CHAPTER 10

GONADECTOMY AND REGENERATION : ALTERATIONS IN VITAMIN C CONTENT DURING TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

It has been established that certain hormones play important roles in metabolism of ascorbic acid (AA). Synthesis of AA in liver is known to be diminished in hypophysectomised rats (Salomon and Stubbs, 1961), and administration of growth hormones to these rats has been known to increase the synthesis of AA (Salomon *et al.*, 1954). It has also been reported that the stimulation of the pituitary-adrenal axis increases the blood ascorbic acid (Allison, 1955). Corticotrophin has been reported to increase the level of ascorbic acid in liver and blood (Stewart *et al.*, 1953; Sinha and Lahiri, 1964).

Sexual influence on biosynthesis and storage of AA, and on hepatic enzyme activities involved in biosynthesis of AA have been previously reported (Stubbs and MacKernan, 1967; Stubbs *et al.*, 1967). The concentration, metabolism and synthesis of AA in liver and adrenal of male and female rats were more androgen sensitive than estrogen sensitive (Chinoy and Rao, 1979). As for the reptiles, AA can be synthesised in their kidney and liver as in the case of turtles (Grollman and Lehninger, 1957) or only in the kidney as in lacertilians (Roy and Guha, 1958). The significance of this vitamin on lacertilian regeneration is by now firmly established (Shah *et al.*, 1971; Shah *et al.*, 1976; Ramachandran *et al.*, 1975; Shah *et al.*, 1980). It is apparent that AA is involved with reproductive hormones on one hand and regeneration on the other. In the present study, an attempt is made to bridge these two lines of observations by studying the alterations in AA content of liver, kidney and regenerate of normal, sham-operated and gonadectomised groups of Gekkonid lizard, *Hemidactylus flaviviridis* during various stages of tail regeneration.

MATERIALS AND METHODS

Adult *Hemidactylus flaviviridis* collected from Baroda were kept in the laboratory for a fortnight for acclimation prior to experimentation. The animals were divided into three groups as mentioned previously (Chapter 7) and maintained on a diet of insects. The three groups of animals *i.e.* normal (C), sham-operated (SGX) and gonadectomised (GX) were autotomised and sacrifised at specific intervals post-autotomy, and the different tissues like liver, tail and kidney, taken out, blotted free of blood, weighed and homogenised in chilled mortars with 6% TCA. The AA content in the tissues was estimated according to the dinitrophenyl method of Roe (1954).

RESULTS

The caudal AA content of the intact normal lizards after autotomy showed a sudden increase on the 3rd day followed by a fall on the 10th day. Thereafter, the AA content showed an increase through 15th day to reach maximal level on the 25th day, followed by a steep fall reaching a near normal level on the 60th day. SGX lizards showed a slightly below normal level of AA prior to autotomy. Subsequent to autotomy, there was an increase by the 3rd day, which further increased by the 10th day. After a decrement between 10th and 15th days, the AA content in SGX lizards again increased to reach maximal level on the 25th day, very much comparable to the controls. Like the controls, SGX lizards also depicted a steep decrease in AA content after the 40th day to reach pre-autotomy level by the 60th day. The GX lizards showed lowest level prior to autotomy and was marked by a more or less unchanged AA content till the 5th day, but its level increased appreciably by the 7th day. With a slight decrement by on the 10th day, the Vitamin content soon increased sharply to reach the maximal

Table **I**. Levels of AA in the tail (mg/100 gm tissue) during its regeneration in the normal, sham operated and gonadectomised lizards, <u>H</u>. <u>flaviviridis</u>.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60
Normal intact (1C)	6.13 ±0.16	16.77 ±1.19	15.82 ±1.26	15.62 ±0.58	8.86 ±0.50	14.19 ±0.49	21.73 ±0.89	11.97 ±1.67	6.48 ±1.79
Sham operated (SGX)	5.31 ±0.83	12.55 ±1.49	10.38 ±0.89	12.09 ±2.47	17.89 ±3.68	11.13 ±2.92	23.93 ±2.54	16.7 ±1.71	7.23 ±0.87
Gonadecto-mised (GX)	4.73 ±0.23 0.005@	6.61 ±1.49	4.91 ±0.23	12.09 ±2.62	9.78 ±1.45	23.03 ±1.94 0.0005*	16.94 ±3.38 0.0005*	14.18 ±2.23	9.79 ±1.39 0.01*

± S. D.

* P values obtained in comparison with SGX

@ P values obtained in comparison with 1C

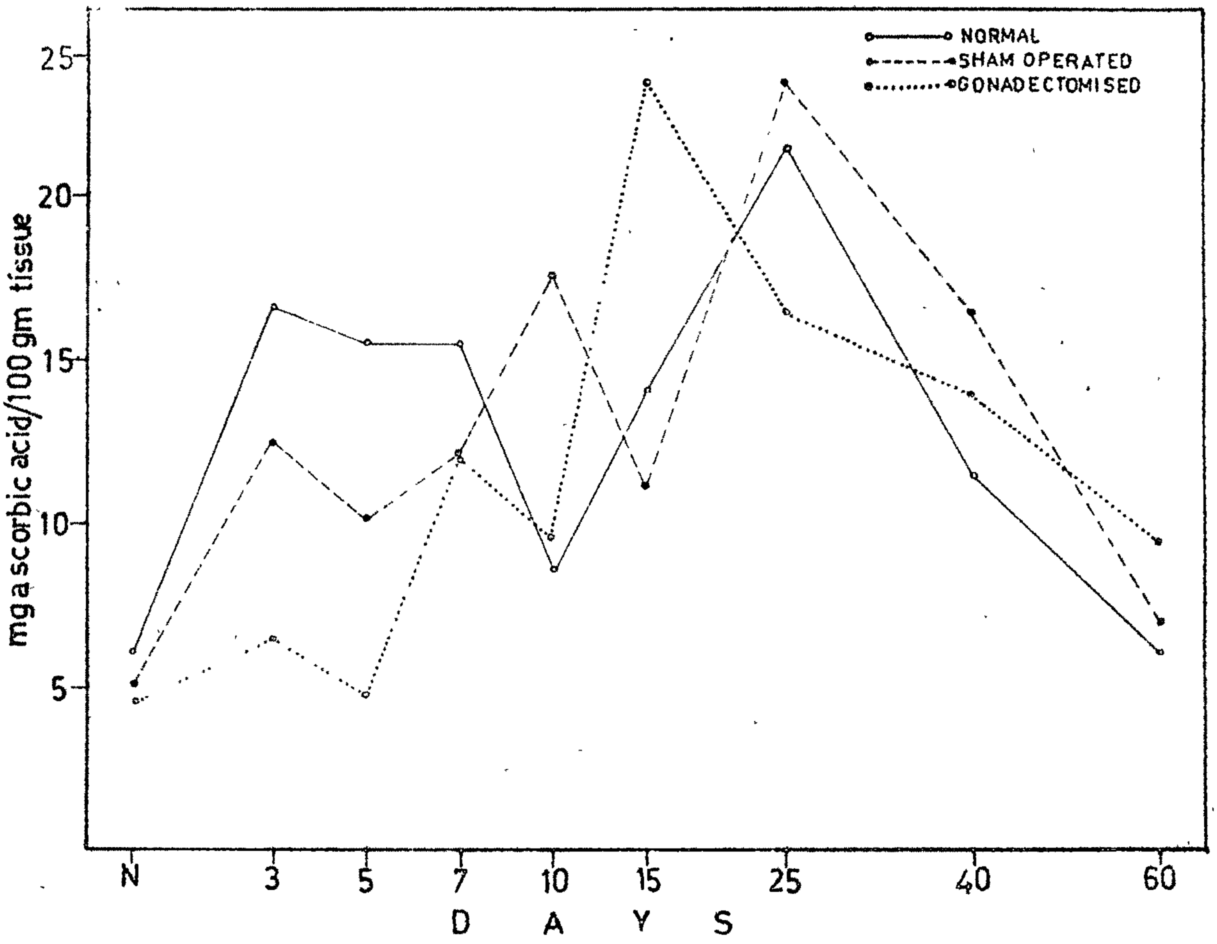

Fig. 1 : Graphic representation of the levels of caudal ascorbic acid during tail regeneration in the normal, sham operated and gonadectomised lizards, <u>H. flaviviridis</u>

Table **1.** Levels of AA (mg/100 gm fresh tissue) in the liver of normal, sham operated and gonadectomised lizards, H. flaviviridis during tail regeneration.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60
Normal intact (IC)	22.16 ±2.82	20.16 ±0.55	22.15 ±1.18	30.1 ±1.122	17.53 ±5.47	30.15 ±6.8	16.34 ±5.24	24.85 ±0.707	23.39 ±5.2
Sham operated (SGX)	20.67 ±1.21 $0.001^{@}$	23.79 ±6.7	16.04 ±0.99	18.71 ±3.8	30.14 ±1.04	17.02 ±3.6	22.21 ±5.7	25.05 ±4.04	24.89 ±3.65
Gonadectomised (GX)	22.36 ±8.9 0.001^{*}	25.25 ±2.19 0.0025^{*}	25.66 ±1.19 $0.001^{@}$	19.96 ±3.4	15.08 ±4.05	17.27 ±1.21 0.0005^{*}	36.15 ±3.7 0.0005^{*}	20.26 ±1.96	20.66 ±1.62

± S.D.

* P values were obtained in comparison with SGX

@ P values expressed in comparison with 1C

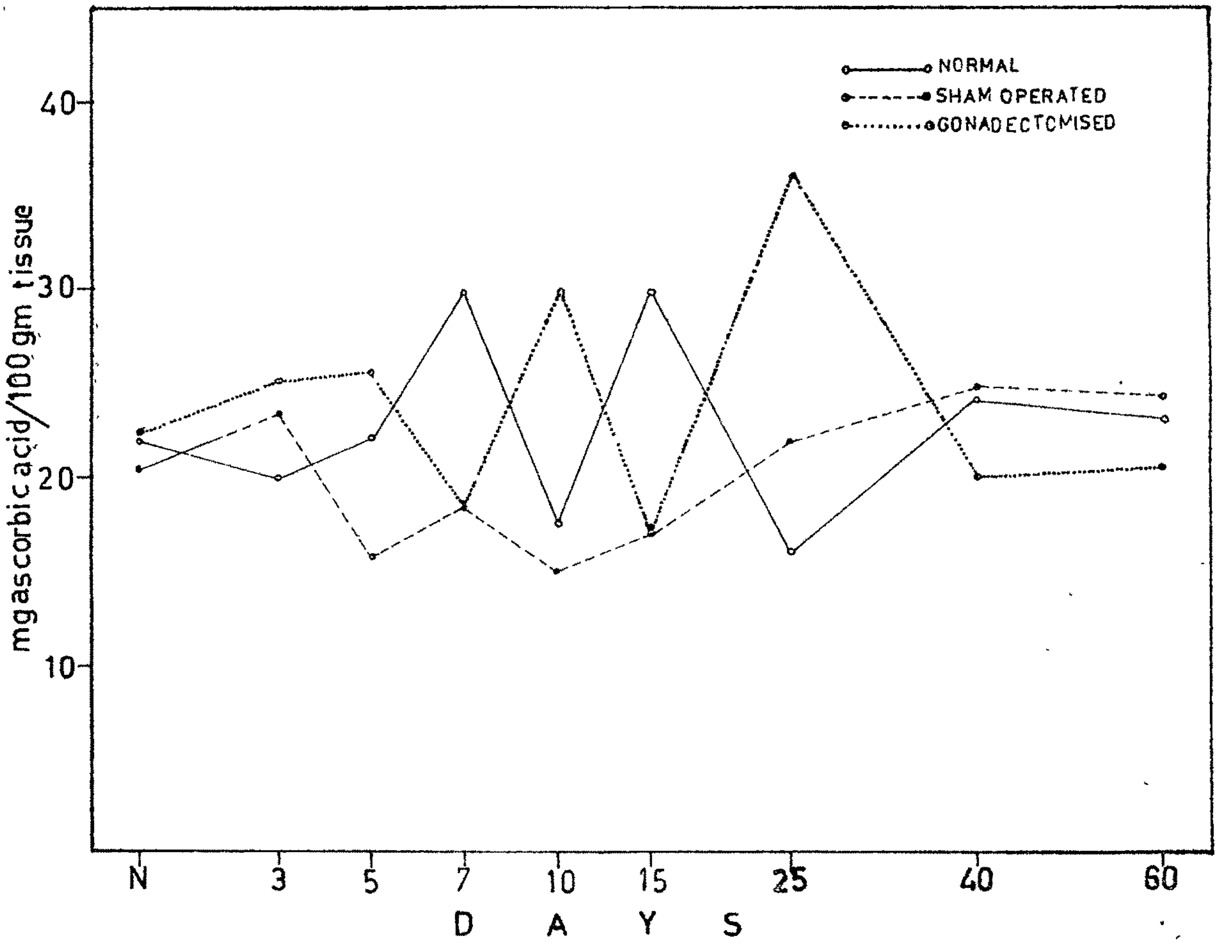

Fig. 2 : Graphic representation of the levels of hepatic ascorbic acid (AA) during tail regeneration in the normal, sham operated and gonadectomised lizards, *H. flaviviridis*.

Table 3. Levels of AA (mg/100 gm tissue) in the kidney of normal, sham operated and gonadectomised lizards, *H. flaviviridis* during tail regeneration.

Periods of regeneration in days	N	3	5	7	10	15	25	40	60
Normal intact (1C)	18.93 ±1.81	34.67 ±9.7	20.86 ±8.37	25.97 ±4.75	23.38 ±2.95	25.56 ±1.80	16.39 ±0.205	19.3 ±1.80	20.09 ±3.24
Sham operated (SGX)	33.7 ±1.57 $0.0005^{@}$	24.79 ±2.59	33.33 ±5.14	24.65 ±3.46	35.05 ±1.57	15.72 ±1.8	14.84 ±4.1	21.96 ±0.758	19.83 ±2.25
Gonadecto-mised (GX)	24.36 ±1.47 $0.0025^{@}$ 0.0005^{*}	36.26 ±0.92	34.33 ±1.28	18.3 ±4.9	11.64 ±1.72	14.16 ±0.73 0.0005^{*}	45.75 ±4.82 0.0005^{*}	34.23 ±5.98	34.28 ±1.61 0.0005^{*}

± S. D.

* P values expressed in comparison with SGX

@ P values expressed in comparison with 1C

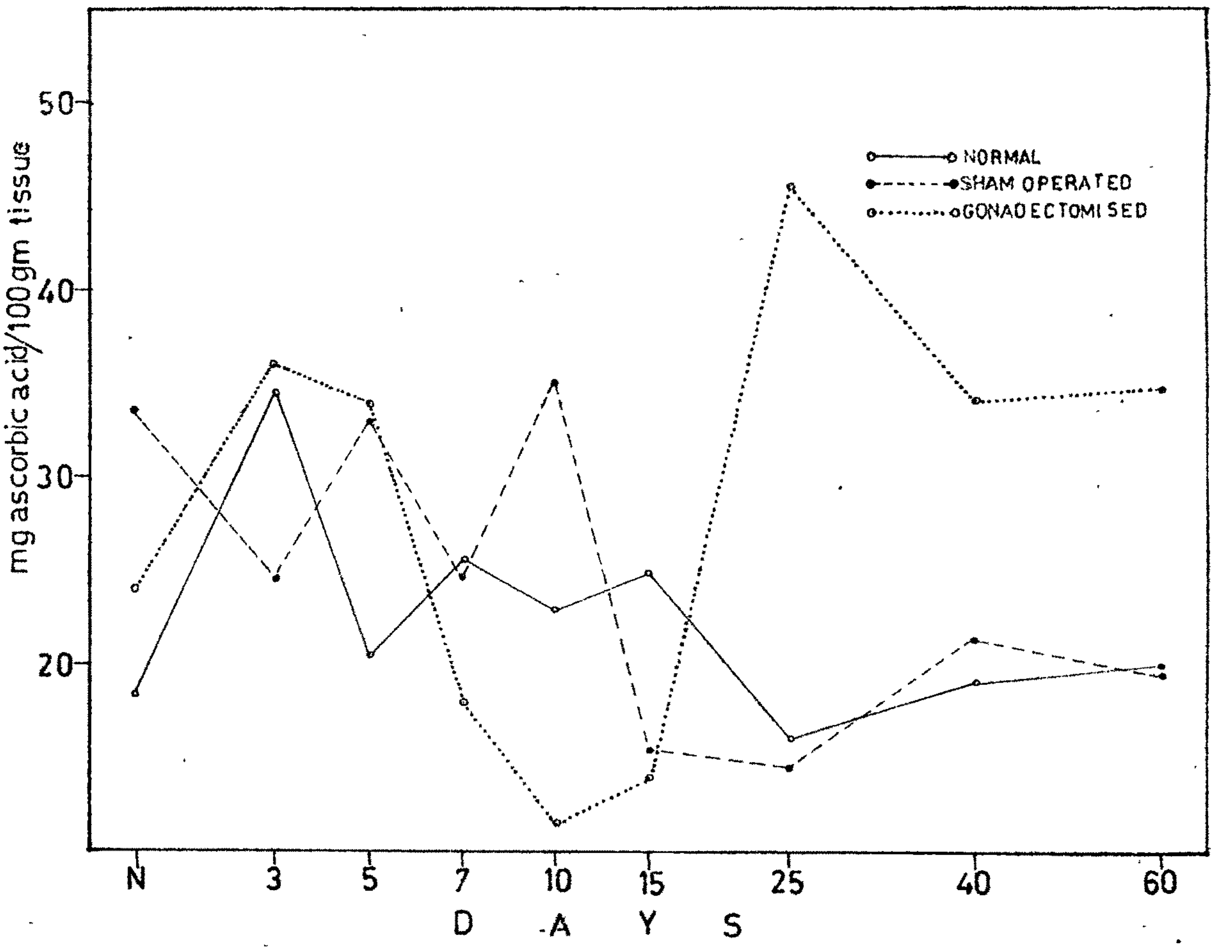

Fig. 3 : Graphic representation of the levels of renal ascorbic acid (AA) during tail regeneration in the normal, sham operated and gonadectomised lizard , *H. flaviviridis*.

level by the 15th day itself. Since then, the AA content depleted gradually towards the pre-autotomy level by the 60th day.

Hepatic AA content of the intact normal lizards post-autotomy showed a slight fall on the 3rd day followed by a gradual increase till the 7th day, whence the first peak was noted. Another peak level was reached on the 15th day with a fall in between on the 10th day. There was a decrease thereafter, and on day 60, reached to a level which was more or less like that of the normal control. The SGX and GX lizards also followed a more or less a similar pattern of changes except for the peak levels. SGX lizards showed an increase on the 3rd day followed by a fall, which brought the level to a subnormal value on the 5th day. Thereafter, there was gradual increase resulting in the only peak on the 10th day. The AA level was again decreased on the 15th day which then gradually increased and attained the normal range by day 60. AA content in GX lizards showed an increase on 3rd and 5th days, followed by a gradual fall till the 10th day. Subsequently, the level increased on the 15th day and was followed by a sudden increase on the 25th day. Between 25th and 40th days there was a steep fall in AA content of liver. The level of AA even on the 60th day post-autotomy was below the normal level.

Renal AA content of the intact normal lizards showed an increase on the 3rd day followed by a fall on the 5th day. There onwards oscillations in AA content were noted till the 15th day. A fall on the 25th day resulted in the attainment of a subnormal level which was followed by a gradual increase to acquire a more or less normal level by the 60th day. The renal AA content of the SGX lizards showed an increased level prior to autotomy. Alternate fall and rise led to two peaks, one on the 5th day, and the other on 10th day. By the 15th day, AA content considerably got depleted, and then onwards the alterations and levels were identical with those of the controls. The slightly raised pre-autotomy level of AA in GX lizards increased further on the 3rd day followed by a slight depletion on the 5th day. There onwards the renal AA content decreased resulting in a subnormal level on the 10th day followed by an increase on the 25th day whence it acquired the second and maximal level. There was a slight decrease on the 40th day which was more or less maintained so even on the 60th day resulting in an above normal AA content in the fully regenerated condition. The changes outlined above are represented in figures 1-3 and tables 1-3.

DISCUSSION

Significance of AA, its phase specific alterations and functional role in tail regeneration of lizards have all been shown by the earlier studies (Shah _et_ _al_., 1971; Shah _et_ _al_., 1976; Ramachandran _et_ _al_., 1975). In a later publication, Shah _et_ _al_. (1980) have also reviewed the participation of systemic sources of AA by studying the changes in the AA content of liver (storage organ) and kidney (synthesising organ) during the various phases of tail regeneration in the scincid lizard, _Mabuya_ _carinata_. Present study has been conducted primarily to evaluate the _in_ _loco_ and systemic changes in AA content of GX lizards during tail regeneration and assess the impact of the same on the progress of tail regeneration. As seen earlier, gonadectomy performed during the breeding period failed to have any impact on the course of the new growth, thus demonstrating the relative indifference of the process of tail regeneration in _H_. _flaviviridis_ to gonadal hormones. Similarly, local and systemic contents of AA have also failed to depict any dramatic change in this condition. In the pre-autotomic condition, gonadectomy induced a reduction in AA content of the tail which was more pronounced than that in SGX ones. A reduction in tissue AA content in the absence of gonadal hormones has been reported by Stubbs _et_ _al_. (1973) and Khandweker _et_ _al_. (1973). However, gonadectomy had no effect on the hepatic AA content, while in the case of renal AA, gonadectomy appeared to have nullifying influence on the surgery induced elevation of AA shown by SGX lizards (see table 1-3).

Subsequent to autotomy, the importance of AA in wound healing, blastema formation, differentiation and growth processes is stressed by the elevated in loco levels of the vitamin in all the three experimental groups of lizards. Evidently, neither sham operation nor gonadectomy hamphered the mobilization capacity of the regenerate. However, the delay in the immediate mobilization of AA during the first 5 days in GX lizards may have a correlation with the observed initial delay in the regenerative growth of these lizards (Chapter 7). After this initial slackness, the AA content in the regenerate of GX lizards depicted a steady increase till the attainment of the maximal level by the 15th day, unlike in intact and sham operated controls where the increment was discontinuous and the peak levels were attained only by the 25th day. The better growth rates recovered for GX lizards during the 2nd and 3rd weeks bear some relation in this context. Again, another parallelism can be drawn from the presently recorded reduced depletion of AA between 25th and 40th days and the corresponding retardation in growth rate in GX lizards.

Changes in the systemic levels of AA (kidney and liver) during the various phases of tail regeneration have registered wave like modulations in control groups as compared to the GX ones (Fig. 1 and 2). The hepatic AA content of control and SGX lizards generally tended to remain in the pre-autotomy range with fluctuations either way all throughout. Though the renal AA content also tended to show wave like modulations, there was, however, a decreasing trend with lowest level being attained on the 25th day. In contrast, the hepatic and renal AA contents of GX lizards depicted identical pattern of changes throughout regeneration with an increase during the first 5 days, decrease between 5th and 10th days and a sharp increase between 10th and 25th days with a decrease thereafter. Interestingly, the modulations were clearcut and devoid of the waviness characteristic of the intact and SGX lizards. The changes do indicate a general increase in synthesis and release of systemic AA in response to the regenerative needs. Greater fluctuations in control lizards and absence of such fluctuations in GX lizards indicate the interference of gonadal hormones and/or their interactions with the factors involved in regeneration specific systemic modulations of AA. Apparently in GX animals with the removal of the gonadal hormones the regeneration specific modulations become more specific and smooth. A very significant observation made herein is the tremendous increase in renal AA content in the GX lizards between the 15th and 25th days of tail regeneration. Quite likely, the increased adrenal activity expected to occur after the removal of the gonads may have something to do with this increment. This suggestion can be substantiated by the observed adrenal hypertrophy as well as the reported ability of adrenalin and corticotrophin in elevating AA content of blood and liver (Stewart et al., 1953; Allison, 1955; Sinha and Lahiri, 1964).

It may be safely concluded from the present observations that gonadal hormones per se have no role in synthesis, storage or mobilization of AA during tail regeneration in lizards. In fact, the absence of gonadal hormones may have a favourable influence on the regeneration induced modulations in AA content and render them more well defined; while in the presence of gonadal hormones the modulations tend to be more fluctuating and renders the pattern obscure.