# CHAPTER 8

## QUANTITATIVE AND HISTOCHEMICAL STUDIES ON ASCORBIC ACID IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

Apart from secondary morphogenetic processes the regenerative process also calls for many biochemical reactions concerned with metabolism, synthesis and tissue lysis stepped up to meet the contigency of the regenerative mechanics. Synthesis of new materials are of paramount importance forming an essential feature of regeneration and for this the raw materials and energy are made available by the breakdown of available metabolites. Such indigenous transformations cause reactivations of specific metabolic pathways which had hitherto remained unimpressive. The stepped up metabolic processes and the synthesis of new materials in a biological system usually involve many cofactors. Ascorbic acid (Vitamin C) is now increasingly being realized as a versatile cofactor involved in multitudes of biochemical reactions. It is presumed to play some role in certain enzyme catalyzed reactions especially that of dehydrogenases (Burns et al., 1951; Meiklejohn, 1953). Gould (1963) has ascribed ascorbic acid a role in collagen synthesis as well. Importance of

this vitamin in wound healing and regeneration has also been hinted (Gould, 1963; Ryvkina, 1940; Bourne, 1942). Apart from the availability of only one isolated report on ascorbic acid during regeneration in Hemidactylus flaviviridis (Shah et al., 1971), the detailed studies undertaken in this laboratory on the metabolic aspects of the normal and regenerating tail of the Scincid lizard, Mabuya carinata, in the light of the above speculated roles of ascorbic acid (AA) have prompted and necessitated the present study on this vitamin.

## MATERIAL AND METHODS

Adult Mabuyas obtained from local animal dealer and maintained in the laboratory on an insect diet were used for the present study. The autotomy of the normal and regenerating tails was induced by pinching them off with necessary force, and the cut surfaces of the tails were blotted to remove blood and tissue fluids. The tail pieces were then immediately fixed on the microtome chuck of a cryostat maintained at -20°C and sectioned at 12-18 $\mu$ thickness.

Histochemical demonstration of ascorbic acid (AA) was carried out according to the method of Giroud and Leblond (1936) as modified by Chinoy (1969a,b). For control, sections were devitaminized by keeping them for 3 hours in 10% neutral formalin before fixation.

For quantitative estimation of total AA content, the autotomized tails were weighed and homogenized in 6% Trichloroacetic acid (TCA) in prechilled mortars. Aliquots of these extracts were utilized for the determination of AA levels in the normal and regenerating tails, employing the dinitrophenyl hydrazine method of Roe et al. (Roe, 1954).

## RESULTS

The quantitative data on the changes of ascorbic acid in the tail tissues during various phases of regeneration is presented in Table 8.1 and Fig. 9.

NORMAL TAIL (Figs. 1 and 2)

Quantitatively, the normal tail had lesser concentration of AA (1.226 ± 0.1092 mg/100 g fresh tissue) than what was reported for the normal tail of the house lizard, Hemidactylus flaviviridis

(4.881 ± 1.307) by Shah and coworkers (1971). The AA was found histochemically present in all the tissues such as epidermis, dermis, muscles, vertebral elements and nerve cord, excepting the adipose tissue, where it was little or almost nil. Of these tissues, the AA concentration was found to be relatively higher, in the skeletal elements viz., bone matter of vertebrae, scutes, scales of the skin and the caudal muscles.

REGENERATING TAIL

Wound healing phase: (Fig. 3)

The cells of the wound epithelium together with the cellular aggregates at the sub-epithelial region revealed a much higher concentration of AA than what was observable in the cells of the epidermis of the skin in the tail stump. The injured regions of the stump tissues also revealed a slight increase in the AA content. This increase of AA in the above mentioned tissues could be well realized from the quantitative data which showed a two fold increase (2.235 ± 0.2640 mg/100 g of fresh tissues) from that of the normal tail (Table 8.1 and Fig. 9).

## EXPLANATIONS FOR FIGURES

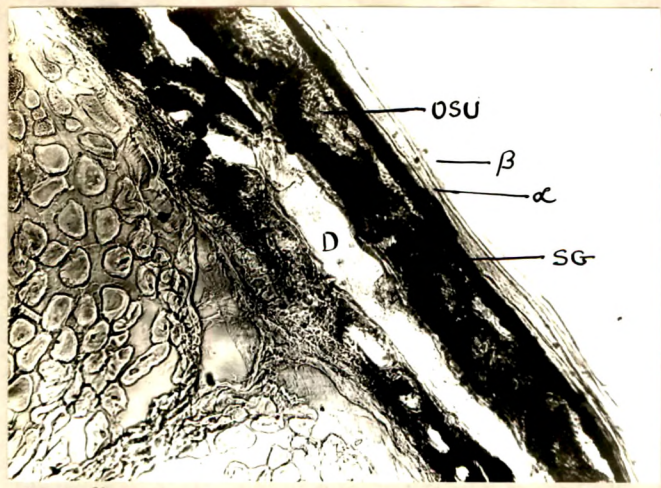
Fig. 1. T.S. of the normal skin revealing the ascorbic acid content in the epidermal and dermal components viz., stratum germinativum, scutes and dermis.

Fig. 2. Photomicrograph of the T.S. of caudal muscles revealing AA content.

Fig. 3. Photomicrograph of an enlarged portion of the wound epithelium and the subapical region revealing high AA content.

## ABBREVIATIONS

| | | |
|---|---|---|
| Œ | - | Alpha cells |
| β | - | Beta cells |
| D | - | Dermis |
| OSU | - | Osteoscutes |
| SG | - | Stratum germinativum |
| WE | - | Wound epithelium |

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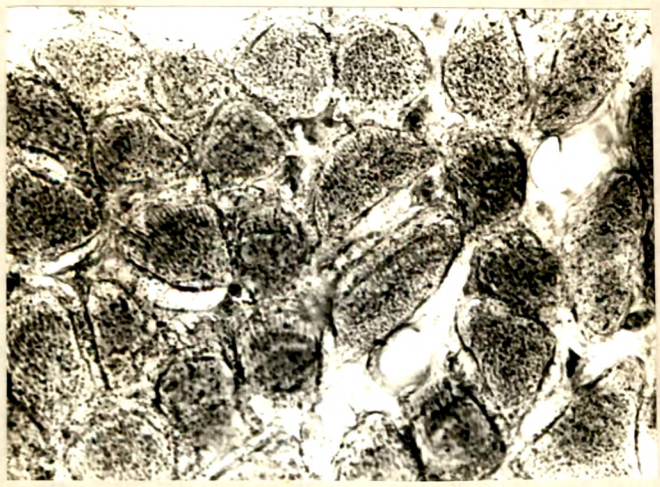


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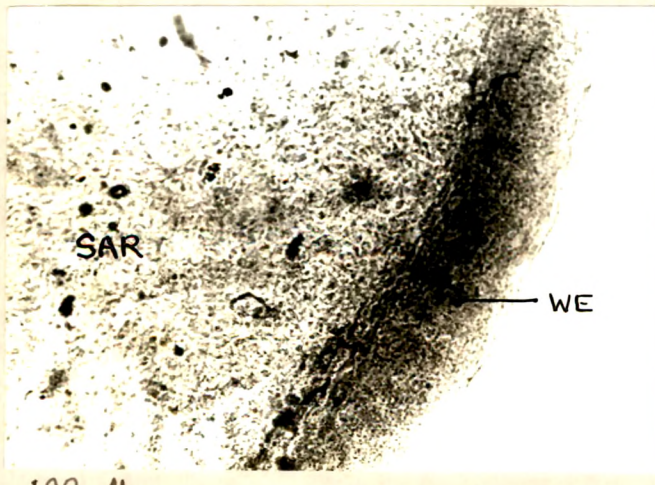


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## EXPLANATIONS FOR FIGURES

Fig. 4. L.S. of the blastema showing the AA distribution. Note the AA granules in the mesenchymal and the blastemic epithelial regions.

Fig. 5. Photomicrograph of the differentiating tail exhibiting AA contents in the various differentiating tissues.

## ABBREVIATIONS

BE - Blastemic epithelium

CNC - Cartilagenous neural canal

DM - Differentiating muscles

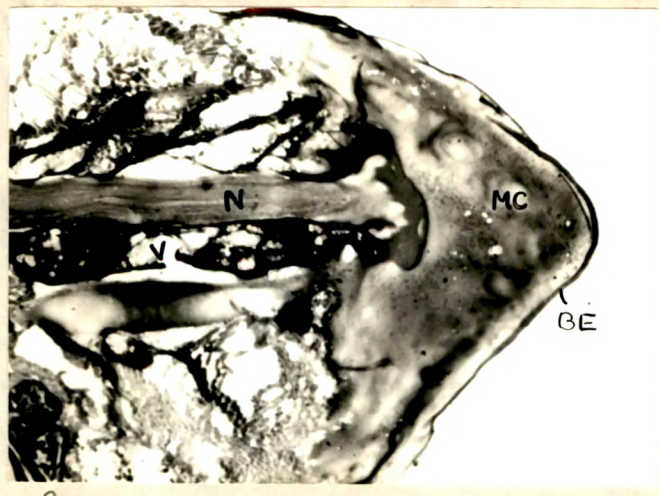
DSC - Differentiating scales

E - Ependyma

MC - Mesenchymal cells

N - Nerve cord

V - Vertebra

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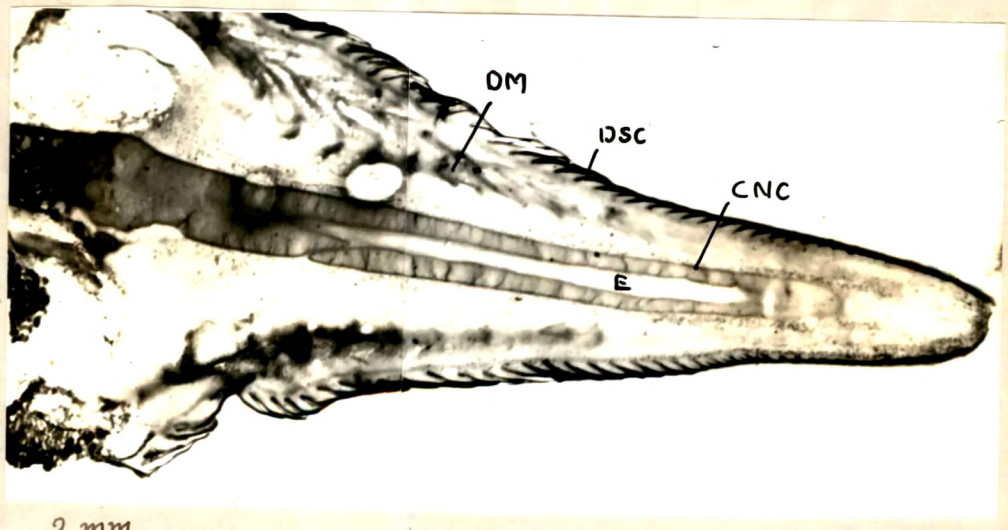


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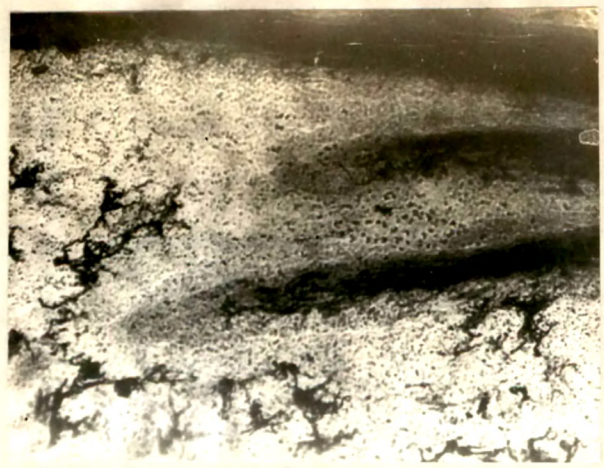
## EXPLANATIONS FOR FIGURES

Fig. 6. Photomicrograph of magnified region of the differentiating scales revealing high content of AA.

Fig. 7. Photomicrograph of magnified region of the differentiating muscles revealing very high content of AA.

Fig. 8. Photomicrograph of magnified region of the differentiating chondrocytes of the cartilagenous neural canal revealing high content of AA.



100 μ

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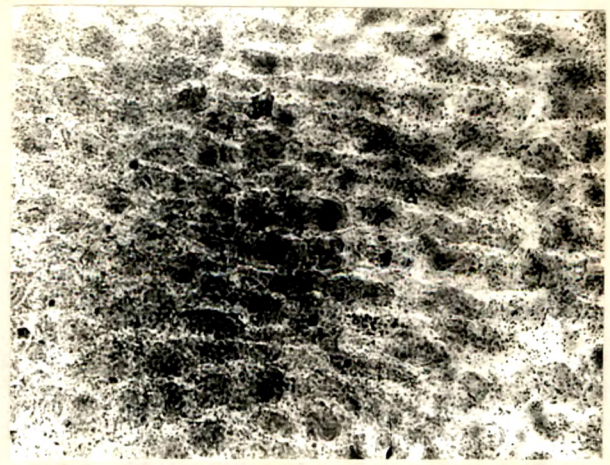

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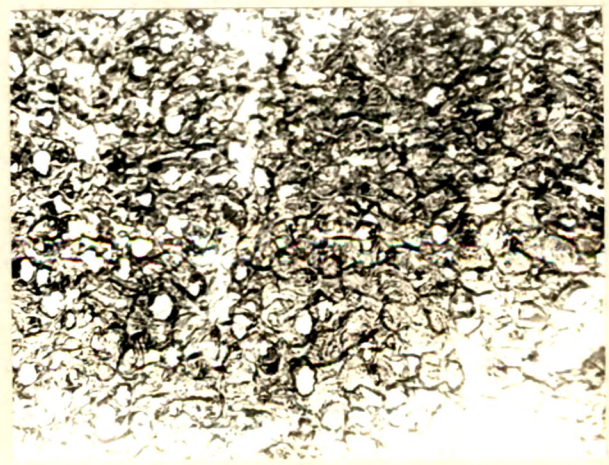

100 μ

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TABLE 8.1

Levels of total ascorbic acid in the normal and regenerating tail of the Scincid lizard, Mabuya carinata

| Normal tail and different phases of regenerating tail | Amount of AA (mg/100 g fresh tissue) | Number of estimations performed |
|---|---|---|
| Normal tail | 1.226 ± 0.1092** | 15 |
| Regenerating tail* | | |
| Wound healing phase (4-6 days)+ | 2.235 ± 0.2640 | 13@ |
| Blastema phase (7-12 days) | 1.269 ± 0.1272 | 12@ |
| Differentiation phase (13-20 days) | 5.251 ± 0.3561 | 12 |
| Growth phase (30-40 days) | 3.670 ± 0.5196 | 12 |
| Fully regenerated tail (60-70 days) | 1.344 ± 0.1936 | 15 |

*The phases of regeneration are arbitrarily defined for the purpose of discussion, though the process of regeneration is a continuous one.

@Tissues from atleast 5-10 animals were pooled for each estimations performed.

+Number of days after autotomy of the tail.

**Mean ± S.D.

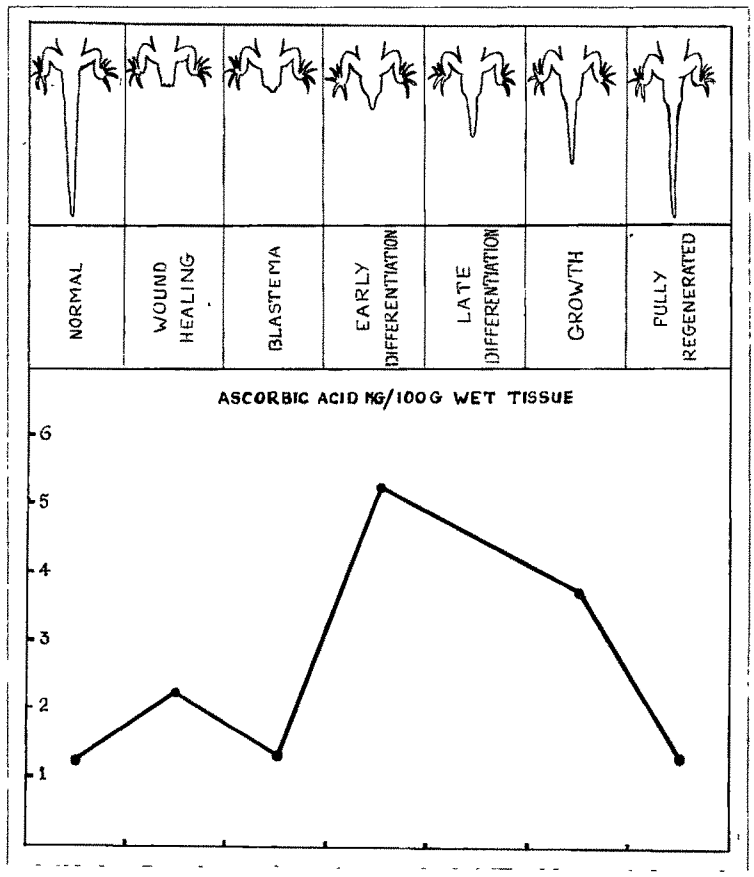


Fig.9. Graphic representation of the levels of total ascorbic acid in the normal and regenerating tail of the Scincid lizard, Mabuya carinata.

Blastemic phase: (Fig. 4)

In this phase the stratified epithelium revealed a higher concentration of AA than the mesenchymal cells of the blastemic cone. However, this concentration of AA in the epithelial cells was noticeably lower than that of the wound epithelium. The amount of AA likewise registered a fall to the level corresponding to that of normal tail (Table 8.1).

Differentiation phase: (Figs. 5,6,7 & 8)

Both quantitative as well as histochemical studies have revealed the highest content of AA at this phase. Increased localization of AA was evident in all the differentiating tissues such as skin, muscles and chondrocytes. A five fold increase of AA content could be well evidenced quantitatively (Table 8.1).

Growth phase to fully regenerated tail:

During this phase the various already differentiated tissues and now undergoing morphological and physiological growth and maturity showed a gradual decline in their AA content, eventually reaching a concentration and localization in the fully regenerated tail, very much

identical and similar to that of the corresponding tissues of the normal tail.

## DISCUSSION

The present histochemical and quantitative studies on AA content in the normal and regenerating tail of the Scincid lizard, Mabuya carinata have revealed ∉ significant fluctuations in its contents. The first sign of increased AA content was evidenced in wound healing phase and a second peak level appeared during the differentiation phase. AA thus appears to have some significant stepped up role during these two phases of regeneration.

The wound healing phase is characterized by the formation of the ground substance covering which in due course gets cast off along with the scab matter when the epithelium is fully formed underneath. The two fold increase in AA noticed at this very phase in the regenerating tail of Mabuya carinata gains validity by the reported association of this vitamin with the wound healing process (Bourne, 1953; Zamanskii and Lopushanskii, 1955; Schauble et al., 1960; Crandon et al.,

1961; and Ksabyan, 1956). A similar increase of AA during wound healing has been reported by Shah et al. (1971) in the tail of Hemidactylus flaviviridis.

A second peak five fold increase in AA level noted during differentiation herein coincided with increased metabolism as reflected by the high activities of the various oxidative enzymes (Ramachandran, 1972) during this period. The parallel increase of AA during the differentiation and the corresponding high activities of TCA cycle enzymes such as succinate dehydrogenase (SDH) isocitrate dehydrogenase (ICDH) and malate dehydrogenase (MDH) (Shah and Ramachandran, 1970 and Ramachandran, 1972) could be explained by taking into account the suggestions of Banerjee et al. (1959) that AA is necessary for the proper functioning of Krebs cycle enzymes.

It is also a known fact that AA dostake part in electron transport chain, by transformation into its free radical; the monodehydroascorbic acid, which is more powerful electron donor than AA itself, thus playing an identical role as cytochrome oxidase (Goodwin, 1960; Mapson, 1953; Meiklejohn, 1953 and Chinoy, 1969a). Thus the reported low level of

cytochrome oxidase in comparison to the TCA cycle enzymes mentioned above (Ramachandran, 1972) may be compensated by the high level of AA in these tissues.

The high level of AA and a concomitant increase of glycogen in the differentiating tissues (Chapter 3) reaching to a maximum level by the late differentiation phase is in agreement with the contention of Banerjee and Ganguli (1962) that the glycogenetic influence of AA is on hexokinase activity, which ultimately facilitates first the uptake of glucose and then the synthesis of glycogen in the tissues. About five fold increase in AA content during differentiation phase strengthens Brachet's view (1950a) that AA promotes differentiation rather than growth.

Once the regenerate reaches its fully grown state, by about 70 days after autotomy, its AA content also comes to a level corresponding to that in the normal tail; which denotes the completion of the process of tail regeneration, where morphological and physiological maturity and metabolic pattern have more or less settled to the normal state.