Mabuya carinata

INTRODUCTION

The term regeneration is a very general one and is applied to comprise all types of restoration of lost or injured parts of an animal body. This process calls for essentially a reactivation of the morphogenetic processes which ultimately restore the lost parts of the body. It appears to be a general rule in biology that in those animals capable of losing their body parts as a result of autotomy, there usually exist provision(s) for a more or less complete replacement of the part(s) lost thereof. This fascinating phenomenon of regeneration has attracted the attention of biologists eversince the time of Aristotle. It was Spallanzani (1768), one of the pioneer experimental zoologists who reported that tadpoles of frogs and toads and larval salamanders could regenerate their tails or limbs if amputated; but as they advance in age, the capacity to regenerate gradually diminishes. Morgan (1901) termed this type of restoration of lost parts as "Epimorphosis". Epimorphic type of regeneration is more characteristic of vertebrates in contrast to the morphollactic one which is more common amongst the invertebrates. The latter group is endowed with a mass of undifferentiated totipotential cells which, when required can undergo differentiation and give rise to a

substitute organ or part more or less similar in structure and function eventhough smaller in size in comparison to the original structure lost. In contrast, in vertebrates it is suggested that the already differentiated and specialized cells, at the time of regeneration, get dedifferentiated and aquire embryonic totipotential characters and such cells then serve as the basic material from which the reconstitution of the regenerate can take place. Schotté (1939) states that regeneration is more or less a recapitulation of the developmental process of the lost parts, progressively getting restricted by age. Schmidt (1968) too agrees with the fact that the process of regeneration is analogous to the developmental process as suggested by Schotté (1939). He further emphasizes that these two processes viz., regeneration and development are analogous and not homologous. It is also commonly believed that higher the systematic position of the animal on the evolutionary ladder, poorer its capacity to regenerate; while lower the organization and systematic position of the animal, higher the capacity to restore the lost parts of the body. However, it is not universally true.

Amongst the vertebrates the highest power of regeneration is exhibited by amphibians and the least

by mammals. It is well recognized that amphibians have a remarkable capacity to restore their lost parts viz., lens, retina, liver, eye, intestine, tail and limbs. During the last two decades, intensive scientific investigations have been initiated and conducted on the different aspects of regeneration of tail and limbs of vertebrates, particularly amphibians, Regeneration in amphibians is well exemplified by the reconstruction of a complex and heterogenous organ like limb consisting of several tissues, to the extent of duplicating structurally and functionally the original one lost. The fundamental essence of regeneration lies in the formation of an anlage of indifferent collection of dedifferentiated cells, the regeneration blastema, from which arises a duplicate of the original structure lost.

Apart from amphibians, some of the reptiles especially the lacertilians have also retained the remarkable capacity of regenerating the tail, though slightly imperfect in nature. However, amongst lacertilians, all lizards are not capable of regenerating the tail. Even amongst those lizards which can regenerate their tails, the process does not proceed to the extent that all the structural and functional features of the lost parts are regained

completely in the regenerate. The tail is an important organ and is in a class by itself especially in lizards. Proficiency of lizards to drop their tails by autotomy is well observed in nature. Unlike the urodele tails, the tails of lizards do possess the preformed anatomical autotomy or breakage planes across the caudal vertebrae and muscles which facilitate autotomy of the tails in an efficient manner.

Lizard tails are one of the best known examples of heteromorphic regeneration in nature; what grows out again is only an apologetic amelioration of what was lost. This statement is extremely befitting when applied to the regenerating skeletal elements of the tail. It is evident from the reports of Woodland (1920); Barber (1944); Kamrin and Singer (1955) and Simpson (1965) that the cartilagenous axial skeleton of the fully regenerated tail does not segment or ossify like the original one. During the last two decades, considerable amount of work on lacertilian tail regeneration has been done. Literature regarding general morphological and histological studies on the normal and regenerating lacertilian tails available are due to the extensive studies of Duges (1829); Hooker (1912); Woodland (1920) on Hemidactylus flaviviridis; Slotopolsky (1922) on Lacerta; Quattrini (1954) on

Lacerta sicula; Kamrin and Singer (1955) on Anolis carolinensis; Huges and New (1959) on Sphaerodactylus; Simpson (1964) on Lygosoma laterale; Moffat and Bellairs (1964) on Lacerta vivipara Jacquin; Bryant and Bellairs (1967) on Anguis fragilis and Lacerta dugesii; Shah and Chakko (1968) on Hemidactylus flaviviridis and Cox (1969a) on Anolis carolinensis.

It was in the wake of these informations on regenerating reptilian tissues of the tail and as a continuation of the work conducted in this laboratory on the normal and regenerating tail of the house lizard, Hemidactylus flaviviridis, it was thought worthwhile to explore some of the histological and histophysiological aspects of the normal and regenerating tail of the Scincid lizard, Mabuya carinata also possessing the capacity to regenerate.

The sequence of regeneration process in Mabuya carinata from the day of autotomy to a fully regenerated state could be arbitrarily divided into six phases viz., (1) Wound healing phase (2) Blastemic phase (3) Early differentiation phase (4) Late differentiation phase (5) Growth phase (6) fully

regenerated tail. Eventhough there is no rigid distinctions between the successive phases, such an arbitrary division of the continuous regenerative process is made merely for the convenience of description.

To understand the histomorphological features of regeneration and also to lay a basic groundwork for the evaluation and elucidation of the various metabolic aspects aimed and planned at by the application of suitable histophysiological techniques, a histological study of the normal and the various above mentioned phases of the regenerating tail of Mabuya carinata was deemed necessary and hence undertaken.

With the establishment of the basic histological features, a path was thus paved for the smooth conduction of biomolecular and enzymological studies. Extensive investigations on the distribution of metabolites, such as glycogen and lipids and associated hydrolytic enzymes and dehydrogeneases have been carried out in the regenerating limbs of urodeles amongst the amphibians (Schmidt, 1960; 1962a,c; 1963a; 1966; 1966a,b: Schmidt and Norman, 1965; Schmidt and Weary, 1962; 1963; Schmidt and Weidman, 1964; Niwelinski, 1960; Wolfe and Cohen,1963).

In this aspect, there is a complete lack of literature as far as the reptilian regenerating systems are concerned. This lack had prompted a series of studies in the regenerating tails of lizards in this laboratory so as not only to understand the metabolic aspects underlying the tail regeneration in reptiles but also to assess the biochemical evolutionary differences if any between the amphibian and reptilian regenerating systems.

Earlier workers from this laboratory (Chakko,1967; Shah and Chakko, 1966a,b; 1967a,b; 1968; 1969; 1971; 1972; Shah and Magon,1969 and Magon, 1970) have studied some of the biochemical and metabolic aspects of the normal and regenerating tail of the Gekkonid lizard, Hemidactylus flaviviridis. Some more work on this lizard has been done recently (Hiradhar, 1972). In the light of the revealations of above studies on amphibian limbs and reptilian tail, it was deemed worthwhile and interesting to have a comparative idea on the similarities and or dissimilarities inherent therein between the Gekkonid lizard, Hemidactylus flaviviridis and the Scincid lizard, Mabuya carinata. Ramachandran (1972) with this perspective in mind had conducted some histochemical studies on enzymes of metabolic importance

chiefly dehydrogenases on the normal and regenerating tail of Mabuya carinata. Based on his studies he has inferred that lipids are not important for the molecular ecology of the normal tail. Further he has revealed the active operation of Hexose monophoshate shunt during the process of regeneration. It was thought desirable in this wake to undertake a histophysiological study on total lipid content and histochemical studies on the associated enzymes, lipase and esterase to not only evaluate the significance of lipids as such but also to complete the pattern speculated above.

Bearing the importance of glycogen as the principal energy yielder of animal tissues in mind, and also the reported high activities of LDH and aldolase (Shah and Ramachandran, 1970, 1972) it was found necessary to determine the glycogen content and phosphorylase localization during the tail regeneration in Mabuya carinata.

Phosphatases are known to play significant roles not only during the lytic processes and in phosphate metabolism but also in the synthesis of many specific proteins such as collagen, fibrous proteins and keratin. Since regeneration encomposes all the above aspects, a study of phosphatases was deemed fit and hence acid and alkaline

phosphatases are histochemically investigated.

Biochemical investigations on the regenerating systems of both amphibians as well as reptiles have indicated a high incidence of glucose-6-phosphatate dehydrogenase (Schmidt and Weidman, 1964; Magon, 1970; Ramachandran, 1972). This is construed to indicate the operation of HMP shunt which in addition to reduced NADP could also yield pentose sugars much needed for nucleic acid synthesis. Moreover, as regeneration calls for generation of additional and new units, a high rate of protein synthesis could be well associated with the process. The validity of the above relevent speculations could be conclusively ascertained by studying the concentration of nucleic acids at different phases. It is in this perspective the histochemical demonstration of DNA and RNA is undertaken in the normal and regenerating tail of Mabuya carinata.

Another interesting aspect found worth investigating is the possible influence of nerve mediators either in the initiation or the progress of regeneration. The localization and distribution of cholinesterases is well in line with this thinking. Such an investigation is rather tempting and all the more pertinent in the light

of reported role of a threshold number of nerves in the initiation of regeneration and the formation of blastema (Singer, 1946; 1947; 1952).

Finally, an evaluation of the content and localization of ascorbic acid is carried out keeping in view the versatility of this vitamin by its reported roles in collagen synthesis (Gould, 1963), wound healing (Bourne, 1953; Zamanskii and Lopushanskii,1955; Schauble et al.,1960; and Ksabyan, 1956), glycogenetic (Banerjee and Ganguli, 1962), activations of krebs cycle enzymes (Banerjee et al., 1959) and also in electron transport (Goodwin, 1960; Mapson, 1953; Meiklejohn,1953 and Chinoy, 1969a). This investigation gains added significance in the light of reported low level of cytochrome oxidase during regeneration in the tail of Mabuya carinata (Ramachandran, 1972) and the high incidence of this vitamin in the regenerating tail of Hemidactylus flaviviridis (Shah et al., 1971).

CHAPTER 1

HISTOLOGICAL OBSERVATIONS ON THE NORMAL AND REGENERATING TAIL OF THE SCINCID LIZARD, MABUYA CARINATA

Problem of regeneration in reptiles has attracted attention of several workers in the past who have studied the problem from the aspects of histomorphological changes that occur in the normal and regenerating tails of lizards. Amongst those who have contributed on such studies are Woodland (1920) on Hemidactylus flaviviridis; Slotopolsky (1922) on Lacerta; Barber (1944), and Kamrin and Singer (1955) on Anolis carolinensis; Huges and New (1959) on Gekkonid lizard, Sphaerodactylus; Simpson (1964) on Lygosoma laterale; Moffat and Bellairs (1964) on Lacerta vivipara Jacquin; Bryant and Bellairs (1967) on Anguis fragilis and Lacerta dugesii; Shah and Chakko (1968) on Hemidactylus flaviviridis and Cox (1969a) on Anolis carolinensis.

Besides the Gekkonid lizard, viz., Hemidactylus flaviviridis; a Scincid lizard, viz., Mabuya carinata, also has the capacity to autotomize its tail and regenerate it. In order to study the histophysiological aspects of the tail regeneration of Mabuya carinata and compare with

similar studies carried out on Hemidactylus flaviviridis by other workers in this laboratory, it was thought necessary to first establish the detailed features of morphology and histology of the normal and the regenerating tail of Mabuya carinata. With this in view, a histological analysis of the normal and the regenerating tail of the Scincid lizard, Mabuya carinata has been carried out.

MATERIAL AND METHODS

The adult lizards, Mabuya carinata obtained from the local animal dealers were maintained in the laboratory on insect diet. The lizards of more or less of the same length and weight and with normal tails were chosen for the present study. Autotomy of the tail at the desired level (distant from the vent, about 4 centimeters) was induced by pinching it off with necessary force. It has been observed that Hemidactylus flaviviridis can autotomize its tail more easily and with less force unlike Mabuya carinata which requires greater force and does not autotomize its tail that easily. The injured end of the autotomized tail, normal or regenerate as the case may be, was blotted free of blood and tissue fluids and fixed on a microtome chuck of a cryostat maintained at -20°C using goat liver as pith. Longitudinal as well

as transverse sections of 8 μ thickness were cut. The sections were transferred on albuminized slides and fixed in cold Bouin's fixative for two hours and were washed thoroughly in cold distilled water till all the yellow colour of the fixative was removed. The sections were stained with haematoxylin and eosin. After usual processing, the sections were mounted in canada balsam for histological observations.

OBSERVATIONS AND DISCUSSION

NORMAL TAIL

Morphology of the normal tail:

Normal tail of *Mabuya carinata* unlike that of the house lizard, *Hemidactylus flaviviridis* does not show external segmentation which in the latter coincides with the planes of autotomy. The tail is more or less cylindrical unlike that of the house lizard, which to a certain extent is dorsoventrally compressed. Normally in an adult *Mabuya carinata*, the tail length is about one half times more than the length of the body (tip of the head to the vent) whereas the tail length in house lizard, *Hemidactylus flaviviridis* is about the same as the length of the body. The scales on the tail of *Mabuya carinata* are relatively larger and highly keratinized

EXPLANATIONS FOR FIGURES

Fig.1. Diagrammatic representation of a longitudinal section of the normal tail passing through the centre of the caudal vertebra revealing the anatomical features.

Fig.2. Photomicrograph of longitudinal section of the normal tail showing the gross anatomical features.

ABBREVIATIONS

CHB - Chevron bone

D - Dermis

DMB - Dorsal muscle belly

LMB - Lateral muscle belly

M - Muscle

N - Nerve cord

NS - Neural spine

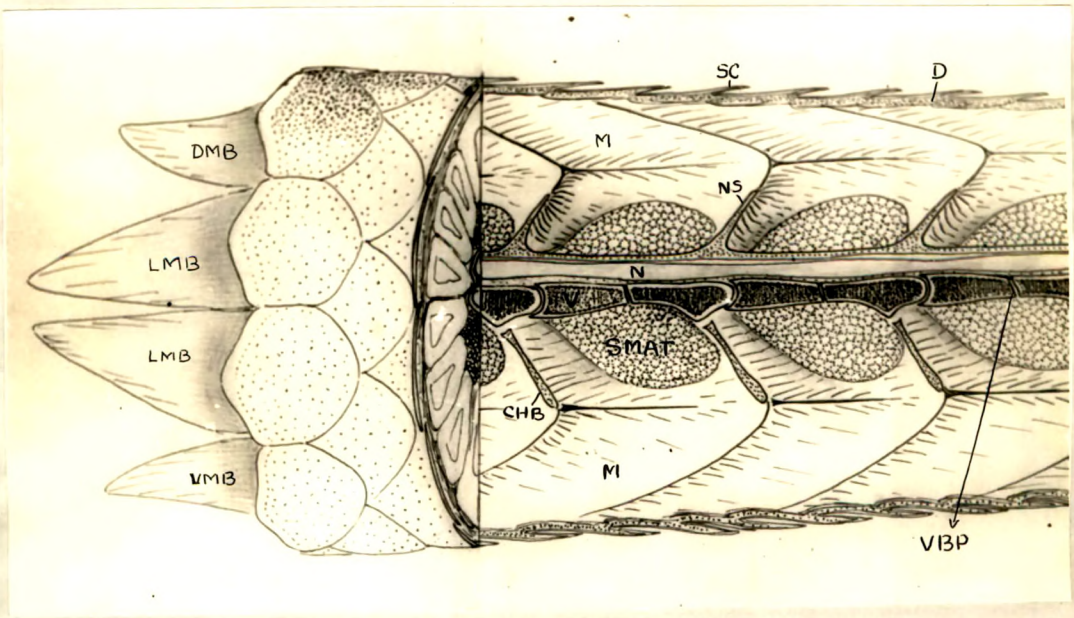
SC - Scale

SMAT - Submuscular adipose tissue

V - Vertebra

VBP - Vertebral breaking plane

VMB - Ventral muscle belly



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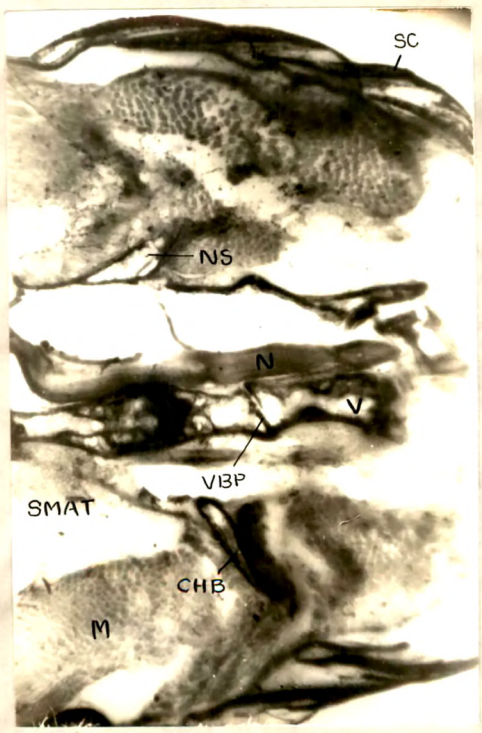

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EXPLANATIONS FOR FIGURES

Fig.3. Cross section of a normal tail (semidiagrammatic drawing) showing 16 muscle bundles just anterior to the level of vertebral autotomy plane. The shaded muscle bundles project out from the proximal region of the autotomized tail following autotomy (see Fig.1) and the nonshaded region represent the muscles that will remain with the tail stump after autotomy.

Fig.4. Photomicrograph of the cross section of a normal tail just anterior to the vertebral autotomy plane showing 16 muscle fasciculi.

Fig.5. An enlarged region of one fasciculus showing arrangement of the muscle fibres.

ABBREVIATIONS

C - Centrum

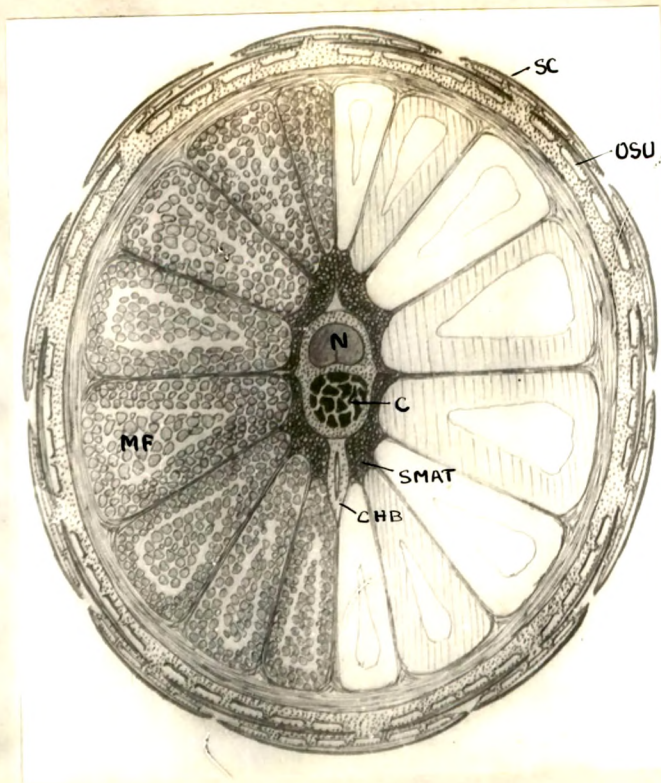
CHB - Chevron bone

MF - Muscle fasciculus

OSU - Osteoscute

S - Scale

SMAT - Submuscular adipose tissue



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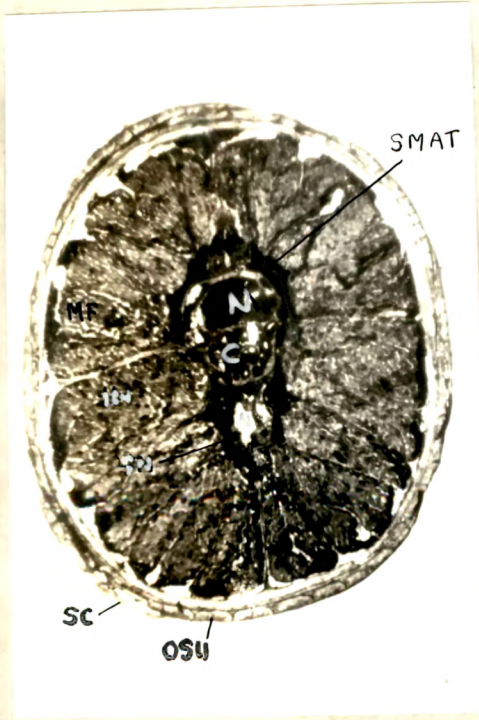

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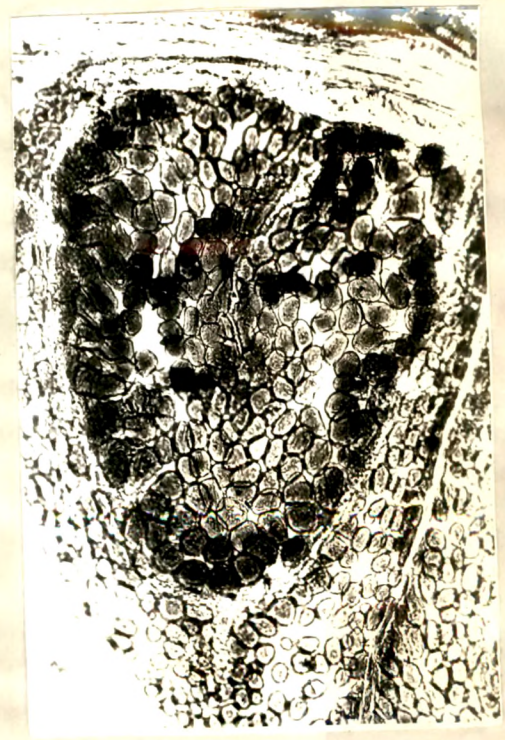

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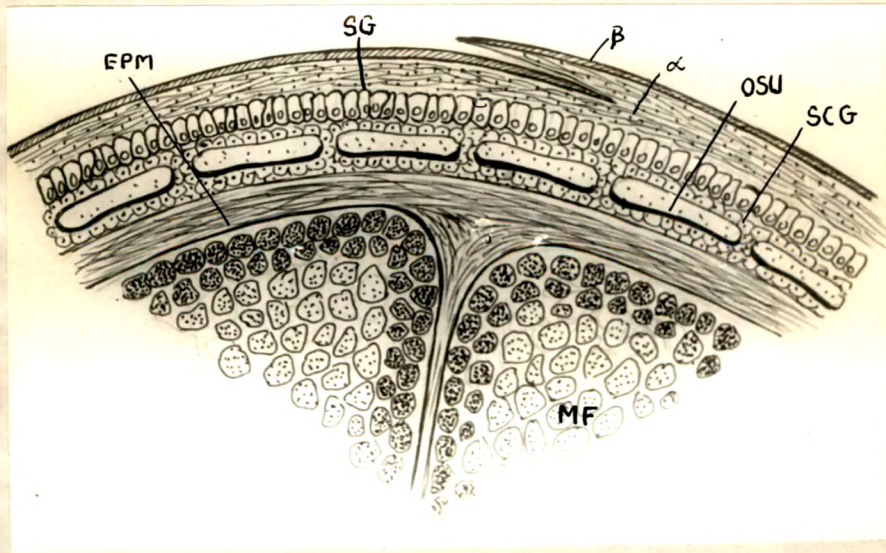
EXPLANATIONS FOR FIGURES

Fig. 6. Diagrammatic representation of the histological details of the normal skin (non-moulting phase).

Fig. 7. Photomicrograph of the normal skin showing the histological features.

ABBREVIATIONS

α	-	Alpha cells
β	-	Beta cells
EPM	-	Epimysium
MF	-	Muscle fasciculus
SCG	-	Scutogenic cells
SG	-	Stratum germinativum



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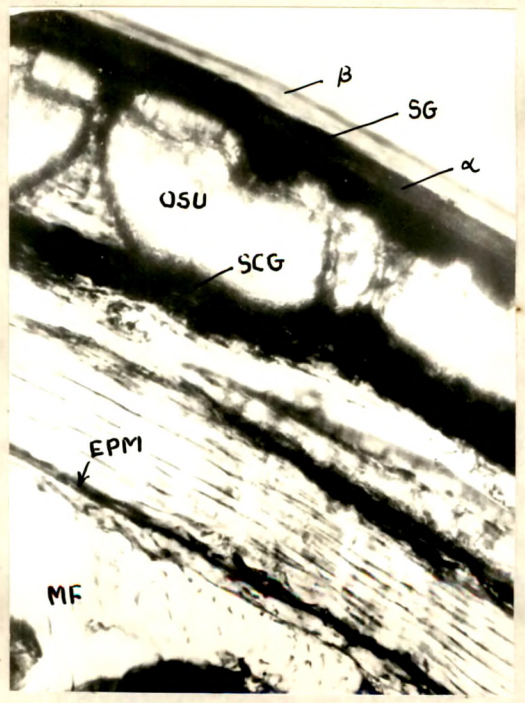

150 μ

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EXPLANATIONS FOR FIGURES

Fig. 8. Photograph of the autotomized tail at wound healing phase.

Fig. 9. Diagrammatic representation of the wound healing phase. Note the completion of the wound epithelium and the scab.

ABBREVIATIONS

CEM - Cut end of the muscle

CHB - Chevron bone

M - Muscle

N - Nerve cord

NS - Neural spine

SAR - Subapical region

SC - Scale

SCB - Scab

SMAT - Submuscular adipose tissue

VBP - Vertebral breaking plane

WE - Wound epithelium

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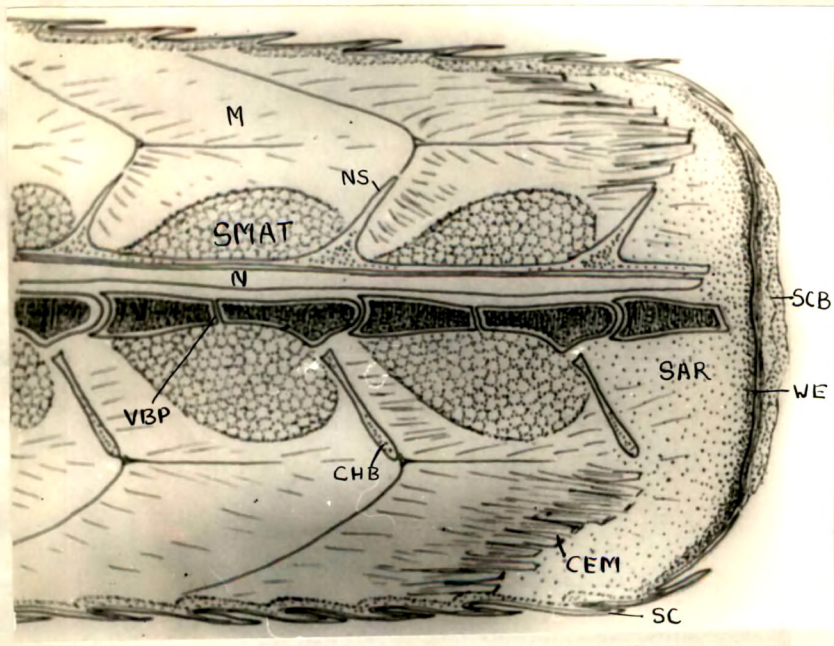

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EXPLANATIONS FOR FIGURES

Fig. 10. Longitudinal section of the tail regenerate at wound healing phase.

Fig. 11. Magnified region of wound epithelium and the scab. Note the stratified wound epithelium and the scab, which is being cast off.

ABBREVIATIONS

CEM - Cut end of the muscle

M - Muscle

N - Nerve cord

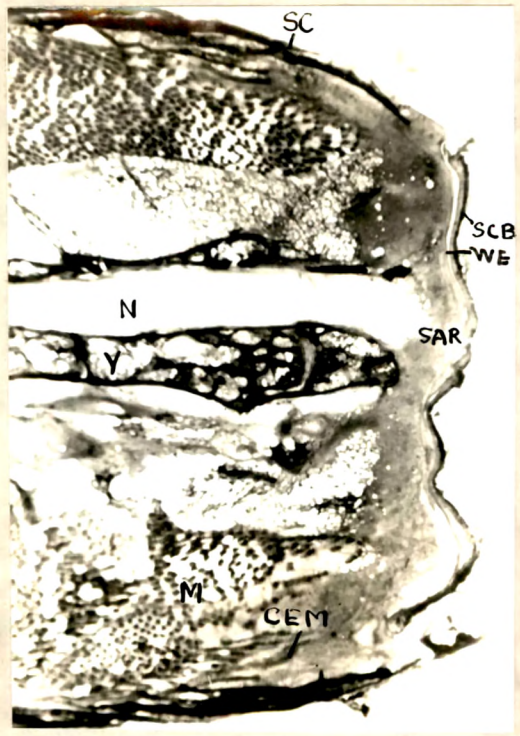
SAR - Subapical region

SCB - Scab

SMAT - Submuscular adipose tissue

V - Vertebra

WE - Wound epithelium



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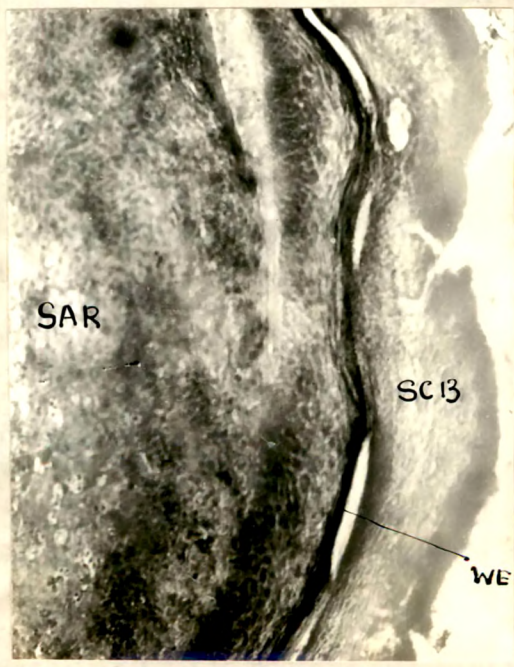

200 μ 11

EXPLANATIONS FOR FIGURES

Fig. 12. Photograph of the tail regenerate at the blastema phase.

Fig. 13. Diagrammatic representation of the longitudinal section of the tail regenerate at blastema phase.

ABBREVIATIONS

BE - Blastemic epithelium

MC - Mesenchymal cells

PRC - Procartilage aggregates

PRM - Promuscle aggregates

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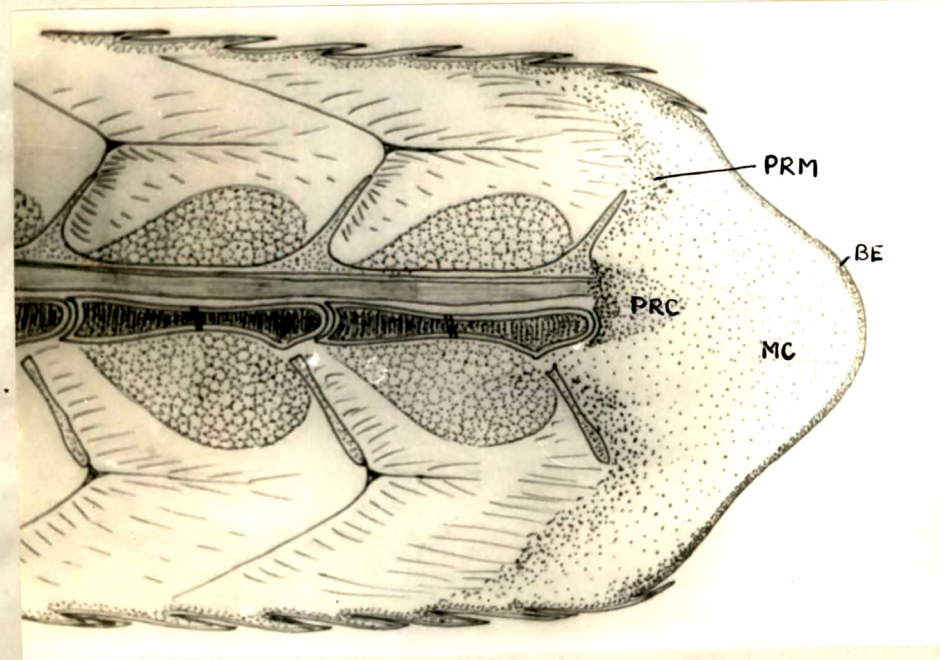

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EXPLANATIONS FOR FIGURES

Fig. 14. Photomicrograph of the L.S. of the tail regenerate at blastema phase.

Fig. 15. Magnified portion of the blastemic core region showing the mesenchymal cells.

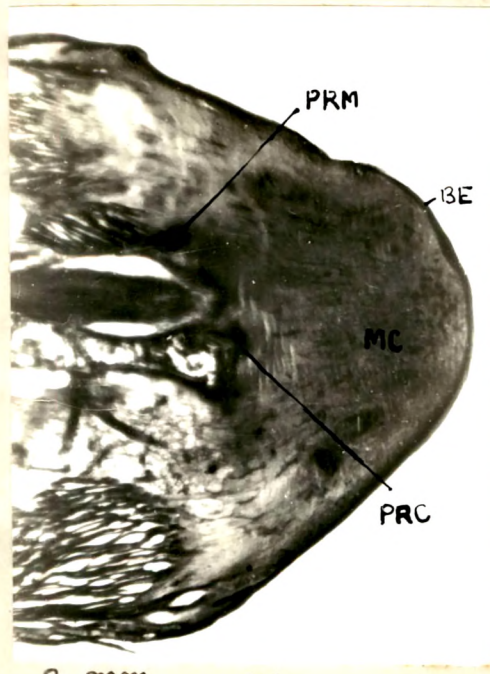
ABBREVIATIONS

BE - Blastemic epithelium

MC - Mesenchymal cells

PRC - Procartilage aggregates

PRM - Promuscle aggregates



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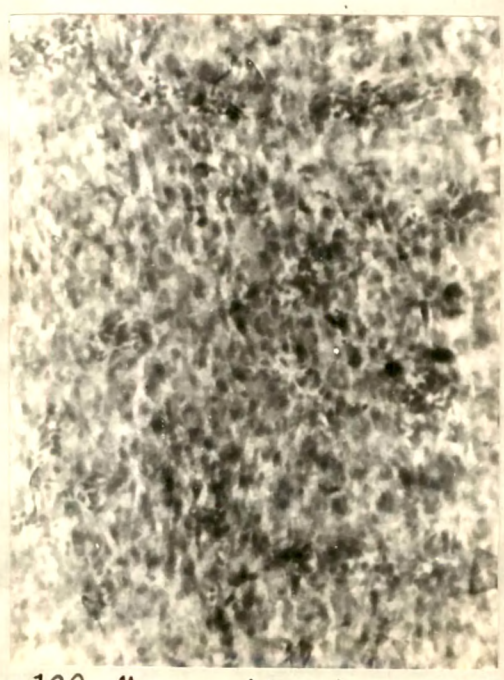

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EXPLANATIONS FOR FIGURES

Fig. 16. Photograph of the tail regenerate at the differentiation phase.

Fig. 17. Diagrammatic representation of the anatomical features of the tail regenerate at differentiation phase.

Fig. 18. Photomicrograph of the proximal region of the tail regenerate during the differentiation phase.

ABBREVIATIONS

CNC	-	Cartilagenous neural canal
E	-	Ependyma
DM	-	Differentiating muscle
DSC	-	Differentiating scale
DSMAT	-	Differentiating submuscular adipose tissue

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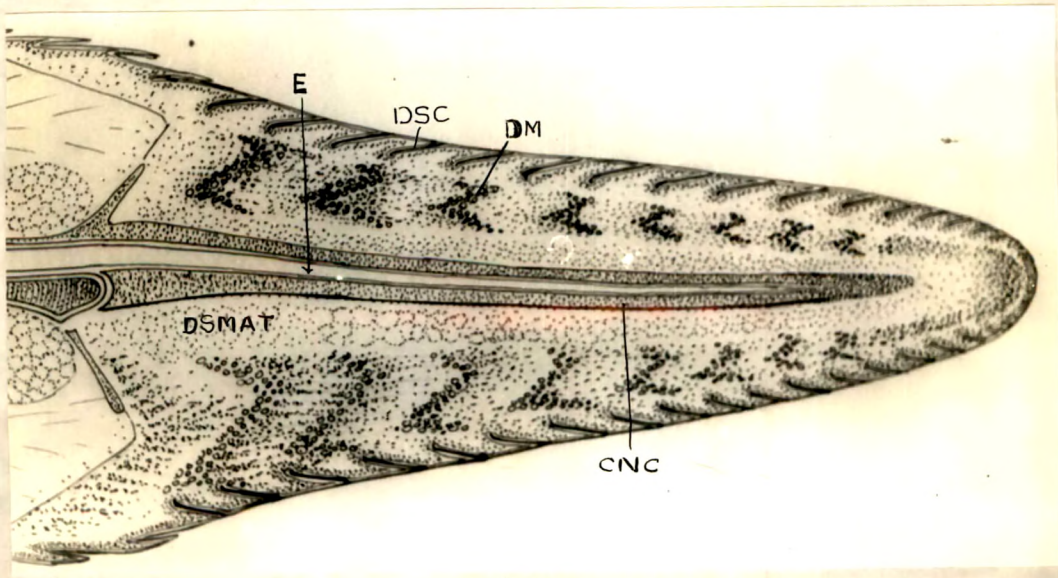

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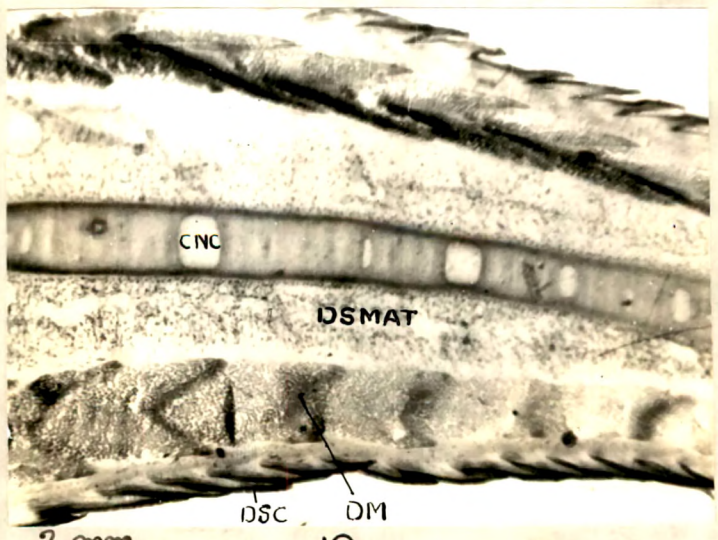

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EXPLANATIONS FOR FIGURES

Fig. 19. Magnified region of the epidermal region. Note the differentiating layers of the epidermis and the linear arrangement of the scutogenic cells which form the future scutes.

Fig. 20. Photomicrograph of the differentiating muscles showing the telescopic arrangement.

Fig. 21. Magnified region of a part of cartilagenous neural canal showing chondrocytes.

ABBREVIATIONS

Œ - Alpha cells

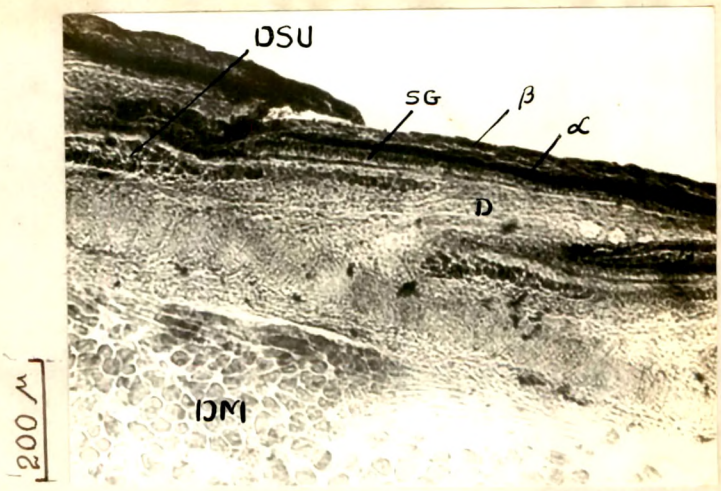
β - Beta cells

CH - Chondrocytes

D - Dermis

DSU - Differentiating scutes

SG - Stratum germinativum



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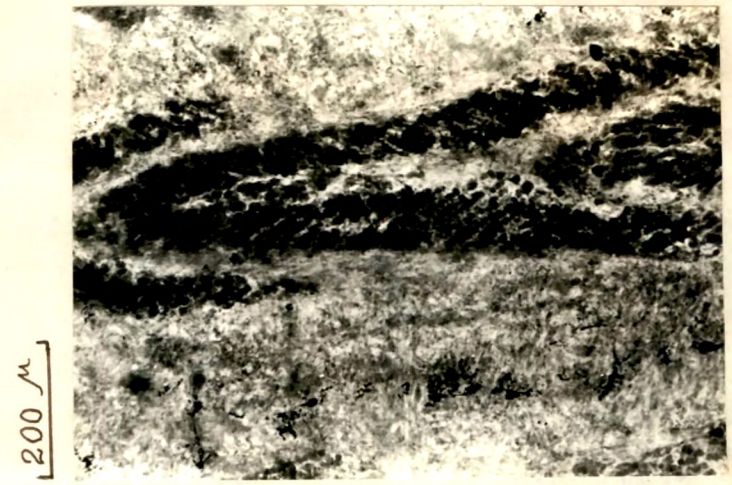

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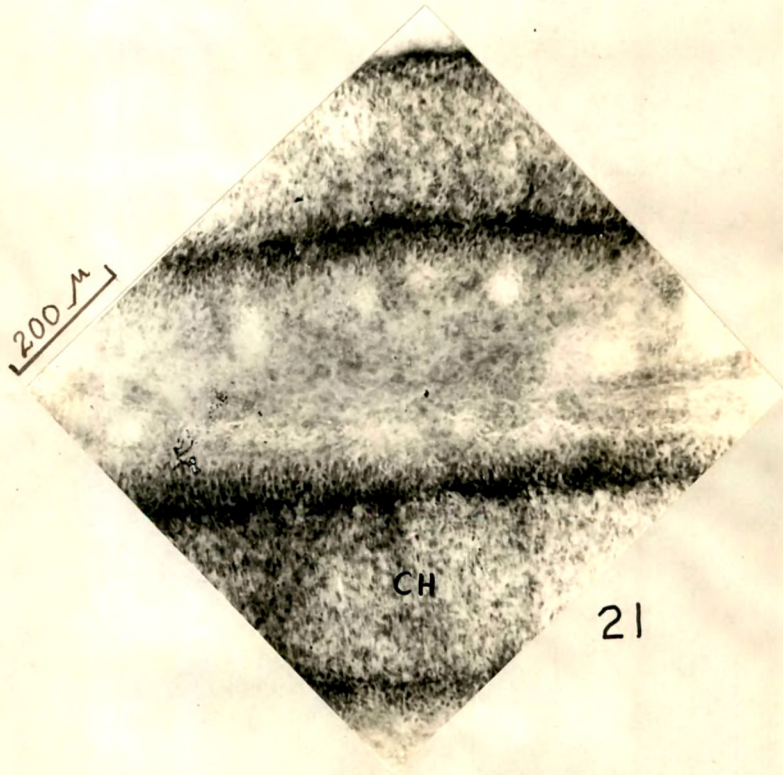

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EXPLANATIONS FOR FIGURES

Fig. 22. Photograph of the tail regenerate at growth phase.

Fig. 23. Photograph of the fully regenerated tail. Note the larger dorsal scales.

Fig. 24. Transverse section of fully regenerated tail revealing the anatomical features. Note the increased number of muscle fasciculi and the cartilagenous neural canal which encloses the ependyma.

ABBREVIATIONS

CNC - Cartilagenous neural canal

E - Ependyma

MF - Muscle fasciculus

OSU - Osteoscute

SC - Scale

SMAT - Submuscular adipose tissue

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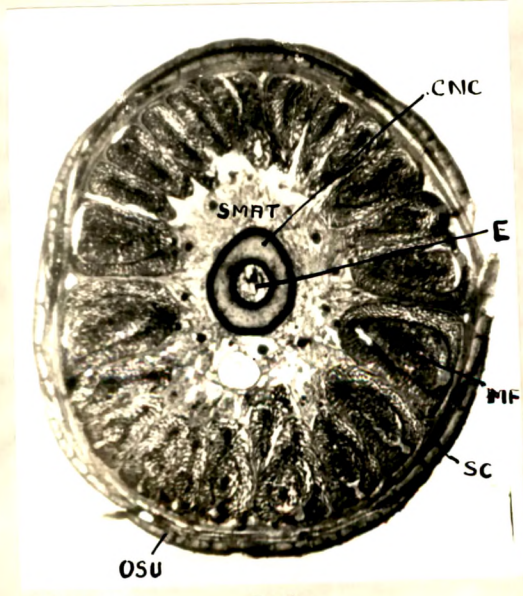

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compared to those present in Hemidactylus flaviviridis. In both the type of lizards, the ventral scales (subcaudals) are relatively larger in size than those present on the dorsal and lateral sides of the tail. Because of high keratinization and greater overlapping arrangement of the scales in Mabuya carinata, some difficulties were encountered in cutting the sections.

Anatomy of the normal tail: (Figs. 1 and 2)

Epidermis: (Figs. 6 and 7)

The main difference between the epidermis of Mabuya carinata and Hemidactylus flaviviridis is that, in the former the beta cells forming the outer most layer of the epidermis are highly keratinized, resulting in the formation of relatively thick scales. As far as the alpha cells are concerned, in both the types of lizards, there are about 3-4 layers of these cells. Immediately below the alpha cell layer, is the layer of stratum germinativum. The general height of stratum germinativum cells in Mabuya carinata is comparatively lesser than that of the similar cells in Hemidactylus flaviviridis. Stratum intermedium layer in both the lizards is noticed below the old generation of alpha layer of cells during the moulting phase when the outer

layers of alpha and beta cells get sloughed off. However, this layer is not clearly obvious in Mabuya carinata as it is so in the house lizard, Hemidactylus flaviviridis.

Prior to ecdysis, the stratum germinativum undergoes a process of rapid cell division, first giving rise to cells of stratum intermedium, which occupy a place between the old and new generation of alpha and beta cells and later with its disintegration, the beta and alpha cells of older generations get sloughed off. The histological changes that take place in the skin of lizards and snakes during ecdysis have been reported by Maderson (1967). Goslar (1958a & b) described the histological changes during the period of moulting and emphasized the hormonal influence in this process. It is likely that during moulting lysosomes in the cells of stratum intermedium may break down and release their lytic enzymes which bring about the disintegration of these cells and cause the separation of the outer beta and alpha cell layers from the newer generation of these cells.

Dermis: (Figs. 6 and 7)

Unlike Hemidactylus flaviviridis, in Mabuya carinata the dermis region is reduced. The outer subepithelial

and inner reticular regions of the dermis which were reported by Shah and Chakko (1968) in Hemidactylus flaviviridis are readily distinguishable in Mabuya carinata. The osteoscutes (osteoderm) which are embedded in the dermis are observed in Mabuya carinata, while they are not present in the house lizard, Hemidactylus flaviviridis.

Subcutaneous adipose tissue:

A well defined subcutaneous adipose tissue, which was reported by Woodland (1920) and Shah and Chakko (1968) in the house lizard, Hemidactylus flaviviridis, is absent in Mabuya carinata. However, a few cells loaded with neutral lipid could be noticed scattered in and below the matter of the dermis.

Caudal muscles:

The muscle bellies in each autotomy segments and those in relation with the adjoining ones are separated from one another by obliquely placed septa of connective tissue. These septal limits indicate the planes of autotomy as far as the myotomes are concerned. The myosepta separating successive myomeres are folded, so that the muscles in each quandrant of the tail assume

roughly cone shape and in cross sections they appear as muscle bundles as shown in the Figs. 3,4,5. The telescopic arrangements of the caudal muscles are more prominent in Mabuya carinata. When autotomy is caused, eight muscle fasciculi (myomeric extensions) of equal length detach off and project from the anterior end of the autotomized portion of the tail, leaving corresponding eight cavities in the autotomized end of the tail stump. The two laterally placed fasciculi of the right half and the left half of the tail are larger and broader in size than the two at the dorsal and the ventral sides. Muscle attachments to the centrum, the neural spine and the chevron bone of the caudal vertebrae are observed in an obliquely radiating manner. When there are sufficient local nervous and or mechanical stimulations, the lateral flexor muscles arranged segmentally around the vertebrae violently contract and cause a break in the vertebra at the level of its breaking plane. The subsequent separation of the soft tissue such as the adipose tissue, surrounding the skeletal axis, effectively detaches the distal part of the tail, which may keep wiggling for a few minutes afterwards. Meanwhile the sphincter in the blood

vessels constrict in the tail stump, preventing the loss of blood.

Submuscular adipose tissue:

The cells of adipose tissue, are loaded with neutral lipids and due to this, their cytoplasm and nuclei are pushed towards the periphery of the cells. The adipose tissue is found segmentally arranged; two bundles placed dorsolaterally and two ventrolaterally (Figs. 1,2,3). During autotomy along the breaking plane these bundles of adipose tissue detach along with the anterior half of the vertebrae.

Vertebral column: (Figs. 1 and 2)

The general morphological features of the tail vertebrae of Mabuya carinata are of typical caudal vertebrae of a reptile. In both the type of lizards, the plane of autotomy is intravertebral i.e., the vertebral cleavage plane divides the whole vertebra into two halves in the middle of its centrum. Thus each autotomy segment of the vertebral column of the tail region contains adjoining halves of the two successive vertebrae. There is no intervertebral cartilage in Mabuya carinata, as present in Hemidactylus flaviviridis, instead a well defined articulating

surfaces of the two successive vertebrae are lined with cartilage. The centra, spines and chevron processes of vertebrae are hollow and in these haematopoietic tissue is lodged.

Spinal cord:

The spinal cord of Mabuya carinata shows more or less the same general features viz., the grey matter surrounded by white matter as seen in the Gekkonid lizard, Hemidactylus flaviviridis.

REGENERATING TAIL

Wound healing phase: (4-6 days after autotomy) (Figs.8,9,10)

The gross change immediately after autotomy that occurs at the stump is the shrinking of the tissues, especially the muscles, at the cut end of the tail, which results in reduction of the wound surfaces. One of the primary manifestations is the formation of a blood clot, at the exposed surface of the wound. The time taken for these changes are about 5-10 minutes. By about 48 hours after autotomy, a large number of lymphocytes invade the undersurface of the clot. This indicates the phagocytosis of the fibrous and cellular debris of injured cells that are beyond repairs at the wound surfaces. The next event

that follows is the migration of the epidermal epithelial cells derived from the stratum germinativum of the epidermis at the cut end of the stump, below the clot (scab). For a complete covering of the wound by the migrating epithelial cells it takes about 96 hours. Such an epithelial covering later through proliferation of its cells assumes a multi-layered feature. To reach this stage it takes about 150 hours (Fig.11). During later stages of wound epithelium formation, the accumulation of dedifferentiated cells is noted between the wound epithelium and the cut end of the original stump tissues. As the dedifferentiation continues at the cut end of the stump tissues, the dedifferentiated mesenchymal cells accumulate at the subapical region of the regenerate. Similar results have been reported by Chalkely (1954, 1959) in the newt, Triturus viridescens; Huges and New (1959) in Sphaerodactylus; and in Hemidactylus flaviviridis (Shah and Chakko, 1968).

Preblastemic phase: (6-8 days after autotomy)

This phase is well distinguished by the appearance of a very small conical projection of the regenerate at the centre of the wound epithelial surface. The mesenchymal cells are arranged compactly towards the apical region of the regenerate but are loosely arranged at its base. In

a 5-6 days old tail regenerate, the wound epithelium increases in depth due to stratification. The preblastemic space (the space between the wound epithelium and the original stump tissues) is now populated with mesenchymal cells which become the blastema cells.

Blastemic phase: (7÷12 days after autotomy)

A well formed regeneration blastema appears in about 10 days after autotomy. The apical wound epithelium in the beginning is transluscent and smooth to the naked eye. But, later the conical cap like projection enlarges to keep pace with the activities at the subapical region and thus a well defined structure appears by about 12 days after autotomy (Fig.12). As the accumulation of dedifferentiated mesenchymal cells progresses, the length of the regenerate increases. At this phase generally two type of cells are noticed in the regenerating blastema; (1) the outer margin which is formed of stratified epithelial cells and (2) the cells of the blastemal core which are formed out of dedifferentiated mesenchymal cells (Figs.13,14,15). Thus there is hardly any difference in preblastemal and blastema phases of the regenerate except in their size.

During the late blastema phase, some of the aggregated mesenchymal cells near the cut end of the vertebra of the original tail foreshadow the formation of the chondroblasts are the procartilage aggregates which surround the extending nerve cord as an ependymal tube. Besides, at the base of the regenerate, condensations of blastemal cells noticed in association with the procartilage aggregates are the promuscle aggregates. They are uninucleated and termed as the mononuclear myoblasts.

Early differentiation phase: (13-20 days after autotomy)

During the late phase of blastema, the changes that are taking place are mainly in the epidermis, dermis, myoblasts and chondroblasts. The differentiation of the tissues listed above begins at the proximal end of the regenerate and progressively proceeds towards the distal end. The time taken for the differentiation of the various tissues varies. Stratified epidermis noticed at the blastema phase, is now in an advanced state having multilayered structure. Such a change is visible at the proximal end of the regenerate, while in the distal region, the epidermis is still only a few layers of cells without differentiation.

Late differentiation phase: (20-30 days after autotomy) (Fig. 16)

About 19 days after autotomy, the initial stage of scale formation at the base of the regenerate results in the formation of the lamellar infolding of the stratum germinativum, due to which the epidermis acquires a wavy contour. The chromatophores appear below the newly formed epidermis at this time. At the base of the regenerate a simultaneous differentiation of epidermal and dermal regions are apparent. The fibroblasts which are packed under the epidermis transform into fibrocytes and laying of the connective tissue fibres (collagen fibres) leads to the increase in the depth of the dermis. Some of the cell aggregates linearly arranged below at the inner ends of the newly formed scales could be seen during the late differentiation phase, which showed affinities towards the alizarin red stain (Fig.19) These cell aggregates (scutogenic cells) give rise to scutes in the dermis of the regenerate. The first noticed myoblasts at the proximal end of the regenerate during the late blastemic phase now transform into myocytes and later get elongated to form the multinucleated myofibres (Fig.20). As the muscle fibres become matured, the striations reapper. The mesenchymal cells surrounding

the ependyma and which are in association with the cut end of the bone matter of the vertebra of the original tail stump get first differentiated into chondroblasts and later chondrocytes constituting the cartilagenous neural canal (Fig. 21). Such sequential changes in the formation of the cartilagenous neural canal progressively occur proximodistally in the regenerate.

The growth of the cut end of the nerve cord in the regenerate which results in the formation of ependyma, keeps pace with the growth of other components of the regenerate (Fig. 18). The ependyma is lodged in the cartilagenous neural canal; no nerves arise from the ependyma.

The nerve supply to the various tissues in the regenerate is from the spinal nerves of the original tail stump. As the differentiation progressed, the fibrocytes surrounding the cartilagenous neural canal transformed into adipose cells. First appearance of the adipose cells is at the base of the regenerate and gradually in proximo-distal direction more and more such cells are formed.

Growth phase: (30-40 days after autotomy) (Fig.22)

Once the differentiation is achieved, the differentiated tissues of the regenerate grow in size

and finally attain histomorphological and physiological maturity characteristic of the corresponding tissues of the original tail. During this phase, the newly formed skin of the regnerate also moults.

Fully regenerated tail: (60-70 days after autotomy)

Morphology: (Fig. 23)

The regenerate, when fully grown and reaches the total tail length as was of the normal original tail, is slightly less in girth than what was the measurement, at any corresponding level of the original tail. The scales of a fully regenerated tail are relatively larger in size than the normal ones. Besides, the number of scales encircling the tail at one region, are only nine instead of ten present in the normal tail. The dorsal scales in the fully regenerated tail are broader, like the ventral ones. Such is not the case of dorsal scales in the normal tail.

Anatomy: (Fig. 24)

The cellular layers of epidermis, namely, beta, alpha and stratum germinativum, the dermis and the layer of scutes is completely formed in a fully regenerated tail. The musculature in the regenerate

is almost similar in most respect to that of the original tail. However, the number of fasciculi in the regenerate are more but the pattern of the muscle arrangement remains more or less similar as was noticed in the normal tail. The adipose tissue in the submuscular region, though present around the cartilagenous neural canal is found to be less than that in the corresponding region in the normal tail. In the fully regenerate, the cartilagenous axial skeleton formed is an unsegmented tube accomodating ependyma in its canal.