CHAPTER 6

HISTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASE IN THE NORMAL AND REGENERATING TAIL OF THE HOUSE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

The histochemical localization of acid phosphatase and its distribution pattern in the regenerating limb and tail of amphibians have been studied in detail. Schmidt (1963) studied the histochemical localization of acid phosphatase activity in the forelimb of the adult newt, *Diemictylus viridescens* and suggested the possible association of this enzyme with the ribonucleic acid, polysaccharide, protein and lipid metabolisms. Schmidt and Weary (1963) demonstrated the histochemical localization of the enzyme in the regenerating limb of the newt, *D. viridescens* and showed that the enzyme was highly active during the early differentiation phase. They also suggested the possible role of the enzyme in protein synthesis which is the most active process during the differentiation phase of the various tissues undergoing regeneration. Earlier, Ghiretti (1950) had shown that the acid phosphatase activity was highest during 15th and 20th day of the tail regeneration in *Triturus cristatus*. This high activity of the enzyme has been correlated with the differentiation phase of various tissues in the regenerating tail. Recently, Hahn (1960) has also shown that the enzyme

activity is considerably high during the period of rapid regeneration and that it declines as the rate of regeneration slows down. On the other hand, Junquiera (1950) had opined that acid phosphatase has no part in the growth and regeneration of the tail of Bufo marinus tadpole. The present investigation on the histochemical localization of acid phosphatase in the normal and regenerating tail of the house lizard, Hemidactylus flaviviridis has therefore been carried out with a view to study the distribution pattern of the enzyme activity so as to obtain a better understanding of the various biochemical events involved in the process of regeneration.

MATERIALS AND METHODS

Adult normal tails and regenerates with at least one or two segments of the original tail stump, were cut out and fixed in cold 10% neutral formalin at 4°C for 24 hours. 10 to 15 μ sections were cut on a freezing microtome. The sections were incubated in an incubation medium for the histochemical demonstration of acid phosphatase activity by two separate methods described below.

1. Gomori's Method (1950): Sections were incubated for 11 to 12 hours at 37°C in an incubation medium containing sodium β-glycerophosphate as substrate, buffered with 0.05M sodium acetate at pH 5.2.

2. Naphthol AS-phosphate method described by Burstone (1958a, 1961): Naphthol AS-BI (Sigma Chemical Company, U.S.A.) was used as substrate and Fast Blue B (BBN) (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 for 30 minutes to one and a half hours at 37°C.

Two separate sets of controls were kept for both the methods: (1) Identical sections incubated in buffered media, devoid of substrate. (2) Sections incubated after keeping the sections in hot water (70 to 80°C) for about 25 minutes. After incubation the samples as well as the controls were thoroughly washed in distilled water and mounted in glycerine jelly.

OBSERVATIONS

According to Gomori (1950) acid phosphatase activity is localized in the nucleus as well as in the cytoplasm. In the present investigation using Gomori's method it was difficult to locate the actual sites of activity due to the artifacts present. The Naphthol AS phosphate method of Burstone (1958a, 1961) was found to be more satisfactory. With this method the precise localization of the enzyme activity was obtained. The sites of the enzyme activity

stained blue and they were found to be extranuclear in distribution.

NORMAL TAIL:

In the epidermis, high acid phosphatase activity was observed in the cytoplasm of the cells of stratum intermedium, stratum germinativum and the basement membrane (Fig. 1). The beta and alpha cells of the old and new generations were devoid of the enzyme activity. The fibroblasts, fibrocytes and the connective tissue fibres of the dermis showed moderate acid phosphatase activity. However, the outer subepithelial region of the dermis possessed a higher enzyme concentration than the inner reticular region. The blood vessels, blood cells and the nerve fibres in the dermis showed very low enzyme reaction. A uniformly distributed high enzyme activity was found in the connective tissue fascia that lies between the dermis and muscle layer.

No enzyme activity was found in the striated muscle. An intense activity of the enzyme was observed in the osteoclasts (Fig. 2). Fat cells showing high enzyme activity could be seen in the marrow in the body of the vertebrae. Other than the osteoclasts and the fat cells, the only other region of the vertebra which showed enzyme activity was the cells of the vertebrae in its breaking

planes. The osteocytes, osteoblasts and chondrocytes seen in between two vertebrae were totally devoid of enzyme activity.

A uniform positive enzyme activity of low intensity was observed throughout the nerve cord. But in the myelin sheath of the nerve fibres the enzyme concentration was quite high. The perineurium showed more enzyme concentration than the endoneurium. The endothelium of capillaries, tunica intima of arteries and veins appeared moderately stained but the cytoplasm of the blood cells showed only low enzyme activity.

REGENERATING TAIL:

Wound healing phase:

The wound epithelium showed very low acid phosphatase activity. However, the enzyme concentration was more towards the stump epidermis than the apex. The phagocytes and blood cells in the subapical region also showed enzyme activity (Fig. 3). High enzyme activity was seen in the scab over the wound healing area.

Preblastemic phase:

An increase in the enzyme activity was noted at the junction of the stump epidermis and stratified epithelium. However, at the apex of the stratified epithelium the

enzyme activity remained the same as in the wound epithelium. The dedifferentiating tissues at the cut area of the tail stump viz; adipose tissue, vertebral column and the spinal cord showed a sharp increase in the enzyme activity.

Blastemal phase:

During this phase the enzyme activity in the stratified epithelium and the differentiating epidermis at the base of the blastema remained low. A considerable increase in the activity of the enzyme was noted at the tip of the cut end of the vertebral column, spinal cord and the adjacent mesenchyme cells of the blastema (Figs. 4 & 5). Towards the periphery the enzyme activity progressively decreased.

Late blastemal phase:

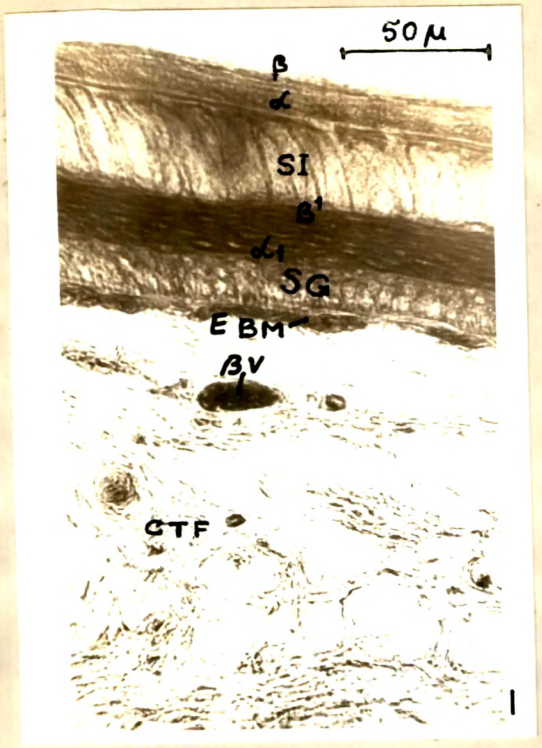
The enzyme activity remained poor in the epidermis. There was a noticeable decrease of the enzyme activity in the fibroblasts which were adjacent to the cut end of the vertebral column and the spinal cord (Figs.6 & 7).

Differentiation phase:

The acid phosphatase activity remained low in the epidermis till the formation of the scales, i.e. about 20 days after amputation (Fig.8). Hereafter, the cells

of the stratum germinativum and the basement membrane showed the enzyme activity which increased gradually as growth continued (Fig. 9). The dermis showed a fairly high uniform enzyme activity in the fibrocytes when the transformation of fibroblasts into fibrocytes took place. However, the enzyme activity was more in the reticular region of the dermis where the transformation of the fibrocytes into connective tissue fibres first took place. The fibrocytes destined to become the fascia between the dermis and muscle layer showed relatively higher enzyme activity than the other regions of the dermis. There was a progressive increase in the activity of the enzyme in these cells till the connective tissue fibres were fully formed. The muscle tissue did not show any enzyme activity during the various stages of myogenesis and thereafter. The submuscular and subcutaneous adipose tissue showed very low enzyme activity during differentiation.

During the differentiation of the cartilagenous neural canal (by about 15 days after amputation) the chondrocytes adjacent to the vertebral column of the tail stump showed acid phosphatase activity. The perichondrial chondrocytes showed higher enzyme activity than the chondrocytes which formed the mass of the cartilagenous neural canal (Fig.10). However, the chondrocytes at the junction between the cut end of the vertebral column and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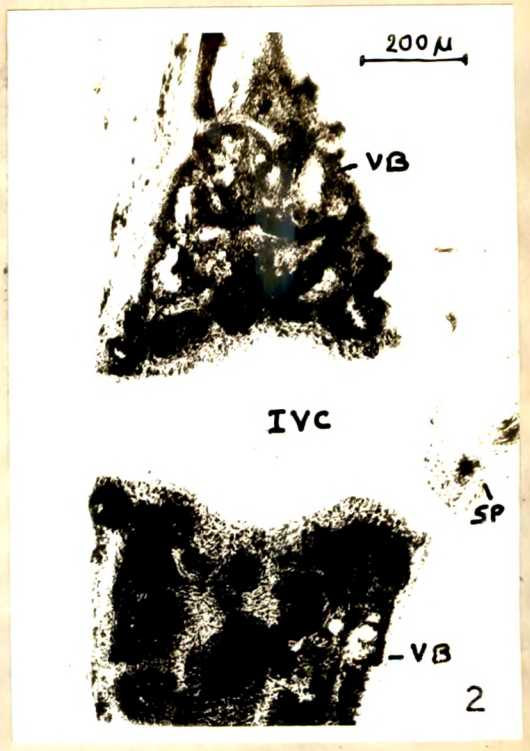
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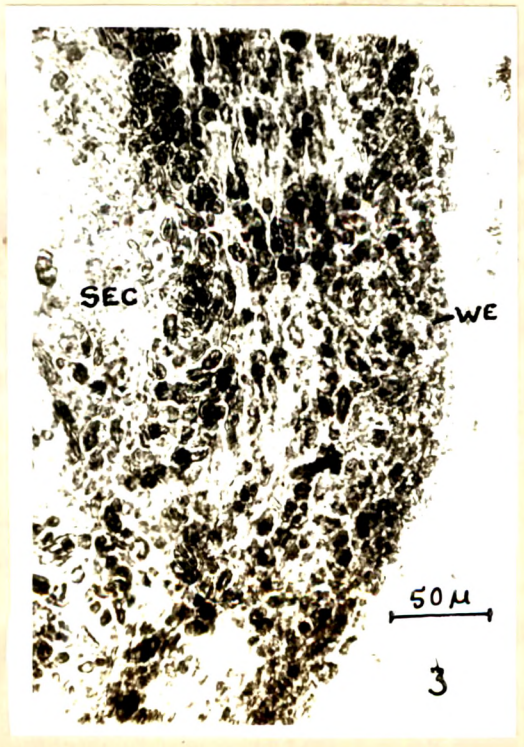
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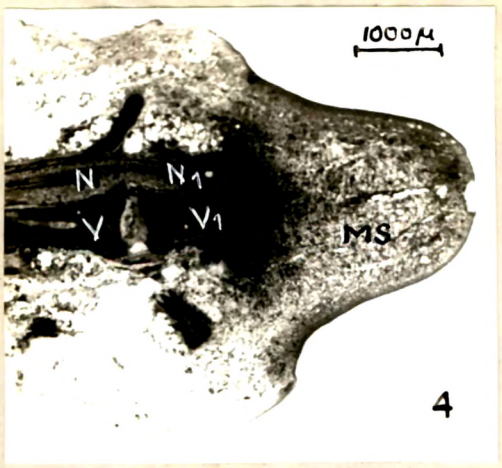
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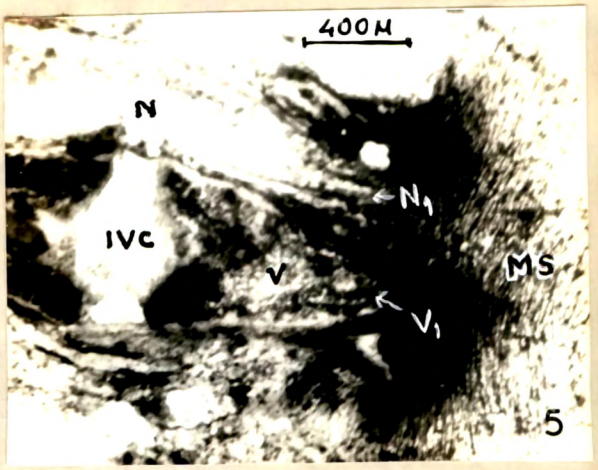
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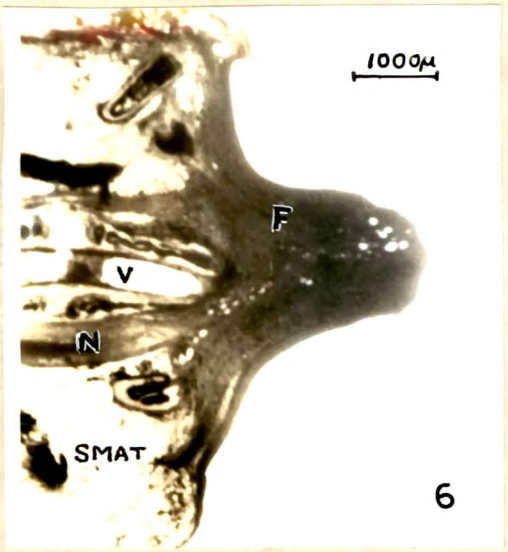
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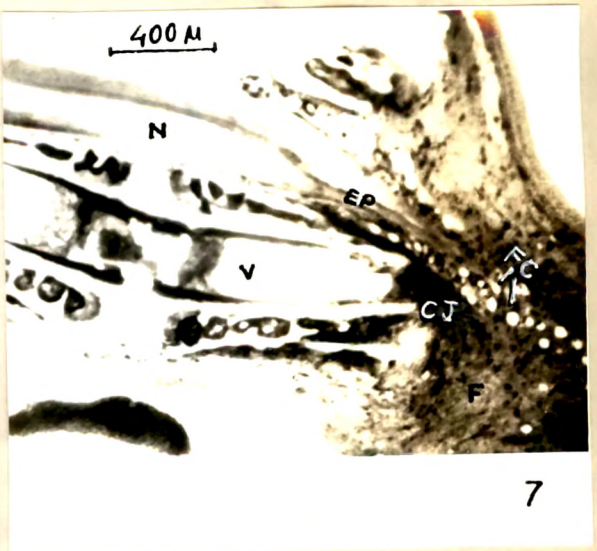
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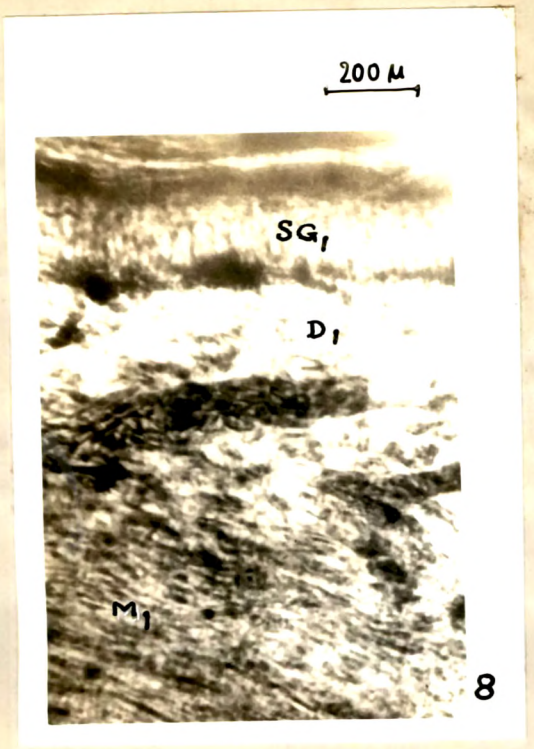
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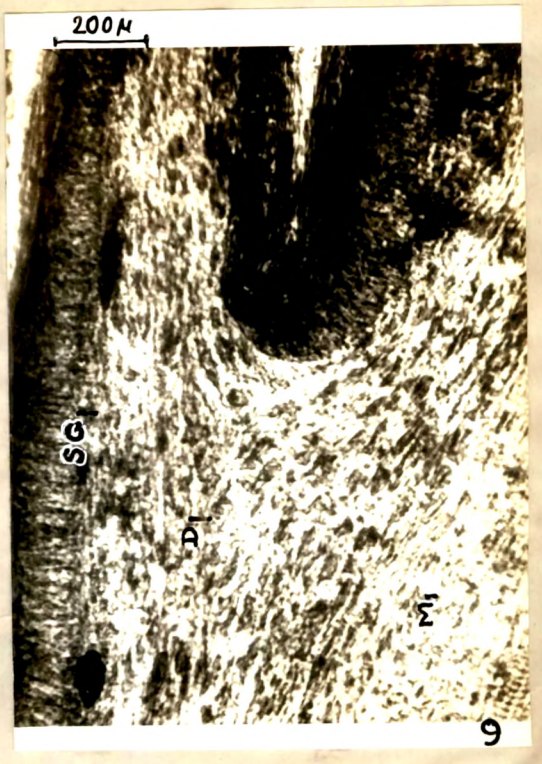
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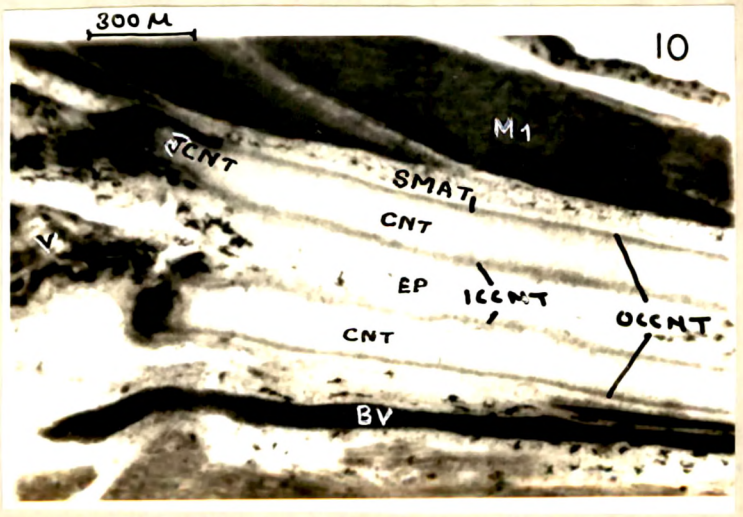
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EXPLANATIONS FOR FIGURES

Fig. 1. L.S. of normal tail skin (ventral side). Note the enzyme activity in the cytoplasm of the cells of stratum germinativum, stratum intermedium and epidermal basement membrane. In the dermis the enzyme activity is noted along the connective tissue fibres and blood vessels.

Fig. 2. L.S. of the caudal vertebrae showing enzyme activity in the osteoclasts.

Fig. 3. Wound epithelium showing moderate enzyme activity on healed wound surface.

Fig. 4. L.S. of blastema showing enzyme activity at the cut end of the vertebral column, spinal cord and mesenchyme cells adjacent to these parts.

Fig. 5. Part of the figure 4 enlarged.

Fig. 6. L.S. of the late blastema showing a decreased enzyme activity adjacent to the cut end of the vertebra and spinal cord when the mesenchyme cells transform into fibroblasts.

Fig. 7. Part of Fig. 6 enlarged.

Fig. 8. L.S. of regenerate (early differentiation phase) showing poor enzyme activity in stratum germinativum.

Fig. 9. L.S. of regenerate skin (late differentiation phase) showing high enzyme activity in the stratum germinativum and fibroblasts in the dermis.

Fig.10. L.S. of the regenerate (late differentiation phase) showing enzyme activity at the junction of vertebral column and cartilagenous neural canal and the chondrocytes at the inner and outer linings of the cartilagenous neural canal. The blood vessels, in the regenerate and original tail stump show high enzyme activity.

ABBREVIATIONS

α - Alpha cells (old generation)

α_1 - Alpha cells (new generation)

β - Beta cells (old generation)

β_1 - Beta cells (new generation)

BV - Blood vessels

CJ - Chondrocytes at the junction of the vertebra with the CNT

CNT - Cartilagenous neural canal

CTF - Connective tissue fibres in dermis

D_1 - Dermis in the regenerate

EBM - Epidermal basement membrane

EP - Ependyma

F - Fibroblasts

FC - Fat cells

ICCNT - Chondrocytes of the inner lining of the CNT

IVC - Intervertebral cartilage

JCNT - Junction of CNT with vertebra column

M_1 - Muscle in the regenerate

MS - Mesenchyme cells

N - Nerve cord (spinal cord)

N_1 - Cut end of the nerve cord

OCCNT - Chondrocytes of the outer lining of the CNT

SEC - Subepithelial cells

SG - Stratum germinativum

SG_1 - Stratum germinativum in the regenerate

SI - Stratum intermedium

SMAT - Submuscular adipose tissue

$SMAT_1$ - Submuscular adipose tissue in the regenerate

SP - Vertebral spine

V - Vertebral column

V_1 - Cut end of the vertebra

VB - Vertebral body

WE - Wound epithelium

the cartilagenous neural canal did show high enzyme activity even though they were fully differentiated(Fig.10). The ependymal tube showed very low acid phosphatase activity throughout the differentiation phase. However, the cells bordering the neurocoel showed comparatively higher enzyme reaction than the other parts of the ependyma. The Schwann cells and the myelin sheath of the nerves showed perceptible enzyme activity.

Growth phase:

During the growth phase all parts except the beta and alpha cells of the regenerated skin showed a high enzyme activity. This high activity was maintained even in the skin of the fully regenerated tail. It is interesting to note that the enzyme activity in the fully regenerated skin was even more than what was seen in that of the original tail. The enzyme activity in the cartilagenous neural canal and the ependyma remained the same as seen during the differentiation phase. The enzyme activity of the other tissues was similar to the corresponding tissues of the normal tail.

DISCUSSION

The presence of acid phosphatase in the skin of different groups of animals has been reported, Reiner

et al. (1957), Moretti and Mescon (1956), in primates; Carranza and Cabrini (1962), in rats; Raekallio (1960), in guinea pig; Kobayashi et al. (1955), in pigeon; Noback and Paff (1951), in mammals. Gomori (1941), reported that the primate epidermal tissue did not show acid phosphatase activity. Junquiera (1950), had demonstrated the presence of cytoplasmic as well as nuclear acid phosphatase activity in the fibroblasts of the Bufo marinus tadpole. Taguchi et al. (1956) observed a higher enzymatic content in the skin of adult Bufo vulgaris than in that of its tadpole. Schmidt (1963), working on the newt, Diemictylus viridescens obtained similar results as those of Kambara (1955), on the newt, Triturus pyrrhogaster. Our observations on the epidermal tissue of the skin of the normal tail of Hemidactylus flaviviridis showed acid phosphatase activity restricted to the germinativum layer and the epidermal basement membrane. Schmidt (1962a), correlated the presence of acid phosphatase in the basement membrane of the epidermis with the metabolism of the non-sulphated polysaccharide or that of the neutral lipids in the adult newt, Diemictylus viridescens. In the lizard intense enzyme activity throughout the structure of the dermis was noted and was mainly found to be localized in the cytoplasm of the fibroblasts, fibrocytes and along the connective tissue fibres. The connective tissue fibres which join the dermis and the

muscle layer showed the highest activity of the enzyme. Carranza and Cabrini (1962) suggested that the acid phosphatase in the connective tissue might be associated with macrophagic function and not with tissue formation. Taguchi et al. (1956), Fujii and Ohnishi (1952), on the basis of their studies on toads and frogs, proposed that some of the phosphatase activity in the skin homogenates are in part due to the dermal enzymes.

An initial increase in the enzyme activity during the period of wound closure may be due to the phagocytic activity of the epithelial cells (Schmidt, 1962; Singer and Salpeter, 1961). According to Raekallio (1960),the production of these enzymes represents one of the first steps in the repair processes starting early in the so-called lag-phase of wound healing. Our observations showed high enzyme activity at the junction between the cut end of the original tail and newly formed epithelium over its surface. This was mainly due to the phagocytic activity of the epithelial cells and the repair of the cut ends of tissues during the wound healing period. The general appearance of the high enzyme activity observed in the pealing-off scab from the healing cut end was mainly due to the enzymatically highly active phagocytes which go through the epithelium and come to separate off the scab from the healed wound. This feature of phagocytic

invasion is in agreement with the findings of Fell and Danielli (1943) on the healing of cuts and burns in the rat's skin. When the apical epithelium over the healed end of the tail was fully formed the enzyme activity in this region decreased.

From our observations we find that there is a fairly high acid phosphatase activity in the tissues wherever the fat is present, viz., in 'fat depot' present between the muscle layer and the cartilaginous neural canal, the subcutaneous fat and the bone marrow of the vertebrae. Presence of acid phosphatase activity in the above stated regions perhaps shows that the acid phosphatase is directly or indirectly involved in fat metabolism in these tissues.

During the regenerative phase till the 20th day after amputation, the skin showed a sharp decrease in the enzyme activity. But by the 20th day the enzyme activity resumed in the germinativum layer, epidermal basement membrane and the dermis of the regenerating skin. In these regions the enzyme activity gradually increased till finally it became almost the same as seen in skin of the normal tail, or even more. However, during this period some basic histological changes were noted in the skin, viz. the increase in the size of the dermal papillae pushing the epidermis with the scale as a projecting body, transformation of the fibroblast into fibrocytes and connective tissue fibres. It is quite

likely that the reappearance of the enzyme activity during the differentiation of the skin as stated above has some definite correlation. The house lizard moults its skin (corneal scale layer) periodically as does an adult newt. Our observations on the high acid phosphatase activity in the epidermis of the skin of the adult lizard, in the normal as well as the fully regenerated tail, support the idea of the probable role of the acid phosphatase in the constant synthesis of the keratin protein in the epidermis connected with the cyclic ecdysis in the adult newts as suggested by Schmidt (1963). During the growth period of the regenerating tail it was observed that ecdysis of the epidermal scales takes place. Prior to ecdysis by about the 20th day after regeneration, an additional layer of cells, the stratum intermedium was formed by the cells of the germinativum layer. This stratum intermedium was arranged between the old generation of the alpha and beta cell layers and the new generation of these cells. The old generation of the alpha and beta cells that form the scales were cast off during ecdysis. The casting-off is brought about by the disintegration of the stratum intermedium. The acid phosphatase activity in the cells of the stratum intermedium is considerably high. The high activity of the enzyme can be explained by considering its role in the lytic processes in the cells which ultimately lead to their disintegration. As the disintegration

of the stratum intermedium took place, the outer most layer of cells viz., old generation of the alpha and beta cell layers got separated off and were pealed off. The new alpha and beta cells were formed by the cells of the germinativum layer. These cells were formed before the casting-off of the old alpha and beta cell layers took place. The acid phosphatase activity in the cells of the germinativum layer was quite high. This was due to continuous synthesis of proteins (keratin) for the formation of the new alpha and beta cell layers.

The striated muscle fibres showed no acid phosphatase activity in the adult normal tail of the lizard. The same was the condition in the different stages of myogenesis. Ogata and Mori (1963), reported from their studies on muscles of various animals like mammals, birds, reptiles and amphibians, that these muscles contained esterase but no other hydrolytic enzymes except a slight activity of acid phosphatase in the white muscle of fish. Their findings suggest that there is a difference in the metabolism of the muscle depending on each species. Gomori (1941) and Dempsey et al. (1946) suggested that there is no acid phosphatase in the muscle of the human, rat and monkey. However, there are other reports such as those of Wolf et al. (1943) recording the activity of the enzyme in the nuclei and occasionally in the cytoplasm of the muscle fibres. Greenstein (1945) obtained a weak activity of the enzyme in the mouse

skeletal muscles. Gomori (1956) got a negative to moderate and at times an intense diffuse activity of the enzyme in the striated muscle of rabbit depending upon the substrates. Schmidt and Weary (1963) noted acid phosphatase activity in the striated muscle of Diemictylus viridescens and also in the differentiating myoblasts which differed a little from that of the basal musculature in activity. The enzyme localization was along the myofibrillae of strap myoblasts. Moog (1944) reported a reduction in acid phosphatase activity during the differentiation of mesoderm into striated muscles. George and Pishawikar (1961) have shown greater acid phosphatase activity in the white fibres of the pigeon pectoralis muscle, while Vallyathan and George (1965) demonstrated its localization in the sarcoplasmic reticulum of both the red and white fibres of the pigeon pectoralis and suggested that this enzyme may be involved in the transport of glycogen to the interior of the cell from the sites of its synthesis.

Burstone (1959a) reported that the acid phosphatase activity of osteoclasts is associated with physiological bone resorption in several species of animals. Further, he has clearly demonstrated that the osteoclasts consistently showed an intensely sharp cytoplasmic acid phosphatase activity and slightly positive reaction of the cells of bone which are adjacent to the osteoclasts in all

species of animals. Cretin (1951) had shown a localized acid pH adjacent to osteoclasts using indicator dyes. Arnold and Jee (1957) had demonstrated an uptake of radioactive plutonium by osteoclasts and suggested their active role in the process of resorption. Macrophages and giant cells associated with lytic phenomena have long been shown to exhibit relatively high acid phosphatase activity. Osteoclasts adjacent to resorbing bone and cartilage also show high enzyme activity (Burstone 1960a). Changus (1957) had shown acid phosphatase activity in the giant cells in fibrous dysplasia. Recently a study of mononuclear phagocytes of inflammatory exudates has been made with reference to acid phosphatase in relation to the phagocytic activity (Danninberg et al., 1960). In the bone tissue of the tail vertebrae of the Hemidactylus flaviviridis acid phosphatase was found to be localized in the osteoclasts, a few osteocytes which are close to the osteoclasts, the cells at the breaking plane of the vertebrae and the fat globules which are seen in the marrow of the vertebrae. During the preblastemic and blastemic period, of the regenerating tail, an intense concentration of the enzyme activity could be seen at the cut end of the vertebra and the adjoining mesenchyme cells in the regenerating tissue. This activity suddenly decreased when the mesenchyme cells got transformed into fibroblasts. The degeneration of the cut end of the vertebral column before the initiation of the differentiation

of the various types of cells from mesenchyme has been reported by Huges and News (1959). From our observations a high concentration of the enzyme was seen when the degeneration of the tip of the cut end of the vertebra was in progress. The initiation of the differentiation could be observed when the changes of the mesenchyme cells into fibroblasts took place. The decrease of the enzyme coincided with the initiation of differentiation. As the differentiation progressed the enzyme activity decreased in the regenerating cartilaginous neural canal day by day and in a 30 days old regenerate the activity is seen only at the junction of the vertebra of the original tail and the regenerating cartilaginous neural canal. In the fully regenerated tail, the enzyme activity in the cartilaginous neural canal was restricted to the inner and outer lining of the canal, the distal terminal end of the canal and the junction of the canal with the vertebra of the original tail stump. The body matter of the wall of the cartilaginous neural canal was almost negative to the enzyme reaction. This suggests that the enzyme activity was restricted to the differentiation period and as soon as this phase was over, the enzyme activity disappeared. The localization of the enzyme in the neutral lipid globules present in the marrow of the vertebrae in the original tail part showed that the enzyme may be involved in the lipid metabolism of this region. The high localization of acid phosphatase

activity at the breaking plane of the tail vertebrae in the Hemidactylus flaviviridis was perhaps due to the degenerating osteocytes and the high phagocytic activity at this point which renders the region of the vertebra easily breakable. Thus as suggested by Fell and Danielli (1943) that where there is high phagocytic activity there is high acid phosphatase activity, we find a similar condition in the 'breaking plane' of the vertebrae in the tail of the house lizard.

Rutenburg and Seligman (1955) have shown that acid phosphatase activity in the peripheral nerves is restricted to the myelin sheaths. Murray (1959) has suggested that the Schwann cells are responsible for myelinization of nerve axons and Schmidt (1963) stressed the localization of acid phosphatase in mylein sheath as a valid evidence in support. In the nerve cord of the original tail stump of the house lizard the enzyme activity was, though low, uniformly distributed in all its parts. However, the myelin sheath and the perineurium showed relatively higher activity. Bartelmez and Bensley (1947) got varying results in the nervous tissue. Enzyme activity was located in the Schwann cell nuclei, cytoplasm and the injured ends of axons. However, in the fixed tissue he could obtain the enzyme activity only in the axons. Lassek (1947) had opined that it was improbable that the enzyme activity would be present in the axons. During the preblastemic and blastemic

periods, a retrogression in the nerve cord had been reported by Huges and News (1959). Degenerative and regenerative activities of the tissues coincide during the preblastemic and blastemic stages of regeneration (Schmidt 1960, 1962). During the preblastemic and blastemic periods of the regenerating tail in the present study it was noticed that the retrogression of the cut end of the nerve cord coincided with the increase in the acid phosphatase activity in this area. The mesenchyme cells in the blastema adjoining the cut end of the nerve cord also showed very high acid phosphatase activity. Again this phase coincided with the early regenerating phase of the nerve cord. These observations are in conformity with the results obtained by Schmidt and Weary (1963), working on the brachial nerve regeneration. They have observed that the phosphatase activity at the injured terminals of the brachial nerve indicate the retrograde degeneration and the similar enzyme activity in the myelinization of axonal sprouts from the same nerve, thereby indicating progressive regeneration. After the preblastemic and blastemic stages in the regenerating tail of the house lizard as the ependyma was being formed the initially high acid phosphatase activity seen during this stage gradually decreased. The initially increased enzyme activity could be correlated with the retrogression of the cut end of the nerve cord and also the early regenerating phase of the

ependyma. As the ependyma formed from the nerve cord, the cells of the inner lining of the ependymal tube retained the high phosphatase activity, but the activity in other parts was at uniformly low intensity. Only Schwann cells in the undifferentiated regions showed high acid phosphatase activity. In the fully regenerated nerve cord, the intensity and the distribution of the enzyme activity was more or less the same as that in the normal nerve cord; the sites of the enzyme activity being the myelin sheath and the Schwann cells at its growing tip. In the mammalian regeneration, a high acid phosphatase activity was reported in axons and myelin sheaths at the transacted region (Bartelmez and Bensley, 1947). Significant increase in acid phosphatase activity in the regions of accelerated growth from the proximal nerve stump has been reported in rabbits (Samorajski, 1956).

The presence of acid phosphatase in the blood vessels and blood cells of mammals has been reported by several investigators (Behrendit, 1943, 1949; Wolf et al., 1943; King, Wood and Delory, 1945; Haight and Rossiter, 1950; Ruttenburg and Seligman, 1955; Heinvaar, 1960). Roche, Thoai and Baudoin (1942) reported the presence of two acid phosphatases in the red cells of the ox and rat, optimally active at pH 4.6-4.8 and at pH 5 - 5.5. Abul Fadl and King (1949) had confirmed the above report by their studies on the red cells of humans and certain animals. Recently

Schmidt (1963) has reported acid phosphatase activity in the blood vessels and blood cells of adult newts. Our observations show that the enzyme activity is present in the epithelium of the capillaries and the tunica intima of the arteries and veins but in the cytoplasm of the blood cells the activity is very low in the normal tail. The enzyme activity in the above stated parts of the vascular system in the regenerating tail showed a similar condition as seen in those parts of the normal tail.

In the cytoplasm of the cells of different tissues of the normal and regenerating tail of the house lizard, the enzyme activity was found to be localized in some granular structures. Appelmann et al. (1955) had observed the same type of localization of the enzyme in the liver cells as a special class of cytoplasmic granules, which are entirely distinct from microsomes and comparable in size to small mitochondria. These granular structures are believed to be the lysosomes (De Duve, 1959; Novikoff, 1960). The lysosomes are believed to be the store house of many lytic enzymes within a lipoprotein envelope, which help in intracellular digestion, autolysis and necrosis (De Duve, 1959). In the nerve cells a direct relationship was suggested between the amount of ribosenucleic acid (RNA) and acid phosphatase activity (Bodian and Mellors 1944). La Velle, et al. (1954) suggested that in nerve cells acid

phosphatase may be part of an enzyme system acting upon a ribonucleoprotein reserve (represented by Nissl substance) to release phosphate for metabolic processes in cell maintenance and function. Schmidt and Weary (1963) has obtained acid phosphatase activity during regeneration in the blastemal cells with a large quantity of RNA. Vorbrodt (1958) had reported some relation between the acid phosphatase activity and protein synthesis. Our observations show that the high acid phosphatase activity in the mesenchyme cells during the preblastemic and blastemic periods may be directly related to protein synthesis activity during the rapidly growing and dividing cells of the mesenchyma. A study of the localization of RNA and its concentration in the mesenchyme cells, which is in progress, might confirm this assumption.

Due to the widespread distribution of acid phosphatase, Schmidt (1963) has hypothesised participation of this enzyme in the metabolism of several substrates like nucleic acid, protein, carbohydrate and lipid. Our observations on the enzyme activity in the different tissues of the adult normal and the regenerating tail of the house lizard, Hemidactylus flaviviridis also show a similar wide distribution. It is conceivable that acid phosphatase may be associated with either synthesis or differentiation, as well as with phagocytosis and dissolution of the tissue components, depending upon the specific tissue involved and its physiological

state (Burstone, 1962). Further, it is understandable that a histochemical reaction *in vitro* which involves hydrolysis of a substrate suggests the existence of the same reaction of the enzyme *in vivo*. However, this concept should be modified in view of increasing awareness of transferase activity of hydrolytic enzymes, like phosphatases, glucosidases, nucleases and proteases (Fischman and Baker, 1956). So it is likely that under the conditions which exist in the cells of living tissue, transfer rather than hydrolysis, is the major activity of the enzyme referred to.