CHAPTER 7

GONADECTOMY AND TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Importance of endocrine participation in the complex process of vertebrate appendage regeneration is gaining increasing attention. Most of the studies todate on endocrine regulation have been restricted to amphibian limb regeneration with lacertilian tail regeneration receiving scant attention. Licht and Howe (1969) reported gonadal activity to be one of the factors for seasonal changes in the rate of tail regeneration in *Anolis carolinensis*. In the recent past, considerable importance has been given to the sex hormones (androgens) due to their effect on oxidative metabolism in reptiles (Chandola *et al.*, 1974a, b). This aspect was thought to be a favourable influence for tail regeneration in *H. flaviviridis* (Shah *et al.*, 1979b). A delay in the formation of blastema during the non-breeding season was also taken to stress the importance of sex steroids. A thyroid- gonad axis has been suggested by Kothari *et al.* (1979) in their studies on tail regeneration in *H. flaviviridis*. Replacement of testosterone was shown to restore the retardation in rate of growth of the tail regenerate in the thyroidectomized lizards.

Taking these facts into account it would be worth studying the involvement of gonads during the process of regeneration. The most direct approach to ascertain the relative importance of sex steroids would be to perform gonadectomy and observe the influence on the course of regeneration. Accordingly, adults of male and female H. flaviviridis were gonadectomized and the effect on the rate of growth of the tail regenerate assessed.

MATERIALS AND METHODS

The experimental animals, H. flaviviridis, procured from the local animal dealer, of 10-12 grams weight were maintained on a diet of insects. The lizards were kept under laboratory conditions for a fortnight to get them acclimatized to the new environment. Gonadectomy under hypothermic anaesthesia was performed by a lateral incision on the ventral side of the abdomen. Sham operations were conducted with the same approach. Males and females were operated for the experiment to see sexual difference, in the rate of tail regeneration, if any, due to gonadectomy. After the operation, the animals were kept for 12 days for healing of the surgical wound prior to tail autotomy. Three different sets of animals were autotomized simultaneously i.e. normal animals with intact gonads (5 males and 5 females), sham

operated (SGX) (5 males and 5 females), gonadectomized lizards (GX) (6 males and 6 females) housed in separate cages. They were kept under watch and the length of the regenerate measured at constant intervals till the regenerate was fully grown. Gonadectomy was conducted during the breeding season so that a true index of the involvement of gonadal hormones, if any, could be obtained.

RESULTS

At the very outset it may be stated that there was no observable sex difference in the regenerative tail growth at any stage, and hence the rate of growth shown in the table 1 and figure 1, is the average of both sexes taken together. Apparently, there was an initial 48 hr delay in the formation of blastema in GX animals. Subsequently, till the 25th day, tail growth was faster in GX animals but between 25th and 40th days post autotomy, GX lizards showed a significantly reduced tail growth. Since then, though the rate of growth was again accelerated, the final length attained on 60th day was, however, less than the controls. Interestingly, from the 10th day of tail regeneration, even SGX lizards showed faster tail elongation than the normal controls, and was more pronounced between the 10th and 15th days. Since then, though the rate of growth was parallel to that of the

Table 1 : Rate of growth in the normal, sham operated and gonadectomised lizards, *H. flaviviridis* during tail regeneration.

Period of regeneration in days	5- 7	7- 10	10- 15	15- 25	25- 40	40- 60
Normal intact (IC)	0.04285 ±0.0017	0.0666 ±0.0032	0.12 ±0.002	0.01 ±0.0002	0.066 ±0.013	0.035 ±0.0018
Sham-operated (SGX)	0.04285 ±0.0015	0.0666 ±0.011	0.22 ±0.01	0.07 ±0.003	0.066 ±0.004	0.035 ±0.005
Gonadectomised (GX)	0.00714 ±0.0003	0.233 ±0.016	0.19 ±0.006	0.1 ±0.001	0.013 ±0.003	0.035 ±0.002

± S.D.

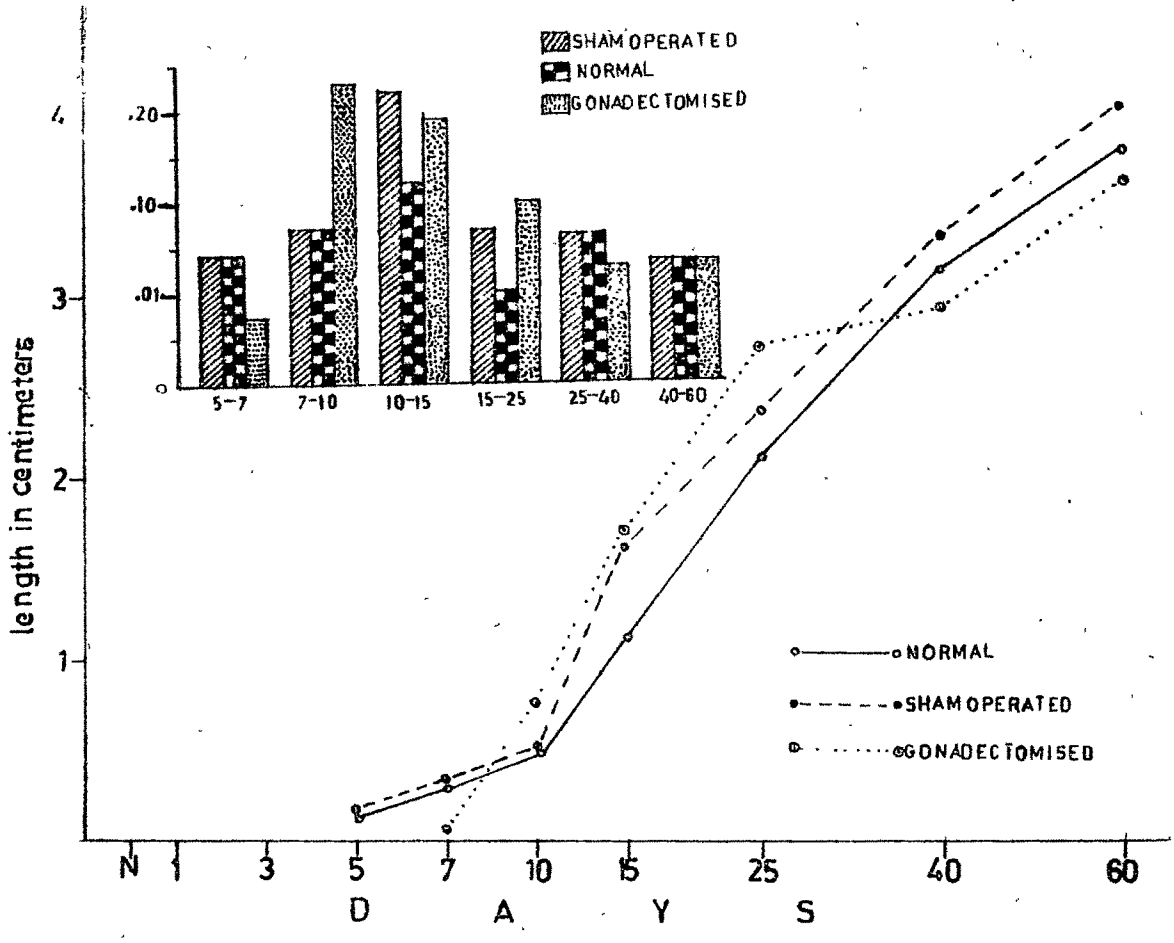

Fig. 1 : Graphic representation of the length of regenerate attained at different time periods.

Inset - Rate of growth during different time periods in the normal, sham operated and unilaterally adrenalectomised lizards,

H. flaviviridis

controls, ultimately on the 60th day, the SGX lizards had longer regenerates in comparison to both GX and normal controls.

DISCUSSION

The 48 hr delay in the formation of the regeneration blastema exhibited by GX lizards could be due to the double stress of surgery and/or absence of gonadal hormone by which the body homeostatasis could be expected to be altered. Since the sham operated animals showed no delay in the formation of blastema it is quite likely that gonadal steroids may have some favourable influence on post autotomic events, especially wound closure. Incidentally, the influence of gonadal steroids on healing process in rats have been studied by Roth et al. (1981) and have shown delayed healing in senescent females as well as in ovariectomized mature rats. Further, administration of estradiol was shown to increase the healing rate in both the above group of rats. Occurrence of a better rate of growth in GX animals till the 25th day indicates that blastemal growth and differentiation are totally independent of the gonadal principles.

Howard and Kitay (1972) have shown that gonadectomy results in an increased adrenal steroid production. In the

absence of functioning gonads, the adrenal is an important source of androgen and estrogen (Santen et al., 1980). GX lizards, in the present study, showed definite enlargement of adrenals. In the Euthyroid birds, plasma concentration of corticosterone was decreased by testosterone and increased by castration (Peczely and Daniel, 1979). Further, gonadectomy was shown to produce adrenal hyperplasia, with these abnormal adrenal cortical cells secreting biologically detectable amounts of steroids in several strains of mouse (Houseby and Dominquez, 1979). From these it could be assumed that the enlargement of adrenals noted herein, after gonadectomy, is a compensatory mechanism to produce adrenal corticosteroids. Based on these reports, increased production of corticosteroids could obviously be expected to occur in GX lizards. Some of the previous observations from this laboratory have highlighted the importance of cortisol in tail regeneration in Mabuya carinata (Ramachandran et al., 1980, 1982). Herein observed accelerated tail regeneration in GX lizards between 7th and 25th days post autotomy may have relevance in this context. Pertinently, Beaupre (1939) and Durand (1963) have shown accelerated process of tail and limb regeneration in axolotl and Triturus alpestris respectively following ovariectomy. Low titres of androgens and estrogens produced by adrenals could, however, be considered insignificant. Such a conclusion finds favourable

supports in the reports of Horah (1939), who was not able to accelerate the rate of regeneration of the tail of axolotl with gonadotropin and that of Durand (1963) who demonstrated a definite supression of limb regeneration in T. alpestris by administration of norethandrolone, a synthetic androgen. In fact, castration was shown to have stimulating influence on regeneration in the above work.

Apart from the adrenals, thyroid was also noted to undergo enlargement in GX condition. This denotes the importance of thyroid in the regenerative process. Thomas (1974) and Thapliyal and Kaur (1976) have demonstrated the stimulatory influence of thyroid hormones on erythropoiesis in reptiles, birds and mammals. Incidentally, increased erythropoietic activity during tail regeneration as well as retardation of regeneration and supressed erythropoietic activity under hypothyroid condition have both been reported in Mabuya carinata (Shah et al., 1980; Ramachandran et al., 1981). Jallageas (1978) showed that thyroxine has no influence on either circulating corticosterone level or corticosterone binding capacity of transcortin and concluded that these two endocrine glands can act independently of the state of the other. Thyroxine can act as an antagonistic hormone to testosterone (Oischi and Konishi, 1978; Jallagaes, 1978). The implication of a definite thyroid

participation in lizard tail regeneration which is inescapable from the above is further emphasised by the recent observations of Ramachandran et al. (1981) on thyroid histology during regeneration in Mabuya carinata. The better tail elongation shown by GX lizards during the first 25 days of regeneration, thus underscores the relative unimportance of gonadal hormones and probably the action of increased adrenal and thyroid output and their favourable effect on regeneration in lizards especially during blastemal and differentiation phases. In Mabuya carinata, no appreciable difference in the growth of tail regeneration could be noted during breeding and non-breeding seasons and in fact earlier phases of regeneration were seen to progress better in the non-breeding season (Ramachandran et al., 1982), once again indicating the insignificance of gonadal status in regeneration. However, the significant retardation in rate of growth noticeable between 25th and 40th days of regeneration implies the probable positive influence gonadal steroids might exert on late differentiative and growth processes. This might be relevant in the context of the known roles of androgens and estrogens in anabolism, nitrogen retention, cell multiplication, tissue growth and accelerated bone maturation (Hall et al., 1974). This is further supported by the observation of a better growth rate of the later phases of tail

regeneration in Mabuya carinata during the breeding season (Ramachandran et al., 1982).

Better rate of tail regeneration in SGX lizards in comparison to normal ones denotes the probable prior gearing up of the machinery for wound healing (a component phase of regeneration) in response to surgical stress and resultant acceleration in tail elongation subsequent to autotomy. Such a conclusion is well founded based on the previous observations on systemic responses elicited post-limb emputation (a non-regenerating system) (Chapters 1-4).

It may be concluded from the present observations and the literature cited, that gonadal steroids per se play no role in tail regeneration upto early differentiation, except for slower wound healing and that they may have some functional involvement in post differentiative growth processes. A string of recent information that could be drawn in favour again are that of (a) Bartke et al. (1973) of a decrease in plasma testosterone level by prostaglandin (b) of Amy et al. (1975); Carol (1976); Harms et al. (1973) and Ojeda et al. (1975) showing influence of prostaglandin on gonadotrophic secretion and, (c) of Steven (1981) of no influence of prostglandin on regeneration in Bufo americans.