CHAPTER V

SERUM T_3 AND T_4 LEVELS DURING TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

It is generally recognised that the thyroid hormone(s) substantially influences the basal metabolism of vertebrates (see Gorbman, 1963). Hyperthyroidism stimulates the body's metabolic rate, whereas hypothyroidism depresses it. The importance of the thyroid gland in amphibian metamorphosis was established by Gudernatsch (1912, 1914). The role of thyroid gland in amphibian regeneration has intrigued many investigators. Richardson (1940), Schotte and Washburn (1954), and Liversage and Brandes (1977) have shown that following concomitant thyroidectomy and forelimb amputation in the adult newt *Notophthalmus viridescens*, regeneration is impeded and abnormal development of skeletal elements ensues, or in some cases, stumping occurs. Korneluk and Liversage (1978) found a significant increase in the localization of labelled thyroid hormone in the regenerating forelimb of adult male newts following the injection of radioactive thyroxine. On the basis of their findings, the possibility existed that the serum thyroxine (T_4) and/or triiodothyronine (T_3) levels might also fluctuate in response to the demands of the regenerat-

ing area. Liversage and Korneluk (1978) have studied serum thyroxine (T_4) and triiodothyronine (T_3) levels in adult newts during forelimb regeneration. In the lizard, Anolis carolinensis, thyroid is attributed to play some role in regulating ependymal growth into the young regenerate (Turner and Tipton, 1971). Previous studies from this laboratory on Hemidactylus and Mabuya (Kothari et al., 1979; Ramachandran et al., 1984) as well as the current study (Chapter 4) have all highlighted the importance of the thyroid hormone in lacertilian tail regeneration. Though the influence of hormones in general on appendage regeneration in vertebrates has been demonstrated by many experimental evaluations, an actual measurement of circulating levels of hormones during regeneration has not been done barring the attempt of Liversage and Korneluk (1978) on T_4 and T_3 in Notophthalmus viridescens. Todate, there is not even a single attempt on measuring the hormonal profile during regeneration in Saurians. Hence an attempt is made in the present study to measure the circulating levels of T_4 and T_3 during tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis.

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 acclimatisation prior to experimentation. Lizards weighing 10-12 gms and having a snout-vent length of 8-10 cms were taken for the study and tail autotomy was done by pinching off the tail two segments distal to the vent. A total of 162 lizards were used for the study. The animals with regenerating tail were sacrificed at fixed intervals of 3, 5, 7, 10, 15, 25, 40 and 60 days post-autotomy along with the normal animals with intact tail. The serum of six lizards were pooled at every time interval specified for each set of estimations. RIA was performed to measure the circulating levels of T_3 and T_4 in the serum, which was cold-centrifuged at 6000 rpm and was assayed using the RIA kit provided by Radiopharmaceuticals division, Bhabha Atomic Research Centre as per their manual (RIAK 4, 4A and RIAK 5, 5A).

RESULTS

The levels of T_3 and T_4 at different time intervals during regeneration are represented in Figure 1 and Table 1.

Definite phase-specific variations in the levels of T_3 and T_4 have been obtained. In general, circulating T_3 level was lesser than that of T_4. at any time, with the T_4 level being ten times higher than that of T_3. During regeneration, whereas T_4 level tended to remain subnormal, the T_3 level remained above-normal. The only period during which T_4 level increased was on the 3rd and 7th days post-autotomy with a drop to a normal level in between on the 5th day. From the maximal level on the 7th day it came down to a subnormal level on the 10th day which was further decreased to a minimal subnormal level by the 15th day. Thereafter, through days 25 and 40 (though the level was still subnormal) the hormone level gradually increased to reach the normal range on the 60th day. On the other hand, T_3 showed a decrease on the 3rd day followed by a swift increase to above-normal level on the 5th day which further increased on the 7th day. The T_3 level reached a maximal level on the 25th day and then decreased gradually through 40th and 60th days, Eventhough the T_3 level tended to decrease between 40th and 60th days, the levels at both these periods were still distinctly above-normal.

TABLE-1 : Levels of serum T_3 and T_4 during tail regeneration in H. flaviviridis.

Periods of regeneration in days	T_3 (µg/100 ml of serum)	T_4 (µg/100 ml of serum)
Zero day	0.238	1.955
3rd day	0.157	2.433
5th day	0.303	1.805
7th day	0.413	2.515
10th day	0.315	1.675
15th day	0.309	1.577
25th day	0.667	1.625
40th day	0.382	1.725
60th day	0.326	2.025

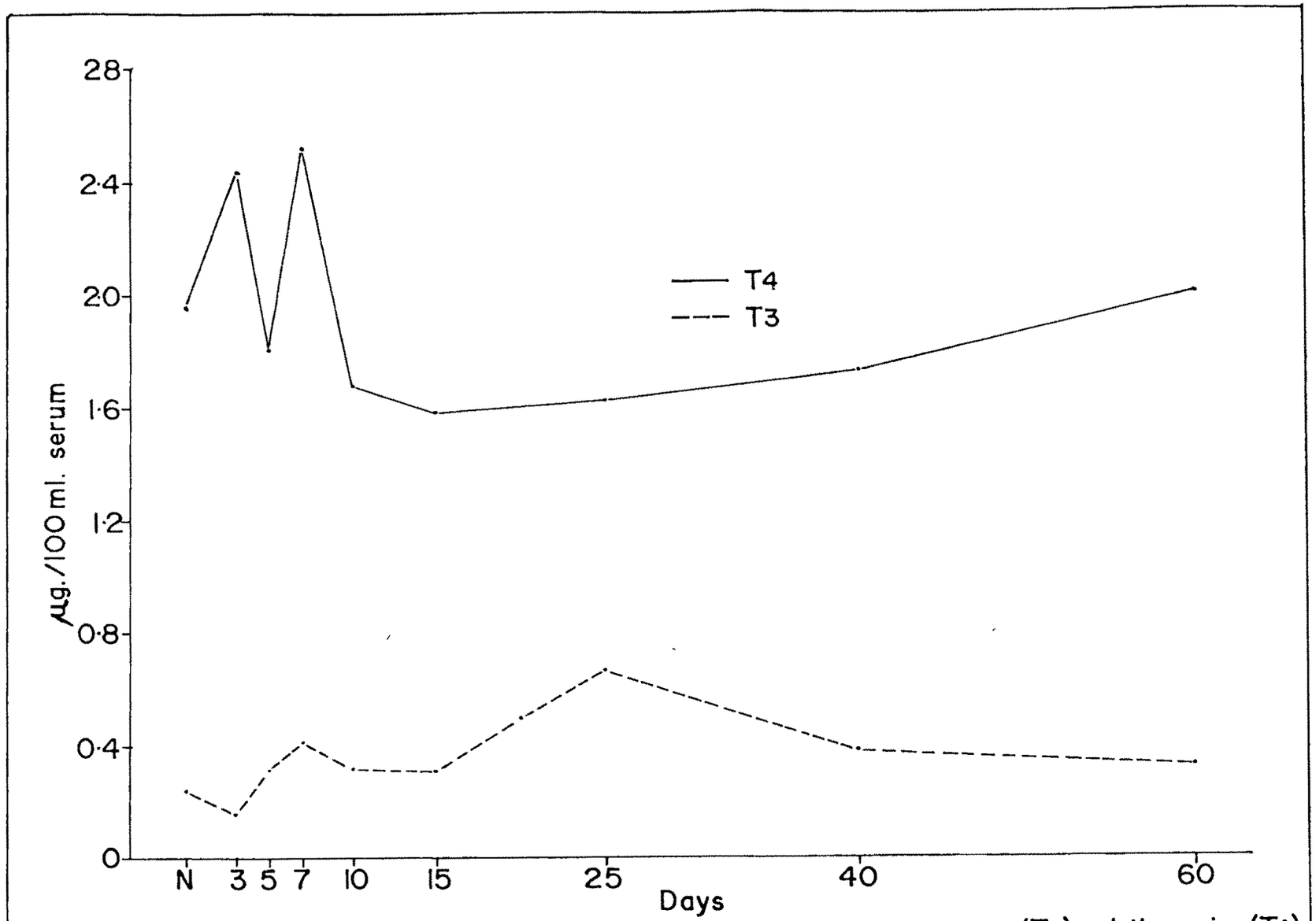

Fig. 1 . Changes in the circulating levels of triiodothyronine(T3)and thyroxine(T4) during regeneration in H. flaviviridis.

DISCUSSION

Present evaluation of serum T_3 and T_4 profile during tail regeneration in H. flaviviridis has shown definite alterations sufficient enough to confirm the involvement of the thyroid gland in lizard tail regeneration. Previous studies from this laboratory as well as elsewhere had implicated the thyroid hormone in the process of regeneration in reptiles (Turner and Tipton, 1971; Turner, 1972; Kothari et al., 1979; Shah et al., 1979b; 1982c; Swamy et al., 1982a, 1983). From the results obtained herein, a biphasic thyroid output could be presumed to occur; an initial one during the first week post-autotomy (as denoted by the increased T_4 level on the 3rd and 7th days and of T_3 on the 7th day) and a later one by about the 3rd week of regeneration (as denoted by the elevated T_3 level on the 25th day). Infact, such a biphasic thyroxine release was inferred by Ramachandran et al. (1981a) based on their preliminary histological observations on the thyroid during tail regeneration in Mabuya carinata. The decreased T_3 titre coupled with the increased T_4 level on the 3rd day suggests the simultaneous action of the autotomy induced stress at the level of peripheral utilisation of T_3 as well as at the level of T_4 secretion from the thyroid. The release of T_4 in the immediate post-autotomy period appears to occur in two

spurts, an initial one during the first three days and a later one between the 5th and 7th days. The latter spurt also seems to be coupled with an increased T_3 release. Obviously, the thyroid hormones can be purported to play important roles both *in loco* as well as systemically during the first week of regeneration. This phase of regeneration marked by wound healing, dedifferentiation and formation of a regeneration blastema might call for both specific local actions as well as supportive systemic modulations which could be under the purview of thyroid hormones. Pertinent to quote in this respect is the report of inhibition of ependymal outgrowth into the blastema of hypothyroidic *A. carolinensis* (Turner and Tipton, 1971) and of inhibition of regeneration specific haematologic adjustments and metabolic alterations in hypothyroidic *M. carinata* (Shah *et al.*, 1982c; Swamy *et al.*, 1982a, 1983; Ramachandran *et al.*, unpublished). It is surmisable that the conglomerate of *in loco* and systemic events characteristic of the immediate post-autotomy phase (first week) which ensures the setting up of requisite conducive state for regeneration, is mediated at least in part by T_4.

The present observations also indicate increased serum T_3 levels during the progressive phase of regeneration. The peak level of T_3 noted to occur by about the

25th day is well corroborated by the histological observations of secretory exhaustion of the thyroid gland at the same period in M. carinata (Ramachandran et al., 1981a). This phase of regeneration is marked by peak differentiative activities in the regenerate and thus the involvement of T_3 in this respect can be presumed especially in the wake of reports of thyroxine in such activities (Siegel and Tabias, 1966; Greenberg et al., 1974). It is known that T_3 is three to five times more potent biologically and T_4 has more affinity for the thyroid hormone receptor protein in the nucleus (Grodsky, 1981). Hence it appears that during the progressive phase of regeneration, T_3 is the preferred form of secretion from the thyroid. In the light of the previously reported alterations in systemic carbohydrate metabolism (Shah et al., 1977a,b and 1982a,c; Menon et al., 1981), the increased T_3 level may also find added correlation. The earlier reported bimodal influence of thyroid hormone(s) in lizard tail regeneration (Ramachandran et al., 1984) in this context gains further credibility.

It is worth mentioning here that a similar study on evaluation of serum T_3 and T_4 levels during limb regeneration in the newt, Notophthalmus viridescens by Liversage and Korneluk (1978) did not reveal any

significant alterations. This had led them to suggest that stimulation of endocrine activity bringing about a hormonal imbalance is not necessary for appendage regeneration and that regeneration can proceed in the background of a normal hormonal balance. This would suggest a differential mechanism of regulation of regeneration to be operative in amphibians and reptiles vis-a-vis hormonal involvement. It is quite likely that the urodeleans with a greater potential for regeneration may have a more labile metabolic set up which could be easily modulated by the mere stress of autotomy/amputation and requiring only transient and minor increases in hormonal output as opposed to lacertilians with reduced regenerative potential and less labile set up thus requiring a stronger hormonal stimulation.

SUMMARY

Circulating levels of serum T_3 and T_4 have been assayed by RIA method during tail regeneration in the Gekkonid lizard, Hemidactylus flaviviridis. In general, the serum T_3 level is lower than that of T_4 and both the hormones did depict phase-specific alterations. Whereas the immediate post-autotomy periods (first week) were marked by elevated T_4 level, the late phases of regeneration corresponding to peak histodifferentiation (15-40 days) were marked by elevated T_3 level. These changes

in serum T_3 and T_4 are taken to indicate the participation of the thyroid gland in lizard tail regeneration and are discussed in relation to the various <u>in loco</u> events and systemic, metabolic and haematologic modulations characteristic of lizard tail regeneration.