

CHAPTER - 8

SECTION: I VITAMIN 'C' IN REGENERATION:
A QUANTITATIVE EVALUATION OF THE CHANGES
IN THE HEPATIC AND RENAL ASCORBIC ACID
CONTENTS DURING TAIL REGENERATION IN
THE SCINCID LIZARD, MABUYA CARINATA

Ascorbic acid (AA) has received more attention in the last ten to fifteen years and with its purported role in many intricate and finer aspects of tissue functioning, is emerging as a versatile vitamin component, whose presence or absence could easily bridge the chasm between health and disease, or even between the normal and abnormal functioning of many biochemical events. As regeneration is a major biological phenomenon entailing a reactivation of developmental and morphogenetic events, and being associated with many intricate biochemical and metabolic aspects, the role of ascorbic acid during tail regeneration in the lizards, Mabuya carinata and Hemidactylus flaviviridis were investigated (Ramachandran et al., 1975; Shah et al., 1971). In the light of the multifarious functions ascribed to

AA, the significant changes observed in the regenerate during tail regeneration in the two lizards, have been correlated with respiratory mechanics, cellular metabolism and, wound healing as well as differentiative processes. The tremendous amount of accumulation and utilization of AA noted during lacertilian tail regeneration (Shah et al., 1971; Ramachandran et al., 1975) may have to be contributed in part or totally by the visceral organs involved in the synthesis and storage of AA. Since in lizards, the kidney and liver serve as the organs of synthesis and storage respectively (Roy and Guha, 1958; Grollman and Lehninger, 1957), it was thought pertinent to investigate the changes if any, in the AA content of these organs during the various periods of tail regeneration in the scincid lizard, Mabuysa Carinata.

MATERIAL AND METHODS

Adult lizards obtained from Karnataka, India, and maintained in the laboratory on a diet of insects served as the experimental animals. After a fortnight's acclimatization to the laboratory conditions the tails were autotomised and subsequently on the periods specified in the present work, the animals with

regenerating tail were sacrificed under mild anaesthesia and the liver and kidney samples were collected for estimating the total AA content as per the method of Roe (1954).

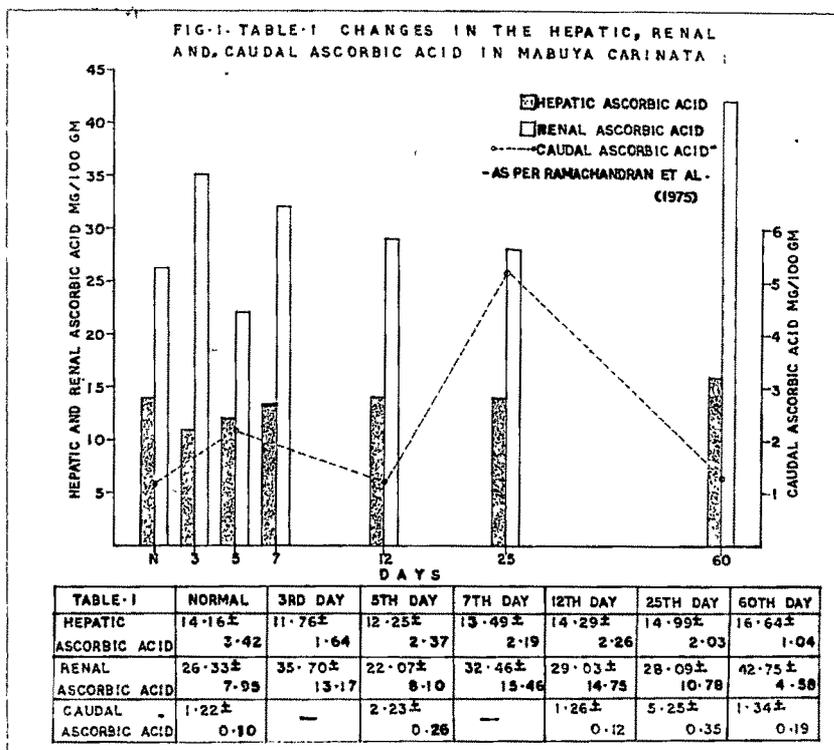
RESULTS

LIVER

Subsequent to autotomy on the 3rd day the AA content of the liver showed slight decline which, however, gradually returned to the normal (preautotomy) level by about the 12th day. The normal level was thereafter maintained all throughout the period of regeneration excepting for the final phase whence the hepatic AA content appeared to show a slightly elevated level as seen on the 60th day when the regenerate had almost reached the fully grown state.

KIDNEY

Unlike in the case of liver, the renal AA content appeared to show an increase on the 3rd day subsequent to tail autotomy. However, on day 5 its level fell significantly and attained a subnormal level. Thereafter the AA content again depicted a swift increase as seen on the 7th day. Since then, through the 12th and 25th



days of regeneration, the renal content though showing very gradual decrease, however maintained an above normal level. On day 60, whence the process of regeneration, had more or less reached towards its end, the AA content of the kidney showed more or less one and half times the normal level. These changes in the AA content of the liver ^{and} kidney during the various periods of tail regeneration are presented in Table - 1, Figure - 1.

DISCUSSION

It becomes apparent from the present study, that in the case of Mabuya, the renal tissue, which serves as a major organ of synthesis of ascorbic acid in lizards, has more or less twice the amount of AA as compared to that in the hepatic tissue which, functions as a storage organ. The changes accrued in response to autotomy and regeneration appears to be more dramatic in the case of renal AA than that of the hepatic AA. The only significant change in the hepatic ascorbic acid is the initial depletion subsequent to autotomy as marked by its reduced level on the 3rd day postautotomy (Table - 1, Figure - 1). On the other hand, the renal tissue at the same time showed an elevated level. Such a set of differential

changes might indicate that, whereas the hepatic tissue responds to autotomy by a depletion as a generalised response to wounding, the renal tissue responds by engaging itself in an increased rate of synthesis. However, since then, the hepatic tissue gradually regained its normal level of AA by about the 12th day (through an intermediary level on the 7th day), as could be visualised from the Table - 1, Figure - 1. In contrast, the renal AA content appears to show a drastic fall on the 5th day to be immediately followed by a swift increase on the 7th day. The drop in the ascorbic acid content of the renal tissue noted on the 5th day (preblastema phase) even in the wake of an already stepped up rate of synthesis is rather significant and corresponds well with the reported doubling of the ascorbic acid content of the regenerating tail at this period (Shah et al., 1971; Ramachandran et al., 1975). Apart from this accumulation at the wound site, the involvement of renal ascorbic acid in the elaboration of hormones of the adrenal cortex as well as in the enhanced activities of various other peripheral organs (as yet unknown) also cannot be overlooked. Thus it might be postulated that the fall in the AA content of the

renal tissue on the 5th day could be due, to both an active release as well as an increased uptake by various tissues such as the healing tail, adrenal gland, other peripheral organs as well as the liver (to regain its normal level). The rise in the AA content of the kidney noted on the 7th day might be attributed to a decreased rate of its utilization and/or even a reduced rate of release. However, with the onset of histogenesis of differentiation there appears to be a gradual but continuous depletion of renal AA though the 12th to 25th days of regeneration (Table - 1, Figure -1) and is paralleled by the early reports from this laboratory of an increase in the AA content of the regenerating tail during this period (Shah et al., 1971; Ramachandran et al., 1975). Though there was a continuous decrease in the AA content of the kidney from the 7th to 25th days postautotomy, its level, however, remained above normal and might reflect the fact that the synthetic machinery remains geared up and keeps pace more or less with the enhanced rate of release as well as utilization. In the wake of the attainment of an elevated functional competence by the renal tissue in meeting adequately the requirement of AA, the

relative insⁿestivity of the hepatic AA is well reflected by the maintenance of a steady and normal level of AA from the 7th day to 25th day of regeneration. Moreover, the maintenance of a steady level of AA at this period of regeneration might also be of a crucial significance as the hepatic **tissue** is noted to be involved in many metabolic and other transformations activated specifically to meet the requirements of the regenerative process (Section - 1 of Chapters - 2, 3, 4, 5, 6, 7, 8; as well as unpublished observations on enzymology of the liver during tail regeneration). It may be appropriate to mention in this connection that a number of workers have associated AA with enzyme catalysed reactions as well as increased metabolic activities of various tissues (Burns et al., 1951; Meiklejohn, 1953; Banerjee et al., 1959; Chinoy, 1969a, 1970, 1971). The most significant aspect of the present study is the very high content of AA noted in the kidney on about the 60th day of tail regeneration whence the regenerative process has more or less come towards its close. Concomitantly, the hepatic AA content too is noted to show a parallel increase though of a lesser magnitude.

These observations though a bit perplexing might be explained on the basis of differential rate of synthesis as well as utilization. It may be presumed that with the completion of the process of regeneration, the requirement and/or utilization by the various tissues in the body comes down, and, concomitantly with the already highly geared^{up} synthetic machinery which is being switched off taking its own time in settling down to its preautotomy pace, as well as the reduced release of AA, there is a sudden accumulation or piling up of AA in the renal tissue, leading to the high level noted in the present study. The increase in the AA content of the liver too noted at this period appears rather self evident and indicates the transport of a part of this extra AA from kidney to the storage organ.

Finally, it may be safely concluded from the facts at hand, that though the liver (storage organ) responds by an initial depletion immediately subsequent to autotomy, for the rest of the period of regeneration, the kidney, (organ of synthesis) takes over the responsibility of satisfying the requirement of AA very effectively, and thus supports regeneration.

SECTION: II VITAMIN 'C' IN WOUND HEALING:
A QUANTITATIVE EVALUATION OF THE CHANGES IN
THE CAUDAL, HEPATIC AND RENAL ASCORBIC ACID
CONTENTS DURING TAIL WOUND HEALING IN THE
AGAMID LIZARD, CALOTES VERSICOLOR

The investigations reported in the previous section together with those conducted earlier at the site of tail regeneration itself (Shah et al., 1971; Ramachandran et al., 1975) have given a good indication about the importance, role and source of supply of ascorbic acid (AA) in the regenerative mechanics of reptiles. The present investigation is a sequel to the studies on regeneration, as it would enable in emphasising the changes observed therein as due to regeneration itself and as not a mere generalised response characteristic of injury and healing. Moreover, in the light of the reported involvement of AA in wound healing in mammals (Gould, 1963; Wolback, 1933; Bartlett et al., 1942a, 1942b; Hertzell and Stone, 1942; Hunt, 1941; Udenfriend, 1966; Kishnani, 1976; Kathuria, 1976), an investigation on these lines in a reptile Calotes

versicolor, an Agamid lizard, after its tail amputation, would go a long way in establishing not only the possible involvement of AA in the healing mechanics of reptiles in general (as no reports exist on submammalian groups), but also help in bringing out the possible similarities or dissimilarities if any that may be inherent between mammals and reptiles in the purported association of AA with healing. At the same time, the role of AA during wound healing as opposed to regeneration in reptiles also needed to be elucidated and clearly differentiated. It was with this view the present study on the changes in the caudal, hepatic and renal AA contents during tail wound healing (after tail amputation) in the Agamid lizard, Calotes versicolor, gained the necessary motivation.

MATERIAL AND METHODS

Adult freshly captured healthy lizards obtained from the local animal dealer and maintained on a diet of insects were used as the experimental animals. After acclimatising the animals to the laboratory conditions for about a fortnight, the tails were

amputated at a fixed distance from the vent. The animals were sacrificed at regular intervals of 3, 5, 7, 12 and 25 days postamputation, and the samples of ^{the} healing tail, liver and kidney were obtained, and in these tissues the content of AA was quantitatively assayed by the method of Roe (1954). The data obtained were then equated with the normal levels (animals with intact tails) of AA within the tissues referred above.

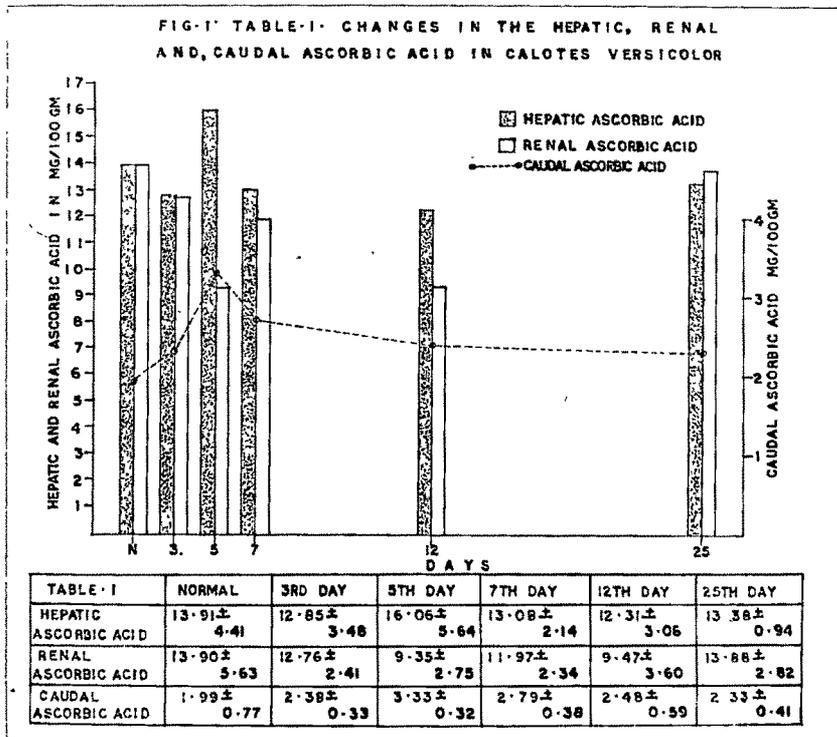
RESULTS

HEPATIC ASCORBIC ACID

The hepatic AA showed an initial decline on the 3rd day followed by the attainment of a maximal level (above normal) on the 5th day. By day 7 however, the AA content touched the normal level and the level was maintained so thereafter excepting for a slight fall on the 12th day.

RENAL ASCORBIC ACID

The renal AA content showed a continuous depletion through the 3rd day upto the 5th day postamputation whence the lowest level was attained. The AA content of the kidney was noted to show a slight recovery on



the 7th day to fall again on the 12th day. By day 25 however, the renal AA content returned to the normal preamputation level.

CAUDAL ASCORBIC ACID

Subsequent to amputation the caudal AA content was noted to show a continuous and gradual increase through the 3rd day to the 5th day whence the highest level was recorded. The AA content after having gone up, since then was noted to show a sudden fall on the 7th day followed by a very gradual decrease on the 12th and 25th days postamputation. The levels of AA observed on the 7th, 12th and 25th days were, however, above normal.

The changes in the content of AA in all the three organs are presented in Table - 1, Figure - 1.

DISCUSSION

The data obtained herein highlight the significant fact that in the case of Calotes unlike in Mabuya, both the kidney (organ of synthesis) as well as the liver (organ for storage) have more or less the same concentration of AA. Another noteworthy feature of

mention again of a comparative nature, that may be brought out at the very outset, is that the level of AA in the kidney never registered an above normal value at any stage during the course of tail wound healing in Calotes. The most significant change observed subsequent to the tail amputation and wound healing was the continuous and gradual accumulation of AA at the wound site through 3rd to the 5th day. Interestingly enough, in the present case too as in Mabuya, the hepatic tissue responded to the stress of tail amputation by an initial depletion of AA recorded on day 3rd post amputation. In this light, it may be tentatively postulated that the hepatic tissue which functions as a store house of AA in reptiles, responds in an identical fashion to the stress of injury (at least of a major nature) by an initial depletion which may vary in degree in various species. It is also rather evident that the renal tissue is more or less the principal source of supply for AA that is noted to accumulate in the tail (wound site) during the early period after injury.

The role of AA in fibroplasia and collagen synthesis associated with the formation of a granulation

tissue, and the resultant gain in tensile strength, which usually serves as an index of normal and proper wound healing have been well established. (Howes et al., 1929; Botsford, 1941; Sandblom, 1944; Localio et al., 1943; Dumphy and Udupa, 1955; Dumphy et al., 1956; Gould, 1963; Wolbach, 1933; Barlett ^tet al., 1942a, 1942b; Hertzeld and Stone, 1942; Hunt, 1941). Moreover, Gould (1963) and Udenfriend (1966) have suggested the effect of AA on collagen synthesis to be in the hydroxylation of proline to hydroxyproline in trophocollagen fibrillogenesis which as shown by Viljanto (1964) depicts a parallel correlation with the increase in the tensile strength. Viewed in this background the present observation of an initial accumulation of AA in the healing tail tissues during the first 5 days postamputation and the subsequent reduction recorded on the 7th day, and the maintenance of the same level since then (Table - 1, Figure - 1), and the fact that the formation of dermal substance subjacent to the wound epithelium and the completion of the wound closure occurs in Calotes round about the 7th day, are rather self explanatory and indicate the definite role of AA in the healing mechanics in this lizard.

At about the same time, the hepatic AA content after the initial fall as noted on the 3rd day, however, soon reversed the trend and attained the highest level on the 5th day. This appears to be quite in contrast to the situation in Mabuya (previous section) wherein the hepatic AA concentration, after the initial fall on the 3rd day was noted on the 5th day, to climb up towards the normal level which was ultimately attained by about the 12th day. Though the hepatic tissue could be considered to draw this extra AA from the kidney, the exact role and the functional significance of this rise in the AA content at this stage appear a bit perplexing and needs elucidation. However, this period of AA accumulation in the liver coincides with the early stages of fatty infiltration and the development of a fatty liver condition (chapter - 2). Moreover, the period from 5th to 12th day postamputation is marked by increasing titres of neutral lipids and glycerides (Section II of chapter - 4) and decreasing level of phospholipids (Section II of chapter - 5). The possibility of phospholipid breakdown and its transformation into neutral lipids was presumed to be probably involved in the etiology

of fatty liver development in Calotes (Section II of chapter - 4). Another factor of significance is the altered enzymatic machinery of the liver of Calotes in conjunction with the development of fatty liver (unpublished data). In this context, the possible involvement of AA in phospholipid breakdown as well as the enhanced enzymatic machinery associated with the development of fatty liver cannot be overlooked, and the reports of Rush and Kline (1941), Abramson (1949), and Chinoy (1969a, 1970, 1971a) who have correlated AA with phospholipid breakdown as well as metabolic activity respectively, are the compelling evidences in favour, which help in stabilising the concept hinted at. Interestingly enough, the changes in the content of hepatic AA is strikingly similar and parallel to that of hepatic phospholipids reported in (Section II of Chapter - 5). Such a parallelism in operation between AA and phospholipids adds further validity and support to the contention.

With the possible withdrawal of AA from the kidney to the liver and the resultant increase in the AA concentration of liver, there is a corresponding depletion of AA in the renal tissue on

the 5th day (Figure - 1, Table - 1). This fall in the renal AA content, possibly by falling in the physiological range of the low threshold level characteristic of the tissue, might be acting as a feed back stimulus thus goading the renal tissue to gear up the synthetic pathway which ultimately results in the elevation of the renal AA content recorded on the 7th day postamputation. However, there appears to be further period of AA utilization, as marked by the reduced content obtained on the 12th day. Though the fall in the AA content of the liver too, noticed at this period might be considered as a continuous one, as could be visualised from Figure - 1, as well as the observed parallel decrease of the hepatic phospholipid content (Chapter - 5, Figure - 1), which are both in line with the development of the fatty liver condition, the fall in the renal AA content observable at this stage is a bit intriguing and rather difficult to explain. By looking at the figure depicting the changes in the hepatic AA content it becomes evident that though the fall in the AA content between the 5th and 7th day is more drastic and sudden, that between the 7th and 12th days is rather low. If it be considered

that there is a substantial requirement of AA, and that the hepatic tissue is dependent on the renal tissue for adequately meeting the requirement, the maintenance of a plateau by the hepatic AA content between the 7th and 12th days (in the wake of a continuous and increased rate of utilization or participation in the pathophysiological mechanics of fatty liver) as well as the concomitant drop in the renal AA content noted during the same period could be easily tied up. Moreover, in the light of the known fact that the formation of the dermal substance and enhanced collagen fibrillogenesis leading to the increase in the tensile strength of the wound take place round about this period, the maintenance of a plateau level of AA in the healing tail as well (see Table - 1, Figure - 1), might be another factor of significance which could also possibly account for the fall in the renal AA concentration. Possibilities of the renal AA playing a role in the altered physiological functioning of various other organs (associated with the pathophysiology of fatty liver) as yet unknown also cannot be overlooked.

With the completion of the healing process as

well as the recovery from the fatty liver condition, the increase in the AA content of both the kidney as well as the liver observed between the 12th and 25th days postamputation, though finding a parallelism with that of Mabuya during its tail regeneration, cannot however be equated together. Whereas in the case of Mabuya in the face of a stepped up rate of synthesis right from the time of tail autotomy, there was, with the completion of regeneration and cessation in the rate of its utilization, a piling up of extra AA leading to the above normal level, in the present case, the increase appears to be due to a triggering of the synthetic process mediated by the prevailing subnormal level thus leading towards the attainment of the normal level. Finally, on a comparative note it may be mentioned that regeneration with its greater and protracted need, entails a higher rate of turnover and/or mobilization of AA, as marked by the attainment of above normal levels of AA in the kidney of Mabuya (as reported in the previous section), in contrast to the mere wound healing process in Calotes, wherein the requirement being modest, the renal AA content never in the course of the healing process rose to an above normal value.