CHAPTER 2

HISTOCHEMICAL OBSERVATIONS ON LIPIDS,LIPASE AND ESTERASE;
AND QUANTITATIVE LEVELS OF TOTAL LIPIDS IN THE NORMAL AND
REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

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 (lipo-
proteins) 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 signi-
ficance 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 histo-physiological 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 $_{\wedge}$AS-D Acetate Naphthol

(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
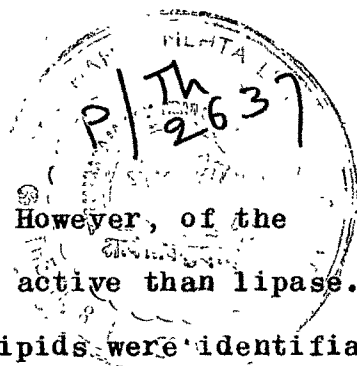
was also evident (Figs. 2 and 3). However, of the

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.
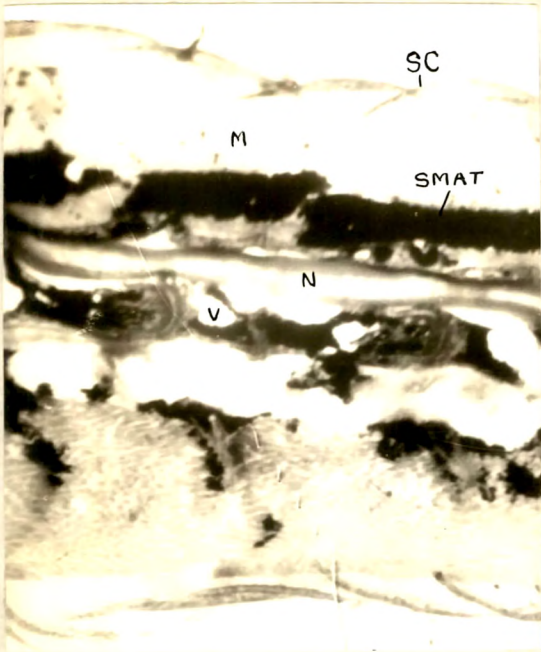
EXPLANATIONS FOR FIGURES

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.
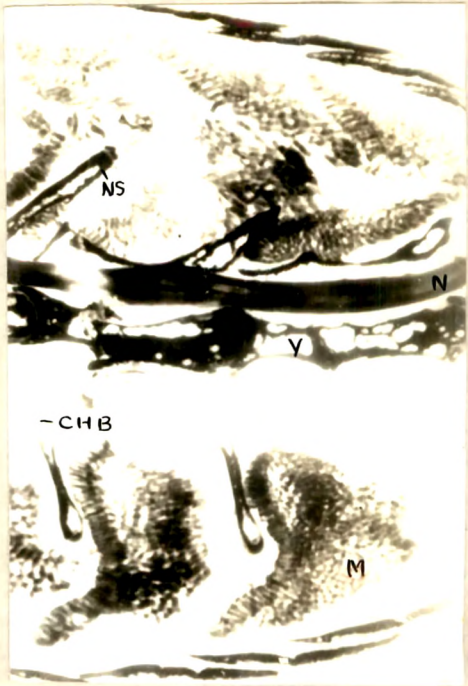
Note the activity in muscle fibres slightly

more than that of lipase.

ABBREVIATIONS

CHB   —   Chevron bone

M   —   Muscle

MF   —   Muscle fasciculus

N   —   Nerve cord

NS   —   Neural spine

SC   —   Scale

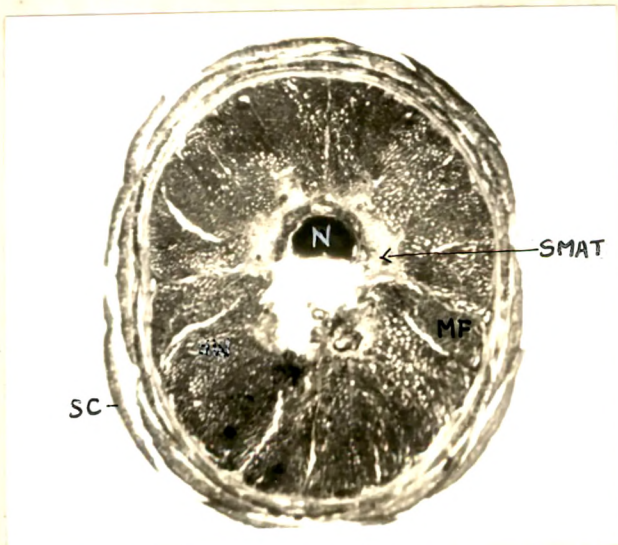SMAT   —   Submuscular adipose tissue

V   —   Vertebra

2 mm   1

2 mm   2

2 mm   3

EXPLANATIONS FOR FIGURES

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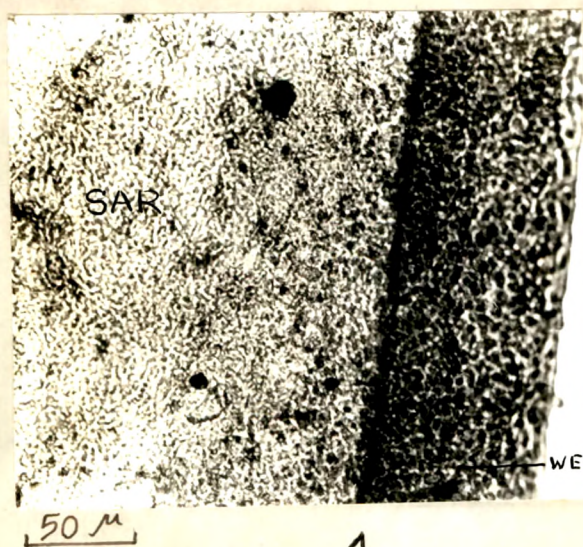lipids.

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
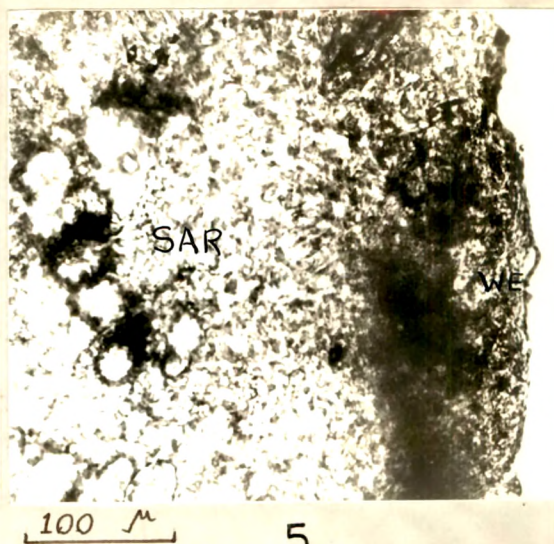phase revealing esterase activity in the wound
epithelium.

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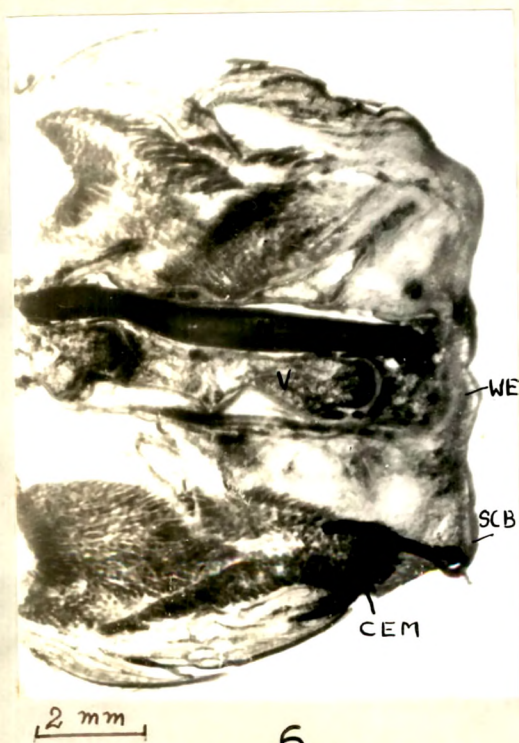and the subapical region revealing esterase
activity.

ABBREVIATIONS

CEM - Cut end of the muscle

N - Nerve cord

SAR - Subapical region

SCB - Scab

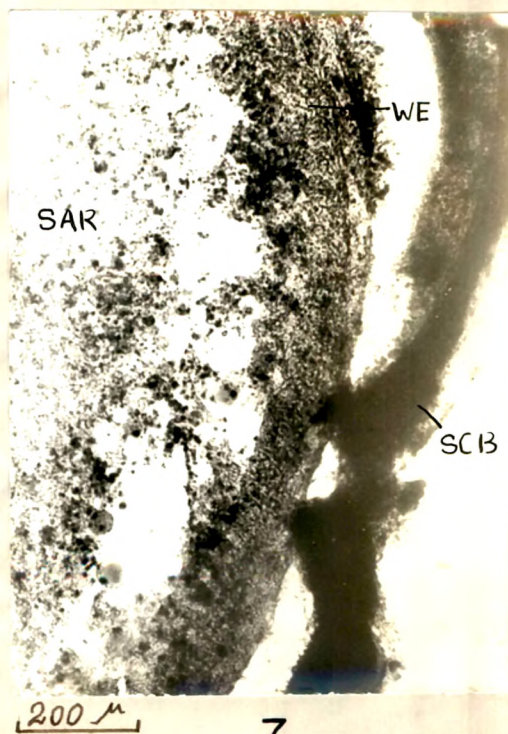V - Vertebra

WE - Wound epithelium

SAR

WE

50 μ

4

SAR

WE

100 μ

5

V

WE

SCB

CEM

2 mm

6

WE

SAR

SCB

200 μ

7

EXPLANATIONS FOR FIGURES

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

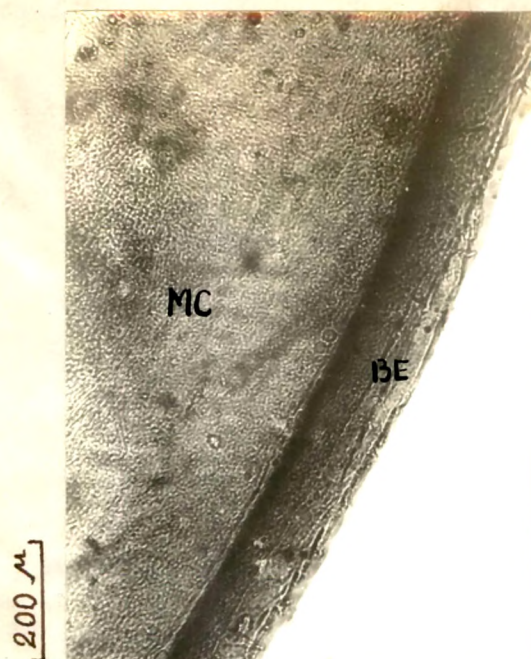Fig. 9. A part of the blastemal cone enlarged to show lipase activity.

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

ABBREVIATIONS

BE – Blastemic epithelium

MC – Mesenchymal cells
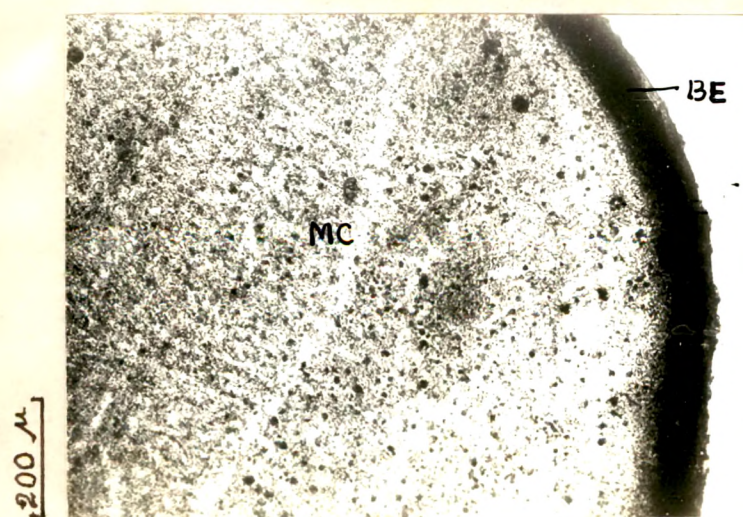
57

MC

BE

8

200 M

BE

MC

9

100 M

BE

MC

10
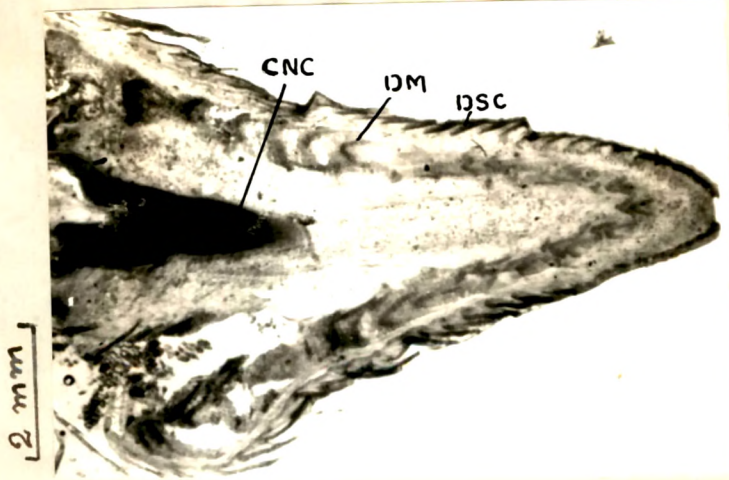
200 M

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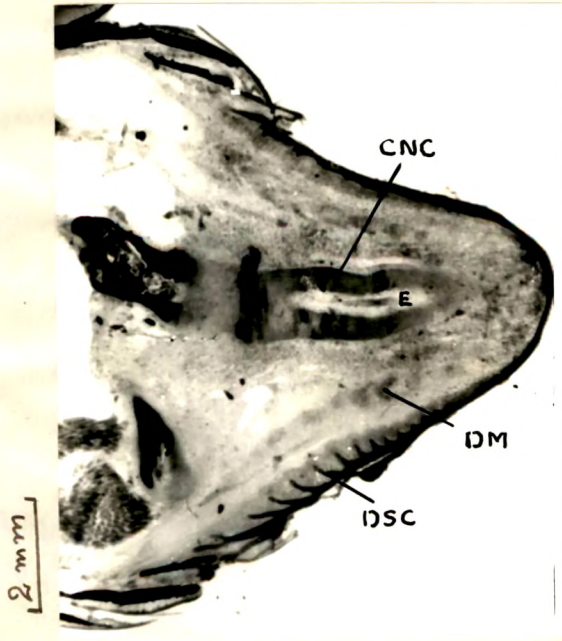
Fig. 13. Photomicrograph of the tail regenerate at the early differentiating phase revealing esterase activity.

ABBREVIATIONS

CNC – Cartilagenous neural canal

CHB – Chevron bone

DM – Differentiating muscle

DSC – Differentiating scale

E – Ependyma

N – Nerve cord

V – Vertebra

11

12
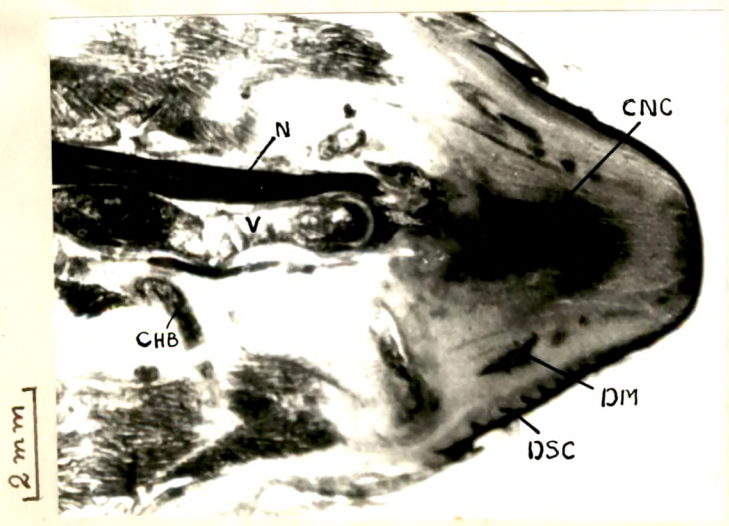
13

EXPLANATIONS FOR FIGURES

Fig. 14. Magnified portion of the differentiating
scales and the dermais 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.

200 μ

14

200 μ

15

200 μ

16

200 μ

17

61
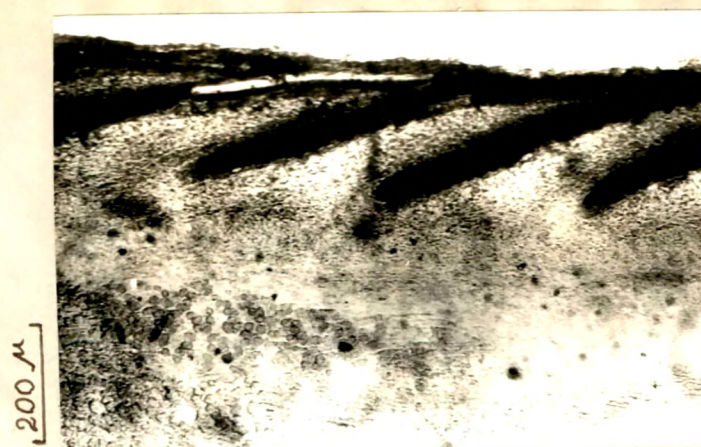
EXPLANATIONS FOR FIGURES

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

ABBREVIATIONS

CNC   -   Cartilagenous neural canal

DM   -   Differentiating muscle

DSC   -   Differentiating scale

DSMAT   -   Differentiating sumuscular
adipose tissue

2 mm

18
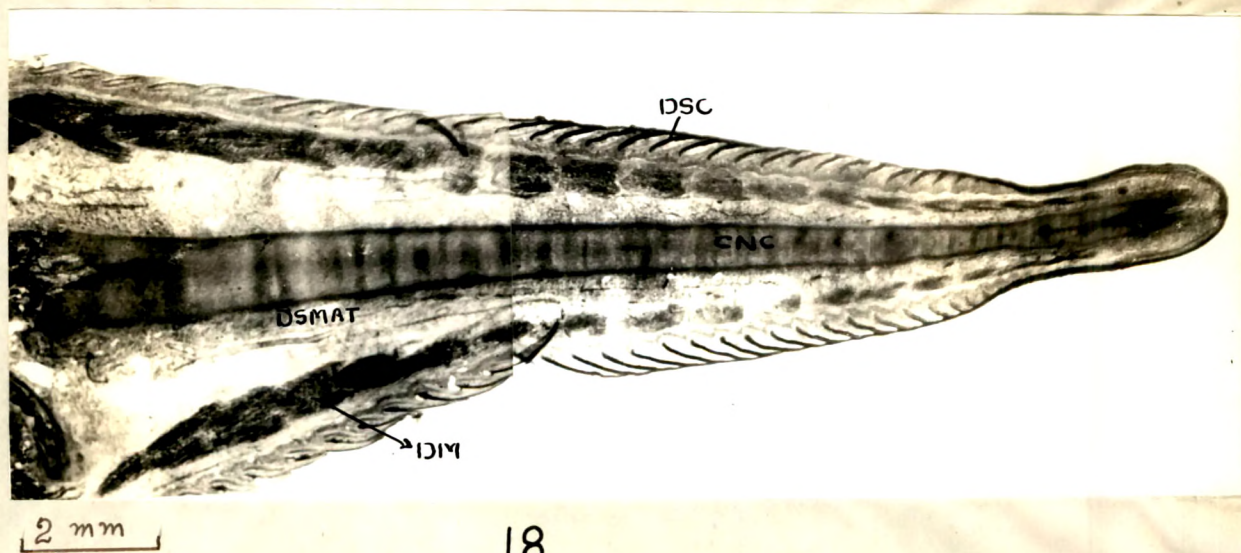
TABLE 2.1

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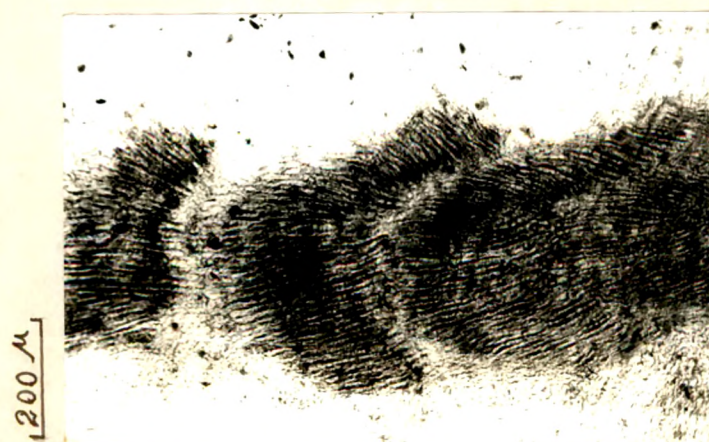
regenerating tail of Mabuya carinata

Normal tail and different phases of regeneration	Amount of total lipids (g/100g fresh tissue)	Nos. of estimations performed
Normal tail	19.53 $\pm$ 2.420**	10
Regenerating tail*		
Blastema phase (7-12 days)[+]	02.68 $\pm$ 0.570	15[@]
Early differentiation phase (13-20 days)	05.00 $\pm$ 0.965	12[@]
Late differentiation phase (20-30 days)	08.07 $\pm$ 1.292	16
Growth phase to fully regenerated tail (30-70 days)	12.48 $\pm$ 2.293	11

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

[@]Tissues of ten animals for each estimations were
 pooled.

[+]Number of days after autotomy.
**Mean $\pm$ 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
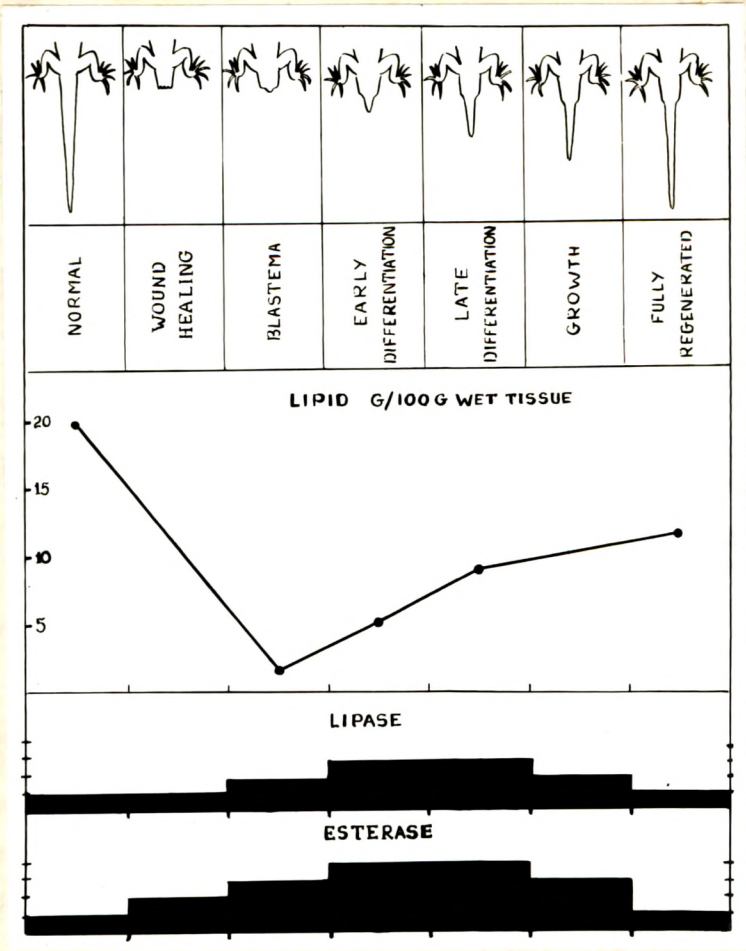lizard, _Mabuya carinata_.

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 localiza-
tion 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 phosphory-
lase (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 dehydrogenenase (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 monophoshate 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 β-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 β-hydroxy butyrate dehydrogenease (BDH), Succinate dehydrogenase (SDH), Isocitrate dehydrogenase (ICDH) and malate dehydrogenease (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

histochemical 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 Ramchandran,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 Ramchandran, 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 dehydrogenease (αC GPDH) during the
tail regeneration in Mabuya carinata (Ramchandran,
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.