CHAPTER IV

EFFECT OF CHEMICAL THYROIDECTOMY AND ADRENO-CORTICAL SUPPRESSION ON TAIL REGENERATION IN THE GEKKONID LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

The vertebrate body is known to respond to physical trauma with characteristic physiological changes in the region of injury as well as systemically. Involved in the latter response are the body tissues, organs, and specifically the endocrine glands. Physiologic adaptation to trauma has been investigated for the most part in mammalian species. In man, observations have been made after accidental injury or surgical operation (Selye, 1950; Moore, 1957,1959a,b; Stoner, 1960; Zimmermann, 1965). The physiological adjustments to injury are numerous and involve most if not the whole body. Epimorphic regeneration of vertebrate appendages is known to be dependent on both neurotropic factors as well as endocrine secretions (Vethamany-Globus and Liversage,1973a,b; Bantle and Tassava, 1974; Jabaily and Singer, 1977; Bast et al., 1979). 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 reptilian tail regeneration. This has provided motivation for explorations on endocrine

participation on lizard tail regeneration. Accordingly, the role of two endocrine glands, the thyroid and adrenal, has been evaluated herein as a sequel to some preliminary investigations undertaken in this laboratory on these lines. The influence of thyroid on tail regeneration of the lizard, Anolis carolinensis has been studied by Turner and Tipton (1971) and Turner (1972). Earlier studies from this laboratory had investigated the effect of thyroid gland on the process of regeneration by surgical thyroidectomy in H. flaviviridis and chemical thyroidectomy in M. carinata. In the present investigation chemical thyroidectomy was induced in H. flaviviridis to study the effect of this endocrine gland on the process of tail regeneration as surgical thyroidectomy may not annihilate extrathyroidal iodinating centres which are known to be present in lower vertebrates (Schmidt, 1958; Chavin and Bouwman, 1965; Evans et al., 1966).

The adrenal cortex has long been considered responsible for what Selye (1950) has called the "stress response"; it is stimulated by increased titres of pituitary ACTH to release large quantities of corticosteroids, during the earliest hours after injury (Cope et al., 1943; Selye, 1950; Moore, 1959a; Zimmermann, 1965). Schotte and Hall (1952) proposed that, in a manner akin to mammalian endocrinology,

the role of pituitary in urodele regeneration was probably confined to the stimulation of adrenal cortex after amputational stress. On the basis of a series of experiments, Schotte and Smith (1961) postulated that the pituitary-adrenal synergism is a major controlling factor especially in the early stages of limb regeneration in urodeles, which includes wound healing and dedifferentiation. As per a previous work done in this laboratory, wherein unilateral adrenalectomy was performed to study the effect of adrenals on the process of regeneration, it was observed that there was a delay in blastema formation in the experimental animals and the rate of regeneration was less in the initial stages. But towards the later stages, it attained near normal regenerative growth rate, which was attributed to the compensatory hypertrophy of the intact adrenal leading to normal requisite output of adrenal hormones. In this light it was deemed fit to investigate the effect of chemical suppression of the adrenals by the synthetic corticosteroid, dexamethasone (DXM) on tail regeneration.

MATERIALS AND METHODS

The lizards, H. flaviviridis, procured from the local animal dealer were maintained in the laboratory on a diet of cockroaches. The animals were kept in the laboratory for a fortnight for acclimatization prior to experimentation.

Lizards weighing 10-12 gms and having a snout-vent length of 8-10 cms were selected for the study.

A total of 120 animals were taken for the experiments on thyroid suppression and were divided into two groups of 60 each. One group served as the euthyroidic controls and the other as the hypothyroidic experimentals. The lizards of the experimental group were force fed with 0.1 ml of 0.2% 6-propyl, 2-thiouracil (PTU) (obtained from Fluka Chemicals, Switzerland; pH adjusted to 8.0-8.2), every alternate day starting 15 days prior to tail autotomy. PTU feeding was continued even after autotomy till the end of the experimentation. The control group received an identical amount of the vehicle for the same period and schedule as the experimental group. Caudal autotomy was done by pinching off the tail 2 segments distal to the vent. The length of the regenerating tail of all the lizards of the 2 groups was measured at the time intervals specified for the present study and compared.

Another lot of 120 lizards was used for the experiments on adrenal suppression. They were divided into two groups of 60 each. One group served as controls and the other was chemically adrenalectomized using the synthetic corticosteroid, dexamethasone. Injections were given intraperitoneally (15 µg/0.1 ml/day/animal) in the evening at 17.00 hrs

(as the evening schedule was found more effective) and was started 10 days prior to tail autotomy. Controls were given the same amount of distilled water intraperitoneally. The injections were continued every alternate day even after autotomy till the end of experimentation. The length of the regenerate of both the groups of animals was measured at the time intervals specified, (i.e., 3, 5, 7, 10, 15, 25, 40 and 60 days post-autotomy) and compared.

The mean and standard error were calculated and Student's 't' test was applied to evaluate statistical significance.

RESULTS

The results of the two experimental set-ups are represented in Tables 1&2 and Figures 1&2. The length of the regenerate in the experimental lizards was significantly less in relation to the length of the controls at all stages.

Thyroid suppression

The euthyroidic lizards regenerated their tail at an average rate of 0.8 mm/day, though the period between 7-10 and 15-25 days depicted maximal growth rate of

TABLE-1 : Regenerative tail elongation and rate of growth in euthyroidic and hypothyroidic *H. flaviviridis.*

	Periods of regeneration in days	3	5	7	10	15	25	40	60	Total % replacement
EUTHYROIDIC	Length of tail in mm.	-	-	2.0 ±0.3	7.0 ±0.5	11.0 ±0.6	26.0 ±0.6	38.0 ±4.0	50.0 ±5.0	65
	Per day rate of growth in blocks of days			1.0	1.6	0.8	1.5	0.8	0.6	
HYPOTHYROIDIC	Length of tail in mm.	-	-	-	1.0 ±0.4	4.0 ±0.5	13.0 ±0.7	23.0 ±3.0	28.0 ±5.0	37
	Per day rate of growth in blocks of days				0.3	0.6	0.9	0.6	0.2	

1.5 mm/day. In contrast, the PTU fed lizards regenerated their

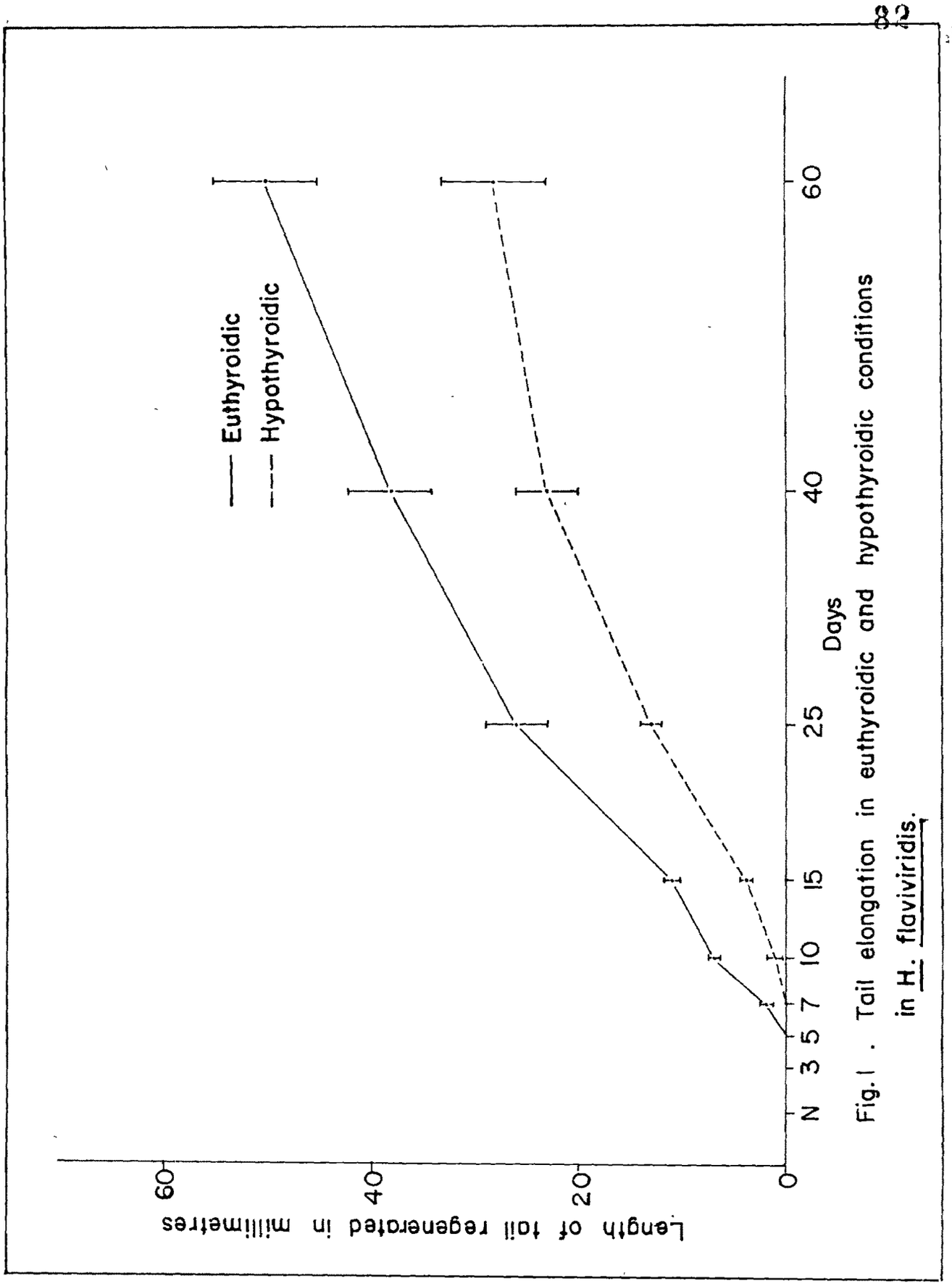

Fig. 1 . Tail elongation in euthyroidic and hypothyroidic conditions in H. flaviviridis.

TABLE-2 : Regenerative tail elongation and rate of growth in normal and adrenal suppressed H. flaviviridis.

	Periods of regeneration in days	3	5	7	10	15	25	40	60	Total % replacement
NORMAL	Length of tail in mm.	-	-	1.0 ±0.1	5.0 ±0.3	9.0 ±0.7	16.0 ±0.3	27.5 ± 2.0	34.3 ±1.0	50
	Per day rate of growth in blocks of days			0.5	1.3	0.8	0.7	0.76	0.34	
ADRENAL SUPPRESSED.	Length of tail in mm	-	-	-	1.0 ±0.1	3.0 ±0.3	9.0 ±0.3	19.0 ±0.3	24.0 ±0.6	34
	Per day rate of growth in blocks of days				0.3	0.4	0.6	0.6	0.25	

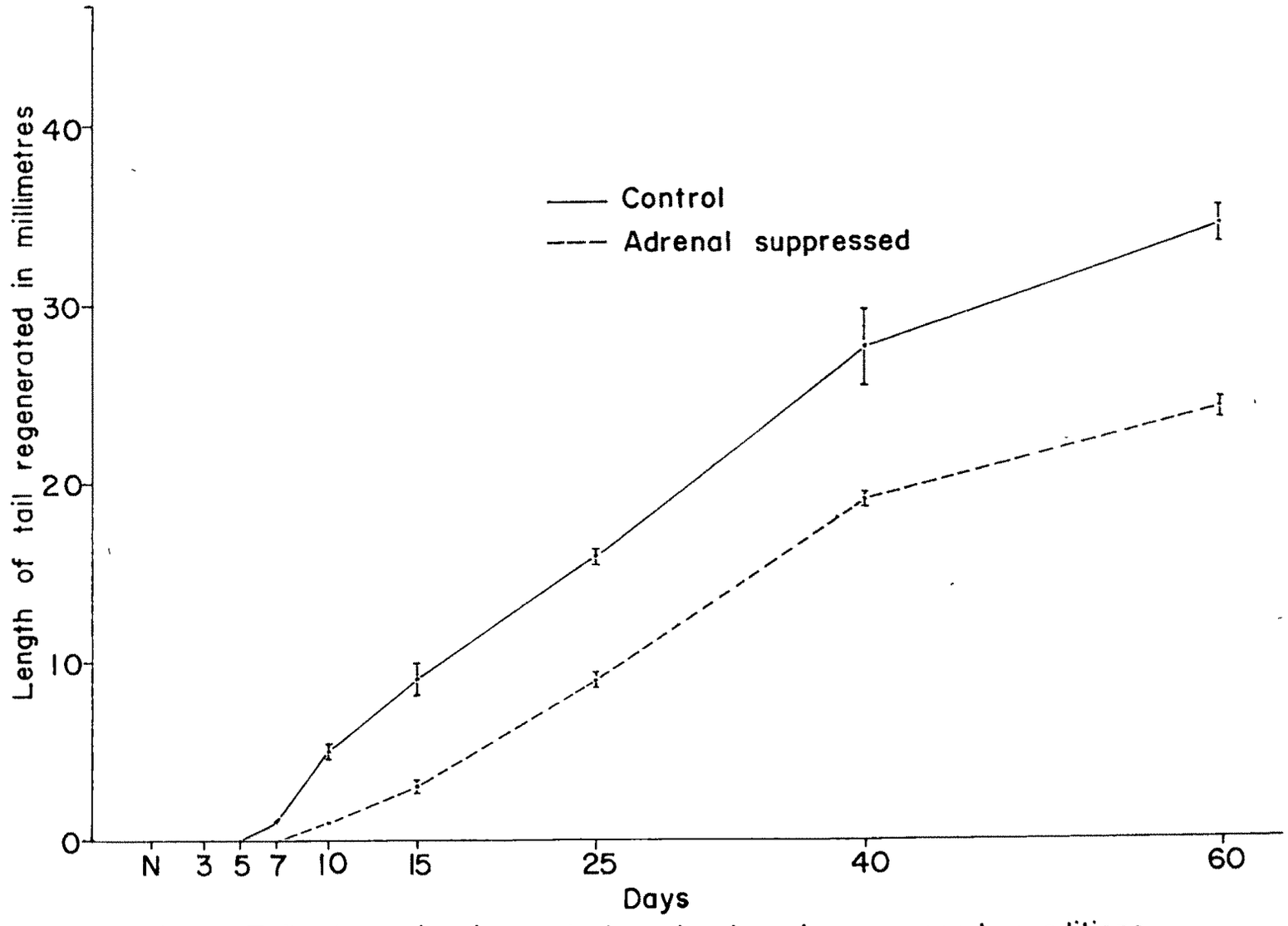

Fig. 2. Tail elongation in control and adrenal suppressed conditions in H. flaviviridis.

1.3 mm/day was shown by the controls between the 7th and 10th days, it steadied to a rate of 0.75 mm/day from 10-40 days. In contrast, the experimental lizards depicted a maximum growth rate of 0.6 mm/day between 15-40 days. In terms of percentage replacement, the control lizards replaced 50% of the cut off tail by 60 days while the DXM treated lizards replaced only 34% during the same time span.

DISCUSSION

Though there is a sizeable but confusing and contradictory literature on thyroid and regeneration in amphibians (see Schmidt, 1968; Liversage and Brandes, 1977; Liversage and Korneluk, 1978), there are only few studies of this type with respect to lizard tail regeneration, and these studies have demonstrated a positive influence of the thyroid gland on lacertilian tail regeneration (Turner and Tipton, 1971; Turner, 1972; Ramachandran et al., 1984). The present results depict a significant initial delay and ultimate retardation of tail regeneration in the PTU fed animals. The regenerative growth in the PTU fed animals was quantitatively and qualitatively poor with a shrunken and stunted, lean, linear growth.

A similar retarded growth observed in thiourea fed hypothyroidic Anolis carolinensis was attributed to an inhibition in growth of the ependyma (Turner and Tipton, 1971). The initial retardation in the present study was about 80% which however gradually got reduced to only 44% by the 60th day. In an earlier study by Kothari et al. (1979) who employed surgical thyroidectomy, had also obtained a 35% retardation by the 60th day. Apparently, lack of thyroid hormone(s) does cause a retardation in tail regeneration to the tune of about 35-45% in H. flaviviridis. However, Ramachandran et al. (1984) in their study on tail regeneration in the Scincid lizard, M. carinata had demonstrated an ultimate 70% retardation by the 60th day with an identical initial delay of 85%. In the same study, they also demonstrated the ability of T_4 to completely nullify the PTU induced retardation in regenerative growth. In this context, it is presumable that there is a difference in the degree of dependence of lacertilian tail regeneration on thyroid hormones. The Scincid lizard, Mabuya is more dependent on the thyroid hormones while the Gekkonid lizard, Hemidactylus is less dependent. Apparently, tail regeneration in Hemidactylus can occur even in the background of a residual thyroxine level though there is a definite retardation in tail elongation. Though it is safe to presume that local events such as dedifferentiation, proliferation and differentiation which underline the process of regeneration

could be programmed within the framework of a normal hormonal balance, a quantitatively and qualitatively good regenerate can be produced only by adaptive systemic support. Accordingly, a bimodal influence of thyroxine on lacertilian tail regeneration was suggested by Ramachandran et al. (1984). In this context, the requirement for major or minor alterations in prevailing endocrine balance may depend on the lability of regeneration specific systemic modulations which might vary in different species of animals. On a comparative basis, Hemidactylus with only 40% retardation in regenerative tail elongation under hypothyroidic conditions as opposed to Mabuya with 70% retardation under similar conditions might be construed to have a more labile mechanism of systemic modulation, requiring quantitatively lesser output from the thyroid.

The second experimental set-up involving adrenocortical suppression has also revealed a retardation in the regenerative outgrowth. However, as compared to thyroid suppression, retardation induced by adrenal suppression was only 30%, though the initial delay as on the 10th day post-autotomy was 80%. With the progression of regeneration, the retardation got gradually minimised. The significant initial delay observed in the present study, finds relevance from the report of Schotte and Wilber (1958) of faster wound repair under induced limb regeneration in adult frogs

implanted with adrenal glands. Though the initial retardation in lean linear growth got minimised during the course of regeneration, the regenerate in the DXM treated lizards was nevertheless quantitatively and qualitatively poor with a shrunken and stunted appearance as in the hypothyroidic lizards. From the present observations, it may be concluded that while adrenocortical hormones *per se* may have little effect on the *in loco* mechanisms associated with regeneration, they may nevertheless exert permissive influences on the adaptive systemic metabolic modulations characteristic of Saurian regeneration (Chapter-8-13).

SUMMARY

Effects of induced hypothyroidism and adrenal insufficiency on the process of tail regeneration in *Hemidactylus flaviviridis* have been investigated. An overall retardation in regeneration has been noticed under both conditions. The hypothyroidic lizards started off with an initial 80% retardation which got reduced to about 40% by the end of regeneration. The adrenal insufficient lizards too started off with an initial delay of 85% which got minimised to only 30% by the end. The formation of blastema showed a delay of 3 days under hypothyroidism while it was 2 days under adrenocortical suppression.

The results indicate that the thyroid and adrenal hormones *per se* have insignificant effect on *in loco* mechanisms while they may exert permissive influence on regeneration specific systemic metabolic modulations.