INTRODUCTION

In the field of Biology the process of regeneration is a fascinating phenomenon. It is well known that the amphibians have remarkable powers to restore their lost parts viz., lens, retina, liver, eye, intestine, limbs and tail. During the last two decades, intensive studies have been carried out on the different aspects particularly the morphological and histological of regeneration in amphibians. However, recently Schmidt (1966) has presented a comprehensive data on the enzyme activities in the regenerating amphibian limb. Unlike amphibians, reptiles possess restricted abilities to reconstitute their lost parts viz., tail and limbs. Amongst amniotes, some reptiles are the only animals known, who have regenerative capacity so as to restore more or less completely (structurally and functionally) the lost parts of their body, while none of the birds or mammals can do so (with the exception of regenerative capacity of the mammalian liver). However,in recent years some investigations in this respect have been carried out to understand the problem of regeneration in reptiles. Kamarin and Singer (1955) and Simpson (1964) have worked on the influence of nerve cord and ependyma respectively on the regenerating lacertilian tail. A histological account of the regenerating lacertilian tail has been given by Woodland (1920), Barber (1944), Huges and News (1959) and

Moffat and Bellairs (1964). Uptil now there is hardly any record of the studies on enzyme histochemistry of the different tissues of the normal and regenerating lacertilian tail. Therefore,the present thesis is an attempt to understand the lacertilian tail regeneration from this point of view.

The sequence of regeneration in the amputated tail could be arbitrarily grouped into six different phases viz., (1) Wound healing (2) Preblastemal (3) Blastemal (4) Late blastemal (5) Differentiation and (6) Growth. It is obvious from the present studies that the successive phases overlap and there is no distinct line of demarcation separating the different phases. However, such an arbitrary division of the continuous regenerative process is done mainly for the convenience of description.

Wound healing phase (24 hours to 4 days after amputation): During this phase the first noticeable event to take place is the shrinkage of the stump tissues and converging of the skin, reducing the surface of the wound area. The wound surface is covered with blood clot. The migration of the epithelial cells to close the wound and sloughing off of the scab is followed. By about 96 hours after amputation the wound is completely healed up.

Preblastemal phase (4 to 6 days after amputation): When the wound is getting healed and also afterwards,

dedifferentiation of the stump tissues start. Some of the injured cells disintegrate and the debris is cleared by macrophages which are present in good number under the epithelial layer over the wound. The dedifferentiated cells form the mesenchyme cells of the blastema.

Blastemal phase (6 to 10 days after amputation): Once the dedifferentiated cells were being formed the blastemal phase started. The dedifferentiated cells increase in number by repeated cell division (mitosis). A small cone like projection appears in the centre of the healed wound. The regenerate at this stage is formed of two types of cells (1) the mesenchyme cells forming the core of the blastemal cone and (2) the epithelial cells forming the cover over the cone.

Late blastemal phase (10 to 15 days after amputation): During this phase the blastemal cone increases in its length and the differentiation of the mesenchyme cells at the base of the regenerate begins. The epithelial cell layers at the base of the regenerate become more stratified and also differentiated into the basic epidermal regions viz., the stratum germinativum and stratum corneum.

Differentiation phase (15 to 30 days after amputation): All the tissues of the regenerate are well differentiated by the end of this phase. Initially the mesenchyme cells at

the base of the regenerate differentiated first. The differentiation progressed proximodistally.

Growth phase (30 days after amputation and onwards till the regenerate reaches the length of the original tail): During this phase the morphologically differentiated cells which are organised into tissues add to their bulk on one hand and become functionally mature on the other.

The animals utilized in the present investigations were kept at room temperature in the laboratory where the temperature and humidity varied according to seasons. Variations in time noted for different phases of regeneration were possibly due to changes in the temperature and humidity. However, a standard diet of young cockroaches was supplied to all the experimental lizards used.

Eventhough Woodland (1920) has given the histological account of the regenerating tail of the lizard, Hemidactylus flaviviridis, it was thought necessary to carry out a detailed study of the histology of the normal and the regenerating tail (at its different phases of regeneration) in order to correlate the study of the enzymes and metabolites. So to start with, in this thesis, a detailed account of the histology of the normal and the regenerating tail of Hemidactylus flaviviridis is presented (Chapter 1).

While studying the histological details of the various tissues of the normal and regenerating tail, large quantities of lipids were found to be loaded in the subcutaneous and submuscular adipose tissues. Lipids being one of the main sources of energy for the metabolic activities of the different tissues, a study was undertaken with the view to characterize these lipids and study their distribution pattern in the normal and regenerating tail (Chapter 2). The presence of lipids in the normal and regenerating tissues of the lizard tail suggests their utilization for obtaining energy. Since the activities of lipase and esterase would be indicative of lipid hydrolysis which is a primary step prior to their utilization, a histochemical study of lipase and esterase was also carried out (Chapter 3).

Histochemical localization of SDH in various tissues of the normal and regenerating tail was studied. In the tissues of the normal tail, SDH could be demonstrated in the cells of the stratum intermedium and stratum germinativum of the epidermis, muscle fibres and the spinal cord. Its significance in the carbohydrate and lipid metabolism of these tissues is discussed (Chapter 4).

Glycogen is the other chief metabolite which is readily available as an energy source for the cell maintenance and

function. A study on the histochemical localization of glycogen and the glycolytic enzyme, phosphorylase in the tissues of the normal and the regenerating tail was carried out (Chapter 5).

The histochemical distribution of acid phosphatase in the various tissues of the normal and regenerating tail of the lizard was studied. The functional significance of the enzyme in relation to phagocytosis, ecdysis and protein synthesis is discussed (Chapter 6). Alkaline phosphatase is said to be absent in the skeletal muscle fibres according to some investigators and if present, it is sarcoplasmic in its location. From the present study it is found that in the skeletal muscle fibres of the normal and the regenerating tail this enzyme is localized in the sarcoplasm and mitochondria. Based on the number of mitochondria and the concentration of the enzyme activity three types of muscle fibres could be identified. A detailed account of the alkaline phosphatase activity in various tissues of the normal and regenerating tail is given (Chapter 7).

The tail regeneration is a continuous process though arbitrarily different histomorphological phases are recognised. It is quite logical to associate the nucleic acids with increased metabolism, cell growth and protein synthesis.

An investigation was carried out to study the localization and concentration of nucleic acids in the tissues of the normal and regenerating tail (Chapter 8).

It is known that the nerve supply plays an important role in initiating and maintaining regeneration in vertebrates (Singer, 1962). The influence of nerves has been suggested to be chemical in nature (Singer, 1957). But the responsible neurochemical agents are not yet known. With this view a study on the histochemical localization of cholinesterases was undertaken (Chapter 9).

Finally, the influence of thyroid gland and its hormone in relation with the growth and differentiation is well established. Therefore, the histological changes in thyroid activity of the normal and regenerating lizards during different phases of regeneration was studied. The alterations in the structure of the thyroid gland in terms of height of the follicular cells and size of the follicles is reported (Chapter 10).

CHAPTER 1

HISTOLOGICAL OBSERVATIONS ON THE NORMAL AND REGENERATING TAIL OF THE HOUSE LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

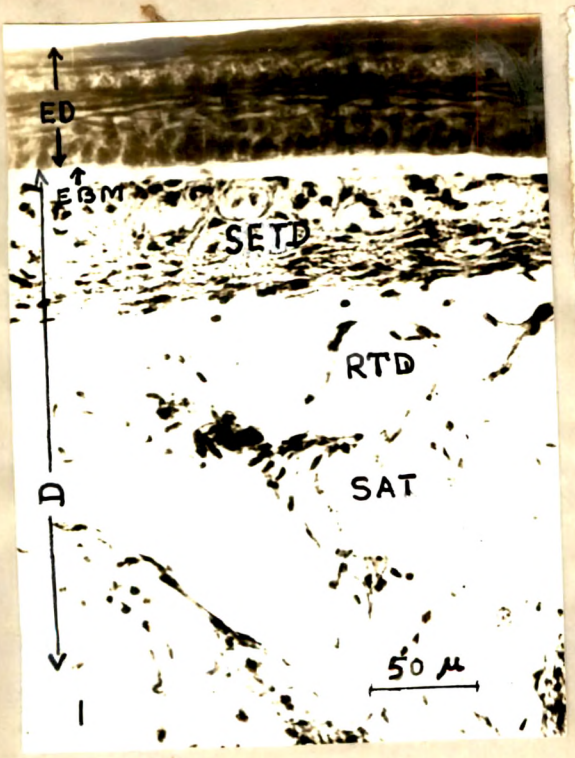
Numerous studies have been carried out on the autotomy and regeneration of the tail in the lizards in the past (few) years, such as those of Woodland (1920) on *Hemidactylus flaviviridis*; Slotopolsky (1922) on *Lacerta*; Kamrin and Singer (1955) on *Anolis carolinensis*; Huges and News (1959) on *Sphaerodactylus*; Simpson (1964) on *Lygosoma laterale* and Moffat and Bellairs (1964) on *Lacerta vivipara jacquin*. Woodland (1920) described in detail the morphology and in general the structure of the normal and the regenerated tail. He further stressed the importance of the caudal flexor muscles and their mode of action in relation to autotomy. However, very little information is available with regard to the cellular details of the different tissues, the microscopical features of the early phases of regeneration and the histogenesis of the various tissues of the regenerating tail of the lizards. The present investigation was therefore an attempt to establish the histology of the normal tail of the house lizard, *Hemidactylus flaviviridis* and trace the cytological and histological changes that take place during regeneration.

MATERIALS AND METHODS

Adult house lizards, H. flaviviridis collected from the University campus and maintained in the laboratory on a diet of young cockroaches were used as experimental animals. All animals were weighed and the lengths from the snout to the vent and to the tip of the tail were measured before amputating the tails, so as to select animals of approximately the same age group. Amputation was carried out along the plane of autotomy keeping two or three segments after the vent intact and the tails were then allowed to regenerate. In the present investigation the histological observations were carried out on the regenerating tail from the day of amputation to 50 days and then on to the fully regenerated tail (grown to that of a normal adult size).

Histological procedure:

The normal tail and the regenerates with atleast one or two segments of the original tail stump were cut and fixed in Bouin's fluid and formalin at room temperature for 24 hours. The washed tissues were fixed on a microtome chuck in a cryostat maintained at -20°C. Longitudinal sections were cut at 5 μ and transferred to ice cold distilled water. Thoroughly washed sections were then fixed on albuminised slides and stained with Haematoxylin-Eosin.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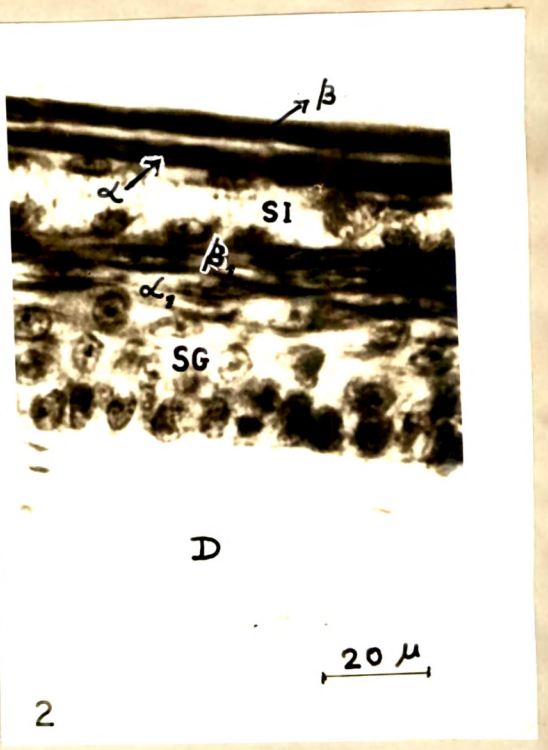
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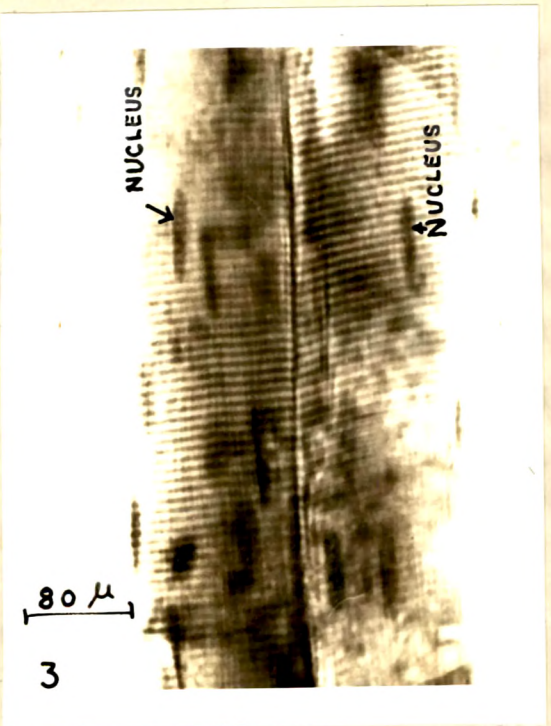
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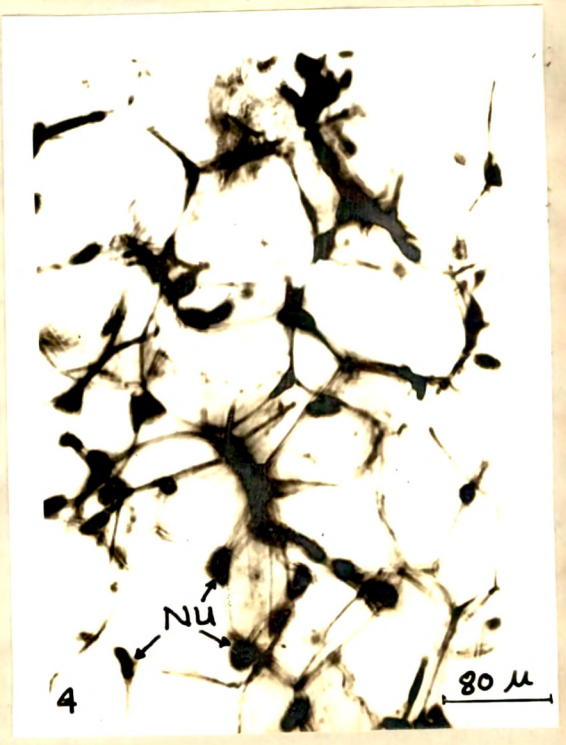
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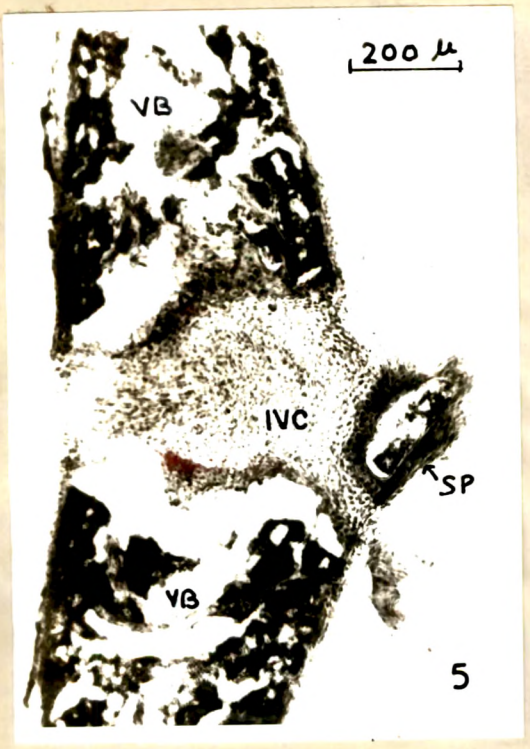
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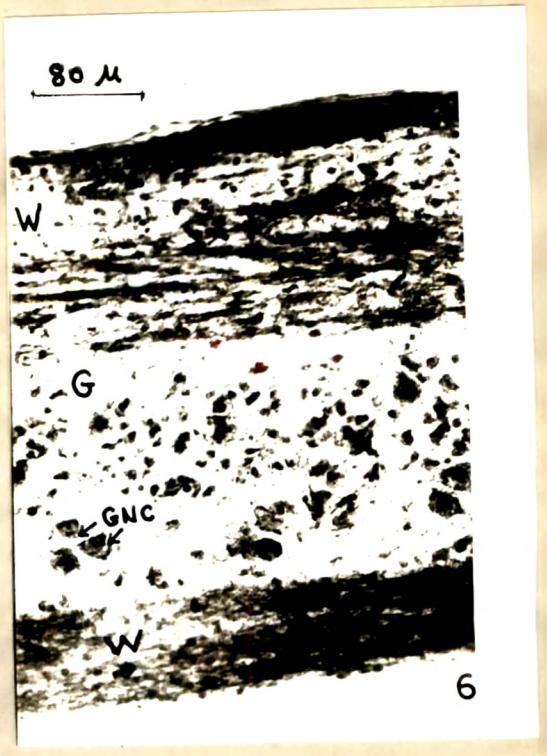
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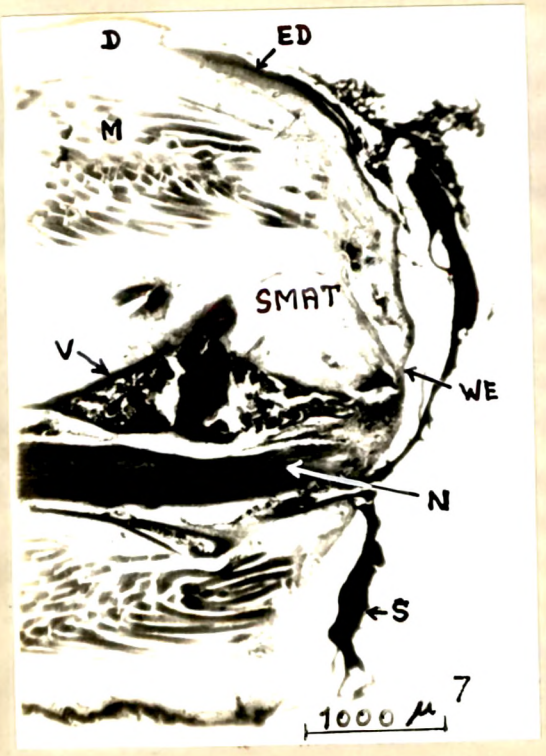
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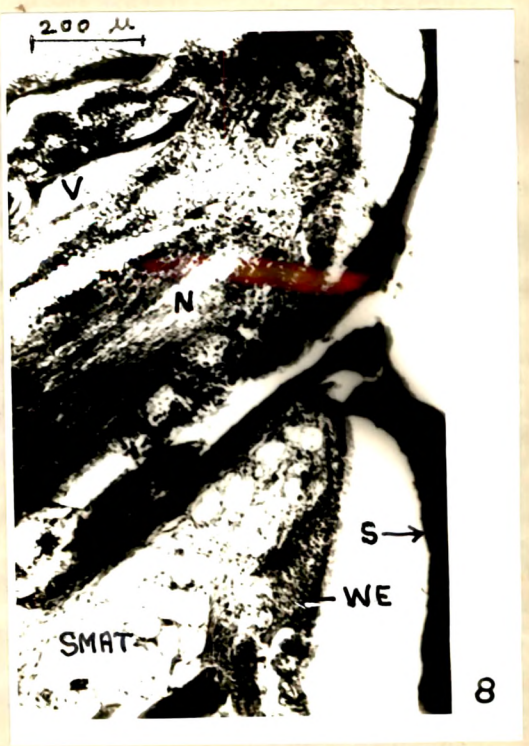
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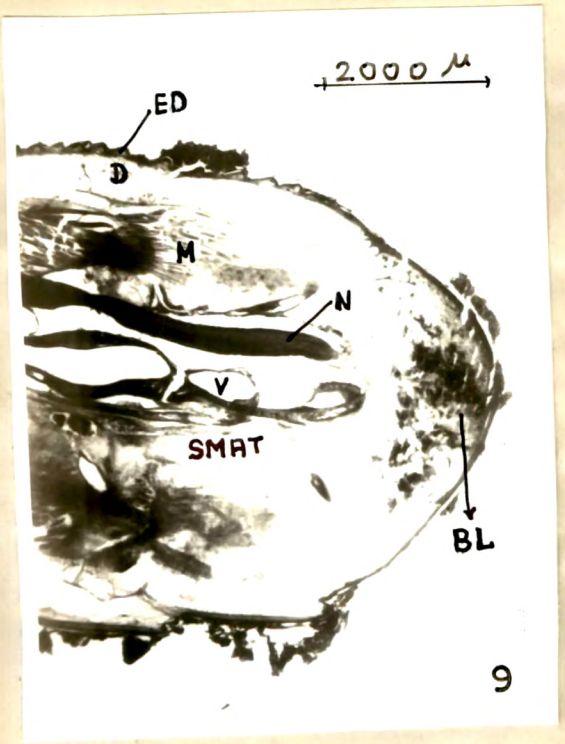

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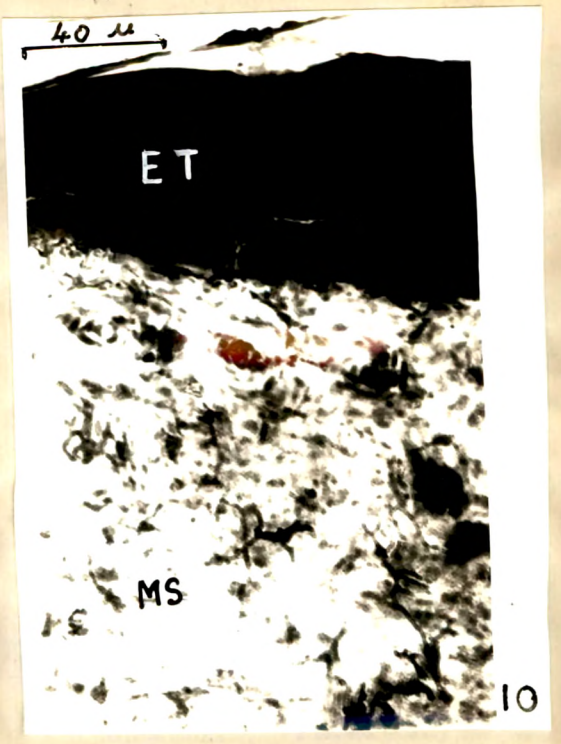

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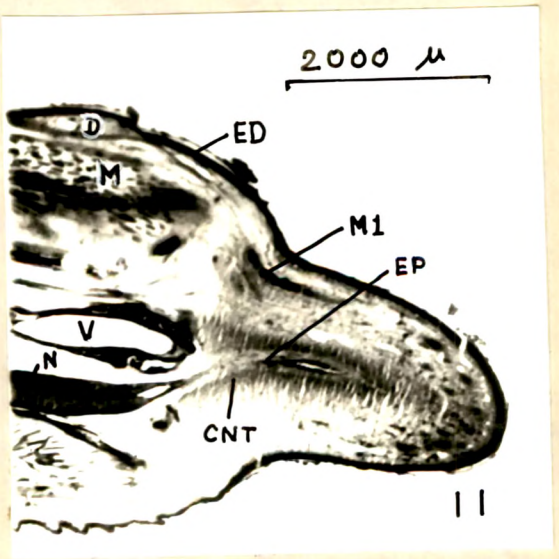

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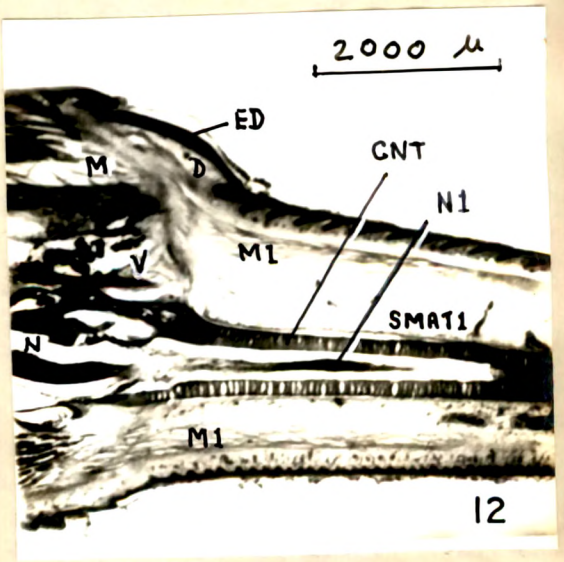

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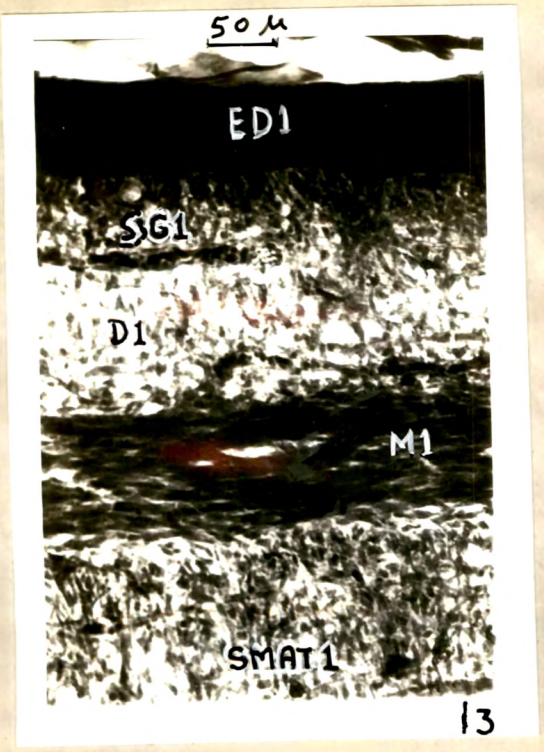

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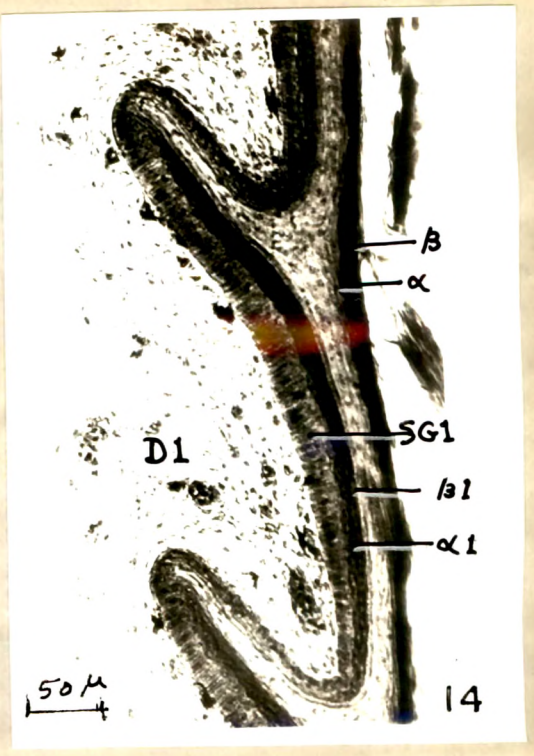

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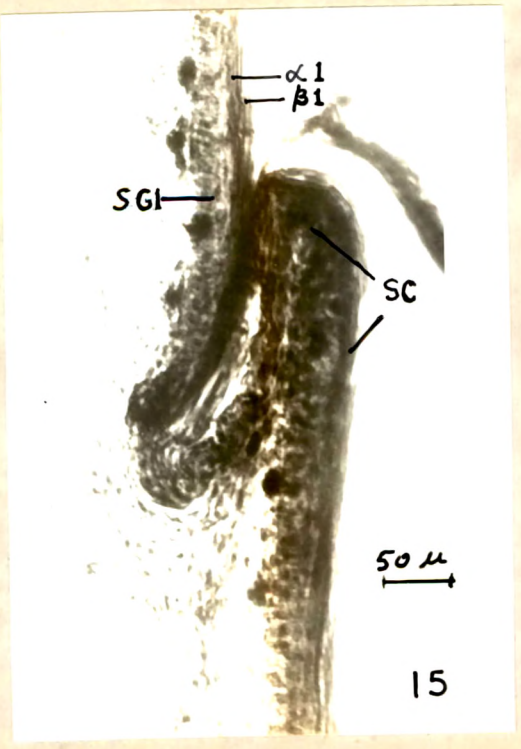

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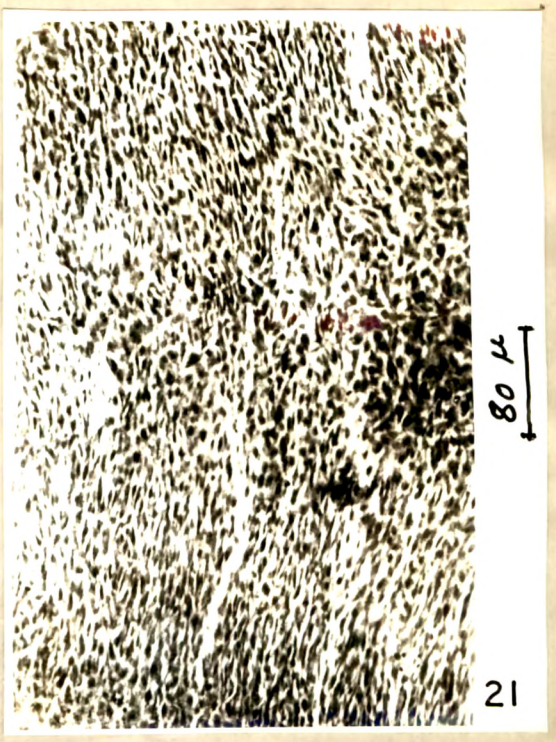

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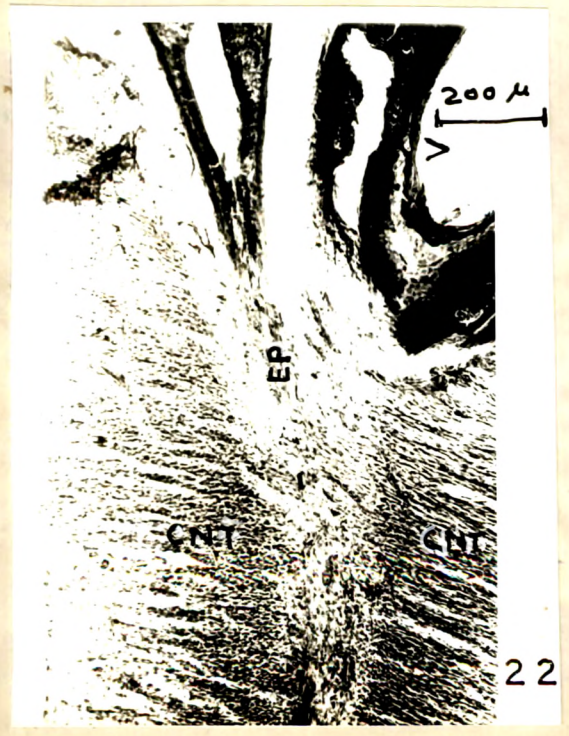

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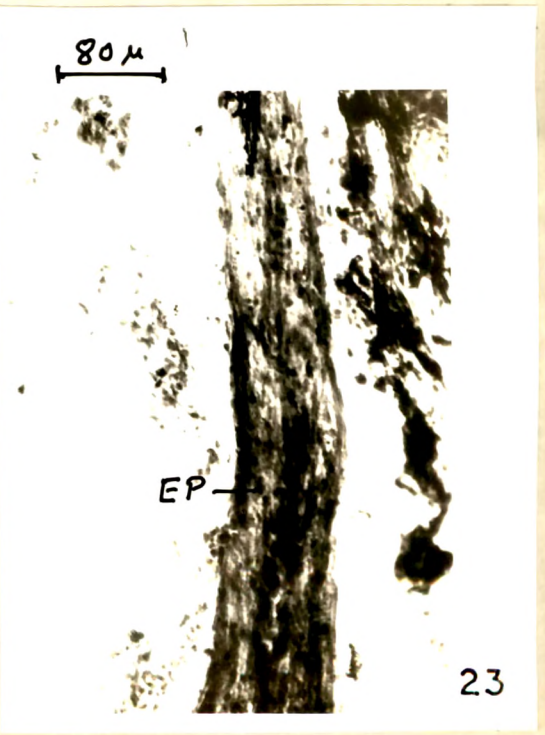

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EXPLANATIONS FOR FIGURES

Fig. 1. L.S. of normal tail skin (ventral side).

Fig. 2. L.S. of normal tail skin; Epidermis magnified.

Fig. 3. Striated muscle fibres (caudal muscle).

Fig. 4. Submuscular adipose tissue showing fat cells and their nuclei.

Fig. 5. L.S. of caudal vertebrae. Note the hollow centra, spine with marrow cells and intervertebral cartilage.

Fig. 6. L.S. of spinal cord (nerve cord).

Fig. 7. L.S. of regenerating tail (wound healing phase). Note the wound epithelium and the scab being pealed off.

Fig. 8. Part of Fig. 7 magnified, showing wound epithelium and pealed scab.

Fig. 9. L.S. of the regenerate (preblastemal phase). Note the formation of the blastemal cone.

Fig.10. L.S. of blastema (enlarged) showing the epithelium and mesenchyme cells.

Fig.11. L.S. of regenerate (late blastemal phase).

Fig.12. L.S. of regenerate (differentiation phase).

Fig.13. L.S. of skin, muscle and submuscular adipose tissue of the regenerate (late blastemal phase).

Fig.14. L.S. of skin in the regenerate(differentiation phase). Note the early stage of scale formation.

Fig.15. L.S. of the skin in the regenerate (late differentiation phase). Note the pealing off of the old generation of beta and alpha cells. Final stage of scale formation.

Fig.16. Mononuclear myoblasts in the regenerate.

Fig.17. Myocytes in the regenerate.

Fig.18. Just differentiated muscle fibres in the regenerate.

Fig.19. Submuscular fibrocytes before deposition of fat in regenerate.

Fig.20. Chondroblasts, a stage prior to formation of chondrocytes.

Fig.21. Chondrocytes in the regenerate.

Fig.22. L.S. of regenerate at the base showing the extention of the spinal cord (ependyma).

Fig.23. Ependyma in the fully regenerated tail.

ABBREVIATIONS

α - Alpha cells (old generation)

α_1 - Alpha cells (new generation)

β - Beta cells (old generation)

β_1 - Beta cells (new generation)

BL - Blastema

CNT - Cartilagenous neural canal

D - Dermis

D_1 - Dermis in the regenerate

EBM - Epidermal basement membrane

ED - Epidermis

ED_1 - Epidermis in the regenerate

EP - Ependyma

ET - Epithelium

G - Grey matter

GNC - Giant nerve cell

IVC - Intervertebral cartilage

M - Muscle

M_1 - Regenerating muscle

MS - Mesenchyme cells

N - Nerve cord (spinal cord)

N_1 - Regenerating nerve cord (ependyma)

Nu - Nucleus

RTD - Recticular dermis

S - Scab

SAT - Subcutaneous adipose tissue

SC - Scale

SETD - Subepithelial dermis

SG - Stratum germinativum

SG_1 - Stratum germinativum in regenerate skin

SI - Stratum intermedium

SMAT - Submuscular adipose tissue

$SMAT_1$ - Submuscular adipose tissue in the regenerate

SP - Vertebral spine.

V - Vertebral column

VB - Vertebral body

W - White matter

WE - Wound epithelium

OBSERVATIONS AND DISCUSSION

NORMAL TAIL

Integument:

The integument on the dorsal and lateral sides of the tail is thinner and bears numerous small scales in comparison to that of the ventral side which is thicker and possesses a few but larger scales. The integument is divided into an outer epidermis and an inner dermis (Fig. 1).

Epidermis:

The histological features of the epidermis of *H. flaviviridis*, show two phases, the resting and the moulting. In the resting stage (non-moulting period) the epidermis is composed of a thick outer layer of stratified epithelium which is divisible into the beta cell layer (the outer five to eight rows of cells) and the alpha cell layer (three to four rows of cells beneath the beta cell layer) and a basal layer of stratum germinativum (a single row of columnar cells) (Fig.2).

The *beta cell layers* form the outer most highly keratinized horny part of the epidermis which consist of about five to eight rows of cells (Fig. 2). The beta cells are highly flattened and their plasma membrane

highly keratinized. The outermost layers form the pavement layers while the innermost layers are more or less similar in height to the outer layers of the alpha cells. The nuclei of the beta cells are highly flattened and their cytoplasm is clear.

The alpha cell layer consists of three to four rows of cells present just above the stratum germinativum (Fig.2). The alpha cells close to the stratum germinativum are taller and a gradual decrease in height is noticed towards the outer rows of cells. The nuclei of the inner rows of cells of the alpha cell layer are rounded, fairly high in chromatic value and placed in the centre of the cells whereas those of the outer rows are somewhat horizontally elliptical and less chromatic. The plasma membranes of these cells are well thickened as a result of keratinization, which is lesser in the cells of the inner rows than those of the outermost rows.

The stratum germinativum is composed of a single row of columnar cells supported by a thin layer of epidermal basement membrane to which they appear to be firmly attached (Fig. 2). The cells are closely packed with their plasma membrane apposed to one another. The nuclei of these cells are ovoid and highly chromatic and placed at their base. Some of them are often seen to be in active phase of cell division.

The histological changes in the skin of lizards and snakes have been reported by Maderson (1967). Goslar

(1958a, b) described the histological changes during the period of moulting and emphasised the hormonal control of this process. During the moulting period, in the house lizard, additional layers in the epidermis appeared, which were the new generation of the beta and alpha cell layers and a layer of stratum intermedium (Fig. 2). Prior to moulting the additional cell layers mentioned above are formed from the cells of the stratum germinativum. The disintegration of the stratum intermedium was noted as an important phenomenon in moulting of the epidermis.

The stratum intermedium is composed of a single row of columnar epithelial cells which are very much similar in structure to those of the stratum germinativum but differ from the latter in that though arranged vertically, they are slightly oblique in position. The nuclei of these cells are always placed at the base of the cells. These nuclei though chromatic do not show mitotic phases. As regards the new generation of the beta and alpha cells the general features are the same as those described for similar cells in the epidermis during the non-moulting phase. The only difference is that keratinization is lesser in the new generation of beta and alpha cells and the latter are not as much flattened as those of the older generation. After the formation of beta and alpha cell layers, the stratum intermedium is formed in between the

old and new generations of beta and alpha cells. Histochemical observations on the changes in the enzyme activities in the cells of the stratum intermedium indicated the possibility of disintegration of the cells of stratum intermedium, which resulted in casting off of the outer scaly beta and alpha cell layers of the old generation.

The epidermal basement membrane is seen as a thin layer of closely woven connective tissue fibres, firmly adhered to the basal end of the cells of stratum germinativum giving it a firm support. The fibres of this tissue differ from those of the underlying dermis in having a high concentration of phosphatases (Chapters 6 & 7).

Dermis:

The layer of connective tissue with blood vessels, nerve fibres, fat cells etc. below the epidermis constitute the dermis. In the dorsal and lateral regions it is relatively thinner than in the ventral. Quite logical is the greater thickness of the dermis in the ventral side which constantly rubs against the ground and thus requires a thick pad of connective tissue whereas the dorsal and particularly the lateral sides have a thin layer of connective tissue which facilitates movements of

the tail during locomotion. The dermis is composed of two parts, (1) the subepithelial and (2) the deeper reticular (Fig.1). The former is thinner and has compactly arranged connective tissue fibres with fibrocytes and a few pigment cells while the latter is loose in texture with fibres, fibrocytes and some spaces between the connective tissue fibres which are filled with fat cells. The fibrocytes are ovoid in shape with centrally placed nuclei. The fibres of the dermis are mainly collagenous fibres with scattered elastic fibres. The reticular portion of the dermis has thick and coarse collagenous fibres criss-crossing and having their main disposition parallel to the surface of the skin. The subepithelial part of the dermis is composed of collagenous fibres mixed with elastic fibres. Fat cells are rarely seen in this region. In this part of the dermis on the dorsal and lateral sides a number of melanocytes are present which give the colour pattern to the skin. Papillary ridges corresponding to the scales of the epidermis push the epidermis outwards to elevate the keratinized surface as scales. Many such ridges, though smaller in size are seen on the dorsal and lateral sides than on the ventral side of the tail. This accounts for the difference in the number of scales on the different sides of the tail skin.

Subcutaneous adipose tissue:

The presence of fat cells in the reticular part of the

dermis is a general feature in the skin all over the tail but the large number of closely packed fat cells forming an adipose tissue in the subcutaneous region of the tail is restricted only to its lateral sides. Fat cells are very closely placed with their cell membrane apposed to one another, however, at places interstitial connective tissue fibres are found. These cells are normally ovoid or spherical in shape but some appear to be polygonal due to the compact arrangement of cells. The main bulk of the cells is due to the accumulated fat. The cytoplasm of the fat cell is displaced to the periphery and the nucleus is shifted to one side.

Muscle:

Next to the dermis and subcutaneous adipose tissue are the caudal flexor muscles which are segmentally arranged. Each muscle segment is separated from the following one by a transversely but obliquely placed septum of connective tissue which also indicates the plane of autotomy of the tail. These muscles are grouped into fasciculi which are eight in myotomal segments. Each fasciculus is comprised of a number of muscle fibres of different diameters. Based on the diameter and the mitochondrial content, three types of fibres can be differentiated. Broad fibres with a few mitochondria, narrow fibres with a large number of mitochondria and a third type of fibres intermediate with respect to size and mitochondrial content. However, the diameter difference is not very pronounced as seen in the muscles of many other vertebrates viz; Varanus, pigeon,

rat etc. The histochemical studies of certain enzymes have also shown that functionally three types of muscle fibres could be differentiated which correspond with the morphological features of the fibres. Fibres of narrow and intermediate type are confined to a narrow band towards the peripheral region of the fasciculi. Each muscle fibre shows distinct striations and bears a number of spindle shaped nuclei (Fig. 3). The myofibrillae of the fibres are clearly seen. At places intercellular and interfascicular droplets of neutral fat are observed.

Submuscular adipose tissue:

A characteristic feature of the house lizard tail is the presence of a thick layer of submuscular adipose tissue. This is arranged in four quadrants around the vertebral column and below the muscle layer. The adipose tissue is divided into segments of septa which were in continuation of the myotomic segmental septa. The general histological features of fat cells and their arrangement are similar to that described for the subcutaneous adipose tissue earlier (Fig. 4).

Vertebral column:

The bony elements in the tail are the caudal vertebrae. The peculiarity of the caudal vertebrae is that the plane of autotomy passes through the middle of the centrum where

it is narrower in girth giving a hour-glass shape. The general morphological features of the vertebra are the same as those of a typical caudal vertebra of a reptile. Histologically the periosteum is a layer of connective tissue tightly adhering to the bone mass. The collagenous fibres and strands of elastic fibres with fibrocytes constitute the endosteum. The solid part of the vertebra showed irregularly placed osteocytes embedded in the ground substance. The osteoclasts and osteoblasts are also present at the outer and innder periphery of the hollow of the centra of the vertebral body and spines (Fig. 5). The centrum and spines of the caudal vertebrae have a cavity containing bone marrow. The cavity of the centrum is divided into two halves with centrally placed cleavage plane of the vertebra where the centrum cavity is obliterated by vertically or obliquely splitted bony tissue. Large number of fat cells loaded with neutral fat are also present. The blood capillaries enter the cavities of the centrum and the spines of the vertebra. Connecting two adjoining vertebrae there is a intervertebral cartilage (Fig. 5), which is of the hyaline type but at the periphery it has a fibrous structure. The arrangement of the chondrocytes does not show grouping of two or four cells in a lacuna, instead they are singly placed in the matrix of the cartilage, which also shows high collagen fibre deposits.

Spinal cord:

The spinal cord in the caudal region shows thickenings at regular intervals along its length, which indicate the plane of origin of the spinal nerves. The thickened areas correspond with the position of the intervertebral cartilage while the narrow part in between the thickenings is in line with the cleavage plane of the vertebra. Two parts of the nerve cord, viz; the grey matter and white matter are distinctly seen (Fig. 6). The nerve cells are numerous, large in size and have relatively bigger nuclei as compared to those in the white matter of the cord. In the former, amongst the nerve cells there are some giant nerve cells too. However, these are not noticed in the latter the major bulk of which is of nerve fibres. A typical nerve cell is usually with branching projections and has a centrally placed nucleus in which there is only one nucleolus. The cytoplasm of the cells shows considerable amount of fat.

REGENERATING TAIL:

Wound healing phase: (Fig. 7)

Soon after amputation, the cut end of the stump tissue shrinks and the skin border converges to reduce the surface area of the wound. As a result, a crater like depression is formed into which the blood clot spreads to cover the wound surface. After 48 hours the soft tissues of the healing

tail stump are covered by a crust of necrotic cellular materials through which the broken surface of the amputated vertebra some times projects. By about 72 hours after amputation, the cut tip of the blood vessels dialates and lymphocytes, RBCs and leucocytes are seen to spread over the inner surface of the crust. The dedifferentiation of various tissues and the retrogression of the cut end of the vertebra could be noted. Different types of cells, viz; lymphocytes, microglial elements of the spinal cord, melanocytes, erythrocytes and macrophages can be seen beneath the crust (Fig. 8). The proliferation of the new epithelium is seen at the cut end of the original tail stump (Fig. 8) and by about 96 hours after amputation a single layer of epithelium is seen over the wound surface. The dedifferentiation and to a certain extent retrogression continues. Accumulation of undifferentiated cells is noted between the wound epithelium and the dedifferentiating tissues.

Preblastemic phase:

Dedifferentiation continues at the cut end of the stump tissues and the undifferentiated mesenchyme cells accumulate at the subapical region. Similar results have been reported by Chalkley (1954, 1959) in Triturus viridescens; Litwiller (1939) in Triturus pyrrhogaster; Bassina (1940) in Triturus taeniatus and Huges and News

(1959) in Sphaerodactylus. The preblastemic phase is seen as a small conical projection in the middle of the wound epithelial surface. The main histological features at this phase are the increase in the thickness of the epithelium by the multiplication and stratification of the epithelial cells (Fig. 8). High mitotic activity is observed among the undifferentiated cells which accumulate below the epithelium. These cells are compactly placed at the subapical region but are loose at the base. In the mass of mesenchyme cells some blood sinuses are seen.

Blastemal phase: (Fig. 9)

Dedifferentiation is an indispensable preliminary to regeneration and provides cells for the blastema (Schotté, 1939; Rose, 1944; Thornton, 1949). The cells are believed to be chiefly derived by dedifferentiation. However, Godlewski (1928) described the migration of epidermal cells and their transformation into blastemal cells. Rose (1948) reviewed Godlewski's original observations and opined that the epidermis might furnish a meagre share of the young blastema during normal regeneration. Observations of Rose (1948) and Manner (1953) showed the accumulation of epidermal cells in the wound area where they remained and never entered into the blastema proper. Heath (1953) observed that the epidermal grafts never participated in the formation of

blastema. Furthermore Hey and Fischman (1961) working with limb tissue of Triturus viridescens, labelled with thymidine, have given direct evidence that apical epidermal cup cells do not contribute to the regeneration blastema. In the present study on the tail regeneration of H.flaviviridis it was observed that the embryonic cells which were the main bulk of the blastema were formed as a result of dedifferentiation of the stump tissues, viz; the connective tissue of the dermis, the muscles and the spinal cord.

During this phase of regeneration the blastema increased due to the mitosis of the undifferentiated cells and further addition of more such cells by dedifferentiation. Besides, two different types of cells could be seen in the blastema, (1) the epithelial cells which formed the outer margin of the blastema and (2) the mesenchyme cells forming the core of the blastemal cone (Fig. 10). Some blood sinuses could be seen here and there in the mass of the mesenchyme cells.

Late blastemal phase: (Fig.11)

During this phase the regenerate increases in length. The differentiation of the epidermal cell layers, viz; stratum germinativum and outer epidermal cells which are not yet differentiated into beta and alpha cells. During this phase the first signs of differentiation are seen at the base of the regenerate. Most of the mesenchyme cells at the base of the blastema change into fibroblasts, which

acquire an oval shape and possess a single large nucleus placed almost in the centre of the cell. A number of blood capillaries are seen to proliferate in almost all regions establishing a profuse blood circulation.

Differentiation Phase: (Fig.12)

Since the differentiation is a continuous process and cannot be demarcated into distinct phases, the cytological changes during the differentiation of the various tissues are described below under differentiation phase. The differentiation of tissues begin at the proximal end of the regenerate and proceed towards the distal end. However, the beginning of differentiation of all tissues are not simultaneous.

The epidermis begins its differentiation as early as when the regenerate is in the blastemal phase. At the proximal end of the blastema the new epithelium gets stratified, the basal layer of the cells having greater height than the outer ones, are vertically placed with their sides closely apposed to one another forming a compact row. A large nucleus is present at the base of the cells of the stratum germinativum. The epidermal cells, outer to the stratum germinativum are in four to five layers. About 8 days after amputation the differentiation of the epidermal cells could be seen all over the blastema and by this time below the epidermis

fibroblasts get arranged to give rise to dermis(Fig.13). Further, after about 15 days, the regenerate skin at the base shows formation of dermal papillae, the beginning of scale formation. Due to papillae formation, the epidermis acquires a wavy contour (Fig. 14). The papillae formation in the dermis is seen at the base of regenerate in the early stage and then gradually progresses towards the distal end. The fibroblasts in the dermis where papillae are found, are transformed into fibrocytes and fibrous tissue is formed with the laying down of the collagenous and elastic fibres. The epidermal cells above the stratum germinativum differentiate further as beta and alpha cells forming the corneal layers of the epidermis. By about 20 days after amputation, the lamellar pattern of the dermal papillae is seen in the skin all over the regenerating tail and histological changes in the dermis and epidermis noted in the basal part of the regenerate, are now seen throughout the length of the regenerate. In the regenerate the epidermal cells sink in to the dermis at regular intervals and later on a split appears in the sunken cell mass separating the epidermal scales. Initially the gap between the scales on the surface are filled with the epidermal cells, the beta and alpha cells of the old generation. Due to this the outer surface of the skin does not show obvious elevated scales projecting, individually and so the skin surface appears smooth. A 30 day old regnerate skin shows

well formed scales projecting all over the regenerate which has well defined keratinized beta and alpha cell layers (Fig. 15). The growth of the skin requires its epidermal keratinized cell layers to be progressively moulted to permit unobstructed growth of the tail in all directions. Therefore, soon after differentiation, the preparation for moulting of the epidermis is being made, when a new generation of beta and alpha cell layers and a layer of stratum intermedium is formed. The stratum intermedium ultimately gets disintegrated leading to casting off of the old generation of highly keratinized beta and alpha cell layers. The epidermal basement membrane now differentiates and forms a support for the stratum germinativum.

The differentiation of the dermis is observed in the late blastemal period. The fibroblasts which are packed under the epidermis transform into fibrocytes. These cells are arranged in an irregular pattern which results in the formation of a meshwork of connective tissue fibres. The fibrocytes have elongated and well defined nuclei. The connective tissue of the dermis in the regenerate is more compact in the subepidermal region than in the deeper part. The subepidermal part, as mentioned earlier, becomes wavy as the epidermal cells at regular intervals grow towards the dermis. In

the mass of the connective tissue of the dermis large numbers of blood capillaries, nerve fibres, pigment cells and a few fat cells scattered here and there are seen.

Between the dermis and the underlying tissue (which will ultimately be changed into muscles) there is a layer of compactly textured connective tissue, the fascia which binds the two together. In the early phases, i.e. in the late blastema this connective tissue is not differentiated but only 20 days after amputation the fibroblasts in this region begin to differentiate into fibrocytes and closely laid collagenous and elastic fibres appear.

The differentiation of the muscle tissue is first observed at the base of the regenerate during the late blastemal phase. It starts with the formation of mononuclear myoblasts from the mesenchyme cells which lie adjacent to the muscle tissue of the original tail stump, thus appearing to be continuous with the latter. The myoblasts are oval in shape with a single big nucleus in their centre. They transform to give rise to myocytes- spindle shaped cells with a single nucleus- which elongate and some of them fuse with one another longitudinally giving rise to multinucleated muscle fibres (Figs. 16,17, 18). The number of nuclei also increases along with an increase in the length and diameter. During this stage

of the maturation of the fibres, striations appear. The nuclei are first oval in shape but later become spindle shaped as the fibres mature. The differentiation of the muscle in the regenerate is seen to proceed proximo-distally. By about 20 days after amputation the muscle fibres could be seen throughout the length of the regenerate.

As early as the blastemal stage scattered fat cells are noticed in the mesenchyme core of the blastema. These cells are modified fibroblasts that accumulate fat and ultimately form the fat cells. The cytoplasm of the fibroblast destined to become the fat cell shows small globules of neutral lipid which grow in size by accumulation of more fat and also by the fusion of fine fat globules. Thus the largely accumulated lipid in the cell pushes its cytoplasm with the nucleus towards the periphery. During the late blastema a somewhat regular arrangement of fat cells appears at the base of the regenerate, close to the ependyma and surrounding the chondroblasts. This is the beginning of the formation of the submuscular adipose tissue. During the differentiation period the connective tissue cells which are present under the muscles and the surrounding cartilagenous neural canal gradually accumulate fat and transform into fat cells of the adipose tissue (Fig. 19). The latter are closely placed but in the interstitial region a number

of blood capillaries are also present. Unlike the muscle tissue which has a segmental arrangement the adipose tissue remains as a single continuous zone around the cartilagenous neural canal. The submuscular adipose tissue progressively increases as more lipids accumulate and new fat cells form, keeping pace with the growth of the cartilagenous neural canal.

The differentiation of the chondrocytes which form the cartilagenous neural canal is noted in the blastemal phase. The initation of the differentiation is noted as a condensation of the mesenchyme cells near the cut end of the vertebra of the stump. Soon after, the mesenchyme cells give rise to the chondroblasts which in turn transform into the chondrocytes (Figs.20, 21). The chondroblasts like the mesenchyme cells are oblong or ovoid in shape with a rounded nucleus. They further elongate and become chondrocytes which secrete the matrix. These arrange themselves so as to form a cartilagenous neural canal. They are closely packed together unlike in the hyaline or fibrous cartilage where there are more spaces in the matrix. The growth of the cartilage takes place in two ways: (1) by forming new chondrocytes from the chondroblasts at the surface and (2) by expansion of the internal mass of wall of the cartilagenous neural canal by division of the chondrocytes. A fully differentiated cartilagenous

neural canal can be seen almost throughout the length of the regenerated tail by about 20 days after amputation. However, a small portion at the distal end of the regenerate is devoid of this skeletal element. The tip of the canal is closed and the chondroblasts at this end remain in an active stage of cell division, till the length of the tail more or less reaches that of the original.

The extension of the amputated spinal cord as an ependymal tube is observed by about 5 days after amputation. The tube has a central lumen and the tissue matter show two parts- a median loose tissue made up of glial cells and an outer layer of compact mass of cells (Fig. 22). By 20 days after amputation the ependymal tube is seen all along the length of the neural canal of the regenerate (Fig. 23).

The main caudal blood vessel ventral to the cartilagenous neural canal becomes well defined during the differentiation phase.

Growth phase:

The growth of the differentiated tissues in the regenerate can be seen from the late blastemal phase onwards till the regenerate more or less attains the size of a normal adult tail. Once the differentiation takes place, the tissues grow in size either by addition of the differentiated cells from the mesenchyme or by the division of the

already differentiated cells. It is very difficult to have a sharp distinction between the end of differentiation and the beginning of growth. In a fully regenerated tail all the tissues except the skeletal and nervous elements show histological features similar to those in the normal tail. Since there is no ossification of the cartilagenous neural canal leading to the formation of caudal vertebrae, a long and continuous canal without any gaps is formed. The ependyma does not increase in girth to give rise to the full structure of the nerve cord similar to what is present in the stump.

In amphibians once the blastema is established the supply of dedifferentiated cells ceases and growth is entirely by mitosis of the cells that are already differentiated (Litwiller,1939). The increase in cell number outstrips the increase in total size of a regenerated amphibian limb. The elongation of the amphibian limb is partly due to the elongation of the developing muscle fibres (Singer and Graven,1948). In the house lizard, H. flaviviridis; the growth of the tail is due to increase in the number of differentiated cells by mitosis, and also by further differentiation of cells of the mesenchyme tissue present at the distal end of the tail, thereby continuously adding to its own length and that of the differentiated tissues. When the growth of the regenerated tail somewhat

reaches the size of the normal adult tail, the mesenchyme cells at the distal end disappears and further elongation of the tail stops.