CHAPTER 7-

THE HISTOCHEMICAL DEMONSTRATION OF ALKALINE PHOPHATASE

IN THE NORMAL AND REGENERATING TAIL OF THE HOUSE

LIZARD, HEMIDACTYLUS FLAVIVIRIDIS

In the developing chick embryo the presence of

phosphatases and their increase during the course of

cellular differentiation was reported by Moog (1943,1944)

and he had observed that the alkaline phosphatase persisted

in the differentiating tissues and its concentration changed

as the differentiation proceeded. Similarly while studying

the regenerating tail of Triturus cristatus, Ghiretti (1950)

had shown that the intensity of alkaline phosphatase acti-

vity was initially low, and reached a maximum in the

regenerating tail after the 10th day of amputation. Later

a decline in the enzyme activity was followed by a second

peak by about 20 days after amputation. The first peak of

rise in the enzyme activity was correlated with the activity

of the blastemal cells, whereas the second peak was correlated

with the onset of the differentiation during regeneration.

Ghiretti (1950) further suggested that the rate of regenera-

tion was rapid in winter and spring but slow in summer.

During the rapid regeneration period there were two peaks showing

high enzyme activity while in summer when the rate of

regeneration was slow, only one peak of rise in the enzyme

activity was observed, which was as late as at about 20th

day after amputation. Junqueira (1950) studied the
alkaline phosphatase distribution in the regenerating
tail of Xenopus laevis tadpoles and suggested that the
enzyme activity always exceeded in regenerates than in
normal tails. Schmidt and Weary (1962) observed that
the alkaline phosphatase activity plays an important role
in the fibrogenesis and that it may also be associated
with the polysaccharide metabolism in regenerating limb
of newt, Diemictylus viridescens. Considerable work has
been done to understand the biochemical events that take
place in the regenerating amphibian appendages but very
little is known in this respect as far as the regenerating
body parts of reptiles are concerned. In this chapter
the histochemical distribution of alkaline phosphatase
activity in the different tissues of the normal and
regenerating tail of the house lizard, Hemidactylus
flaviviridis was studied.

MATERIALS AND METHODS

The normal tail and the regenerates with at least
one or two segments of the original tail stump were cut
and fixed in cold 10% neutral formalin (neutralized with
sodium hydroxide) at 4°C for 24 hours. The tissues were
then washed in distilled water for about 8 hours and
sectioned on a freezing microtome at 10 to 15 μ thickness.

The sections were washed in distilled water and placed
in an incubation medium for the demonstration of alkaline
phosphatase activity by two separate methods as described
below.

(1)   Fredricsson's method (1952) which is a modification
      of Gomori's technique, as described by Pearse (1960).
      Sections were incubated for 13 to 14 hours at 37°C
      in an incubation medium containing sodium β-glycero-
      phosphate as the substrate, buffered at pH 9.2 with
      2% sodium barbitol.

(2)   Naphthol AS phosphate method described by Burstone
      (1958a, 1961): Naphthol AS-MX (Sigma Chemical
      Company, U.S.A.) was used as the substrate and Fast
      Blue B (BBN) (Sigma Chemical Company, U.S.A.) as
      the diazonium dye. The incubation medium buffered
      at pH 9.2 with 0.2M tris, was freshly prepared as
      required. Sections were incubated for 30 minutes
      to 12 hours at 37°C.

Two separate sets of controls were kept for both
the methods: (1) Identical sections incubated in the
buffered media devoid of substrate and (2) Sections
incubated after keeping them in hot water at 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.

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OBSERVATIONS

Microscopic examination of the samples as well as controls revealed that the naphthol AS phosphate method described by Burstone (1958a,1961) was preferable to Fredricsson's (1952) modification of Gomori's technique as described by Pearse (1960). By the latter method the enzyme localization was not precise and was seen in the cytoplasm as well as in the nucleus of cells. Besides, the enzyme activity in the nucleus was seen only on a prolonged incubation for about 17 to 19 hours. By this method, the high enzyme activity was denoted as black precipitates of the cobalt sulphide and the low activity appeared as grey colour. The control sections also showed this grey colour almost all over the tissue. Thus it was difficult to localize the exact site and intensity of the enzyme activity. As the grey colour appeared in the control sections when the enzyme activity has been inhibited, it was difficult to distinguish the enzymatically active tissues from the nonactive ones. The Naphthol AS phosphate method described by Burstone (1958a,1961) on the other hand, gave better clarity for the sites of enzyme activity and its intensity. The highly coloured diazonium salt, Fast Blue B (BBN) gave a deep colour at the sites of high enzyme activity while a pale blue colour suggested a low level of enzyme activity. By this method the enzyme was found to be

extranuclear in distribution and was localized in the
cytoplasm as well as ⁁*in the* mitochondria. For the above stated
advantages, in the present study we have followed the
✗ naphthol AS phosphate method.

NORMAL TAIL:

In the epidermis a high alkaline phosphatase
activity was observed in the cytoplasm and mitochondria
of the cells of the stratum intermedium, stratum germi-
nativum and along the connective tissue fibres of the
basement membrane (Fig.1). However, the beta and alpha
cells of the old and new generations were devoid of enzyme
activity. In the dermis the enzyme was highly active only
in the nerve fibres, blood vessels and the blood cells
while it was very low in the connective tissue fibres.

In the skeletal muscle fibres the alkaline phosphatase
activity was fairly high and of varied intensity. The
enzyme was found to be localized in the sarcoplasm and the
mitochondria of the muscle fibres (Fig.2). Based on the
number of mitochondria and the intensity of the enzyme
activity in the mitochondria and in the sarcoplasm, three
types of muscle fibres could be differentiated - one type
with numerous mitochondria and high mitochondrial as well
as sarcoplasmic enzyme activity, a second type with very
few mitochondria and low enzyme activity in their sarcoplasm

as well as mitochondria and a third type with intermediate values of mitochondrial content and enzyme activity.

The subcutaneous and the submuscular adipose tissue showed moderate enzyme activity. The osteoblasts and the osteocytes of the bone tissue of the vertebrae and the fat cells present in the hollows of the centra of the vertebrae showed very high enzyme activity. The intra-vertebral and intervertebral cartilage also showed fairly well distributed enzyme activity in its chondrocytes (Fig.3). In the spinal cord, the enzyme activity was low and uniformly distributed. The enzyme activity in the tunica intima and adventitia of the arteries and veins, the endothelium of capillaries and the cytoplasm of the blood cells was fairly high.

REGENERATING TAIL:

Wound healing phase:

A very low but perceptible enzyme activity was observed in the wound epithelium. However, the lymphocytes, leucocytes and histocytes at the subepithelial region showed fairly high enzyme activity (Fig.4).

Preblastemal phase:

During this phase the cells of the epithelium remained poor in the enzyme activity. In the subepithelial region

the RBCs, leucocytes, lymphocytes and histocytes showed

a high enzyme activity. The localization of the enzyme

in these cells was cytoplasmic. The enzyme activity in

the stump tissues remained the same as in those of the

normal tail described earlier.

Blastemal phase:

The enzyme activity was low in the epithelial and

the mesenchyme cells (Figs. 5 & 6).

Late blastemal phase:

During this phase an increased enzyme activity was

noted in the mesenchyme cells in the blastemal cone as

(the) differentiation commenced (Figs. 7 & 8). Fairly high

enzyme activity was observed in the blood capillaries and

blood cells present in the blastemal core. Fat cells of

different size with high enzyme activity were seen localized

uniformly surrounding the ependyma at the proximal region

and unevenly distributed in the distal region of the blastema

(Figs. 8 & 9).

Differentiation phase:

Stratum germinativum of the epidermis showed a very

low enzyme activity during the differentiation phase (Fig.10).

The cells of the beta and alpha layers did not show any

enzyme activity. A further increase in the enzyme activity

was noted in the cells of stratum germinativum when the formation of the scales took place (Fig.11). The formation of the stratum intermedium and the cells of the new generation of beta and alpha layers occurred at the time of moulting. High enzyme activity was observed in the stratum intermedium but in the beta and alpha cells of the new generation it was negligible. The differentiated epidermal basement membrane retained its high enzyme activity.
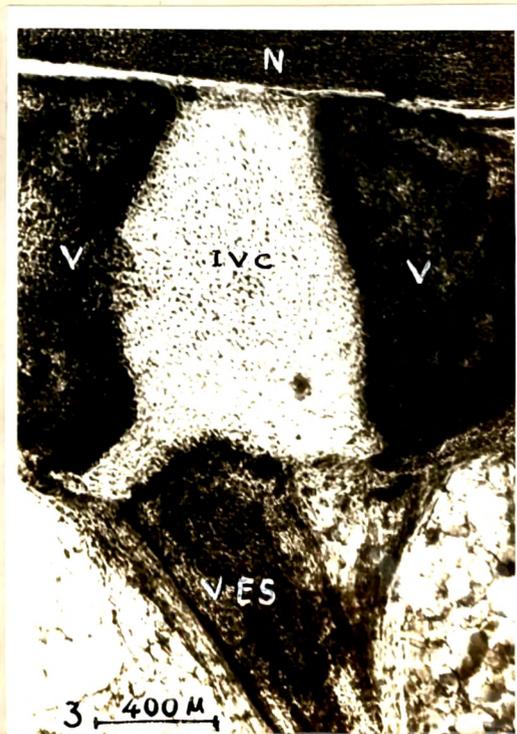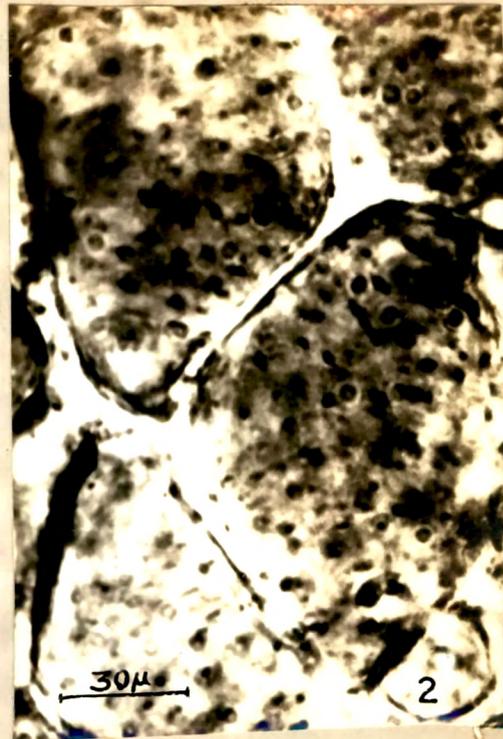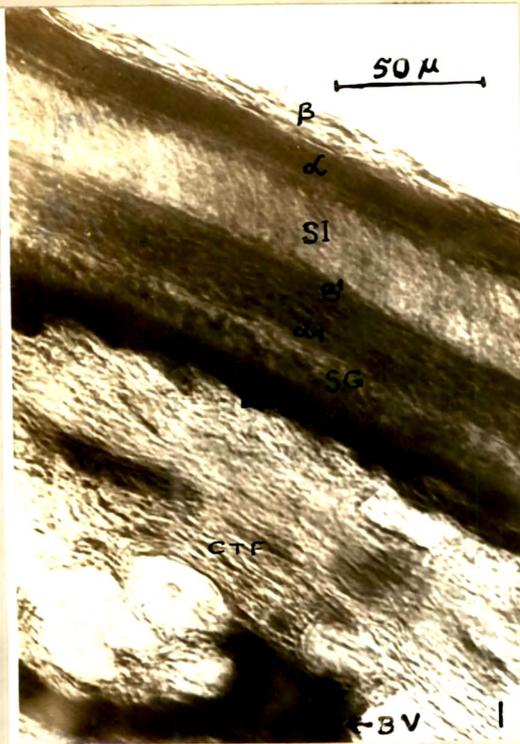
In the dermis region fibroblasts with high enzyme activity were observed before differentiation. The enzyme activity decreased as the fibroblasts transformed into the connective tissue fibres. Thus differentiated connective tissue fibres showed a low enzyme activity. However, the blood vessels and nerve fibres of the dermis showed a high enzyme activity. The fascia between the dermis and the muscle was devoid of enzyme activity. However, the undifferentiated fibroblasts in this region still showed fairly high enzyme concentration before differentiating into connective tissue fibres.
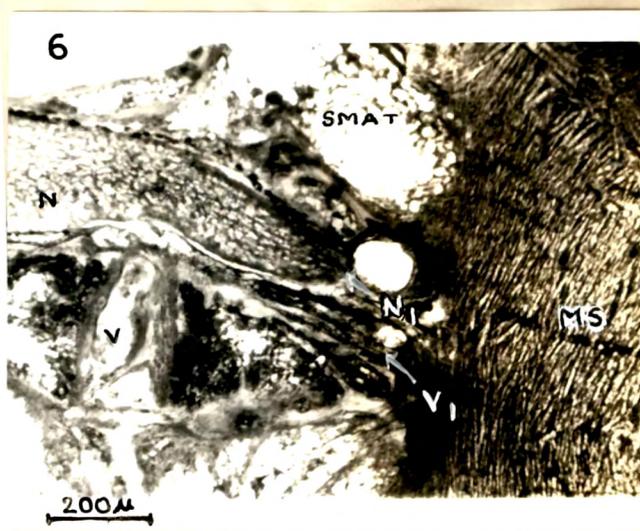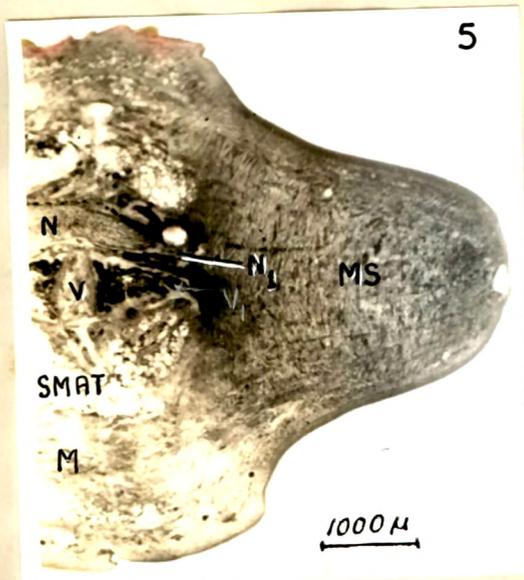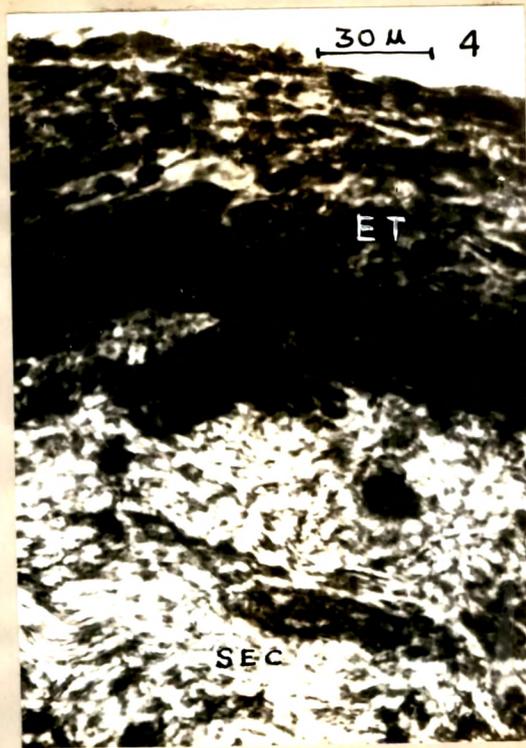
During myogenesis the mononuclear myoblasts, myocytes and the just differentiated myofibres showed poor enzyme activity. The enzyme activity was first noted in the cytoplasm of the myofibres soon after the differentiation. Fairly high alkaline phosphatase activity was observed in the cells of the subcutaneous and submuscular adipose tissues.
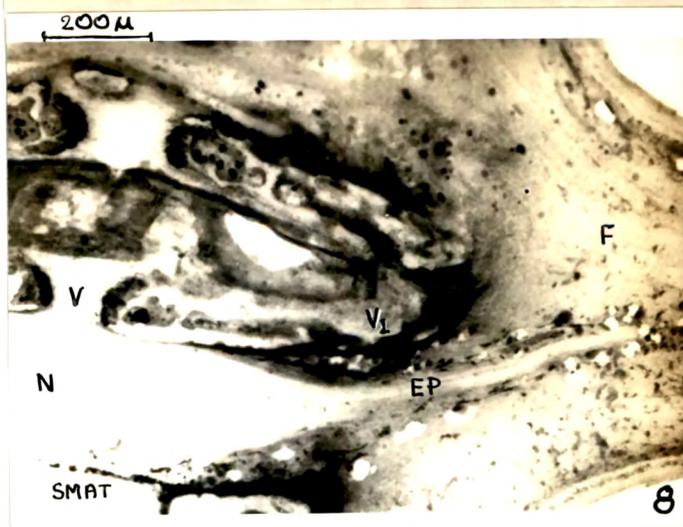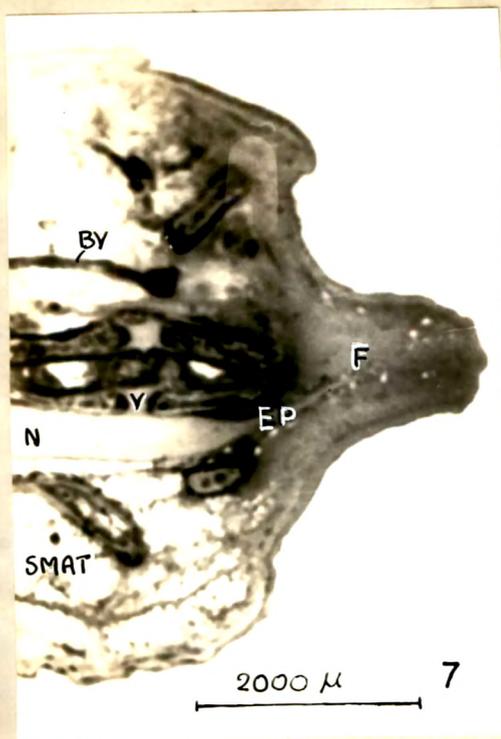
An increase in the enzyme activity was observed when the mesenchyme cells transformed into the chondroblasts and the chondroblasts in turn to chondrocytes. The perichondrial chondrocytes showed higher enzyme activity than those which formed the mass of the cartilagenous neural canal (Figs. 12 & 13). The ependymal tube showed very poor enzyme activity during the differentiation phase. However, the cytoplasm of the Schwann cells showed some enzyme activity.
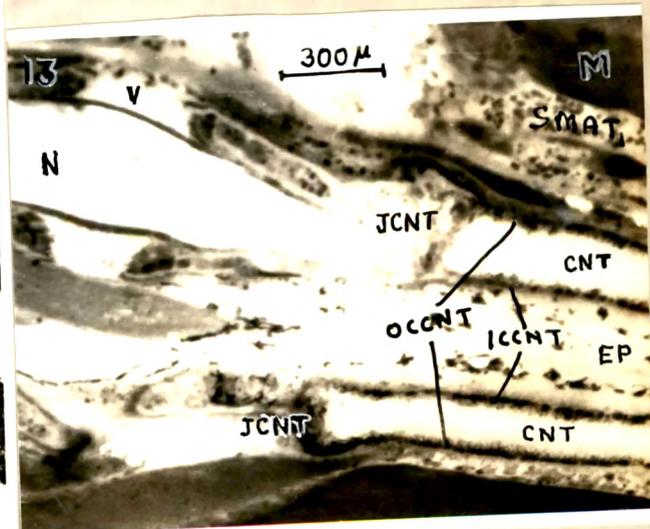
Growth phase:

During the growth phase all cellular layers except the beta and alpha cells of the epidermis, showed high enzyme activity which continued to be the same even in the fully regenerated tail. In the dermis, the blood vessels, blood cells and the nerve fibres showed high enzyme activity while the connective tissue fibres of the dermis and fascia were poor in the enzyme activity. The cytoplasmic enzyme activity in the muscle fibres which was observed during the differentiation phase increased gradually as the muscle fibres matured. The mitochondrial localization of the enzyme first appeared during the growth phase and its intensity increased as the muscle fibres attained morphological and functional maturity. The regenerated muscle fibres also showed three types of fibres as in the normal tail. The subcutaneous and the submuscular

Figure 7. Scale bar 2000 μ. Labels: BY, V, N, EP, F, SMAT.

Figure 8. Scale bar 200 μ. Labels: V, V₁, N, EP, F, SMAT.

Figure 9. Scale bar 200 μ. Labels: ED, FC, F.

10. 30μ · β · α · SG₁ · F

11. D₁ · β · α · SG₁ · 30μ

12. ED · D₁ · M₁ · CNT · C · 300μ

13. V · N · 300μ · M · SMAT · JCNT · CNT · OCCNT · ICCNT · EP · JCNT · CNT
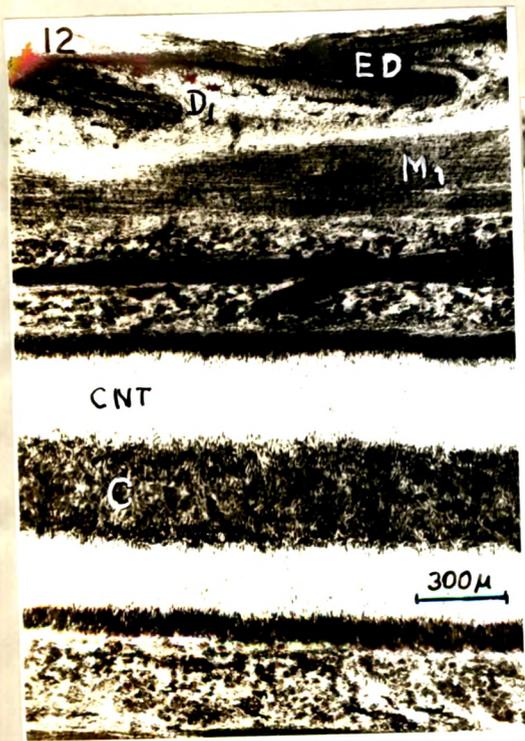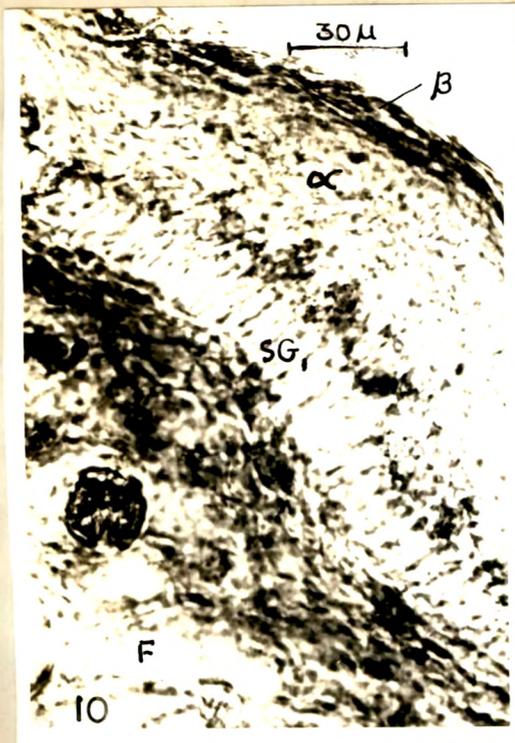
EXPLANATIONS FOR FIGURES

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

Fig. 2.   T.S. caudal muscle fibres showing localization
of sarcoplasmic and mitochondrial enzyme activity.

Fig. 3.   L.S. of vertebral column at the intervertebral
junction. Note the enzyme activity in the bone
cells, marrow cells and the cells of the inter-
vertebral cartilage. Part of the spinal cord with
enzyme activity is also seen.

Fig. 4.   Wound epithelium and subapical cells showing
enzyme activity.

Fig. 5.   L.S. of blastema showing low enzyme activity in
the blastemal cells. However, fairly high con-
centration of the enzyme in the cells adjacent
to the cut end of the vertebra and spinal cord
could be noticed.

Fig. 6.   Portion of the cut end of the vertebra and spinal
cord with adjacent blastemal cells enlarged.

Fig. 7.   L.S. of late blastema showing an increase of the
enzyme activity in all cells of the blastema.

Fig. 8.   Portion of Fig.7 (basal portion of the regenerate
enlarged).

Fig. 9.   Distal part of Fig.7 enlarged. Note the increased
enzyme activity in the tissues forming the core
of the blastema but low enzyme activity in the
epidermis.

Fig.10.   A portion of 8 day old blastema, showing the just
differentiated epidermal cells with low enzyme
activity in the germinativum layer.

Fig.11.   L.S. of the skin (the scale region) of a 20 day old
regenerate showing the increased enzyme activity
in the germinativum layer.

Fig.12. L.S. of the regenerate showing enzyme activity
in the epidermis, muscle, submuscular adipose
tissue, cartilagenous neural canal and blood
vessels.

Fig.13. L.S. of the regenerate (late differentiation
phase) showing enzyme activity at the junction
of the vertebral column and cartilagenous neural
canal and in the chondrocytes at the inner and
outer margin of the cartilagenous neural canal.

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

C – Chondrocytes

CNT – Cartilagenous neural canal

CTF – Connective tissue fibres in dermis

D_1 – Dermis in the regenerate

EBM – Epidermal basement membrane

ED – Epidermis

EP – Ependyma

ET – Epithelium

F – Fibroblasts

FC – Fat cells

ICCNT – Chondrocytes at the inner lining of the CNT

IVC – Intervertebral cartilage

JCNT — Junction of CNT in the vertebral column

M — Muscle

M_1 — Muscle in the regenerate

MS — Mesenchyme cells

N — Nerve cord (spinal cord)

N_1 — Regenerating nerve cord (ependyma)

OCCNT — Chondrocytes at 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

V — Vertebral column

V_1 — Cut vertebra

VES — Vertebral spine

adipose tissue showed high enzyme activity throughout
the growth phase. There was no change in the intensity
of the enzyme activity in the cartilagenous neural canal
from that seen during the differentiation phase. The
ependyma, the glial cells, Schwann cells and the myelin
sheath showed high enzyme activity, whereas, in the other
parts of the ependyma the enzyme concentration was
uniformly low.

DISCUSSION

Kambara (1955) reported the distribution of alkaline
phosphatase in the epidermis of Triturus pyrrhogaster.
A higher enzyme activity was observed in the epidermis on
the dorsal side of the body than that on the ventral side.
Karczmar and Berg (1951) found that this enzyme was absent
in the epidermis of the larval Amblystoma limbs. They
opined that the absence of alkaline phosphatase may be
due to the age difference or the functional difference
of the larval tissue. Schmidt and Weary (1962) noted a
gradual decrease in the enzyme activity from the inner to
the outer cell layers of the epidermis of the newt,
D. viridescens. In the H. flaviviridis, the enzyme was
localized in the cytoplasm and the mitochondria of the
cells of the stratum germinativum, stratum intermedium
and along the connective tissue strands of the basement

membrane. The cells of beta and alpha layers were devoid of the enzyme activity. As the new beta and alpha cells were being formed the alkaline phosphatase activity of the stratum germinativum registered an increase. Thus the activity of the enzyme in this layer of cells must be related with the process of keratinization similar to the one suggested by Schmidt and Weary (1962) in the newt, D. viridescens. Before the old layer of beta and alpha cells were ecdysed it was noticed that a layer of stratum intermedium with very high enzyme activity was formed from the stratum germinativum. At the time of ecdysis the cells of stratum intermedium got autolysed and so the outer layers (older generation) of beta and alpha cells got casted off, leaving behind the new beta and alpha cell layers exposed. Thus in the processes of ecdysis also it seems that alkaline phosphatase must be playing some significant role. Schmidt and Weary (1962) have also suggested that the enzyme may be involved in the process of ecdysis of the skin in the newt. Several workers have suggested that alkaline phosphatase is associated with the formation of fibrous proteins and the passage of metabolites across the cell membrane, (Verzar and McDowgall, 1936; Moog,1941; Bradfield, 1950; Danielli, 1954; Vallyathan and George, 1965). The high concentration of alkaline phosphatase in the basement membranes of many tissues supports the above suggestion. Our observations on the high concentration

of the enzyme activity in the epidermal basement membrane in the house lizard, H. flaviviridis are in favour of the above view.

Presence of alkaline phosphatase in the connective tissue fibres of the dermis had been reported by Glenner and Burstone (1958) in Necturus maculosus and Schmidt and Weary (1962) in Diemictylus viridescens. In mammals the presence of this enzyme in the dermis was found only during the process of wound healing (Fell and Danielli, 1943). In the lizard the enzyme was very low in concentration along the connective tissue fibres.

There are varied reports on the alkaline phosphatase activity in skeletal muscle. Dempsey et al. (1946), using Gomori's technique have found that the enzyme reaction was positive in the muscle fibres on prolonged incubation. Greenstein (1945) reported very low enzyme activity in the mouse muscle. However, according to Gomori, 1941; Kabat and Fouth, 1941; Menheimer and Seligman, 1948; Becket and Bourne, 1958, there is no alkaline phosphatase activity in the muscles. Nevertheless, successful histochemical demonstration of the presence of the alkaline phosphatase activity in the pigeon breast muscle was claimed by George et al. (1958), and George and Pishawikar (1961). Recently Vallyathan and George (1965) using the azo coupling method have shown the localization of alkaline phosphatase activity

in the sarcoplasmic reticulum of the pigeon breast
muscle fibres. Karczmar and Berg (1951) had shown a low
level of enzyme activity in the striated muscle fibres
of the Amblystoma larvae, while Schmidt and Weary (1962)
could show the enzyme activity only in a few injured
muscle fibres of the limb muscles of <u>Diemictylus</u> <u>viridescens</u>
by using the method described by Burstone (1958a,1961).
Our findings on the alkaline phosphatase activity in the
muscle fibres of the normal tail muscles of the house
lizard, <u>Hemidactylus</u> <u>flaviviridis</u> showed that the enzyme
was localized in the sarcoplasm as well as ⁀in the⁀ mitochondria.
On the basis of the intensity of the sarcoplasmic and
mitochondrial enzyme and the number of mitochondria, three
types of muscle fibres could be ~~differentiated~~ distinguished.

It was observed that a moderate alkaline phosphatase
activity was obtained in the tissues where fat is stored,
viz., in the subcutaneous adipose tissue, submuscular
adipose tissue surrounding the vertebral column and the
fat cells present in the hollow of the centra of the caudal
vertebrae. From these observations it appears that the
enzyme may be directly or indirectly involved in fat meta-
bolism of these fatty tissues.

Association of alkaline phosphatase activity with the
calcification of bones in mammals and birds had been reported
by a number of workers (Robison, 1923; Fell and Robison,

1929, 1934; Moog, 1944). Lorch (1947) had observed
presence of the enzyme in the endosteum, the inner layer
of periosteum, some osteocytes, and the matrix and
osteoblasts of bone. It has become evident that the
phosphatase in the bone is concerned with the production
of a high local concentration of phosphate ions, which
will favour the deposition of calcium phosphate (Robison,
1923). Gomori (1943b) concluded from a survey of developing
bones of chick and developing embryos of mammals that all
cartilage which calcify became phosphatase positive and
the deposits of bone salts appeared in the enzyme active
region. Phosphatase negative regions were never observed
to calcify. Similar results were obtained by Moog (1944)
in a day to day study of the developing chick embryo which
revealed that the moderate phosphatase activity of the
mesenchymal rudiment of the bones faded as cartilage
differentiation proceeded, to reappear with greater inten-
sity in the perichondrial zone as the cartilage cells have
hypertrophied. Bourne (1943) found that when holes were
bored in living bones they regenerate a phosphatase rich
fibrous matrix as the bone salts deposition began. Karczmar
and Berg (1951) had reported alkaline phosphatase activity
particularly during calcification of cartilagenous skeletons
of the Amblystoma larva and Schmidt and Weary (1962) observed
a similar condition in regenerating limbs of Diemictylus

<u>viridescens</u>. In the bone tissue of the tail vertebrae
of the house lizard, alkaline phosphatase was found to
be localised in osteoblasts, osteocytes and the chondro-
cytes of the intervertebral cartilage. These observations
suggest the role of the enzyme in the metabolism of calcium
in the bone tissue of the tail vertebrae. Newman <u>et</u> <u>al</u>.
(1950) demonstrated histochemically the presence of alkaline
phosphatase in the axons and epineurium of the nerves of
mouse, rabbit and guinea pig. Samarajski (1957) opined that
the alkaline phosphatase activity in the peripheral nerves
must be primarily associated with the growth of nerve fibres.
Marchant (1949) had reported strong alkaline phosphatase
activity in the sciatic nerves of rabbit, cytoplasm of
Schwann cells, debris of myelin and nerve fibres. However,
Schmidt and Weary (1962) found that the enzyme was restricted
to the neutral sheath. In the non-amputated adult tail of
the house lizard, a low enzyme activity was uniformly present
in all the parts of the spinal cord. Alkaline phosphatase
activity of moderate high intensity was observed in the blood
vessels of amphibians (Junqueira, 1950; Glenner and Burstone,
1958) and mammals and birds (Gomori <u>et</u> <u>al</u>., 1959; Scheen and
Winkelmann, 1961). Monis and Lutenberg (1959) and Haight
and Rossiter (1950) have shown that the alkaline phosphatase
is absent in the mammalian erythrocytes. However, the
presence of cytoplasmic alkaline phosphatase activity in the
leucocytes and nucleated erythrocytes in adult newts had

been reported by Schmidt and Weary (1962). In blood vessels of the house lizard the localisation of the enzyme was found to be in the tunica intima and adventitia of arteries and veins, the endothelium of capillaries and the cytoplasm of the blood cells. Dean et al.(1960) suggested that alkaline phosphatase in the blood vessels and blood cells promote transport and absorption of glucose. Since the enzyme activity is fairly high in these parts of the non-amputated tail of the house lizard, it is likely that here the enzyme may also be involved in the active transport and absorption of glucose.

The wound epithelium was mostly devoid of alkaline phosphatase activity in adult Triton (Preda et al.,1962) and in mammals (Fell and Danielli, 1943; Hanke, 1962; Johnson and McMinn, 1958). Karczmar and Berg (1951) have also observed that the enzyme activity was nil in the epithelium as well as in epidermis between the sixth and twelfth days of regeneration in the larval Amblystoma. Schmidt and Weary (1962) found high cytoplasmic alkaline phosphatase present in the wound epithelium, which remained consistent in the limb stump epidermis. In the regenerating tails of the house lizard the alkaline phosphatase activity in the wound epithelium was very low, whereas the lymphocytes, histocytes and blood cells collected under it showed

a high enzyme concentration in their cytoplasm. The
enzyme activity was somewhat restricted to the histocytes
and blood cells in the subapical region of the preblastemal
period of regeneration, until enzyme rich blastemal cells
began to accumulate. A similar observation has been
reported by Schmidt and Weary (1962) in the regenerating
limb of newt and by Karczmar and Berg (1951) in Amblystoma
larva.

An increase in the alkaline phosphate activity during
the blastemal phase of regeneration had been reported in
the regenerating tail of <u>Triturus</u> (Ghiretti,1950), <u>Bufo</u>
<u>marinus</u> tadpoles (Junqueira, 1950), <u>Rana</u> tadpoles (Moyson,
1946), <u>Triton</u> (Preda <u>et al</u>., 1962), <u>Amblystoma</u> (Karczmar
and Berg,1951) and the adult newt, <u>Diemictylus viridescens</u>
(Schmidt and Weary, 1962). Karczmar and Berg (1951) have
made a suggestion that the high alkaline phosphatase acti-
vity of the blastemal cells may reflect a transition through
which the differentiated cells must pass. An intra blastemic
fibrogenesis had been reported during the blastemal phase
of regeneration (Schmidt,1962a). Jackson (1958) suggested
that if the blastemal cells were responsible for the fibro-
genesis, they are functioning as fibroblasts. Moreover,
the connective tissue cells were supposed to be the prime
source of the regeneration blastema (Mettetal, 1939; Manner,
1953; Schmidt, 1962a). In the regenerating tail of the

house lizard an increase in the enzyme activity during the blastemal phase of regeneration was seen. This increase was noticed in the cytoplasm of the fibroblast during this phase. In this animal also it is possible that as suggested by Karczmar and Berg (1951) the fibroblast cells may reflect a transition through which differentiating cells may have to pass, and so during the transition, the high alkaline phosphatase activity must be playing some significant role in cell differentiation.

Alkaline phosphatase increases during the differentiation phase of regeneration (Ghiretti, 1950; Karczmar and Berg, 1951; Schmidt and Weary, 1962). During this phase, in the regenerating tail of Hemidactylus flaviviridis we observed varying intensity of the enzyme activity in different tissues. The enzyme activity in the epidermal tissue was very low but as soon as the differentiation of the cells of stratum germinativum and stratum intermedium started, the enzyme activity reached a maximum when the scales were being formed. This high enzyme activity was maintained thereafter during the growth phase and even in the fully regenerated epidermis. Thus in these layers of the epidermis, the enzyme activity is related to their functional activity viz., keratinization and ecdysis. During myogenesis, initially there was a decrease in the

enzyme activity when the fibroblasts transformed into myoblasts. The myocytes and the just formed myofibres showed very low enzyme activity. After this there was a gradual increase in the enzyme activity in myofibres during their growth phase. It first increased in the cytoplasm and then in the mitochondria. Our observations on the decrease in enzyme activity in the fibroblasts which were transformed into myoblasts, agree with the finding of Moyson (1946) that in the developing limb, the mesenchyme that became muscle lost their enzyme activity. The cells of the fat depot around the vertebral column and those of the subcutaneous adipose tissue in the lizard showed moderate enzyme activity right from the beginning and remained so even when they were fully loaded with fat. The differentiating nerve cord showed low enzyme activity throughout the differentiation phase. The differen-tiating skeleton was phosphatase reactive through chondro-genesis. As the differentiation of the chondrocytes was complete the enzyme activity was slightly low. Schmidt and Weary (1962) have demonstrated alkaline phosphatase during the chondro- and osteogenesis of the regenerating limb. Karczmar and Berg (1951) found the blastemal cells and the hypertrophic chondrocytes of the skeletal osteo-genesis to be notably high in this enzyme. The association of alkaline phosphatase with calcification is well established (Pritchard, 1952). During the growth phase and in the fully

regenerated tails the enzyme activity was similar to
that observed in the chondrocytes of the neural canal
at the differentiation phase.

It is a well established fact that there are many
similarities between regeneration and embryonic development
(Schotté, 1939). Borghese (1957) studying the developing
limbs of birds and mammals suggested that phosphatase
usually preceeds organ differentiation and another secondary
increase in the enzyme activity was supposed to have a role
in the functioning of the organs or structures. Ghiretti
(1950) had reported two peaks in the alkaline phosphatase
activity in the regenerating tail of Triturus cristatus.
The first peak was at the blastemal phase and the second
at the onset of differentiation, whereas Moyson (1946)
reported only one peak (blastemal) of the enzyme in the
tadpole regeneration. Our study on the enzyme distribution
in the regenerating house lizard tail has revealed that
there is an initial increase of the enzyme during differen-
tiation and a secondary peak of the enzyme activity during
the functional phase of some of the regenerating tissues
like muscles and epidermis.

Alkaline phosphatase activity corresponds with the
presence of glycogen (Schmidt, 1960) in the cells of the
epidermis, apical epithelium, Schwann cells of the

regenerating nerves, hypertrophied chondrocytes of ossification centres and the cells of the articular cartilage. From this study he suggested that alkaline phosphatase may be involved in the anaerobic utilization of glycogen leading to lactic acid, a metabolic activity suggested by the depletion of glycogen by transected muscles during the early stages of regeneration. Our studies on glycogen in the regenerating tail of Hemidactylus flaviviridis (Chapter 5) showed an identical histochemical localization of glycogen and of the enzyme, alkaline phosphatase. We have observed that in the muscle fibres the enzyme is localized in their sarcoplasm as well as in mitochondria. Vallyathan and George (1965) have suggested that the alkaline phosphatase present in the sarcoplasmic reticulum of the pigeon breast muscle may be involved in transport of glycogen from the sites of its synthesis. Thus identical localization of glycogen and alkaline phosphatase in the normal and regenerating tail of house lizard suggests the correlation of the enzyme activity and active transport and utilization of glycogen.