# CHAPTER 4

## HISTOCHEMICAL OBSERVATIONS ON ACID PHOSPHATASE IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

Particular attention has been given to phosphatases since Gomori (1939, 1941) introduced histochemical methods for their demonstration in various tissues. Acid phosphatase is known to hydrolyze phosphate esters optimally in an acidic environment. Since its identification with other hydrolases in a group of cytoplasmic bodies, the lysosomes (Duve, 1959; Novikoff, 1961, 1963) many further studies have been carried out. Some information has been obtained regarding phosphatases in regenerating tissues of amphibians. Kambara (1955) has demonstrated phosphatase activity in the epidermis, dermis, subcutaneous glands and muscles of the adult newt, Triturus pyrrhogaster. Ghiretti (1950) found two peaks of activities of acid phosphatase in the regenerating Triturus cristatus tail homogenates and has correlated them with growth of the blastema and the differentiative process during regeneration. However, Hahn (1960) could detect only a single peak of acid phosphatase activity during the blastemic

phase in the regenerating tail of the tadpole, Xenopus laevis; and subsequently a gradual decline in its activity as the rate of regeneration slowed down. Schmidt (1963a) studied the histochemical localization of acid phosphatase in the forelimb of the adult newt, Diemictylus viridescens and suggested the possible role of this enzyme in association with ribonucleic acid, polysaccharide, protein and lipid metabolism. However, little information is available regarding the acid phosphatase activity in regenerating tissues of lacertilians. Recently, Shah and Chakko (1966a) studied histochemically the distribution pattern of acid phosphatase in the normal and regenerating tail of the adult house lizard, Hemidactylus flaviviridis.

The present study on the histochemical localization of acid phosphatase in the normal and regenerating tail of the Scincid lizard, Mabuya carinata has therefore been undertaken not only for the understanding of the role of this enzyme in the regenerating lacertilian tails in particular but also during vertebrate regeneration in general, and also to note points of similarities and or differences that may exist between the similarly regenerating

tails in the two lizards belonging to two different groups *viz.*, Gekkonidae and Scincidae.

## MATERIAL AND METHODS

The adult lizards, *Mabuya carinata* maintained in the laboratory on insect diet were used as the experimental animals. The autotomy was induced as described in Chapter 1. Sections of 12-18 μ thickness were cut in a cryostat maintained at -20°C and were fixed in chilled acetone for one hour. The sections were thoroughly washed in cold distilled water and the histochemical demonstration of acid phosphatase was carried out according to the method of Burstone (1958a). Naphthol AS-BI Phosphate (Sigma Chemical Company, U.S.A.) was used as the substrate and Fast Blue B or Red Violet LB (Sigma Chemical Company, U.S.A.) as the coupling salt. The incubation medium was freshly prepared and buffered at pH 5.2 with 0.2M sodium acetate. Sections were incubated at room temperature (29-32°C) in a medium devoid of substrate served as controls. After the incubation, both the sample and the control sections were thoroughly washed in distilled water and mounted in glycerine jelly.

## OBSERVATIONS

Immediately after the sections were transferred to the incubation medium, the vertebral elements of the normal and the regenerating tails showed response towards the acid phosphatase activity. Within one hour of incubation the chondrocytes revealed the maximum enzyme activity. A prolonged incubation of about 6-8 hours was required for the skin, nerve cord and the connective tissues to respond towards acid phosphatase so as to reach a maximum at the 8th hour of incubation. Incubation for more than 8 hours did not have any further change in the activity of this enzyme.

### NORMAL TAIL

The cells of the alpha layer and the stratum germinativum showed fairly high activity of acid phosphatase with no activity what-so-ever in the beta cell layers of the skin (Fig.1). The scutogenic cells which are embedded in the dermis and the dermal connective tissues showed moderate activity of the enzyme. A granular localization of the enzyme was noticed at the site of its activity. The caudal muscles and the submuscular adipose tissue were both enzyme negative. An intense activity of acid phosphatase was observed

in the osteocytes, some of the bone marrow cells and the chondrocytes at the articular surfaces of the caudal vertebrae. The enzyme was very active in the bone cells at the breaking plane (plane of autotomy) of the vertebra. An activity of low intensity could be observed in the grey matter of the nerve cord whereas in the white matter it tended to be negligible.

REGENERATING TAIL

Wound healing phase: (Fig. 2)

The cells of the wound epithelium showed a very high activity of acid phosphatase. The cellular aggregate underlying the wound epithelium was also noted to show a response towards the enzyme activity, though slightly less than the wound epithelium. The cut end of the original stump tissues showed a slight increase in activity of the enzyme than that observed in the corresponding tissues of the normal tail.

Preblastemic and blastemic phase: (Fig. 3)

Though an increased level of the enzyme activity could be noticed in the mesenchymal cells underlying the stratified blastemic epithelium, the epithelium as

## EXPLANATIONS FOR FIGURES

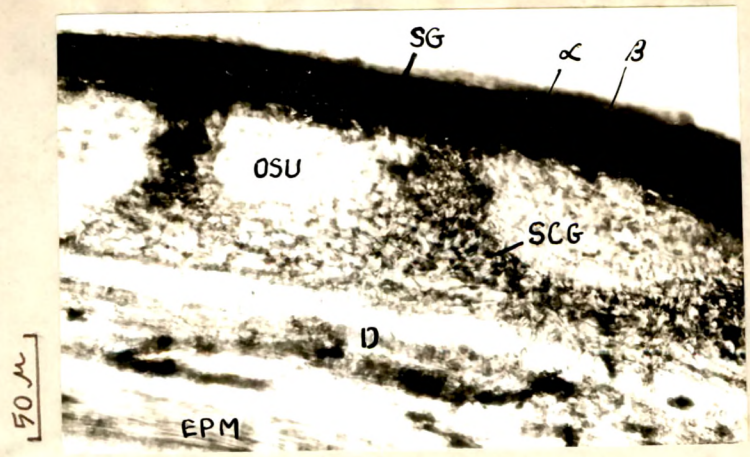
Fig.1. Magnified region of the normal skin showing acid phosphatase activity in the alpha cell layer and stratum germinativum.

Fig.2. Photomicrograph of the longitudinal section of tail regenerate at the wound healing phase. Note the high acid phosphatase activity in the wound epithelium, cut end of the muscles and the cut end of the vertebra.

Fig.3. Longitudinal section of the blastema showing high content of acid phosphatase in the mesenchymal cells and the blastemic epithelium.

## ABBREVIATIONS

| | | |
|---|---|---|
| $\alpha$ | - | Alpha cells |
| $\beta$ | - | Beta cells |
| BE | - | Blastemic epithelium |
| CEM | - | Cut end of the muscle |
| CEV | - | Cut end of the vertebra |
| D | - | Dermis |
| EPM | - | Epimysium |
| MC | - | Mesenchymal cells |
| OSU | - | Osteoscute |
| PRC | - | Procartilage aggregate |
| SAR | - | Subapical region |
| SCB | - | Scab |
| SCG | - | Scutogenic cells |
| WE | - | Wound epithelium |

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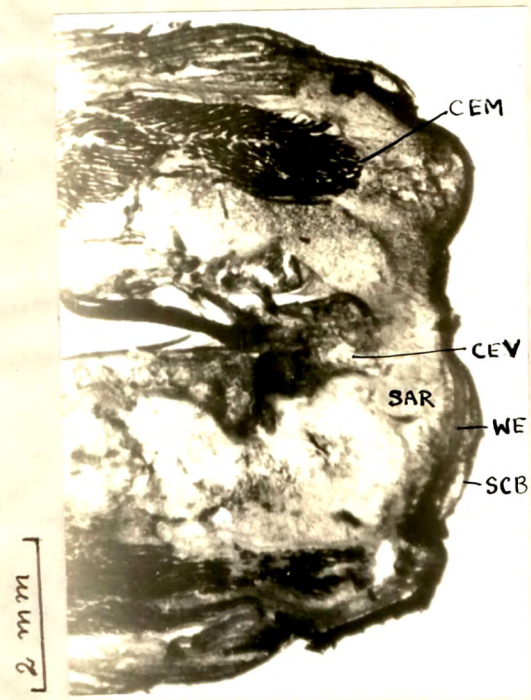


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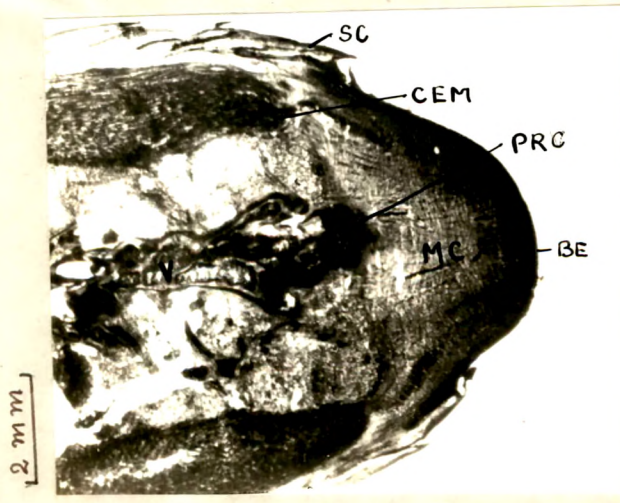


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## EXPLANATIONS FOR FIGURES

Fig. 4. L.S. of the tail regenerate at the late differentiation phase revealing acid phosphatase activity in the differentiating scales, and the cartilagenous neural canal. Note the total absence of acid phosphatase in the differentiating muscles.

Fig. 5. Photomicrograph of the differentiating scale and the dermis region revealing the acid phosphatase activity.

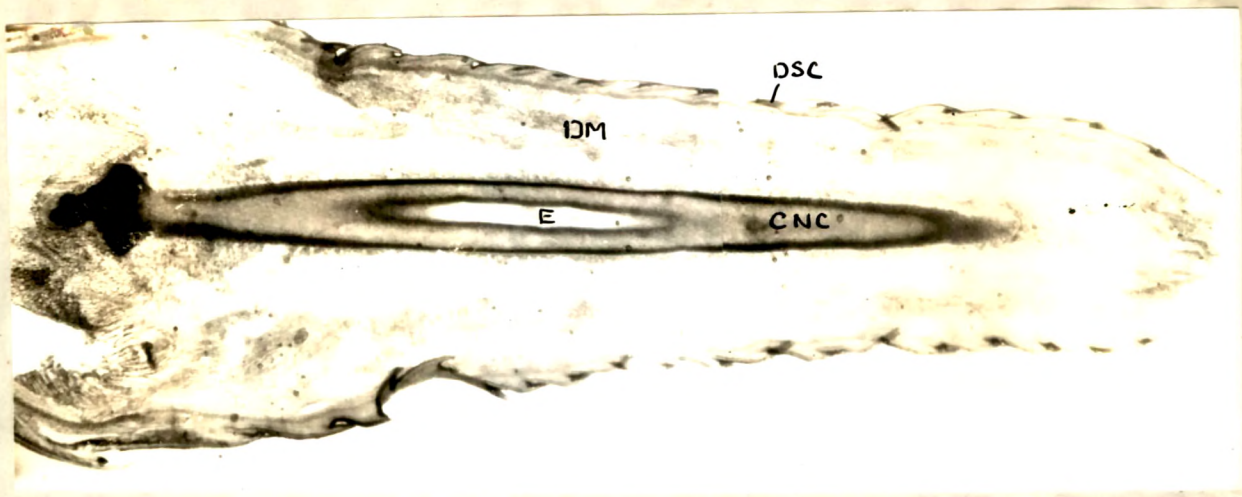
Fig. 6. Magnified region of the cartilagenous neural canal revealing the high enzyme activity in the chondrocytes.

## ABBREVIATIONS

CNC - Cartilagenous neural canal

DM - Differentiating muscle

DSC - Differentiating scale




2 mm

4

200 μ

5

200 μ

6

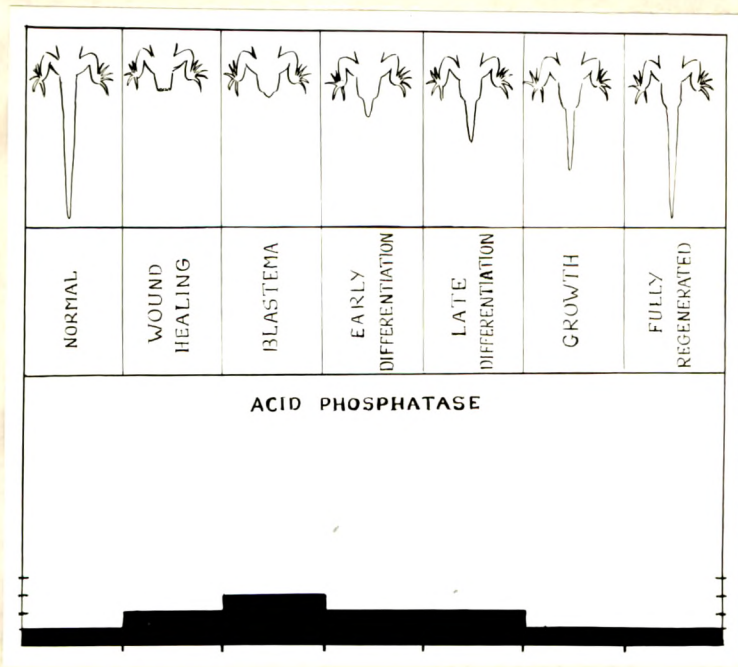



Fig.7. Graphic representation of acid phosphatase activity in the normal and regenerating tail of the Scincid lizard, Mabuya carinata.

such when contrasted with the wound epithelium did not reveal any differences in the enzyme activity. The dedifferentiating tissues at the cut end of the tail stump viz., vertebral elements, muscles, connective tissue and the dermis region showed an increased level of enzyme activity.

Differentiation phase:

During the differentiation phase, the acid phosphatase in the various differentiating complements such as epidermis, and dermis which represented a lack of the enzyme activity. However, the chondrocytes did show a slight acid phosphatase activity. A gradual reaquisition of the enzyme activity by the various cellular elements those listed above (which did not show the enzyme activity) was noticeable; while the chondrocytes of the cartilagenous neural canal represented the maximum activity, in the late differentiation phase (Figs. 5, 6). An important observation of significance however, was that acid phosphatase remained unrepresented in the muscles all throughout myogenesis (Fig. 4).

Growth phase:

During the growth phase the enzyme activity was noted to increase further towards the normal level in

the various, now well differentiated, tissues which are undergoing a process of morphological and physiological maturity. In the cartilagenous neural canal, the inner and outer peripheral chondrocytes revealed a higher enzyme activity than those centrally placed in the wall matter of the canal.

Fully regenerated tail:

With the attainment of the fully grown state, the various tissues of the regenerate demonstrated more or less exactly identical pattern of localization and intensity of the enzyme activity as was seen in the corresponding normal tail tissues.

## DISCUSSION

The presently observed acid phosphatase reactivity of the epidermis chiefly exhibited by the stratum germinativum layer of Mabuya carinata compares well with that of Kambara's (1955) report on Triturus pyrrhogaster and of Schmidt's (1963a) on Diemictylus viridescens. Taguchi et al. (1956) found that tadpole skin has a higher concentration of acid phosphatase than the adult Bufo vulgaris formosus. Epidermal reactivity of acid

phosphatase has been reported in rat (Carranza and Cabrini, 1962; Rutenberg and Seligman, 1955), in guinea pig (Carranza and Cabrini, 1962; Raekallio, 1960), and in pigeon (Kobayashi et al., 1955). A correspondence between acid phosphatase activity and protein synthesis has been suggested by Vorbrodt (1958). The high enzyme action may reflect the need for ortho-phosphate in some fundamental metabolism associated with epidermis, perhaps in the synthesis of tonofilaments and keratin proteins which are constant features of the skin undergoing ecdysis. Hence, it could be true that in the case of Mabuya carinata too this enzyme is in good association with the constant necessity for keratin, a feature well reflected in the cyclic ecdysis.

Wolf et al. (1943) in their studies of acid phosphatase in muscle tissue has noted a nuclear localization with an occasional muscle fibre reactivity. But Beckett and Bourne (1958) reported a granular enzyme response at the poles of the muscle nuclei during their studies on normal and diseased human muscles. The presence of acid phosphatase in the striated muscle of the forelimb of the adult newt,

Diemictylus viridescens (Schmidt, 1963a) has been associated with the distribution of histochemically detectable glycogen and other polysaccharides in them (Schmidt, 1962a & c). Eventhough, the glycogen loaded pigeon breast muscles have been reported to be acid phosphatase active (George and Pishawikar, 1961) and caudal muscles of Mabuya carinata are also glycogen loaded and very much comparable to the white fibres of the pigeon breast muscles (Chapter 3), the absence of acid phosphatase in them and the functional or nonfunctional significance involved herein cannot as yet be explained. It is also to be noted that similar absence of acid phosphatase has been reported by Shah and Chakko (1966a) in the normal tail muscles of Hemidactylus flaviviridis and by Gomori (1941) in the muscles of mammals.

The high activity of acid phosphatase at the planes of autotomy in the tail vertebrae in Mabuya carinata could be due to the high phagocytic activity at this points, a factor which makes these region easily breakable during autotomy. Similar explanation has been put forth by Shah and Chakko (1966a) in

Hemidactylus flaviviridis. It may be noted in this connection that Fell and Danielli (1943) had suggested the acid phosphatase activity to be a must in association with phagocytosis.

Since the lysosomes are known to store lytic enzymes especially acid phosphatase, within a lipo-protein envelope (Duve, 1959; Weber and Niehus, 1961) amongst whose functions are included intracellular digestion, autolysis and necrosis and since Raekallio (1960) has opined that the production of acid phosphatase represent one of the first steps in the repair process starting early in the so called lag-phase of wound healing, the functional significance of the presently observed high acid phosphatase activity at the wound surface of the regenerating tail of Mabuya carinata became rather self explanatory. The role of acid phosphatase in the process of wound healing has been indicated by Carranza and Cabrini (1962). An increased initial activity of this enzyme during the period of wound healing noted herein may be not only due to the phagocytic activity of the epithelial cells as suggested by Schmidt (1962a) and Singer and

Salpeter (1961) but also partly due to the migrating macrophages towards the wound surface.

Though Ghiretti (1950) has reported two peaks of acid phosphatase activity during the course of tail regeneration in adult Triturus cristatus; the first during the growth of the regeneration blastema and the second between the 15th and the 20th day in association with differentiation, the present work on the tail regeneration of Mabuya carinata revealed only a single peak of the enzyme activity extending from wound healing to blastema (Fig.7). Hahn (1960) could also notice only one peak of acid phosphatase activity during the blastemic phase of regeneration in the tail of the tadpole Xenopus. As it is known that the preblastemic and blastemic environments are acidic (Okuneff, 1928, 1929 and Schmidt, 1960) the hydrolytic function of acid phosphatase can be presumed to thrive well during this phase. This presumption gets strengthened by the reported high activity of cathepsin during these phases (Deuchar et al., 1957 and Jensen et al., 1956). The presently observed acid phosphatase along with the concomitant large quantities of ribonucleic acid (Chapter 6) observed during the blastemic phase of regeneration finds a possible

significance in the proposed relationship between acid phosphatase and ribonucleic acid in the nerve cells for the release of phosphate for cell maintenance and function (Bodian and Mellors, 1944; Lavelle et al., 1954), and draws further support from the investigations of Schmidt (unpublished) in Diemictylus viridescens and Shah and Chakko (1972) in the house lizard, Hemidactylus flaviviridis.

The activity of acid phosphatase associated with chondrocytes during chondrogenesis in Mabuya carinata during late differentiation phase seems to be in good correlation with a similar activity of acid phosphatase along with skeletogenesis reported by Ghiretti (1950) during regeneration in Triturus cristatus. Observations of a similar nature regarding acid phosphatase activity are those of Burstone (1958b) in the matrix of young bones of mammals, Ruyter (1964) in cartilage and osteogenic cells of rat, and of Schmidt and Weary (1963) in the regenerating skeleton of the forelimb of the adult newt, Diemictylus viridescens.

The reappearance of acid phosphatase in the epidermal region during the growth phase leading to

fully regenerated condition could be explained with the possible role of this enzyme in synthesis of keratin and lytic properties including intracellular digestion and phagocytosis which are to be expected during the moulting process, which would ultimately lead to an attainment of the level of enzyme complement observable in the normal tail.

Due to the widespread distribution of this enzyme in the regenerating tissues, Schmidt (1963a) has hypothesised the participation of acid phosphatase(s) in the metabolism of several substrates like nucleic acids, proteins, carbohydrates and lipids. Further, the observations of Shah and Chakko (1966a) on Hemidactylus flaviviridis and the present observations regarding the enzyme activity in different tissues of adult normal and regnerating tails of Mabuya carinata also show a similar pattern of distribution. It may be speculated in this wake that acid phosphatase may be associated with both synthetic activities as well as for phagocytosis during regeneration, depending upon the physiological state of the specific tissues involved in this process as has been envisaged by Burstone (1962).