CHAPTER XII

RENAL AND HEPATIC ASCORBIC ACID CONTENTS DURING TAIL REGENERATION IN THE SCINCID LIZARD, *MABUYA CARINATA* UNDER EUTHYROIDIC, HYPOTHYROIDIC AND T4 REPLACED CONDITIONS

Ascorbic acid has been implicated in multiple biological activities (Banerjee *et al.*, 1959; Chinoy, 1969, 1972; Nickels *et al.*, 1973; Lewin, 1974, 1976; Meiklejohn, 1953). Besides its roles in metabolic and other molecular events of adult organisms, this vitamin is also known to influence the process of development (Barnett and Bourne, 1942; Mazur *et al.*, 1961; Gould, 1963; Barnes and Kodicek, 1972). Previous studies of Shah *et al.* (1971) and Ramachandran *et al.* (1975) have demonstrated the functional association of ascorbic acid with lacertilian tail regeneration. Later, Shah *et al.* (1980 c) studied the ascorbic acid content of liver and kidney of *Mabuya carinata* and thereby showed the adaptational alterations in both these organs in response to caudal regeneration. Though ample literature on biological actions of ascorbic acid in animal systems are available, apart from a few reports (Stubbs and McKernan, 1967; Stubbs *et al.*, 1967; DeNicola *et al.*, 1968; Dieter, 1969; Biswas, 1969; Ishii *et al.*, 1970; Padhi and Patnaik, 1979) endocrine

regulation of synthesis, storage and release of this vitamin has received scant attention. Since ascorbic acid is shown to be intimately associated with lizard tail regeneration, and as hypothyroidic inhibition of tail regeneration in Mabuya carinata is being evaluated in terms of many physiological parameters, assessment of alterations in hepatic and renal ascorbic acid contents under euthyroidic, hypothyroidic and thyroxine replaced conditions was deemed fit. Such a study, apart from providing information on the functional relation between thyroid, ascorbic acid and tail regeneration may also be useful in understanding the possible role, if any, of thyroid hormone in ascorbic acid turnover, release etc.

MATERIALS AND METHODS

Healthy Mabuyas of both sexes obtained from Hyderabad, India and allowed to get acclimated to the laboratory conditions were kept on insect diet. Animals were subjected to chemical thyroidectomy and thyroxine replacement therapy as described in Chapter I. Tails were autotomized by pinching off at about 1.5-2.0 cms distal to the vent. Animals were then sacrificed under mild anaesthesia at fixed intervals of 3, 5, 7, 10, 12, 15, 25, 40 and 60 days post-autotomy.

Liver and kidney were removed quickly and quantitative estimation of ascorbic acid content was done following the

method of Roe (1954). The values are expressed as mg ascorbic acid/100 gms of tissue weight.

For each day and each tissue specified a total of five to seven estimations were made. The mean and standard deviation were obtained and students' 't' test was used to determine statistical significance.

RESULTS

Comparative data recorded in Figs. 1-3 and Table 1 denote very little change in the contents of hepatic and renal ascorbic acid in the three groups of animals in the pre-autotomic condition. Marginal but nonsignificant increase in the liver and decrease in the kidney were apparent in the PTU induced hypothyroidic group. Marked feature of tail regeneration was an initial depletion of hepatic ascorbic acid content during the 3rd to 5th days post-autotomy and gradual rise back to above normal level by 10th day in group A. There appeared to be a slight fall between 12th and 15th day. Since then hepatic ascorbic acid content showed an increasing trend and by the 60th day the ascorbic acid content was about 33% above normal. In contrast the renal ascorbic acid content depicted a continuous increase right from the time of autotomy till a maximal level of 17.65 mg/100 gms, was attained by 7th day post-autotomy. Thereafter, renal ascorbic acid content showed a

Table 1. Quantitative levels of Ascorbic acid in Liver and Kidney during tail regeneration under euthyroidic, hypothyroidic and T4 replaced conditions in M. carinata.

(Values are expressed as mg ascorbic acid/100 gms of tissue)

Periods of tail regeneration in days	LIVER			KIDNEY		
	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)
N	10.24 ±0.66	11.59 ±0.89	11.43 ±0.99	9.01 ±0.84	8.27 ±0.38	10.01 ±0.01
3	10.61 ±1.63	13.87 ±1.93	12.89 ±1.87	10.91 ±1.29	11.91@ ±1.88	10.37 ±1.72
5	7.95 ±2.04	12.99 ±1.31	14.77@ ±0.62@	12.74@ ±1.64	10.78* ±1.25	11.61 ±2.08
7	9.14 ±1.61	11.54 ±1.23	12.46 ±1.11	17.65*@ ±2.34@	7.95 ±0.91	12.45 ±2.88
10	13.22 ±2.35	11.49 ±1.35	10.62 ±1.13	14.81@ ±2.16	9.68 ±1.31	12.25 ±2.02
12	13.19 ±1.83	20.01*@ ±1.69@	16.26*@ ±2.66@	12.02 ±2.71	15.34*@ ±2.94@	14.51*@ ±1.84@
15	11.61 ±1.63	12.34 ±1.73	15.88*@ ±2.01@	9.71 ±2.11	13.08*@ ±1.94@	15.58*@ ±1.38@
25	11.81 ±1.35	19.71*@ ±1.32@	11.04 ±1.74	8.61 ±1.58	15.07*@ ±0.91@	9.78 ±2.38
40	12.85* ±1.94	12.56 ±2.39	13.21 ±2.17	7.58 ±2.16	9.38 ±2.45	8.45 ±1.26
60	13.58@ ±2.46	10.06 ±1.81	12.51 ±1.41	8.81 ±1.64	7.93 ±1.19	10.95 ±1.45

* $P < 0.01$; *@ $P < 0.005$; @ $P < 0.0025$; @@ $P < 0.001$; *@@ $P < 0.0005$

N - Normal (Pre-autotomy state)

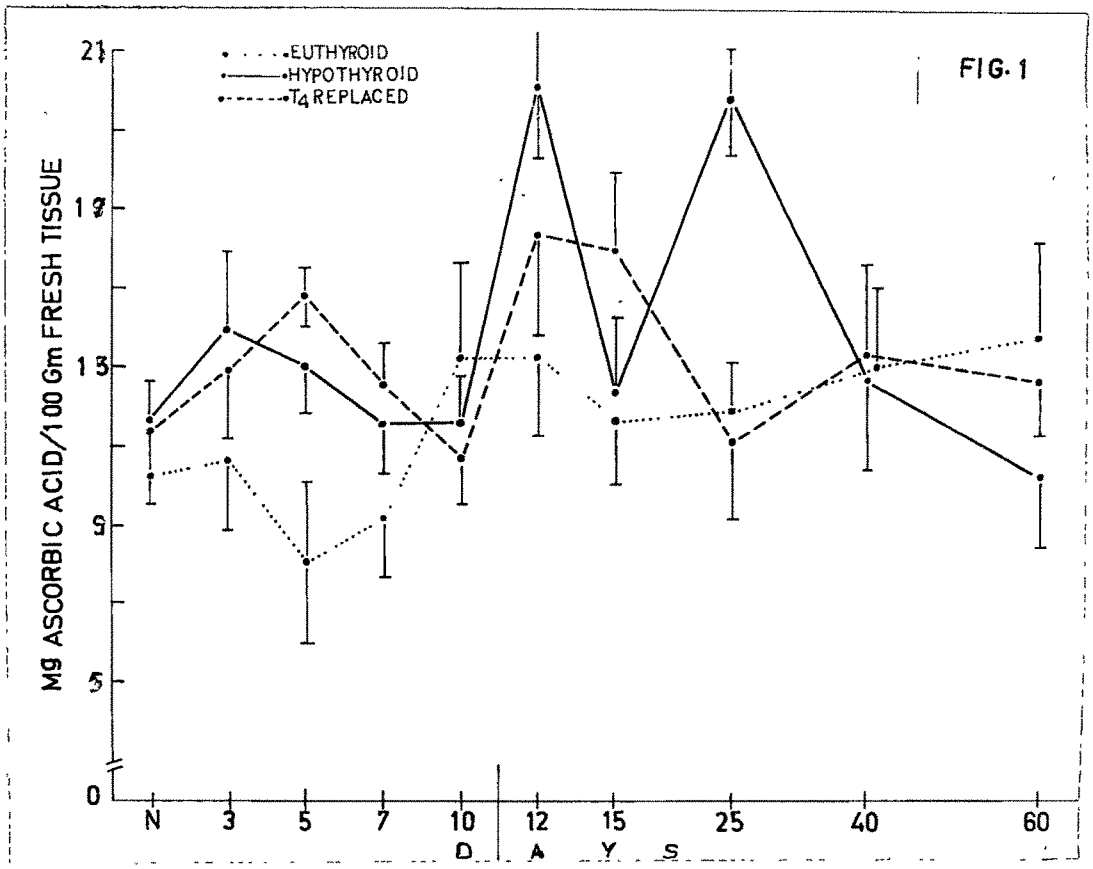

Fig. 1. Changes in hepatic ascorbic acid content during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

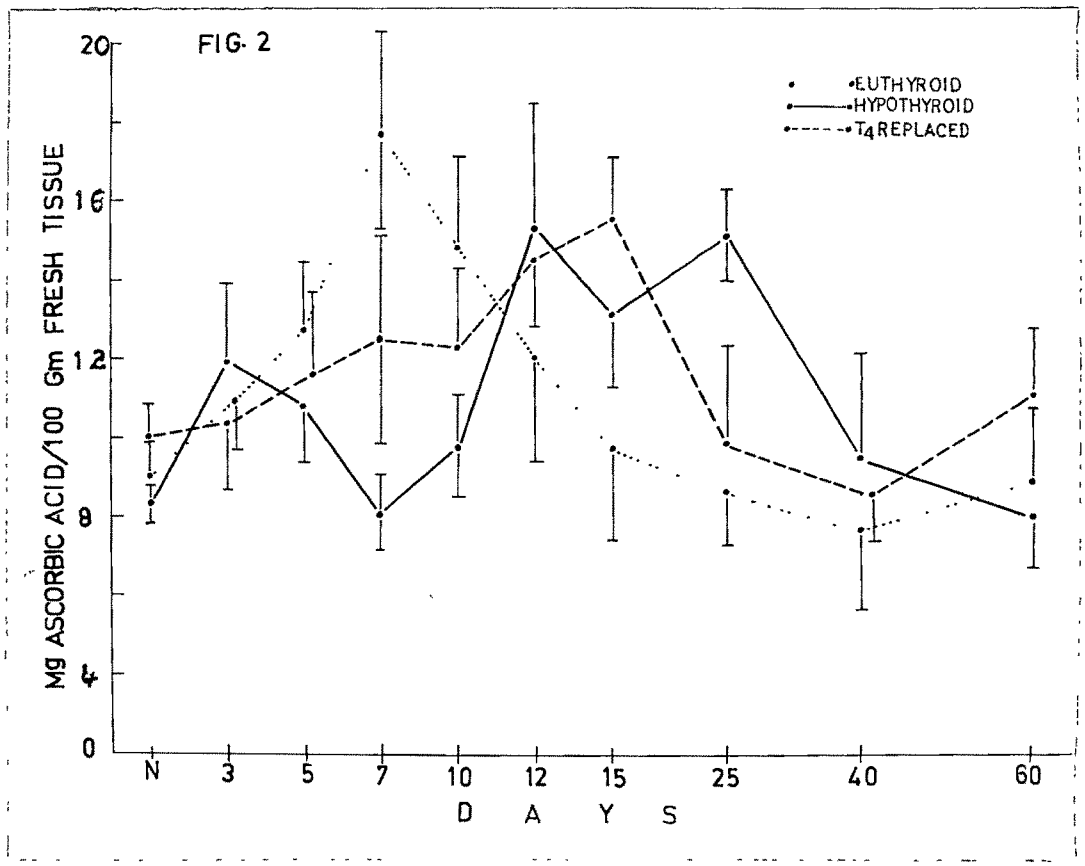

Fig. 2. Changes in renal ascorbic acid content during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

continuous and steady depletion till the lowest subnormal level of 7.5 mg/gms was reached on 40th day; which by 60th day had increased to 8.81 mg, still subnormal.

A comparison of the pattern of changes of hepatic and renal ascorbic acid contents in hypothyroidic animals reveals a complete parallelism between the two. Both the organs depicted an increase on the 3rd day, fall to normal levels by 7th day and a gradual increase thereafter to attain peak, above normal levels, on 12th day. The ascorbic acid levels again nearly touched the same high levels on 25th day by an abrupt and sharp decline on the 15th day. Since 25th day, the ascorbic acid content of both the organs decreased sharply to near normal levels on the 40th day and slightly below normal levels by 60th day. The clear cut difference between euthyroidic and hypothyroidic conditions was, however, greatly reduced in T4 replaced animals and the pattern of changes tended to be more similar to the euthyroidic controls. Most similar change was noted with respect to renal ascorbic acid content, which increased gradually but protractedly to reach a maximal level on the 15th day after caudal autotomy. Thereafter a sharp depletion was evidenced during 25th and 40th days to reach lowest subnormal level. Thence the ascorbic acid content in kidney rose up to slightly above normal level by 60th day of tail regeneration. The pattern of alterations in hepatic ascorbic acid content too in T4 replaced animals

was very much comparable to that of group A, except for sharper increases and decreases in content and temporal shift to the right in the regenerative time scale. Accordingly there was continuous increase during the first 5 days, decrease to below normal level by 10th day through 7th, and another increase to maximal above normal level by 12th day and a decrement to slightly below normal level by 25th day through 15th, and a subsequent increase to above normal level by 40th day which was more or less maintained so even on 60th day of tail regeneration.

DISCUSSION

Shah et al. (1980 c) have shown hepatic tissue to be the immediate responder for supply of ascorbic acid to the site of regeneration. Increased biosynthetic activity in the renal tissue as well as its active participation in meeting the vitamin C requirements of the regenerating system, during the later phases, have also been inferred thereat. These conclusions appear to be validated by the presently observed fall in hepatic ascorbic content during the first 5 days and the continuous increase in the renal ascorbic content during the first 7 days post-autotomy followed by the pronounced depletion thereafter in the euthyroidic group of animals. Current experimental results reveal that hypothyroidism per se does not affect the systemic turnover of ascorbic acid induced by the

regeneration specific milieu internae. However, the exact parallelism shown by the hepatic and renal ascorbic acid contents during the various periods of tail regeneration as well as the regular wave like pattern of changes which are both unlike the euthyroidic animals tend to suggest the loss of regeneration associated purposeful and adaptive differential alterations due to PTU induced thyroxine lack. This is further emphasized by the partial rectification brought about by T4 replacement in PTU fed C group of animals. This inability of T4 replacement to transform the PTU type of pattern completely to the euthyroidic one may either be due to the continuous presence of T4 in the system as opposed to the phase specific spurt in plasma and tissue thyroxine levels reported to occur in the euthyroidic state (Ramachandran et al., 1981) or the presence of PTU somehow interfering with the proper action of T4 either directly or indirectly.

Though the few studies of Stubbs and McKernan (1967); Stubbs et al. (1967); DeNicola et al. (1968); Dieter (1969); Biswas (1969); Ishii et al. (1970); Bratcher and Kent (1971) and Majumder and Chatterjee (1974) have all indicated the influence of various hormonal principles in synthesis, storage and transport of ascorbic acid, no definite humoral control of ascorbic acid synthesis and turnover has yet been elucidated. Analysis of the present observations though disfavour any direct involvement of the thyroid hormone in

ascorbic acid metabolism, does however, indicate the requirement of thyroxine in purposeful and differential responses of organs of synthesis and storage of ascorbic acid in lacertialians towards the specific controlling principle(s). A conglomerate of hormonal and other factors could be purported to be commissioned to modulate the various systemic responses of the lizard towards the stress of automy and ensuing regeneration. Though in such a setup, it is difficult to pinpoint any specific factor as the controlling factor, it may however, be safely concluded that one or more of these factor/s do integrally interact with thyroxine to alter the systemic turnover of ascorbic acid to meet the exigencies of tail regeneration in an efficient fashion. The absence of thyroxine appears to throw the whole process into disorganised, displaced and wasteful modulations as evidenced by the pattern of changes recorded in the hypothyroidic state.

Recently, Padhi and Patnaik (1979) have shown increased incidence of ascorbic acid in the kidney of the male garden lizard, Calotes versicolor in response to somatotrophin. In this light, the 90% increment in hepatic and renal ascorbic acid contents noted during 12th and 25th days of tail regeneration probably represents the hypersensitive response to growth hormone in the absence of thyroxine. This is all the more pertinent as growth hormone can well be purported

to play an important integral role in the regenerative mechanics of vertebrates. In view of the reported hypothyroidic inhibition of tail regeneration (Chapter I) the loss of ascorbic acid content of both liver and kidney observed in the present investigation might in all probability represent a wasted pool of systemic ascorbic acid. The slightly delayed temporal disposition with more pronounced modulations in T4 replaced group correlate well with the earlier reported delay in tail regeneration in Mabuya carinata and its enhancement during the later periods (Chapter I).

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