CHAPTER 3

INVOLVEMENT OF RENAL AND HEPATIC ASCORBIC ACID IN THE TAIL REGENERATION OF THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Metabolic interactions and structural changes during lizard tail regeneration have been the major aspects of investigations from this laboratory (Shah, 1975). These studies have significantly contributed towards our comprehension of reparative regeneration in reptiles. It is quite justifiable to anticipate excessive physiological demands which would be impinged upon the animal following partial or total loss of an appendage. Subsequently, reorientations and compensations of metabolism could be expected to occur throughout the body. These would be largely in terms of participation of systemic factors and metabolic fluctuations in distant organs.

Ascorbic acid (AA) has been known to play significant role in metabolic interactions (Rusch and Kline, 1941; Banerjee *et al.*, 1959; Banerjee and Ganguli, 1962) and structural lay out of normally developing and repairing tissues (Brachet, 1950; Mazur *et al.*, 1961; Gould, 1963; Rasmussen, 1967; Prasad, 1971). However, literature on

involvement of AA in regenerating systems, which are attractive models to study metabolic and structural changes in adult organisms has been consistently poor (Ryvkina, 1940; Shah et al., 1971; Ramachandran et al., 1975). Liver and kidney are considered to be the major sites of storage and synthesis of AA, respectively, in most of the vertebrate species (Grollman and Lehninger, 1957; Bublitz et al., 1958; Isherwood et al., 1960; Chatterjee, 1973). Among reptiles, both liver and kidney of turtles can synthesise AA (Grollman and Lehninger, 1957). However, in lacertilians the biosynthetic mechanism for AA resides only in kidney and not in liver (Roy and Guha, 1958) where it is only stored. Nevertheless, its concentration in liver depends upon renal synthesis (Mohanty and Patnaik, 1968). Keeping in view the sparseness of literature and broad spectral involvement of AA in various vital processes of animals, it will be interesting to examine the fluctuations exhibited in AA content at the sites of storage and synthesis during tail regeneration. The current chapter reports the changes in hepatic and renal AA contents during various phases of tail regeneration of the house lizard, Hemidactylus flaviviridis.

MATERIALS AND METHODS

The house lizards, H. flaviviridis, collected from the University campus were maintained in the laboratory on a diet of insects. Fifty adult lizards of about the same weight (12 ± 2 gms) were selected. Estimations of AA in liver and kidney were carried out in the normal lizards (with intact original tail) as well as in those with regenerating tails, which were sacrificed at specific intervals in accordance with the various phases of tail regeneration following autotomy (Shah and Chakko, 1968a). The liver and kidney were removed immediately after decapitation under general hypothermia. Extracts were prepared in 6% TCA (Trichloro acetic acid) and aliquots were used to estimate AA by Dinitrophenyl hydrazine method of Roe (1954).

RESULTS

The data of quantitative estimations of AA in liver and kidney of H. flaviviridis in the normal and lizards with regenerating tail have been presented in Table 1 and Fig. 1.

The level of AA content in liver (16.64±0.09) of the normal lizard (with the intact original tail) was found

Table 1 : Levels of Ascorbic acid (AA) content in liver, kidney and tail* during tail regeneration in the house lizard, Hemidactylus flaviviridis

Normal tail and different phases of tail regeneration	Ascorbic acid content (mg/100 gm fresh tissue)		
	Liver	Kidney	Tail
Normal tail	16.64±0.09@ (15)	11.50±0.04@ (15)	4.881±1.30@ (12)
Regenerating tail$			
Wound healing	28.74±1.23 (14)	22.11±1.06 (14)	8.196±2.02 (12)
Blastema	27.10±1.95 (15)	19.01±0.08 (15)	4.925±1.74 (10)
Differentiation	28.54±1.72 (15)	23.10±2.00 (14)	25.36±2.27 (11)
Growth	22.21±1.17 (14)	19.45±0.02 (15)	14.14±1.10 (10)
Fully regenerated	19.45±0.02 (15)	16.00±2.30 (15)	5.312±1.21 (12)

Figures in the parentheses indicate the number of experiments done.

$ The phases of regeneration are arbitrarily chosen for the purpose of discussion.

@ Mean ± S.D.

* Data after Shah et al. (1971)

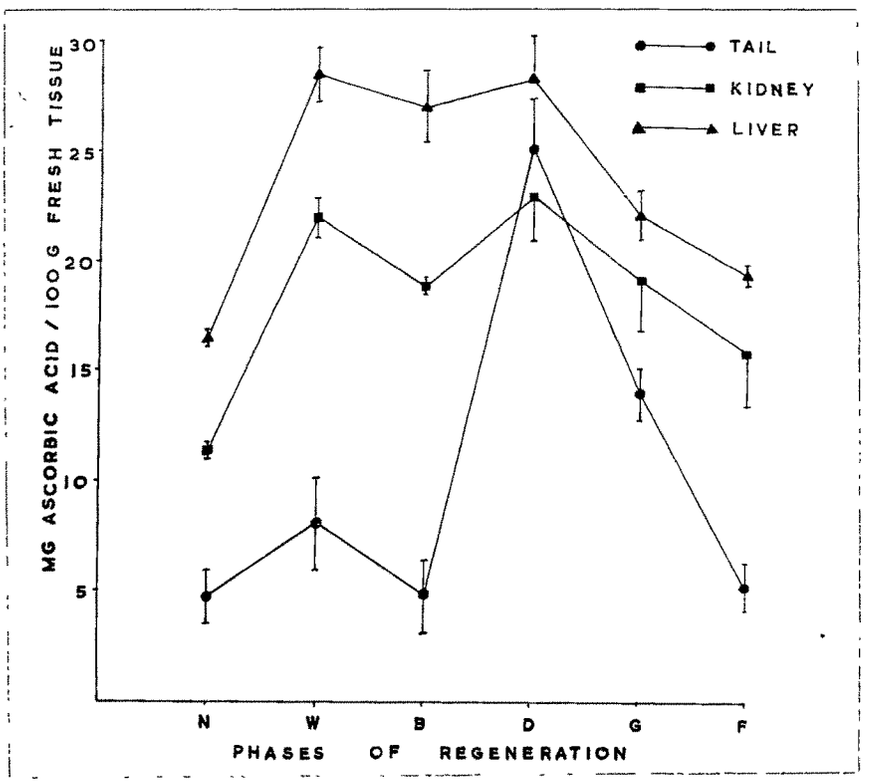

Fig. 1 : Graphic representation of levels of ascorbic acid (AA) tail, kidney and liver during different phases of tail regeneration in H. flaviviridis.

N - Normal tail
W - Wound healing phase
B - Blastema phase
D - Differentiation phase
G - Growth phase
F - Fully regenerated tail.

to be more than that in the kidney (11.50±0.04). Hepatic AA rose to one and a half times the normal level during wound healing; this higher level was maintained throughout the course of regeneration. In the lizard with fully regenerated tail, the liver AA attained its preautotomy level, but was slightly higher than that observed in the lizards with original intact tail. As compared to liver, the renal AA presented greater fluctuations during different phases of tail regeneration; two fold increase during wound healing after autotomy was seen, which declined during blastema phase to half the elevated level, nevertheless, it was higher than the normal level of AA in the kidney of the lizards with intact original tail. Again a two fold rise (twice the normal value) during differentiation phase was recorded. A fall towards normal level was seen in the lizards with fully grown regenerate. However, like its hepatic level, its renal level was also slightly higher than in the normal lizard.

DISCUSSION

AA has been implicated in proper wound healing of epithelial and visceral tissues (Bartlett *et al.*, 1942a; b; Bourne, 1953; Ksabyan, 1956; Schauble *et al.*, 1960;

Abt et al., 1959; Crandon et al., 1961; Zamanskii and Lopushanskii, 1955; Pilo et al., 1971). During the healing of the autotomy wound of lizard tail, two fold increase in AA content at the site has been reported by Shah et al. (1971) and Ramachandran et al. (1975). Present study has revealed a one and half time increase in hepatic AA content and two fold increase in renal AA content. These elevations suggest a gearing up of synthetic machinery residing in the distant organs like kidney in the event of increased demand for the vitamin at the healing site. It seems plausible that during the early healing of the exposed caudal surface, AA is mobilised from the liver as well as kidney which are known to be the sites for storage and synthesis respectively (Roy and Guha, 1958; Grollman and Lehninger, 1957). Support to such a contention can be found in the investigations of Lauber and Rosenfeld (1938), Schilling et al. (1953) and Candlish and Chandra (1967) who have reported such mobilisation of AA from distant organs prior to healing of cutaneous wounds in mammals.

With the completion of healing and onset of blastemic phase, a considerable fall in renal AA content was noticed; while no change could be observed in the hepatic AA level.

Blastema is characterised by active cell division and cellular proliferation (Shah and Chakko, 1972) and prevalence of anaerobic environment (Shah and Hiradhar, 1974). These conditions are not conducive to collagen fibrillogenesis, hence this protein is not synthesised during blastema formation (Shah et al., 1971; Schmidt and Jasch, 1971). On the contrary, collagenolytic activities prevail as reported by Grillo et al. (1968) in regenerating amphibian limb at this stage. Shah and Hiradhar (1975) have also reported lack of glycosaminoglycans in blastema, characteristic of collagen. Thus, overall requirement of AA during this phase is lower compared to that during wound healing. This is evident from the reduction in renal AA content denoting that the synthetic activity of the organ in this regard is lowered. Maintenance of more or less the same increased level of AA in the liver could be due to greater metabolic activities of this organ itself concerning the carbohydrate and lipid metabolism (Chapter 1) and also increased dehydrogenase activities (Chapter 2) at this stage. Association of AA with mobilisation of lipids (Rusch and Kline, 1941), carbohydrate metabolism (Banerjee and Ganguly, 1962) and proper functioning of Krebs cycle enzymes (Banerjee et al., 1959) can be recalled at this juncture.

Differentiation of tissues in the regenerate calls forth greater metabolic activities and its structural organization. Liver maintains its high level of AA, and kidney which had turned its synthetic machinery to a slightly low gear during blastemic phase, is once again geared to high activity which is evident in another peak of two fold increase in AA content during differentiation. Maintenance of a considerably high content of AA in the kidney (organ of synthesis) and liver (storage organ) during the differentiation phase, in spite of a five fold increase in the vitamin content of the regenerating tail tissue (Shah et al., 1971) indicates that synthesis of AA far exceeds the rate of its utilization during this phase. Laying down of connective tissue matrix and collagen fibres for successful integration, and incorporation of amino acids for protein synthesis of differentiating tissues is a prominant feature of this phase and is greatly influenced by AA intake (Yew, 1973). Studies on glycosaminoglycans and ascorbic acid content (Shah and Hiradhar, 1975; Shah et al., 1971) during tail regeneration of H. flaviviridis have amply shown that AA is well implicated in the differentiation process of the regenerate. Thus, the ascorbic acid synthesising activity of kidney

which is at a high gear at this stage suggests the involvement of this visceral organ in meeting with the high demand of AA by the regenerate.

As the regenerate progresses towards growth phase, a gradual depletion of AA in both, the hepatic and renal tissues is anticipated. Higher than the normal values of AA in these organs during growth phase can be appreciated when the importance of AA as a growth promoting factor (Sen, 1974) is taken into consideration. However, slightly higher levels of the vitamin in both these organs in the lizards with almost fully grown regenerated tail may be due to slow trend in returning to normal preautotomy levels.