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 μm 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 antrone 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
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
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
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
TABLE 3.1

Quantitatively estimated levels of total glycogen in the normal and regenerating tail of the Scincid lizard, *Mabuya carinata*

<table>
<thead>
<tr>
<th>Normal tail and different phases of regenerating tail</th>
<th>Amount of glycogen (g/100 g fresh tissue)</th>
<th>Number of estimations performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tail</td>
<td>0.0570 ± 0.0064**</td>
<td>12</td>
</tr>
<tr>
<td>Regenerating tail*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastema phase (7-12 days)*</td>
<td>0.0333 ± 0.0040</td>
<td>10@</td>
</tr>
<tr>
<td>Early differentiation phase (13-20 days)</td>
<td>0.1693 ± 0.0095</td>
<td>13®</td>
</tr>
<tr>
<td>Late differentiation phase (20-30 days)</td>
<td>0.3070 ± 0.0401</td>
<td>15</td>
</tr>
<tr>
<td>Growth phase (30-40 days)</td>
<td>0.1638 ± 0.0033</td>
<td>15</td>
</tr>
<tr>
<td>Fully regenerated tail (40-70 days)</td>
<td>0.8648 ± 0.0060</td>
<td>11</td>
</tr>
</tbody>
</table>

* Stages of regeneration are arbitrarily defined for the purpose of discussion even though 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 histochemical 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 observations it could be suggested that the normal tissues of the *Mabuya carinata* tail predominantly utilize carbohydrate 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 muscle: 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 preblastemal phase of the limb regeneration in axolotl was reported by Okunoff (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 preblastemal...
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
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 phosphorylase 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 *Mabuya carinata* 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, *Dienictylus viridesens*. 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 precedence 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 *Mabuya carinata* and also of Chakko (1967), Magon (1970) and Hiradhar (1972) in *Hemidactylus flaviviridis*.

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 Ramachandran, 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 Ramachandran, 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.