CHAPTER 7

EFFECTS OF TESTOSTERONE ON THE RATE OF GROWTH OF THE REGENERATING TAIL OF NORMAL AND THYROIDECTOMISED GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

The effect of gonadal hormones in the process of repair and regeneration in vertebrates seems to be enigmatic (Schmidt, 1968). In the recent past, a few studies on the effect of androgen on amphibian limb regeneration (Durand, 1963) and healing processes in mammals (Tsmura and Ishida, 1963; Tsmura, 1963; Yoshikava, 1963) have been carried out. However, no attempts have been made to evaluate the effect of exogenous administration of male hormones on reptilian tail regeneration. Observations of a differential sex response on the rate of growth of the regenerating tail in normal and thyroidectomised house lizards, Hemidactylus flaviviridis (Chapter 6) have suggested the possibility of influence of the male hormone on the process of tail regeneration, which prompted to undertake the present study aimed at evaluating the effect of the exogenous hormone, testosterone, on the rate of growth of the regenerating tail in the normal and thyroidectomised lizards, H. flaviviridis.

MATERIALS AND METHODS

Present study was carried out during the breeding season i.e. during the months of November to April (Sanyal and Prasad, 1967). Adult lizards of both the sexes of more or less in the same weight group (12-14 gms) with snout to vent length of 8-10 cms were selected. Total 160 lizards were utilized for the experiments and were divided into groups described as under.

Group I : In the first group of forty normal lizards (with intact original tail) of both the sexes, 0.2 mg testosterone (Aquaviron aqueous adsorbate testosterone, Indian Schering, Ltd.) was injected intramuscularly on every alternate day with 0.1 ml of 0.9% saline as the vehicle. Autotomy was induced on the 7th day (by then 0.6 mg testosterone was already administered). Administration of testosterone was continued till the animals acquired almost the full length of the regenerating tail (for about 60 days in both the sexes). The rate of growth of the regenerate was measured at every 10 day interval starting from the 10th day after autotomy till the regenerate was almost fully grown. The lizards had received totally 6 mg testosterone. Another forty lizards injected with 0.1 ml of 0.9% saline

served as controls for the group I. Rest of the experiment was as described for animals of the first group.

Group II : In the second group of forty lizards, thyroidectomy was performed under hypothermic anaesthesia. Operative procedure followed was as described in Chapter 6. In so operated animals, 0.2 mg testosterone was injected intramuscularly on every alternate day from the day of operation with 0.1 ml of 0.9% saline as the vehicle. Autotomy was induced on the 7th day (by then 0.6 mg testosterone was already administered and the wound of the operation was well healed). The rate of growth of the regenerating tail was measured. In another group of forty lizards thyroidectomy was performed and only 0.9% saline was injected intramuscularly on every alternate day from the day of operation. This group served as control to the group II. Rest of the experiment was as described for group II.

RESULTS AND OBSERVATIONS

All the control lizards to group I survived the entire experimental period, however, a few deaths did occur in the experimental lizards of both the groups as well as

in those of the control of the second group. Percentage mortality rate was about 26.6 and 11.9 in two experimental groups respectively; and 13.5 in the controls to the second group. However, in general surviving lizards had good health and served the experimental purpose. The data for the rate of growth of the regenerate and the time taken (in days) for attainment of each stage of regeneration are presented in Tables 1, 2, 3, 4 and Figs. 1, 2, 3 and 4.

In the males of the first group, administration of testosterone slightly enhanced the growth of the regenerate as compared to that in the control lizards. However, in females the testosterone administration had considerably enhanced the growth of the regenerate as compared to that in experimental males and to that in the animals of the same sex treated with only saline which served as controls. It took about 6.5 ± 1.713 days in testosterone treated male lizards for the autotomy wound to heal as compared to 7.1 ± 1.13 days in the controls. On the other hand, the female lizards receiving testosterone took about 7.00 ± 1.04 days as compared to 9.03 ± 1.57 days in the females treated as controls. The time taken for the blastema formation in the lizards of both the sexes treated with testosterone was about 4 days after the autotomy wound was healed, which was relatively earlier than their respective controls. As

Table 1 : Rate of growth (in mm) of the regenerating tail of testosterone and saline treated normal (euthyroidic) lizards, H. flaviviridis

Days after autotomy	Testosterone treated		Saline treated	
	Male	Female	Male	Female
10th day	3.51±1.09	3.75±1.01	2.66±1.26	1.51±0.50
20th day	15.0 ±2.64	14.1 ±1.28	13.3 ±1.05	9.81±1.71
30th day	26.3 ±1.76	24.2 ±3.75	21.2 ±2.23	17.3 ±1.01
40th day	34.4 ±3.20	30.4 ±1.91	29.6 ±1.53	23.1 ±2.01
50th day	46.9 ±2.05	42.0 ±2.47	39.9 ±2.82	32.1 ±3.13
60th day	59.8 ±2.91	58.7 ±3.57	49.3 ±1.37	42.9 ±2.91
70th day			59.8 ±2.13	51.3 ±2.13
80th day				59.7 ±2.98

Table 2 : Rate of growth (in mm) of the regenerating tail of testosterone and saline treated thyroidectomised lizards, H. flaviviridis.

Days after autotomy	Thyroidectomy + testosterone		Thyroidectomy + saline	
	Male	Female	Male	Female
10th day	3.97±0.57	3.87±1.31		2.01±0.71
20th day	14.0 ±2.82	14.6 ±2.51	3.10±1.13	6.21±1.97
30th day	23.6 ±3.00	24.0 ±1.82	5.07±1.70	11.3 ±2.01
40th day	31.1 ±1.85	31.3 ±1.08	12.3 ±2.07	18.0 ±2.93
50th day	39.5 ±2.23	39.8 ±1.54	21.0 ±2.13	27.1 ±3.07
60th day	45.7 ±2.13	45.1 ±1.52	29.1 ±1.94	36.3 ±2.43
70th day	52.3 ±1.31	51.0 ±2.13	37.4 ±2.73	45.3 ±3.04
80th day	57.3 ±1.91	58.9 ±1.31	45.0 ±1.51	52.0 ±2.04
90th day			50.5 ±2.07	55.4 ±2.04
100th day			55.1 ±1.31	

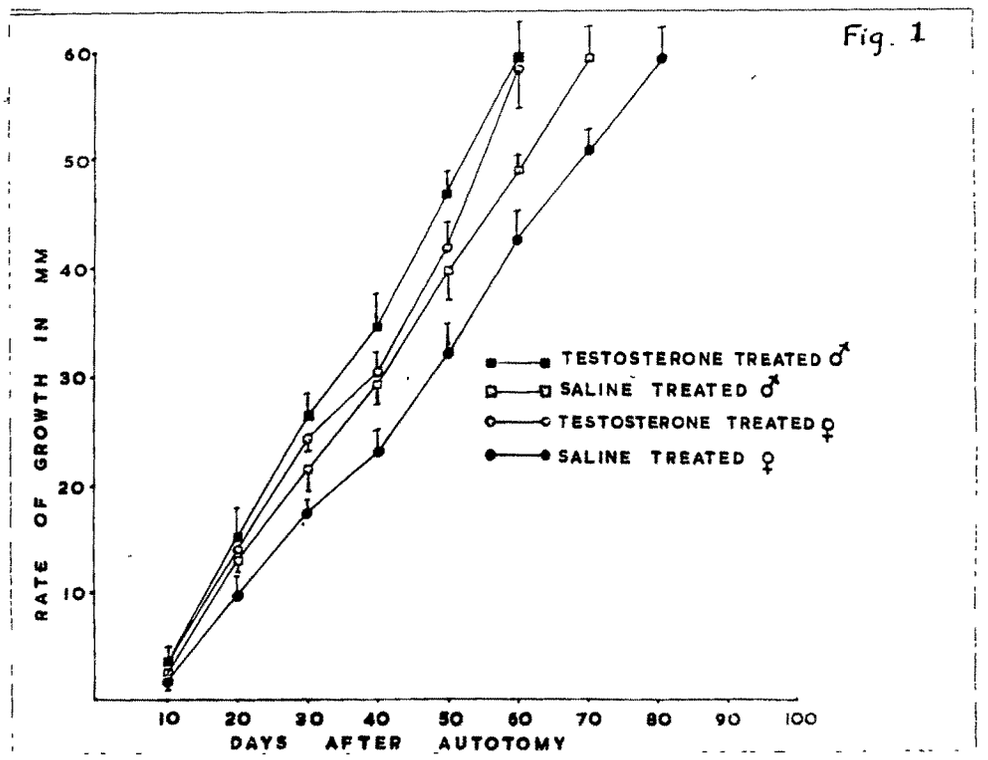
Fig. 1
RATE OF GROWTH IN MM
60
50
40
30
20
10
10 20 30 40 50 60 70 80 90 100
DAYS AFTER AUTOTOMY
TESTOSTERONE TREATED ♂
SALINE TREATED ♂
TESTOSTERONE TREATED ♀
SALINE TREATED ♀

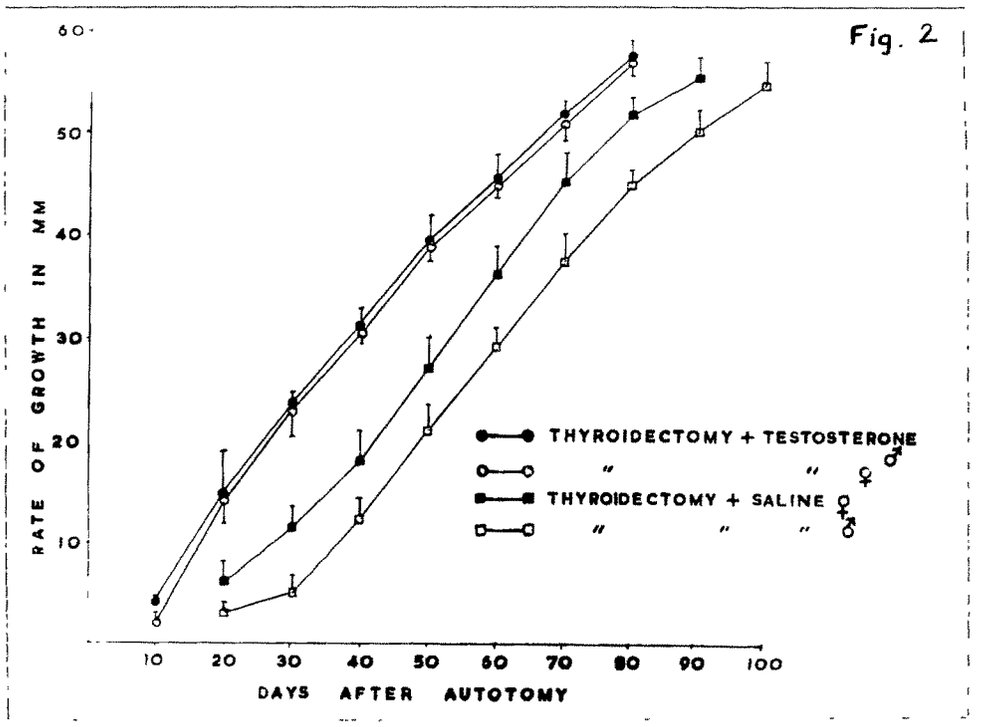
Fig. 2
RATE OF GROWTH IN MM
60
50
40
30
20
10
10 20 30 40 50 60 70 80 90 100
DAYS AFTER AUTOTOMY
THYROIDECTOMY + TESTOSTERONE ♂
" " ♀
THYROIDECTOMY + SALINE ♀♂
" " "

Fig. 1 : Graphic representation of the rate of growth of the regenerating tail of testosterone and saline treated normal (euthyroidic) lizards, H. flaviviridis.

Fig. 2 : Graphic representation of the rate of growth of the regenerating tail of testosterone and saline treated thyroidectomised lizards, H. flaviviridis.

Table 3 : Number of days taken by regenerate to reach different stages of tail regeneration in testosterone and saline treated normal (euthyroidic) lizards, H. flaviviridis.

Stages of regeneration	Testosterone treated		Saline treated	
	Male	Female	Male	Female
Wound healing	6.50±1.71	7.00±1.04	7.10±1.13	9.03±1.57
Blastema	10.3 ±2.03	11.1 ±1.47	12.1 ±0.97	15.0 ±1.94
Differentiation	14.0 ±1.57	15.9 ±1.09	17.5 ±1.94	22.1 ±2.13
Growth	16.9 ±1.01	17.5 ±1.91	20.9 ±2.31	25.0 ±2.51
Fully regenerated tail	60.5 ±3.46	59.9 ±2.93	70.4 ±3.91	78.1 ±2.02

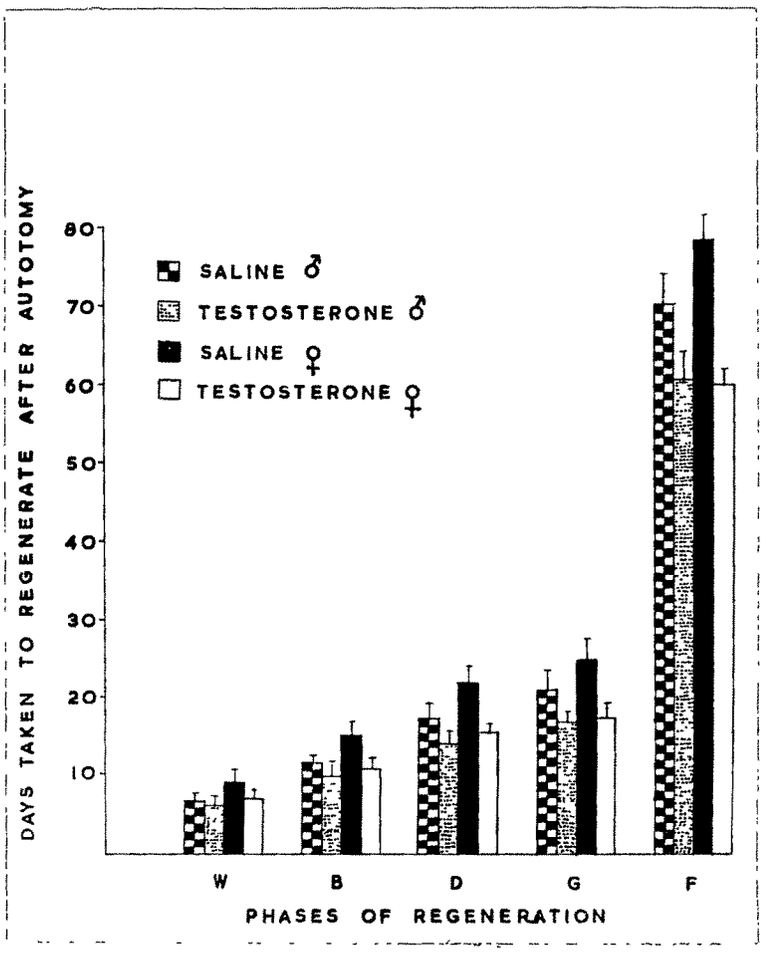

Fig. 3 : Graphic representation of time taken (in days) for attainment of various stages of tail regeneration after autotomy in saline and testosterone treated normal (euthyroidic) lizards, H. flaviviridis.

W - Wound healing phase
B - Blastema phase
D - Differentiation phase
G - Growth phase
F - Fully regenerated tail

Table 4 : Number of days taken by regenerate to reach different stages of tail regeneration in testosterone and saline treated thyroidectomised lizards, H. flaviviridis.

Stages of regeneration	Thyroidectomy + testosterone		Thyroidectomy + saline	
	Male	Female	Male	Female
Wound healing	8.10±1.07	7.42±1.31	12.3±1.97	9.50±1.07
Blastema	14.3 ±1.53	13.9 ±1.79	21.2±2.07	17.5 ±1.53
Differentiation	22.0 ±2.13	21.5 ±2.31	29.0±1.64	23.0 ±0.47
Growth	26.5 ±1.97	25.1 ±1.53	35.6±1.97	27.1 ±2.14
Fully regenerated tail	80.5 ±4.53	79.4 ±3.91	96.5±4.59	90.5 ±3.74

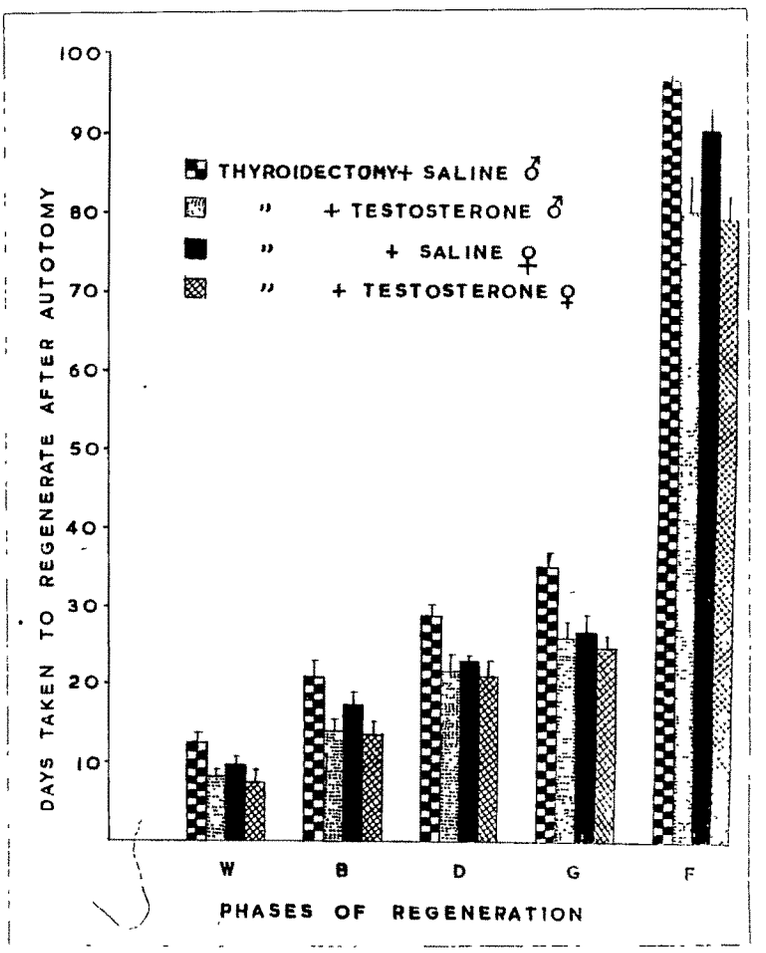

Fig. 4 : Graphic representation of time taken (in days) for attainment of various stages of tail regeneration after autotomy in saline and testosterone treated thyroidectomised lizards, H. flaviviridis.

W - Wound healing phase
B - Blastema phase
D - Differentiation phase
G - Growth phase
F - Fully regenerated tail

the regeneration progressed further, differentiation in the regenerate also occurred earlier in both the sexes, however, the rate of growth in female lizards receiving testosterone was faster (15.9 ± 1.09 days) as compared to that in females used as controls (22.1 ± 2.13 days). Testosterone treated, both the males and females acquired more or less full length of their regenerate in about 60.5 ± 3.46 and 59.9 ± 2.93 days respectively, which is earlier than that in control lizards (70.4 ± 8.91 days in males and 78.1 ± 2.02 days in females) (Table 3; Fig. 3).

The growth of the regenerate in thyroidectomised lizards of both the sexes (group II) receiving exogenous testosterone was not completely restored to a normal pace, nevertheless, it was improved compared to the controls where thyroidectomised lizards were injected with saline. The days taken for the autotomised wound to heal in testosterone administered thyroidectomised male and female lizards were 8.10 ± 1.07 and 7.42 ± 1.31 respectively, which were less than that in thyroidectomised lizards receiving only saline (controls) where males and females took about 12.3 ± 1.97 and 9.50 ± 1.07 days respectively. Similarly blastema formation also was observed earlier in testosterone administered thyroidectomised lizards

compared to those receiving only saline. Differentiation of the regenerate in so treated thyroidectomised male and female lizards was observed by about 22.0 ± 2.13 and 21.5 ± 2.31 days respectively which was earlier than the control group where males and females took about 29.0±1.64 and 23.0 ± 0.47 days respectively. The total days taken to acquire the full length of their tail regenerate in testosterone administered thyroidectomised lizards were about 80.5 ± 4.53 and 79.4 ± 3.91 in males and females respectively. The rate of growth was faster than that noticed for respective sexes in controls, where males took about 96.5 ± 4.59 and females about 90.5 ± 3.74 days (Table 4; Fig. 4).

DISCUSSION

Androgens, have been shown to increase an overall growth rate and tissue regeneration in mammals (Brody, 1945; Gaunt, 1954; Dyson and Joseph, 1968). In the present study, testosterone administration in normal (euthyroidic) female lizards increased the rate of growth of the regenerate considerably. This finding of an accelerated growth of the tail regenerate in females lends credulence to the assumption that male sex hormone profoundly influences the anabolic reactions during tail regeneration. The

assumption of anabolic influence of androgens gains support from the works of Jørgensen and Schmidt (1962), Barbera et al. (1962) and Joseph and Dyson (1966) in mammals where androgen administration enhanced the healing process and regeneration. However, in case of males, the growth of the regenerate was not affected significantly with testosterone administration. At this juncture, it may be suggested that there might be an 'optimum' level of androgen already established endogenously in response to the regenerative activities and hence exogenously administered testosterone could result in inducing reduction in androgen production in the animal itself. Inhibiting effects of administration of exogenous testosterone on production of androgens in the male rabbit has been reported by Dyson and Joseph (1968) which is believed to maintain the physiological level of androgens in the body. Extrapolating these facts, it could be considered logical that there may not be any significant effect on the rate of growth of the tail regenerate on administration of androgens in male lizards. Despite the complexities of defining hormone action on tail regeneration in lizards (Licht, 1967), an enhanced growth of the regenerate in androgen treated female lizards H. flaviviridis underscores the anabolic

action of this hormone, and its subsequent influence on regenerating system. It would not be out of place to recall that the growth rate of regenerating tail in male lizards is higher than that in females which is probably due to higher androgen level in the former (Chapter 6).

It has been implied in the house lizards, H. flaviviridis, that male hormone enhances oxidative processes of the body, which is considered to be a result of its direct influence on the process or through the mediation of thyroid (Thapliyal et al., 1975; Chandola et al., 1974b). Thus, in the present study it appears reasonable to suggest that administration of testosterone in the house lizards enhances the general metabolic rate of the body, may be through the mediation of thyroid gland or directly which is reflected in a faster rate of growth of the regenerating tail.

Relatively more delay in growth rate of the regenerating tail of thyroidectomised male lizards as compared to that in thyroidectomised female lizards was rectified only to certain extent when such males were treated with testosterone. These observations led to believe that the enhanced delay in tail regenerative

activities of males could be due to adverse effects of thyroidectomy on androgen production by male gonads. The delay in growth rate of thyroidectomised female lizards was rectified almost totally on administration of testosterone. These facts once again suggest and indirectly confirm the influence of testosterone in promoting repair and regeneration of tail in house lizards.

Role of thyroid gland in oxidative processes of lizards has been reported by Maher (1965). Ability of testosterone to restore the lowered respiratory rate in thyroidectomised snakes to a normal level was reported by Thapliyal et al. (1975). With these facts in view, it may be presumed that testosterone administration elevates lowered oxidative processes of the body in thyroidectomised lizards, which in some way becomes responsible for the enhanced growth of the tail regenerate.

From the present study it becomes clear that the androgens play significant role in the process of tail regeneration by providing necessary measure for enhanced growth of the regenerate which may be partly by direct action of this hormone or through the mediation of the thyroid gland.