CHAPTER 3

COMPARATIVE EVALUATION OF *IN LOCO* AND SYSTEMIC ALTERATIONS IN ASCORBIC ACID DURING POST-CAUDAL AUTOTOMY AND POST-LIMB AMPUTATION IN *HEMIDACTYLUS FLAVIVIRIDIS*

Although ascorbic acid(AA) is undoubtedly widely required in metabolism, it can be synthesised by a variety of plants and by all animals studied except, primates and guinea pigs. Kidney and liver are known to be the sites of AA production in reptiles (Grollman and Lehninger, 1957). But in lacertilians, the necessary mechanism to synthesis AA lies only in the kidney (Roy and Guha, 1958). Vitamin C is readily oxidised to the dehydro form. Both forms are physiologically active and both are found in the body fluids. The enediol group of ascorbic acid may be involved in the physiologic functions of this vitamin. Liver and kidney are considered to be the major sites of storage and biosynthesis of AA respectively in most of the vertebrate species (Grollman and Lehninger, 1957; Chatterjee, 1973). But, its concentration in the liver depends upon the renal synthesis (Mohanty and Patnaik, 1968). The biochemical function of ascorbic acid is still not known. Probably the most clearly established functional role is in maintaining the normal intercellular material of cartilage, dentine, bone and in collagen synthesis. It is also shown to influence certain

biological activities, like maintaining levels of cAMP and cGMP (Lewin, 1976; Tisadale, 1975), production of adrenalin, serotonin and noradrenalin (Lewin, 1976), detoxification of endogenous and exogenous toxic substances (Heacook and Powell, 1973), phagocytosis (Nungester and Ames, 1948) and structural layout of normally developing and repairing tissues (Barnes and Kodicek, 1972).

AA is known to be intimately involved in wound healing associated with regeneration as well as in other events basic to regeneration (Shah et al., 1971, 1976, 1980; Ramachandran et al., 1975). Since the wound healing associated with regeneration and the normal wound healing have obvious difference, a comparative analysis of AA in these two types of healing process occurring post-amputation of two different body extremities (one regenerating and other non-regenerating) in the same animal would be appropriate. It was in this wake, that a comparative quantitative evaluation of caudal (regenerating) and limb (non-regenerating) AA contents post-autotomy and post-amputation respectively in Hemidactylus flaviviridis has been attempted. Moreover, the evaluation has been extended to liver and kidney too, as systemic stores of AA have been shown to be involved with the mechanics of tail regeneration (Shah et al., 1980). This is with the idea that such a study

might probably throw some light on the ability of one part of the body to regenerate (tail), and the lack of this ability in another part (limb) in lizards.

MATERIALS AND METHODS

H. flaviviridis collected from Baroda were kept in the laboratory for a fortnight to get them acclimatized to the new environment. The animals were kept on a diet of insects. The lizards were divided into two groups, one with their tail autotomised two to three segments away from the vent, and the other with their forelimb amputated from the distal part of the humerus. Lizards from both the groups were sacrifised at regular time intervals (i.e. during different phases of regeneration) and liver, kidney, limb amputated. Stump and tail regenerate were used for the estimation of ascorbic acid by the method of Roe (1954).

RESULTS

The changes in the hepatic ascorbic acid content were more or less similar under both the conditions (tail autotomy and limb amputation), with an early increase reaching a peak level on 2nd and 3rd days post-caudal autotomy and post-limb amputation respectively. Thereafter,

Table 1. Levels of AA (mg/100 gm fresh tissue) during tail regeneration in H. flaviviridis.

Periods of regeneration in days	N	1	2	3	5	7	10	20	25	40	60
Liver	23.2 ±3.28	30.3 ±9.23 0.0005*	35.7 ±1.23 0.001*	24.3 ±0.451 0.005*	16.7 ±0.7995 0.001*	23.39 ±0.2329	16.2 ±0.577	18.9 ±0.451	16.3 ±0.1258	26.8 ±0.5 0.0005*	20.3 ±0.147
Kidney	28.4 ±1.22	45.5 ±2.36 0.0005*	28.4 ±1.98	16.06 ±0.7979 0.0001*	32.4 ±2.1985 0.0005*	26.5 ±0.1544	21.8 ±0.130	22.8 ±1.054	14.7 ±0.2715	15.5 ±0.4027	23.1 ±0.204
Tail	5.82 ±0.334	7.5 ±0.214 0.01*	8.0 ±0.341	7.5 ±0.4984	14.0 ±0.3874	21.3 ±0.450	8.7 ±0.0488	13.3 ±0.1794	9.6 ±0.0128	8.12 ±0.0217	7.00 ±0.0185
Hand	25.7 ±1.218	21.7 ±1.378 0.001*	14.8 ±0.985	11.3 ±0.8536	12.0 ±0.830	13.0 ±0.7173	15.3 ±0.18	28.3 ±0.2865 0.0005*	19.6 ±0.952	35.3 ±1.0117	28.7 ±0.1042

± S. D.

* P value

Table 2. Levels of AA (mg/100 gm fresh tissue) post-limb amputation in H. flaviviridis.

Periods of regeneration in days	N	1	2	3	5	7	10	20	25	40	60
Liver	23.2 ±3.28	35.2 ±1.47 0.0005*	40.1 ±10.8 0.0005*	41.5 ±0.5053	25.3 ±0.8031	19.5 ±0.7173 0.001*	26.5 ±0.1098	23.8 ±0.3041	12.1 ±0.1083 0.01*	21.7 ±0.447	22.7 ±0.328
Kidney	28.4 ±1.22	46.7 ±2.45 0.0005*	25.4 ±1.61	14.18 ±0.6516	40.7 ±1.081 0.0005*	19.63 ±0.6554	21.7 ±0.294	25.0 ±0.873	19.5 ±0.185	24.5 ±0.5904	26.4 ±0.415
Tail	5.82 ±0.334	2.2 ±0.0149 0.01*	4.6 ±0.198	4.0 ±0.162	8.1 ±0.12	9.5 ±0.567	4.65 ±0.1356 0.001*	3.3 ±0.3054	3.5 ±0.3754	3.0 ±0.1914	4.5 ±0.2182
Limb	25.7 ±1.218	30.58 ±1.365 0.001*	14.5 ±0.187	11.4 ±0.5319	17.9 ±1.0016 0.001*	16.1 ±0.374	13.2 ±0.507	16.5 ±0.442	24.5 ±0.6586	10.6 ±0.1485	20.1 ±0.248

± S. D.

* P value

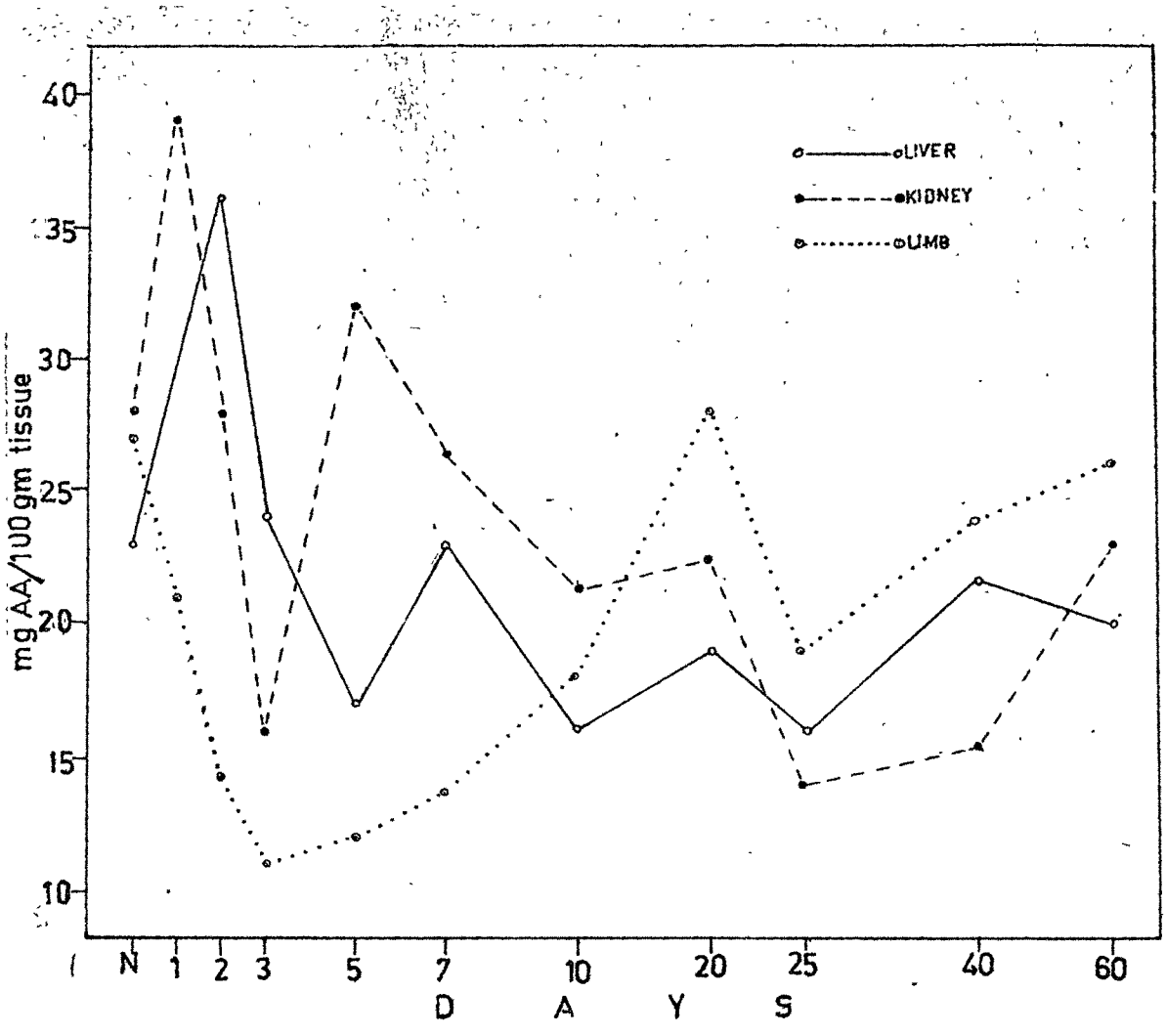

Fig. 1 : Graphic representation of the levels of tissue ascorbic acid during tail regeneration in H. flaviviridis

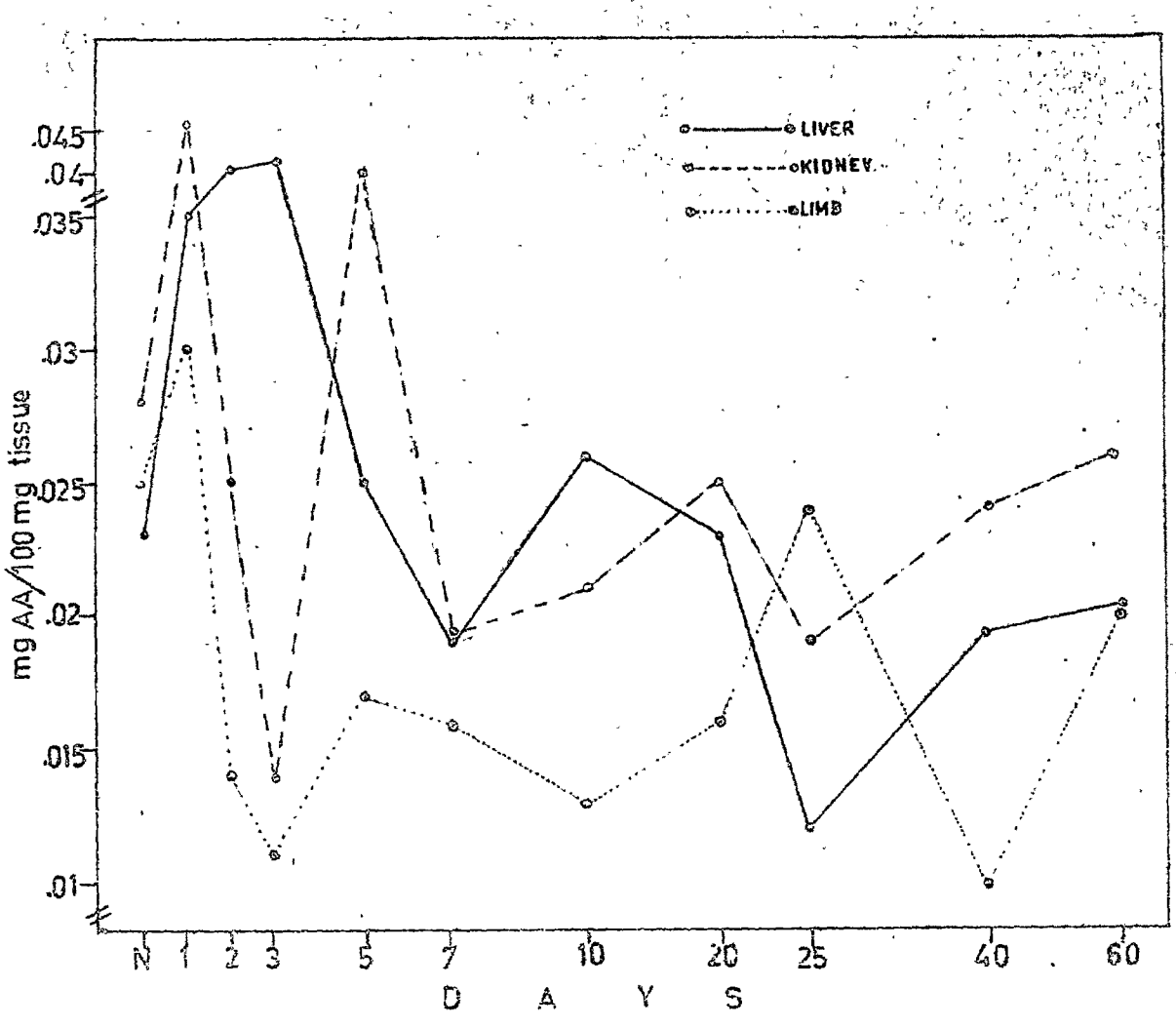

Fig. 2 : Graphic representation of the levels of ascorbic acid in the liver, kidney and limb post-limb amputation in the lizard, H. flaviviridis.

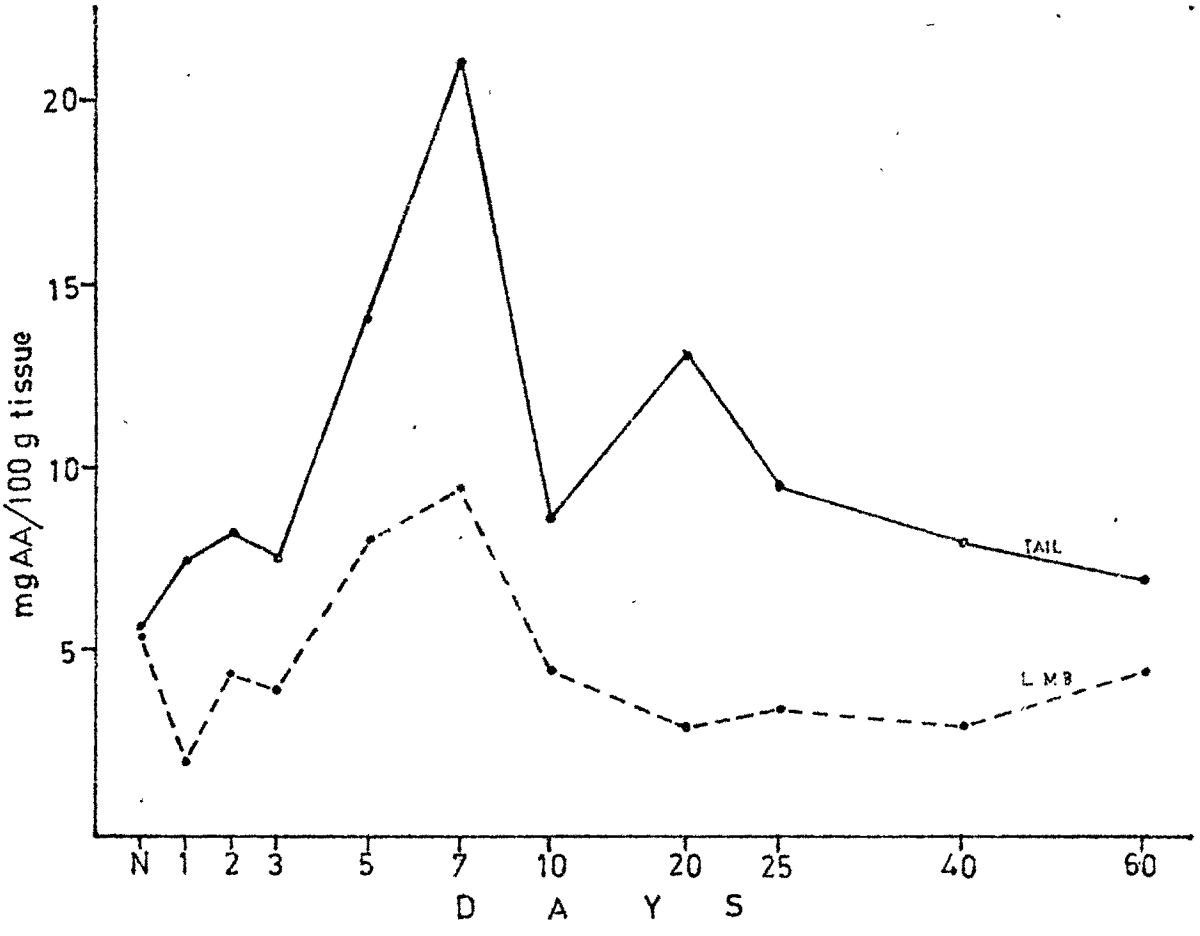

Fig. 3 : Graphic representation of the comparative levels of ascorbic acid in the tail during tail regeneration and post-limb amputation in the lizard, H. flaviviridis

the hepatic ascorbic acid content showed a zig zag pattern of changes during tail regeneration all the while remaining below the normal level. While in the case of limb amputation, the ascorbic acid content of the liver decreased to a subnormal level by 7th day and then increased to the normal range by 10th day. Once again the AA content decreased to the lowest subnormal level by about 25th day and then gradually commenced rising towards normal level. In the case of renal ascorbic acid, the pattern of changes were exactly identical during the first 5 days. Sharp increase within the first 24 hours followed by a steep fall to subnormal level by 3rd day, and further increase to above normal by about 5th day. Since then, whereas in the case of tail regeneration the renal ascorbic acid content decreased more or less continuously till 25th day, in the case of limb amputation, the level fell sharply by 7th day and then showed a gradual increase between 7th and 20th days to show further depletion by 25th day. After the 25th day, the renal AA content showed a continuous increase in both the cases. A comparative evaluation of the local AA contents under the two conditions revealed a continuous increase and attainment of a peak by 7th day and a sharp decline by 10th day in the case of tail autotomy. A second phase of increase by 20th day with subsequent continuous decrease towards the normal level was the feature in the tail regenerate. On the

other hand, the limb amputated stump showed an initial increase 24 hrs post-amputation and a sharp decline to the lowest subnormal level by 3rd day. Thereafter, the AA content of the limb depicted a more or less continuous increase to reach a near normal level by 25th day. This was followed by a second depletion during the 25th and 40th days, after which the limb ascorbic acid gradually increased towards the normal level. Limb AA content, during tail regeneration showed a tremendous decrease by 3rd day post-caudal autotomy. The level was, however, restored gradually to the normal by the 20th day. There was once again a decrease in the AA content during the 20th to 25th days following which there was recovery towards normal level. Caudal AA content post-limb amputation showed an attainment of peak level by the 7th day post-limb amputation with an initial depletion during the first 24 hrs. Between the 7th and 20th days there was a fall to sub-normal level, which was more or less maintained so till 40th day, and was followed by gradual recovery towards the normal level (Tables 1 and 2; Figures 1 - 3).

DISCUSSION

AA has been implicated in various activities associated with lizard tail regeneration (Shah *et al.*,

1971, 1976, 1980; Ramachandran et al., 1975). Further, systemic stores of AA (kidney and liver) have also been shown to undergo adaptive quantitative alterations in response to the stress of caudal autotomy and ensuing regeneration in Mabuya carinata (Shah et al., 1980). As is evident from the results recorded herein (Fig. 1 - 3), not only is the earlier reports of increased in loco elevations in AA content in Hemidactylus confirmed, but even the participation of kidney (synthesising organ) and liver (storage organ) reported in Mabuya seems a feature applicable to Hemidactylus. These indicate a common pattern of in loco and systemic response in both the lizards during their caudal regeneration. The results obtained also show more significant depletion in AA content of the synthesising organ (kidney), than the storage organ (liver).

Interestingly, the changes in AA content of kidney and liver post-limb amputation are near replica of those obtained post-caudal autotomy. However, in terms of local changes, those recorded for limb are very much distinct from those recorded for tail and are more or less like mirror images. Whereas in the case of tail the AA content was supra-normal all throughout regeneration, the AA content of limb was all throughout sub-normal. Evidently the AA content of the amputated limb stump gets depleted and

further shows inability to mobilize the vitamin. Since the systemic response appears identical in both the conditions, it is safe to presume that in an animal with a restricted power of regeneration, a loss of any part of the body, may it even be a part not capable of regeneration, evokes identical systemic modulations. It would then seem that the inability of the local site to favourably respond to the systemic one and react in an expected fashion for regeneration are probably the reasons for the absence of regenerative potential. Finally, a comparison of the alterations in AA content of the tail, post-limb amputation and content of limb, post-caudal autotomy again emphasize the differential responsiveness of the two sites. Whereas the post-caudal autotomy changes in limb were more or less similar to the changes shown by limb after its amputation, the changes in the caudal tissue after its autotomy and those after limb amputation were quite different. This underscores the ability of the caudal tissue to respond differentially under the two conditions unlike the limb which has only a fixed stereotyped possibility. Could it mean that the regeneration specific responses are super imposed over the general type of responses and is evoked at times of injury or loss of a part possessing the power of regeneration? As an offshoot, it would imply that the loss of this specific response by a part will lead to the eliciting of only the general type of response at times of injury/loss of a part.