Lipids, being of high energy value, serve as important metabolite for various energy linked reactions in animal tissues. Lipids laid down as adipose tissue, and as a coating around certain organs, apart from functioning as store houses, also aid in biological insulation. Combinations of lipids and proteins (lipoproteins) are important cellular constituents and also serve as an excellent means for lipid transport.

Woodland (1920), based on his studies on the histology of the normal and regenerating tail of the house lizard, Hemidactylus flaviviridis, emphasized the deposition of large amount of lipids in the subcutaneous and submuscular regions. Schmidt (1966a,b) studied the distribution pattern of sudanophilic lipids in the regenerating limb of the adult newt, Diemictylus viridescens, and discussed their significance in the metabolism of the blastema cells. Recently Chakko (1967) from this laboratory studied, histochemically, the distribution pattern of lipids and lipolytic enzymes viz., lipase and esterase in the normal and regenerating
tail of the house lizard, *Hemidactylus flaviviridis* and discussed their role in regeneration. A similar study on another lizard would prove interesting, and reveal the species difference, if any, at the histophysiological level; and also it would add to our knowledge of reptilian regeneration which is very meagre at present. It was with these views and also with an intention to enhance our knowledge regarding the importance of lipids during the molecular ecology of regeneration in general, that it was decided to study the distribution of lipids and lipolytic enzymes (lipase and esterase) in the normal and regenerating tail of the Scincid lizard, *Mabuya carinata*.

**MATERIAL AND METHODS**

Adult lizards, *Mabuya carinata*, obtained from the local animal dealer and maintained in the laboratory on insect diet, were selected for the present study. The autotomy of the normal and regenerating tails at the desired length from the vent (about 4 centimeters distal to the vent), was induced by pinching them off with required force. The autotomized tails were immediately blotted to remove blood and tissue fluids.
and were mounted on a microtome chuck of a cryostat maintained at -20°C. Transverse as well as longitudinal sections of 12-18 μ thickness were cut and processed for the histochemical demonstration of different types of lipid. For the histochemical demonstration of sudanophilic lipids the sections were stained with Sudan black B, for the neutral lipids with Fettrot 7 B and for acidic lipids with Nile blue sulphate. Formol-calcium fixed sections were stained with Sudan black B and Fettrot 7 B according to the method described by Pearse (1960). For the demonstration of acidic lipids in the tissues, the Nile blue sulphate method of Cain (1947) as cited by Pearse (1960) was employed. For the demonstration of phospholipids the acid haematin method of Baker (1946) was employed. Sections treated with a mixture of methanol-chloroform (1:2 v/v) at 55°C for three hours prior to staining served as controls.

Quantitative estimations of the total lipids in the normal and regenerating tails were carried out by extracting the lipids from oven dried tissues using a mixture of methanol-chloroform (1:2 v/v). The amount of lipids was calculated in grams per 100 grams of wet tissues.
The histochemical demonstration of lipase was carried out by the modified method of Gomori as described by George and Ambadkar (1963) using 'Tween 85' as the substrate. Sections were incubated for 8-10 hours at room temperature (29-32°C). Sections incubated in the medium devoid of the substrate and those incubated after treating them with warm distilled water (80°C) for five minutes served as controls.

For the histochemical demonstration of esterase the method of Burstone (1957b, 1958) using AS-D Acetate (Sigma Chemical Company, U.S.A.) as the substrate, was employed. Incubation was done at room temperature (29-32°C) for 6-10 hours. Sections incubated in the medium devoid of the substrate served as suitable controls.

OBSERVATIONS

NORMAL TAIL (Fig.1)

Except for the presence of minute traces in the stratum germinativum layer of the epidermis and the peripheral muscles in each of the faciculi, the various tissues of normal tail taken as a whole showed a general lack of lipids of all types presently investigated. A correspondingly low activity of both lipase and esterase
was also evident (Figs. 2 and 3). However, of the two, esterase was found to be more active than lipase. Sudanophilic, neutral and acidic lipids were identifiable in both stratum germinativum and peripheral muscles in minute traces but the former showed only a very poor response towards Nile blue sulphate. The only components of the normal tail that showed the presence of neutral lipids indicated by the positive response towards Fettrot 7 B and Nile blue sulphate were the subcutaneous and submuscular adipose tissue and the marrow cells of the vertebrae. The present observations with the various lipid stains have revealed that the adipose tissues and the marrow cells in the vertebrae of the normal tail of *Mabuya carinata* are composed entirely of neutral lipids. A poor localization of both lipase and esterase could also be observed in the peripherally situated cytoplasm of the adipose tissue cells, and in the marrow cells. The acidic lipids could be noticed in the chondrocytes at the articulating surfaces of the vertebrae. The lipase and esterase activities were, however, negligible in these cells. In the nerve cord, the grey matter responded more favourably towards acidic lipids as compared to the white matter which had more neutral lipids. Both lipase and esterase were equal but moderate in their activities in both the parts of the nerve cord.
EXPLANATIONS FOR FIGURES

Fig. 1. Photomicrograph of the longitudinal sections of a normal tail stained for lipids (Sudan black B). Note the negligible response of the various tissues except the submuscular adipose tissues towards the stain.

Fig. 2. Photomicrograph of the longitudinal section of the normal tail denoting low lipase activity.

Fig. 3. Photomicrograph of the transverse section of the normal tail revealing esterase activity. Note the activity in muscle fibres slightly more than that of lipase.

ABBREVIATIONS

CHB - Chevron bone
M - Muscle
MF - Muscle fasciculus
N - Nerve cord
NS - Neural spine
SC - Scale
SMAT - Submuscular adipose tissue
V - Vertebra
EXPLANATIONS FOR FIGURES

Fig. 4. Photomicrograph of the wound epithelium and the subapical region revealing the sudanophilic lipids.

Fig. 5. Photomicrograph of the wound epithelium revealing lipase activity. Note the cells of the subapical region showing less activity of the enzyme.

Fig. 6. L.S. of the tail regenerate at the wound healing phase revealing esterase activity in the wound epithelium.

Fig. 7. Higher magnification of the wound epithelium and the subapical region revealing esterase activity.

ABBREVIATIONS

CEM - Cut end of the muscle
N - Nerve cord
SAR - Subapical region
SCB - Scab
V - Vertebra
WE - Wound epithelium
EXPLANATIONS FOR FIGURES

Fig. 8. Magnified region of blastema cone revealing the acidic lipid (Nile blue sulphate stained).

Fig. 9. A part of the blastemal cone enlarged to show lipase activity.

Fig. 10. Photomicrograph of a region of blastema cone revealing esterase activity. Note the activity in the blastemal epithelium and in the mesenchymal cells.

ABBREVIATIONS

BE - Blastemal epithelium
MC - Mesenchymal cells
EXPLANATIONS FOR FIGURES

Fig. 11. L.S. of differentiating tail regenerate
showing the sudanophilic lipid in the various
differentiating tissues.

Fig. 12. L.S. of the tail regenerate at early
differentiating phase revealing lipase
activity.

Fig. 13. Photomicrograph of the tail regenerate at
the early differentiating phase revealing
esterase activity.

ABBREVIATIONS

CNC - Cartilagenous neural canal
CHB - Chevron bone
DM - Differentiating muscle
DSC - Differentiating scale
E - Ependyma
N - Nerve cord
V - Vertebra
EXPLANATIONS FOR FIGURES

Fig. 14. Magnified portion of the differentiating scales and the dermis showing sudanophilic lipids.

Fig. 15. Higher magnification of the differentiating muscles revealing sudanophilic lipids.

Fig. 16. Photomicrograph of an enlarged region of the differentiating scales and the dermis region revealing lipase activity.

Fig. 17. Differentiating muscles showing lipase activity.
EXPLANATIONS FOR FIGURES

Fig. 18. Photomicrograph of the tail regenerate at the late differentiation phase exhibiting esterase activity in the various differentiating tissues.

ABBREVIATIONS

CNC - Cartilagenous neural canal
DM - Differentiating muscle
DSC - Differentiating scale
DSMAT - Differentiating sumuscular adipose tissue
TABLE 2.1

Quantitative data of total lipids in normal and regenerating tail of *Mabuya carinata*

<table>
<thead>
<tr>
<th>Normal tail and different phases of regeneration</th>
<th>Amount of total lipids (g/100g fresh tissue)</th>
<th>Nos. of estimations performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tail</td>
<td>19.53 ± 2.420**</td>
<td>10</td>
</tr>
<tr>
<td>Regenerating tail*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastema phase (7-12 days)*</td>
<td>02.68 ± 0.570</td>
<td>15@</td>
</tr>
<tr>
<td>Early differentiation phase (13-20 days)</td>
<td>05.00 ± 0.965</td>
<td>12@</td>
</tr>
<tr>
<td>Late differentiation phase (20-30 days)</td>
<td>08.07 ± 1.292</td>
<td>16</td>
</tr>
<tr>
<td>Growth phase to fully regenerated tail (30-70 days)</td>
<td>12.48 ± 2.293</td>
<td>11</td>
</tr>
</tbody>
</table>

*Phases of regeneration are arbitrarily defined for the purpose of discussion even though the process of regeneration is a continuous one.

@Tissues of ten animals for each estimations were pooled.

+. Number of days after autotomy.

**Mean ± S.D.
Fig. 19. Graphic representation of the total lipid contents; and the activities of lipase and esterase in the normal and regenerating tail of the Scincid lizard, Mabuya carinata.
REGENERATING TAIL

Wound healing phase: (Figs. 4, 5, 6 and 7)

The wound epithelium showed the presence of neutral, acidic as well as phospholipids. Droplets of neutral lipids scattered in the region below the epithelium could also be noticed. Both lipase and esterase were detectable in both wound epithelium as well as the cells lying subjacent to it with latter tending to be more than the former.

Preblastemic and blastemic phase:

The cells of the stratified blastemic epithelium as well as the dedifferentiated mesenchymal cells forming the core of the blastema showed considerable amount of lipids (Fig. 8). Lipid globules could also be seen in the intercellular spaces of the blastema. Though, both neutral and acidic lipids were observable, the latter appeared to be more than the former. An increased activity of both lipase and esterase was very much apparent during this phase in comparison to the wound healing phase (Figs. 9 and 10).

Differentiation phase: (Figs. 11, 14 and 15)

During the early period of differentiation, all the differentiating elements such as epidermis, myoblasts
and chondroblasts at the base of the regenerate showed increased content of lipids. A concomitant increase in the lipase and esterase activities were too observable (Fig. 12, 13, 16, 17). But with the advent of the late differentiation phase the lipids tended to decrease gradually in the various differentiating components of the regenerate with a steady increase in the levels of lipase and esterase. Though both lipase and esterase were active during the differentiation phase, a comparison easily revealed the esterase activity to be much more than that of lipase (Fig. 15). Towards the completion of the differentiation phase neutral lipids were found to be more in association with the proximodistally developing submuscular adipose tissue with a decreasing content in the various other cellular elements. Though both neutral as well as acidic lipids were in fairly high concentrations during this phase, the latter tended to be slightly more than the former.

**Growth phase:**

The gradual fall of the lipid content in the various tissues of the regenerate observed during the previous phase continued during the growth phase as well. On the other hand further accumulation of neutral lipids could
be observed in the well defined submuscular adipose tissue. A simultaneous decrease in lipase and esterase activities too could be observed in the various, by now well differentiated tissue. The enzyme activities as well as the lipid contents by a gradual and steady decrease during the growth finally attained, in the fully regenerated tail, a level of concentration and localisation characteristic of the corresponding normal tail. However, the submuscular adipose tissue present in the fully regenerated tail is less than that noticed in the normal tail.

Quantitative estimation of total lipids in the normal and regenerating tail has been carried out. The results obtained are presented in Table 2.1 and Fig. 19.

DISCUSSION

The histochemical and quantitative studies on lipids in the adult normal and regenerating tail tissues of the Scincid lizard, *Mabuya carinata* have shown that there are significant fluctuations in the amount of lipids during different phases of regeneration. The blue oxazine reaction with the Nile blue sulphate shown by the stratum germinativum, connective tissues and
caudal muscles of the normal tail may be due to the free fatty acids and acidic lipids as was envisaged by Schmidt (1966b) in the adult newt, Diemictylus viridescens. Since the normal tail tissues of Mabuya carinata failed to show any response towards lipase or esterase in appreciable concentration it may be said that the acidic lipids which are reported in some tissues in the normal tail may be of only structural importance rather than of any metabolic importance. The histochemical observations on glycogen and phosphorylase (Chapter 3) and high activity of aldolase in the normal tail tissues of Mabuya carinata (Shah and Ramachandran, 1972) are indicative of the fact that the normal tissues of the adult tail are predominantly carbohydrate dependant rather than lipid dependant for their energy requirements. Similar observations were reported by Shah and Chakko (1967b) and Magon (1970) in the tail of the house lizard, Hemidactylus flaviviridis, and Schmidt (1962a) in the tissues of the normal appendages of the adult newt, Diemictylus viridescens.

There is a significant variation in the total lipid content of the normal and fully regenerated tail when compared with that of the different phases of
regeneration. From the histochemical studies it could be stated here that most of the quantitatively estimated total lipid content is contributed by the subcutaneous, submuscular adipose tissues and the marrow cells of the caudal vertebrae.

The submuscular adipose tissue showed a slight activity of lipase and esterase in the peripherally situated cytoplasm of its cells but not as high as reported by George and Eapen (1959) in the adipose tissues of some vertebrates. This low level activity of these enzymes could be correlated with functional inefficiency of the adipose tissue in the normal tail for the purpose of energy yield. It may be further said that the adipose tissue may be giving an anatomical and structural integrity for the tail. It is interesting to note that some of the lizards incapable of regenerating their tails like Calotes and Uromastix do not have either the submuscular or the subcutaneous adipose tissues. It could be said that the presence of adipose tissues in the tail of those lizards which could autotomize and regenerate the tails under natural conditions may have some hitherto unknown important functions. However, lizards are well known for hibernation and or aestivation,
it could be presumed that the adipose tissue may have some role in providing energy during such periods of starvation.

Increased levels of lipids and lipolytic enzymes noticed during the wound healing phase gradually mounted to a higher level in the blastemic phase. Similar observations were reported by Chakko (1967) in the regenerating tail of *Hemidactylus flaviviridis*, Schmidt (1966a, b) in the forelimb of the adult newt *Diemictylus viridescens* and Hess (1959) in the tail of the tadpole *Xenopus laevis*. The presently noticed increased lipid contents in the preblastemic and blastemic phases of regeneration is well correlated with the observations of Ramachandran (1972) of the increased activities of Glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme and Isocitrate dehydrogenase (ICDH) in the blastemal cells of *Mabuya carinata* as these enzymes are important sources of reduced NADP, an important cofactor for lipogenesis. Similar observations were reported by Chakko (1967) and Magon (1970) in *Hemidactylus flaviviridis*. Siperstein (1958) has shown that NADPH$_2$ generated through Hexose monophosphate shunt (HMP) pathway could be utilized for the synthesis of fatty acids in the tissues. The
utilization of lipids at this stage seems to be far outweighed by the synthetic process as could be surmised from the increasing levels of lipids during the blastemic to differentiation phases by both the histochemical as well as quantitative studies (Table 2.1). A concomitant increase of lipase and esterase which were noticed in *Mabuya carinata* from blastemic to early differentiation phase is in conformity with the observations of Chakko (1967) and increased activity of \( \beta \)-hydroxy butyrate dehydrogenase (BDH) in *Hemidactylus flaviviridis* (Magon, 1970) indicative of the utilization of lipids for energy. This is further strengthened by the presently noticed gradual depletion of lipids from the various differentiating tissues and the high activities of lipase as well as esterase noted herein and those of \( \beta \)-hydroxy butyrate dehydrogenase (BDH), Succinate dehydrogenase (SDH), Isocitrate dehydrogenase (ICDH) and malate dehydrogenase (MDH) (Shah and Ramachandran, 1970 and Ramachandran, 1972).

The quantitatively observed increased lipid contents during the late differentiation could be explained by the fact that it is at this stage the adipose tissue is being laid proximodistally. The
histrochemical picture at the same time reveals that the response towards lipid stains is shown only by the differentiating adipose tissue cells, while the other differentiating tissues showed only a negligible response. Thus the whole picture when viewed together brings out the fact that from late differentiation phase there is tremendous amount of lipid utilization which is well supported by the observed increased activities of concerned enzymes viz., BDH, SDH, and ICDH (Ramachandran, 1972 and Shah and Ramachandran, 1970) in Mabuya carinata. The corresponding increase of glycogen noticed during this phase (Chapter 3) is clearly indicative of the fact that some of the lipids are also being diverted towards the channels of glyconeogenesis, as was also envisaged by Shah and Ramachandran (1970, 1972 and Ramachandran, 1972) based on their studies on the various enzymes of intermediary metabolism. Along with the increase of above mentioned lipids a simultaneous increase of phospholipids was also evidenced by the present study, all throughout the blastema and differentiation phases. The functional significance of increased phospholipids, apart from the laying down of structural integrity of the various
organelles of the proliferating cells and may also lie in its possible utility as energy source. The increased phospholipid content observed herein is in good correlation with the discussed role of alpha glycerophosphate dehydrogenase (αGPDH) during the tail regeneration in *Mabuya carinata* (Ramachandran, 1972).

The growth phase is marked by a decreased level of lipids and the associated enzymes (present chapter and Ramachandran, 1972) are indicative of a process aimed at the normalization of physiological and biochemical setup in the fully regenerated tail. This normalization process appears to terminate with the attainment of a level of lipid content, lipase and esterase activities in the fully regenerated tail characteristic of the corresponding normal tail.