CHAPTER 5

HISTOCHEMICAL LOCALIZATION OF ALKALINE PHOSPHATASE IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

Alkaline phosphatase is known to hydrolyze phosphorylated metabolites optimally in an alkaline medium. Moyson (1946) was the first to report the nuclear localization of alkaline phosphatase in the regenerating tissues of Rana temporaria, using the method of Gomori (1939). Similar results were obtained with this method by Junquiera (1950) and Preda et al. (1962). However, Pearse (1960) characterized this intense nuclear alkaline phosphatase response as merely an artifact of methodology. A predominant cytoplasmic activity of alkaline phosphatase was reported by Karczmar and Berg (1951) during limb development and regeneration in Amblystoma opacum and Amblystoma punctatum and Schmidt and Weary (1962) in the forelimb of the urodele, Diemictylus viridescens. These investigators found the blastemal cells and the hypertropic chondrocytes to be highly active. Moog (1943, 1944) observed the presence of phosphatases and their increase during the course of cellular differentiation in developing chick embryos. She further noted a persistent alkaline

phosphatase activity in the differentiating tissues and a change in its concentration along with the progress of differentiation. Junquiera (1950) studied the distribution of alkaline phosphatase in the regenerating tail of Xenopus laevis tadpoles and reported the high incidence of the enzyme activity in the regenerating tail. Ghiretti (1950) studied the alkaline phosphatase activity in the regenerating tail of adult Triturus cristatus and reported two maximal activities of this enzyme, one during the blastemic and other during differentiation phase. Schmidt and Weary (1962) correlated the alkaline phosphatase activity in the regenerating forelimb of the adult newt, Diemictylus viridescens with fibrogenesis and polysaccharide metabolism. Recently Shah and Chakko (1967a) studied the histochemical localization of alkaline phosphatase in the normal and regenerating tail of the house lizard, Hemidactylus flaviviridis. Apart from this, there is no other report bearing on the activity of alkaline phosphatase in regenerating reptilian appendages. This prompted the present study on the role of alkaline phosphatase in the regenerating tail of Mabuya carinata, a Scincid lizard.

MATERIAL AND METHODS

Selected adult Mabuyas were obtained from the local animal dealer and maintained in the laboratory on insect diet. Autotomy of the tail was carried out as described in (Chapter 1). The autotomized tail surfaces were blotted to remove blood and other tissue fluids and were immediately mounted of a microtome chuck of a cryostat maintained at -20°C. Sections of 12-18 μ thickness were cut and fixed in cold acetone for two hours. The sections were then washed thoroughly in cold distilled water and processed for the histochemical studies.

The histochemical demonstration of alkaline phosphatase was carried out according to the method described by Burstone (1958a, 1961) using Naphthol AS-MX Phosphate (Sigma Chemical Company, U.S.A.) as the substrate and Fast blue B (BBN) (Sigma Chemical Company, U.S.A.) as the diazonium salt. The incubation medium buffered at pH 9.2 with 0.2M Tris was freshly prepared. The sections were incubated for 30 minutes to 10 hours at room temperature (29-32°C).

Sections incubated in a buffered medium devoid of the substrate served as the controls. After

incubation, the sample as well as the control sections were thoroughly washed in distilled water and mounted in glycerine jelly.

OBSERVATIONS

Immediately after keeping the sections for incubation, the skeletal elements of the normal and the different phases of the regenerating tail showed the enzyme response and reached to a maximum by 60 minutes of incubation, but the muscles and epidermal cells took relatively longer time that varied from 8-10 hours of incubation. The muscle took about 10 hours to show the maximum activity of alkaline phosphatase.

NORMAL TAIL (Fig. 1)

The outer beta and alpha layers of the skin were enzyme negative, while the cells of the stratum germinativum showed appreciable enzyme localization (Fig. 2). A diffused activity of alkaline phosphatase could also be noticed in the connective tissue elements of dermis. The caudal muscles depicted both sarcoplasmic as well as mitochondrial localization of alkaline phosphatase whereas there was no activity of the enzyme in the submuscular adipose tissue (Fig.3).

In the vertebrae, the cartilage cells at the articulating surfaces of the centra showed a high enzyme response, however, the marrow cells demonstrated a mild reactivity. The enzyme could be noticed in the nerve cord also with the activity tending to be higher in the grey matter than in the white matter.

REGENERATING TAIL

Wound healing phase: (Fig. 4)

The highest level of enzyme activity during this phase was noticed at the cut end of the vertebra in the stump of the tail with an appreciable activity in the wound epithelium and a slightly lesser level in the cells present subjacent to the epithelium.

Blastemic phase: (Fig. 5)

The cut end of the original tail tissues continued to show a high activity of alkaline phosphatase even at this stage. The transformation of the wound epithelium into the blastemic epithelium was marked by an increase of enzyme activity. The mesenchymal cells forming the core of the blastema though showing lesser than the epithelium were nevertheless enzyme reactive.

EXPLANATIONS FOR FIGURES

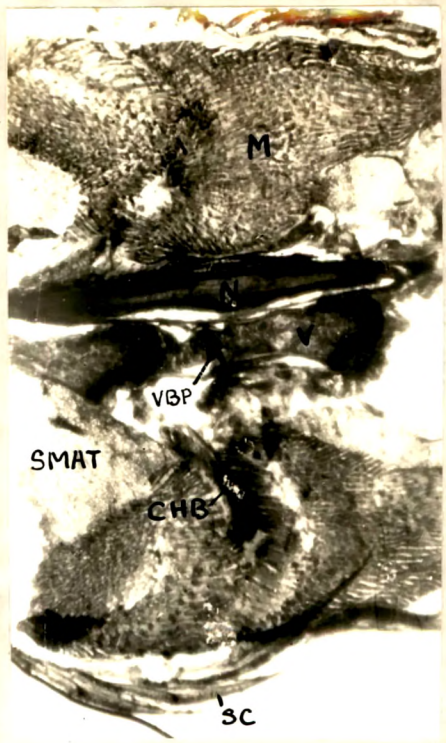
Fig. 1. Photomicrograph of the longitudinal section of the normal tail exhibiting the alkaline phosphatase activity in caudal muscles, vertebral elements and in nerve cord.

Fig. 2. T.S. of normal skin revealing the enzyme activity in the stratum germinativum, and the scutogenic cells.

Fig. 3. T.S. of the caudal muscles showing the enzyme localization in sarcoplasm as well as in mitochondria.

ABBREVIATIONS

α	-	Alpha cells
β	-	Beta cells
EPM	-	Epimysium
M	-	Muscle
N	-	Nerve cord
SCG	-	Scutogenic cells
SG	-	Stratum germinativum
SMAT	-	Submuscular adipose tissue
V	-	Vertebra
VBP	-	Vertebral breaking plane



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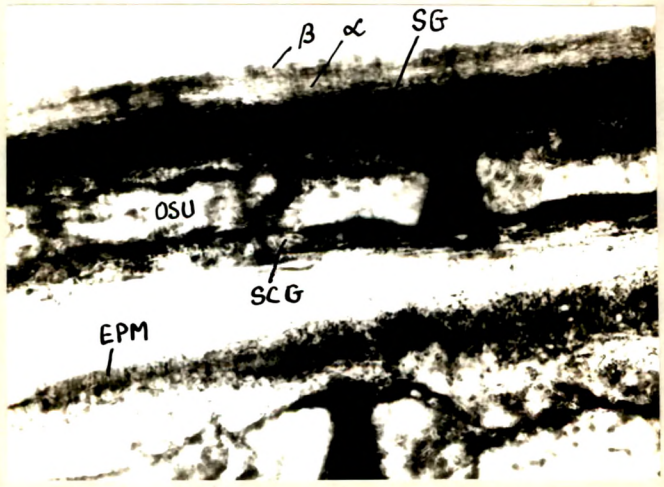

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EXPLANATIONS FOR FIGURES

Fig. 4. Photomicrograph of the longitudinal section of the tail regenerate at wound healing phase. Note the enzyme activity in the wound epithelium and cut end of the muscles.

Fig. 5. Longitudinal section of blastema showing alkaline phosphatase activity in the blastemic epithelium and the mesenchymal cells.

ABBREVIATIONS

BE - Blastemic epitheliu

CEM - Cut end of the muscle

CHB - Chevron bone

MC - Mesenchymal cells

NS - Neural spine

PRC - Procartilage aggregates

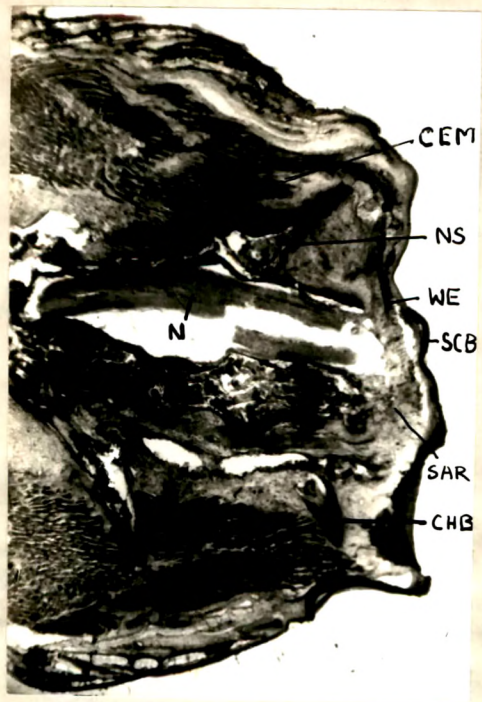
PRM - Promuscle aggregates

SAR - Subapical region

SC - Scale

SCB - Scab

WE - Wound epithelium



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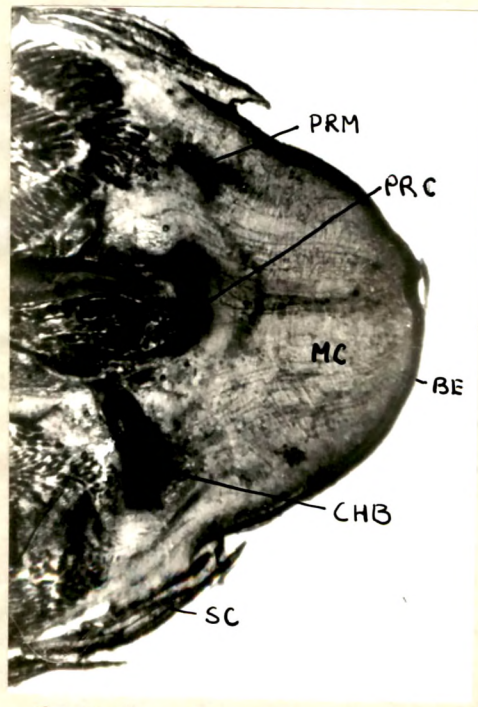

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EXPLANATIONS FOR FIGURES

Fig. 6. Longitudinal section of the tail regenerate at the differentiation phase. Note the high activity of the enzyme alkaline phosphatase in the differentiating muscles, scales and the chondrocytes of the cartilagenous neural canal.

Fig. 7. Magnified region of the differentiating scale and the dermis region revealing the enzyme activity.

Fig. 8. Higher magnification of the differentiating muscles exhibiting alkaline phosphatase activity.

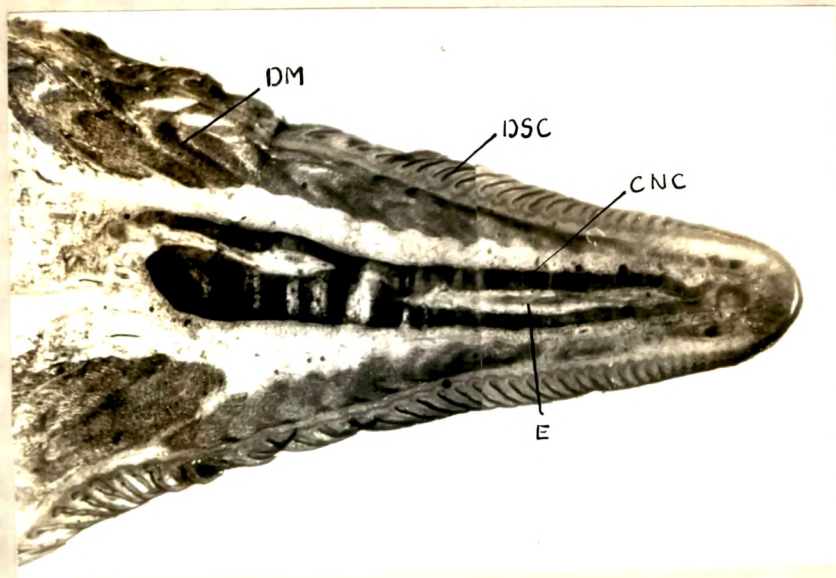
ABBREVIATIONS

CNC - Cartilagenous neural canal

E - Ependyma

DM - Differentiating muscle

DSC - Differentiating scale



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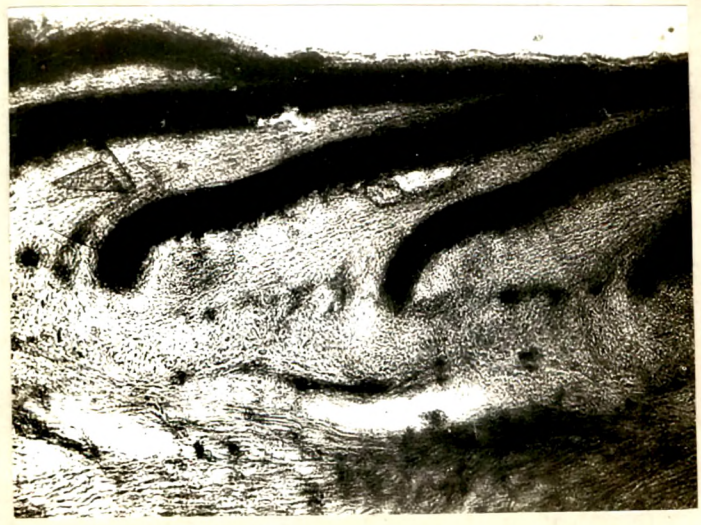

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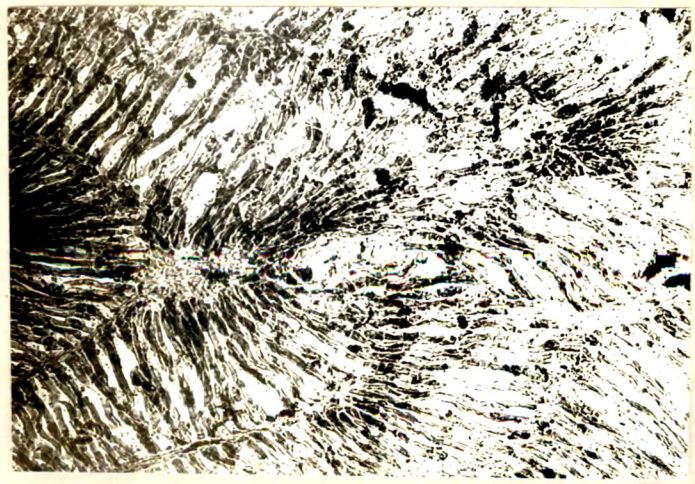

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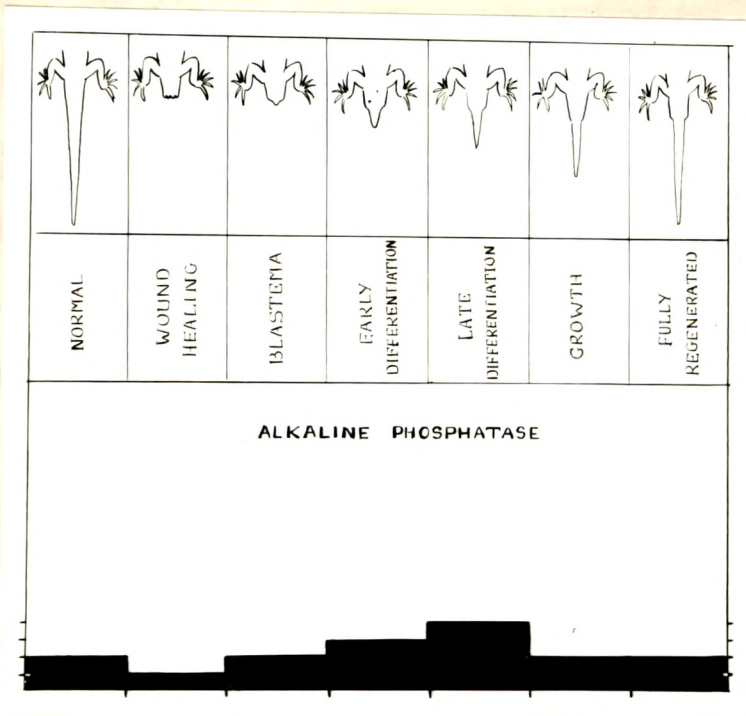

Fig.9. Graphic representation of alkaline phosphatase activity in the normal and the regenerating tail of the Scincid lizard, Mabuya carinata.

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

Right from the blastemic phase, all throughout differentiation, there was a steady rise in alkaline phosphatase activity in the various differentiating tissue components, such as skin, muscles and cartilage of the regenerate with an ultimate high value being attained during the late differentiation phase (Fig 9).

Growth phase:

This phase witnessed a gradual decline in enzyme activity, from the peak value attained during the late differentiation onwards the normal level with the result along with the attainment of the fully regenerated condition, the various cellular elements of the regenerate aquired a level of enzyme complement and localization characteristic of the corresponding cellular elements of the normal tail.

DISCUSSION

In the normal tail a cytoplasmic localization of alkaline phosphatase was evidenced in the epidermis, muscles and the vertebral elements. A similar localization of the enzyme in the epidermal region has been reported by Shah and Chakko (1967a) in

Hemidactylus flaviviridis, Kambara (1955) in Triturus pyrrhogaster and Schmidt and Weary (1962) in Diemictylus viridescens. Karczmar and Berg (1951) reported a negative epidermal phosphatase activity in the larval Amblystoma limbs and opined that the absence of alkaline phosphatase may be due to the age difference or functional difference of the larval tissues or most likely both. Presently observed alkaline phosphatase activity in the epidermis of Mabuya carinata which undergoes constant keratinization and ecdysis is indicative of the involvement of the enzyme in these processes. Such a conclusion has been drawn by Schmidt and Weary (1962) too, in the adult newt, Diemictylus viridescens. Though there are reports of absence of alkaline phosphatase in the muscles (Gomori, 1941; Schmidt and Weary, 1962) in the present investigation, the caudal muscles of Mabuya carinata responded favourably well towards the enzyme and the reports bearing on the activity of alkaline phosphatase in the caudal muscles of Hemidactylus flaviviridis (Shah and Chakko, 1967a), in the muscles of Amblystoma larvae (Karczmar and Berg, 1951) and in the pigeon breast muscles (George et al., 1958; Vallyathan and George, 1965 and Khan and George, 1967)

lend strong support to the present observations. An exact role of alkaline phosphatase in the caudal muscles of Mabuya carinata though not discernible at this juncture, it might be worth reflecting in this connection the suggestion of an important role for this enzyme in the transport of phosphorylated metabolites such as glycogen in pigeon breast muscles by Vallyathan and George (1965). By the reported association of alkaline phosphatase and calcification of bone in mammals and birds (Robison, 1923; Fell and Robison, 1929, 1934; Moog, 1944) and in regenerating limbs of Amblystoma larvae (Karczmar and Berg, 1951) and in Diemictylus viridescens (Schmidt and Weary, 1962) and the suggestion of Robison (1923) that alkaline phosphatase in bone is concerned with the production of a high local concentration of phosphate ions which favour the deposition of calcium phosphate, it could be suggested that this enzyme might be playing a role in the calcium metabolism of the skeletal elements of the normal tail of Mabuya carinata.

The presently observed appreciable enzyme activity in the wound epithelium as much as in the adjacent stump epidermis during wound healing in Mabuya carinata and a similar observation by Schmidt and Weary (1962) in

Diemictylus viridescens appear to be unique in comparison to the negative enzyme response in the regenerating epidermis of tadpoles (Junquiera, 1950; Moyson, 1946), and adult Triton (Preda et al., 1962) and Mammals (Fell and Danielli, 1943; French and Benditt, 1954; Raekallio, 1961). The glycogen depletion from the cut end of the original tail stump muscles from 24 hours onwards to 96 hours (Chapter 3) and the increased activity of alkaline phosphatase noted herein at identical loci and the suggestion of Vallyathan and George (1965) are rather tempting, and may be construed to indicate that alkaline phosphatase might be playing an important role in the transport of phosphorylated glycogen from the cut end to the wound surface thus facilitating the anaerobic utilization of glycogen to provide the energy required for the wound epithelium during the active process of cell division.

The increased blastemic response towards alkaline phosphatase in the tail of Mabuya carinata is in complete agreement with that of Ghiretti (1950) in the tail of adult Triturus, Junquiera (1950) in the tail of Bufo marinus tadpole, Moyson (1946) in Rana tadpoles,

Preda et al. (1962) in the tail of Triton, Karczmar and Berg (1951) in Amblystoma larvae and Schmidt and Weary (1962) in the limb of Diemictylus viridescens. Schmidt and Weary (1962), Jackson (1957, 1958) and Fell and Danielli (1943) have all correlated the alkaline phosphatase activity of the blastemic cells with fibrogenesis during this and later stages of regeneration. But it is of interest to note at this juncture, that Shah and Ramachandran (1972) based on their studies on aldolase in the regenerating tail of Mabuya carinata have surmised that in the absence of glycogen there might be an uptake of monosaccharide molecules in the form of glucose from the circulating blood by the blastemal cells. In the light of this suggestion it may be presumed that in the blastema of Mabuya carinata, the increased alkaline phosphatase activity is somehow involved either in the transport and or in the uptake and utility of monosaccharide molecules between the blood and the blastemal cells.

A second time increase in alkaline phosphatase activity reported by Ghiretti (1950) in Triturus cristatus corresponds well with the maximum enzyme activity attained during the differentiation phase

in the regenerating tail of Mabuya carinata. Karczmar and Berg (1951) and Schmidt and Weary (1962) have also reported a similar increased activity of alkaline phosphatase during differentiation, particularly during skeletogenesis. Whereas the presence of this enzyme in the differentiating scales and epidermis is understandable in the light of known occurrence of keratinization and ecdysis; its presence in the cartilagenous neural canal in the absence of ossification (Woodland, 1920; Barber, 1944; Kamrin and Singer, 1955 and Simpson, 1965) is a matter of conjucture. The pronounced enzyme response from the dermal region of the differentiating tail could be well correlated with the synthesis of collagen and fibrous proteins (Bradfield, 1950). The differentiating muscles too depicted an increased enzyme concentration. This when equated with the parallel increase in glycogen content (Chapter 3) is once again indicative of a possible significant role for alkaline phosphatase in the transport and or utilization of glycogen. This involvement of alkaline phosphatase in glycogen metabolism became all the more evident by the observed parallel decrease in alkaline phosphatase activity and

the glycogen content (Chapter 3) during the growth phase in the various by now well differentiated components of the tail. With the attainment of the fully regenerated condition, the enzyme activity attained a level characteristic of the normal tail.

Finally it is interesting to note that Saev (1963) and Morton (1965) have suggested a number of functions for alkaline phosphatase such as the maintenance of an orthophosphate pool, transfer of phosphoryl groups and the hydrolysis and esterification of metabolites moving across cell membranes, within the cells, between the cells and the extracellular spaces. This attribution of a number of functions to alkaline phosphatase has raised speculations in terms of heterogenity of this enzyme.

In fact, based on electrophoretic motility and a number of other methods, existence of different types of alkaline phosphatases have been demonstrated in enzyme preparations from a variety of mammalian tissues such as rat liver, intestine, kidney, bone, lung and blood serum (Fishman et al., 1962) and human placenta, sera (Boyer, 1961), bone, intestine, kidney,

and liver (Moss, 1963; Moss and King, 1962). In a preliminary study on regenerating limb of the adult newt, Diemictylus viridescens, Schmidt (unpublished) could separate electrophoretically at least six alkaline phosphatase active bands, suggestive of the fact that more than one alkaline phosphatase(s) might be taking part during amphibian limb regeneration. The present high alkaline phosphatase activity in comparison to acid phosphatase (Chapter 4) during reptilian tail regeneration could be a manifestation of its versatility in the wake of above observations.