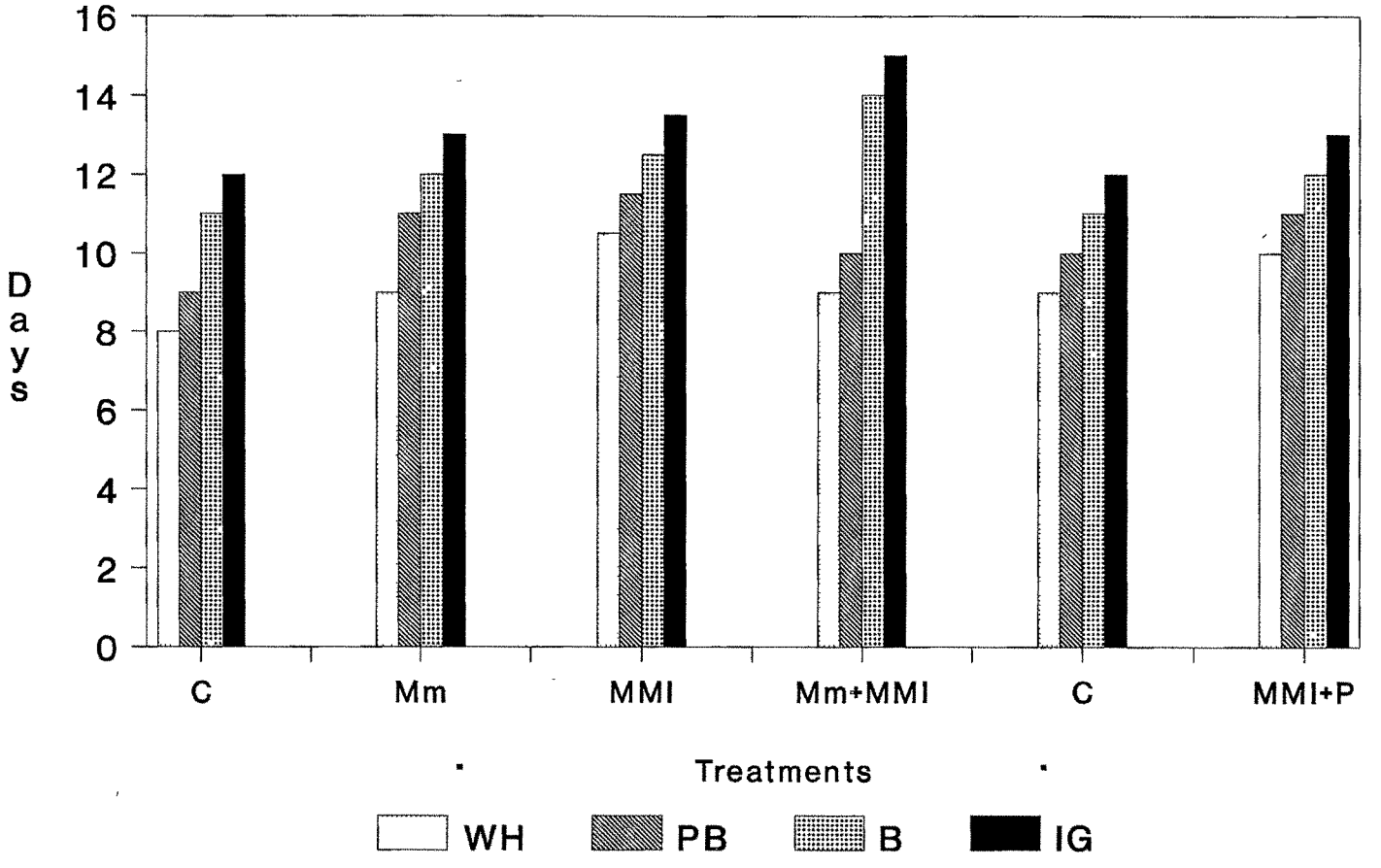
Set-up - III

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
Mm	-	0.33	0.68	0.78	0.43
MMI	-	0.40	0.63	0.30	0.20
Mm + MMI		0.22	0.08	0.17	0.28

CONTROL	-	0.56	0.70	0.77	1.12
MMI + Pimozide	-	0.41	0.66	0.62	0.66

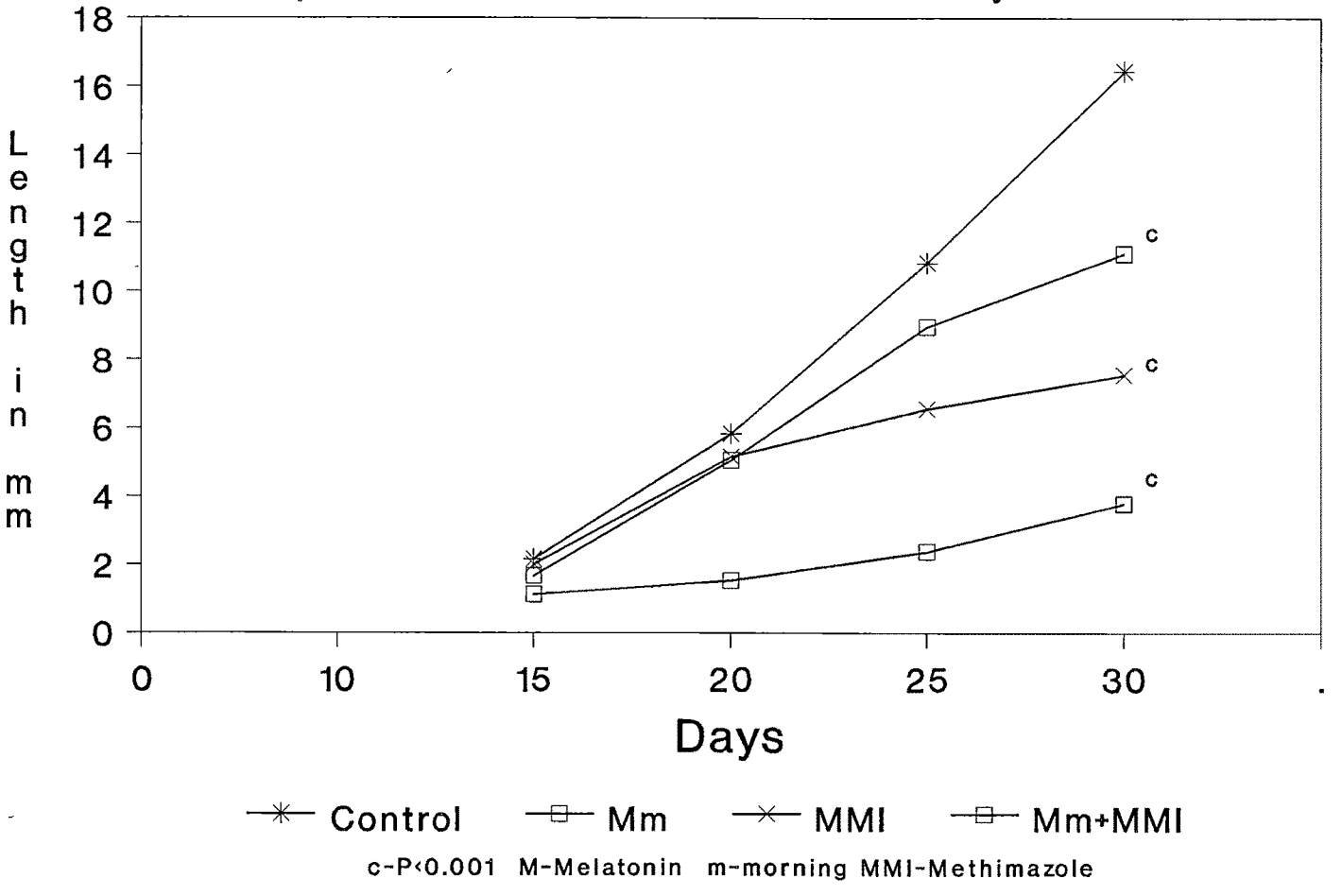
Mm - Morning melatonin, MMI - Methimazole.

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



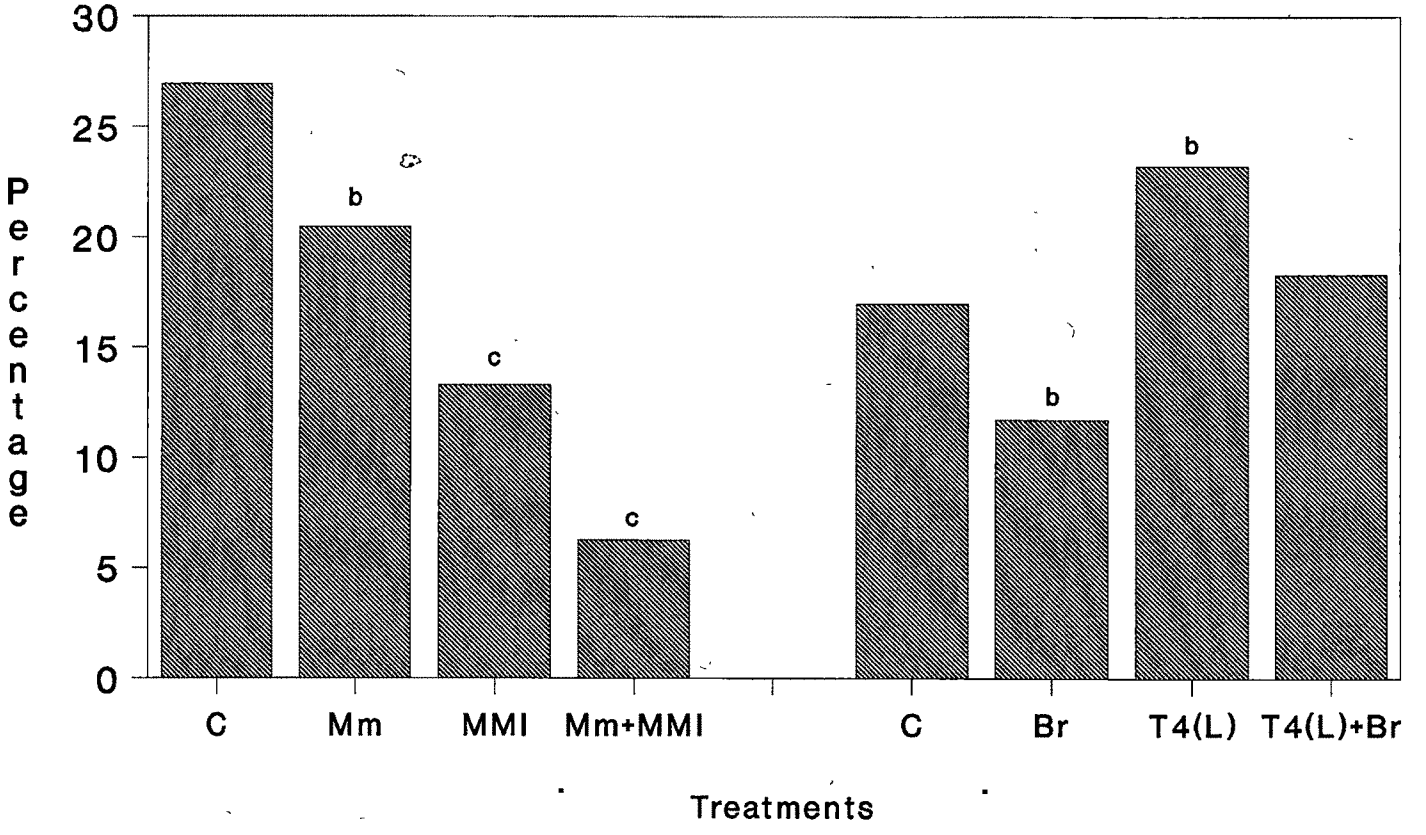
Mm-morning melatonin MMI-Methimazole P-Pimozide

Fig.8 The length of tail regenerated in control(C) and experimental lizards at the end of 30 days in monsoon.



c-P<0.001 M-Melatonin m-morning MMI-Methimazole

Fig.9&12 The percentage of tail replaced in control(C) and experimental lizards in monsoon and winter respectively.



T4-Thyroxine (L)-local MMI-Methimazole Br-Bromocriptine Mm-morning melatonin

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

However, lizards treated with both thyroxine and pimozide attained the stages a day earlier and, also produced a much longer new growth at the end of 30 days. These are represented in tables 9-11

Set up 2 :

Both the groups of lizards receiving thyroxine or thyroxine and bromocriptine showed and advancement by one day in the attainment of the various arbitrary stages, while brimocriptine alone treated lizards showed a delay by two days. The final length of tail replaced at the end of 30 days was significantly greater in thyroxine administered groups of lizards while, it was less in bromocriptine alone administered groups of lizards. A comparison of the growth rates revealed that thyroxine plus bromocriptine administered lizards had consistently low growth rates in relation to thyroxine administered lizards. The data are represented in tables 12-14 and Figs. 10-12.

Set up 3 :

The MMI plus pimozide treated lizards showed no difference in any of the parameters compared to the controls. However, thyroxine plus pimozide treated lizards formed a blastema 2 days earlier and produced a marginally longer new growth, though insignificant at the end of 30 days (tables 12-14).

TABLE -9

Showing the number of days taken to attain the various arbitrary stages and the length of tail regenerated and total percentage replacement in control and experimental lizards.

Schedule - III (Winter)

Setup - I

Manipulations	Total length	Percentage replacement	Number of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	3.00 ± 0.24	4.90 ± 0.99	22	23	24	25
Bromocriptine	2.60 ± 0.15	4.26 ± 0.86	23	24	25	26
Thyroxine (S)	2.80 ± 0.12	4.59 ± 0.52	22	23	24	25
Pimozide	2.76 ± 0.18	4.52 ± 0.64	23.5	24	25	26
Thyroxine (S) + Pimozide	5.50^{b} ± 0.68	9.01^{b} ± 1.02	21	22	23	24

S - Systemic, MMI - Methimazole; L - Local; WH - Wound healing ; PB - Preblastema; B-Blastema; IG - Initiation of Growth.

b - $P < 0.005$.

TABLE - 10
Length of tail regenerated at different time periods post autotomy in control and experimental lizards.

Schedule-III (WINTER)

Set-up I

Manipulations	DAYS				
	10	15	20	25	30
CONTROL	-	-	-	-	3.00 ± 0.20
Bromocriptine	-	-	-	-	2.60 ± 0.06
Thyroxine (S)	-	-	-	-	2.80 ± 0.12
Pimozide	-	-	-	-	2.76 ± 0.14
MMI	-	-	-	-	2.50 ± 0.21
Thyrozine (S) + Pimozide	-	-	-	1.50 ± 0.08	5.50^{b} ± 0.62

S - Systemic, MMI - Methimazole.
b - $P < 0.001$

TABLE - 11

Per day growth rate (mm) in blocks of 5 days in control and experimental lizards.

Schedule-II (Winter)

Set up I

Manipulations	PER DAY RATE OF GROWTH DAYS				
	5-10	10-15	15-20	20-25	25-30
CONTROL	-	-	-	-	0.60
Bromocriptine	-	-	-	-	0.52
Thyroxine (S)	-	-	-	-	0.56
Pimozide	-	-	-	-	0.55
MMI	-	-	-	-	0.50
Thyroxine (S) + Pimozide	-	-	-	0.30	0.80

S - systemic, MMI - Methimazole

TABLE - 12

Showing the number of days taken to attain the various arbitrary stages and the length of tail regenerated and the total percentage replacement at the end of 30 days in control and experimental lizards.

Schedule - III (Winter)

Set-up-II

Manipulations	Total length	Percentage replacement	Number of days taken to attain various arbitrary stages			
			WH	PB	B	IG
CONTROL	10.40 ± 1.01	17.00 ± 2.12	14	15	16	17
Bromocriptine	7.16[b] ± 0.88	11.73[b] ± 1.49	15	16	17	18
Thyroxine (L) Alternate after 15 days	14.20b ± 1.20	23.27[b] ± 2.02	13	14	15	16
Thyroxine (L) + Bromocriptine	11.36 ± 0.96	18.34 ± 2.42	13	14	15	16

Set-up III

CONTROL	5.75 ± 0.61	9.42 ± 0.99	24	25	26	27
Thyroxine(S) + Pimozide	6.60 ± 0.73	10.81 ± 1.02	22	23	24	25
MMI + Pimozide	5.00 ± 0.58	8.19 ± 1.00	24	25	26	27

L - Local, S - Systemic, MMI - Methimazole, WH - Wound healing, PB - Pre-blastema, B - Blastema, IG - Initiation of growth.

b - $P < 0.005$.

TABLE - 13
Showing the length of tail regenerated at different time periods (days).

Schedule-III (Winter)

Setup - II

Manipulations	DAYS				
	10	15	20	25	30
CONTROL	-	-	3.25 ± 0.44	6.47 ± 0.63	10.47 ± 1.56
Bromocriptine	-	-	1.80[b] ± 0.05	5.20[b] ± 0.74	7.85[c] ± 0.44
Thyroxine (L) Alternate after 15 days	-	-	3.90 ± 0.60	7.25 ± 0.80	11.34 ± 1.05
CONTROL	-	-	-	-	5.75 ± 0.32
Thyroxine (S) + Pimozide	-	-	-	-	6.60[a] ± 0.58
MMI + Pimozide	-	-	-	-	5.00 ± 0.61

L - Local, S - Systemic, MMI - Methimazole
a - $P < 0.01$, b - $P < 0.005$, c - $P < 0.001$.

TABLE - 14
Showing per day rate of growth in block of 5 days in the control and experimental lizards.

Schedule - III (Winter)

Setup - II

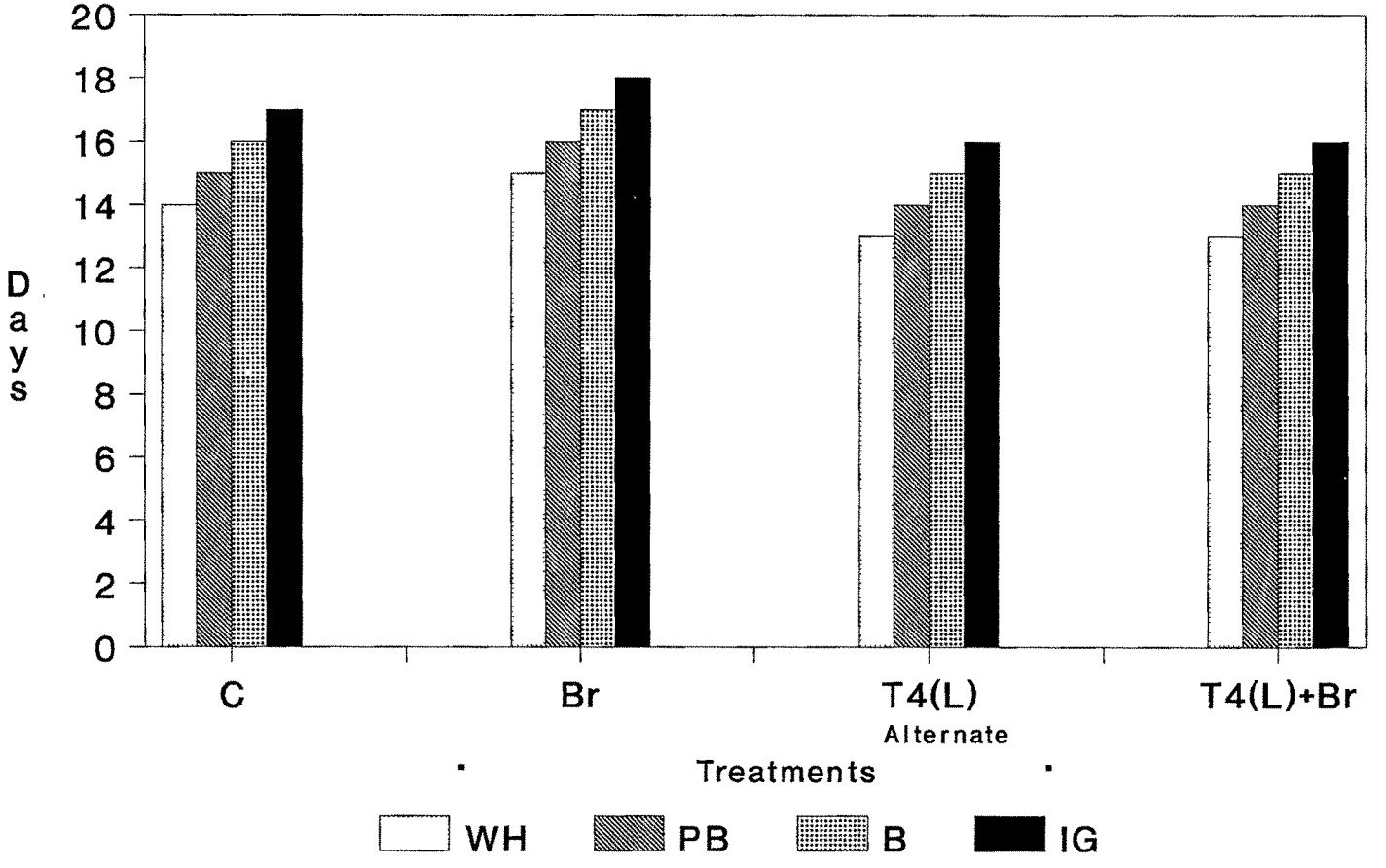
Manipulations	PER DAY RATE OF GROWTH DAYS				
	5-10	10-15	15-20	20-25	25-30
CONTROL	-	-	0.65	0.64	0.80
Bromocriptine	-	-	0.36	0.68	0.53
Thyroxine (L) Alternate after 15 days	-	-	1.00	0.80	1.04
Thyroxine (L) + Bromocriptine	-	-	0.78	0.67	0.81

Set-up III

CONTROL	-	-	-	-	1.15
Thyroxine (S) + Pimozide	-	-	-	-	1.32
MMI + Pimozide	-	-	-	-	1.00

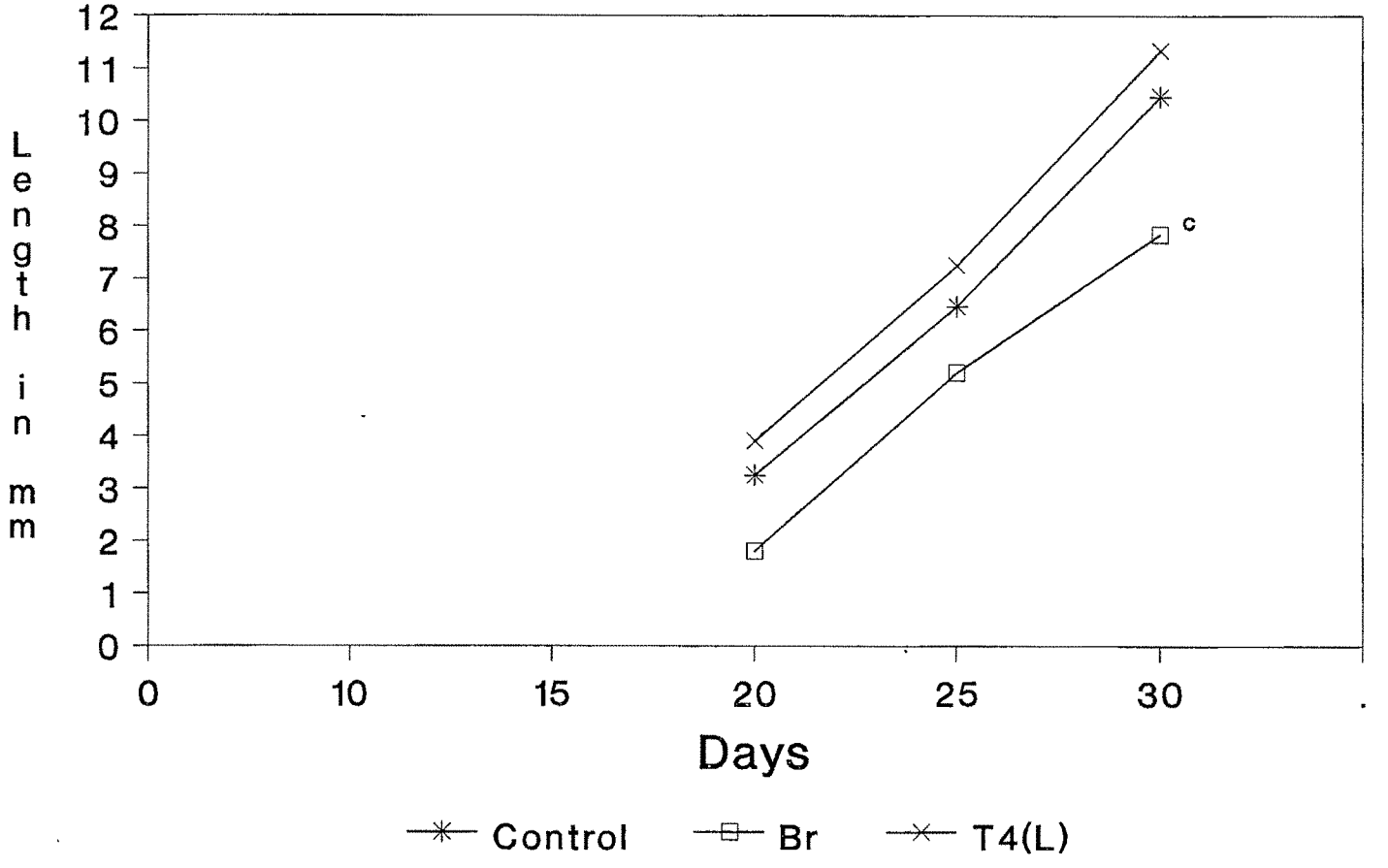
L - Local, S - Systemic, MMI - Methimazole.

Fig.10 Number of days taken to attain the arbitrary stages in control (C) and experimental lizards.



Br-Bromocriptine T4(L)-Thyroxine(local)

Fig.11 The length of tail regenerated in control(C) and experimental lizards in winter.



c-P<0.001 Br-Bromocriptine T4(L)-Thyroxine,local-alternate

DISCUSSION

For the sake of clarity, discussion is taken up under individual experimental schedules and an overall consideration is made finally.

Schedule I : (Summer months, higher temperatures) :

Thyroidectomy can create a state of athyreosis; however Tx has been combined with MMI treatment to ensure an athyroidal state in case of subtotal thyroidectomy in some cases. This has been essentially done as a double check and the results show that in the absence of thyroid hormone, formation of a regeneration blastema and regeneration *per se* are completely inhibited. It is obvious from the previous studies on PTU or MMI induced hypothyroidism (Ramachandran *et al.*, 1984; Ramachandran and Abraham, 1990 and Chapter 6), as well as from the present observations that, only the initiation of regeneration is delayed and the regenerative growth retarded, but regeneration *per se* not inhibited. This can be attributed to the role of thyroxine in inducing the outgrowth of the ependyma, a *priori*, for the formation of a blastema (Simpson, 1964, 1965, 1969, 1970; Cox, 1969 a,b,; Turner and Tipton, 1971; Turner, 1972) as well as in promoting regenerative growth. Since PRL is also implicated in regenerative growth in both amphibians and reptiles (Schauble and Tyler, 1972; Schauble and Nentwig, 1974; Licht and Jones, 1967), pimozide, a DA antagonist was used to raise the endogenous PRL level in lizards made athyroidic. The efficacy of

pimozide in *H. flaviviridis* has already been demonstrated (Ndukuba and Ramachandran, 1989). The fact that athyroidic lizards failed to form a blastema despite elevated PRL secretion, attests to the primary importance of thyroxine in triggering off regeneration and setting up a favourable conducive environment for PRL controlled tail elongation. The importance of both thyroxine and PRL in the maximal expression of regenerative potential is evident by the inability of PRL alone to initiate regeneration or, thyroxine replacement in athyroidic lizards to better the regenerative growth in control lizards and, an enhanced regenerative response in thyroxine plus pimozide treated athyroidic lizards. The importance of both thyroxine and prolactin was also inferred by the hypophysectomy, hypothyroidism and thyroxine replacement studies in *Anolis carolinensis* (Turner and Tipton 1971). A synergistic action between thyroxine and PRL was deemed necessary for maximal regenerative response, a was also shown in newts (Tassava, 1969b).

Schedule II : (Monsoon months, intermediate temperatures)

Despite the inferred favourable influence of thyroxine, continuous daily administration of thyroxine systemically retarded tail regeneration considerably. Co-administration of bromocriptine, a potent DA agonist which is known to decrease PRL release, did not further lower the regenerative potential and took same time periods for the attainment of arbitrary stages, regenerated an identical length of tail with similar growth rates, as with thyroxine administration alone. Apparently, bromocriptine had

activity and thyroid hormone levels during tail regeneration in lizards again, attest to the need for optimum levels and duration of presence of thyroid hormones for full regenerative expression (Ramachandran *et al.*, 1981b, 1993). Viewed in this context, the presently observed ineffectiveness of bromocriptine to exert additional retardative influence in thyroxine administered animals is understandable.

The need for synergistic action of both thyroxine and PRL in maximal expression of the regenerative potential, is further emphasised by the inability of thyroxine administration to athyroidic hypoprolactinemic lizards to elicit a regeneration response equivalent to that of euthyroidic controls. Moreover, increased PRL secretion induced by pimozide in a background of hypothyroidism also reduced the regenerative potential more than that in the athyroidic hypoprolactinemic lizards receiving thyroxine. Obviously, under induced suboptimal levels of thyroxine, even increased PRL level is not able to maintain the full regenerative efficiency. This may also substantiate the need for an optimum thyroid hormone priming for the synergistic action of PRL. Further proof for the need of both thyroxine and PRL in eliciting optimum regenerative growth is provided by the very drastically reduced regenerative performance by hypothyroid lizards administered melatonin in the morning, which was significantly more than the retardatory influence exerted by either hypothyroidism or Mm alone. Whereas retardation induced by hypothyroidism could be accredited to reduced thyroid hormone levels, Mm induced retardation is related with reduced PRL

secretion, a duration effect of melatonin which makes the animal to read it as an extended scotophase (Ramachandran and Ndukuba, 1993; Chapters 4 and 5). The minimal regenerative growth (hardly 23% of the controls) shown by hypothyroidic Mm treated lizards provides compelling evidence for the dependence of lizard tail regeneration on both thyroxine and prolactin.

Schedule III : (Winter months, lower temperature)

A generalised projection from the experiments under this schedule is the unresponsiveness to any of the manipulations tending to alter PRL and thyroid hormone levels and, the unaltered more or less lethargic regenerative response. Neither elevation of thyroxine or PRL by thyroxine administration or by pimozide treatment respectively, made even an iota of difference in terms of regenerative performance. However, simultaneous elevation of both resulted in meagre hastening of the regenerative process. The response to the combined hormonal assault was nevertheless a minimal acceleration, considering the already espoused potentially synergistic effect of these two hormones. Interestingly, continuous thyroxine administration daily for the first 15 days and every other day thereafter, produced an improved regenerative growth, which was minimised by simultaneous induction of hypoprolactinemia, again attesting to the synergistic action of both thyroxine and PRL. Treatment with bromocriptine alone (hypoprolactinemia) significantly reduced the regenerative growth. An earlier study on thyroxine administration and, even the

present study on bromocriptine administration at lower temperatures registered no response compared to the controls. As against this, the improved regenerative performance shown by thyroxine administration as well as the retardation induced by bromocriptine appears contradictory. This apparent discrepancy becomes clear when the temperature ranges are compared. Whereas the responsiveness to thyroxine and bromocriptine was manifested at a slightly higher average temperature range of 20-23°C, the unresponsiveness to treatments occurred at the lower average temperature range of 17-19° C. The temperature effect becomes very clear when a comparison is made between the regenerative performance of lizards made hypoprolactinemic by bromocriptine administration in the months of August-September, November-December and January-February at the end of 30 days. At a higher temperature of about 25-26° C in August-September, the control lizards replaced 16.12mm of tail as against 7.16mm by the bromocriptine treated lizards (Chapter 5). At about 20-30° C in November-December, the controls regenerated 10.4mm as against 7.85mm by the bromocriptine treated lizards while, at 17-19° C in January-February, the length of the regenerate in control lizards was only 3mm as against 2.6mm in bromocriptine treated ones. Clearly at lower temperatures, both, the circulating titres of thyroxine and PRL as well as the responsiveness towards them are both greatly reduced as had been inferred earlier (Chapter 6). The fact that even experimental manipulations involving hypothyroidism coupled with

hypoprolactinemia or even hypothyroidism alone with hypoprolactinemia could not deter the regeneration course in either way, further strengthens the low temperature influenced insignificant response to these hormones and the winter sluggishness in regenerative performance.

Finally, it can be generalized from the entire gamut of observations made herein, that both PRL and thyroid hormones are the essential mediators of initiation of regeneration and maintenance of tail elongation. Since temperature seems to play very important role in the levels of the hormones as well as their responsiveness, with higher temperatures exerting a positive influence and lower temperatures a negative influence, a synergistic action of these two hormones is an essential aspect for maximal tail elongation. Complete absence of thyroid hormone can inhibit tail regeneration, possibly by preventing the growth of the ependyma. In this respect, the initial target for thyroid hormone at the local site could be the ependyma of the spinal cord. In another investigation in the present study, it is revealed that *in loco* administration of NGF (nerve growth factor) in hypothyroidic animals could prevent the delay in the initiation of regeneration (Chapter 8). Apparently, thyroxine induces the formation and release of some growth promoting substance from the spinal cord/ ependyma which leads to its outgrowth as well as the organisation of a blastema. Just as thyroxine is the principal hormone in initiation of regeneration, PRL seems to be the principal evokator of tail elongation. However, neither could function in total

absence of the other. This is justified by the ability of each of these two hormones to compensate for the other in situations of its reduced level. The importance of both thyroxine and PRL for regeneration is supported by the observation of increased localization of both labelled thyroxine and PRL in the regenerating limbs of adult newts (Korneluk and Liverage, 1978; Furlong *et al.*, 1987) and, in fact, the localization of PRL in the wound epithelium was shown to induce morphological alterations which were inferred to account for the functional and associated biochemical alterations in the regenerating tissue. This is further substantiated by the recently observed increase in PRL level and PRL provoked alterations in molecular mechanisms favouring cell proliferations after partial hepatectomy (Buckley *et al.*, 1991). Considering the synergistic action of these two hormones during regeneration, a likely possibility of release of one hormone triggering the release of the other cannot be discounted. In this respect, there are evidences from mammals to show the ability of TRH and TRH analogues to induce PRL release (Login *et al.*, 1991; Scbuiling *et al.*, 1993) and thyroidectomy resulting in reduced PRL release and, also the need for thyroxine in TRH induced PRL release (Ramalbo *et al.*, 1990, 1992; Mizukani *et al.*, 1993; Ozawa and Kurosumi, 1993; Yang and pan, 1994). Another observation that merits consideration is the ability of exogenously administered melatonin in the morning in hypothyroid lizards to decrease the regenerative performance to remarkably low levels; this suggests Mm to be more potent in decreasing PRL

secretion, a conclusion which was already arrived at (Chapters 4 and 5). This may have relevance in the observed sluggishness of regenerative performance in the winter season as, both lower temperatures and short photoperiods are known to increase melatonin level and its scotophase duration, which might reduce PRL level and responsiveness drastically in the winter season.

SUMMARY

Both thyroxine & PRL are implicated in lizard tail regeneration. In order to understand the relative roles of these two hormones, and to seek a possible relation between these two hormone and, the observed seasonal variations in regenerative performance, a combination of surgical and neuropharmacological manipulations have been carried out in *H. flaviviridis*. The various experiments were carried out in the summer, monsoon and winter months. The results revealed that, athyriosis inhibits tail regeneration while, thyroxine replacement overcame the inhibitory effect and the regenerative growth was potentiated by pimozide treatment. In the monsoon months both, thyroxine administration as well as bromocriptine treatment retarded tail regeneration. Athyroidic, hypoprolactinemic lizards receiving thyroxine or, hypothyroid lizards recieving pimozide, both, showed improved regenerative growth but significantly less than the control. Hypothyroid lizards treated with melatonin in the morning depicted a much attenuated regenerative growth. In the winter months neither hypothyroidism or hypoprolactine nor, hyperthyroidism or hyperprolactin caused any effect on the regenerative growth. It is concluded from the observations that, both thyroxine and PRL are important and exert synergistic action with some interrelationship. It is also concluded that, thyroid and lactotroph activity as well as sensitivity towards thyroxine and PRL are both temperature dependent with, higher temperatures increasing and lower temperatures decreasing them.