

CHAPTER - 9

HISTOENZYMOLGY OF REGENERATING LACERTILIAN TAIL:

I ATP PHOSPHOHYDROLASE (ATPase; E.C. 3.6.1.4.)

ACTIVITY DURING REGENERATION IN THE

SCINCID LIZARD, MABUYA CARINATA

ATPase is a hydrolytic enzyme which brings about the splitting up of the biological energy currency molecules or ATP molecules liberating high energy pyrophosphates. Distribution of this enzyme in various tissues especially muscles, has been studied by a number of workers (Singer and Barron, 1944; Newman et al., 1950; Maengwyn Davies et al., 1952; Sommer and Spach, 1964; Riley, 1973).

Many of the works aimed chiefly towards an understanding of the specificities and nonspecificities of the enzyme have revealed significant physiological polymorphism in terms of pH optima, ionic cofactor or activator and the sites of intracellular localization (Singer and Barron, 1944; Newman et al., 1950; Maengwyn-Davies et al., 1952; Chappell and Perry, 1955; Padykula and Herman,

1955; Freiman and Kaplan, 1960; Azzone et al., 1961).

An analysis of Mg dependent ATPase, whose occurrence is of a general nature, could easily give an insight into the mechanics of energy metabolism that is taking place in any particular tissue.

Earlier histophysiological and histoenzymological studies in *Mabuya* have more or less chalked out the various metabolic intricacies and interconversions that underly the process of tail regeneration in the reptilian species (Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975; Ramachandran et al., 1975; Radhakrishnan and Shah, 1973; Radhakrishnan, 1972). With this background, the study of ATPase activity would be profitable in understanding the dynamics of energetics during tail regeneration in *Mabuya carinata* and as such is undertaken presently.

MATERIAL AND METHOD

The adult *Mabuyas* obtained from Karnataka, India, and maintained in the laboratory on a diet of insects served as the experimental animals. The autotomy of the normal and regenerating tails during different periods of regeneration was performed as outlined earlier. Immediately after autotomy, the wound surfaces of the

cast off tails were blotted to remove blood and tissue fluids and were immediately fixed on a chuck of a cryostat microtome maintained at -20° C. Longitudinal and transverse sections of 12 - 18 μ thickness were cut and incubated for about 1 hour at 37° C in the incubation medium prepared as per the method of Wachstein and Meisel (1957) and were further processed as described by them. Sections incubated in a substrate blank medium served as the controls.

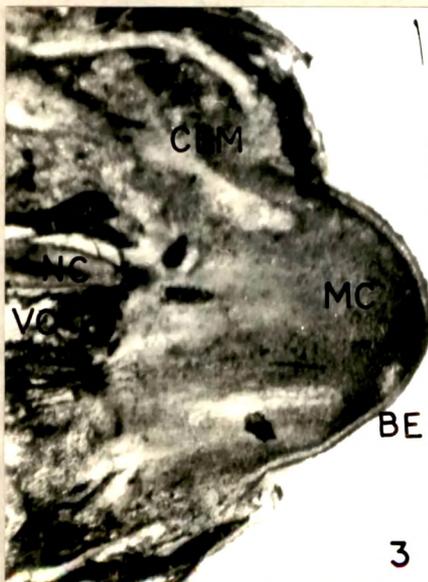
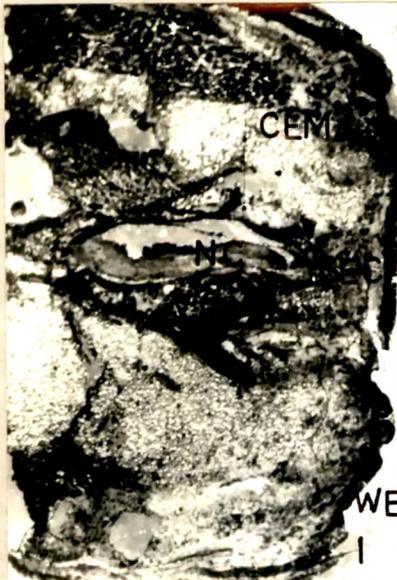
OBSERVATION

NORMAL TAIL

Of the various normal tail tissues, the maximum enzyme concentration was in the muscle fibres, followed, by the stratum germinativum and the scutogenic cells in the integument, the bone matrix and elements together with the intervertebral cartilagenous cells in the vertebral column, and, the grey matter in the nervecord. Slight enzyme activity could be noticed in the white matter, in the peripheral cytoplasmic component of the adipose tissue, and to a certain extent in the innermost layer of α cells in the epidermis. β cells as well

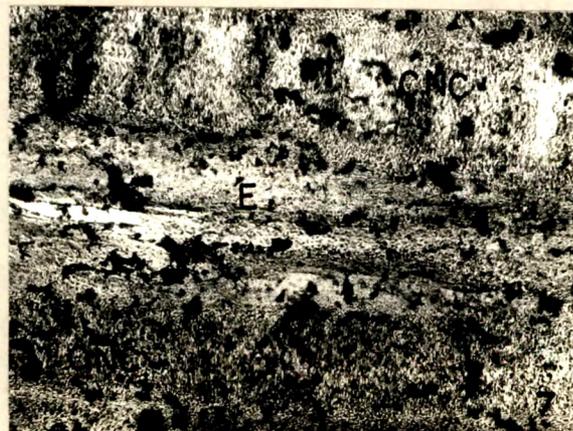
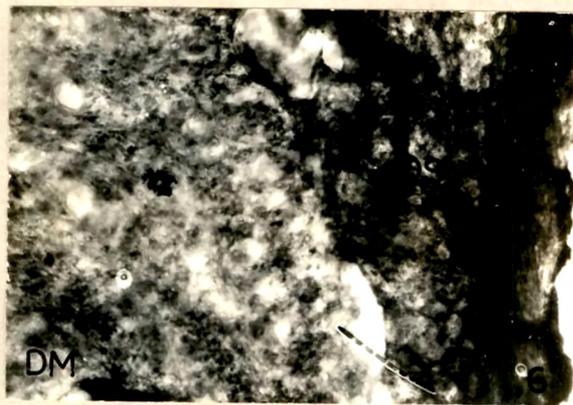
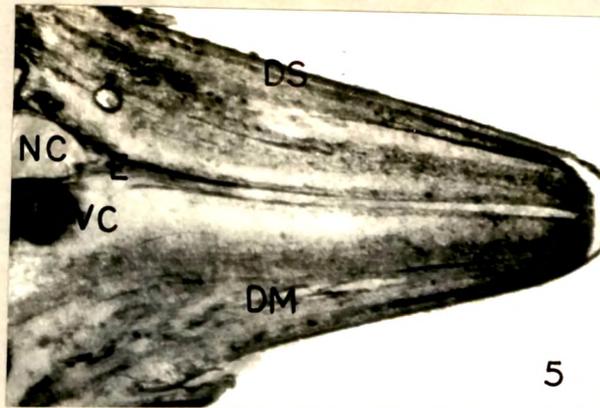
EXPLANATION TO FIGURES

- Fig. 1. Photomicrograph of L.S. of wound healing tail showing ATPase activity. CEM - cut end of muscles; NC - Nerve cord; SEC - Subepithelial cells; VC - Vertebral column; WE - Wound epithelium.
- Fig. 2. Wound epithelium (WE) together with the subepithelial cells (SEC) depicting ATPase activity. 50 X.
- Fig. 3. L.S. of Blastema denoting ATPase activity. BE - Blastemic epithelium; CEM - Cut end of muscles; MC - Mesenchymal cells; NC - Nerve cord; VC - Vertebral column.
- Fig. 4. Blastemic epithelium (BE) with the mesenchymal cells (MC) showing enhanced ATPase activity. 125 X.



EXPLANATION TO FIGURES

- Fig. 5. L.S. of differentiating tail showing ATPase activity. DM - Differentiating muscles; DS - Differentiating scales; E - Ependyma; NC - Nerve cord; VC - Vertebral column.
- Fig. 6. Differentiating scales (DS) and differentiating muscles (DM) depicting enzyme activity. 50 X.
- Fig. 7. Cartilagenous neural canal (CNC) with the ependyma (E) showing ATPase activity. 50 X.



as outer layer of DC cells together with the scutes and the various dermal elements recorded an almost total lack of the enzyme activity. The localization of ATPase in the muscles was both in the mitochondria as well as sarcoplasm (though more prominently in the former) and almost of equal intensity in all the muscle fibres. In other tissues where the enzyme reactivity was discernible, was localized chiefly in the cytoplasm of their cells.

WOUND HEALING PHASE

Increased activity of ATPase could be easily visualised in the wound epithelium. The cut end of the stump tissues also recorded a high enzyme activity.

PREBLASTEMIC AND BLASTEMIC PHASES

A remarkable increase of the enzyme activity in the cells of blastemic epithelium was the characteristic feature of this phase. The mesenchymal cells of the blastema also showed adequate enzyme activity though of a lower level as compared to that in the epithelium. Even at this stage the cut end of the stump tissues continued to exhibit a high enzyme activity.

DIFFERENTIATION PHASE

During differentiation, in addition to the apical mesenchymal cells underlying the epidermis the extending ependyma at the basal part of the blastema as well as the cells differentiating into chondroblasts and myoblasts too presented a relatively higher enzyme content. As the differentiation progressed proximo-distally, the differentiating chondroblasts ^{and} myoblasts at the base of the regenerate showed progressive increase in the enzyme concentration as they transformed into chondrocytes and, myocytes and my^ofibres. All throughout differentiation, the differentiating scales and epidermis, along with the differentiating scutogenic cells and muscle fibres, were found to maintain a high enzyme activity.

GROWTH PHASE

During this phase, the differentiated chondrocytes and myofibres attained a level of enzyme activity characteristic of the corresponding cells of the normal tail. The cartilagenous neural canal showed appreciable activity whereas the enzyme in the ependyma was not very active. But during the final

stages of growth phase, the ependyma and the glial cells became enzyme active and in the wall of the cartilagenous neural canal, only the middle core of chondrocytes remained enzyme active. The fully grown regenerated tail revealed an identical intensity and pattern of enzyme activity and localization as seen in the corresponding tissues of the normal tail.

DISCUSSION

The most significant feature of the present investigation is the increasing activity of ATPase from the regressive to the early progressive phases of regeneration. Whereas the comparatively lower level of the enzyme in the normal tail tissues is understandable, and indicates a low steady level of functioning, the increasing content of the enzyme noted from the regressive to early progressive phases of regeneration highlights the dominant role of energetically associated reactions during these periods. The above normal level of ATPase observed during wound healing phase and its increased concentration at the cut end of the stump tissues could be well correlated with the similar activity

and concentration of a number of other enzymes, metabolites and vitamin 'C' studied in the healing wounds of the autotomised tail of *Mabuya* (Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975, 1976; Ramachandran et al., 1975; Radhakrishnan, 1972). These changes are all indicative of an overall increased energy flux during wound healing, and hence an increase in ATPase activity. Previous studies on enzyme and metabolites during tail regeneration in *Mabuya carinata* have well established the fact that the blastema phase is an actively synthetic phase elaborating new lipid molecules both neutral as well phospholipid (Shah and Ramachandran, 1972, 1973, 1974, 1975, 1976, Radhakrishnan, 1972). Similarly, during the differentiation phase too, synthesis of a number of substances such as glycogen, mucopolysaccharides, proteins, and lipids had been suggested (Shah and Ramachandran, 1970, 1972, 1973, 1975, 1976; Ramachandran et al., 1975; Radhakrishnan, 1972). The tremendous amount of energy needed during the synthesis of such macromolecules is known to be supplied by the preferential catabolism of both carbohydrates as well as lipids. The effective

building up of energy production during catabolic reactions with its incorporation into anabolic reactions is of utmost importance to a metabolically active tissue, thus helping to strike an economic balance between the output and input of chemical energy. In this respect, the regenerating system can be looked upon as a metabolically active system regulating and synchronising the various anabolic and catabolic reactions as per need from time to time, thus maintaining an effective energy economy. This becomes very evident with the increase in ATPase activity noticed during the blastemic and differentiation phases of tail regeneration. With its known function of hydrolytic breakdown of ATP and release of chemical energy, it could be purported to play an effective role in channelising the flow of energy from the catabolic to the anabolic reactions. Thus, concurrent to the increased synthetic activity during these phases, the correspondingly increased activity of ATPase recorded herein could be construed as an indication of the establishment of a machinery for the effective transfer of energy to the many endergonic reactions characteristic of the regenerative process at this time. Another significant aspect, is the importance of ATPase in providing the requisite energy at the cell surface for the

active transport of chemical moieties; as, such transport mechanisms could be expected to be operative in a regenerating system where active processes such as division, differentiation, growth and metabolic transformations are all at play. Moreover, the Mg dependent ATPase is known to be sensitive to the concentration of Na and K ions and is presumed to be concerned with electron transport (Skou, 1957; Jarnefelt, 1961, 1962; Deul and McIlwain, 1961; Alridge, 1962). With the reported increase in these two cations during regeneration in the postblastemic phase (Shah and Hiradhar, 1974) a similar involvement of ATPase in the regenerative mechanisms could also be considered within the realms of possibility.

With the completion of the process of differentiation and the commencement of growth phase, the gradually decreasing ATPase activity is suggestive of decreasing levels of energy flux which could well be compared with the decreasing metabolic activity noticed in earlier studies during this terminal phase of regeneration. As the regenerate attains a more or less

fully grown condition, the various metabolic processes settle down to a steady state level as in the case of the tissues of the normal tail, and hence, ATPase activity too settles down to a more or less normal level.