CHAPTER 6

THYROID-GONAD INTERRELATIONSHIP AND ITS EFFECT ON THE RATE OF GROWTH OF THE REGENERATING TAIL OF THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Although the influence of hormones on regenerative processes in amphibia has received considerable attention (Tassava *et al*., 1968; Tassava, 1969; Liversage and Scadding, 1969; Vethamany-Globus and Liversage, 1973a & b) very little is known about this aspect on saurian tail regeneration (Licht, 1967). The influence of thyroid on tail regeneration of lizard, *Anolis carolinensis* has been studied by Turner and Tipton (1971) and Turner (1972). Changes in histological features of thyroid gland during various phases of tail regeneration in the house lizard, *Hemidactylus flaviviridis* have been reported by Shah and Chakko (1968b) and Magon (1970).

A relationship between thyroid activity and testosterone has been established in reptiles by Tam *et al*. (1967), Chiu *et al*. (1969) and Chandola *et al*. (1974a, b). It is reported by Joseph and Dyson (1965, 1966) that the rate of tissue regeneration is greater in males than that in females in case of mammals which is considered to be due to the higher androgen level in the former. However, in case of reptilian regeneration an isolated report by Congdon *et al*. (1974)

indicates that males of lizard, Coleonyx regenerate the tail faster than females of the same species. With these facts in mind, it was deemed worthwhile to study the growth of the tail regenerate in the normal as well as thyroidectomised lizards alongwith certain histophysiological changes in the gonads following thyroidectomy.

MATERIAL AND METHODS

All experiments were carried out during the months November to April (breeding season for the house lizard, Hemidactylus flaviviridis, Sanyal and Prasad, 1967). Lizards of the same weight groups (12-14 gms) and snout to vent length 8-10 cms, were selected in order to use only adult animals. A total number of 150 lizards were utilized for the experiments and were divided in groups as under.

Group-I (Euthyroidic) : First group of fifty lizards of both the sexes were subjected to autotomy by pinching off the tail leaving two to three segments intact to vent. The rate of growth of the regenerating tail was measured at every 10 day interval starting from the 10th day after autotomy till the regenerate reached almost the length of the original tail. The time taken (in days) for attainment of each stage of the tail regeneration viz., wound healing, blastema, differentiation and growth as described by Shah and Chakko (1968a) were noted.

Group-II (Thyroidectomised) : In the second group of fifty lizards of both the sexes, thyroidectomy was performed under hypothermic anaesthesia. Operative procedure included a transverse incision on the ventral surface of the throat anterior to pectoral girdle, and careful removal of the thyroid gland.

Group-III (Sham operated) : Third group of fifty lizards of both the sexes were subjected to sham operation.

Incisions in groups II and III were sutured with silk thread. Necessary post operative care was taken.

The lizards of groups II and III were subjected to autotomy 10 days after operation, as by this time the operation wound gets healed. Rate of growth of the regenerating tail in both the groups was measured.

Ten lizards (five each of both the sexes) from each of the above mentioned groups including those with original as well as regenerating tails were sacrificed. Testis or ovary, epididymis or oviduct, as the case may be, and kidneys from male lizards were removed, weighed and fixed in Bouin's fixative for histological processing. Seven μ thick paraffin sections of these organs were cut and stained with haematoxylin-eosin (Gurr, 1956).

Height of the cells of the sexual segment of kidney and that of the cells of epididymis was measured with the help of an ocular micrometer. Post-mortem examination of the throidectomised lizards which were sacrificed for the study was carried out in order to ascertain complete removal of the thyroid gland.

RESULTS AND OBSERVATIONS

All sham operated animals survived the entire experimental period, however, mortality in the thyroidectomised lizards was about 14.5%; but by and large the post operative condition of the surviving thyroidectomised animals was good. 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 and Figs. 1, 2, 3 & 4.

In group I (euthyroidic lizards), the rate of growth of the regenerate in male lizards was faster than that in females. Male lizards took about 7.2 ± 1.51 days for the autotomised wound to heal, while in females it took about 9.61±1.91 days. Blastema cone appeared by about 12.30±2.46 and 15.09±2.98 days in males and females respectively. As the regeneration progressed, differentiation phase of the regenerate in case of males was noticed by 20.71±2.91 days, while that in females was seen by about 24.56±1.76 days. Duration of the regenerate to reach almost the full grown length was 70.5±6.59 and 80.3±5.17 days in males and females respectively (Table 2; Fig. 3).

Rate of growth of the regenerating tail in groups I (euthyroidic) and III (sham operated) was almost similar but a delay was noticed in the regenerative process of group II (thyroidectomised). In group II the delay in wound healing due to thyroidectomy in females was almost nil, whereas that in males it was by about five days. Similarly in blastema formation also, males without thyroid showed a delay by about nine days and that in females, it was only of about three days. The regenerate in thyroidectomised males acquired the length of 55.6±2.47 mm by about 100th day after autotomy as compared to control male lizards where the regenerate reached the length of 59.5±3.76 mm by 70th day post autotomy. Thus, there was a

Table 1 : Rate of growth (in mm) of the regenerating tail of normal and thyroidectomised lizards, *H. flaviviridis*.

Days after autotomy	Normal		Thyroidectomised		Sham operated	
	Male	Female	Male	Female	Male	Female
10th day	2.66±0.26	1.5±0.07	-	1.97±0.57	2.43±0.48	1.31±0.51
20th day	13.3 ±0.05	10.1±1.41	3.20±1.05	6.01±2.03	13.1±1.56	9.50±1.31
30th day	21.6 ±2.23	17.6±1.53	5.37±1.98	10.5 ±2.91	20.5±2.41	16.5 ±2.16
40th day	30.9 ±2.56	28.3±2.17	11.8 ±2.91	18.1 ±1.64	28.9±3.13	27.4 ±2.51
50th day	41.5 ±3.09	37.4±1.41	20.5 ±2.64	26.8 ±2.54	39.5±2.78	36.1 ±2.53
60th day	50.7 ±2.73	47.7±2.01	29.0 ±2.56	35.4 ±3.57	49.3±3.57	46.5 ±3.27
70th day	59.1 ±2.37	53.0±2.52	36.5 ±3.07	44.9 ±3.09	59.5 ±3.76	53.5 ±2.90
80th day		59.6±3.58	44.8 ±2.97	51.5 ±2.96		58.9 ±3.47
90th day			50.1 ±2.65	56.8 ±2.04		
100th day			55.6 ±2.47			

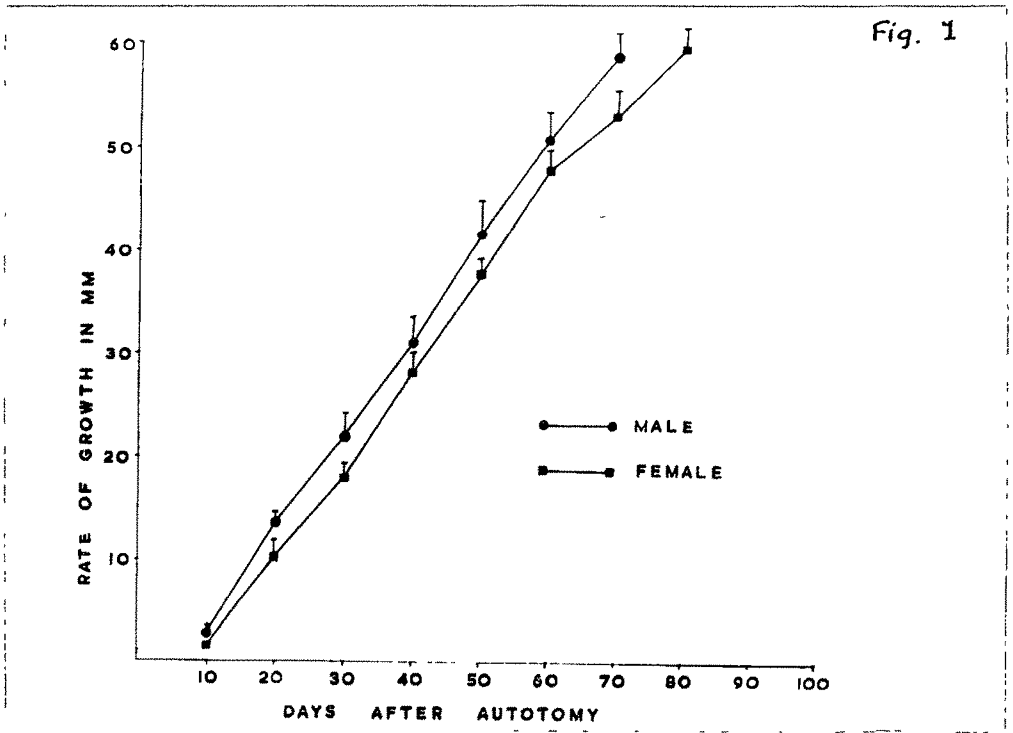
Fig. 1
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50
40
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RATE OF GROWTH IN MM
MALE
FEMALE
10 20 30 40 50 60 70 80 90 100
DAYS AFTER AUTOTOMY

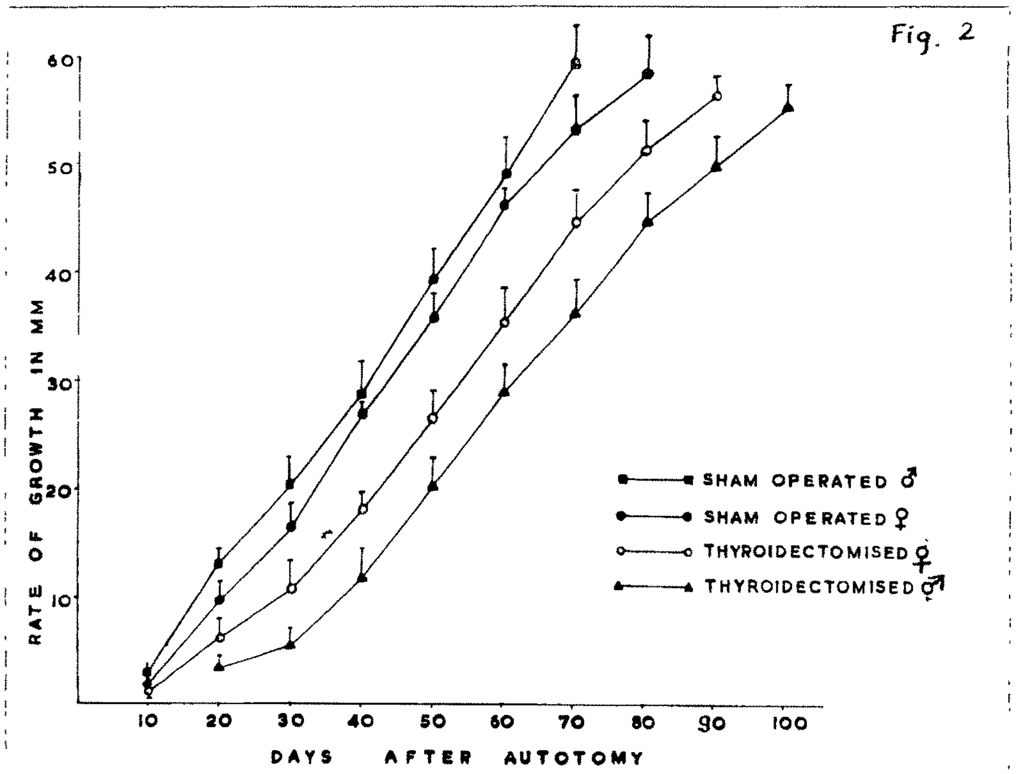
Fig. 2
60
50
40
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RATE OF GROWTH IN MM
SHAM OPERATED ♂
SHAM OPERATED ♀
THYROIDECTOMISED ♀
THYROIDECTOMISED ♂
10 20 30 40 50 60 70 80 90 100
DAYS AFTER AUTOTOMY

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

Fig. 2 : Graphic representation of the rate of growth of the regenerating tail of sham operated and thyroidectomised lizards, H. *flaviviridis*.

Table 2 : Number of days taken by regenerate to reach different stages of tail regeneration in normal and thyroidectomised lizards, *H. flaviviridis*.

stages of regeneration	Normal		Thyroidectomised		Sham operated	
	Male	Female	Male	Female	Male	Female
Wound healing	7.20 $\pm$ 1.51	9.61 $\pm$ 1.91	12.5 $\pm$ 1.91	9.71 $\pm$ 1.56	7.90 $\pm$ 1.69	9.30 $\pm$ 1.58
Blastema	12.3 $\pm$ 2.46	15.0 $\pm$ 2.98	22.7 $\pm$ 3.14	18.6 $\pm$ 2.57	13.0 $\pm$ 2.63	15.5 $\pm$ 2.84
Differen-tiation	20.7 $\pm$ 2.91	24.5 $\pm$ 1.76	37.5 $\pm$ 2.59	28.3 $\pm$ 2.31	20.5 $\pm$ 2.51	23.6 $\pm$ 2.48
Growth	25.3 $\pm$ 2.56	30.2 $\pm$ 2.98	44.9 $\pm$ 3.67	35.5 $\pm$ 3.80	26.3 $\pm$ 2.78	30.4 $\pm$ 2.67
Fully regenera-ted tail	70.5 $\pm$ 6.59	80.3 $\pm$ 5.17	98.5 $\pm$ 5.64	90.5 $\pm$ 3.47	70.6 $\pm$ 4.59	79.7 $\pm$ 6.59

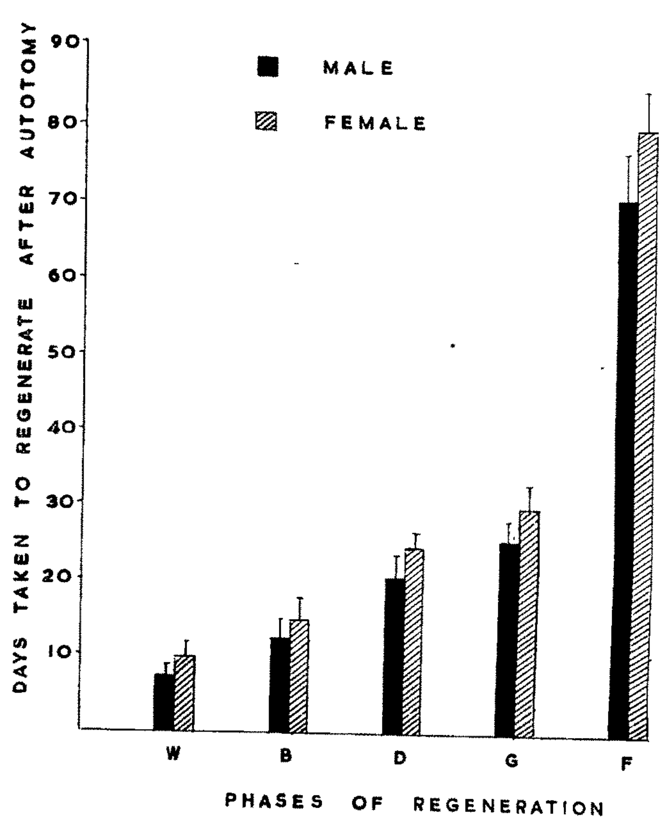


Fig. 3 : Graphic representation of time taken (in days) for attainment of various stages of tail regeneration after autotomy in normal (euthyroidic) lizards, H. flaviviridis.

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

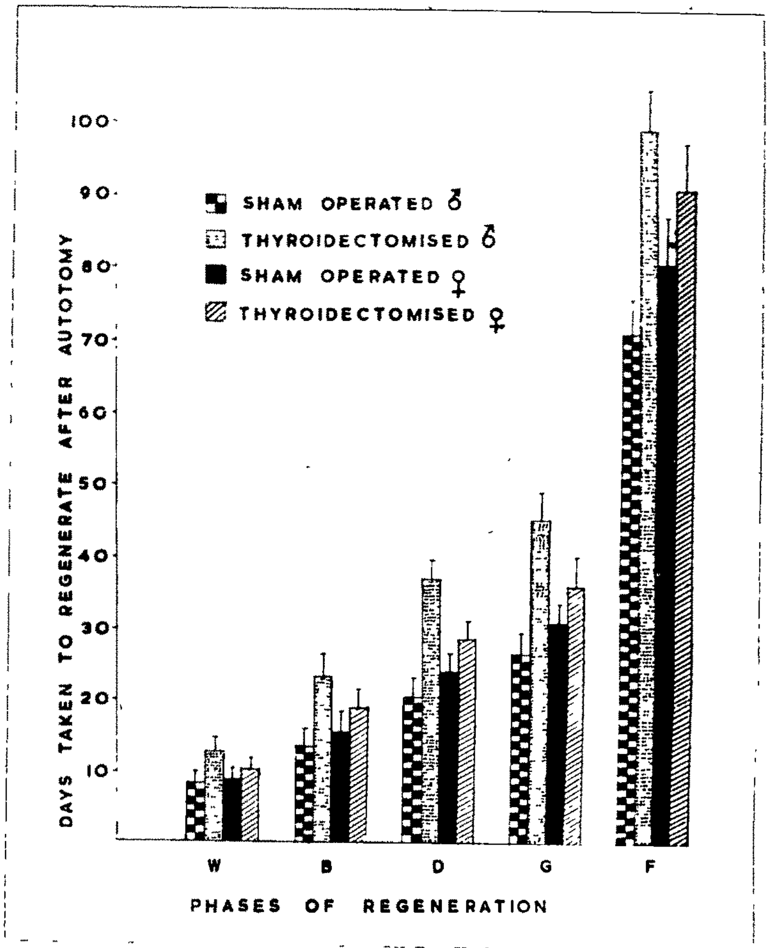


Fig . 4 : Graphic representation of time taken (in days) for attainment of various stages of tail regeneration after autotomy in sham operated and thyroidectomised lizards, H. flaviviridis.

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

delay of about 30 days and also the full length was not achieved in those males subjected to thyroidectomy. The regenerate in thyroidectomised females achieved the length of 56.8±2.04 mm by about 90 days after autotomy as compared to control female lizards where the regenerate reached the length of 58.91±3.47 mm by about 80th day post autotomy which shows that there was a delay of about 10 days in thyroidectomised female lizards. Thus from the comparative data it becomes amply clear that due to thyroidectomy, male lizards are more affected with regard to their tail regeneration as compared to females (Tables 1 & 2; Figs. 2 & 4).

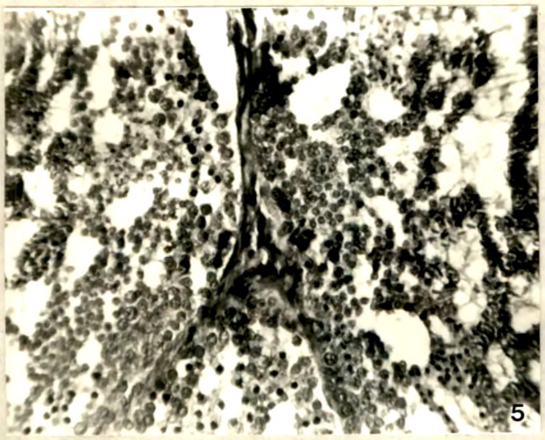
Histology of gonads in normal lizards (euthyroidic) of group I and sham operated of group III did not reveal any difference during the course of their tail regeneration.

Data for gonadosomatic index ($GSI = \frac{\text{Gonad wt. X 100}}{\text{Body wt.}}$) in both the sexes of lizards, height of the cells of sexual segment of kidney and that of epididymis in males after thyroidectomy are presented in Table 3.

Gonadosomatic index of thyroidectomised male lizards had decreased considerably compared to that observed in the controls (sham operated), while that in the

Table 3 : Data for gonadosomatic index and cell height of epididymis and sexual segment of kidney in thyroidectomised and sham operated lizards, H. *flaviviridis*.

	Male		Female	
	Thyroidectomised	Sham operated	Thyroidectomised	Sham operated
Gonadosomatic index GSI	0.76 ± 0.03	1.12 ± 0.08	0.23 ± 0.02	0.24 ± 0.01
Cell height in μ epididymis	12.1 ± 1.59	42.1 ± 3.59	-	-
Cell height in μ sexual segment of kidney	11.5 ± 1.07	36.8 ± 3.07	-	-



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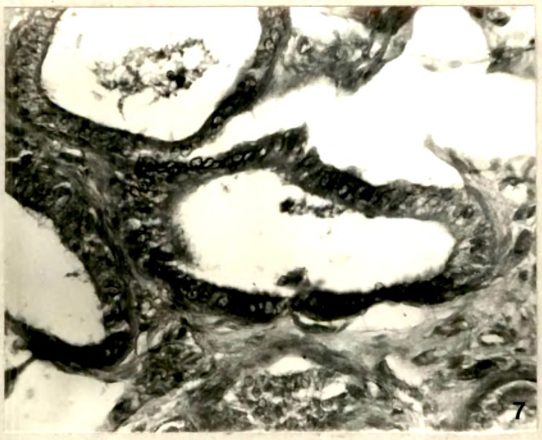

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EXPLANATION FOR FIGURES

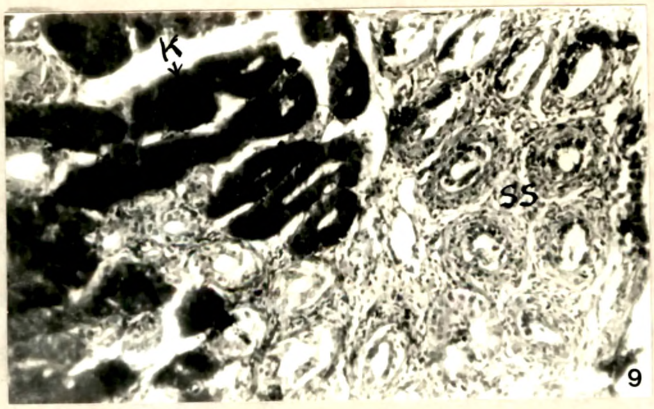
Fig. 5 : Photomicrograph showing section of testis of thyroidectomised lizard with reduced spermatogenesis.

Fig. 6 : Photomicrograph showing section of testis of sham operated lizard with active spermatogenesis.

Fig. 7 : Photomicrograph showing section of epididymis of thyroidectomised lizard. Note decrease in the size of tubules and cells lining the tubules and also absence of spermatozoa in the lumen.

Fig. 8 : Photomicrograph showing section of epididymis of sham operated lizard with spermatozoa in the lumen of tubules.

Magnification 250 X.


K
SS
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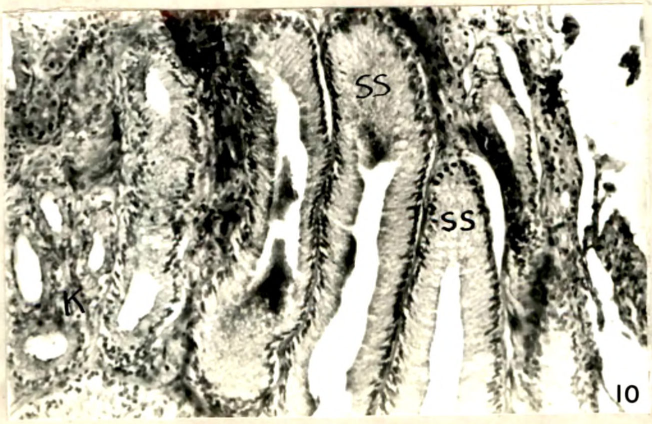
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EXPLANATION FOR FIGURES

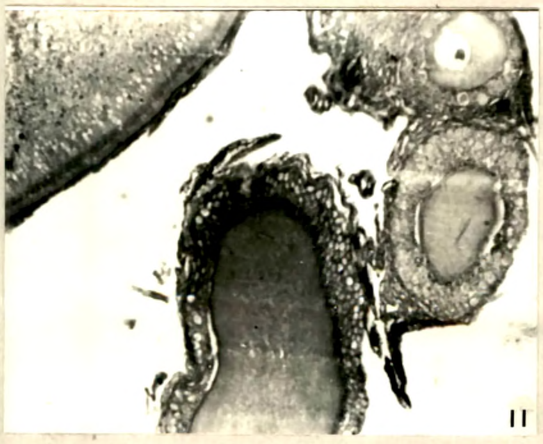
Fig. 9 : Photomicrograph showing section of sexual segment of kidney of thyroidectomised lizard. Note decrease in the size of tubules and cells lining the sex segment.

Fig. 10 : Photomicrograph showing section of sexual segment of kidney of sham operated lizard. Magnification 160 X.

ABBREVIATIONS

K - Kidney

SS - Sexual segment of kidney



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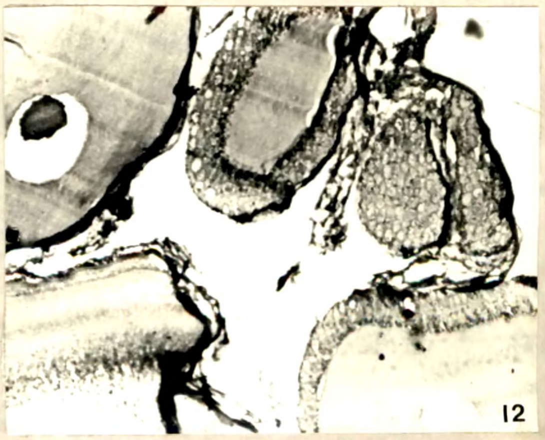

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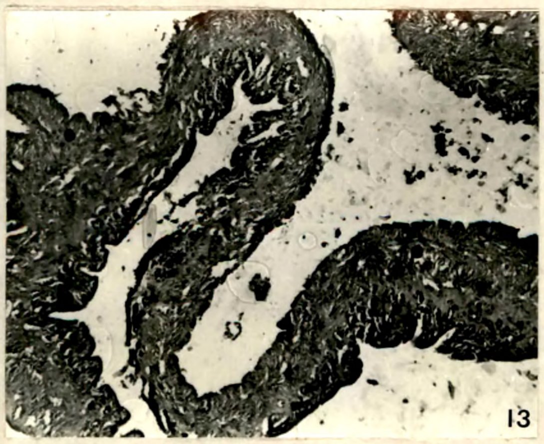

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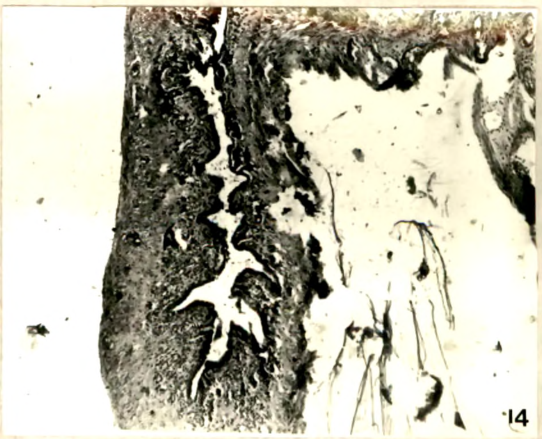

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EXPLANATION FOR FIGURES

Fig. 11 : Photomicrograph showing section of ovary of thyroidectomised lizard.

Fig. 12 : Photomicrograph showing section of ovary of sham operated lizard.

Fig. 13 : Photomicrograph showing section of oviduct of thyroidectomised lizard.

Fig. 14 : Photomicrograph showing section of oviduct of sham operated lizard.

Magnification 160 X.

thyroidectomised females it did not show any significant change. General histological examination of testis in thyroidectomised male lizards revealed degenerative changes viz., shrinkage of seminiferous tubules with reduced spermatogenic activity, and virtually no spermatozoa were seen in the lumen of the testis as well as epididymis tubules (Figs. 5 & 7). Whereas the testis of the control lizards (sham operated) revealed all the stages of spermatogenesis, besides, mature spermatozoa in the seminiferous tubules as well as in the epididymis were also observed (Figs. 6 & 8). This state indicated an active condition of the gondas. Height of the cells of sexual segment of kidney and that of the cells of epididymis in the thyroidectomised male lizards was observed to be decreased (Figs. 7 & 9) to about one third their original values compared to that in the control lizards (Table 3). Histological observations on the ovary and oviduct did not reveal any degenerative changes after thyroidectomy (Figs. 11 & 13) as compared to those in sham operated animals (Figs. 12 & 14).

DISCUSSION

The present investigations on the rate of growth of the tail regenerate in normal lizards have revealed a distinct difference between two sexes, where males regenerate their tails faster than the females. Such a difference could be attributed to greater anabolic effects of testosterone reflected in the wound healing and reparative processes of the male lizards. The results of the present study are in agreement with those of Congdon _et al_. (1974) in _Coleonyx_ lizards where it has been shown that males regenerate their tails faster than the females. According to these authors the difference is due to different selection pressure between two sexes. Presently observed slower rate of regeneration of the tail in female lizards may be an adaptive compensatory measure so as to strike a compromise between energy allocation for the restoration of the lost tail and the extra energy expenditure usually associated with reproductive functions, besides being an inherent differential response for the regeneration in two sexes. Joseph and Dyson (1965) also have reported a faster rate of ear hole regeneration in male rabbits than in the female ones.

Relatively more delay in the rate of growth of tail regenerate in thyroidectomised male lizards as compared to that in females was noticed in the present study. Observations of Hopper (1965) and Hopper and Wallace (1970) where adult male guppies fed on thiouracil failed to regenerate the anal fin, whereas there was no adverse effect of thiouracil administration on the regeneration of the fin in females, lend credulence to present findings of differential sex response to the thyroid hormone in tail regeneration of lizards. In thyroidectomised female lizards, the delay in the growth of the regenerate may be due to the effect of thyroidectomy on all tissues of the body since thyroid hormones are essential for the maintenance of all tissues (Catt, 1970; Greenberg et al., 1974). However, in the thyroidectomised male lizards the pronounced delay observed at all stages of regeneration indicates something more than a general effect of thyroidectomy on the body which could be perhaps its involvement in functional aspects of male gonads.

An assumption that a differential response of gonads to thyroidectomy exists which is reflected in differences in rate of tail regeneration, warrented histological examination of gonads and gonoducts in both the sexes of thyroidectomised lizards. Thyroidectomy resulted in degenerative changes in male gonads such as reduction in the size of seminiferous tubules and reduced spermatogenesis, while in female gonads and gonoducts no such degenerative changes were observed under similar experimental condition.

Here it may be presumed that gonadal function in male lizards is more thyroid dependent than that in the females. And the presently observed differential effect of thyroidectomy on gonads and tail regeneration in two sexes provides the basis for comparing the relative thyroid-gonad interrelationship influencing tail regeneration in H. flaviviridis.

Eyeson (1970) and Plowman and Lynn (1973) have reported reduced androgen production in thyroidectomised male lizards. Extrapolating this fact and from the observed degenerative changes in the testis of thyroidectomised male house lizards, it can be well assumed that in such animals there may be considerable reduction in androgen production. Reduced metabolic rate in absence of thyroid hormone (Maher, 1965) and testosterone (Chandola et al., 1974a) in lizards has been implicated which might be reflected in a greater delay in the rate of growth of the regenerate in male lizards. Here it may also be suggested that the metabolic reactions that underlie the process of tail regeneration are more under the influence of thyroid and/or gonadal hormones in male lizards than in the female ones.

Epididymis of the lizards which is used as a model to study the state of androgen production of testis (Dufaurg and Gigon, 1975) shows considerable shrinkage of the tubules which contained virtually no sperms after thyroid removal. Development of the sexual segment of kidney in H. flaviviridis is synchronous with the spermatogenic activity of testis (Sanyal and Prasad, 1966) and it is androgen dependent (Prasad and Sanyal, 1969). Hence, degenerative changes observed in epididymis as well as in the sexual segment of kidney could be considered due to decreased output of male gonadal hormones. Thapliyal et al. (1974) and Choubey (1974) also have reported degenerative changes in the sexual segment of kidney after thyroidectomy in the water snake Natrix piscator and garden lizard Calotes versicolor respectively.

From these facts and the data obtained in the present study, it becomes quite logical to state that in males, the pronounced delay in tail regeneration is due to thyroidectomy because of, (i) lowering of the general metabolic rate, and (ii) poor production of androgens. In females, it could be only the first factor in play viz., lowering of the general metabolic rate. Thus, it can be easily suggested that thyroid and gonadal (male) hormones effectively influence tail regeneration in H. flaviviridis.