CHAPTER 3

HISTOCHEMICAL OBSERVATIONS ON GLYCOGEN AND PHOSPHORYLASE AND QUANTITATIVE GLYCOGEN CONTENT IN THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

It is well established that glycogen is the chief stored carbohydrate in the animal tissues that could serve as the prime reservoir of immediate energy by its catabolism through the intermediary reactions of glycolysis. Due to this importance of glycogen as the primary energy source, both glycogen as well as the various glycolytic enzymes have been extensively studied in the various vertebrate tissues (Mancini, 1948; Montagna, 1949; Bergman, 1960; Grillo, 1961; Falin, 1961 and Cosmos, 1966). Okuneff (1933) was the first to report the existence of glycolysis in the regenerating limb of 8-10 months old axolotl. Later some workers (Needham, 1952; Hess, 1959; and Schmidt, 1960, 1966b) have discussed the energy problems of the regenerating amphibian appendages. Apart from these no other reports pertaining to the distribution of glycogen and phosphorylase in the regenerating vertebrate tissues are available. Moreover, studies of this nature have not received much attention in the regenerating reptilian appendages. The only report bearing on the histochemical

localization of glycogen and phosphorylase available
to date is that of Shah and Chakko (1967b) in the
normal and regenerating tail of the house lizard,
Hemidactylus flaviviridis.

Therefore, it was thought desirable to study
histochemically the distribution and localization of
glycogen and phosphorylase in the normal and
regenerating tail of the Scincid lizard, Mabuya
carinata so as not only to expand our knowledge
regarding the metabolic adaptations in the regenerating
reptilian appendages but also to enhance our knowledge
of regeneration in general and particularly in reptiles.
For further support to histochemical observations,
quantitative estimations of glycogen in the normal and
regenerating tail of the same lizard were carried out.

MATERIAL AND METHODS

Adult Mabuyas maintained in the laboratory on a
diet of young insects were selected as the experimental
animals. The autotomy of the tail was induced as
described in Chapter 1. The autotomized tails were
fixed on a microtome chuck in a cryostat maintained

at -20°C. They were sectioned at 12-18 μ thickness and processed for the histochemical demonstration of glycogen and phosphorylase.

For the study of glycogen, the sections were mounted on a slide without any adhesive and fixed in alcoholic picroformol for 2-3 hours at -20°C. After fixation, the sections were thoroughly washed in absolute alcohol in order to remove the yellow colour of the fixative and then processed for the histochemical demonstration of glycogen using PAS technique as described by Pearse (1960). Sections treated with salivary amylase or diastase before the staining served as controls.

Quantitative studies of the total glycogen content in the normal and regenerating tails were carried out according to the method of Seifter et al., (1950), using anthrone reagent.

For the histochemical demonstration of phosphorylase the method of Takeuchi and Kuriaki (1955) was adopted. The sections were directly transferred to the incubation medium and incubated at room temperature (29-32°C) for about 30-60 minutes.

Glucose-1-phosphate was used as the substrate and glycogen as the primer and pH of the medium was adjusted to 5.5. After incubation, the sections were treated with ten times diluted Gram's iodine with distilled water. The sections were mounted in a mixture of Gram's iodine and glycerol (1:9 v/v). Observations and microphotographs were taken immediately. Sections incubated in a substrate blank medium served as controls.

OBSERVATIONS

NORMAL TAIL (Fig.1)

Excepting for the stratum germinativum wherein a moderately high content of glycogen and phosphorylase could be noticed, all the other cellular elements of the epidermis together with the dermis failed to show any localization. Of the various other normal tail components, the caudal muscles depicted the highest concentration of glycogen and phosphorylase with the peripheral fibres in each fasciculus revealing a comparatively high level than the inner ones (Figs. 2 and 3).

The subcutaneous and submuscular adipose tissue and the vertebral elements were all devoid of glycogen and phosphorylase (Fig.1). However, some of the marrow cells and the cartilage cells at the articulating surfaces of the centra showed slight response towards both glycogen and phosphorylase. In the nerve cord glycogen and phosphorylase were noticeable at a low level in the grey matter whereas in the white matter they were completely absent.

REGENERATING TAIL

Wound healing phase: (Figs. 4 and 5)

Localization of both glycogen and phosphorylase in appreciable concentration was noticed in the cells of the wound epithelium, but this concentration was relatively less in the cellular aggregates underlying the wound epithelium. During the wound healing phase the depletion of glycogen from the cut ends of the original stump tissues was noticed.

Blastemic phase:

Along with the progressive stratification of the wound epithelium and attainment of the blastemic epithelium
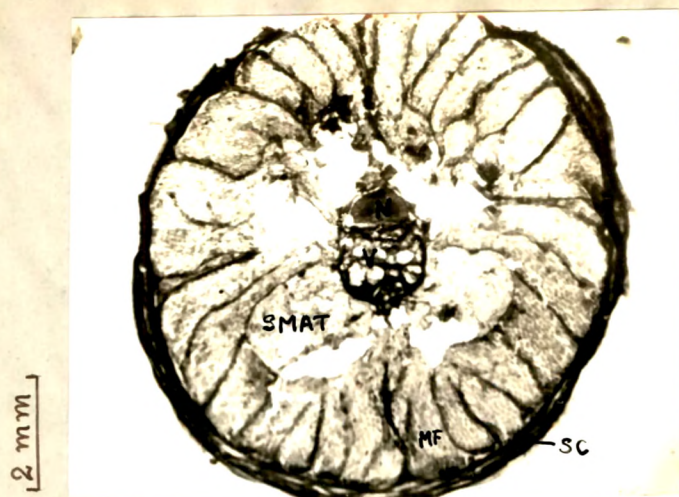
EXPLANATIONS FOR FIGURES

Fig. 1. T.S. of the normal tail revealing the glycogen content in muscles and the skin region.

Fig. 2. T.S. of the normal skin revealing the glycogen content. Note the higher content of glycogen in the peripheral muscle fibres than those in the centre of the fasciculum.
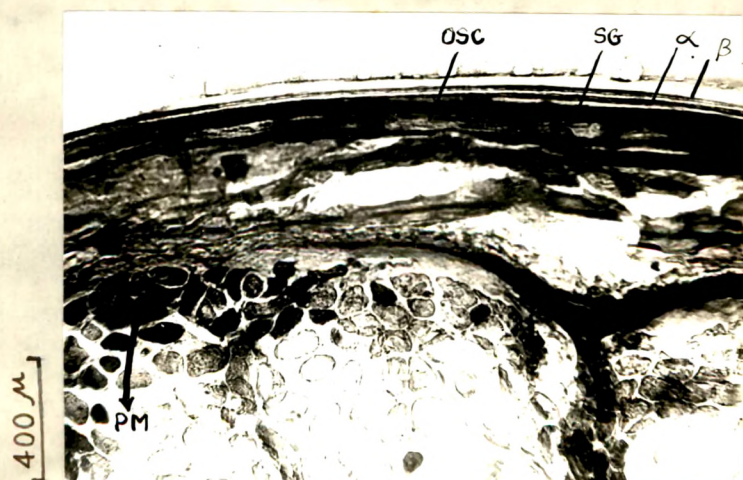
Fig. 3. L.S. of the normal tail showing the high activity of phosphorylase in muscle and the nerve cord.
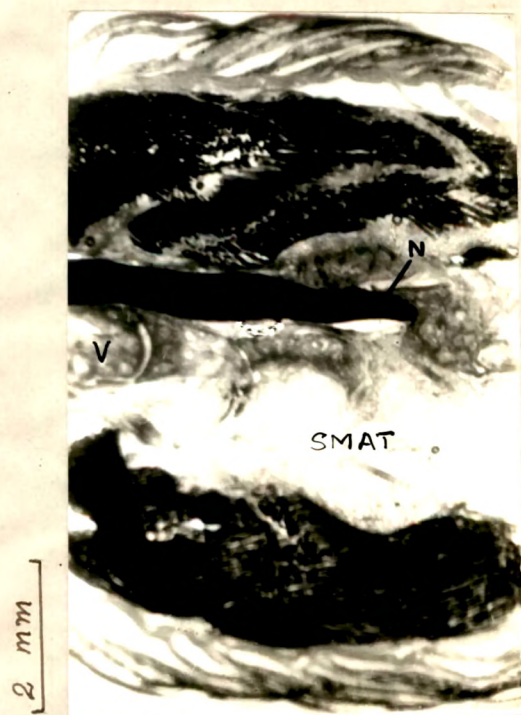
ABBREVIATIONS

α	-	Alpha cells
β	-	Beta cells
MF	-	Muscle fasciculus
N	-	Nerve cord
OSU	-	Osteoscute
PM	-	Peripheral muscle fibres
SG	-	Stratum germinativum
SMAT	-	Submuscular adipose tissue
V	-	Vertebra

1

2

3

EXPLANATIONS FOR FIGURES

Fig. 4. Wound epithelium showing the glycogen
content.

Fig. 5. Photomicrograph of the wound epithelium
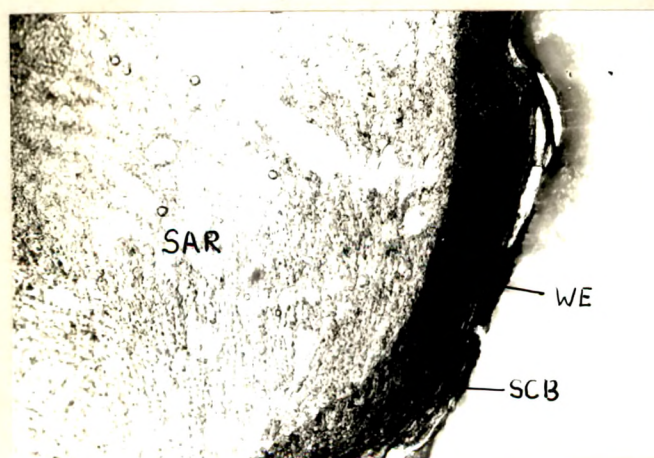showing phosphorylase activity.

ABBREVIATIONS

SAR — Subapical region

SCB — Scab

WE — Wound epithelium

4

200 μ

5
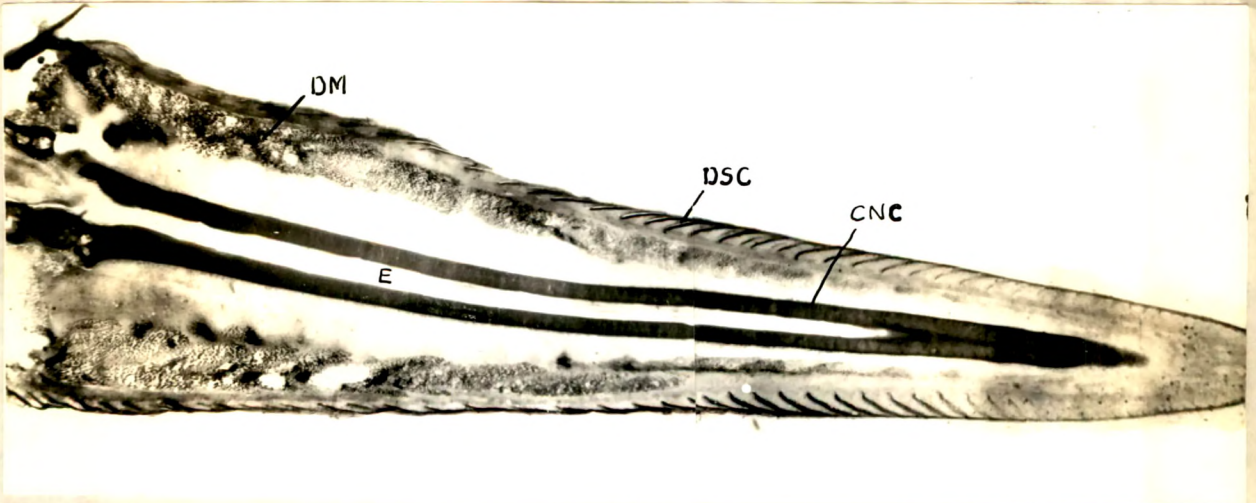
200 μ

EXPLANATIONS FOR FIGURES

Fig. 6. Photomicrograph of the late differentiation phase of the tail regenerate revealing high content of glycogen in various tissues.

Fig. 7. Magnified region of the differentiating scale and the muscles revealing the glycogen content.

Fig. 8. Photomicrograph of the longitudinal section of the tail regenerate at the differentiation phase revealing phosphorylase activity in the various tissues.

ABBREVIATIONS
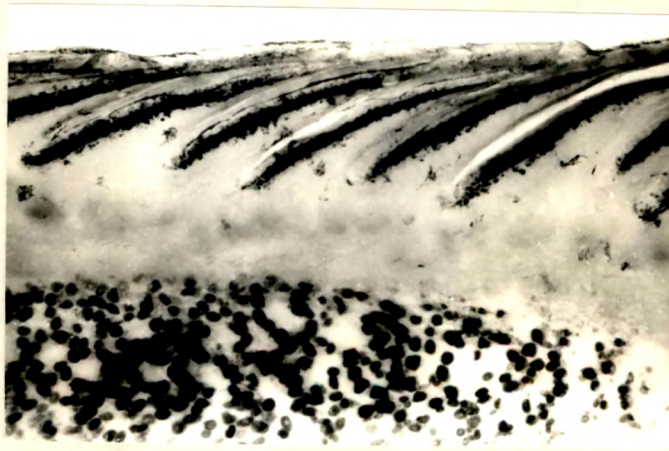
CNC - Cartilagenous neural canal

DM - Differentiating muscle

DSC - Differentiating scale

E - Ependyma

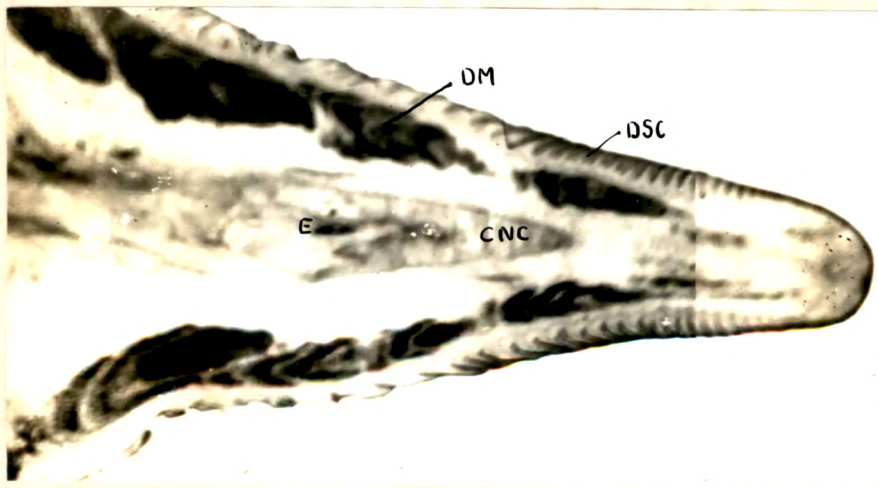2 mm   6

200 μ   7

2 mm   8

EXPLANATIONS FOR FIGURES

Fig. 9. Magnified view of the differentiating scale revealing phosphorylase activity.

Fig. 10. Higher magnification of the differentiating muscles exhibiting maximum activity of phosphorylase.

Fig. 11. Photomicrograph of a portion of the cartilagenous neural canal magnified to show phosphorylase activity in the chondrocytes.
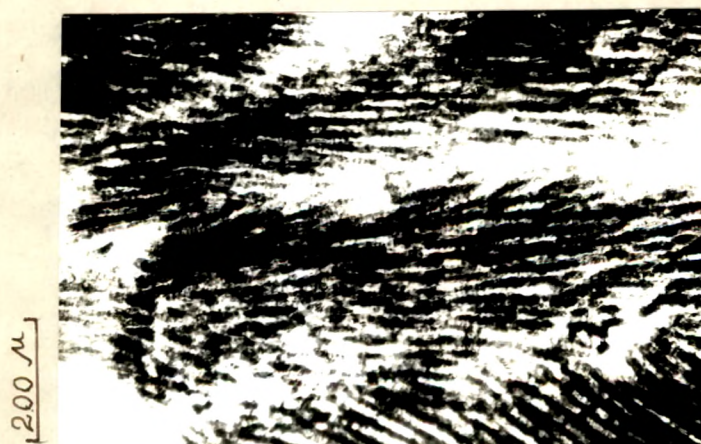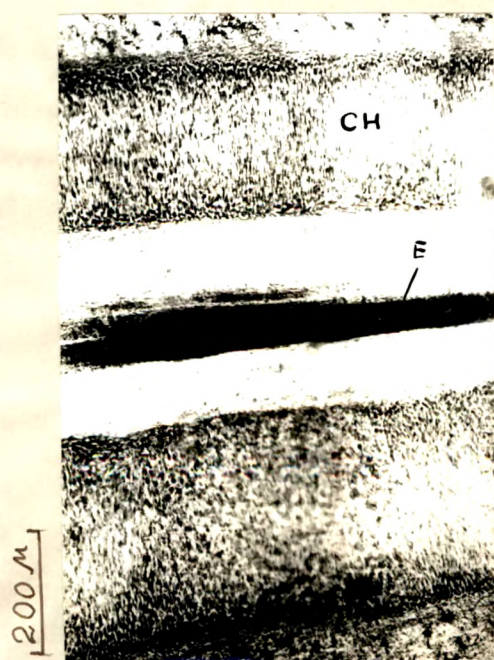
ABBREVIATIONS

CH — Chondrocytes

E — Ependyma

9

200 μ

10

200 μ

CH

E

11

200 μ

TABLE 3.1

Quantitatively estimated levels of total glycogen in the

normal and regenerating tail of the Scincid lizard,

Mabuya carinata

Normal tail and different phases of regenerating tail	Amount of glycogen (g/100 g fresh tissue)	Number of estimations performed
Normal tail	0.0570 ± 0.0064**	12
Regenerating tail*		
Blastema phase (7-12 days)[+]	0.0333 ± 0.0040	10[@]
Early differentiation phase (13-20 days)	0.1693 ± 0.0095	13[@]
Late differentiation phase (20-30 days)	0.3070 ± 0.0401	15
Growth phase (30-40 days)	0.1638 ± 0.0033	15
Fully regenerated tail (40-70 days)	0.0648 ± 0.0060	11

* Stages of regeneration are arbitrarily defined for the purpose of discussion eventhough the process is a continuous one.

@ Tissues from five animals for each estimations were pooled.
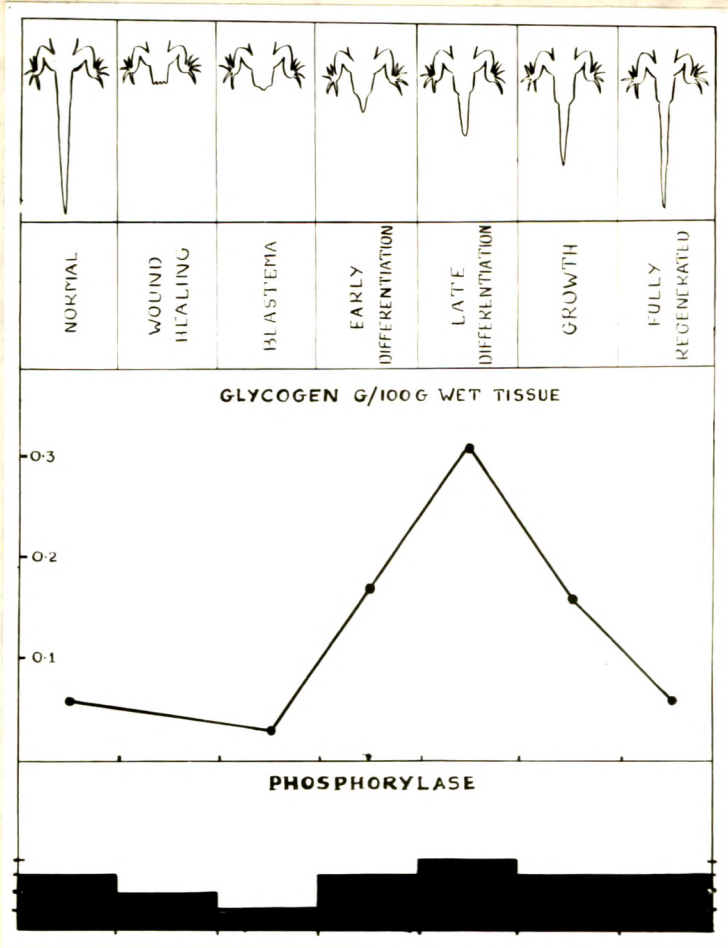
+ Number of days after autotomy
** Mean ± S.D.

Fig.12. Graphic representation of the total
glycogen content; and phosphorylase
activity in the normal and regenerating
tail of the Scincid lizard, Mabuya
carinata.

there was a concomitant progressive increase of both

glycogen and phosphorylase. However, mild activity

of phosphorylase and presence of glycogen in the form

of fine granules amongst the mesenchymal cells forming

the core of blastema could be observed.

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

All throughout the differentiation, the various

differentiating tissues viz., epidermal elements,

muscles and the cartilage cells were seen to record

concomitant rise and parallel levels of glycogen (Fig.12 and

Table 3.1) and phosphorylase. The highest levels of

glycogen and phosphorylase were noted to be during

this phase when compared to both the normal and other

various phases of regeneration of the tail. It was

rather evident from both the quantitative and histo-

chemical studies that the gradual increase of glycogen

and phosphorylase noted from early differentiation

onwards, attained a peak value during the later

differentiation phase, when the various tissues such

as skin, muscles, ependyma and cartilage cells of the

cartilagenous neural canal were well differentiated.

(Figs. 9, 10 & 11).

Growth phase and fully regenerated tail:

With completion of differentiation, the various
well differentiated tissues of the regenerate showed a
gradual decrease in glycogen content as well as
phosphorylase activity during the growth phase, which
was marked by the attainment of morphological and
physiological maturity of the differentiated tissues.
Along with this attainment of maturity which coincides
with the fully regenerated condition of the tail, the
levels of both glycogen and phosphorylase were found
to attain a level characteristic of the various tissues
of the normal tail.

DISCUSSION

The present studies on glycogen and phosphorylase
in the normal and regenerating tail of the Scincid
lizard, Mabuya carinata have shown that there exist
significant fluctuations in their distribution pattern
and total quantity during the various phases of
regeneration. From the observations made in the course
of the present study it becomes very clear that in the
normal tail of Mabuya carinata glycogen and phosphorylase
have a dominant distribution in comparison to lipid and

lipolytic enzymes, lipase and esterase (Chapter 2).
It may be noted here that identical observations have
been reported with regard to glycogen and phosphorylase
by Shah and Chakko (1967b) in the normal and regenerating
tail of the house lizard, Hemidactylus flaviviridis.
Schmidt (1962a) also reported the presence of appreciable
amount of glycogen in the forelimb of the newt,
Diemictylus viridescens. In the wake of these observa-
tions it could be suggested that the normal tissues of
the Mabuya carinata tail predominantly utilize carbo-
hydrate as a chief energy source. This is amply
confirmed by the reported high activity of aldolase in
the normal tail of Mabuya carinata and Hemidactylus
flaviviridis by Shah and Ramachandran (1972) and Magon
(1970) respectively and in the forelimb of the adult
newt, Diemictylus viridescens by Schmidt and Weidman
(1964). The histochemically observed pattern of
localization of glycogen and phosphorylase in the skin
of Mabuya carinata is in conformity with an identical
localization reported by Montagna and Ellis (1958) and
Bradfield (1951) in the skin of man and other animals.
They had suggested the ability of these epithelial cells
to synthesize and utilize glycogen for the energy

requirements during the synthesis of keratin and for cellular proliferation. At this stage it may be presumed that since the stratum germinativum contains an appreciable amount of glycogen and phosphorylase, the metabolite might be undergoing glycogenolysis through glycolytic pathway for the required energy for the cellular proliferation which could be expected during the active state of moulting.

The support for the presently reported high content of glycogen in the caudal muscles in the Mabuya comes from the studies of Shah and Chakko (1967b) in Hemidactylus flaviviridis and Schmidt (1962a) in the muscles of the forelimb of the newt, Diemictylus viridescens. Identical localization of high aldolase activity was also reported in the caudal muscles of Mabuya carinata (Shah and Ramachandran, 1972) and in the tail muscles of Hemidactylus flaviviridis (Magon, 1970) and in the limb muscles of Diemictylus viridescens (Schmidt and Weidman, 1964). George and Naik (1958) suggested that glycogen loaded broad muscles of the pigeon breast muscles are of the quick contracting type, compared to the narrow ones. The caudal muscle fibres of Mabuya carinata could be compared with the

broad muscle fibres of the pigeon breast muscles as
they are chiefly glycogen loaded and also of quick
contracting type, as could be surmised from the quick
and violent contractions of the muscles noticeable
during autotomy. Putting together all the above
observations it may be suggested here that carbohydrates
(glycogen and or glucose) are being utilized chiefly
for the energy requirements by the normal tail.

Presently observed glycogen in the wound
epithelium could be attributed to its phagocytic
properties which was postulated by Singer and Salpeter
(1961) during their studies on regeneration in the
limb of the adult newt. This phagocytic activity of
the wound epithelial cells was later confirmed by
electron microscopic studies of Norman and Schmidt
(1967). An increase in the lactic acid content in
the tissues during the preblastemic phase of the limb
regeneration in axolotl was reported by Okuneff (1933).
The glycogen depletion from the cut end of the original
tail tissues observed in Mabuya carinata is supported
by the studies of Schmidt (1960, 1962a) who correlated
the lactic acid accumulation during the preblastemic

phase with the depletion of glycogen from the cut end
of the muscle fibres noted from 24-96 hours after
amputation of the forelimb of the adult newt, Diemictylus
viridescens.

The quantitative studies on glycogen (Table 3.1)
has clearly indicated a reduced or minimal level of
the metabolite (glycogen) in the blastema of the
regenerating tail of Mabuya carinata. Eventhough, a
reduced content of glycogen could be noted quantitatively,
both glycogen and phosphorylase were found to remain
histochemically insensitive. Low levels of glycogen
and phosphorylase was very much apparent in the blastema
of the regenerating tail of Mabuya carinata. Shah and
Chakko (1967b) have also agreed on a similar pattern of
glycogen and phosphorylase distribution in the blastema
of Hemidactylus flaviviridis. Identical observations
regarding glycogen have also been made by Schmidt (1962a)
and Wolfe and Cohen (1963) in the regenerating forelimb
of newt. These observations when viewed in the light
of increasing levels of lipid and lipase in the blastema
of all the above mentioned regenerating systems (Chapter 2,
Chakko, 1967 and Schmidt, 1966a) tend to highlight the

significance of lipids at this phase of regeneration.
It could be easily surmised that lipids are being
synthesized actively during the blastemic phase due to
its high energy value in comparison to glycogen. This
is well confirmed by the reported high activity of
Glucose-6-phosphate dehydrogenase (G6PDH) and malic
enzyme in the blastema of Mabuya carinata (Ramachandran,
1972) and in Hemidactylus flaviviridis by Magon (1970)
and Hiradhar (1972) respectively. Schmidt and Weidman
(1964), Johnson and Singer (1964) and Wolfe and Cohen
(1963) have also reported high incidence of G6PDH in
the regenerating blastema of newt forelimbs. On the
whole, the mesenchymal cells of the blastema appear to
engage themselves in the biosynthesis of lipids by
actively and economically oxidising the available low
levels of glycogen at this phase or even glucose as
hinted by Shah and Ramachandran (1972) and Ramachandran
(1972) based on the studies of aldolase and other
dehydrogenases, respectively in the regenerating tail
of Mabuya carinata.

From the early differentiation phase onwards
there was a gradual increase of glycogen content

reaching ultimately its peak value during the late
differentiation (Table 3.1). Concomitant increase of
phosphorylase was noted in the differentiating tissues
mainly epidermis, muscles and the chondrocytes.
Phosphorylase activity which was at first weak in the
myoblasts appeared to register a gradual increase
during myogenesis with the maximum activity being attained
in the myofibrils. Conformity for this observations
comes from the reports of Shah and Chakko (1967b) on
the phosphorylase activity during myogenesis in the
regenerating tail of the house lizard, Hemidactylus
flaviviridis. So it is likely as suggested by Schmidt
et al. (1959) and Robbins et al. (1959) that during the
early phases of myogenesis, in the absence of phosphory-
lase glycogen is being synthesized by the mediation of
glycogen synthetase (UDPG glycogen transferase). An
evidence in favour of this suggestion is the reported
presence of glycogen synthetase during differentiation
in the tail of Hemidactylus flaviviridis (Hiradhar,1972).

It is of interest to note here that Shah and
Ramachandran (1970, 1972) based on their studies on
lactate dehydrogenase (LDH) and aldolase respectively,

in the differentiating tail of <u>Mabuya</u> <u>carinata</u> have also concluded in favour of a process of glyconeogenesis. During chondrogenesis too as in myogenesis, there was a gradual increase of both glycogen and phosphorylase, similar to the observations of Schmidt (1962a) during skeletogenesis in the regenerating forelimb of the adult newt, <u>Diemictylus</u> <u>viridescens</u>. From the present observations on glycogen and phosphorylase during differentiation it becomes amply clear that during the early differentiation phase glycogen synthesis predominates (corresponding to the lipid utilization, Chapter 2) ~~takes preceeding~~ over its utilization while during the late differentiation phase both the processes are at work simultaneously. Supports in favour of this contention can be drawn from the work of Shah and Ramachandran (1970, 1972) and Ramachandran (1972) in <u>Mabuya</u> <u>carinata</u> and also of Chakko (1967), Magon (1970) and Hiradhar (1972) in <u>Hemidactylus</u> <u>flaviviridis</u>.

With the completion of differentiation and the onset of growth there was a gradual decrease in glycogen content from the peak level observed during the late differentiation phase and a concomitant increase of

phosphorylase activity. This observation along with the reported high activity of aldolase (Shah and Ramachandran, 1972) and Lactate dehydrogenase (LDH) activity, (Shah and Ramchandran, 1970) in Mabuya carinata are indicative of the fact that glycogen is being metabolized for meeting the energy requirements of the growth phase to attain morphological and functional maturity. Towards the end of the growth phase, there was a gradual decrease in the levels of the metabolite (glycogen) and the associated enzymes (Shah and Ramachandran, 1970, 1972 and Ramchandran,1972), finally reaching the state in the fully regenerated tail, which is characteristic of the normal tail. Nevertheless, the level of glycogen seems to be slightly higher in the fully regerated tail than in the normal one (Table 3.1).

All these facts when viewed together, it may be said that during the early phases of tail regeneration in Mabuya carinata, the metabolic pattern is mainly of anaerobic type, but later, especially during the differentiation phase, slowly shifts to strike the balance between the anaerobic and aerobic metabolism, depending upon the energy requirements which are necessary for growing tissues.